Examining the effector mechanisms of Xuebijing Injection on COVID-19 based on network pharmacology

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Research

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Abstract

Objective To examine the potential effector mechanisms of Xuebijing (XBJ) on coronavirus disease 2019 (COVID-19) based on network pharmacology.

Methods We searched Chinese and international papers to obtain the active ingredients of XBJ. Then, we compiled COVID-19 disease targets from the GeneCards gene database and via literature searches. Next, we used the SwissTargetPrediction database to predict XBJ's effector targets and map them to the abovementioned COVID-19 disease targets in order to obtain potential therapeutic targets of XBJ. Cytoscape software version 3.7.0 was used to construct a "XBJ active-compound–potential-effector target" network and protein–protein interaction (PPI) network, and then to carry out network topology analysis of potential targets. We used the ClueGO and CluePedia plugins in Cytoscape to conduct gene ontology (GO) Biological Process (BP) analysis and Kyoto Encyclopedia of Genes and Genomes (KEGG) signaling pathway enrichment analysis of XBJ's effector targets.

Results We obtained 147 potential COVID-19 effector targets of XBJ. Fourteen of these targets—glyceraldehyde 3-phosphate dehydrogenase (*GAPDH*), albumin (*ALB*), tumor necrosis factor (*TNF*), epidermal growth factor receptor (*EGFR*), mitogen-activated protein kinase 1 (*MAPK1*), Caspase-3 (*CASP3*), signal transducer and activator of transcription 3 (*STAT3*), *MAPK8*, prostaglandin-endoperoxide synthase 2 (*PTGS2*), *JUN*, interleukin-2 (*IL-2*), Estrogen Receptor 1 (*ESR1*), and *MAPK14*—had degree values > 40 and therefore could be considered key targets. They participated in extracellular signal–regulated kinase 1 and 2 (*ERK1*, *ERK2*) cascade, the T-cell receptor signaling pathway, activation of *MAPK* activity, cellular response to lipopolysaccharide (LPS), and other inflammation- and immune-related BPs. XBJ exerted its therapeutic effects through the renin–angiotensin system (RAS), nuclear factor κ-light-chain-enhancer of activated B cells (NF-κB), *MAPK*, phosphatidylinositol-4,5-bisphosphate 3-kinase (*PI3K*)–protein kinase B (*Akt*)–vascular endothelial growth factor (VEGF), toll-like receptor (TLR), TNF, and inflammatory-mediator regulation of transient receptor potential (TRP) signaling pathways to ultimately construct a "ingredient–target–pathway" effector network.

Conclusion: The active ingredients of XBJ regulated different genes, acted on different pathways, and synergistically produced anti-inflammatory and immune-regulatory effects, which fully demonstrated the synergistic effects of different components on multiple targets and pathways. The results of this study validated current pharmacological mechanistic studies of XBJ in the treatment of sepsis and severe pneumonia and could better explain XBJ's effector mechanisms in the clinical treatment of COVID-19.

1. Introduction

In December 2019, the Huanan Seafood Wholesale Market in Wuhan, Hubei Province, China, became the epicenter of an outbreak of pneumonia of unknown etiology, which attracted a great deal of attention in China and the rest of the world. Chinese scientists quickly isolated a novel coronavirus from patients, severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2), the causative agent of coronavirus

disease 2019 (COVID-19) [1, 2]. As of March 29, 2020, the global number of confirmed cases was 634,835 and the global death toll was 29,891. Currently, scientists around the world are conducting an exhaustive search for effective antiviral drugs. However, the only feasible method at this writing is the use of various broad-spectrum antivirals, such as nucleoside analogs and HIV protease inhibitors [3]. Up to now, no COVID-19–specific antiviral drugs or vaccines have been developed, and the aforementioned drugs can only reduce viral infection [4, 5].

Many results from clinical practice show that TCM plays an important role in COVID-19 treatment and has brought new hope for controlling this disease [6]. Xuebijing (XBJ) was included in the *Diagnosis and Treatment Plan for Coronavirus Disease 2019* (interim 7th edition) [7] that was jointly released by the National Health Commission and National Administration of Traditional Chinese Medicine. XBJ is composed of extracts of *Carthamus tinctorius*, *Paeonia anomala*, *Ligusticum striatum*, *Salvia miltiorrhiza*, and *Angelica sinensis* [8]. This compound can boost circulation, relieve stasis, and clear blocked meridians, and it is widely used in China to treat active inflammation [9, 10]. In 2004, XBJ was approved by the National Medical Products Administration (formerly the China Food and Drug Administration) for the treatment of systemic inflammatory-response syndrome, sepsis, and multiple-organ dysfunction syndrome (MODS) [11, 12]. Studies show that XBJ treatment can reduce the secretion of pro-inflammatory cytokines such as interleukin (IL)-6, IL-13, and tumor necrosis factor alpha (TNF-α) to alleviate inflammation and thereby inhibit liver damage [13]. Chen et al. showed that XBJ treatment can decrease oxidative stress (OS) and levels of pro-inflammatory cytokines [14]. Li et al. showed that XBJ can regulate immune response, including reducing inflammatory mediators and bacterial load, and plays a protective role in bacterial infections, particularly those caused by drug-resistant bacteria such as methicillin-resistant *Staphylococcus aureus* (MRSA) [15]. A real-world study also pointed out that the incidence of adverse reactions from XBJ in clinical practice is low (0.3%), and most adverse reactions are mild. Therefore, XBJ could be a safe and effective drug for treating COVID-19, but its specific molecular mechanisms are still unknown.

