

Uncovering the underlying mechanisms of Compound Yuxingcao Mixture in the treatment of COVID-19 based on network pharmacology

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Research

Keywords: network pharmacology, COVID-19, Compound Yuxingcao Mixture, inflammatory response, mechanism

Posted Date: June 8th, 2020

DOI: <https://doi.org/10.21203/rs.3.rs-32916/v1>

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Abstract

Background and objective: The novel coronavirus named COVID-19 emerged in Wuhan, China in December, 2019 and has spread rapidly in China and around the world. The traditional Chinese medicine Compound Yuxingcao Mixture (CYM) has been recommended in recent editions of the national guideline while the underlying mechanisms are still unclear. In this study, we analyzed the effectiveness and potential mechanisms of CYM on COVID-19 based on network pharmacology and molecular docking approach.

Methods: The active ingredients and potential targets of CYM were screened using TCMSP and STITCH databases. Genes related severe acute respiratory syndromes (SARS) and Middle East respiratory syndrome (MERS) were queried on the DisGeNET and MalaCards databases. CYM-COVID-19 common target protein interaction network was established by STRING database. Gene Ontology (GO) and Kyoto Encyclopedia of Genes and Genomes (KEGG) pathway enrichment analyses were conducted to generate the relative pathways based on KOBAS databases. In addition, the possible binding site of screened compounds were also predicted by Autodock vina software.

Results: A total of 103 active ingredients and 205 putative targets were screened from CYM, of which 32 overlapped with the targets of COVID-19 and were considered therapeutic targets. The analysis of the network diagram demonstrated that the CYM activity of ingredients of quercetin, luteolin, β -sitosterol and kaempferol may play a crucial role in treating COVID-19 by regulating TNF, IL-6, IL-1 β , etc. The analysis of molecular binding energy showed that β -sitosterol had the lowest binding energy with COVID-19 3CLpro (-8.63 kJ/mol). GO and KEGG enrichment analysis revealed that these targets were closely associated with inflammatory responses and immune defense processes.

Conclusion: In summary, our study identified the potential mechanisms and targets of CYM for the prevention of COVID-19, providing directions for further clinical research.

Introduction

Since December 2019, the large-scale spread of the new coronavirus disease (COVID-19), officially known as severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2), is a major epidemiological event(1) [1]. As of April 14, 2020, COVID-19 has affected more than 1,948,617 patients in 210 countries and regions around the world, resulting in approximately 121,846 deaths worldwide(2, 3) [2,3]. Since there are no effective control measures, the World Health Organization (WHO) eventually requested member countries to expand their emergency response mechanisms to find new vaccines and effective drugs(4) [4].

Traditional Chinese medicine (TCM), as a core component of the national healthcare system, has been recommended in recent editions of the national guideline for the treatment of COVID-19(5) [5]. COVID-19 belongs to the category of “pestilence” in TCM and is named “pulmonary pestilence”. Clinically, it has radiological characteristics of pneumonia, and presenting as fever and respiratory symptoms(6) [6].

Subsequently, the Sichuan Provincial Health Commission recommended the Chinese patent medicine of Compound Yuxingcao Mixture (CYM) for clinical treatment(7) [7]. This prescription consists of *Houttuyniae Herba*, *Isatidis Radix*, *Scutellariae Radix*, *Lonicerae Japonicae Flos*, *Forsythiae Fructus*. Among them, *Houttuyniae Herba* is regarded as sovereign medicine which has the functions of clearing heat and detoxification, diuresis and dehumidification(8, 9) [8, 9]. The CYM has been used in the clinical treatment of COVID–19 and achieved expected results, but its underlying mechanisms remains to be clarified.

Network pharmacology is an interactive network based on the theory of system biology, including cheminformatics, bioinformatics, network biology and pharmacology. It clearly reveals the underlying mechanisms of active ingredients acting on human body by constructing drug-active ingredient-target-disease network, which is consistent with the general view of TCM. The purpose of this study is to explore the underlying mechanism of CYM on COVID–19 disease through network pharmacology, drug targeting interaction database and biological analysis methods. Our flowchart is shown in Figure 1.

Materials And Methods

Construction of the herb-property-flavor-meridian tropism network for CYM prescription

The five herbs in the CYM prescription, and the corresponding information about their TCM properties (warm, cold and neutral), flavors (sweet, bitter and pungent) and meridian tropism (lung, heart, stomach, spleen, liver, gallbladder, large intestine and small intestine) were compiled from the Traditional Chinese Medicines Integrated Database (TCMID) (<http://119.3.41.228:8000/tcmid/search/>) and Encyclopedia of Traditional Chinese Medicine (ETCM) platform (<http://www.tcmip.cn/ETCM/index.php/Home/Index/>). A network of herb-property-flavor-meridian tropism was constructed using R package.

Collection of active ingredients

The active ingredients of CYM were collected from the Traditional Chinese Medicine Systems Pharmacology (TCMSP) platform (<http://tcmispw.com/tcmisp.php>). Oral bioavailability (OB) refers to the percentage of unmodified drugs that enter the circulatory system after oral administration. Drug likeness (DL) is a vague concept that refers to the similarity between compounds and known drugs. According to the relevant parameters of the pharmacokinetic properties, the active ingredients of CYM were screened using the $OB\% \geq 30\%$ and $DL \geq 0.18$ as parameters(10, 11) [10, 11].

Screening of target genes for active ingredients

The names and numbers of active ingredients obtained from the TCMSP database were gathered and retrieved on the PubChem database (<https://pubchem.ncbi.nlm.nih.gov/>), and the corresponding SMILE numbers were recorded as well. Subsequently, the following two databases DrugBank

(<http://www.drugbank.ca>) and STITCH (<http://stitch.embl.de/>) were used to predict the target information of active ingredients. Finally, all the target information was standardized using UniProt (<http://www.UniProt.org/>).

Screening of potential targets for COVID–19

No data on COVID–19-related genes were available in the DisGeNET (<https://www.disgenet.org/>) and MalaCards (<https://www.malacards.org/>) databases in the present study. Because the new coronavirus is very similar to SARS-CoV and MERS-CoV, Severe Acute Respiratory Syndrome (SARS) and Middle East Respiratory Syndrome (MERS) was used in our research to gather the target genes that may be related to the new coronavirus.

PPI network construction

To explain the interaction between target genes, the selected genes were uploaded to STRING database (<http://string-db.org>) to obtain the information of protein-protein interaction (PPI). In the present study, the species was set as *Homo sapiens*, the minimum required interaction score was set as medium confidence 0.400, then the remaining parameters remained the default settings. Subsequently, the information of PPIs was downloaded and visualized using Cytoscape 3.7.1. In all nodes, the size and color of the nodes were used as the criteria for screening hub genes.

Drug-active ingredient-target network construction

A Venn diagram was used to visualize the amount of overlap between the genes related to the CYM ingredients and COVID–19-related genes. After removing the redundant genes, the drug-active ingredient-target network was constructed by cytoscape 3.7.1 software. In the network diagram, the nodes represent five traditional Chinese medicines, active ingredients, and therapeutic targets respectively. The edges in the network were used to connect drugs-active ingredients-therapeutic targets and the amount of edges were measured in degrees.

Gene ontology and KEGG enrichment analysis of target genes

To elucidate the potential biological function of CYM in the treatment of COVID–19, GO (Gene Ontology) functions and KEGG (Kyoto Encyclopedia of Genes and Genomes) pathway enrichment analysis were performed. The gene list of targets were inputted into STRING (<http://string-db.org>) and KOBAS (<http://kobas.cbi.pku.edu.cn/>) to perform GO and pathway analysis. The P -value<0.05 were considered to

be statistically significant, then the bubble plot of pathways were drawn via imageGP (<http://www.ehbio.com/>) platform.

