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Network Pharmacology Analysis of the Targets and Mechanisms of Sang Ju Yin for COVID-19 Treatment

Isabelle Li[1]

[1]Laurel Springs School, USA

ABSTRACT

As the 2019 coronavirus disease (COVID-19) establishes its prevalence as a global health emergency, novel drugs and medications have become increasingly necessary for prevention and treatment. Traditional Chinese medicine (TCM) has played a pivotal role in containing the virus, and herbal formulas such as Sang Ju Yin have held substantial promise in reducing mortality rates and promoting recovery. However, the exact targets and mechanisms of Sang Ju Yin in COVID-19 treatment have not been extensively explored. Therefore, the objective of this study was to use network pharmacology to identify the interactions between Sang Ju Yin and COVID-19. A total of 170 active ingredients and 1476 targets of Sang Ju Yin were retrieved from multiple databases and combined with 1313 COVID-19 targets to obtain 233 common targets. These core targets were integrated into a protein-protein interaction (PPI) network and active-ingredient-disease-target network using STRING and Cytoscape 3.9.1 software. Gene ontology (GO) and Kyoto Encyclopedia of Genes and Genomes (KEGG) pathway analysis was performed using DAVID and Metascape to determine related biological processes and signaling pathways. The results indicate that Sang Ju Yin likely regulates inflammatory and immune responses in COVID-19 by targeting AKT1, IL-6, and MAPK3. The active ingredients luteolin, kaempferol, and quercetin physically bind to and inhibit the angiotensin converting enzyme 2 (ACE2) and 3-chymotrypsin-like protease (3CLpro) required for viral invasion and replication. The results of this study provide evidence for the therapeutic effects of Sang Ju Yin on COVID-19 and support the application of TCM for treatment worldwide.

Introduction

With nearly 600 million cases worldwide, the 2019 coronavirus disease (COVID-19) pandemic currently dominates the forefront of scientific and clinical research (World Health Organization, 2022). From its first reported case in Wuhan, Hubei Province, China, the SARS-CoV-2 virus continues to spread rapidly due to its contagious respiratory transmission, invasive spike protein, rapid replication, and strain mutation. While there is no formal cure, recent advancements in Western medicine have effectuated the rise of mRNA vaccines, oral antiviral pills, monoclonal antibody treatments, and palliative drugs such as remdesivir and baricitinib (U.S. Food and Drug Administration, 2022). Despite its status as the place of viral origin, China has kept a relatively stable control over the virus and its cases. This is likely attributed to the policies and guidelines implemented by the country's National Health Commission. The latest edition of China's COVID-19 Diagnosis and Treatment Program offers a dual integrative approach, combining both Western and traditional Chinese medicine (TCM). The TCM plan revolves around six most effective prescriptions and medicines, namely the Jinhua Qinggan Granule (JHQGG), Lianhua Qingwen Capsule (LHQW), Xuebijing Injection (XBJ), Qingfei Paidu Decoction (QFPD), Huashi Baidu Formula (HSBDF), and Xuanfei Baidu Granule (XFBD) (Niu et al., 2021). The inclusion of TCM into COVID-19 treatment presents an additional advantage compared to other treatment plans as TCM formulas and prescriptions can be modified based on the severity of cases.

Unlike the reductionism of Western medicine, TCM is defined by its theoretical and philosophical basis: the constant maintenance of a dynamic balance (Luo et al., 2020). As such, TCM emphasizes holistic treatment based on syndrome differentiation as opposed to symptomatic treatment (Yu et al., 2021). Syndrome differentiation is defined

as the comprehensive analysis of the individual pathogenesis and conditions of patients (Luo et al., 2020). Since patients vary in disease stage, degree, and symptoms, different prescriptions are recommended and modified for personalized treatment (Yu et al., 2021). The majority of TCM is composed of herbal medicine in which therapeutic natural products are combined into specific formulas to treat certain diseases (Capodice & Chubak, 2021). These herbal remedies have been present throughout Chinese history and were implemented in more than 300 large epidemic outbreaks (Yu et al., 2021). TCM has treated other infectious diseases such as the herpes simplex virus, human immunodeficiency virus (HIV), hepatitis B, hepatitis C, dengue virus, and Ebola virus (Huang et al., 2021). During the 2003 outbreak of severe acute respiratory syndrome (SARS) in Guangdong, China, TCM treatment helped to relieve dyspnea and malaise, shortening hospitalization time for patients (Huang et al., 2021). Similarly, the TCM herbs Huangqin and Huangqi were notably used for inhibition of the 2009 H1N1 influenza A virus as the compounds significantly reduced autophagosome formation (Capodice & Chubak, 2021). TCM has been particularly effective against COVID-19 and the mounting clinical evidence demonstrates that TCM can prevent disease progression and relapse, reducing mortality rates and increasing recovery numbers (Li et al., 2021). The LHQW formula, in particular, is one of the most commonly used treatments as it can inhibit viral replication and reduce pro-inflammatory cytokines at a transcriptomic level (Capodice & Chubak, 2021). Specific formulas have also been developed for each stage of disease progression, ranging from onset and replication to inflammatory response and organ damage (Li et al., 2021).

Of the recommended TCM prescriptions for COVID-19, Sang Ju Yin has emerged as a potential drug candidate. The formula includes a combination of mulberry leaf, chrysanthemum, forsythia, mint, reed root, and licorice (Kumari & Kumar, 2020). Sang Ju Yin is primarily used as a relief for cough, colds, sore throat, mild thirst, and fever and can be applied to diseases such as influenza, hand, foot, and mouth disease, whooping cough, acute bronchitis, pneumonia, etc. (Kumari & Kumar, 2020). Despite being a household remedy, the formula has also been used in treatment of large epidemics such as the 2009 swine flu and SARS virus. Sang Ju Yin possesses immunomodulatory functions against SARS, regulating T cells and increasing CD4/CD8 levels in the blood (Poon et al., 2006). For COVID-19, Sang Ju Yin has been prescribed for mild and moderate cases as well as patients with dyspnea and severe cough (Arghal & Hafsal, 2020; Kumar et al., 2020).