Network pharmacology uses drug, compound, gene, and disease database information to construct drug–target, target–disease, and drug–disease interaction networks in order to reveal the complex mechanisms of TCM formulations that have multiple targets and multiple component characteristics [16]. The concepts of network pharmacology share many similarities with the holistic view of TCM, as both use systemic methods to treat complex diseases such as cancer. This provides a basis for the transition from empirical medicine to evidence-based medicine [16, 17]. Based on chemical-matteromics study results for XBJ, we employed network pharmacology to construct a “component–target–pathway” network model in order to comprehensively and systematically predict potential effector targets and pathways of this compound’s main chemical components in COVID-19 treatment. These findings will provide a scientific basis for further research into effective substances and mechanisms in XBJ treatment of COVID-19.

2. Materials And Methods

2.1 Collection of potential active ingredients of XBJ

We searched the global scientific literature to determine the active ingredients of XBJ. The PubChem database (https://pubchem.ncbi.nlm.nih.gov/) was used for cross-validation and to acquire the molecular structures of potential active chemical components, which we stored in canonical simplified molecular-input line-entry system (SMILES) format.

2.2 Prediction of potential effector targets of XBJ

To predict potential effector targets, we used the SwissTargetPrediction database (http://www.swisstargetprediction.ch/index.php) [18]. We input the aforementioned potential active ingredients in SMILES format into this database, with “humans” (*Homo sapiens*) as the study species, to obtain the potential effector targets of the component compounds; results were stored in .csv format. After compilation and removal of repetitions, we obtained the potential effector targets of XBJ.

2.3 Screening of potential therapeutic targets of COVID-19

Three sources were used to obtain potential therapeutic targets of COVID-19. First, we obtained the COVID-19 disease target set by searching the GeneCards gene database (http://www.genecards.org) [19] on the phrase “coronavirus pneumonia”. Second, we searched the literature to collect potential therapeutic targets of COVID-19. Angiotensin-converting enzyme 2 (ACE2) is reported to be the receptor for SARS-CoV [20] and is also believed to be that for SARS-CoV-2. We used single-cell sequencing results for colon epithelial cells [21] to extract genes that are co-expressed with ACE2, which we matched with human targets as potential therapeutic targets for COVID-19. Third, we downloaded human coronavirus (HCoV)-related host proteins from the appendices of one study [22]. The relevant coronaviruses included SARS-CoV, Middle East respiratory syndrome coronavirus (MERS-CoV), infectious-bronchitis virus (IBV), mouse hepatitis virus (MHV), HCoV-229E, and HCoV-NL63; the host proteins were considered potential therapeutic targets for COVID-19. Finally, we obtained intersections between the active-ingredient-related targets and the disease-related targets from the three abovementioned sources. Intersectional targets were considered potential therapeutic targets of XBJ in COVID-19.

2.4 Construction of a network of active ingredients and effector targets

We input the abovementioned potential active compounds of XBJ and their potential effector targets into Cytoscape software version 3.7.0 (http://www.cytoscape.org) [23] to plot a “XBJ active-compound–potential effector target” network analysis map. On this map, different nodes represented potential active compounds and effector targets of XBJ, and the map’s edges showed relationships between these two factors. Next, we used the NetworkAnalyzer plugin [24] for topology analysis of the network.

2.5 Protein–protein interaction (PPI) analysis and network topology analysis

We input the potential therapeutic targets of XBJ into the Search Tool for the Retrieval of Interacting Genes/Proteins (STRING; https://string-db.org/) [25] and selected humans as the species to obtain PPI data. Next, we input this data into Cytoscape to plot a PPI network map. The Cytohubba [26] plugin in Cytoscape was used for network topology analysis, and results were sorted by degree.

2.6 Gene functional annotation of potential effector targets

Using the ClueGO [27] and CluePedia [28] plugins in Cytoscape software and setting humans as the species, we performed gene ontology (GO) Biological Process (BP) enrichment analysis [29] and Kyoto Encyclopedia of Genes and Genomes (KEGG) pathway analysis [30] of the potential effector targets, and then saved the results. BPs with $P < 0.01$ and signaling pathways with $P < 0.05$ were selected. We used Cytoscape for visualization.

3. Results

3.1 Network analysis of active ingredients and effector targets

We selected 30 active ingredients of XBJ that had been detected by liquid chromatography–mass spectrometry (LC–MS) [31]. Results are shown in Table 1. We obtained 251 potential therapeutic targets of COVID-19 from the GeneCards database. Single-cell sequencing was used to obtain 3558 gene targets that were co-expressed with ACE, and 119 therapeutic targets were obtained from the systemic literature search. There were 147 potential COVID-19 therapeutic targets and XBJ potential effector targets (Fig. 1). We used Cytoscape to plot a “component–target” network relationship map (Fig. 1). This map included 170 nodes, which were comprised of 26 XBJ components matched to 144 disease-related targets.

Table 1
Potential active ingredients of XBJ.