Molecular docking

The protein COVID-19 3CL^{Pro} (PDB ID:6LU7) required for docking was obtained from PDB (<https://www.rcsb.org/>) database. The AutoDockTools 1.5.6 software was used to remove water molecules, perform hydrogenation and calculate the charge of the protein. Finally, it was saved as pdbqt format file. The structure of small molecule ligands of the TCM that need to be docked were obtained from Pubmed (<https://www.ncbi.nlm.nih.gov/Pubmed>) and ZINC (<https://zinc.docking.org/>). Subsequently, the Autodock vina software was used for molecular docking (the parameters were set as num_modes = 10, energy_range = 4, exhaustiveness = 100).

Results

The herb-property-flavor-meridian tropism network for CYM prescription

Based on TCM theory, the five herbs in CYM were classified according to their property, flavor and meridian tropism (Table 1 and Figure 2). A network was constructed among elements of the TCM classification system (Table 1) and the five herbs of the CYM prescription via R software. The connection degree of lung, heart, stomach and small intestine is the highest in the meridian tropism group, and the corresponding connection values are four, three, two and two, respectively. Four herbs (*Houttuyniae Herba*, *Scutellariae Radix*, *Lonicerae Japonicae Flos*, *Forsythiae Fructus*) in CYM are associated with the lung meridian. Among the flavor group, the greatest degree of connection is bitter, with three degrees of association.

Screening potential targets of CYM

To identify the active compounds of CYM, two classical ADME parameters, $OB\% \geq 30\%$ and $DL \geq 0.18$, were used for screening. Through mining the TCMSP, a total of 594 potential compounds were screened from five traditional Chinese medicines (Supplemental Table 1). After that, we inputted the molecular name and SMILE number of the active ingredients into the Drugbank and STITCH databases to obtain 151 and 80 putative targets of CYM, respectively. The putative targets included PTGS1, IL-6, JUN, TNF, IFNG, IL-2 and so on. Detailed information about the potential targets of CYM was shown in (Figure 3A, Supplemental Table 2).

The acquisition of known therapeutic targets for COVID-19

Genes related to COVID-19 were retrieved from the DisGeNET database and MalaCards database using the keywords “SARS” and “MERS”. In the study, a total of 260 known therapeutic targets were collected from DisGeNET database and 144 known therapeutic targets were collected from MalaCards database. After eliminating redundant targets, a total of 351 known therapeutic targets were collected (Figure 3B). Then the potential targets in CYM was mapped to the COVID-19 targets to obtain 32 therapeutic genes using the ImageGP platform, and a Venn diagram was drawn (Figure 3C and Table 2).

PPI networks construction and core genes screening

PPI network was used to explore the function of diverse targets in CYM and COVID-19. A acquisition of 32 targets were inputted into the STRING database, the medium confidence score was set as 0.400 for further analysis, and then visualized by Cytoscape 3.7.1. There were 32 protein nodes and a total of 225 interactive connecting lines in the PPI network. The average node degree of freedom is 14.1, and the avg. local clustering coefficient is 0.758. Among all core targets, the bigger the deeper, the more important it is. Further analysis from cytoHubba revealed that the degree of tumor necrosis factor (TNF), interleukin 6 (IL-6) and interleukin 1 β (IL-1 β) is 30, 28 and 25 respectively, which were closely related to the inflammatory response (Figure 4).

Drugs-Compounds-Targets network analysis

To elucidate the potential mechanism of CYM in the treatment of COVID-19, we used Cytoscape 3.7.1 software to build the Drugs-Active compounds-Targets network. The hexagon nodes represent five traditional Chinese medicines, the ellipse nodes represent 103 active ingredients and the diamond nodes represent 32 overlapping targets for disease. The edges indicate that nodes can interact with each other. Further analysis of the network topology shows that the centralization and heterogeneity are 0.339 and 1.602 respectively, which indicates that the compound-target space has a tendency for certain compounds and targets. Therefore, the network contains some core ingredients with multi-targets, such as Quercetin (degree = 75), Luteolin (degree = 24), Beta-sitosterol (degree = 28), Wogonin (degree = 18), Acacetin (degree = 10), Kaempferol (degree = 24), which may play a crucial role in the treatment of COVID-19 (Figure 5).

Gene ontology enrichment analysis

To verify whether the 32 target genes are related to COVID-19, we entered the candidate targets into the STRING platform for GO functional enrichment and annotation. In the study, a total of 750 biological processes, 52 molecular functions, and 25 cellular components were obtained. The top 10 entries were selected based on false discovery rate (FDR) <0.05 and the number of enriched genes, and then visualized by Cytoscape (Figure 6). The X-axis indicates the number of enriched genes for the term, and the Y-axis indicates the GO term. The results demonstrated that the biological processes mainly involved

response to stimulus (32 targets), cellular process (32 targets), biological regulation (30 targets) (Figure 6A, B); the molecular functions mainly involved Binding (31 targets), Protein binding (26 targets), Catalytic activity (17 targets) (Figure 6C, D); the cellular components mainly involved Intracellular (30 targets), Extracellular region (24 targets), Extracellular space (20 targets) (Figure 6E, F). The GO enrichment analysis results showed that the active compounds of CYM could participate in various cellular processes, so as to treat COVID-19.

The KEGG pathway enrichment analysis

The selected targets were inputted into KOBAS database to carry out pathway enrichment analysis. A total of 183 signaling pathways were obtained, and the top of 20 entries were screened according to the number of enriched targets (Figure 7A, B). The signaling pathways were listed as follows: Pathways in cancer, Inflammatory bowel disease (IBD), IL-17 signaling pathway, Chagas disease (American trypanosomiasis), AGE-RAGE signaling pathway in diabetic complications, C-type lectin receptor signaling pathway, Yersinia infection, Fluid shear stress and atherosclerosis, Tuberculosis, Cytokine-cytokine receptor interaction, Leishmaniasis, Th1 and Th2 cell differentiation, T cell receptor signaling pathway, Th17 cell differentiation, Jak-STAT signaling pathway, NOD-like receptor signaling pathway, Human cytomegalovirus infection, Pertussis, Toll-like receptor signaling pathway, TNF signaling pathway. These results suggest that the effective pharmacological ingredients in CYM may treat COVID-19 through alleviating inflammatory responses and enhancing immune pathways. The inflammatory bowel disease (IBD) and IL-17 signaling pathway, which play a crucial role in treating disease were annotated (Figure 7C, D).

Analysis of molecular docking and binding modes results

It is worth noting that we have made several milestone discoveries, including the crystal structure of 3CL^{PRO}, which can serve as candidate molecular target for SARS therapy(12) [12]. To evaluate the targeting of several active ingredients in CYM to COVID-19 3CL^{PRO}, we selected the top six ingredients with larger degrees from the network diagram for molecular docking and binding energy analysis (Table 3 and Figure 8). The binding energy of the core active ingredients of CYM are all less than -5 kJ/mol, of which the lowest binding energy of active compounds with COVID-19 3CL^{PRO} is beta-sitosterol (-8.63 kJ/mol).