Despite the efficacy of TCM for COVID-19 treatment, therapeutic drugs must be consistently refined through research. Current studies have used network pharmacology to understand the molecular mechanisms underlying TCM treatment. Network pharmacology is defined as a research method for visualizing the multi-gene, target, pathway, and disease network interactions in drug treatment (Xing et al., 2020). This focus on the overall network and inclusion of multiple drug targets synchronizes well with TCM which uses the synergistic effects of compounds to restore the balance of biological networks (Liu et al., 2022; Yu et al., 2021). However, network pharmacology studies have not been applied to Sang Ju Yin and its treatment of COVID-19. This study aims to identify the pharmacological effects of Sang Ju Yin on COVID-19 and the specific active ingredients, targets, and pathways involved.

Methods

The network pharmacology approaches outlined in this study were largely modeled after (Yu et al., 2021). The methodology involved [1] Sang Ju Yin and COVID-19 target prediction, [2] network construction and analysis, and [3] functional enrichment analysis. A workflow diagram of the procedure is outlined in Figure 1.

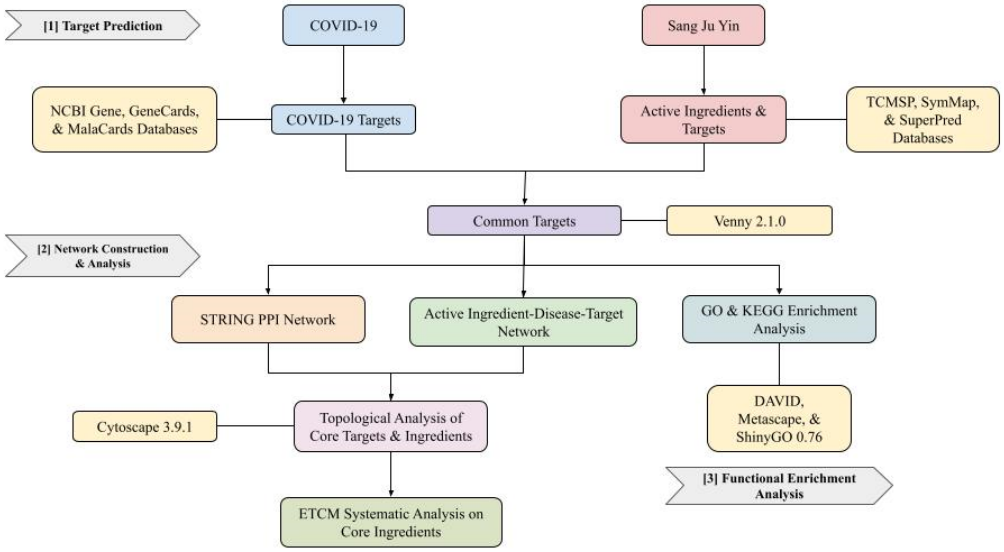

Figure 1. Flowchart of target prediction, network construction, and functional enrichment procedures.

Screening of Sang Ju Yin components and Targets

The herbal components of Sang Ju Yin were found on Bio Essence Health Science and entered into the Traditional Chinese Medicine Systems Pharmacology Database and Analysis Platform (TCMSP) to find active ingredients. The pharmacokinetic parameters were set as oral bioavailability (OB) ≥ 30% and drug-likeness (DL) ≥ 0.18. OB indicates the percentage of drug absorbed into systemic circulation while DL displays the optimal drug potential for a compound (Liu et al., 2022; Yu et al., 2015). High OB and DL values are recommended for drugs with effective biological activity. Additional putative drug targets were screened from the SymMap and SuperPred databases with $p < 0.01$. The official gene names of all targets were standardized using UniProtKB after duplicate removal.

Screening of COVID-19 related and Common Targets

The keyword "novel coronavirus pneumonia" was entered into the GeneCards, MalaCards, and NCBI Gene databases. All gene targets were consolidated, and duplicates were removed. The COVID-19 targets and Sang Ju Yin targets were combined, and the intersecting targets were visualized using Venny 2.1.0.

PPI Network Construction and Analysis

The common targets were uploaded to the STRING database to form a protein-protein interaction (PPI) network. Parameters were selected as follows: Homo sapiens as organism, no combined score screening, and high confidence value > 0.7. The PPI network was then imported into Cytoscape 3.9.1 for further topological analysis. The targets were first filtered using the "network analyzer" plug-in for all targets with values greater than the mean betweenness centrality, closeness centrality, and degree. Nodes with larger parameter values were likely more important to the network. The targets were then filtered using the CytoNCA plug-in based on betweenness centrality, closeness centrality, Eigenvector centrality, local average connectivity-based method (LAC), and network centrality values. The targets were filtered for a third time using the CytoHubba plug-in to find the top 5 and top 10 targets based on degree; these hub genes were generated as subnetworks.

Active Ingredient-Disease-Target Network Construction and Analysis

Three networks (herb-active ingredient, active-ingredient-target, and disease-target) were constructed using Cytoscape and merged into an overall network. The network was analyzed based on betweenness centrality, closeness centrality, and degree to find the top 3 active ingredients. The top 3 active ingredients were uploaded to The Encyclopedia of Traditional Chinese Medicine (ETCM) database for systematic analysis on related targets, pathways, and diseases.

Gene Ontology (GO) and Kyoto Encyclopedia of Genes and Genomes (KEGG) Enrichment Analysis

GO and KEGG enrichment analysis was performed on the common targets using the DAVID and Metascape databases. “Homo sapiens” was selected for species, and results were sorted according to the p-value ($p < 0.05$) and false discovery rate (FDR). Enrichment results were visualized as bubble charts and bar graphs using ShinyGO 0.76. The top 10 GO biological processes (BP), cellular components (CC), and molecular functions (MF), and KEGG pathways, including the top 3 genes for each term, were screened out.

