Original Article

Network pharmacology and molecular docking analysis reveals the mechanism of asiaticoside on COVID-19

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Background: Asiaticoside (AS) is a saponin extracted from the traditional Chinese herbal medicine *Centella Asiatica*, which has the effects of reducing inflammatory infiltration and anti-oxidation in pneumonia and combating pulmonary fibrosis. We hypothesize that AS might have therapeutic potential for the treatment of the coronavirus disease 2019 (COVID-19). With the help of network pharmacology and molecular docking techniques, this study discussed the underlying molecular mechanism of AS in the treatment of COVID-19.

Methods: The molecular structure of AS was obtained from the Traditional Chinese Medicine Systems Pharmacology Database and Analysis Platform (TCMSP) system. The targets of AS were achieved using PharmMapper, SwissTargetPrediction, and the Comparative Toxicogenomics Database (CTD). The targets corresponding to COVID-19 were obtained using GeneCards, Online Mendelian Inheritance in Man (OMIM), and CTD database. Then, a target protein-protein interaction (PPI) network was formed using the Search Tool for the Retrieval of Interacting Genes/Proteins (STRING) database. A network of AS, COVID-19, and their co-targets was built using Cytoscape. Afterwards, the co-targets were analyzed by Gene Ontology (GO) and Kyoto Encyclopedia of Genes and Genomes (KEGG) pathway enrichment. Moreover, the predictions of crucial targets were further investigated by performing molecular docking with AS.

Results: A total of 45 core targets of AS were found to be engaged in the pathogenesis of COVID-19. The KEGG enrichment analysis indicated that AS might be protective against COVID-19 through inflammation- and immune-related signaling pathways, including interleukin-17 (IL-17) signaling, T helper 17 (Th17) cell differentiation pathway, Coronavirus disease-COVID-19, MAPK, the PI3K-Akt signaling pathway, and so on. The results of molecular docking showed that AS had a high affinity with those core targets.

Conclusions: The beneficial effect of AS on COVID-19 might be through regulating multiple immune or inflammation-related targets and signaling pathways.

Keywords: Coronavirus disease 2019 (COVID-19); asiaticoside (AS); network pharmacology; molecular docking

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Introduction

Coronavirus disease 2019 (COVID-19) was induced by severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2), a haunting coronavirus discovered in humans. The initial outbreak of SARS was caused by the peer of the same species of SARS-CoV-2, a SARS-CoV (1). Later, the middle east respiratory syndrome-related coronavirus (MERS-CoV) caused the MERS outbreak in 2015 (2). Like its predecessors, COVID-19 has wreaked severe outbreaks of acute respiratory diseases and provoked great panic worldwide (3). Since its first report in Wuhan, Hubei, China, COVID-19 has spread to the whole world with almost 100 million diagnosed patients and caused more than 2,141,468 deaths by 11:55 am Central European Time (CET) 27 January 2021, as reported by the World Health Organization (WHO). Patients with COVID-19 exhibited hyperinflammatory response, also called a cytokine storm, which has been observed and is suspected of causing the detrimental progression of COVID-19 (4). The common symptoms of COVID-19 patients have been dry cough, fever, upper airway congestion, shortness of breath, sputum production, fatigue, arthralgia, and myalgia (5). Older populations and those with reduced immunity have proven more likely to exhibit severe symptoms like pneumonia, acute respiratory distress syndrome (ARDS), acute respiratory failure, kidney failure, and even death (5). Currently, the disease's exact pathogenesis is not fully understood, and effective disease-specific drugs are greatly anticipated.