Discussion

From the perspective of TCM, the medication paradigm for the treatment of COVID-19 is based on a comprehensive and dialectical understanding of the pathological evolution of COVID-19. As the epidemic disease outbreak is urgent, serious and highly infectious, the pathogen is generally attributed to dampness toxin according to the main symptom characteristics of this disease(13) [13]. TCM deduces

that COVID-19 is located in lung and closely related to stomach and spleen. Its pathological changes involve the heart, liver and kidney in the later stage(14) [14]. Dampness pathogen can slowly evolve into cold-dampness or dampness-heat, and it depends entirely on the patients' body constitution. Clinical observation reveals that patients will present some symptoms of Qi and Yin deficiency(15, 16) [15, 16]. Therefore, TCM recommends that the patients should gradually restore sufficient health Qi to dispel pathogen from the body, so that they will enter the recovery period. The CYM has the functions of clearing heat and detoxification, enhancing the body's immunity, and stimulating the body's immune defense system to indirectly exert anti-viral effects. Among them, *Houttuyniae Herba* and *Scutellariae Radix* is regarded as sovereign and assistant medicine respectively. And the rest are adjuvants, which cooperates with together to exert the efficacy.

In this study, the network pharmacology method was used to screen active ingredients in CYM, we obtained their target genes and established a visual network analysis of drugs-active ingredients-target genes. Preliminary analysis indicated that 103 potential active ingredients in CYM might play a crucial role in regulating 32 targets through multiple cellular processes mainly involved response to stimulus (32 targets), cellular process (32 targets), biological regulation (30 targets). Further analysis demonstrated that these 32 targets were roughly divided into three categories: inflammatory cytokines, mitogen-activated protein kinases and others. Among them, the inflammatory cytokines are mainly TNF, IL-6, IL-1 β , CCL2, IL-10, IL-2, which concentrate on inflammatory responses. As reported, IL-6 is an important cytokine that initially expressed through the immune system in response to injurious or infectious processes(17) [17]. In the early stage, it activates the JAK/STAT signal pathway, and initiates B cell differentiation, plasma cell production and a series of acute reactions(18, 19) [18, 19]. The increase of IL-6 occurs early in the initiation of cellular stress and has a long duration, so it can be used as an indicator for the early diagnosis of acute infection as well as the severity and prognosis of infection(20) [20]. The Yi JH team analyzed 69 severe COVID-19 patients and found that the level of IL-6 in severe patients was significantly higher than in non-severe patients, which was closely related to the patients' maximum temperature and CT results(21) [21].

According to the characteristics of network topology, the core nodes including Quercetin (degree = 75), Luteolin (degree = 24), Beta-sitosterol (degree = 28), Wogonin (degree = 18), Acacetin (degree = 10), Kaempferol (degree = 24) were screened in the network diagram, which might be the potential ingredients of CYM for the treatment of COVID-19. Since antiviral activity of some flavonoids is known, Jo et al applied a flavonoid library to probe inhibitory compounds against MERS-CoV 3C-like protease (3CL^{Pro}) and demonstrated that quercetin 3- β -D-glucoside had high efficiency on blocking the enzymatic activity of MERS-CoV 3CL^{Pro}, which was also confirmed independently using a tryptophan-based fluorescence method(22) [22]. In addition, several studies have demonstrated that quercetin produced its anti-inflammatory activity by acting on TLR4 and cytokines. In LPS-triggered signaling via TLR4, quercetin suppressed the nuclear factor of kappa light polypeptide gene enhancer in B degradation, and subsequently stimulated the phosphorylation of AKT and p38/MAPK in bone marrow-derived macrophages(23) [23]. In the present study, the results of molecular docking and binding energy analysis

demonstrated that the selected CYM ingredients also had high binding degree with the active site of COVID-19 3CL^{Pro} in the virus (Figure 8).

Using the KEGG pathway enrichment tool, we can obtain some important pathways that may be regulated by the drugs studied in the process of treating diseases, thus helping us to understand the mechanisms of drugs in treating diseases. In this study, the selected targets were analyzed by KOBAS platform, and the top 20 entries were screened out including the inflammatory bowel disease (IBD), Jak-STAT signaling pathway, TNF signaling pathway and IL-17 signaling pathway, which demonstrated that CYM could indirectly exert anti-viral effect by enhancing the body's immunity and stimulating the body's immune defense system.

Conclusion

In summary, the network pharmacology method was used in our study to predict the therapeutic targets and underlying mechanisms of CYM in the treatment of COVID-19. Although the predicted results were consistent with recent researches, more clinical trials are warranted for our findings to be confirmed.

Abbreviations

CYM: Compound Yuxingcao Mixture; MERS: Middle East respiratory syndrome; SARS: severe acute respiratory syndromes; GO: Gene Ontology; KEGG: Kyoto Encyclopedia of Genes and Genomes; COVID-19: the new coronavirus disease; TCM: Traditional Chinese medicine; TCMID: Traditional Chinese Medicines Integrated Database; ETCM: Encyclopedia of Traditional Chinese Medicine; TCMSP: Traditional Chinese Medicine Systems Pharmacology; OB: Oral bioavailability; DL: Drug likeness; PPI: protein-protein interaction; TNF: tumor necrosis factor; IL-6: interleukin 6; IL-1 β : interleukin 1 β .

Declarations

Author contributions

J. G. Zhu conceived and designed the studies. X. Y. Chen and Y. Q. Zhang completed the data collection and analysis. All authors participated in drafting of the manuscript and revising it before final submission.

Conflict of interest

The authors declare that they have no conflicts of interest.

Finding

This work was supported by grants from The National Natural Science Foundation of China (81660426), the Science and Technology Project of Guizhou Province in 2017([2017]5803), The High-level innovative talent project of Guizhou Province in 2018 ([2018]5639), The Science and Technology Plan Project of Guiyang in 2019 ([2019]2–15), Science and Technology Plan Project of Guizhou Province in 2019 ([2019]1203).

References

1. Singhal T. A Review of Coronavirus Disease–2019 (COVID–19). *Indian Journal of Pediatrics* 2020;87(4):281–6.
2. Abd ET, Stockand JD. Recent progress and challenges in drug development against COVID–19 coronavirus (SARS-CoV–2) - an update on the status. *Infection Genetics and Evolution* 2020;83:104327.
3. Coronavirus Outbreak. Available at: <https://www.worldometers.info/coronavirus/>. Accessed 23 Feb 2020.
4. World Health Organization. WHO Director-General’s opening remarks at the media briefing on COVID–19 - 11 March 2020. Geneva: World Health Organization; 2020.
5. Ren JL, Zhang AH, Wang XJ. Traditional Chinese medicine for COVID–19 treatment. *Pharmacological Research* 2020;155:104743.
6. Yang Z, Shi J, He Z, Lu Y, Xu Q, Ye C, Chen S, Tang B, Yin K, Lu Y, Chen X. Predictors for imaging progression on chest CT from coronavirus disease 2019 (COVID–19) patients. *Aging (Albany NY)* 2020;12(7):6037–48.
7. QH W, X Q, TX Z, LF H, W L, J P. Analysis of Key Varieties and Rational Allocation of Traditional Chinese Medicine Resources Under COVID–19 Epidemic Situation. *Chin J Exp Tradit Med Form* 2020;p.1–7.(<http://kns.cnki.net/kcms/detail/11.3495.R.20200410.1723.005.html>).
8. Dai W, Hu L, Ji L, Li J, Bi K, Li Q. A Comprehensive Method for Quality Evaluation of *Houttuynia* Herba by a Single Standard to Determine Multi-components, Fingerprint and HPTLC Method. *Analytical Sciences* 2015;31(6):535–41.
9. Zhu CS, Nie AZ, Zhang B, Lin ZJ. [Medication rules of Professor Zhang Bing in treatment of skin itching based on data mining]. *Zhongguo Zhong Yao Za Zhi* 2019;44(3):597–601.
10. Wang ZY, Jiang ZM, Xiao PT, Jiang YQ, Liu WJ, Liu EH. The mechanisms of baicalin ameliorate obesity and hyperlipidemia through a network pharmacology approach. *European Journal of Pharmacology* 2020;878:173103.
11. Zhao X, Hao J, Chen S. Network Pharmacology-Based Strategy for Predicting Therapy Targets of Traditional Chinese Medicine Xihuang Pill on Liver Cancer. *Evid Based Complement Alternat Med* 2020;2020:6076572.
12. Serseg T, Benarous K, Yousfi M. Hispidin and Lepidine E: two Natural Compounds and Folic acid as Potential Inhibitors of 2019-novel coronavirus Main Protease (2019-nCoV_{Mpro}), molecular docking

