Since January 2020 Elsevier has created a COVID-19 resource centre with free information in English and Mandarin on the novel coronavirus COVID-19. The COVID-19 resource centre is hosted on Elsevier Connect, the company's public news and information website.

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•Research article•

Traditional Chinese medicine network pharmacology study on exploring the mechanism of Xuebijing Injection in the treatment of coronavirus disease 2019

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[ABSTRACT] As a representative drug for the treatment of severe community-acquired pneumonia and sepsis, Xuebijing (XBJ) injection is also one of the recommended drugs for the prevention and treatment of coronavirus disease 2019 (COVID-19), but its treatment mechanism for COVID-19 is still unclear. Therefore, this study aims to explore the potential mechanism of XBJ injection in the treatment of COVID-19 employing network pharmacology and molecular docking methods. The corresponding target genes of 45 main active ingredients in XBJ injection and COVID-19 were obtained by using multiple database retrieval and literature mining. 102 overlapping targets of them were screened as the core targets for analysis. Then built the PPI network, TCM-compound-target-disease, and disease-target-pathway networks with the help of Cytoscape 3.6.1 software. After that, utilized DAVID to perform gene ontology (GO) function enrichment analysis and Kyoto Encyclopedia of Genes and Genomes (KEGG) pathway enrichment analysis to predict the action mechanism of overlapping targets. Finally, by applying molecular docking technology, all compounds were docked with COVID-19 3 CL protease(3CLpro), spike protein (S protein), and angiotensin-converting enzyme II (ACE2). The results indicated that quercetin, luteolin, apigenin and other compounds in XBJ injection could affect TNF, MAPK1, IL6 and other overlapping targets. Meanwhile, anhydrosafflor yellow B (AHSYB), salvianolic acid B (SAB), and rutin could combine with COVID-19 crucial proteins, and then played the role of anti-inflammatory, antiviral and immune response to treat COVID-19. This study revealed the multiple active components, multiple targets, and multiple pathways of XBJ injection in the treatment of COVID-19, which provided a new perspective for the study of the mechanism of traditional Chinese medicine (TCM) in the treatment of COVID-19.

[KEY WORDS] Network pharmacology; Molecular docking; Xuebijing injection; COVID-19
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Introduction

Novel coronavirus–infected pneumonia (NCIP) was first discovered in Wuhan, Hubei Province, China in December 2019. On March 11, 2020, the World Health Organization (WHO) declared the 2019 coronavirus disease (COVID-19) is a pandemic [1]. Clinical symptoms such as fever, dyspnea, normal or decreased white blood cell count, organ dysfunction (such as shock, acute heart injury, and acute kidney injury) appear in patients, which can cause death in severe cases [2]. The incubation period of the patients from infection to onset is generally about 5.2 days and approximately 2.3% fatality rate among confirmed cases. Olderly patients as well as patients with other underlying diseases are more likely to die [3, 4]. Due to the high infectivity of the virus, it has a huge negative impact on people's lives and the economic development of the country, but there is still a lack of special drugs and related vaccines [5]. For thousands of years, traditional Chinese medicine (TCM) has accumulated a lot of experience in the treatment of epidemics and has shown a certain effect in the treatment of infectious diseases such as severe acute respiratory syndrome and influenza A virus. Therefore, the National Health Commission and the State Administration of Traditional Chinese Medicine jointly formulated corresponding Chinese medicine diagnosis and treatment plans for the epidemic, and the TCM did show a clinical effect that was not weaker than Western medicine [6, 7].

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XBJ injection is the Category 2 of Traditional Chinese Medicines refined from the ancient prescription "Xuefu Zhuyu Decoction". It is composed of five kinds of TCMs: Safflower (Honghua), Paeoniae Radix Rubra (Chishao), Ligusticum chuanxiong rhizomes (Chuanxiong), Angelica (Danggui), and Salvia miltiorrhiza (Danshen), and is mostly used clinically to treat sepsis [8], acute lung injury (ALI) [9], severe pneumonia [10], multiple organ dysfunction syndrome (MODS) [11] and other critical diseases. Recent studies have shown that XBJ injection can attenuate the excessive production of various inflammatory mediators such as IL-6, TNF-α, MCP-1, MIP-2, and IL-10 in serum and the activation of signaling pathways initiated by Pam3CSK4 [12], which played a protective role in sepsis mice. Combined with XBJ injection to treat septic shock can improve prognosis by reducing the body's inflammatory response [13]. Besides, XBJ injection can stimulate Treg differentiation *in vitro*, moderately inhibit Th17 differentiation, prevent cytokine storms, and improve the survival of patients with septic shock to a certain extent [14]. In addition, XBJ injection can prevent inflammatory reaction after Hepato-Pancreato-Biliary surgery and treat rheumatoid arthritis by regulating the inflammatory reactions of the body [15, 16]. In summary, XBJ injection has a significant anti-inflammatory effect.

XBJ injection has repeatedly appeared in the national diagnosis and treatment plan for major epidemics [17]. In the published "Diagnosis and Treatment of Novel Coronavirus Pneumonia (Trial Version Fifth, Sixth)" [18, 19], XBJ injection was listed as one of the treatment measures for severe and critical cases, and it was recommended for the mid-term clinical treatment period and severe stage. For the conventional type of COVID-19 cases, XBJ injection combined with common antiviral treatment could reduce lung lesions, improve the efficacy, and cut down the incidence of severe cases [20]. Long Wen *et al*. have studied the treatment of 60 severe patients with COVID-19 admitted to the North Hospital of the First Hospital of Changsha City and found that XBJ injection can effectively improve the inflammation markers and prognosis of severe COVID-19 patients [21]. XBJ injection has a positive therapeutic effect on COVID-19 cases, but its underlying mechanism remains unclear.