NO	Compound	Herbs
1	5-hydroxymethyl-furfural	Carthami Flos
2	Albiflorin	Radix Paeoniae Rubra
3	Apigenin	Radix Salviae, Carthami Flos
4	Benzoylpaeoniflorin	Radix Paeoniae Rubra
5	Butylidenephthalide	Chuanxiong Rhizoma, Angelicae Sinensis Radix
6	Caffeic acid	Chuanxiong Rhizoma
7	Catechinic acid	Radix Paeoniae Rubra
8	Chlorogenic acid	Radix Salviae, Carthami Flos
9	Cryptotanshinone	Radix Salviae
10	Ethyl ferulate	Chuanxiong Rhizoma, Angelicae Sinensis Radix
11	Ferulic acid	Angelicae Sinensis Radix, Carthami Flos
12	Gallic acid	Radix Paeoniae Rubra
13	Galuteolin	Radix Salviae, Carthami Flos
14	Hydroxysafflor yellow A	Carthami Flos
15	Hyperoside	Carthami Flos
16	Luteolin	Radix Salviae, Carthami Flos
17	Naringenin	Carthami Flos
18	Oxypaeoniflorin	Radix Paeoniae Rubra
19	Paeonol	Radix Paeoniae Rubra
20	Protocatechuic acid	Radix Salviae
21	Protocatechuic aldehyde	Radix Salviae
22	Quercetin	Carthami Flos
23	Rosmarinic acid	Radix Salviae
24	Rutin	Carthami Flos
25	salvianolic acid A	Radix Salviae
26	Salvianolic acid B	Radix Salviae
27	Senkyunolide I	Chuanxiong Rhizoma, Angelicae Sinensis Radix

NO	Compound	Herbs
28	Sodium Danshensu	Radix Salviae
29	Tanshinol	Radix Salviae
30	Tanshinone II A	Radix Salviae

3.2 Network analysis of potential therapeutic targets

We input the PPI information obtained from STRING into Cytoscape to plot the PPI network, and then we used Cytohubba for network topology analysis. Table 2 shows the results, Fig. 4 shows the visualization results, and both show the top 50 potential therapeutic targets by degree values. Glyceraldehyde 3-phosphate dehydrogenase (*GAPDH*), *TNF*, mitogen-activated protein kinase 3 (*MAPK3*), Caspase-3 (*CASP3*), epidermal growth factor receptor (*EGFR*), *MAPK1*, prostaglandin-endoperoxide synthase 2 (*PTGS2*), signal transducer and activator of transcription 3 (*STAT3*), and *MAPK8* all had degree values > 40, showing that these proteins occupied an important position in the PPI network.

Table 2
Parameter information for network topology analysis of XBJ's potential therapeutic targets.

NO	Targets	Degree	NO	Targets	Degree
1	GAPDH	77	26	MCL1	30
2	ALB	73	27	LCK	27
3	TNF	70	28	PARP1	27
4	EGFR	63	29	GSK3B	27
5	MAPK1	61	30	PIK3R1	27
6	CASP3	58	31	SERPINE1	26
7	STAT3	55	32	ABCB1	25
8	MAPK8	49	33	SYK	25
9	PTGS2	48	34	XIAP	25
10	JUN	47	35	SELE	24
11	IL2	43	36	MET	24
12	ESR1	40	37	PRKCA	24
13	MAPK14	40	38	BTK	23
14	RELA	39	39	HSPA5	23
15	BCL2L1	39	40	ABCG2	22
16	ICAM1	38	41	PRKCB	22
17	CTNNB1	38	42	CD38	21
18	MPO	37	43	AGTR1	21
19	FGF2	35	44	FLT3	20
20	PIK3CA	34	45	NOS2	20
21	CASP8	33	46	NFE2L2	19
22	ACE	32	47	ALOX5	19
23	F2	32	48	CTSB	18
24	ITGB1	32	49	HNF4A	18
25	PPARG	31	50	PRKCE	18

3.3 GO gene function and KEGG signaling pathway enrichment analyses

GO BP enrichment analysis showed that the potential therapeutic targets of XBJ involved 228 BPs. This indicated that the active ingredients of XBJ could exert their effects through multiple BPs (Table 3).

Table 3
GO Biological Process enrichment analysis.

NO	GO ID	GO Term	% Associated Genes	Nr. Genes
1	GO:0042542	response to hydrogen peroxide	5.00	10.00
2	GO:1990776	response to angiotensin	13.89	5.00
3	GO:0035924	cellular response to vascular endothelial growth factor stimulus	8.64	7.00
4	GO:0002224	toll-like receptor signaling pathway	5.03	10.00
5	GO:0061756	leukocyte adhesion to vascular endothelial cell	13.16	5.00
6	GO:0001936	regulation of endothelial cell proliferation	6.17	10.00
7	GO:0031663	lipopolysaccharide-mediated signaling pathway	8.33	7.00
8	GO:0050727	regulation of inflammatory response	5.42	24.00
9	GO:0010634	positive regulation of epithelial cell migration	4.74	9.00
10	GO:0030335	positive regulation of cell migration	4.04	26.00
11	GO:0070371	ERK1 and ERK2 cascade	4.33	19.00
12	GO:0050852	T cell receptor signaling pathway	4.20	12.00
13	GO:0000187	activation of MAPK activity	4.32	12.00
14	GO:0071222	cellular response to lipopolysaccharide	5.43	15.00
15	GO:0002526	acute inflammatory response	5.33	9.00
16	GO:0050900	leukocyte migration	4.64	30.00
17	GO:0001780	neutrophil homeostasis	17.39	4.00
18	GO:0036092	phosphatidylinositol-3-phosphate biosynthetic process	14.71	5.00
19	GO:2000106	regulation of leukocyte apoptotic process	8.00	10.00
20	GO:0019369	arachidonic acid metabolic process	11.11	7.00

KEGG signaling pathway enrichment analysis showed that XBJ acted on COVID-19 mainly through 95 pathways. This indicated that the targets of the active ingredients of XBJ were distributed across different pathways and that XBJ might use multiple pathways to carry out its synergistic effects (Table 4).

Table 4
KEGG signaling pathways in which the potential therapeutic targets of XBJ were involved.