Results

Screening of Sang Ju Yin and COVID-19 Targets

After screening through the TCMSP, SymMap, and SuperPred databases, 170 active ingredients and 1476 targets of Sang Ju Yin were identified. After screening through the NCBI Gene, GeneCards, and MalaCards databases, 1313 targets for COVID-19 were identified. The targets were then pooled and deduplicated, and 233 common targets were identified. A Venn diagram of the intersecting targets is shown in Figure 2.

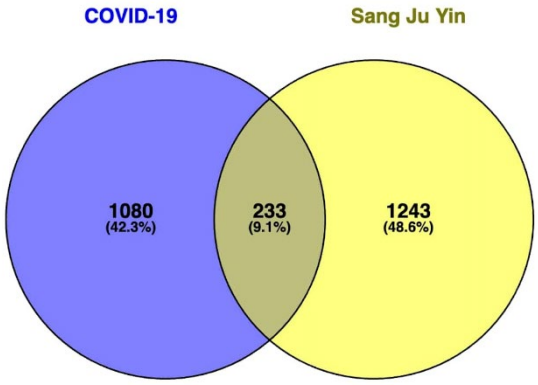

Figure 2. Intersection of COVID-19 and Sang Ju Yin gene targets.

PPI Network Analysis

The common targets were uploaded to the STRING database, and a PPI network (Figure 3) with 238 nodes and 2153 edges and an average degree value of 18.1 ($p < 0.01$). Topological analysis on the PPI network revealed 2 subnetworks. The top 10 targets were used to construct a subnetwork containing IL-6, CASP3, ACTB, STAT3, JUN, AKT1, TP53, MAPK3, IL1B, and TNF (Figure 4A). The top 5 targets were used to construct a subnetwork containing IL-6, STAT3, TNF, MAPK3, and ACTB (Figure 4B).

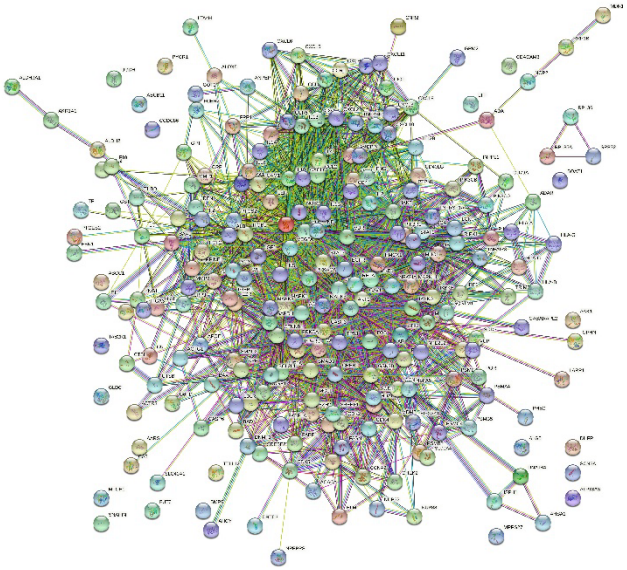

Figure 3. STRING PPI Network ($p < 0.01$).

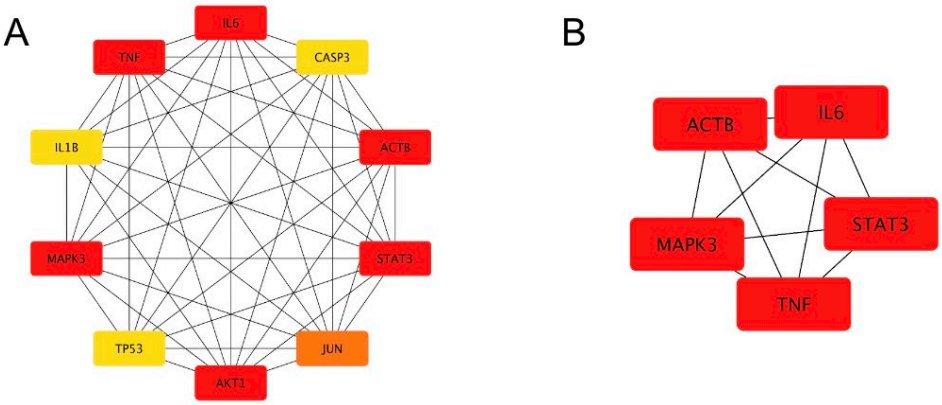

Figure 4. Top 10 hub genes (Figure 4A) and top 5 hub genes (Figure 4B). Genes with darker colors have a higher degree ranking and are likely more important to the network.

Active Ingredient-Disease-Target Network Analysis

Three networks (herb-active ingredient, active-ingredient-target, and disease-target) were constructed and merged into an overall network with 1593 nodes and 1644 edges (Figure 5). Topological analysis on the network revealed that the top 3 active ingredients of Sang Ju Yin were luteolin, quercetin, and kaempferol. Further ETCM systematic analysis was conducted on these compounds to show the related targets, pathways, and diseases. Luteolin targeted AHR, AKR1C1, and ACTB; quercetin targeted AHR, AKT1, and ACTB while kaempferol targeted AHR, ACTB, and AKR1C1.

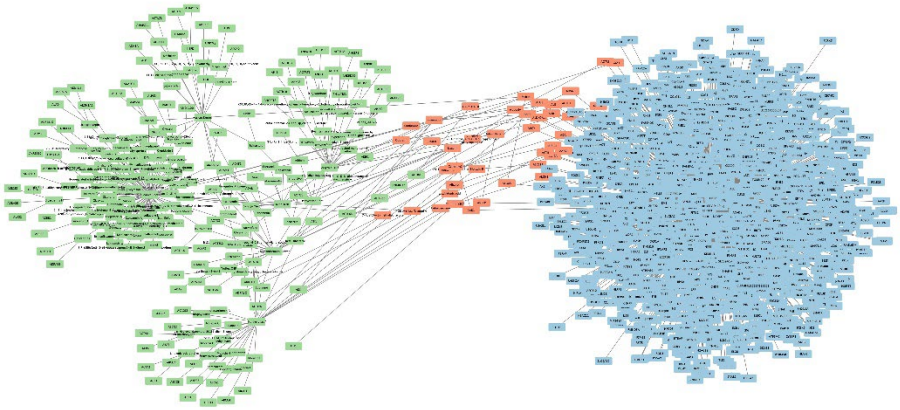

Figure 5. Active-ingredient-disease-target network. COVID-19 targets are depicted in blue while Sang Ju Yin herbs, active ingredients, and targets are depicted in green. Overlapping ingredients or targets are depicted in orange.