- and SAR study. *Curr Comput Aided Drug Des* 2020.
13. Ho L, Chan K, Chung V, Leung TH. Highlights of traditional Chinese medicine frontline expert advice in the China national guideline for COVID–19. *European Journal of Integrative Medicine* 2020;101116.
 14. Zhang YS, Cong WH, Zhang JJ, Guo FF, Li HM. [Research progress of intervention of Chinese herbal medicine and its active components on human coronavirus]. *Zhongguo Zhong Yao Za Zhi* 2020;45(6):1263–71.
 15. Jin YH, Cai L, Cheng ZS, Cheng H, Deng T, Fan YP, Fang C, Huang D, Huang LQ, Huang Q, Han Y, Hu B, Hu F, Li BH, Li YR, Liang K, Lin LK, Luo LS, Ma J, Ma LL, Peng ZY, Pan YB, Pan ZY, Ren XQ, Sun HM, Wang Y, Wang YY, Weng H, Wei CJ, Wu DF, Xia J, Xiong Y, Xu HB, Yao XM, Yuan YF, Ye TS, Zhang XC, Zhang YW, Zhang YG, Zhang HM, Zhao Y, Zhao MJ, Zi H, Zeng XT, Wang YY, Wang XH. A rapid advice guideline for the diagnosis and treatment of 2019 novel coronavirus (2019-nCoV) infected pneumonia (standard version). *Mil Med Res* 2020;7(1):4.
 16. Luo E, Zhang D, Luo H, Liu B, Zhao K, Zhao Y, Bian Y, Wang Y. Treatment efficacy analysis of traditional Chinese medicine for novel coronavirus pneumonia (COVID–19): an empirical study from Wuhan, Hubei Province, China. *Chin Med* 2020;15:34.
 17. Akkaya M, Kwak K, Pierce SK. B cell memory: building two walls of protection against pathogens. *Nature Reviews Immunology* 2020;20(4):229–38.
 18. McInnes IB, Schett G. Pathogenetic insights from the treatment of rheumatoid arthritis. *Lancet* 2017;389(10086):2328–37.
 19. Kaur S, Bansal Y, Kumar R, Bansal G. A panoramic review of IL–6: Structure, pathophysiological roles and inhibitors. *Bioorg Med Chem* 2020;28(5):115327.
 20. Li H, Chen K, Liu M, Xu H, Xu Q. The profile of peripheral blood lymphocyte subsets and serum cytokines in children with 2019 novel coronavirus pneumonia. *J Infect* 2020.
 21. Liu T, Zhang J, Yang Y, Ma H, Li Z, Zhang J, Cheng J, Zhang X, Zhao Y, Xia Z, Zhang L, Wu G, Yi J. The potential role of IL–6 in monitoring severe case of coronavirus disease 2019. *medRxiv* 2020(<https://doi.org/10.1101/2020.03.01.20029769>).
 22. Jo S, Kim H, Kim S, Shin DH, Kim MS. Characteristics of flavonoids as potent MERS-CoV 3C-like protease inhibitors. *Chemical Biology & Drug Design* 2019;94(6):2023–30.
 23. Kaneko M, Takimoto H, Sugiyama T, Seki Y, Kawaguchi K, Kumazawa Y. Suppressive effects of the flavonoids quercetin and luteolin on the accumulation of lipid rafts after signal transduction via receptors. *Immunopharmacol Immunotoxicol* 2008;30(4):867–82.

Table

Table 1 Property, flavor and meridian tropism of herbs in CYM.

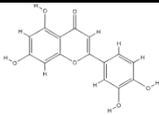
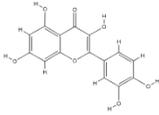
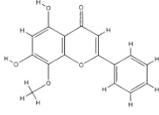
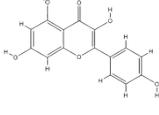
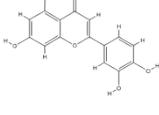
Herb		Property	Flavor				Meridian tropism						
Latin name	Acronym	Chinese name	Cold	Sweet	Bitter	Pungent	Lung	Stomach	Heart	Spleen	Gallbladder	Large Intestine	Small Intestine
Houttuyniae Herba	HH	Yuxingcao	√			√	√						
Scutellariae Radix	SR	Huangqin	√		√		√			√	√	√	√
Isatidis Radix	IR	Banlangen	√		√			√	√				
Lonicerae Japonicae Flos	LJF	Jinyinhua	√	√			√	√	√				
Forsythiae Fructus	FF	Lianqiao	√		√		√		√				√
Total			5	1	3	1	4	2	3	1	1	1	2

Table 2 Information of 32 therapeutic genes.

Number	Gene name	Gene symbol	Uniprot-ID
1	Prostaglandin G/H synthase 1	PTGS1	P23219
2	Tumor necrosis factor	TNF	P01375
3	Interleukin-6	IL6	P05231
4	Transcription factor AP-1	JUN	P05412
5	C-C motif chemokine 2	CCL2	P13500
6	Beta-2 adrenergic receptor	ADRB2	P07550
7	Dipeptidyl peptidase IV	DPP4	P27487
8	Mitogen-activated protein kinase 14	MAPK14	Q16539
9	Serum paraoxonase/arylesterase 1	PON1	P27169
10	Thrombin	F2	P00734
11	Acetylcholinesterase	ACHE	P22303
12	Mu-type opioid receptor	OPRM1	P35372
13	Myeloperoxidase	MPO	P05164
14	Mitogen-activated protein kinase 1	MAPK1	P28482
15	Cathepsin D	CTSD	P07339
16	Interferon gamma	IFNG	P01579
17	Interleukin-2	IL2	P60568
18	Interleukin-1 beta	IL1B	P01584
19	Tissue-type plasminogen activator	PLAT	P00750
20	Thrombomodulin	THBD	P07204
21	Tissue factor	F3	P13726
22	Arachidonate 5-lipoxygenase	ALOX5	P09917
23	Heme oxygenase 1	HMOX1	P09601
24	Retinoblastoma-associated protein	RB1	P06400
25	Superoxide dismutase [Cu-Zn]	SOD1	P00441
26	Epidermal growth factor receptor	EGFR	P00533
27	Maltase-glucoamylase, intestinal	MGAM	O43451
28	Signal transducer and activator of transcription 1-alpha/beta	STAT1	P42224
29	Interleukin-5	IL5	P05113
30	Interleukin-13	IL13	P35225
31	Peroxisome proliferator-activated receptor alpha	PPARA	Q07869
32	Interleukin-10	IL10	P22301

Table 3 Molecular docking results of key targets.