Traditional Chinese medicine (TCM) is a comprehensive system with multicomponent and multi-target characteristics that exert synergistic effects on many diseases with fewer side effects. The potential of TCM in the prevention and treatment of COVID-19 has attracted mounting interest. Based on the classification system of TCM, the core pathogenesis of COVID-19 is a wet epidemic caused by cold and humidity outside the lung and spleen, which transforms into heat and leads to heat stagnation (4). Throughout the thousands of years of Chinese civilization, TCM has acquired rich clinical experience in diagnosing and treating epidemics and has been shown to reduce the mortality rate and improve the prognosis of a range of diseases. It has been demonstrated that TCM could shorten fever duration and accelerate symptomatic relief in patients with severe COVID-19 (6,7). In the Diagnosis and Treatment Program of COVID-19 (trial version 7; available at: http://www.nhc.gov.cn/xcs/zhengcwj/202003/46c9294a7dfe4cef80dc7f5912eb1989.shtml), jointly issued by the National Health Commission and the National Administration of Traditional Chinese Medicine, Chinese herbal decoctions, and Chinese patent medicine, TCM has been suggested as a possible option for the treatment of COVID-19. More than 85% of SARS-CoV-2-infected patients have received TCM treatment in China (8). Early intervention with TCM could delay the disease progression, shorten the disease course, improve the cure rate, and reduce the mortality rate (7,9). However, the complicated ingredients of TCM make it challenging to understand the mechanisms.

Asiaticoside (AS) is a natural Chinese medicine monomer extracted from *Centella Asiatica*, which has been used for many years to treat dermal disorders, venous insufficiency, and microangiopathy (10). In 2008, AS was launched in China with the approval of the State Food and Drug Administration (Z20083081). It has two pharmaceutical forms, including AS tablet and AS ointment. It has been reported that AS has potent pharmacological activity as well as broader pharmacological effects including anti-oxidant and scavenging free radicals, antidepressant and anti-anxiety, immune regulation and anti-inflammatory, anti-ulcer, hepato-protective, and antitumor activities (11,12). Researchers have found that AS exerts therapeutic effects on diarrhea, asthma, tuberculosis, atherosclerosis, wound healing, as well as antifungal and antibacterial (13,14). It has the functions of relieving asthma, clearing heat, and detoxifying dampness (15). It is effective against pulmonary infection and prevents lipopolysaccharide (LPS)-induced acute lung injury through potent anti-inflammatory effects (16). Also, AS has been shown to have an impact on the prevention and treatment of H5N1/H1N1 by inhibiting the mTOR pathway (17). Based on the evidence above, we proposed that AS could exert a positive therapeutic effect on COVID-19.

In regard to the working mechanism, TCM regulates the human body synergistically through multiple targets, although it is challenging to identify specific action mechanisms. Network pharmacology is an emerging method that merges computer science and clinical medicine. Meanwhile, it constructs and visualizes the 'multi-gene, multi-target, and multi-pathway' interaction network to assess the molecular mechanism of medicine (18). This approach is especially suitable for researching multicomponent medicines such as TCM due to their complex matrix nature (19). Molecular docking is a pathway for structural molecular biology and computer-aided drug design in novel medicines (20). The targets found by network pharmacology are considered as receptors.