Network pharmacology is based on the theory of system biology. It constructs an interaction network for drugs, targets, pathways, and diseases, and then analyzes the internal correlation between them by screening key nodes. Therefore, its emphasis is the balance network (or robustness), perturbations in the network, and the biological state of drug effects rather than examining the role of a single or a few scattered targets [22]. The core ideas of the systematization and integrity of network pharmacology are consistent with the characteristics of multi-component, multi-target, and multi mechanism of TCM [23, 24]. It has a wide range of applications in screening active ingredients, explaining the mechanism of drug action, and studying the pathogenesis of diseases [25, 26].

In this study, we aimed to screen the bioactive ingredients of XBJ injection, clarify its therapeutic targets and pathways based on a comprehensive network pharmacology-based strategy. In addition, the molecular docking method was also employed to predict the binding mode and affinity of the pharmacologically active compounds and the potential target proteins of COVID-19: 3CLpro, ACE2, and S-protein. This provides a theoretical basis for the in-depth discussion of the pharmacological mechanism of XBJ injection for COVID-19. The procedures of article were shown in Fig. 1.

Materials and Methods

XBJ injection ingredients collection and targets fishing

The ingredients and the corresponding target genes of XBJ injection were first collected through multiple databases. XBJ injection is composed of 5 kinds of TCM extracts of Honghua, Chishao, Chuanxiong, Danggui, and Danshen. Because of the special nature of the injection, XBJ injection does not contain all the ingredients of these five TCMs. In the early stages, other research groups used UPLC-QTOF/MS and other techniques to identify the composition of XBJ injection [27-29]. Combined with literature reports, we have screened a total of 45 compounds with high content, accurate biological activity report, and important pharmacological activity. With the help of the Traditional Chinese Medicine Systems Pharmacology Database and Analysis Platform (TCMSP, http://tcmspw.com/tcmsp.php), Traditional Chinese Medicine Integrated Database (TCMID, http://119.3.41.228:8000/tcmid/search/), Encyclopedia of Traditional Chinese Medicine (ETCM, http://www.nrc.ac.cn:9090/ETCM/index.php/Home/Index/index.html), We searched putative targets of 45 compounds.

Owing to the characteristics of the database, the related targets of some compounds have not been updated or included in time. Accordingly, in the meantime, we searched literatures with the compound name plus "mechanism" or "target" as the keywords in PubMed (https://pubmed.

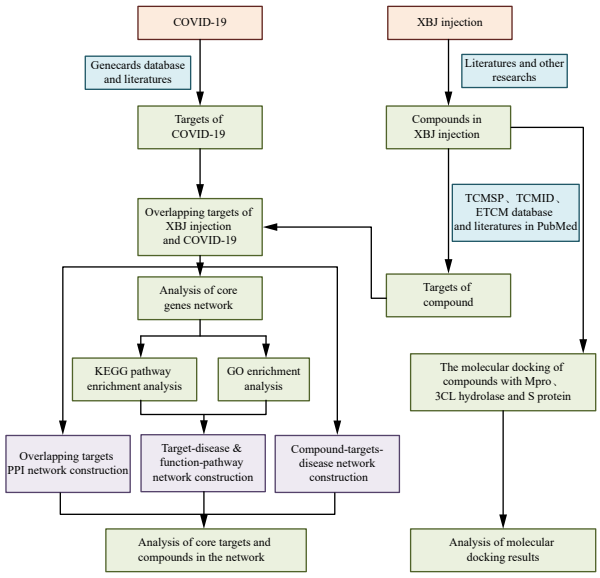

Fig. 1 The workflow of mechanism of XBJ Injection in the treatment of COVID-19

ncbi.nlm.nih.gov). Artificial screening of targets directly affected by compounds shown in recent five-year literatures which were related to the mechanism research about this compound. Targets with unclear meaning and indirect effects of compounds were not adopted. Removed duplicate targets collected from literatures and databases, and applied the Uniprot (http://www.uniprot.org) database to unify the targets into standard gene names. Thus, a target database of compounds was formed.

Collection of COVID-19 related targets

To construct networks, we next collected target genes related to the COVID-19 disease. The potential targets for COVID-19 were obtained from two resources: the GeneCards database (http://www.genecards.org/) and PubMed. GeneCards [30] is a comprehensive bioinformatics database, which provides researchers with platforms and tools to effectively explore human genes, diseases, cells, proteins, *etc*. Used "Novel Coronavirus Pneumonia" as a keyword to search for potential target genes of COVID-19 in the GeneCards database. Meanwhile, "COVID-19" was applied as a keyword to retrieval in the PubMed database and manually sorted out the COVID-19 related genes in literatures. After that, removed duplicate values and organized these data as a COVID-19 target database. The online Venn diagram tool was employed for identification of the overlapping targets of XBJ injection and COVID-19, thus obtaining the core targets of XBJ injection for COVID-19 treatment.

Enrichment analysis

In order to acquire a deeper understanding of the functions of the obtained core target genes and their roles in the signaling pathway, we imported the screened core targets into the DAVID database (https://david.ncifcrf.gov/home.jsp) and set the corresponding species as "Homo Sapiens". DAVID is a bioinformatics database that integrates biological data and analysis tools to associate genes with biological annotations to find the most significantly enriched annotation pathways. Therefore, it can help researchers quickly extract meaningful biological information from large-scale genes [31]. We utilized the Functional Annotation Tool in the DAVID database to obtain Gene Ontology (GO) analysis and Kyoto Encyclopedia of Genes and Genomes (KEGG) analysis results. Sorted according to the "FDR" value and screened out the top ten KEGG and GO results into networks for further study.