Figures

MOL ID	Name	CAS No.	Structural Formula	Binding energy (kJ/mol)
				COVID-19 3CL ^{pro}
MOL000006	Luteolin	491-70-3		-7.16
MOL000098	Quercetin	73123-10-1		-5.97
MOL000173	Wogonin	632-85-9		-7.45
MOL000358	Beta-sitosterol	83-46-5		-8.63
MOL000422	Kaempferol	520-18-3		-6.26
MOL001689	Acacetin	480-44-4		-6.28

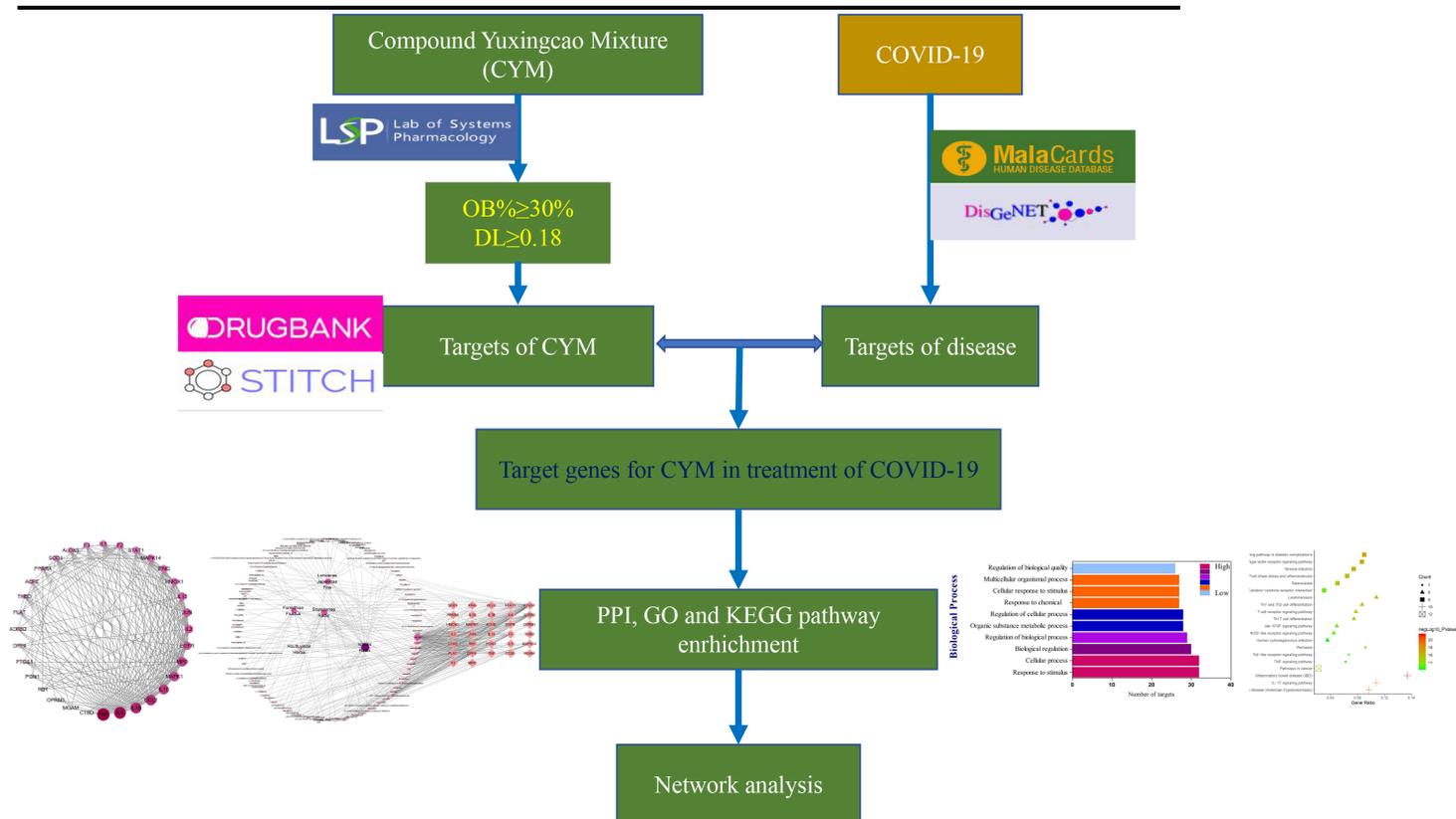


Figure 1

The flowchart of a systematic network pharmacology-based strategy to explore the pharmacological mechanisms of CYM for the treatment of COVID-19.