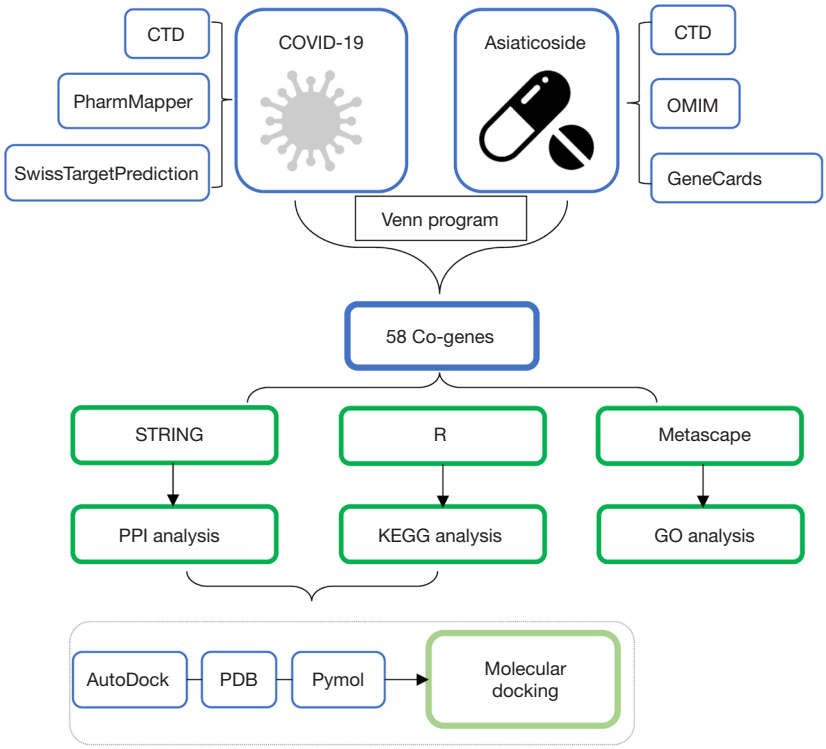

Figure 1 The flow chart of this whole analysis for this study. CTD, Comparative Toxicogenomics Database; COVID-19, coronavirus disease 2019; OMIM, Online Mendelian Inheritance in Man; STRING, Search Tool for the Retrieval of Interacting Genes/Proteins; PPI, protein-protein interaction; KEGG, Kyoto Encyclopedia of Genes and Genomes; GO, Gene Ontology; PDB, Protein Data Bank.

Therefore, the integration of network pharmacology and molecular docking helps accelerate experimental verification and target discovery (4). With the help of network pharmacology and molecular docking, we explored the molecular mechanism of how AS improves COVID-19 and laid the foundation for investigating the molecular basis of COVID-19 treatment with AS. The detailed procedure is shown in *Figure 1*. Although AS has not been applied for COVID-19 in China at present, our study would show it was beneficial in the treatment of COVID-19 and it may be a potential agent. The research also provided a rapid channel for the discovery and application of new anti-CoV therapeutics.

Methods

Predicting the targets of AS

The study was conducted in accordance with the Declaration of Helsinki (as revised in 2013). The protein targets related to AS were retrieved from SwissTargetPrediction (http://www.swisstargetprediction.ch/), Comparative Toxicogenomics Database (CTD; (https://ctdbase.org/;jsessionid=84E0B5D0353A4B9A77545215380F0CA7), and the PharmMapper database (http://www.lilab-ecust.cn/pharmmapper/). After the line notations (from Simplified Molecular-Input Line-Entry System) of AS were imported into the SwissTargetPrediction database, target information was then obtained. The SwissTargetPrediction database is a web server that accurately predicts the similarity in the targets of bioactive molecules based on their chemistry (21). PharmMapper (22) could predict potential biological targets for a given small molecule against all the experimentally determined three-dimensional (3D) structures of proteins available on PharmTargetDB. The candidate targets of AS were predicted by PharmMapper after the 3D structures had been submitted, and all the parameters were kept as default. The CTD is a publicly available database providing curated core information about chemical genes/protein interactions, chemical diseases, and gene-disease relationships from peer-reviewed literature (23). The potential targets of AS were

predicted using CTD with default setting parameters.

After removal of duplicated data, the desired targets were obtained. Then, the target proteins screened from the three databases were standardized in The Universal Protein Resource (UniProt; https://www.uniprot.org/), and the targets of "Homo sapiens" were reserved for further analysis.

Predicting the targets of COVID-19

The COVID-19-related target proteins were screened from the following three sources: (I) GeneCards database (https://www.genecards.org/), which is an online database of human genes and genetic diseases enabling navigate gene-disease linkages (24); (II) Online Mendelian Inheritance in Man database (OMIM; https://www.omim.org/), which possesses over 15,500 gene entries, and focuses on explaining the relationships of gene-phenotype (25); (III) CTD database. We established a COVID-19-related gene set by combining these search results.

Intersection of drug targets and disease targets

Using the Venn diagram intersection, we mapped the COVID-19 targets to those of AS and located the overlapping drug-disease targets. These targets were considered potential targets of AS action in the treatment of COVID-19.

Protein-protein interactions (PPIs) network construction

We gathered the common targets of COVID-19 and AS as the co-targets of AS for COVID-19. The Search Tool for the Retrieval of Interacting Genes/Proteins database (STRING; https://cn.string-db.org/cgi/input.pl) was used to integrate all publicly available sources of PPI information and complement these with computational predictions. The co-targets were input into STRING and a PPI network was built to construct a comprehensive global network, including physical and functional interactions. The cutoff of the PPI confidence score was 0.4. Degree stood for the number of connections of the node in the whole network, reflecting the interaction information between nodes and the importance of the core targets. The targets were sorted by degree, and the top 30 nodes were displayed.