Network construction

Network construction can filter out key nodes from the complex interaction of drugs, target genes and diseases. All networks were visualized in Cytoscape 3.6.1 software. To explain the latent pharmacological mechanism of XBJ injection on the treatment of COVID-19, TCM-compound-target-disease, and disease-target-pathway networks were established. Imported the names of diseases, compounds, core targets, and pathways into Cytoscape 3.6.1 software to separately construct TCM-compound-target-disease and disease-target-pathway networks.

Moreover, the "network analyzer" plug-in was applied to calculate the network topology parameters of the nodes, mainly considering the Betweenness Centrality, Closeness Centrality, and degree. It is generally believed that nodes with larger parameters might be meaningful compounds or targets and act a pivotal part in the entire biological network.

PPI network construction

Proteins tend to form macromolecular complexes through interactions to perform biological functions. Protein-protein interaction (PPI) network analysis helps us study the molecular mechanisms, biological processes, and functions of disease treatment from a systematic perspective. Inputted the overlapping target genes of compounds and disease into the STRING database (https://string-db.org/) to analyze the PPI between the target proteins. Set the organism to "Homo sapiens" and the scoring value (confidence) > 0.7. In order to determine the core targets of XBJ injection for COVID-19 treatment, the obtained PPI network data was imported into Cytoscape 3.6.1 software for further analysis.

Composition-target molecular docking

The studies have found that the key to the infection of human cells by the novel coronavirus (SARS-CoV-2) is the combination of the coronavirus S protein and the human ACE2 protein, which makes the virus invade the human body and cause disease [32]. 3CLpro is a key protein for viral RNA replication. Inhibiting the function of 3CLpro can block viral replication with a high probability, and indicate the direction for the development of antiviral drugs. The active ingredients in XBJ injection were docked with these three proteins. Meanwhile, drugs (remdesivir, lopinavir, ritonavir) that might be effective for the COVID-19 were considered as positive controls. First, we downloaded the pdb format files (PDB ID: 1R42, 6LU7, 6VSB) of the protein 3D structure from the RSCB PDB database (https://www.rcsb.org/), and applied the Protein Preparation Wizard module in Meastro10.1 software to process them with operations such as hydrogenation and dewater. After that, saved the files as the pdb suffix, and used the Receptor Grid Generation module to generate the grid files for docking. Downloaded the sdf files of the 3D / 2D structure of the active compounds and positive controls from the PubChem database (https://pubchem.ncbi.nlm.nih.gov/), and pretreated ligands with the aid of the Ligprep Preparation module in Meastro10.1 software. Imported the generated protein grid files and the pre-processed ligands into the Ligand Docking module for extra precision (XP) docking. Using docking scores of the positive drugs as the reference values, screened for compounds that were superior to the reference values.

Results

Common targets of XBJ injection and COVID-19

We searched the literature using "XBJ injection constituents" as the keyword. Based on the chemical composition of XBJ injection measured in other studies, we finally determined 45 chemical components with high content, definite bio-

Table 1 Basic information of active compounds in XBJ injection

Traditional Chinese Medicine	Number	Molecule Name	Relative molecular mass
Honghua	CHEM1	Luteoloside	448.38
	CHEM2	Luteolin	286.25
	CHEM3	Apigenin	270.25
	CHEM4	Uridine	244.20
	CHEM5	Quercetin	302.25
	CHEM6	Protocatechuic acid	154.12
	CHEM7	Caffeic acid	180.17
	CHEM8	Rutin	610.57
	CHEM9	Kaempferol	286.23
	CHEM10	Gallic acid	170.13
	CHEM11	5-hydroxymethyl furfural	126.20
	CHEM13	Crocetin	976.96
	CHEM14	Safflower yellow A	610.518
	CHEM25	Guanosine	283.24
	CHEM26	Hydroxysafflor yellow A	612.59
	CHEM27	Chlorogenic acid	354.31
	CHEM29	Naringenin	272.25
	CHEM45	Anhydrosafflor yellow B	1044.9
Chishao	CHEM6	Protocatechuic acid	154.12
	CHEM9	Kaempferol	286.23
	CHEM10	Gallic acid	170.13
	CHEM12	Paeonol	166.19
	CHEM16	Paeoniflorin	480.45
	CHEM17	Albiflorin	480.46
	CHEM18	Galloylpaeoniflorin	632.57
	CHEM19	Oxidized paeoniflorin	496.51
	CHEM29	Naringenin	272.25
	CHEM31	Catechin	290.28
	CHEM32	Matrine	248.36
	CHEM33	Benzoylpaeoniflorin	584.57
Danshen	CHEM2	Luteolin	286.25
	CHEM3	Apigenin	270.25
	CHEM6	Protocatechuic acid	154.12
	CHEM7	Caffeic acid	180.17
	CHEM15	Protocatechuic aldehyde	138.13
	CHEM31	Catechin	290.28
	CHEM34	Salvianolic acid B	718.66
	CHEM35	Cryptotanshinone	296.39
	CHEM36	Danshensu	198.17
	CHEM37	Salvianolic acid A	494.48
	CHEM38	Salvianolic acid C	492.44
	CHEM39	Tanshinone IIA	294.34
	CHEM40	Tanshinone I	276.29
	CHEM42	Rosmarinic acid	360.34
	CHEM43	Danshensu sodium	220.16
Chuanxiong	CHEM3	Apigenin	270.25
	CHEM6	Protocatechuic acid	154.12
	CHEM7	Caffeic acid	180.17

Continued

Traditional Chinese Medicine	Number	Molecule Name	Relative molecular mass
	CHEM10	Gallic acid	170.13
	CHEM21	*n*-Butylidenephthalide	188.22
	CHEM22	Levistolide A	380.48
	CHEM23	Ligustrazine	136.19
	CHEM30	Senkyunolide H	224.25
	CHEM44	Senkynolide I	204.24
Danggui	CHEM4	Uridine	244.20
	CHEM7	Caffeic acid	180.17
	CHEM20	Hypericin	504.4
	CHEM24	Ferulic acid	194.20
	CHEM25	Guanosine	283.24
	CHEM28	Ethyl ferulate	222.24
	CHEM41	Ligustilide	190.24

logical activity reports, and important pharmacological activities. The information about compounds in each TCM is shown in Table 1.