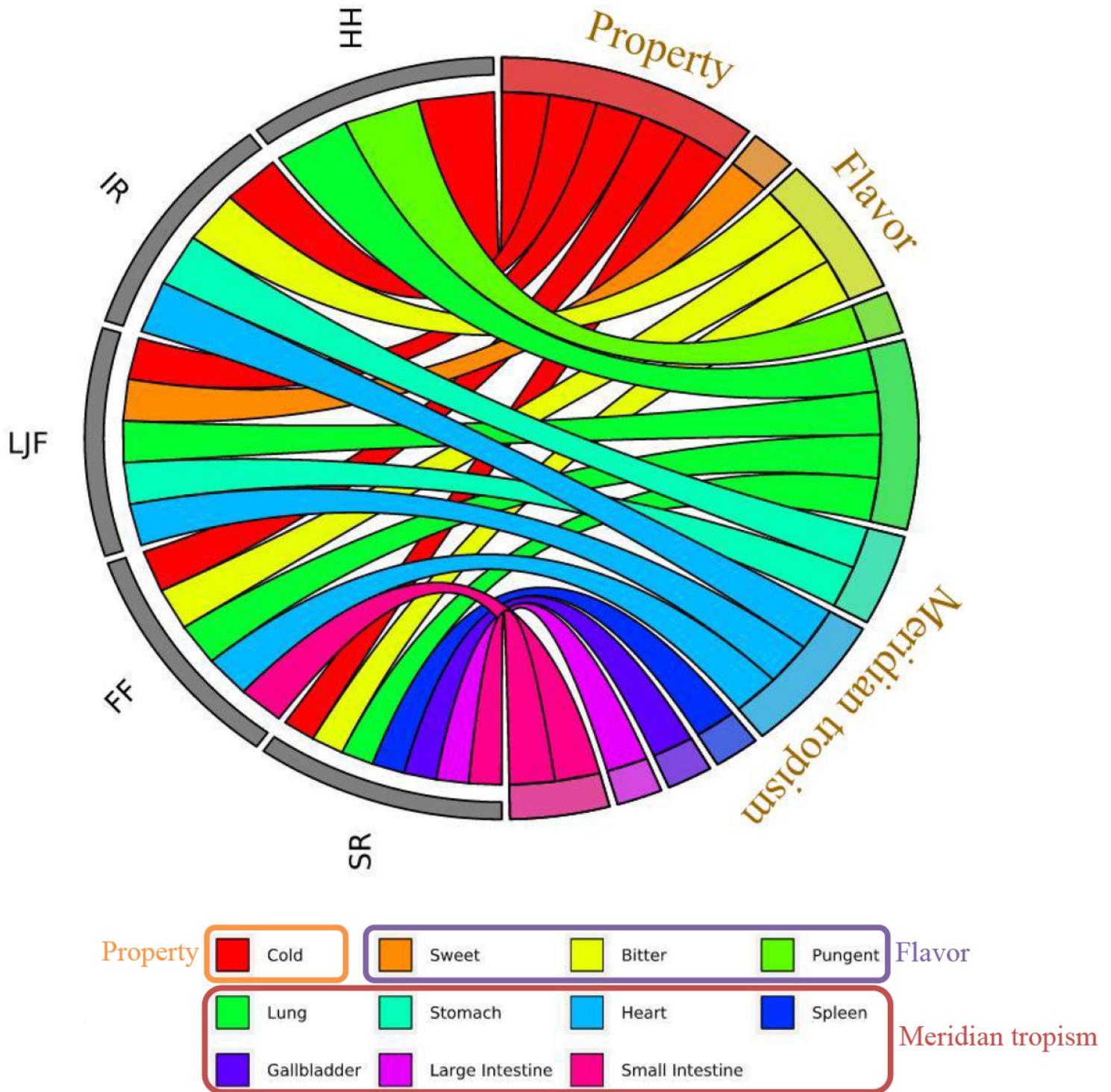


Figure 2

The herb-property-flavor-meridian tropism network of the CYM prescription. The network indicates the interconnection of the five herbs in CYM and corresponding information about their TCM property (cold), flavors (sweet, bitter and pungent) and meridian tropisms (lung, stomach, heart, spleen, gallbladder, large intestine and small intestine). The herbs are linked to their assigned terms via colored ribbons. HH:

Houttuyniae Herba; SR: Scutellariae Radix; IR: Isatidis Radix; LJF: Lonicerae Japonicae Flos; FF: Forsythiae Fructus.

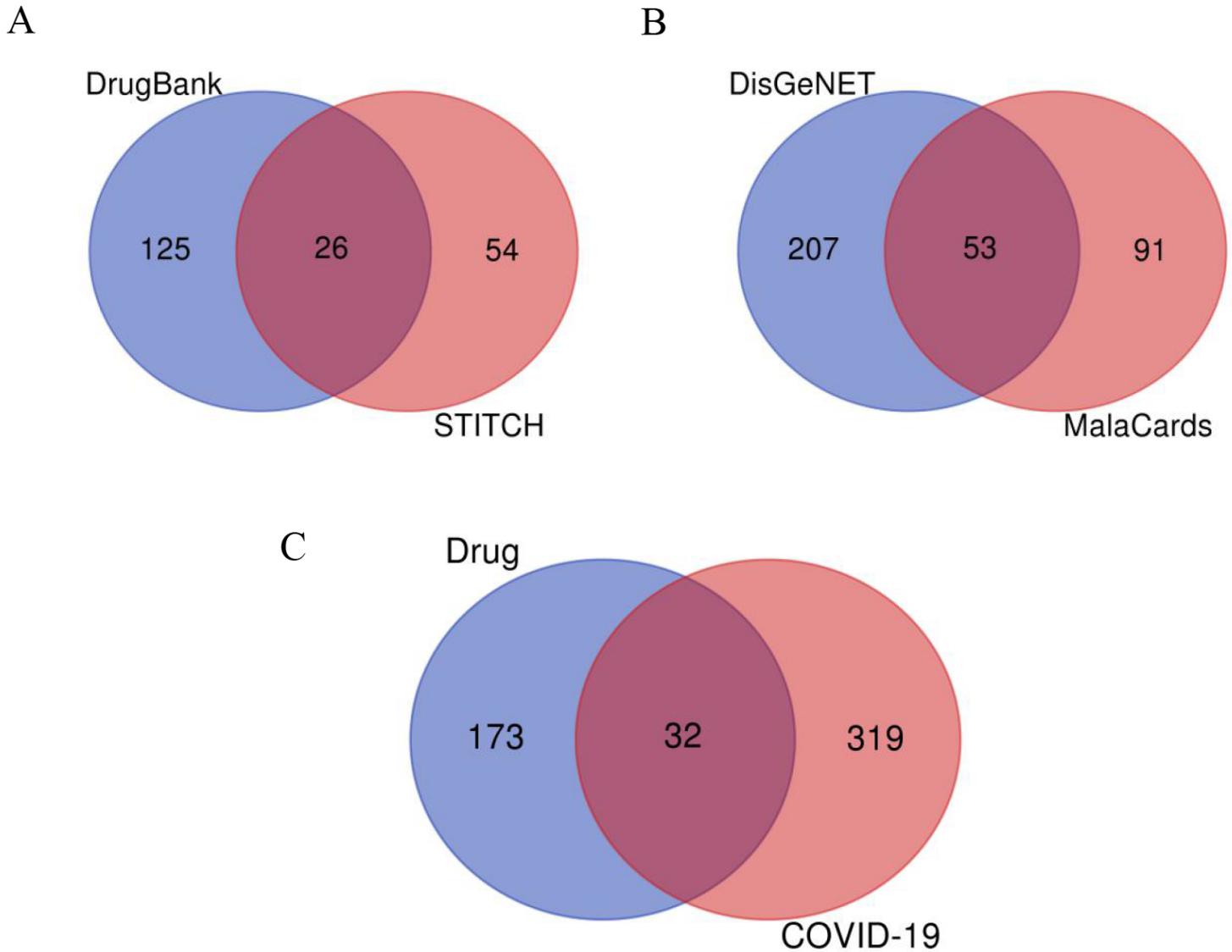


Figure 3

The potential targets of CYM and the overlapping therapeutic target genes of COVID-19 were mapped in the Venn diagram. (A) A total of 151 and 80 putative targets of CYM were obtained from Drugbank and STITCH databases. (B) A total of 351 known therapeutic targets were collected from DisGeNET and MalaCards databases. (C) The 32 overlapping genes between the CYM and COVID-19.

