Original Article

Molecular Insight into the Therapeutic Promise of Xuebijing Injection against Coronavirus Disease 2019

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Abstract

Background: The potential human-to-human transmission of the coronavirus pneumonia (coronavirus disease 2019 [COVID-19]) has caused an outbreak of acute respiratory illness. Xuebijing injection is recommended as first-line treatment for the severe and critical patients with COVID-19. The aim of present study is to interpret the pharmacological mechanisms and molecular connections of Xuebijing injection against COVID-19 by utilizing the approaches of network pharmacology and molecular docking. **Materials and Methods:** Active ingredients of Xuebijing injection were collected by Traditional Chinese Medicine Systems Pharmacology (TCMSP) database, and putative therapeutic targets were screened from TCMSP, SwissTargetPrediction, and STITCH databases. Moreover, the protein–protein interactions, topological analysis, and pathway enrichment were established to distinguish the hub targets and pathways by employing STRING database, Cytoscape software, DAVID database, respectively. In addition, the potential interaction and binding activity of candidate ingredients in Xuebijing injection with core targets were revealed by molecular docking simulation (AutoDock software). **Results:** A total of 115 bioactive components in Xuebijing injection were collected, and416 targets including AKT1, TP53, VEGFA, ALB, TNF and so on were responsible for treating COVID-19. Bioinformatics analysis revealed that matching core targets were closely associated with the inhibition of cytokine storm for its clinical beneficial effects in severe cases. The results of enrichment analysis indicated that PI3K-Akt signaling pathway, human T-cell leukemia virus type 1 infection, mitogen-activated protein kinase signaling pathway, tuberculosis, focal adhesion, TNF signaling pathway, and small-cell lung cancer were represented pathways of Xuebijing injection against COVID-19 in terms of lung inflammation, virus infection, and lung injury. Meanwhile, the active ingredients of Xuebijing injection exerted superior binding activities with 3CLpro and angiotensin-converting enzyme 2 as observed via molecular docking simulation. **Conclusions:** Through the comprehensive analysis of network pharmacology, the current research preliminarily elaborated the molecular regulation of therapeutic mechanisms for Xuebijing injection against COVID-19 and binding activity between active components and core targets, which provided scientific evidence to facilitate the development of Xuebijing injection and clinical treatment for COVID-19.

Keywords: Coronavirus disease 2019, molecular docking, network pharmacology, therapeutic mechanism, Xuebijing injection

Introduction

Since December 2019, coronavirus disease 2019 (COVID-19) became an enormous threat to public health, and 2019 Novel Coronavirus (2019-nCoV) was responsible for the outbreak of emerging infectious pneumonia.[1-5] The World Health Organization (WHO) announced that the official named severe acute respiratory syndrome caused by 2019-nCoV as COVID-19; subsequently, the WHO Emergency Committee declared this outbreak as the sixth public health emergency of international concern.[6,7] Remarkably, 2019-nCoV that infected population via respiratory droplets, human-to-human and other transmission, posed pandemic potential with unfortunate characteristics of strong infectiousness, rapid dissemination, long incubation period, and general susceptibility for different

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crowds.[8-10] Except for asymptomatic carrier, the most common clinical manifestations of patients suffered from COVID-19 were fever, cough, and myalgia or fatigue; furthermore, some serious cases with rapid progression were affected by life-threatening organ dysfunctions or complications including acute respiratory distress syndrome (ARDS), metabolic acidosis, coagulation disorders, septic shock, and multiply organ dysfunction syndrome (MODS) and even death.[11-13] According to official release of 21 March, 2020, by the National Health Commission of the People's Republic of China, more than 80,000 cases had been confirmed as COVID-19 with 3261 death cases; besides, a total of 1845 cases were diagnosed as severe cases currently.[14]

In the theory of Traditional Chinese Medicine (TCM), 2019-nCoV-infected pneumonia was deemed to the category of "pestilence," the disease mainly located in lung and related to the spleen, and the basic pathogenesis is characterized by "dampness, toxin, stasis, and closure."[15,16] Based on the guiding principle of syndrome differentiation and treatment, it had been confirmed that TCM could bring promising benefits for the prevention, treatment, and recovery of COVID-19 among Chinese population due to its unique therapeutic principles and various therapies in first-line clinical treatment.[17,18] Given that TCM achieved satisfactory therapeutic superiority during the accumulation of clinical experience and medical evidence in diagnosis and treatment of COVID-19, the therapy regimen of TCM was first recommended in the Guideline on Diagnosis and Treatment of Coronavirus Disease 2019 (Trial 3th version).[19] Through continuous improvement and revision of the guidelines, there were some respective TCM prescriptions, Chinese patent medicines, and Chinese herbal injections with regard to individuals in different stage and type of syndrome, and it also highlighted that the curative advantages of TCM should play an indispensable role in the treatment of COVID-19, especially severe and critical patients.[20,21] Meanwhile, the TCM schemes had also been issued by some provinces and regions of China to exert advantages in preventive treatment of diseases, comprehensive therapies, and rehabilitation.[22,23]

Xuebijing injection, which was approved as second-grade national new medicine for treating sepsis in China over 15 years (medicine manufacturing approval number: Z20040033), was composed of Hong Hua (*Carthami Flos*), Chi Shao (*Paeoniae Radix Rubra*), Chuan Xiong (*Chuanxiong Rhizoma*), Dan Shen (*Salviae Miltiorrhizae Radix Et Rhizoma*), and Dang Gui (*Angelicae Sinensis Radix*).[24,25] In particular, the commercialized injectable prescription of Xuebijing injection was based on therapeutic principles of TCM that was proposed by a famous integrative medicine emergency expert, Professor Jin-Da Wang; it possessed functions of activating blood circulation to dissipate blood stasis, relaxing tendons to remove obstruction, and eliminating pathogenic heat to collapse toxins, and had been widely used for treating patients with critical diseases including sepsis, severe pneumonia, severe acute pancreatitis, infection-induced systemic inflammatory response syndrome, chronic obstructive pulmonary disease (COPD), ARDS, and MODS.[26,27] In this clinical fight against COVID-19, the considerable effects of Xuebijing injection had been widely investigated; therefore, it was recommended as adjuvant therapy for severe and critical cases in aforementioned guidelines of COVID-19 (Trial 4th, 5th, 6th, and 7th version) (http://www.nhc.gov.cn/yzygj/pqt/new_list.shtml).[28-30] In the light of notable role of Xuebijing injection against COVID-19, we aimed to elucidate the underlying mechanism and binding activity via network pharmacology and molecular docking. On the one hand, a multilevel bioinformatics network was conducted to explore the relationship between candidate components, hub targets, and core pathways from a holistic perspective.[31,32] On the other hand, molecular docking technology was applied to illustrate the binding mode and site between dominant components and critical receptor proteins (3CLpro, angiotensin-converting enzyme 2 [ACE2]) at molecular level,[33-35] providing the scientific basis for clinical application and pharmacological mechanism of Xuebijing injection against COVID-19.