Functional Enrichment Analysis

Metascape enrichment clusters showed that targets were significantly related to immune-related cytokine signaling, lipid and atherosclerosis, inflammatory response, Kaposi sarcoma-associated herpesvirus infection, and the IL-18 signaling pathway (Figure 6). The top Metascape GO terms included cellular response to cytokine stimulus, regulation of apoptotic processes, chemokine receptor binding, cytokine activity, etc. (Figure 7). Metascape KEGG pathways were centered around inflammation and oxidative stress; notable results included the ACE-RAGE, rheumatoid arthritis, IL-17, and TNF signaling pathway (Figure 8). These results were similar to enrichment terms generated by DAVID (Tables 1 and 2). Overall, enrichment analysis showed that the most prominent targets were AKT1, BAX, CXCLs, CCLs, BCLs, CCRs which were involved in inflammation and immune response processes.

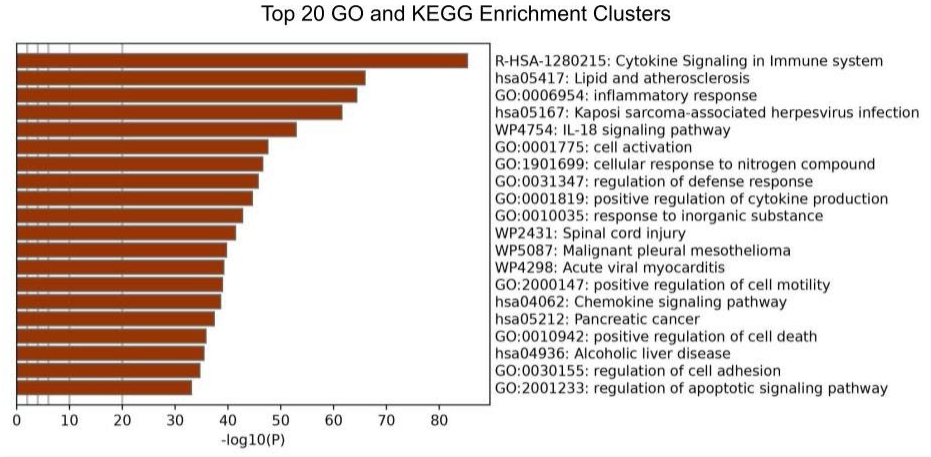

Figure 6. Top 20 GO and KEGG enrichment analysis clusters from Metascape.

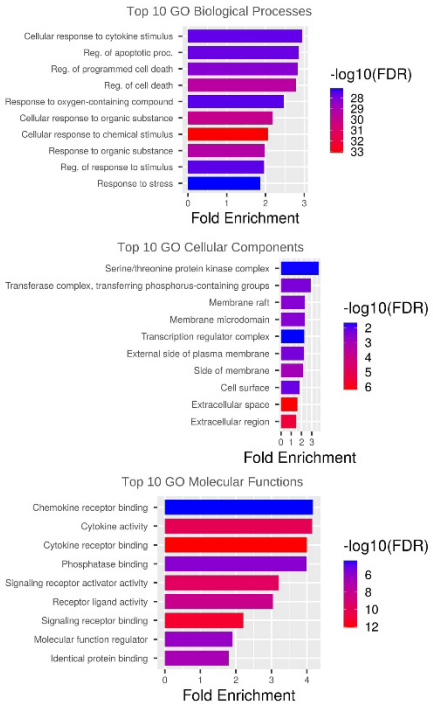

Figure 7. Top 10 GO terms from Metascape ($p < 0.05$) sorted by false discovery rate (FDR) and fold enrichment.

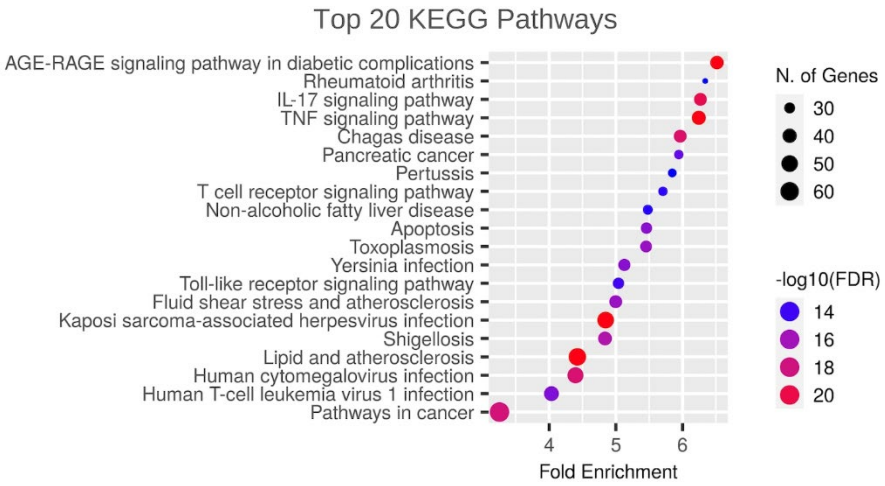

Figure 8. Top 20 KEGG pathways from Metascape ($p < 0.05$) sorted by false discovery rate (FDR), fold enrichment, and number of genes.

Table 1. Top 10 GO terms and related genes from DAVID. GO terms and genes for biological processes (BP), cellular components (CC), and molecular functions (MF) are listed.