NO	GO ID	GO Term	Associated Genes Found
1	KEGG:00910	Nitrogen metabolism	[CA1, CA13, CA2, CA4, CA6]
2	KEGG:04614	Renin-angiotensin system	[ACE, AGTR1, KLK1, MME]
3	KEGG:04064	NF-kappa B signaling pathway	[BCL2, BCL2L1, BTK, ICAM1, LCK, PARP1, PRKCB, PTGS2, RELA, SYK, TNF, XIAP]
4	KEGG:00590	Arachidonic acid metabolism	[AKR1C3, ALOX5, CBR1, CYP2C19, CYP2C9, PLA2G1B, PLA2G5, PTGS1, PTGS2]
5	KEGG:04010	MAPK signaling pathway	[CASP3, DUSP1, EGFR, FGF2, FLT3, JUN, MAPK1, MAPK14, MAPK8, MAPKAPK2, MET, NR4A1, PRKCA, PRKCB, RELA, TNF]
6	KEGG:04012	ErbB signaling pathway	[EGFR, GSK3B, JUN, MAPK1, MAPK8, PIK3CA, PIK3CB, PIK3CD, PIK3R1, PRKCA, PRKCB]
7	KEGG:04014	Ras signaling pathway	[BCL2L1, EGFR, FGF2, FLT3, MAPK1, MAPK8, MET, PIK3CA, PIK3CB, PIK3CD, PIK3R1, PLA2G1B, PLA2G5, PRKCA, PRKCB, RELA]
8	KEGG:04015	Rap1 signaling pathway	[ADORA2A, ADORA2B, CTNNB1, EGFR, FGF2, ITGB1, MAPK1, MAPK14, MET, PIK3CA, PIK3CB, PIK3CD, PIK3R1, PRKCA, PRKCB]
9	KEGG:04062	Chemokine signaling pathway	[CCR1, CCR5, CCR8, CXCR1, GSK3B, MAPK1, PIK3CA, PIK3CB, PIK3CD, PIK3CG, PIK3R1, PRKCB, RELA, STAT3]
10	KEGG:04066	HIF-1 signaling pathway	[BCL2, EGFR, EGLN1, GAPDH, HK2, LDHA, MAPK1, NOS2, PIK3CA, PIK3CB, PIK3CD, PIK3R1, PRKCA, PRKCB, RELA, SERPINE1, STAT3]
11	KEGG:04068	FoxO signaling pathway	[EGFR, MAPK1, MAPK14, MAPK8, PIK3CA, PIK3CB, PIK3CD, PIK3R1, STAT3]
12	KEGG:04151	PI3K-Akt signaling pathway	[BCL2, BCL2L1, EGFR, FGF2, FLT3, GSK3B, IL2, ITGAV, ITGB1, MAPK1, MCL1, MET, NR4A1, PIK3CA, PIK3CB, PIK3CD, PIK3CG, PIK3R1, PKN1, PRKCA, RELA, SYK, YWHAG]
13	KEGG:04370	VEGF signaling pathway	[MAPK1, MAPK14, MAPKAPK2, PIK3CA, PIK3CB, PIK3CD, PIK3R1, PRKCA, PRKCB, PTGS2]
14	KEGG:04620	Toll-like receptor signaling pathway	[CASP8, JUN, MAPK1, MAPK14, MAPK8, PIK3CA, PIK3CB, PIK3CD, PIK3R1, RELA, TNF]

NO	GO ID	GO Term	Associated Genes Found
15	KEGG:04621	NOD-like receptor signaling pathway	[BCL2, BCL2L1, CASP8, CTSB, JUN, MAPK1, MAPK14, MAPK8, RELA, TNF, XIAP]
16	KEGG:04630	JAK-STAT signaling pathway	[BCL2, BCL2L1, EGFR, IL2, MCL1, PIK3CA, PIK3CB, PIK3CD, PIK3R1, PIM1, STAT3]
17	KEGG:04657	IL-17 signaling pathway	[CASP3, CASP8, GSK3B, JUN, MAPK1, MAPK14, MAPK8, PTGS2, RELA, TNF]
18	KEGG:04668	TNF signaling pathway	[CASP3, CASP7, CASP8, ICAM1, JUN, MAPK1, MAPK14, MAPK8, PIK3CA, PIK3CB, PIK3CD, PIK3R1, PTGS2, RELA, SELE, TNF]
19	KEGG:04750	Inflammatory mediator regulation of TRP channels	[MAPK14, MAPK8, PIK3CA, PIK3CB, PIK3CD, PIK3R1, PRKCA, PRKCB, PRKCE, PTGER4]
20	KEGG:04915	Estrogen signaling pathway	[BCL2, EGFR, ESR1, JUN, MAPK1, PIK3CA, PIK3CB, PIK3CD, PIK3R1]

4. Discussion

XBJ has been approved for the treatment of severe infection (sepsis). For a long period of time in China, it was believed that XBJ could improve prognosis in severe lung infection [32] as well as 28-day mortality rate, Acute Physiologic Assessment and Chronic Health Evaluation (APACHE) II score, white blood cell (WBC) count, and temperature in sepsis patients without causing serious adverse events. A prospective, randomized controlled trial in 33 hospitals in China, published in September 2019, proved that XBJ can significantly improve the primary endpoint of pneumonia severity in patients with severe community-acquired pneumonia, as well as secondary endpoints such as mortality rate, mechanical-ventilation duration, and length of intensive-care unit (ICU) stay [33]. Since the start of the COVID-19 outbreak, XBJ has been recommended as a Chinese patent medicine in local COVID-19 diagnosis and treatment plans released by many provincial health commissions due to its rapid onset and significant efficacy in critically ill patients. This shows that XBJ might have important clinical value in COVID-19 treatment. However, the material bases and molecular effector mechanisms are still unclear. Therefore, analysis of the potential effector mechanisms of XBJ in the treatment of COVID-19, elucidating its potential active ingredients and their potential effector targets, and demonstrating the network effector mechanisms of XBJ on COVID-19, can provide a scientific basis for using XBJ in the clinical treatment of COVID-19.