Materials and Methods

Acquisition of candidate components and putative targets

The Mandarin Chinese of five herbs in Xuebijing injection including *Hong Hua*, *Chi Shao*, *Chuan Xiong*, *Dan Shen,* and *Dang Gui* was used as the keywords to collect available data of candidate components from the Traditional Chinese Medicine Systems Pharmacology (TCMSP) database (TCMSP, http://lsp.nwu.edu.cn/tcmsp.php).[36] In addition, the characteristic principles were recruited in the literature review through the electronic databases of PubMed, the China National Knowledge Infrastructure Database, the Wanfang Database, and the China Biology Medicine disc (SinoMed) and *Chinese Pharmacopoeia* (2015 Edition). In this study, an oral availability (OB) >30% and a pharmacokinetic value of drug-like (DL) >0.18 were selected as the limiting conditions to identify potentially active compounds of Xuebijing injection.[37] OB could represent the physicochemical ability of bioactive compounds throughout circulate in the body after oral administration.[38] As a considerable indicator for determining the molecular similarity according to its physicochemical properties compared with conventional drugs, DL might be helpful to determine the therapeutic activities of active ingredients from multitudinous compounds in the medicinal materials.[39] Subsequently, the chemical information including MOL2 structure, canonical name, and SMILES number of the identified dominant components were downloaded for further bioinformatics analysis from the PubChem Compound Database (http://pubchem.ncbi.nlm.nih.gov/).[40]

By referring to the putative targets of Xuebijing injection, the predicted target profiles for these selected compounds were obtained from TCMSP, SWISS (http://www.swisstargetprediction.ch/), and STITCH database (Version 5.0, http://stitch.embl.de/) with probability score ≥0.7, and species were limited as *Homo sapiens*.[41,42] After discarding duplicates,

all of these targets were uniformed into corresponding gene names by UniProt sites (http://www.uniprot.org/).[43]

Construction of component–target networks

The visualization and topological analysis of component –target (C-T) network were undertaken through Cytoscape software (version 3.6.1, http://www.cytoscape.org/).[44] The integrative network consisted of numerous nodes and edges, among this, nodes represented candidate components and corresponding targets, edges referred to interactions between these elements. Besides, the topological analysis of every node was employed to identify the pivotal components and hub genes within the interaction network according to the topological properties such as degree centrality (DC), closeness centrality (CC), and betweenness centrality.[45] Next, the obtained proteins were decomposed into the Search Tool for the Retrieval of Interacting Genes/Proteins (STRING, https://string-db.org/) to forecast the protein–protein interactions (PPI),[46] and Molecular Complex Detection algorithm with the default parameters in Cytoscape Apps was conducted to discover biological modules of dense clusters in PPI networks based on complex connection data.[47,48]

Gene ontology and pathway enrichment analysis

To evaluate the biological functions and bioinformatics annotation of potential core targets and pathways within the constructed network, the gene ontology (GO) functional enrichment analysis involving there modules, namely biological process (BP), cellular component (CC), and molecular function (MF), and Kyoto Encyclopedia of Genes and Genomes (KEGG) pathway enrichment analysis were performed via the Database for Annotation, Visualization, and Integrated Discovery (DAVID Bioinformatics Resources, https://david.ncifcrf.gov/, version 6.8).[49,50] The top 20 results of enrichment analysis were selected to illuminate in Omicshare 3.0 (http://www.omicshare.com/).

Molecular docking simulation

The ligand–receptor interaction between pharmacodynamic components in Xuebijing injection and core proteins of COVID-19 was established through AutoDock Tools 1.5.6 (The Scripps Research Institute, Santiago, California and the United States).[51] With regard to ligands, their 3D molecular structure was captured from PubChem website and then imported to AutoDock for removing water molecules, adding hydrogenate, and charging energy; the files of optimized small molecules were saved as mol2 format ultimately. Furthermore, the core protein conformations of 3CLpro (PDB ID: 6 LU7, 0.21 nm) and ACE2 (PDB ID: 1R42, 0.22 nm) were acquired in the RCSB Protein Data Bank database (PDB, https://www.rcsb.org/).[52] The original ligands and water molecules in protein receptors were removed by PyMOL software and saved in PDB format.[53] Briefly, the results of molecular docking calculations by Lamarckian genetic algorithm were presented as binding energy (ΔGbind/kcal·mol-1), there was negative correlation between the values of binding energy and binding affinity, and PyMOL software was wielded to visualize the results of molecular docking simulation for dominant components.

Results

Composite ingredients of Xuebijing injection

We investigated the chemical compounds that contained in the five herbal medicines of Xuebijing injection from TCMSP database. Consequently, a total of 824 chemical ingredients were obtained, including 189, 119, 189, 202, and 125 compounds in Hong Hua, Chi Shao, Chuan Xiong, Dan Shen, and Dang Gui, respectively. After screening on the conditions of OB ≥30% and DL ≥0.18, and duplicate removal, we found 115 compounds of Xuebijing injection, of which 22, 29, 7, 65, and 2 dominants separately in Hong Hua, Chi Shao, Chuan Xiong, Dan Shen, and Dang Gui, respectively. The pharmacokinetic properties of some active compounds in Xuebijing injection are summarized in Table 1.