Employing the TCMSP, TCMID, ETCM, and GeneCards platforms, the known and predicted targets of 45 compounds and COVID-19 were retrieved. Then, through the search of literature, experimentally proven human targets directly affected by 45 compounds and COVID-19 were also included. The original data source is shown in Fig. 2A. The specific target gene data sources of XBJ injection are shown in supplemental file 1, and details of COVID-19 related target genes are shown in supplemental file 2. After removing the duplicate data, a total of 408 targets related to XBJ injection and 265 targets related to COVID-19 were obtained. Among them, XBJ injection and COVID-19 had a total of 102 coincident targets (Fig. 2B), which became the focus of our follow-up analysis.

Go and KEGG pathway enrichment analysis

102 overlapping targets were performed for enrichment analysis using the DAVID online tool. GO functional enrichment analysis yielded 217 GO results ($P < 0.05$), including 162 biological process (BP) results, 22 cell composition (CC) results, and 33 molecules function (MF) results, accounting for 75%, 10%, and 15%, respectively. The top-ranked GO enrichment pathways including extrinsic apoptotic signaling pathway in absence of ligand, inflammatory response, lipopolysaccharide-mediated signaling pathway, intrinsic apoptotic signaling pathway in response to DNA damage, *etc*. 117 pathways were screened by KEGG enrichment results ($P < 0.05$). Screened the top 20 KEGG results and the top 10 BP, CC, MF results in GO annotation analysis according to the *P*-value and visualized them separately with the R software package. The results are shown in Figs. 3A, B.

As shown in Figs. 3A, B, a variety of pathways which related to inflammation and immune response were significantly enriched, such as the TNF signaling pathway, inflammatory bowel disease (IBD) pathway, the Toll-like receptor signaling pathway, the Hypoxia-inducible factor (HIF)-1 signaling pathway, the P13K-AKT signaling pathway, the MAPK signaling pathway, apoptosis, and the Nucleotide-binding oligomerization domain (NOD)-like receptor signaling pathway. The enrichment level of pathways related to

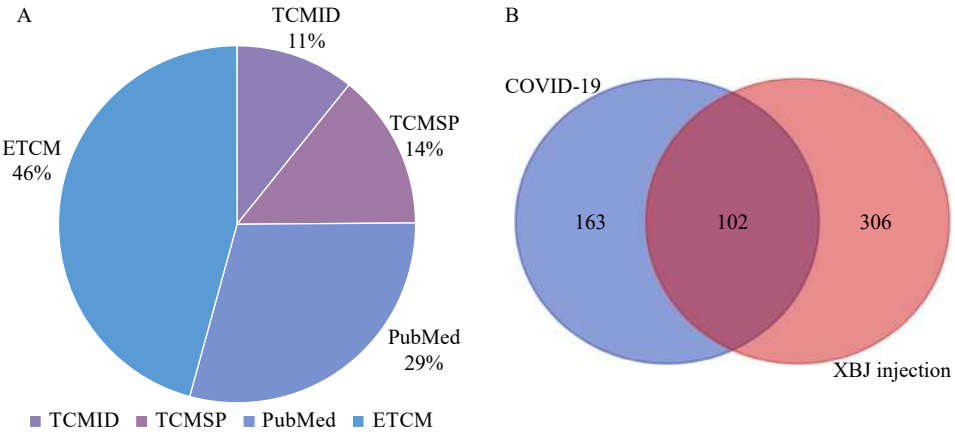

Fig. 2 (A) Data sources of active compounds in XBJ injection. 29%, 14%, 46% and 11% of the data were separately from PubMed, TCMSP, ETCM and TCMID. (B) Identification of overlapping targets of COVID-19 and XBJ injection. 102 overlapping targets were obtained from XBJ injection and COVID-19