Rank	BP	Top 3 Genes	CC	Top 3 Genes	MF	Top 3 Genes
1	inflammatory response	AKT1, CCL11, CCL2	extracellular space	CCL11, CCL2, CCL3	identical protein binding	AKT1, BCL2, BAX
2	positive regulation of gene expression	AKT1, CCL3, CXCL8	cytoplasm	AKT1, AAK1, BCL2	enzyme binding	AKT1, JUN, MDM2
3	cellular response to lipopolysaccharide	CCL2, CCR5, CXCL1	cytosol	AKT1, AAK1, BCL2	protein binding	AKT1, AAK1, ABCB11
4	positive regulation of transcription from RNA polymerase II promoter	AKT1, CXCL10, CXCR3	extracellular region	CCL11, CCL2, CCL3	chemokine activity	CCL11, CCL2, CCL3
5	immune response	CCL11, CCL2, CCL3	macromolecular complex	AK1, BCL2, BLM	CXCR chemokine receptor binding	CXCL1, CXCL10, CXCL11
6	chemotaxis	CCL11, CCL2, CCL3	extracellular exosome	ABCB11, ABCC1, BAX	cytokine activity	CXCL9, CD40LG, BMP6
7	response to drug	ABCC1, BCL2, BAD	cell surface	ABCB11, CCR5, CCR6	RNA polymerase II sequence-specific DNA binding transcription factor binding	FOS, JUN, NFE2L2
8	negative regulation of apoptotic process	AKT1, BCL2, BCL2L1	membrane	ALG5, ABCC1, ATP13A3	ubiquitin protein ligase binding	BCL2, JUN, MDM2
9	negative regulation of gene expression	AKT1, CCL3, CCR1	nucleoplasm	AKT1, BCL2, BLM	protein kinase binding	AKT1, AAK1, CCL2
10	apoptotic process	BCL2, BAX, BAD	external side of plasma membrane	CCR1, CCR5, CCR6	receptor binding	CCL2, CXCL1, CXCL10

Table 2. Top 10 GO terms and genes from DAVID.

Rank	Term	Top 3 Genes
1	lipid and atherosclerosis	AKT1, BCL2, BAX
2	Kaposi sarcoma-associated herpes-virus infection	AKT1, BAX, CCR1
3	TNF signaling pathway	AKT1, CCL2, CXCL1
4	AGE-RAGE signaling pathway in diabetic complications	AKT1, BCL2, BAX
5	IL-17 signaling pathway	CCL11, CCL2, CXCL1
6	Chagas disease	AKT1, CCL2, CCL3
7	Human cytomegalovirus infection	AKT1, BAX, CCL2
8	Pathways in cancer	AKT1, BCL2, BAX
9	Influenza A	AKT1, BAX, CCL2
10	Measles	AKT1, BCL2, BAX

Discussion

Active Ingredients

Topological and systematic analysis of the active-ingredient-disease-target network revealed that the top 3 active ingredients in Sang Ju Yin that interact with COVID-19 are luteolin, kaempferol, and quercetin. This is consistent with molecular docking studies confirming that all three compounds directly bind and inhibit 3-Chymotrypsin-like protease (3CLpro) and angiotensin converting enzyme 2 (ACE) which are required for SARS-CoV-2 replication and entry (Jo et al., 2020). Quercetin, in particular, plays a more specific role by directly downregulating SARS-CoV-2 ACE2 through transcription factors and miRNAs (Huang et al., 2021). Furthermore, luteolin, kaempferol, and quercetin are classified as flavonoids which are phytochemical compounds with anti-inflammatory and anti-oxidative effects (Tao et al., 2020). This is congruous with the ETCM analysis of targets: all three compounds targeted AHR which modulates immune function and chronic inflammation. Severe COVID-19 is characterized by hyperinflammation; hence, Sang Ju Yin likely treats the virus through the regulation of inflammatory response. Moreover, luteolin, kaempferol, and quercetin possess the same functions in the QFPD formula, inhibiting inflammation, regulating lung injury, and protecting nerve function (Zhao et al., 2021). Luo et al. found that all three compounds increase T cell differentiation and downregulate inflammatory factors including interleukin, TNG, and integrin (Luo et al., 2020). Additionally, quercetin and luteolin also target the inflammatory proteins TNF, MAPK1, IL6, etc. (Nguyen et al., 2012). This further supports the role of Sang Ju Yin in negatively modulating immune and inflammatory processes for COVID-19 treatment.

Kaempferol and quercetin, in particular, also mediate apoptosis by changing the cell cycle. Kaempferfol increases the number of cells in G0, G1, and S phases, thereby inhibiting viral proliferation (Poon et al., 2006). However, it is not nearly as effective as quercetin which can inhibit the infection and replication of various viruses in monolayer cell culture. Regardless, these findings suggest that Sang Ju Yin also utilizes apoptosis regulation to treat COVID-19.

Notable Targets

The PPI subnetworks consolidated the top gene targets: IL-6, CASP3, ACTB, STAT3, JUN, AKT1, TP53, MAPK3, IL1B, and TNF. This is consistent with previous studies that suggest Sang Ju Yin is regulated by AKT1, BAX, IKBKB, IL-6, and STAT3 (Wu et al., 2020). Additionally, the majority of Sang Ju Yin targets are similar to those of other TCM. AKT1, IL-6, MAPK1 and 8, JUN were core targets of the QFPD and LHQW formulas, suggesting that most TCM prescriptions target the same key proteins for COVID-19 treatment (Wu et al., 2020).