This study preliminarily demonstrated XBJ's "multiple component–multiple target–multiple pathway" effector characteristics, and our network topology analysis of potential effector targets identified some critical effector targets of the compound. GO and KEGG enrichment analyses found that the potential

targets of XBJ involved multiple inflammation- and immune-related gene functions and signaling pathways, which might be one basis for XBJ treatment in COVID-19.

From a potential active-ingredient perspective, XBJ possesses potential anti-inflammatory and immune-boosting effects [34]. The three active ingredients of *Carthamus tinctorius* have protective effects in lipopolysaccharide (LPS)–induced acute lung injury (ALI) [35]. The potential anti-inflammatory components in XBJ inhibit NF-κB activity; decrease expression of TNF-α, IL-1β, and IL-6 [36]; alleviate inflammatory responses; and inhibit secretion of pro-inflammatory cytokines mediated by the high-mobility group box 1 protein (*HMGB1*)–receptor for advanced glycation endproducts (*RAGE*) axis, thereby decreasing mortality rate in a mouse model [37]. XBJ also upregulates toll-interacting proteins in septic rats to protect the lungs from permeable leakage and injury [38]. It also prevents cytokine storm, inhibits inflammatory responses, and regulates regulatory T-cell (Treg)–T helper 17 cell (T_h17) balance to improve survival in septic shock [39]. In addition, XBJ promotes the expression of Annexin A1 to inhibit cleavage of pro-inflammatory cytokines and decreases IL-8 and TNF-α levels to protect rats from damage due to *Acinetobacter baumannii* sepsis [40]. SARS-CoV-2 replicates in respiratory-tract epithelial cells to cause acute inflammation and severe respiratory disease. During infection, local production of pro-inflammatory cytokines exacerbates disease progression. Therefore, the anti-inflammatory activity and cytokine-inhibitory effects of XBJ might constitute its potential mechanism in COVID-19 treatment.

In GO BP analysis, the 147 potential therapeutic targets of XBJ involved multiple inflammation- and immune-related BPs such as extracellular signal–regulated kinase 1 and 2 (*ERK1*, *ERK2*) cascade, the T-cell receptor signaling pathway, activation of *MAPK* activity, and cellular response to LPS. This suggests that the significant anti-inflammatory effects of XBJ are its therapeutic effects in inflammation. Virus–host interactions are an important aspect of viral replication. Ribonucleic acid (RNA) viruses such as influenza, Ebola virus, and SARS-CoV can induce *Raf*–mitogen-activated protein kinase kinase (*MEK*)–*ERK* signal transduction in the *MAPK* cascade, which is associated with replication of pathogenic RNA viruses in humans and allows for cell differentiation and proliferation [41]. This is consistent with the fact that XBJ targets *ERK1*/*ERK2* cascade.

Extensive proteinoid and serous exudates are present in the alveoli of COVID-19 patients. These cases also present bilateral diffuse alveolar damage accompanied by fibromyxoid exudates, and both lungs show apparent pulmonary edema, alveolar epithelial detachment, and hyaline-membrane formation [42]. In terms of infection-related serum markers, studies show that C-reactive protein (CRP), IL-6, and erythrocyte sedimentation rate (ESR) are significantly increased in many patients [43]. Severe cytokine storm can appear in severely to critically ill patients, resulting in excessive immune activation and excess production of IL-7, IL-10, granulocyte colony-stimulating factor (GCSF), interferon gamma inducible protein 10 kD (IP-10), monocyte chemoattractant protein 1 (MCP-1), macrophage inflammatory protein 1A (MIP1A), and TNF-α [44]. From this, it can be seen that SARS-CoV-2–mediated inflammation plays an important role in COVID-19 progression, and uncontrollable lung inflammation may be the main cause of death in COVID-19. Hence, intervention measures to reduce inflammation might help decrease the mortality rate [45].

With regard to effector targets, the degree values of *GAPDH*, albumin (*ALB*), *TNF*, *EGFR*, *MAPK1*, *CASP3*, *STAT3*, *MAPK8*, *PTGS2*, *JUN*, *IL-2*, EStrogen Receptor 1 (*ESR1*), and *MAPK14* were all > 40, suggesting that in COVID-19 these could be the main therapeutic targets of XBJ's active ingredients. KEGG signaling pathway enrichment analysis showed that the 147 potential XBJ therapeutic targets involved many inflammation- and immune-related signaling pathways.

The renin–angiotensin system (RAS), OS and cell death, cytokine storm, and endothelial dysfunction are four main pathways in COVID-19 pathogenesis. ACE is a receptor in the airway, alveoli, and vascular endothelium. COVID-19 uses ACE to enter type II pneumocytes or intestinal epithelial cells in order to induce ACE2 internalization and shedding, resulting in the occurrence and development of acute respiratory distress syndrome (ARDS) [46]. Many of XBJ's active ingredients act on multiple targets in the RAS, which could potentially interfere with ACE receptors. NF-κB activation exacerbates lung inflammation caused by SARS-CoV infection, and inhibition of NF-κB signaling significantly reduces such inflammation and increases the survival rate of SARS-CoV–infected mice [47, 48]. In addition, NF-κB is an important transcription factor that induces expression of viral genes, and inhibition of NF-κB activation is an immune evasion mechanism of SARS-CoV [49]. Therefore, we speculate that XBJ's active ingredients might interfere with the NF-κB pathway and regulate innate immunity and inflammation during viral infection to alleviate lung inflammation during COVID-19.