From the Venn diagram of compounds' distribution for herbs displayed in Figure 1, there were some identical compounds in different medicinal materials in Xuebijing injection, for example, luteolin was a dominant component both in Hong Hua and Dan Shen simultaneously and beta-sitosterol was distributed among Hong Hua, Chi Shao, and Dang Gui, suggesting that these herbs shared some common material basis for exerting efficacy in clinical treatment. As the monarch herb in the prescription, it is noteworthy that Hong Hua had the majority of common ingredients [Table 2].

Construction of component–target network

The interactive network between active phytochemical compounds of Xuebijing injection and corresponding targets

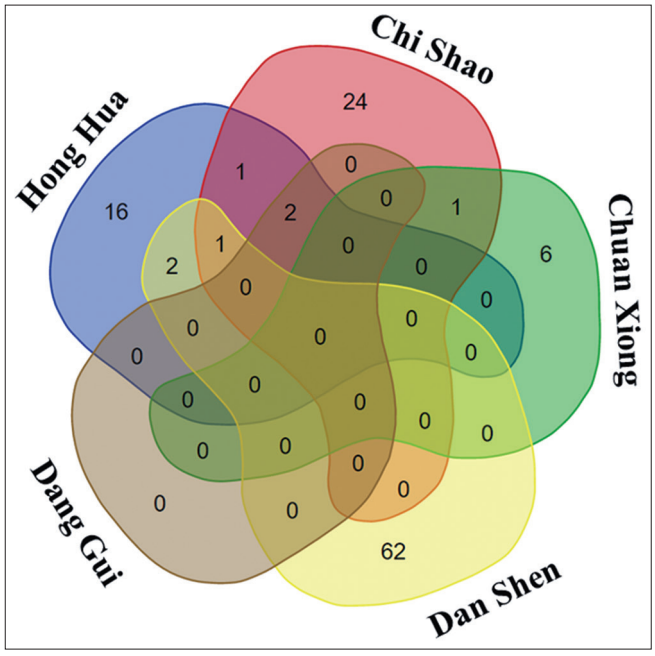


Figure 1: Venn Diagram of distribution for common compounds in Xuebijing injection

Table 1: Basic information of some active compounds in Xuebijing injection

Herb	Number	Chemical Composition	Molecular weight	OB (%)	DL
Hong Hua	MOL002773	Beta-carotene	536.96	37.18	0.58
	MOL002721	Quercetagetin	318.25	45.01	0.31
	MOL002719	6-Hydroxynaringenin	288.27	33.23	0.24
	MOL002710	Pyrethrin II	372.5	48.36	0.35
	MOL002707	Phytofluene	543.02	43.18	0.5
	MOL002706	PHYTOENE	545.04	39.56	0.5
	MOL002698	Lupeol-palmitate	665.26	33.98	0.32
	MOL002680	Flavoxanthin	584.96	60.41	0.56
	MOL001771	Poriferast-5-en-3beta-ol	414.79	36.91	0.75
Chi Shao	MOL001002	Ellagic acid	302.2	43.06	0.43
	MOL001918	Paeoniflorgenone	318.35	87.59	0.37
	MOL001921	Lactiflorin	462.49	49.12	0.8
	MOL001924	Paeoniflorin	480.51	53.87	0.79
	MOL000359	Sitosterol	414.79	36.91	0.75
	MOL004355	Spinasterol	412.77	42.98	0.76
	MOL000449	Stigmasterol	412.77	43.83	0.76
	MOL006992	(2R,3R)-4-methoxyl-distylin	318.3	59.98	0.3
Chuan Xiong	MOL002135	Myricanone	356.45	40.6	0.51
	MOL002140	Perlolyrine	264.3	65.95	0.27
	MOL002151	Senkyunone	326.52	47.66	0.24
	MOL002157	Wallichilide	412.57	42.31	0.71
	MOL000359	Sitosterol	414.79	36.91	0.75
Dan Shen	MOL001601	1,2,5,6-tetrahydrotanshinone	280.34	38.75	0.36
	MOL007061	Methylenetanshinquinone	278.32	37.07	0.36
	MOL007071	Przewaquinone f	312.34	40.31	0.46
	MOL007077	Sclareol	308.56	43.67	0.21
	MOL007079	Tanshinaldehyde	308.35	52.47	0.45
	MOL007081	Danshenol B	354.48	57.95	0.56
	MOL007082	Danshenol A	336.41	56.97	0.52
	MOL007085	Salvilenone	292.4	30.38	0.38
	MOL007088	Cryptotanshinone	296.39	52.34	0.4
	MOL007093	Dan-shexinkum d	336.41	38.88	0.55
	MOL007101	Dihydrotanshinone I	278.32	45.04	0.36
	MOL007108	Isocryptotanshi-none	296.39	54.98	0.39
	MOL007111	Isotanshinone II	294.37	49.92	0.4
	MOL007122	Miltirone	282.41	38.76	0.25
	MOL007123	Miltirone II	272.32	44.95	0.24
	MOL007141	Salvianolic acid g	340.3	45.56	0.61
	MOL007145	Salviolone	268.38	31.72	0.24
	MOL007156	Tanshinone VI	296.34	45.64	0.3
Dang Gui	MOL000358	Beta-sitosterol	414.79	36.91	0.75
	MOL000449	Stigmasterol	412.77	43.83	0.76

OB: oral availability, DL: drug-like

was depicted in Figure 2a, except for 20 of selected compounds did not participate in network construction, others were all related to multiple targets, resulting in 1957 associations between 95 active ingredients and 416 potential targets. The blue nodes represented potential targets, and nodes with other colors were compounds in different herbs of Xuebijing injection. In addition, the results of C-T network indicated that each compound distributed pharmacological functions to 20.6 potential targets on average; conversely, each target was interacted with 7.65 compounds within the biological network, suggesting that the pharmacological mechanism of Xuebijing injection was associated with synergistic action of multiple ingredients responded to multiple targets simultaneously. Remarkably, the results substantiated that the curative effects of Xuebijing injection in the treatment of severe patients with COVID-19 could attribute to inhibit cytokine storm including interleukin (IL) 2, IL4, IL6, IL8, tumor necrosis factor (TNF), mitogen-activated protein kinase 1 (MAPK1), MAPK8, MAPK14, TP53, vascular endothelial growth factor A (VEGFA), STAT3, JUN, PIK3CA, EGFR, ERBB2, ERBB3, and IKBKB. From the aspect of chemical compounds, there were 11 (12.22%) active ingredients that attributed to establish