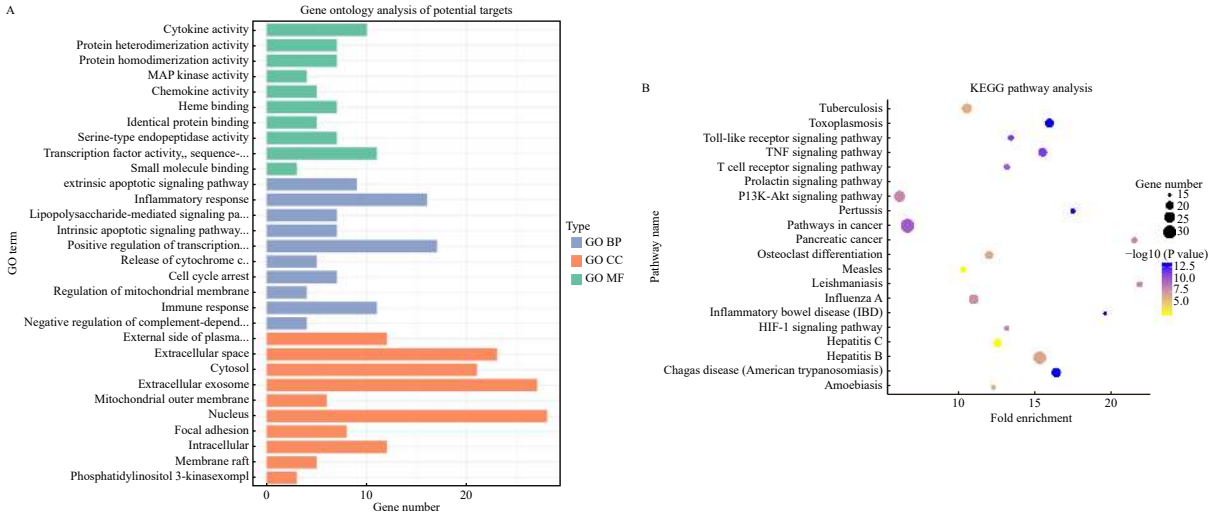

Fig. 3 Enrichment analysis results of core targets in XBJ injection. (A) Top 10 BP, CC and MF results in GO annotation analysis. The y-axis stands for enriched GO of the targets, and the x-axis stands for the gene number in the GO annotation results. (B) Top 20 KEGG pathway enrichment bubble diagram ($P < 0.05$).The y-axis stands for enriched pathways of the targets, and the x-axis stands for the values of fold enrichment

regulating viral infections was also unignored. Those pathways involved hepatitis B, hepatitis C, influenza A, and Epstein-Barr virus infection. Furthermore, it also showed a certain degree of enrichment in cancer pathways and other infectious lung diseases such as pertussis, tuberculosis. Therefore, we inferred that XBJ injection might be beneficial to the treatment of COVID-19 by regulating inflammation and immune regulation related pathways, viral infection related pathways.

Network construction and analysis

Cytoscape 3.6.1 software was employed to draw the TCM-compound-target-disease and disease-target-pathway networks (See Figs. 4A, B). The TCM-compound-target-disease network comprised a total of 153 nodes (1 disease node, 5 medicinal materials nodes, 42 compound nodes, 105 target nodes) and 599 edges, of which compounds such as danshensu sodium, galloylpaeoniflorin and anhydrosafflor yellow B had not found their corresponding targets. These inter-

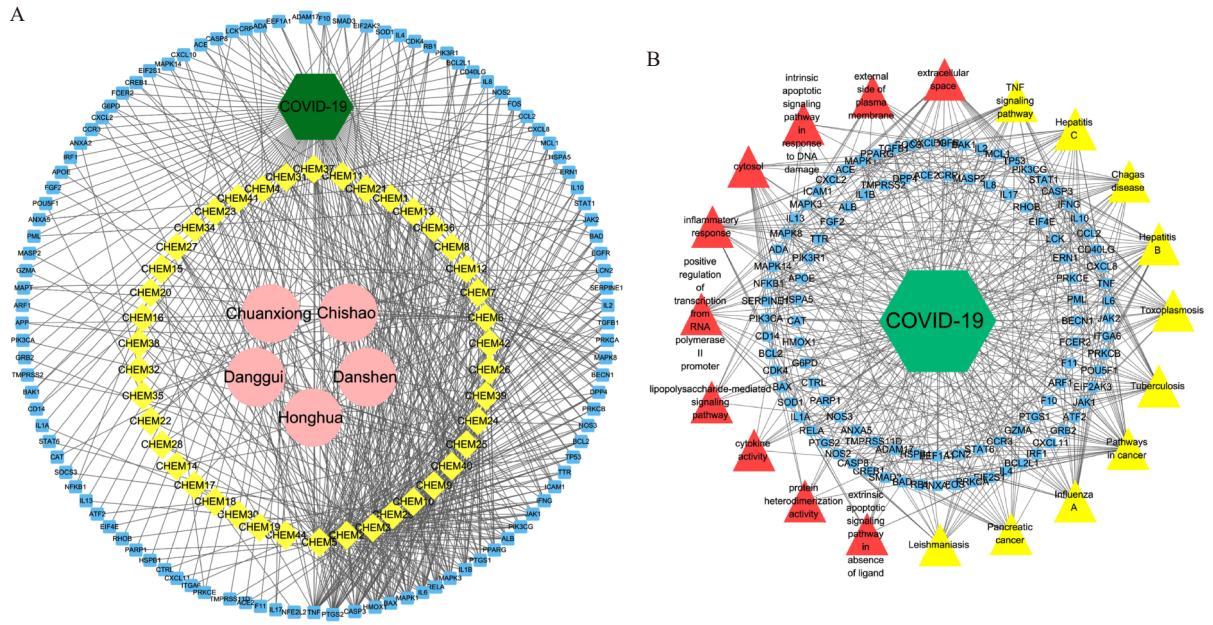

Fig. 4 Network construction. (A) TCM-compound-target-disease network of core targets of XBJ injection and COVID-19. The pink circle represents five TCM in XBJ injection, and the yellow diamond represents the main active compounds contained in five TCM. The blue rectangle represents the core targets, while the green hexagon represents COVID-19. (B) Disease-target-pathway network of core targets. The blue rectangle represents the core targets, and the green hexagon represents COVID-19. The red triangle represents the top 10 GO annotation results, while the yellow triangle represents the top 10 KEGG enrichment results

actions indicated that one compound was able to modulate many targets, and one target was capable of being modulated by multiple compounds simultaneously. According to the node degree parameters of the nodes in the network (shown in Table 2), the top five compounds in the network were quercetin (CHEM5), luteolin (CHEM2), apigenin (CHEM3), Naringenin (CHEM29), Gallic acid (CHEM10), and could interact with 52, 35, 34, 26, 25 targets respectively. From the perspective of the target, the top 5 were TNF, PTGS2, CASP3, BAX, and HMOX1, which could interact with 22, 21, 21, 19, 19 compounds respectively.