One study has shown that XBJ inhibits *MAPK* and NF-κB expression and has protective effects in ALI [50]. The compound regulates the NF-κB, *MAPK*, and *PI3K–Akt* pathways in mouse macrophages and downregulates inflammatory cytokines such as IL-6, TNF-α, MCP-1, MIP-2, and serum IL-10 to increase the survival rate of septic mice [15]. XBJ downregulates toll-like receptor 4 (*TLR-4*) and NF-κB expression to carry out its anti-inflammatory effects. *MAPK* activation can promote the expression and release of pro-inflammatory cytokines such as TNF-α and IL-1β, -6, and − 8; it is a core factor in inflammation regulation. Viruses usually directly or indirectly affect the *PI3K–Akt* pathway to control intracellular-signaling pathways. EGFR aggregation and binding of influenza virus to cell surfaces might activate *Akt*. The *PI3K–Akt* signaling pathway might synergize with the RAS to promote viral entry, which has significant effects in viral infection in humans [51]. TLR-2, TLR-3, and TLR-4 activation by COVID-19 causes the release of inflammatory cytokines such as IL-1β. The binding of SARS-CoV-2 to TLRs causes release of pro–IL-1β, inflammasome activation, and production of mature IL-1β. Pro-inflammatory cytokines are important mediators of local and systemic inflammation. Viral particles first invade the respiratory mucosa before infecting other cells, thereby inducing a series of immune responses leading to cytokine storm [52]. Therefore, XBJ could be used to treat COVID-19 patients due to its anti-inflammatory effects, anti-immune apoptosis, and alleviation of pneumonia-induced multi-organ damage. In addition, we found that critical nodes on our "XBJ active-compound–potential effector target" network analysis map participated in the aforementioned pathways, suggesting that the predictions made in this study are somewhat accurate.

5. Conclusions

XBJ is made up of five medicinal materials and contains 30 active ingredients that regulate different genes, act on different pathways, and synergize anti-inflammatory and immunoregulatory effects. This fully demonstrates the synergy of multiple targets and multiple pathways between different components, as well as the holistic concept of TCM formulations. In this study, we employed network pharmacology to examine the “multiple component–multiple target–multiple pathway” effector mechanisms of XBJ in COVID-19. Our results suggested existing studies on the pharmacological mechanisms of XBJ in the treatment of sepsis and severe pneumonia, could better explain the effector mechanism of XBJ in COVID-19 treatment, and provided a preliminary examination of the potential effector mechanism of this compound in this disease.

Declarations

Ethics approval and consent to participate

Not applicable in this section. This manuscript does not report on or involve the use of any animal or human data or tissue.

Consent for publication

Not applicable in this section. This manuscript does not contain data from any individual person.

Availability of data and materials

All data are available in the manuscript and the they are showed in tables, figures and supplement file.

Competing interests

The authors declare that they have no conflict of interest.

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Authors' contributions

Conceptualization, LXH, and JY; methodology, ZWJ, and YQ; software, NYS; formal analysis, ZSF and YLL; investigation, ZWJ; resources, ZHF; data curation, NYS; writing— original draft preparation, ZWJ, and YQ; writing—review and editing, JY; supervision, LXH; project administration, JY; funding acquisition, LXH. All authors have read and agreed to the published version of the manuscript.