AKT1 was included in the top targets from all PPI, ETCM, and KEGG analyses and is, therefore, likely crucial to the molecular mechanism of Sang Ju Yin. Xia et al. determined that AKT1 is activated during COVID-19 infection, regulating lung injury, fibrogenesis, and immune cell development (2020). IL-6 was another key target, and it is often upregulated in the plasma of severe COVID-19 patients (Wang et al., 2020). As a cytokine, IL-6 has a versatile function in immunoglobulin secretion, regulating immune response, and preventing tissue damage (Poon et al., 2006). In the case of COVID-19, IL-6 serves as the major component in fatal cytokine storms. Cytokine release syndrome (CRS) or a cytokine storm is an excessive immune response and release of interferons, interleukins, chemokines, and other immune mediators (Huang et al., 2021). This aggressive immune activity causes acute inflammation and oxidative damage which can degenerate into serious conditions such as acute respiratory distress syndrome (ARDS) and multiple organ dysfunction syndrome (MODS) (Qiao et al., 2021). CRS is the main cause of COVID-related morbidity as immune function becomes ineffective and suppressed after cytokine storms. CRS is mainly associated with elevated IL-6 levels as IL-6 stimulates immune activation and amplifies inflammation (Huang et al., 2021). Currently, IL-6 inhibitors and steroids are already used to treat COVID, signifying the importance of this cytokine to the virus's fatality (Capodice & Chubak, 2021). Therefore, regulation of IL-6 by Sang Ju Yin is necessary for effective treatment.

Qiao et al. discovered that the cell destruction and inflammation of SARS-CoV-2 promotes increased chemokine activity (2021). This coincides with the top targets found in this study: CXCLs, CCLs, CCRs. All three families consist of chemokine ligands or receptors which recruit immune cells. This further elucidates the role of Sang Ju Yin in chemokine signaling.

In order to reduce proinflammatory cytokine and chemokine activity, regulatory pathways must be activated. The MAPK3/MAPK1 pathway decreases cell inflammation and damage by reducing cytokine and inflammasome levels (Tong et al., 2020). As a notable target in this study, MAPK3 activity is likely modulated by Sang Ju Yin to control hyper inflammation and immune responses.

Pathways and Processes

GO and KEGG enrichment analysis revealed that Sang Ju Yin most likely treats COVID-19 through regulation of cytokine signaling and inflammatory and immune response pathways. The TNF, IL-17, and IL-18 pathways were significantly enriched which aligns with the results of previous studies showing the TCM regulation of IL-17, IL-18, and TNF pathways to inhibit inflammation and immune response (Yu et al., 2021). Furthermore, clinical evidence confirms the presence of TNFα in the blood and tissues of severe COVID patients; TNFα activates cytokines such as IL-6, IL-17, and IL-18, linking the three pathways together (Xia et al., 2020) On a broader note, the enrichment analysis for this study included herpesvirus, influenza A, human cytomegalovirus infection, substantiating the role of Sang

Ju Yin in the treatment of other viral diseases. In conclusion, COVID-19 may be treated by Sang Ju Yin through the regulation of inflammatory and immune response pathways.

Limitations

The largest limiting factor in this study was insufficient consideration for the content, concentration, and dosage of Sang Ju Yin. Although pharmacokinetic parameters were considered, the effect of Sang Ju Yin concentration on COVID-19 cannot be easily determined as TCM does not have quality and proportion standards (Yu et al., 2021). This is critical to evaluate the true therapeutic performance of TCM as a certain amount of drug compound must reach the active site of COVID receptors to be effective (Yu et al., 2015). Despite the emergence of patented formulas, TCM remains difficult to apply to clinical trials because it fundamentally emphasizes the idiosyncrasies of individual conditions which may be incompatible with the homogenized conditions of randomized control trials (Capodice & Chubak, 2021). Dosages and concentrations are constantly modified depending on disease stage and severity which varies in patients. Regardless, the efficacy of TCM for COVID-19 treatment must be verified using *in vivo* or *in vitro* experiments, imaging and molecular docking studies, and standard clinical trials in order for it to be adopted for international use (Wang & Qi, 2020).

Conclusion

This study utilized network pharmacology to explore the active ingredients, targets, and molecular mechanisms of Sang Ju Yin for COVID-19 treatment. It was found that Sang Ju Yin likely regulates the TNF, IL-17, and IL-18 inflammation and immune response-related pathways by acting on AKT1, IL-6, MAPK3, AHC, and other targets, thereby resisting COVID-19. The active ingredients luteolin, kaempferol, and quercetin modulate this process and directly inhibit viral invasion and replication via ACE2 and 3CLpro. The results of this study provide evidence for the pharmacological effects of Sang Ju Yin on COVID-19.

Future Direction

Efforts should be placed into the experimentation and clinical application of Sang Ju Yin. Purification of the active compounds luteolin, quercetin, and kaempferol is required for the development of novel COVID-19 targeted therapies (Huang et al., 2021). Other non-oral intake methods and dosages for Sang Ju Yin such as intravenous injections should be explored. Though more in-depth research is necessary, TCM currently remains one of the most effective COVID prevention and treatment options and should be applied worldwide in the future (Zhao et al., 2021).

Acknowledgements

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References

Arghal A., Hafsa S. (2020). A Concise Review of the Popularised Drugs Used For The Management of COVID-19 (SARS-CoV-2). *Archives of Clinical and Biomedical Research 4: 421-425.* Retrieved from https://www.fortuneonline.org/articles/a-concise-review-of-the-popularised-drugs-used-for-the-management-of-covid19-sarscov2.html

Bio Essence Health Science. *Sang Ju yin- 桑菊飲- Mulberry Leaf & Chrysanthemum decoction*. Bio Essence Health Science. (2022). Retrieved August 31, 2022, from https://www.bioessence.com/home/productdetail/100155

Bock K. W. (2020). Aryl hydrocarbon receptor (AHR)-mediated inflammation and resolution: Non-genomic and genomic signaling. *Biochemical pharmacology*, *182*, 114220. https://doi.org/10.1016/j.bcp.2020.114220

Capodice, J.L., Chubak, B.M. (2021). Traditional Chinese herbal medicine-potential therapeutic application for the treatment of COVID-19. *Chin Med* 16, 24. https://doi.org/10.1186/s13020-020-00419-6