Construction of the whole network

The intersection of PPI target and drug-disease common targets were taken for further analysis. A visual drug-disease-target network was established based on data mentioned above through Cytoscape (version 3.8.0) to visualize the complicated relationships between drug-disease and their potential targets (26). In the network, nodes represented the drug, targets, and disease, while the connections between them represented these biological interactions. The top 16 targets were listed in the central panel, which was screened based on the degree value. The molecular degree value reflected the number of connections between the molecular and target in the network (27). A larger value indicated a greater possibility for the component to become the critical target of AS for COVID-19.

Gene Ontology (GO) and Kyoto Encyclopedia of Genes and Genomes (KEGG) pathway enrichment analysis

Metascape (https://metascape.org/gp/index.html#/main/) is a portal website designed to provide an extensive gene list annotation and analysis resource. Metascape integrates gene annotation, functional enrichment, membership search, and interactome analysis to leverage over 40 independent knowledgebases within one integrated portal in the field of design features. Therefore, Metascape was useful in processing data for GO analysis on following aspects: biological process (BP), molecular function (MF), and cellular component (CC) (28).

To understand gene functions and signaling pathways of potential targets, KEGG pathway analysis (29) was carried out using the clusterProfiler package and org.Hs.eg.db (30,31) to locate the gene ID of the potential targets. The statistical significance threshold of enrichment analysis was $P<0.05$ and adjusted $P<0.05$. The top 20 enriched terms were shown on a chord plot if exist.

AS-target molecular docking

After taking the intersection of genes in the whole network and involved in the KEGG pathways, the top 9 targets (ranked by their degree values) were chosen for molecular docking analysis. In detail, the 9 targets and AS were used as receptors and ligands, respectively. The 3D structure of these targets was obtained from the Protein Data Bank (PDB) database (https://www.rcsb.org/). AutoDock 1.5.6 (https://autodock.scripps.edu/) was used to remove the water molecules, add the nonpolar hydrogen, isolate proteins, calculate Gasteiger charges for the structure, and perform molecular docking using local search parameters.

The generated conformation with the best affinity was then selected and visualized in Pymol 2.3 (https://pymol.org/2/).

Statistical analysis

The cutoff of the PPI confidence score was 0.4 by STRING. GO analysis was performed by Metascape and KEGG pathway analysis was conducted using R as mentioned above. The statistical significance threshold of enrichment analysis was P<0.05 and adjusted P<0.05. Molecular docking was carried out using local search parameters. The generated conformation with the best affinity was then selected and visualized in Pymol.

Results

Screening of potential drug-disease targets

As shown in *Table 1*, 97 potential targets for AS action were mined. Additionally, a total of 7,415 COVID-19 associated genes were acquired from the CTD, GeneCards, and OMIM databases (online available: https://cdn.amegroups.cn/static/public/atm-22-51-01.pdf). The mapping of the AS targets with the COVID-19 targets yielded 58 genes, which were the possible targets of the AS action in treating the disease (*Figure 2* and *Table 2*).

PPI network of the co-targets

To understand the mechanism of AS in treating COVID-19, it was necessary to determine the interactive effect of the co-target proteins. By overlapping the AS targets with those involved in COVID-19, 58 potential co-targets were identified and fed into the STRING database to obtain the interlaced network containing correlations among targets. After hiding the disconnected nodes, 45 targets were presented in the whole network (*Figure 3A* and *Table 3*). The top 30 targets are shown in *Figure 3B*, which were expected as the core targets in the PPI network. They probably participate in the treatment mechanism of AS for COVID-19. It was assumed that AS carries out its medicinal effect and treats COVID-19 by acting on these core targets.

The construction of the disease-target-drug network

After taking the intersection of drug-disease co-targets and PPI targets, we condensed the disease-target-drug network containing 47 nodes (including AS, COVID-19, and 45 genes) and 233 edges (*Figure 3C*). A larger node reflected greater importance. Interestingly, the genes in the network were precisely consistent with those in PPI, which illustrated the accuracy of the analysis results. The top 16 genes with a degree above 9 were involved in transcription 3 (*STAT3*), jun proto-oncogene (*JUN*), mitogen-activated protein kinase 14 (*MAPK14*), mechanistic target of rapamycin (*mTOR*), brain-derived neurotrophic factor (*BDNF*), catalase (*CAT*), matrix metallopeptidase 2 (*MMP2*), BCL2 like 1 (*BCL2L1*), nerve growth factor (*NGF*), heat shock protein 90 alpha family class a member 1 (*HSP90AA1*), *MAPK9*, Bruton tyrosine kinase (*BTK*), nuclear factor of activated T cells 1 (*NFATC1*), interferon regulatory factor 4 (*IRF4*), MMP1, and nuclear receptor coactivator 3 (*NCOA3*).