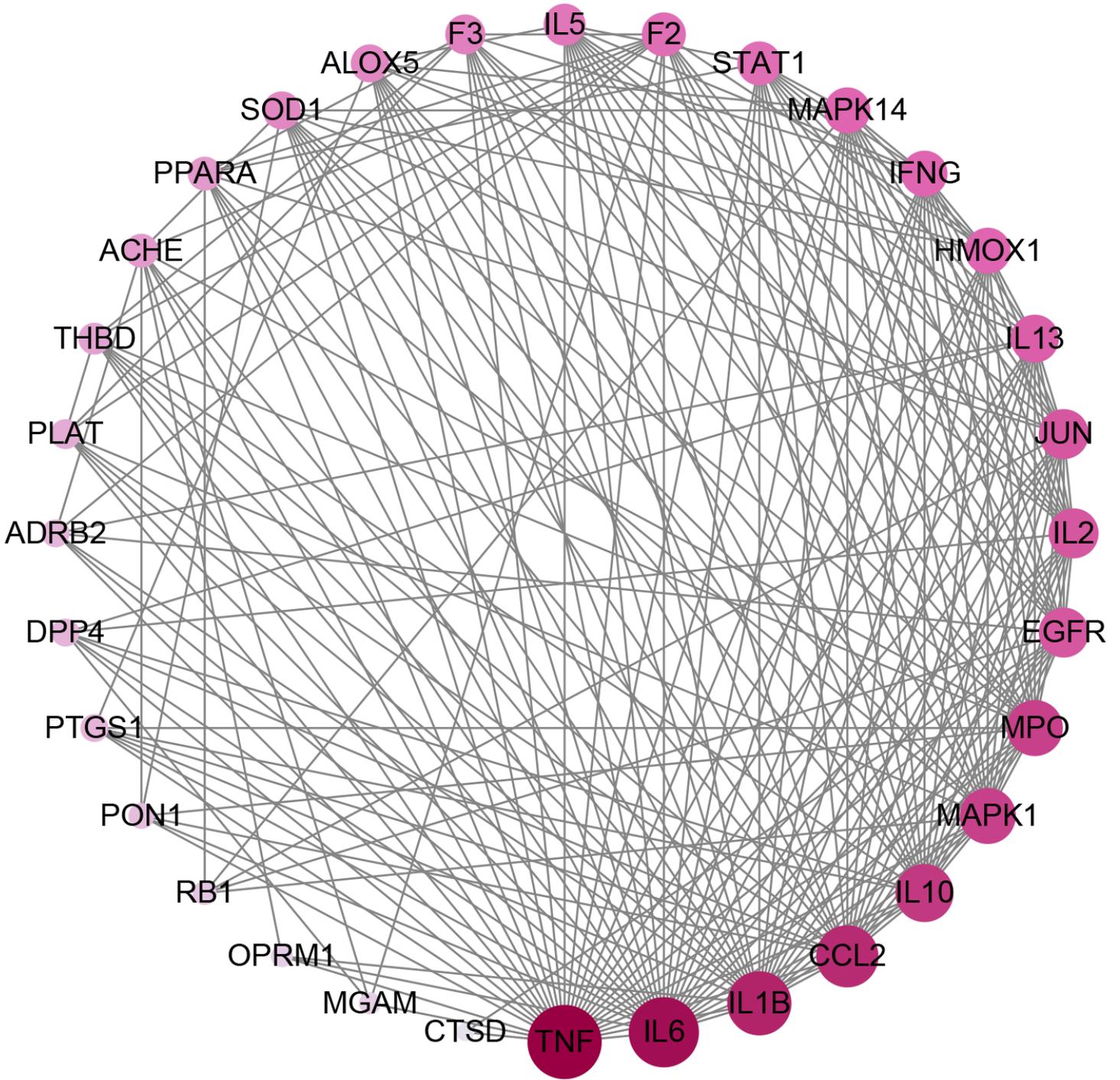


Figure 4

Common target PPI network between CYM and COVID-19. The nodes represent target genes, and the connections represent the interaction between two targets. The bigger the deeper, the more important it is.

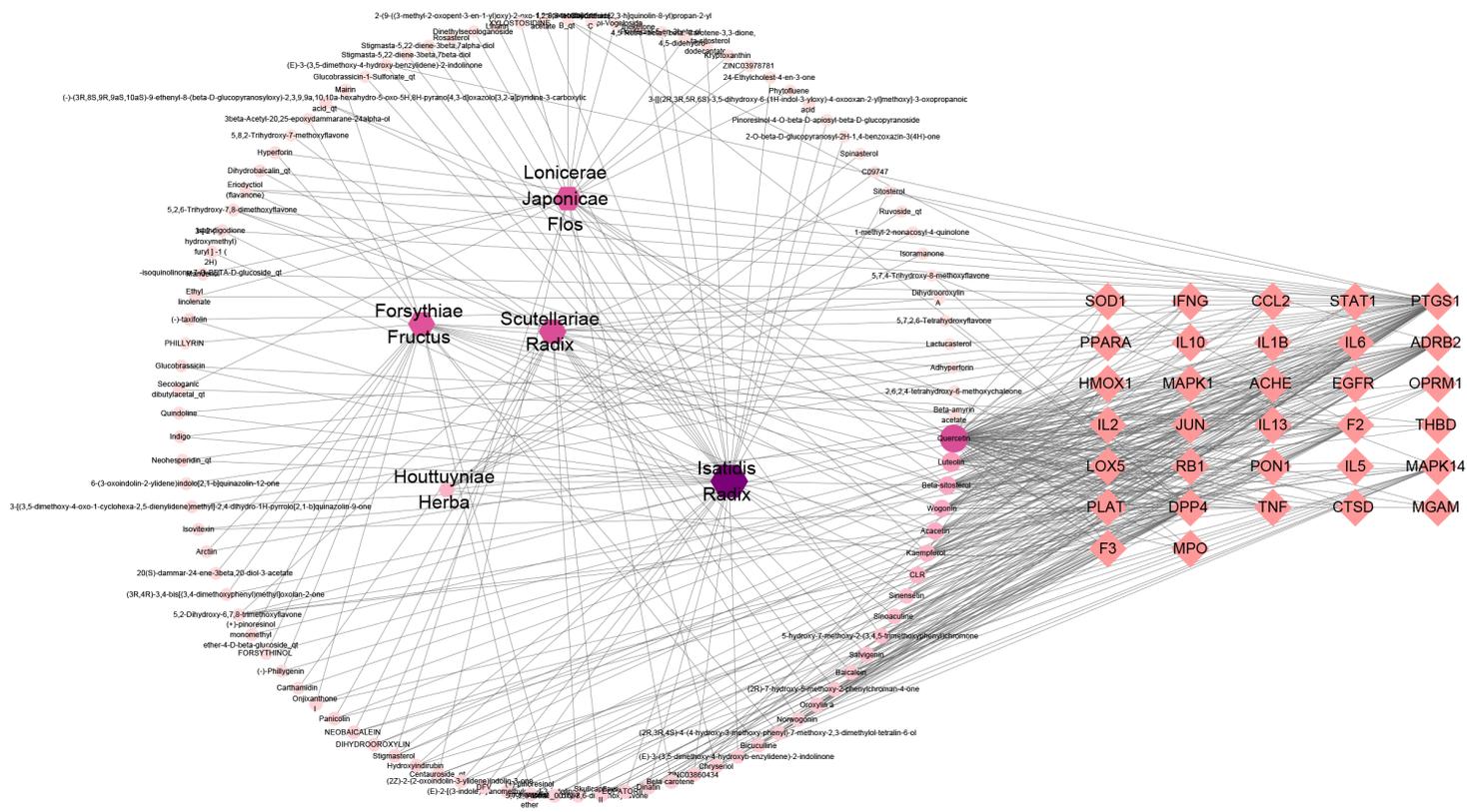


Figure 5

The drug-active ingredient-target network. The hexagon nodes represent traditional Chinese medicines, the ellipse nodes represent active ingredients and the diamond nodes represent overlapping targets for disease.