Deng Y., Liu B., He Z., Liu T., Zheng R., Yang A. & Xu Y. (2020) Based on network pharmacology and molecular docking method to explore the active compounds of Huoxiangzhengqi oral liquid in the prevention of new coronavirus pneumonia (COVID-19). *Chinese Herbal Medicine*. Retrieved from https://kns.cnki.net/kcms/detail/12.1108.R.20200224.2202.002.html

Hernández C.C., Payrol J.A., Laime S.G., García A.M., Miche L., et al. (2021) Diabetes COVID-19, potentialities of *Morus alba* L. (mulberry) and *Stevia rebaudiana* Bertoni (stevia). Mini-review. *Glob J Rare Dis* 6(1): 006-009. DOI: 10.17352/2640-7876.000031

Huang, K., Zhang, P., Zhang, Z., Youn, J. Y., Wang, C., Zhang, H., & Cai, H. (2021). Traditional Chinese Medicine (TCM) in the treatment of COVID-19 and other viral infections: Efficacies and mechanisms. *Pharmacology & therapeutics*, *225*, 107843. https://doi.org/10.1016/j.pharmthera.2021.107843

Jo, S., Kim, S., Shin, D. H., & Kim, M. S. (2020). Inhibition of SARS-CoV 3CL protease by flavonoids. *Journal of enzyme inhibition and medicinal chemistry*, *35*(1), 145–151. https://doi.org/10.1080/14756366.2019.1690480

Kumar, G., Afaq, U., Pathak, B.C., Gupta, B.K. (2020). Complementary Herbal Remedies Currently Used Against COVID-19. *National Webinar on Impact of COVID-19 Pandemic and Future Prospects*. Retrieved from https://www.researchgate.net/profile/Gyanendra-Kumar-14/publication/350579953_COMPLEMENTARY_HERBAL_REMEDIES_CURRENTLY_USED_AGAINST_COVID-19/links/6066c0cd92851c91b1988b46/COMPLEMENTARY-HERBAL-REMEDIES-CURRENTLY-USED-AGAINST-COVID-19.pdf

Kumari, R., Kumar, S. (2020). Pharmacological Approaches to Investigate the Potential Herbal Medicine (Decoction) in Treating COVID-19. *ACTA Scientific Agriculture* 4, 7. Retrieved from https://www.researchgate.net/profile/Sanjay-Kumar-97/publication/348182128_Pharmacological_Approaches_to_Investigate_the_Potential_Herbal_Medicine_Decoction_in_Treating_COVID-19/links/5ff2bab5299bf140886c6d10/Pharmacological-Approaches-to-Investigate-the-Potential-Herbal-Medicine-Decoction-in-Treating-COVID-19.pdf

Li, P., Hu, S., Qian, C., Yao, Y., Li, L. Y., Yang, J. F., Yang, L., Yang, C. C., Zhou, H., Wang, S. X., Hu, Y., Zhu, X. Y., Zhou, J., Pan, L. X., Shen, C. P., & Zhou, H. (2021). The Therapeutic Effect of Traditional Chinese Medicine on Inflammatory Diseases Caused by Virus, Especially on Those Caused by COVID-19. *Frontiers in pharmacology*, *12*, 650425. https://doi.org/10.3389/fphar.2021.650425

Liu, J., Yuan, S., Yao, Y., Wang, J., Scalabrino, G., Jiang, S., & Sheridan, H. (2022). Network Pharmacology and Molecular Docking Elucidate the Underlying Pharmacological Mechanisms of the Herb *Houttuynia cordata* in Treating Pneumonia Caused by SARS-CoV-2. *Viruses*, *14*(7), 1588. https://doi.org/10.3390/v14071588

Luo, H., Gao, Y., Zou, J. *et al.* (2020) Reflections on treatment of COVID-19 with traditional Chinese medicine. *Chin Med* 15, 94. https://doi.org/10.1186/s13020-020-00375-1

Mirzaie, A., Halaji, M., Dehkordi, F. S., Ranjbar, R., & Noorbazargan, H. (2020). A narrative literature review on traditional medicine options for treatment of corona virus disease 2019 (COVID-19). *Complementary therapies in clinical practice*, *40*, 101214. https://doi.org/10.1016/j.ctcp.2020.101214

Nguyen, T.T.H., Woo, HJ., Kang, HK. *et al.* (2012)Flavonoid-mediated inhibition of SARS coronavirus 3C-like protease expressed in *Pichia pastoris* . *Biotechnol Lett* 34, 831–838. https://doi.org/10.1007/s10529-011-0845-8

Niu, W. H., Wu, F., Cao, W. Y., Wu, Z. G., Chao, Y. C., & Liang, C. (2021). Network pharmacology for the identification of phytochemicals in traditional Chinese medicine for COVID-19 that may regulate interleukin-6. *Bioscience reports*, *41*(1), BSR20202583. https://doi.org/10.1042/BSR20202583

Poon, P. M., Wong, C. K., Fung, K. P., Fong, C. Y., Wong, E. L., Lau, J. T., Leung, P. C., Tsui, S. K., Wan, D. C., Waye, M. M., Au, S. W., Lau, C. B., & Lam, C. W. (2006) Immunomodulatory effects of a traditional Chinese medicine with potential antiviral activity: a self-control study. *The American journal of Chinese medicine*, *34*(1), 13–21. https://doi.org/10.1142/S0192415X0600359X

Qiao, L., Huang, W., Zhang, X. *et al.* (2021). Evaluation of the immunomodulatory effects of anti-COVID-19 TCM formulae by multiple virus-related pathways. *Sig Transduct Target Ther* 6, 50. https://doi.org/10.1038/s41392-021-00475-w

Tao, Q., Du, J., Li, X., Zeng, J., Tan, B., Xu, J., Lin, W., & Chen, X. L. (2020). Network pharmacology and molecular docking analysis on molecular targets and mechanisms of Huashi Baidu formula in the treatment of COVID-19. *Drug development and industrial pharmacy*, *46*(8), 1345–1353. https://doi.org/10.1080/03639045.2020.1788070