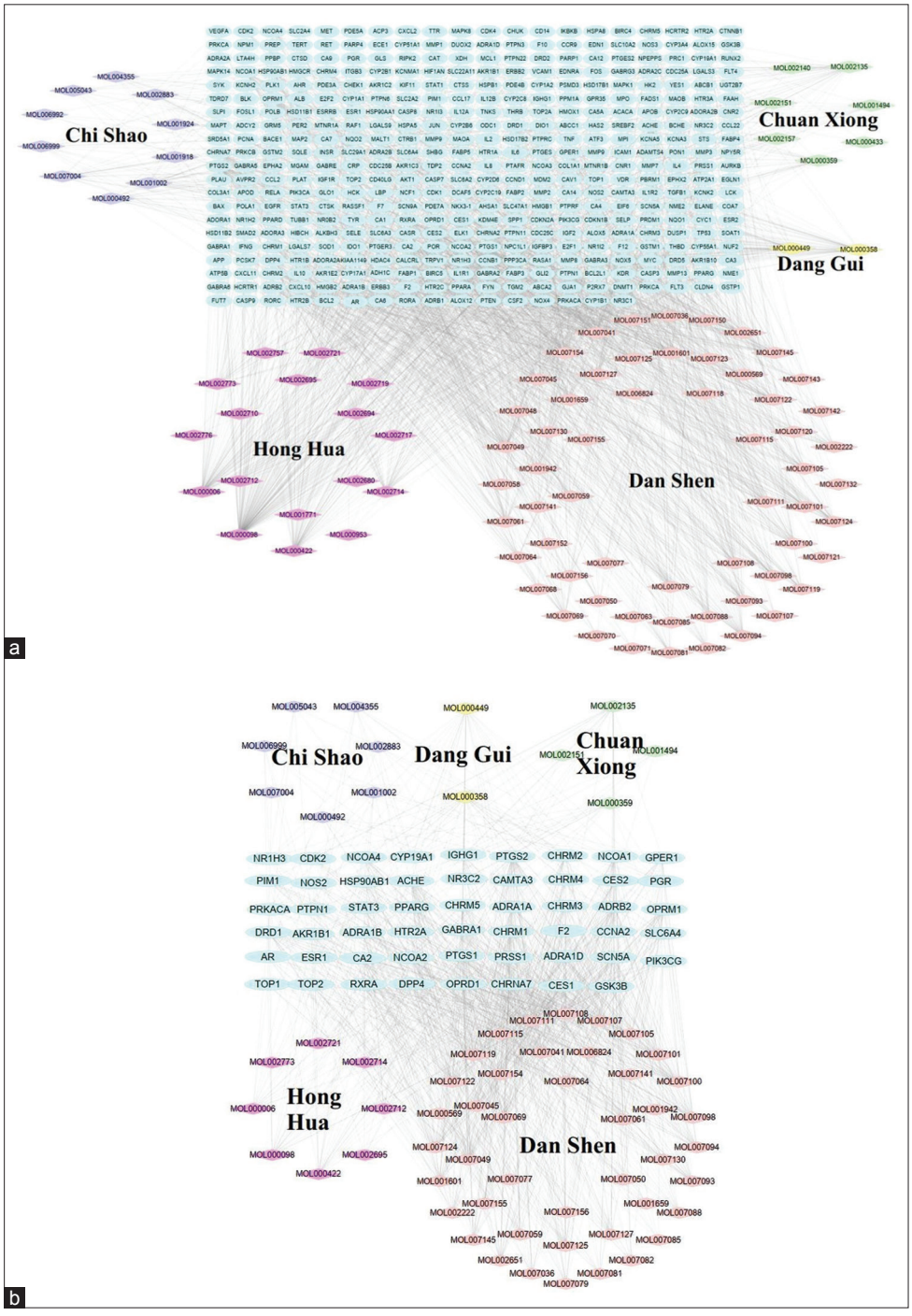


Figure 2: The compound-target network diagram (a) and compound-target network diagram with degree ≥10 of Xuebijing injection (b)

links with more than 20 targets; the results demonstrated clearly that the critical compounds including lignans, quercetin, flavoxanthin, 6-hydroxy-kaempferol in Hong Hua, and sitosterol in Hong Hua and Dang Gui played considerable roles in biological regulation of targets' groups.

According to the results of topological analysis in Figure 2b, the network diagram was displayed the relationship between the nodes with their DC >10. Among them, a total of 67.37% was dominate compounds and others belonged to therapeutic targets. The results again accurately described the complex mechanism of the gene regulatory networks of Xuebijing injection against COVID-19 based on the unique advantages of multiple components, multiple links, and multiple targets.

Protein–protein interactions network and module analysis

As presented in Figure 3, PPI network analysis of the target groups was conducted to identify hub genes and reveal potential mechanism of Xuebijing injection against COVID-19, the potential core targets with greater size were procured, and the top 6 genes were AKT1 (AKT serine/threonine kinase 1), TP53 (cellular tumor antigen p53), VEGFA, ALB (Fas-binding

factor 1), and TNF. Moreover, the hub genes including IL6, VEGFA, TNF, MAPK1, JUN, STAT3, EGFR, and TP53 all had a close relationship with cytokine storm.

The module analysis was applied to discover the hidden biological information of highly interconnected clusters; based on the results listed in Table 3, PPI network was divided into 6 clusters with K-core >4 ultimately. In brief, the BP of these clusters involved in positive regulation of transcription from RNA polymerase II promoter, cell proliferation, response to drug, oxidation-reduction process, intracellular receptor signaling pathway, and cellular lipid metabolic process, respectively.