GO enrichment analysis

Currently, a problem exists in most GO analyses. The redundancies in ontologies and descriptors can sometimes complicate the interpretation of the output. For instance, ontology terms found in GO form a hierarchical structure of increasing granularity, which makes the terms unnecessarily redundant. Besides, terms from different ontology sources can be closely related. As the functional enrichment analysis can identify overlapping or related terms, it is not effortless to extract non-redundant and representative processes to report in the analysis output. To further understand the core genes in different GO terms, Metascape, which could cluster the redundant terms, was used to perform GO enrichment analysis. The GO chord plots which could represent the core targets related to GO annotations were constructed.

In the right panel of *Figure 4* (P<0.05), the color transition from red to purple represents the increasing P value and the decreasing significance of a term. In the left panel, the genes from top to bottom implies the descending degree and importance of the core genes. As shown in *Figure 4A* (P<0.05), the 20 top-ranking terms about BP were chosen. The positive regulation of transferase activity, response to acid chemical, reactive oxygen species metabolic process, muscle cell proliferation, positive regulation of CC biogenesis, leukocyte activation, and so on, were included. Based on CC enrichment analysis, the targets contained serine/threonine protein kinase complex, presynapse, perinuclear region of cytoplasm, Golgi lumen, and ficolin-1-rich granule lumen, RNA polymerase II transcription factor complex, and mitochondrial matrix (*Figure 4B*; P<0.05). Simultaneously, MF terms mainly contained protein kinase

Table 1 Target prediction result for AS

Symbol	Source
RARG	PharmMapper
NF2	PharmMapper
CSDE1	PharmMapper
PRPS1	PharmMapper
MAPK9	PharmMapper
UAP1	PharmMapper
PCNA	PharmMapper
CCNE1	PharmMapper
NUDT18	PharmMapper
FES	PharmMapper
ZEB2	PharmMapper
MUC1	PharmMapper
ITPKC	PharmMapper
IQUB	PharmMapper
TIMM9	PharmMapper
HBEGF	PharmMapper
ARHGAP5	PharmMapper
MSN	PharmMapper
TRIM21	PharmMapper
NAGK	PharmMapper
RND1	PharmMapper
BTK	PharmMapper
FABP2	PharmMapper
RARB	PharmMapper
VAV2	PharmMapper
HSCB	PharmMapper
IGHV4-59	PharmMapper
GALM	PharmMapper
KYNU	PharmMapper
SULT2A1	PharmMapper
ACADVL	PharmMapper
NR1I3	PharmMapper
NR3C2	PharmMapper
NR3C2	PharmMapper
MAP3K3	PharmMapper
AOC3	PharmMapper
HBB	PharmMapper
MAPKAPK2	PharmMapper
PDK3	PharmMapper
MYSM1	PharmMapper
NEO1	PharmMapper
PDK2	PharmMapper
HIBCH	PharmMapper
HFE	PharmMapper
GALK1	PharmMapper
DTYMK	PharmMapper
ACVR2B	PharmMapper
ASAP1	PharmMapper
NFATC1	PharmMapper
NAE1	PharmMapper
CUX2	PharmMapper
HSP90AA1	PharmMapper
TUT1	PharmMapper
CDK5R1	PharmMapper
MAGEA4	PharmMapper
NMRK1	PharmMapper
RRM1	PharmMapper
MMP2	PharmMapper
HLA-E	PharmMapper
RAN	PharmMapper
MAPK6	PharmMapper
POU2F1	PharmMapper
PAH	PharmMapper
HMGCS1	PharmMapper
IRF4	PharmMapper
MMP1	PharmMapper
FCGR2A	PharmMapper
NCOA3	PharmMapper
PRPSAP2	PharmMapper

Table 1 (*continued*)