Tong, T., Wu, YQ., Ni, WJ. *et al.* (2020). The potential insights of Traditional Chinese Medicine on treatment of COVID-19. *Chin Med* 15, 51. https://doi.org/10.1186/s13020-020-00326-w

U.S. Food and Drug Administration. (2022, May). *FDA has approved two drugs and authorized others for emergency use*. U.S. Food and Drug Administration. Retrieved August 31, 2022, from https://www.fda.gov/consumers/consumer-updates/know-your-treatment-options-covid-19#:~:text=What%20treatments%20are%20available%20for,hospitalized%20adults%20with%20COVID%2D19

Ullah, A., Munir, S., Badshah, S. L., Khan, N., Ghani, L., Poulson, B. G., Emwas, A. H., & Jaremko, M. (2020). Important Flavonoids and Their Role as a Therapeutic Agent. *Molecules (Basel, Switzerland)*, *25*(22), 5243. https://doi.org/10.3390/molecules25225243

Wang, J., Qi, F. (2020). Traditional Chinese medicine to treat COVID-19: the importance of evidence-based research. *Drug Discoveries & Therapeutics. 2020; 14(3):149-150.* https://doi.org/10.5582/ddt.2020.03054

Wang, M. Y., Zhao, R., Gao, L. J., Gao, X. F., Wang, D. P., & Cao, J. M. (2020). SARS-CoV-2: Structure, Biology, and Structure-Based Therapeutics Development. *Frontiers in cellular and infection microbiology*, *10*, 587269. https://doi.org/10.3389/fcimb.2020.587269

World Health Organization. (2022). *WHO coronavirus (COVID-19) dashboard.* World Health Organization. Retrieved August 31, 2022, from https://covid19.who.int/

Wu, Z., Chen, X., Ni, W., Zhou, D., Chai, S., Ye, W., Zhang, Z., Guo, Y., Ren, L., & Zeng, Y. (2021). The inhibition of Mpro, the primary protease of COVID-19, by *Poria cocos* and its active compounds: a network pharmacology and molecular docking study. *RSC advances*, *11*(20), 11821–11843. https://doi.org/10.1039/d0ra07035a

Wu H., Wang J., Yang Y., Li T., Cao Y., Qu Y. & Sun Y. (2020). Preliminary exploration the mechanism of "Qingfei Paidu Decoction" against novel coronavirus pneumonia based on network pharmacology and molecular docking technology. Chinese Journal of *Pharmacy* (03), 374-383. https://doi.org/10.16438/j.0513-4870.2020-0136

Xia, Q. D., Xun, Y., Lu, J. L., Lu, Y. C., Yang, Y. Y., Zhou, P., Hu, J., Li, C., & Wang, S. G. (2020). Network pharmacology and molecular docking analyses on Lianhua Qingwen capsule indicate Akt1 is a potential target to treat and prevent COVID-19. *Cell proliferation*, *53*(12), e12949. https://doi.org/10.1111/cpr.12949

Xing, Y., Hua, Y. R., Shang, J., Ge, W. H., & Liao, J. (2020). Traditional Chinese medicine network pharmacology study on exploring the mechanism of Xuebijing Injection in the treatment of coronavirus disease 2019. *Chinese journal of natural medicines*, *18*(12), 941–951. https://doi.org/10.1016/S1875-5364(20)60038-3

Xu, J., Zhang, Y. (2020). Traditional Chinese Medicine Treatment of COVID-19. *ScienceDirect*, 39, 101165. https://doi.org/10.1016/j.ctcp.2020.101165

Yang, Y., Islam, M. S., Wang, J., Li, Y., & Chen, X. (2020). Traditional Chinese Medicine in the Treatment of Patients Infected with 2019-New Coronavirus (SARS-CoV-2): A Review and Perspective. *International journal of biological sciences*, *16*(10), 1708–1717. https://doi.org/10.7150/ijbs.45538

Yu, B., Lin, F., Ning, H., & Ling, B. (2021). Network pharmacology study on the mechanism of the Chinese medicine Radix Isatidis (Banlangen) for COVID-19. *Medicine*, *100*(32), e26881. https://doi.org/10.1097/MD.0000000000026881

Yu, J. W., Wang, L., & Bao, L. D. (2020). Exploring the active compounds of traditional Mongolian medicine in intervention of novel coronavirus (COVID-19) based on molecular docking method. *Journal of functional foods*, *71*, 104016. https://doi.org/10.1016/j.jff.2020.104016

Zhang, Z. Diagnosis and treatment protocol for COVID-19 patients (Trial Version 9). *Health Care Sci*. 2022; 1: 14–28. https://doi.org/10.1002/hcs2.1

Zhang, Z. J., Wu, W. Y., Hou, J. J., Zhang, L. L., Li, F. F., Gao, L., Wu, X. D., Shi, J. Y., Zhang, R., Long, H. L., Lei, M., Wu, W. Y., Guo, D. A., Chen, K. X., Hofmann, L. A., & Ci, Z. H. (2020). Active constituents and mechanisms of Respiratory Detox Shot, a traditional Chinese medicine prescription, for COVID-19 control and prevention: Network-molecular docking-LC-MSE analysis. *Journal of integrative medicine*, *18*(3), 229–241. https://doi.org/10.1016/j.joim.2020.03.004

Zhao, Z., Li, Y., Zhou, L., Zhou, X., Xie, B., Zhang, W., & Sun, J. (2021). Prevention and treatment of COVID-19 using Traditional Chinese Medicine: A review. *Phytomedicine : international journal of phytotherapy and phytopharmacology*, *85*, 153308. https://doi.org/10.1016/j.phymed.2020.153308

Zong Y., Ding M., Jia K., Ma S. & Ju W. (2020) Based on network pharmacology and molecular docking method to explore the active compounds of Dayuan drink in the treatment of new coronavirus (2019-nCoV) pneumonia. *Chinese Herbal Medicine*. Retrieved from https://kns.cnki.net/kcms/detail/12.1108.r.20200209.1038.002.html