Functional enrichment analysis

We acquired 638 entries ($P < 0.05$) of GO enrichment analysis for treating COVID-19 with Xuebijing injection. Of which, numerous BPs (471 terms, 73.82%) were involved in Xuebijing injection for treating COVID-19; the significant terms were associated with positive regulation of transcription from RNA polymerase II promoter, response to drug, negative regulation of apoptotic process, positive regulation of cell proliferation, and signal transduction. A total of 54 (8.46%) related items were identified as cellular component (CC), covering plasma membrane, cytoplasm, nucleus, cytosol, and so on. Besides, 113 (17.71%) items involved in MF, suggesting the targets of Xuebijing injection, were enriched in protein binding, ATP binding, enzyme binding, zinc ion binding, protein homodimerization activity, etc.

There were 108 potential signal pathways ($P < 0.05$) in the KEGG pathway enrichment screening. The relevant gene symbols that correspond to 95 components of Xuebijing injection interacted closely with the critical signaling pathways involving PI3K-Akt, human T-cell leukemia virus type 1 (HTLV-I) infection, MAPK, tuberculosis, focal adhesion, TNF, small-cell lung cancer, etc. Through these associated pathways, Xuebijing injection may exert satisfactory effects for treating

Table 2: Distribution of common compounds in Xuebijing injection

Number	Chemical composition	Herbs
MOL000006	luteolin	Hong Hua, Dan Shen
MOL000358	beta-sitosterol	Hong Hua, Chi Shao, Dang Gui
MOL000359	sitosterol	Chi Shao, Chuan Xiong
MOL001771	poriferast-5-en-3beta-ol	Hong Hua, Dan Shen
MOL002714	baicalein	Hong Hua, Chi Shao,
MOL002776	baicalin	Hong Hua, Chi Shao, Dan Shen

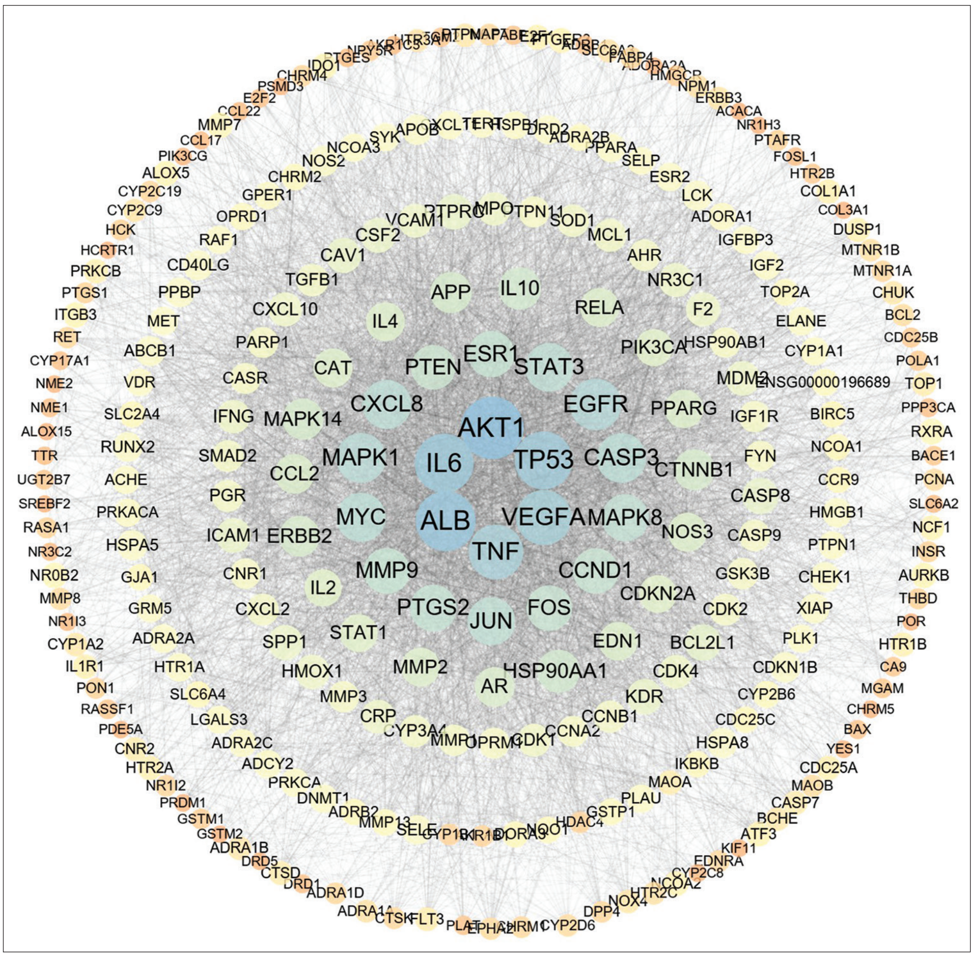
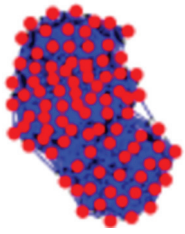
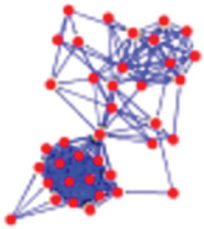
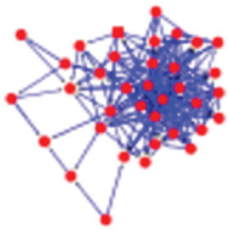
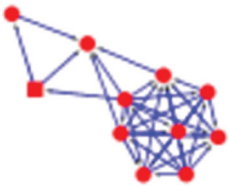
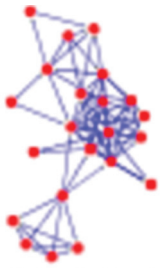


Figure 3: The protein-protein interaction network of potential targets in Xuebijing injection

Table 3: The center gene ontology biological process in each cluster

Number	Cluster	Biological process of cluster	*P*	MCODE scores	Nodes	Edges
1		Positive regulation of transcription from RNA polymerase II promoter	6.3E-15	45.136	89	1986
2		Cell proliferation	3.3E-9	10.811	38	200
3		Response to drug	2.6E-6	10.171	36	178
4		Oxidation-reduction process	6.8E-10	7.000	11	35
5		Intracellular receptor signaling pathway	8.4E-4	6.667	22	70
6		Cellular lipid metabolic process	1.2E-4	5.143	8	18

MCODE: Molecular complex detection

COVID-19 in aspects of lung inflammation, virus infection, and lung injury, especially for severe and critical cases. The top 20 of each category are described in Figure 4. The size of the dots reflected the number of corresponding targets, and the different colors of the dots donated the different *P*-adjusted value.