Table 1 (*continued*)

Symbol	Source
ZCWPW1	PharmMapper
BCL2L1	SwissTargetPrediction
HSD11B2	SwissTargetPrediction
HSD11B1	SwissTargetPrediction
JUN	SwissTargetPrediction
PTPA	SwissTargetPrediction
GLI1	SwissTargetPrediction
F2	SwissTargetPrediction
GLRA1	SwissTargetPrediction
GLRA2	SwissTargetPrediction
PTAFR	SwissTargetPrediction
STAT3	SwissTargetPrediction
RORC	SwissTargetPrediction
FDFT1	SwissTargetPrediction
PTPN1	SwissTargetPrediction
BDNF	CTD
NGF	CTD
PDPK1	CTD
CAT	CTD
CYP2C19	CTD
CYP3A4	CTD
MAPK14	CTD
MTOR	CTD
PEBP1	CTD
PIK3R6	CTD
SLC18A2	CTD
STX1A	CTD
SYNJ1	CTD
TH	CTD

AS, asiaticoside; CTD, Comparative Toxicogenomics Database.

binding, transcription factor binding, kinase activity, MAPK binding, lipid binding, oxygen binding, nuclear receptor activity, protein homodimerization activity, growth factor activity, protein tyrosine kinase binding, protein domain specific binding, protein phosphatase binding, and serine-type endopeptidase activity (*Figure 4C*; P<0.05).

KEGG enrichment analyze

To comprehensively clarify the numerous mechanisms of AS on COVID-19, KEGG pathway enrichment analyses were conducted using org.Hs.eg.db and the clusterProfiler package of R. The primary pathology of COVID-19 is viral pneumonia with patchy inflammatory cellular infiltration and pulmonary edema. From the results of the 20 top-ranking pathways that were screened out (*Figure 4D*; P<0.05), those were enriched in: (I) immune- and inflammation-related signaling pathways such as T cell receptor signaling pathway, T helper 17 (Th17) cell differentiation, and interleukin-17 (IL-17) signaling pathway. (II) Cancer related pathways included proteoglycans in cancer, programmed death-ligand 1 (PD-L1), and programmed cell death protein 1 (PD-1) checkpoint pathway in cancer. (III) Virus infection-related pathways included hepatitis B, measles, herpes virus infection-associated with Kaposi sarcoma, and even directedly targeting COVID-19. Beyond that, as shown in *Table 4*, the Epstein-Barr virus infection, Yersinia infection, Th1 and Th2 cell differentiation, relaxin signaling pathway, human T-cell leukemia virus 1 infection, apoptosis, tumor necrosis factor (TNF) signaling pathway, MAPK signaling pathway, PI3K-Akt signaling pathway, and so on, were also significantly enriched.

Results of molecular docking

The intersection of the top 16 genes in the disease-target-drug network and the top 16 genes in the KEGG pathways were taken for molecular docking. The result showed that AS had a strong affinity with the chosen proteins STAT3, JUN, MAPK14, MTOR, MMP2, HSP90AA1, MAPK9, BTK, and NFATC1. As shown in *Figure 5*, the drug and target proteins could form a stable complex by binding different hydrogen bonds with the residues at very close distance.

Discussion

The COVID-19 pandemic is a public health emergency causing worldwide concern, which has had an enormous impact on the global health economy (32). The pathogenesis of COVID-19 is extremely complicated. Patients who are susceptible to this pathogenic coronavirus face a risk of

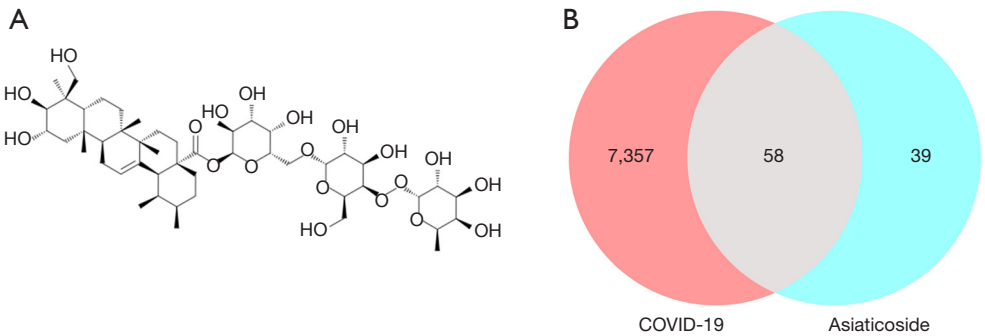

Figure 2 The structure of AS obtained from TCMSP system (A) and venn diagram for the targets of COVID-19 and AS (B). (The detailed are listed in *Table 2*). AS, asiaticoside; TCMSP, Traditional Chinese Medicine Systems Pharmacology Database and Analysis Platform; COVID-19, coronavirus disease 2019.