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Figures

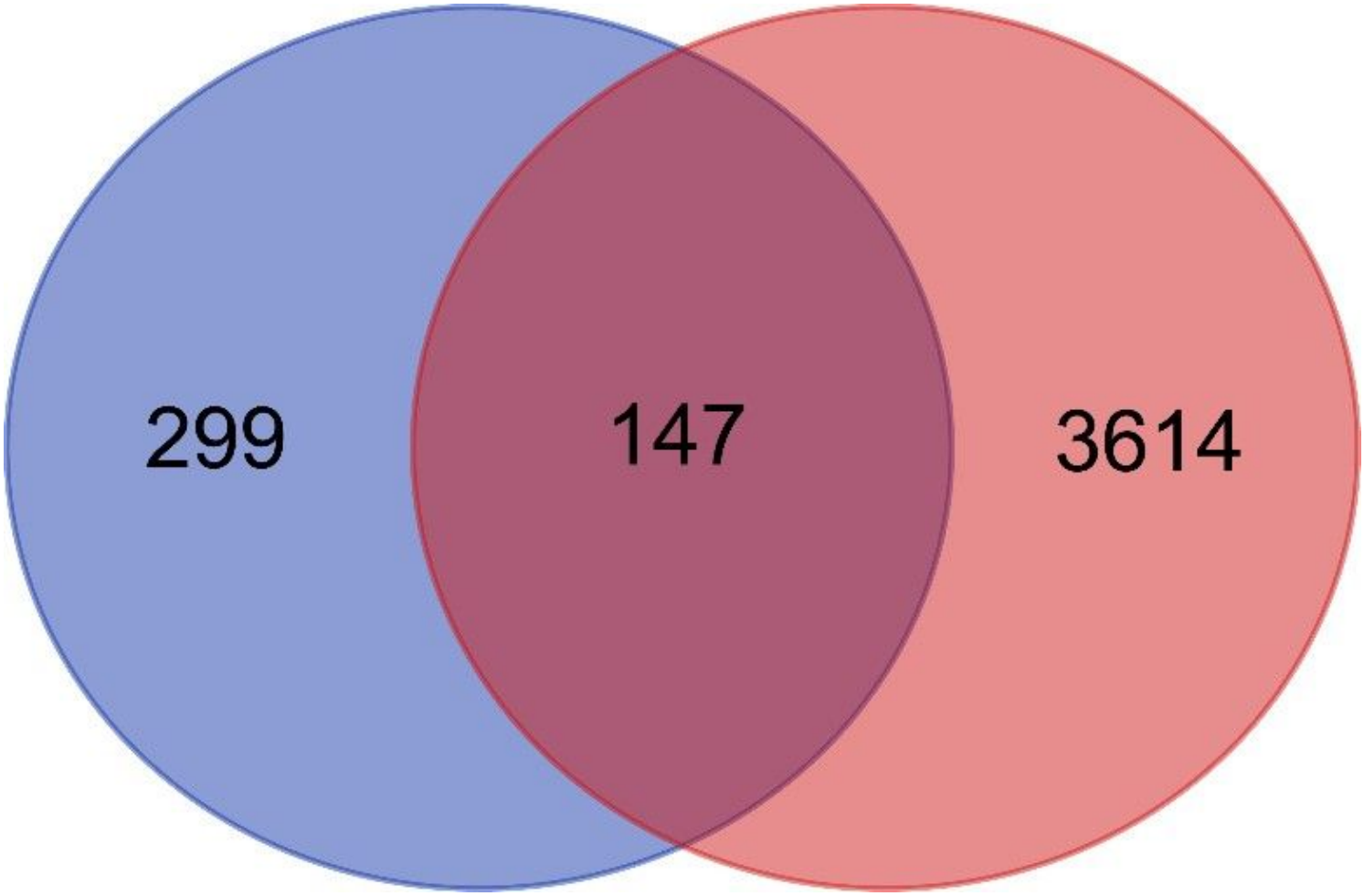

Figure 1

Venn diagram of potential COVID-19 therapeutic targets and potential XBJ effector targets.

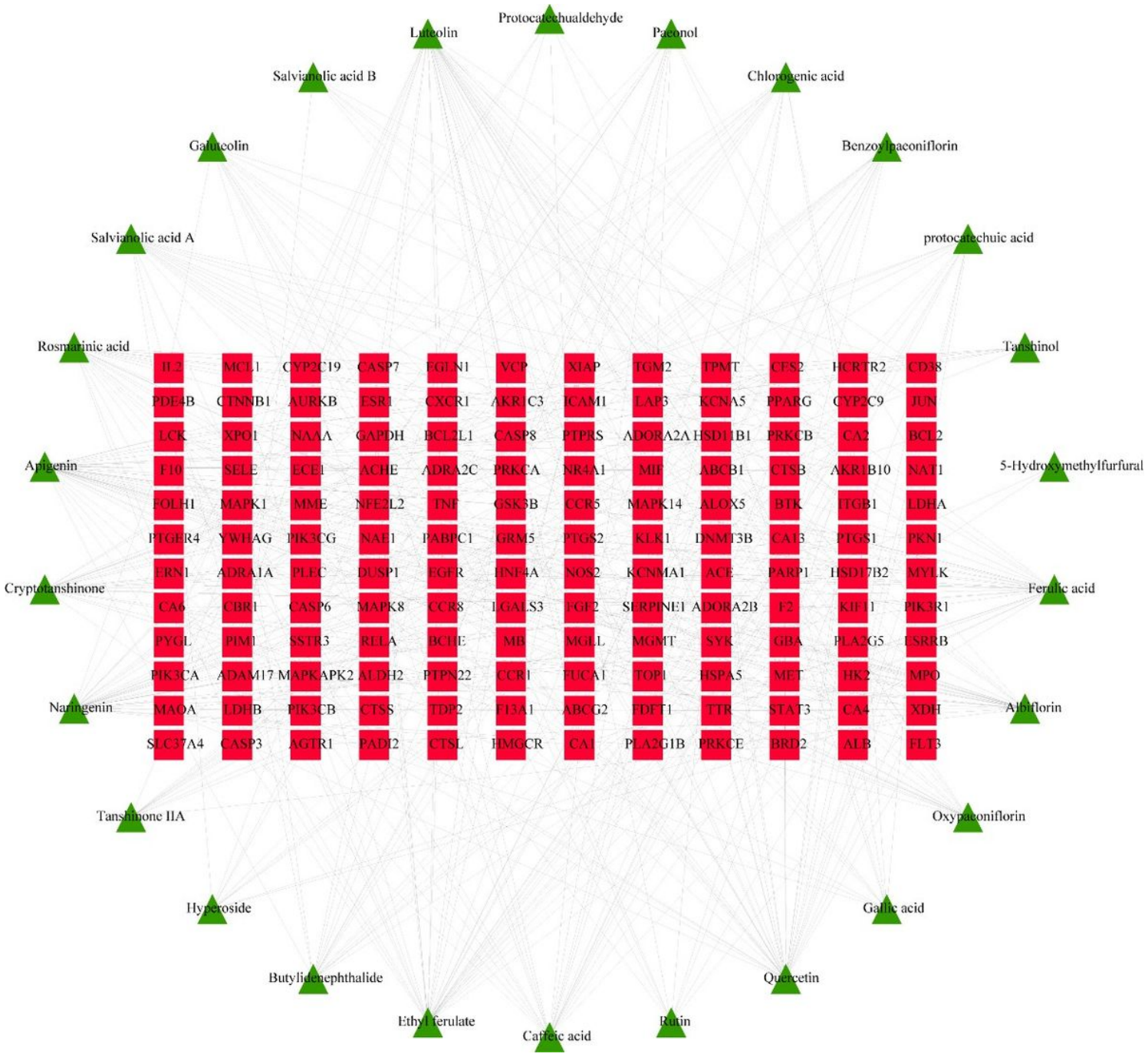

Figure 2

Potential active-ingredient–potential therapeutic-target network analysis.