For further analysis, sorted by the corrected *P*-value, that was, FDR value, the top 10 KEGG pathways and GO annotation results were added into disease-target-pathway networks. As can be seen from the network, the targets of COVID-19 were coordinated and controlled by multiple pathways. The pathway with the most enriched genes was the pathways in cancer, with 32 genes, followed by the Hepatitis B pathway and extracellular space annotation with 28 genes and 23 genes respectively.

Table 2 Node parameters of TCM-compound-target-disease network for XBJ injection

Names on the network	Betweenness Centrality	Closeness Centrality	Degree	Name
CHEM5	0.0851	0.4750	52	Quercetin
CHEM2	0.0299	0.4222	35	Luteolin
CHEM3	0.0456	0.4294	34	Apigenin
CHEM29	0.0277	0.4086	26	Naringenin
CHEM10	0.0335	0.4108	25	Gallic acid
CHEM9	0.0170	0.3979	24	Kaempferol
CHEM40	0.0158	0.3838	20	Tanshinone I
CHEM25	0.0166	0.3819	18	Guanosine
CHEM24	0.0134	0.3838	18	Ferulic acid
CHEM42	0.0101	0.4234	17	Rosmarinic acid

PPI network analysis

Studying the interaction network between proteins helps to mine the core regulatory genes. Entered 102 overlapping targets related to COVID-19 and XBJ injection into the STRING database to construct the PPI network (See Fig. 5A). The network had 94 nodes and 779 interaction relationships, with an average degree value of 16.6. Then used Cytoscape 3.6.1 for visual processing, and applied the Degree descending arrangement in the CytoHubba plugin to filter out 40 core targets that were greater than the average degree value. The top ten target proteins are shown in Fig. 5B. Combining PPI network, TCM-compound-target-disease, and disease-target-pathway networks through Merge function in Cytoscape 3.6.1(results see Table 3), the results suggested that TNF, MAPK1, IL6, and other genes might play an important role in the treatment of COVID-19.

Analysis of molecular docking results

Molecular docking results revealed that most of the active compounds in XBJ injection had a good binding activity with ACE2, 3CLpro, and S protein. Table 4 evinced the

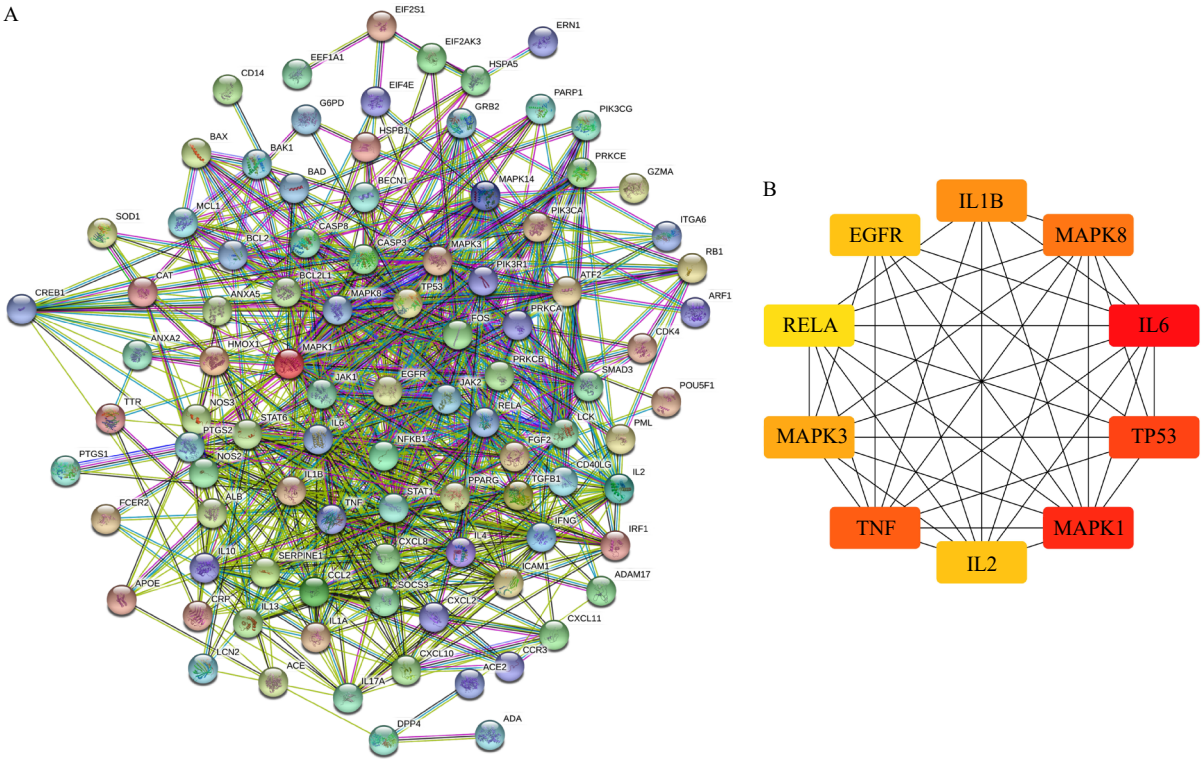

Fig. 5 PPI network construction. (A) PPI network of overlapping targets of XBJ injection and COVID-19. (B) Core genes of identified targets. Among all the core targets, the darker the red, the more important it was