Table 2 Co-targeted genes for AS and COVID-19

Symbol
NF2, CSDE1, MAPK9, PCNA, CCNE1, NUDT18, ZEB2, MUC1, ITPKC, TIMM9
HBEGF, MSN, TRIM21, RND1, BTK, FABP2, RARB, VAV2, GALM, ACADVL
NR3C2, HBB, PDK3, PDK2, HFE, ACVR2B, ASAP1, NFATC1, NAE1, HSP90AA1
TUT1, CDK5R1, MMP2, POU2F1, HMGCS1, IRF4, MMP1, NCOA3, PRPSAP2, BCL2L1
HSD11B2, HSD11B1, JUN, F2, GLRA1, PTAFR, STAT3, PTPN1, BDNF, NGF
CAT, CYP2C19, CYP3A4, MAPK14, MTOR, PEBP1, SLC18A2, TH

AS, asiaticoside; COVID-19, coronavirus disease 2019.

developing many fatal complications, such as organ failure, pulmonary edema, septic shock, severe pneumonia, and ARDS (33,34). However, a drug that can effectively treat COVID-19 has not yet been identified.

When exposed to human coronaviruses pathogens, the host immune system reacts immediately by triggering related defense mechanisms to achieve a more efficacious shield. This process can be characterized by the increase of inflammatory cytokines and chemokines (35). The deteriorated clinical presentation of COVID-19 is also associated with arrantly elevated pro-inflammatory cytokines, including IL-1β, IL-6, IL-8, and IL-17 (36). As reported, Chinese medicine with anti-inflammatory function has exhibited a tremendous beneficial effect in treating COVID-19 by markedly relieving primary symptoms like fever and cough, and could accelerate recovery (7). For example, Lian Hua Qing Wen notably inhibited the replication of SARS-CoV-2 in Vero E6 cells as well as reduced pro-inflammatory cytokine TNF-α and IL-6 expression at the messenger RNA (mRNA) level (37). Shen Fu injection has been shown to reduce the lung inflammation and decrease the expression levels of IL-1β and IL-6 in COVID-19 patients (9).

AS, which is well tolerated with minimal side effects and superior efficacy as many other herbal medicines, has a broad-spectrum anti-inflammatory and anti-oxidant effect and has the functions of clearing heat, detoxifying dampness, and relieving asthma (11,12,15). Moreover, it is likely to have value as a potential intervention for ARDS (38).

In this study, 45 potential co-targets (*Table 3*) and 67 significantly enriched signaling pathways (*Table 4*) were identified by network pharmacology in treating COVID-19 with AS, among which many were involved in the cytokine storms and ARDS. The results of the PPI network showed that AS might weaken cytokine storms by regulating *STAT3*, *JUN*, *MAPK14*, *mTOR*, *MMP2*, *HSP90AA1*, *MAPK9*, *BTK*, *NFATC1*, and other genes (*Figure 3*). Among them, *mTOR* is a critical factor elevated during a cytokine storm of COVID-19 and participates in cell metabolism and proliferation (39). As reported, AS has an influence on the treatment of H5N1/H1N1 by inhibiting the mTOR