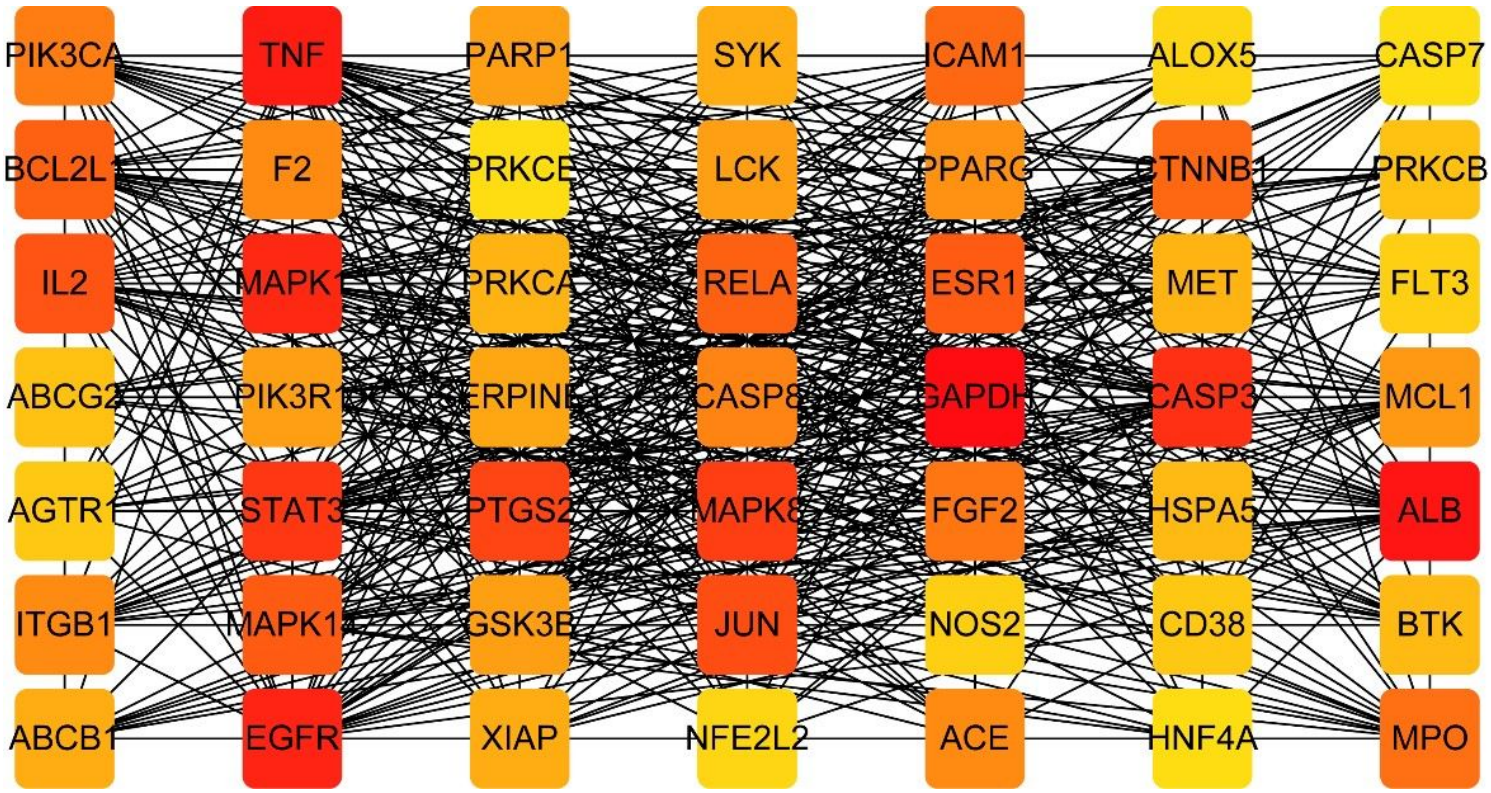

Figure 3

Network topology analysis. Red circles represent COVID-19–related potential effector targets. Black lines represent protein–protein interactions present. Deeper colors represent higher degree values.

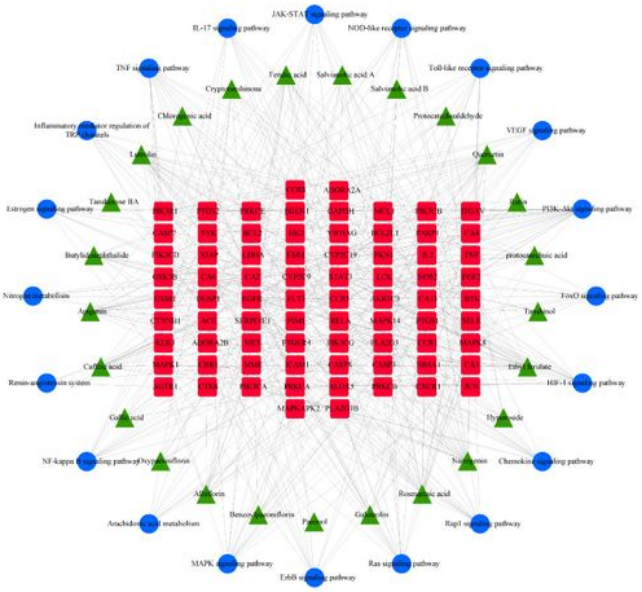

Figure 4

“Active ingredient–effector target–signaling pathway” network plot. Green diamonds represent the potential active ingredients of XBJ. Red squares represent COVID-19–related potential effector targets. Blue circles represent signaling pathways in which the target is involved.

Supplementary Files

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