Molecular docking simulation

In the current study, the analysis of molecular docking simulation provided a visual interpretation of the interaction between key compounds of Xuebijing injection and crucial targets (3CLpro, ACE2); the results of dominative ligands with superior binding

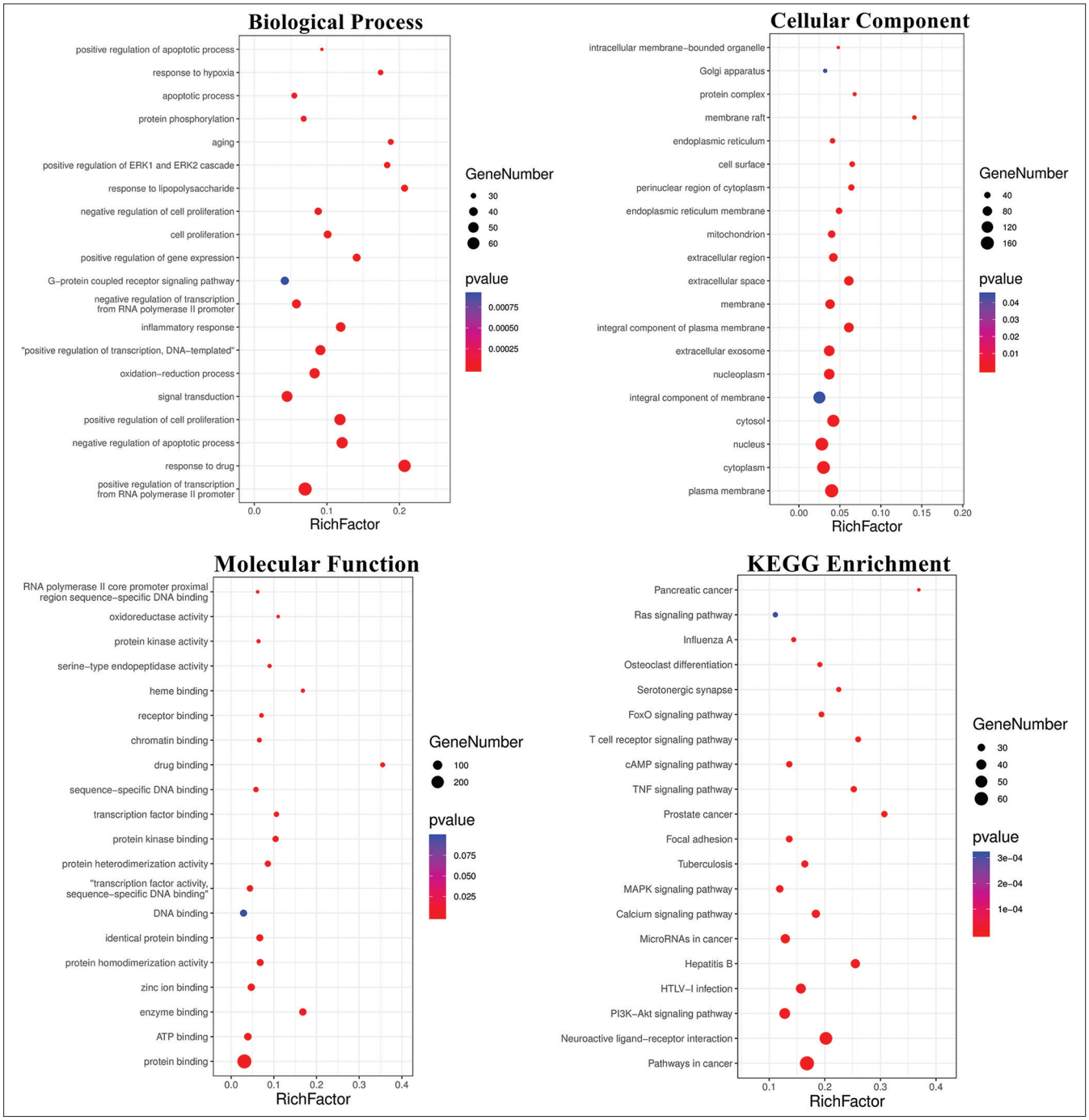


Figure 4: Gene ontology enrichment analysis and Kyoto Encyclopedia of Genes and Genomes pathway analysis of potential targets in in Xuebijing injection

activity are summarized in Table 4. Approximately 80% of them formed optimal conformation between 3CLpro and ACE2 with binding energy <−5.0 kJ/mol. Thus, our predicted results suggested that the possible molecular mechanism of Xuebijing injection in the treatment of COVID-19 was on the basis of its bioactive ingredients which entered the active pocket of the core proteins with preferable binding activity. The characteristic constituents, Hydroxysafflor yellow A and Danshenol B, were considered as examples for emerging the diagrams of molecular docking in Figure 5; the results indicated that Danshenol B may bind to 3CLpro and ACE2 and form stable complex with strong hydrogen bonds.