Table 3 Node parameters of Merge network for XBJ injection

Names on the network	Betweenness Centrality	Closeness Centrality	Degree	Name
TNF	0.2156	0.6705	78	Tumor necrosis factor
MAPK1	0.0536	0.6201	75	Mitogen-activated protein kinase 1
IL6	0.0487	0.6201	73	Interleukin-6
MAPK3	0.0334	0.6092	63	Mitogen-activated protein kinase 3
RELA	0.0307	0.5904	62	Transcription factor p65
PTGS2	0.0395	0.5904	58	Prostaglandin G/H synthase 2
CASP3	0.0338	0.5825	57	Caspase-3
TP53	0.0310	0.5786	57	Cellular tumor antigen p53
MAPK8	0.0301	0.5786	56	Mitogen-activated protein kinase 8
IL1B	0.0200	0.5805	51	Interleukin-1 beta

Table 4 Molecular docking score of active components in XBJ injection and positive drugs

Chemical Name	ACE2	3CLpro	Spike Protein
Ritonavir	−5.092	−4.228	−7.954
Lopinavir	−5.561	−6.114	−8.594
Remdesivir	−6.03	−8.1	−9.714
Quercetin	−5.898	−7.775	−8.572
Luteolin	−6.258	−6.84	−7.838
Apigenin	−4.34	−6.112	−6.322
Anhydrosafflor yellow B	−10.796	−11.184	−17.205
Salvianolic acid B	−7.576	−11.261	−14.563
Rutin	−7.069	−9.888	−14.044

docking scores of the three positive drugs, the top three compounds in the network, and the top three compounds with the best binding with the three proteins. The docking results of them are shown in Fig. 6. The results of molecular docking of all compounds are shown in supplemental file 3. Taking the docking score of the positive drugs as a control, the more negative the score, the better the docking result between the compound and the protein. The top three compounds in the network analysis results, which were quercetin, luteolin, and apigenin, all had similar docking scores as positive drugs but not reached the ideal value. The docking scores of AHSYB, SAB, and rutin were significantly higher than those of the positive control. It demonstrated that the compounds that were not ranked very high in the network may also be the active ingredients of XBJ injection for COVID-19.

Discussion

Since the outbreak of COVID-19 in December 2019, a total of more than 80 000 people has been diagnosed in China. As of May 11, 2020, there were more than 3 million confirmed cases outside China [33]. The effectiveness of TCM on the treatment of epidemics is well documented. In the SARS period, TCM as a supplementary means made a significant contribution. Moreover, in the treatment of COVID-19, TCM has played a role that cannot be ignored. It has been reported that the use of TCM to treat 102 patients with mild symptoms has shortened the disappearance of clinical symptoms and the recovery time of body temperature. The average length of hospitalization and the incidence of severe cases were significantly reduced, and the improvement rate of CT image was increased [34].

The initial symptoms of COVID-19 are not obvious, mainly manifested as no symptoms or fever, dry cough, and fatigue. However, about 75% of the patients can develop into serious diseases, which accompanied by dyspnea and severe

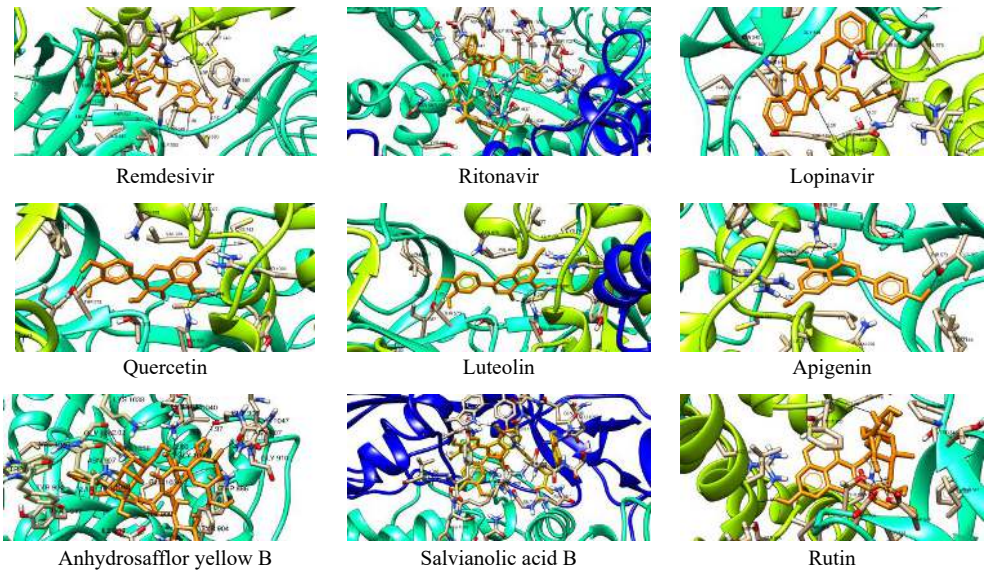

Fig. 6 Molecular docking results of the three positive drugs, the top 3 compounds in the network, and top 3 compounds with the best binding. A yellow object represents a compound and The surrounding chain represents an anti-inflammatory target

pulmonary infection, such as acute respiratory distress syndrome (ARDS), sepsis and so on. So, at this stage, the death rate of patients is very high, which is about 15% [35]. XBJ injection is listed as one of the treatment measures for the severe and critical case and a lot of evidence has shown that XBJ injection has a significant anti-inflammatory effect. For instance, XBJ injection is the only Chinese patent medicine approved in China for the treatment of sepsis which can treat sepsis by reducing inflammatory reaction [8, 12]. Meanwhile, ARDS and multiple organ failure in severe patients of COVID-19 were reported to be associated with excessive inflammation and cytokine storms [36]. Therefore, XBJ injection may intervene to treat COVID-19 by regulating inflammation and immune response. Among the 5 Chinese herbal medicine ingredients of XBJ injection, the main components of Honghua include apigenin, safflower yellow A, quercetin, *etc*. It is reported that Honghua can play a pivotal role in the prevention of ALI and other inflammatory lung diseases though regulating Nrf2 [37]. The main components of Chishao are paeoniflorin, paeonol, gallic acid, *etc*. In particular, paeoniflorin is the most abundant compound in XBJ injection. Paeoniflorin could exert therapeutic effect on sepsis *via* decreasing plasma sTREM-1 level and inflammatory response [38]. Chuanxiong contains components such as ligustrazine, apigenin, butylphthalide, and so on. Among them, Ligustrazine could ameliorate the inflammatory damage through inhibiting the Rho/ROCK pathway, thereby interfering with the endothelial dysfunction treatment in ALI [39]. Besides, cryptotanshinone, as a component of Danshen, had antitumor activity by inducing the immune response of the body, and also had a certain therapeutic effect on pulmonary fibrosis [40, 41]. In summary, XBJ injection can exert anti-inflammatory effects through a variety of components, which has a significant impact on lung inflammatory diseases.