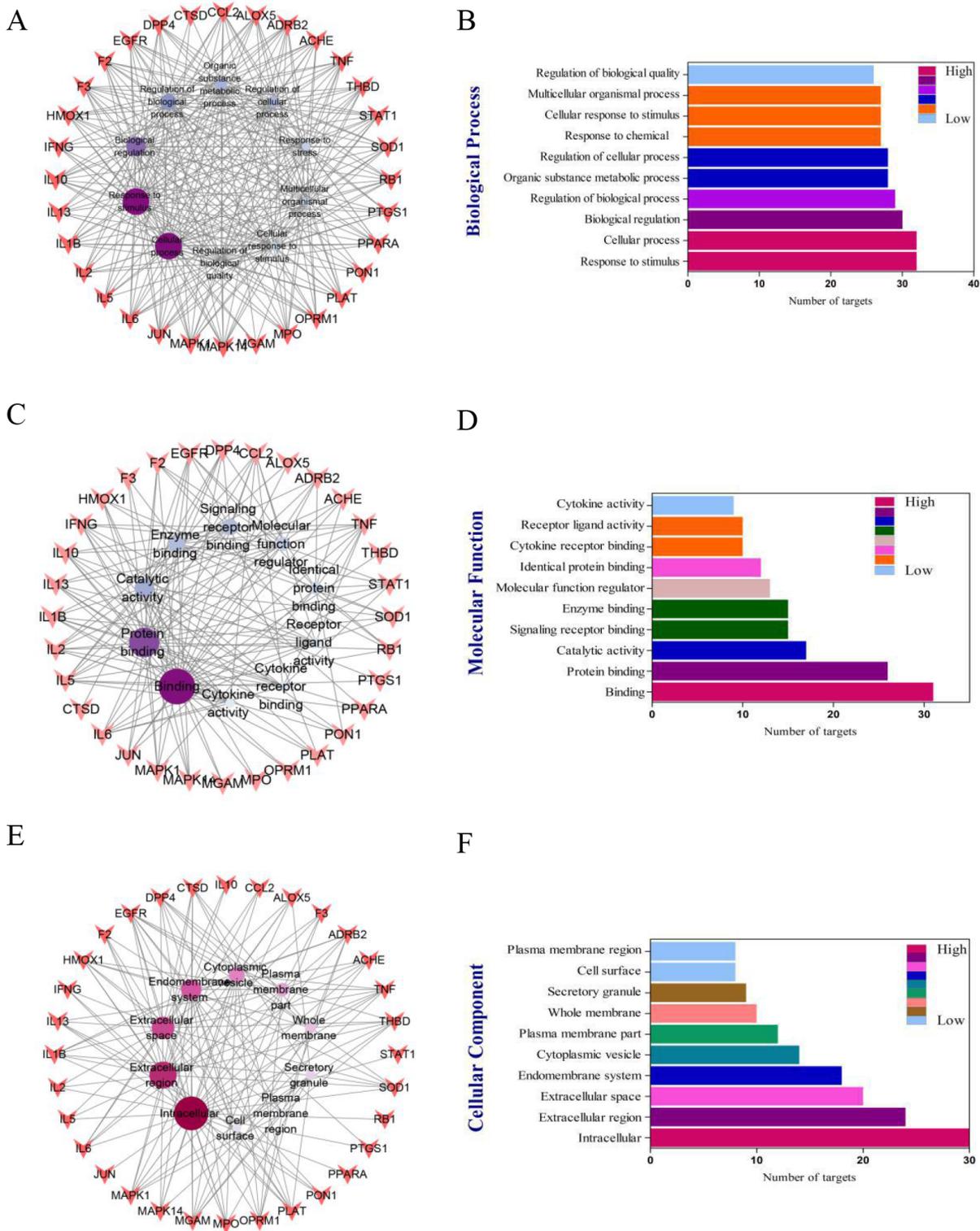


Figure 6

Gene ontology (GO) term enrichment for hub genes. (A,B) The top 10 entries of biological functional analysis of the core targets. (C,D) The top 10 entries of molecular function among candidate targets. (E,F) The top 10 entries of cellular component of the core targets. The X-axis indicates the number of enriched genes for the term, and the Y-axis indicates the GO term.

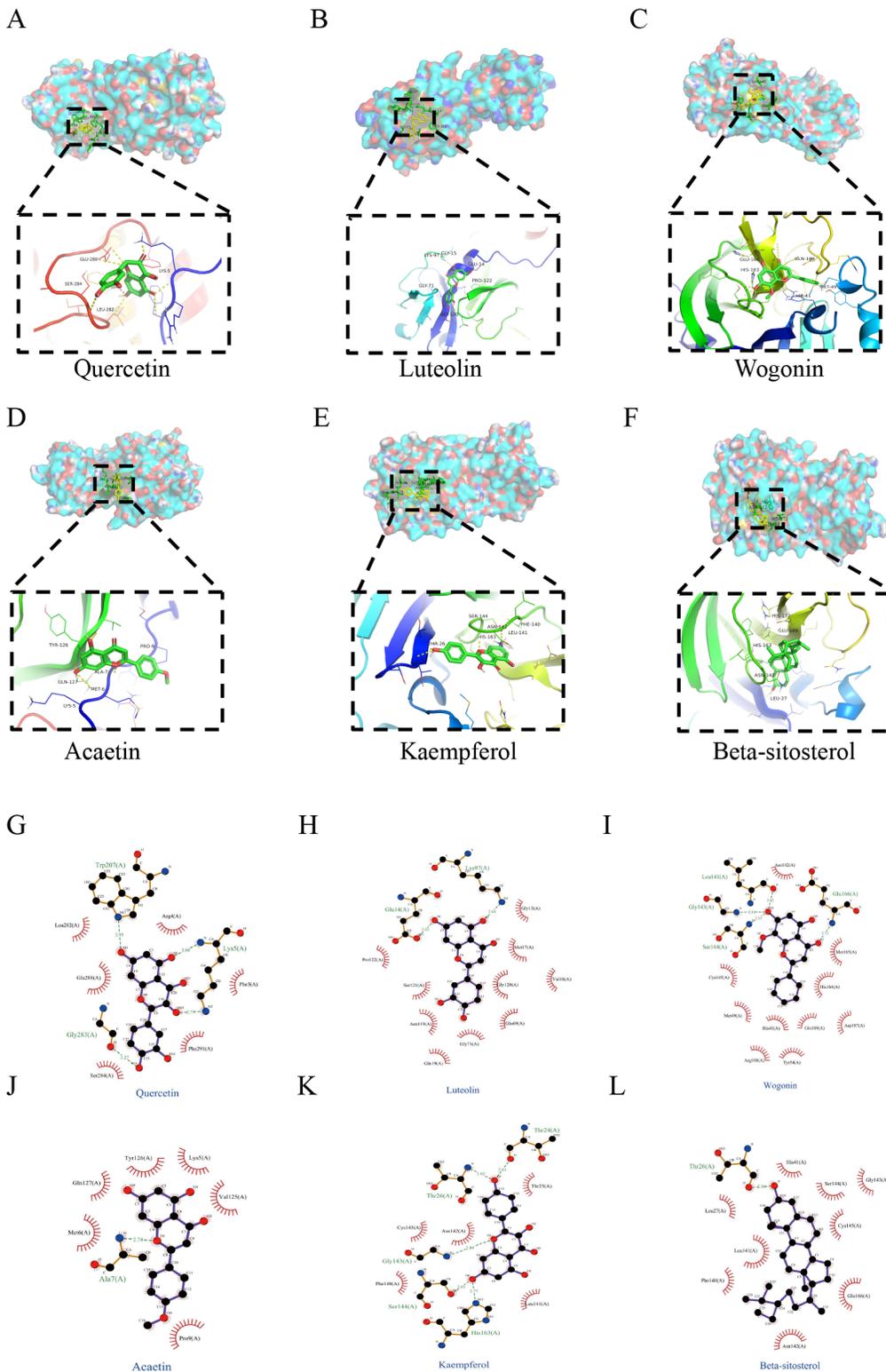


Figure 8

Molecular docking analysis between the selected ingredients and COVID-19 3CLpro. (A,B,C,D,E,F) A ball&stick represents a compound and a cartoon chain represents the active site of COVID-19 3CLpro. (G,H,I,J,K,L) Schematic diagrams represented the 3CLpro binding site and proximate affinity of candidate compounds in CYM. Black dots: carbon atoms; blue dots: nitrogen atoms; red dots: oxygen atoms; green dotted lines: hydrogen bonds; red combs: amino acid residues.

Supplementary Files

This is a list of supplementary files associated with this preprint. Click to download.

- [SupplementalTable1.docx](#)
- [SupplementalTable2.docx](#)