Discussion

New coronavirus pneumonia caused by highly pathogenic 2019-nCoV was an acute infectious disease with the population's susceptibility, had contributed to large outbreaks, and evolved into great threats to global public. In China, implementing "attach equal importance to TCM and Western medicine" policy in clinical practice exerts positive effects in the whole rescue process of treating COVID-19 toward the end of December 2019.[17,54-57] For now, the latest official data show that 91.5% (74,187 cases) received TCM among the

Table 4: Docking results of candidate compounds in Xuebijing injection

Herbs	Molecule name	△Gbind (3CLpro)	△Gbind (ACH2)	Herbs	Molecule name	△Gbind (3CLpro)	△Gbind (ACH2)
Chi Shao	(+)-catechin	−5.29	−5.19	Dan Shen	Salvilenone	−6.72	−6.65
	Ellagic acid	−5.5	−6.68		Cryptotanshinone	−6.34	−7.54
	Lactiflorin	−6.02	−5.12		Danshenspiroketallactone	−5.2	−6.25
	Paeoniflorin	−5.43	−3.2		Deoxyneocryptotanshinone	−6.52	−6.22
	Ethyl oleate	−2.5	−2.67		Dihydrotanshinone I	−7.5	−6.68
	Spinasterol	−8.87	−7.15		Epidanshenspiroketallactone	−7.17	−6.63
	Campest-5-en-3beta-ol	−7.79	−5.97		Isotanshinone II	−8.8	−6.89
	Stigmast-7-en-3-ol	−7.35	−6.56		Manool	−6.26	−6.03
	Albiflorin	−2.97	−3.68		Microstegiol	−6.71	−7.13
	Paeoniflorigenone	−5.62	−5.8		Miltipolone	−8.9	−6.96
	EvofolinB	−3.97	−3.09		Miltirone	−7.33	−6.78
Chuan Xiong	Sitosterol	−7.27	−6.05		Miltirone II	−5.67	−6.73
	Mandenol	−3.91	−2.29		Neocryptotanshinone	−5.65	−5.98
	Myricanone	−6.3	−5.9		Salviolone	−6.11	−6.75
	Perlolyrine	−6.04	−5.96		Tanshindiol B	−6.18	−6.01
	Senkyunone	−6.46	−4.94		Przewaquinone E	−6.7	−6.17
	Wallichilide	−6.25	−6.24		Tanshinone iia	−6.85	−6.44
Dan Shen	1,2,5,6-tetrahydrotanshinone	−8.44	−7.07	Hong Hua	Luteolin	−5.92	−5.63
	Poriferasterol	−7.4	−6.19		Quercetin	−6.86	−4.8
	Isoimperatorin	−8.19	−5.57		Beta-sitosterol	−6.72	−6.28
	Sugiol	−6.95	−6.19		Kaempferol	−5.53	−5.1
	Dehydrotanshinone II A	−8.21	−7.04		Stigmasterol	−7.27	−6.63
	α-amyrin	−8.97	−7.94		Poriferast-5-en-3beta-ol	−7.53	−5.8
	2-isopropyl-8-methylphenanthrene-3,4-dione	−7.0	−6.08		Flavoxanthin	−7.18	−6.37
	3α-hydroxytanshinone II A	−7.16	−6.68		Lupeol-palmitate	−5.64	−4.69
	4-methylenemiltirone	−6.42	−6.57		Phytoene	−4.85	−3.52
	Formyltanshinone	−6.9	−6.3		Phytofluene	−2.99	−3.88
	Methylenetanshinquinone	−7.88	−6.84		Pyrethrin II	−4.08	−4.74
	Sclareol	−6.66	−5.58		6-Hydroxykaempferol	−5.02	−4.98
	Tanshinaldehyde	−7.51	−6.92		Baicalein	−5.44	−5.51
	Danshenol B	−8.27	−6.55		6-Hydroxynaringenin	−5.01	−4.91
	Danshenol A	−6.24	−6.55		Hydroxysafflor yellow A	−1.57	−1.07
	Salvilenone	−6.72	−6.65	Dang Gui	Beta-sitosterol	−6.57	−6.5
	Cryptotanshinone	−6.34	−7.54		Stigmasterol	−7.27	−6.63

confirmed cases with COVID-19 in China, and increasing treatment results of clinical observation indicated that the total effective rate of TCM had reached more than 90%.[58] Various clinical studies had confirmed that the clinical superiority of TCM, including alleviation the clinical symptoms, prevention the progression of disease, improvement the curative rate, and promotion the physical recovery for individuals in convalescent period.[59,60] A prospective cohort study on the efficacy of Xuebijing injection in the treatment of COVID-19 was urgent established in January 21, 2020, which led by academician Nan-Shan Zhong and cooperated with the First Affiliated Hospital of Guangzhou Medical University, Guangzhou Respiratory Health Research Institute.[61] As representative intravenous herbal preparation, it had been listed as recommended drugs against COVID-19, especially bringing new hope for severe and critical cases. There was accumulating evidence to suggest that Xuebijing (XBJ) injection had the preferable benefits and promising potentials to patients with critically illness including sepsis, severe community-acquired pneumonia, ARDS, MODS, and so on.[62,63] Besides, extensive research indicated that Xuebijing could exert broad range of pharmacological activities via inhibiting proinflammatory cytokine secretion, attenuating the crosstalk between inflammation and coagulation, protecting endothelial cells, preventing oxidative stress, neutralizing endotoxins, ameliorating sepsis-induced lung injury, improving microcirculation, relieving cumulative immunosuppression, etc.[64-66]

Based on the approach of network pharmacology, the present study aimed to reveal the possible molecular mechanism involved in the effects of Xuebijing injection on COVID-19 from multiple angles. First, the top protein genes such as IL6, VEGFA, TNF, MAPK1, JUN, STAT3, EGFR, and TP53 with the highest degree in PPI network were responsible for cytokine storm, which was induced by virus infection and led