According to the results of network analysis, TNF, MAPK1, and IL-6 might be the crux in the treatment of COVID-19, and all of them were the targets of quercetin, luteolin, and apigenin. IL-6 is a versatile cytokine with a wide range of functions. It has the functions of regulating immune response, acute phase response, and tissue damage, and participates in the anti-infective immune response of the body [42]. Drugs targeting IL-6 or down-regulating IL-6, such as tocilizumab and sarilumab, might be effective in blocking inflammatory storms, so they could become the promising treatment for patients with COVID-19 [43, 44]. Mitogen-activated protein kinase (MAPK) has been reported as a key regulator of ALI [45]. Mechanistically, MAPK1 could achieve the purpose of treating cell injury and lung injury by decreasing cell inflammation and apoptosis. At the same time, studies have suggested that medicines targeting the MAPK3/MAPK1 signaling pathway may has crucial value in the treatment of various inflammatory diseases [46, 47]. Tumor necrosis factor alfa (TNFα) is present in the blood and diseased tissues of patients with COVID-19, and TNFα levels are much higher in patients with severe diseases [42]. TNF could activate IL-6 and IL-1, meanwhile, anti-TNF could decrease leucocyte traffick to inflamed tissues by reducing adhesion molecules and chemokines [48, 49]. Thus TNF, as an essential target, is able to contribute to the immunotherapy of COVID-19 [50, 51]. Besides, in severe patients, serum levels of IL-2, IL-7, IL-10, GSCF, IP10 (CXCL10), MCP1 (CCL2), and MIP1A (CCL3)were significantly higher than that of non-severe patients [35, 52, 53]. Therefore, the ingredients in XBJ injection are capable of subsiding the inflammatory response by reducing the concentration of the following factors in serum: IL-1B, IL-2, IFNG, TNF-α, CXCL10, CCL2, IL10, IL-17, IL-8. KEGG enrichment results showed that TNF, MAPK1, and IL-6 can be enriched to the following pathways: TNF signaling pathway, Influenza A, Tuberculosis, Toll-like receptor signaling pathway, PI3K-Akt signaling pathway, and NOD-like receptor signaling pathway, which suggested that XBJ injection might target IL6, MAPK1, and TNF through quercetin, luteolin, and apigenin, and then affected inflammation, immune regulation, and viral infection related pathways, played an anti-inflammatory effect to treat COVID-19.

The interaction between the viral S protein and ACE2 on the surface of the host cell is important because ACE2 can participate in the regulation of the renin-angiotensin system to trigger lung inflammation. 3CLpro, a viral protease, was regarded as the target of COVID-19 and used to find many potential compounds, such as lopinavir. Therefore, these three are regarded as ideal targets for the treatment of COVID-19 [54]. Among the molecular docking results of all compounds with 3CLpro, S protein, and ACE2, the three compounds with the highest scores were AHSYB, SAB, and rutin. After searching the database, we found that there was little information about the potential target of AHSYB, which indicated that there were few studies on its pharmacological activities. However, with safflor yellow A and hydroxysafflor yellow A, it can ameliorate the lung inflammation and damage caused by lipopolysaccharide through inhibiting the formation of the neutrophil extracellular trap (NET) [55]. SAB, a water-soluble component of Danshen, usually exists in the form of magnesium salt, namely magnesium lithospermate B. It has been reported that magnesium lithospermate B and rosmarinic acid can an exert antiviral effect through reducing the propagate of Enterovirus 71(EV71) particles [56]. In the past studies, rutin, in addition to its well-known antioxidant activity, has shown a good inhibitory effect on hepatitis C virus (HCV), EV71, and other viruses [57, 58]. The above results indicated that AHSYB, SAB, and rutin in XBJ injection might be useful in the treatment of COVID-19 by inhibiting virus invasion and replication.

However, due to the lack of understanding of viruses, disease complications, there are some deficiencies in our study, such as the lack of in-depth research on the predictive ingredients and core targets and pathways. Thus, further investigation and relevant validation *in vivo* and *in vitro* are needed.

Conclusion

In this study, network pharmacology and molecular

docking technology were used to explore the potential regulatory mechanism of XBJ injection in the treatment of COVID-19. It is suggested that XBJ injection may affect IL-6, TNF, and MAPK1 targets through quercetin, luteolin, and apigenin to inhibit excessive inflammation and cytokine storm caused by COVID-19. The role of XBJ injection involves a variety of biological processes, mainly signaling pathways such as the TNF signaling pathway, Influenza A, Tuberculosis, Toll-like receptor signaling pathway, PI3K-Akt signaling pathway, and so on. Meanwhile, AHSYB, SAB, and rutin suppressed the invasion and replication of novel coronavirus, which will play a role in the treatment of COVID-19. It embodies the therapeutic effect of XBJ injection on COVID-19 with the multi-component, multi-target, and multi-channel.

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