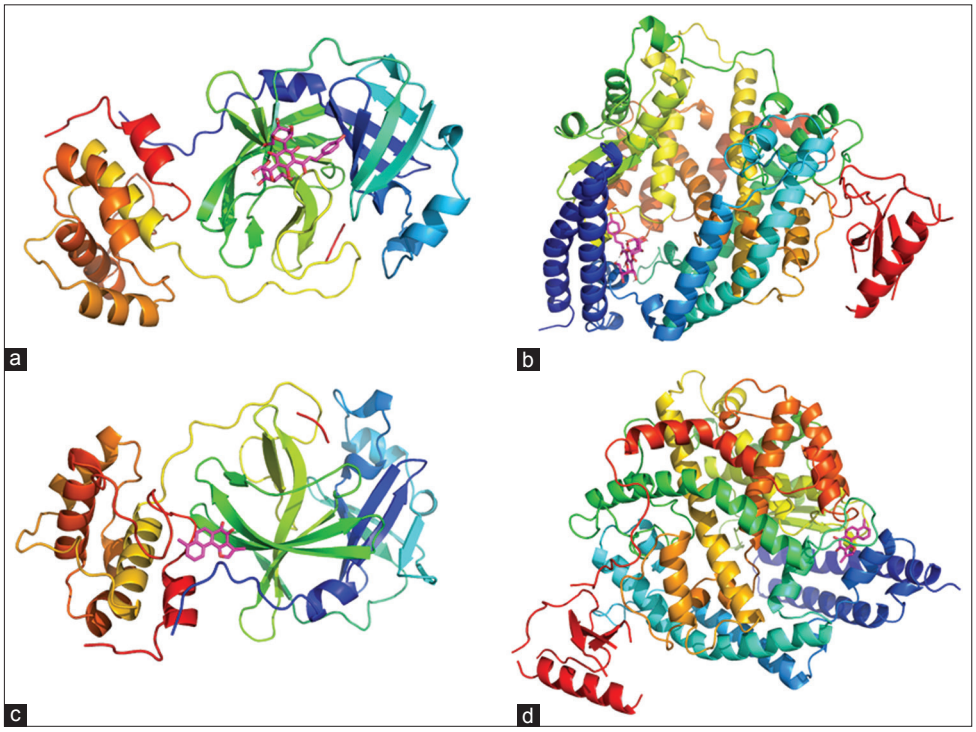


Figure 5: Molecular docking diagram of Hydroxysafflor yellow A and Danshenol B. (a) Hydroxysafflor yellow A and 3CLpro, (b) Hydroxysafflor yellow A and angiotensin-converting enzyme 2, (c) Danshenol B and 3CLpro, (d) Danshenol B and angiotensin-converting enzyme 2

to severe acute lung injury, ARDS, and even multiple organ failure until death.[67,68] Significantly, it had been reported that the profile of cytokine storm was associated with COVID-19 disease severity, characterized by increased TNF-α, IL-2, IL-7, IL-10, interferon-α, and monocyte chemoattractant protein-1, and a subgroup of patients with severe COVID-19 might have the syndrome of cytokine storm.[69-70] Hence, Xuebijing injection had a positive influence on inhibition of cytokine storm to reduce rising mortality for some severe and critical COVID-19 cases. Second, these aforementioned genes with higher degree, AKT1, encoded a serine/threonine kinase that had best-known substrates glycogen synthase kinase-3. It was found that AKT1 can not only modulate pulmonary fibrosis through inducing IL-13 production by macrophages, but also play unique roles in inflammation, cell growth, proliferation, migration, and survival.[71-73] Especially, the active principles of Xuebijing injection involved in the regulation of upstream and downstream effectors can probably prevent and treat COVID-19. Third, based on the results of functional enrichment analysis, these high-degree pathways including PI3K-Akt signaling pathway, HTLV-I infection, MAPK signaling pathway, tuberculosis, focal adhesion, TNF signaling pathway, and small-cell lung cancer all played important parts in lung inflammation, virus infection, and lung injury, suggesting that clinical benefits of Xuebijing injection against COVID-19 are closely related to resist pulmonary infection, suppress the production of inflammatory markers in the lungs, and relieve COVID-19-associated lung injury. For example, the MAPK signaling pathway can control the strength and duration of inflammatory responses by regulating both the expression and function of multiple inflammatory factors.[74,75]

With regard to key compounds of Xuebijing injection within C-T network, the majority of these active ingredients were related to multiple targets. As one of representative bioactive and water-soluble compounds of Xuebijing injection, Hydroxysafflor yellow A can exhibit broad and effective pharmacological activities including lung protection, metabolism regulation, and endothelium cell protection as for patients with COVID-19.[76] Indeed, animal studies demonstrated that Hydroxysafflor yellow A could attenuate bleomycin-induced pulmonary fibrosis via inhibiting the expression of α-SMA, Smad3 phosphorylation, and improving the morphological changes in lung tissue.[77] In addition, quercetin exhibited effective antioxidant and anti-inflammatory, antiviral properties, for example, prevented the progression of rhinovirus-induced lung disease in mice with COPD phenotype, induced apoptosis in the lungs by modulation of p53 posttranslational modifications, and attenuated the hyperoxic lung injury through the upregulation of CYP1A1/CYP1B1/NQO1 mRNA, proteins and the reducing the levels of NF-kB and MDA-protein adducts in lung tissues of neonatal mice.[78-80] According to the results of molecular docking, Danshenol B possessed the lower binding energy with 3CLpro and ACE. Moreover, the compounds from the herbal plant, Danshen, including Salvianolic acid B and Tanshinone IIA, were closely related to reverse alveolar structural destruction/loss and regulate the proliferation and migration of lung cell via JAK2/STAT3/VEGF signaling

pathways, attenuate paraquat-induced acute lung injury in rats by modulating ACE2 and Ang-(1-7) in terms of increasing neutrophil infiltration, lung wet/dry weight ratio.[81,82]

Towards aforementioned common compounds, Chinese herbs with high content of luteolin was applied ethnopharmacologically to treat inflammation-related symptoms, and results of pharmacological research revealed that the protective effects of luteolin against mercuric chloride-induced lung injury in mice were involved AKT/Nrf2 and NF-κB pathways.[83-84] Baicalin could alleviate acute lung injury by inhibiting NF-κB pathway activation in chicken model, regulating the crosstalk between the CX3CL1-CX3CR1 axis and NF-κB pathway in CX3CL1-knockout mice. [85-86]

Taken together, Xuebijing injection had confirmed its beneficial effects against the pathogenesis of COVID-19 which were associated with suppressing cytokine storm, attenuating lung inflammation, alleviating lung damage, and antiviral activities via multiple components, multiple targets, multiple pathways, multiple levels, and multiple connections by utilizing the bioinformatics methods of network pharmacology and molecular docking. Nevertheless, the further experiments both *in vitro* and *in vivo* were warranted to verify the main active components, key targets, and pathways Xuebijing injection against COVID-19 to fully understand the related material basis and pharmacological activities.

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Conflicts of interest

There are no conflicts of interest.

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