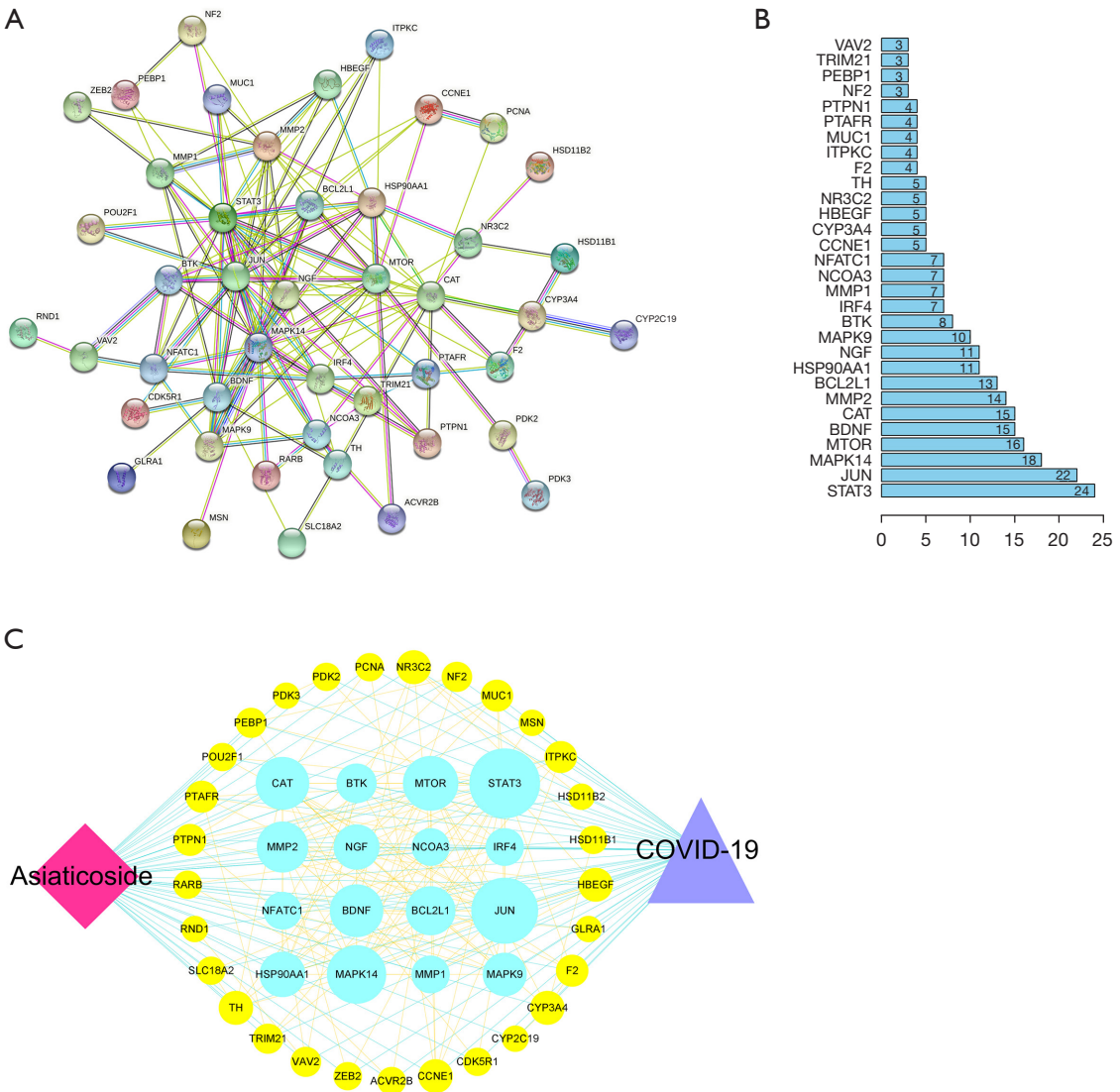

Figure 3 An association network of AS-targeted proteins associated with COVID-19. (A) The PPI network of overlapping targets of COVID-19 and AS. (B) The top 30 co-target proteins (ranked by their degree values). (C) Interaction between COVID-19, AS and co-targets (constructed using Cytoscape). The proteins were ranked by their degree values, and the larger 16 hub nodes in the inner ring represent greater importance. AS, asiaticoside; COVID-19, coronavirus disease 2019; PPI, protein-protein interaction.

pathway (17), which shows the potential of AS in treating COVID-19 by inhibition of mTOR.

The KEGG enrichment analysis indicated that the core targets were mostly enriched in inflammation- and immune-related signaling pathways: Th17 cell differentiation, IL-17, TNF, MAPK, and the PI3K-Akt signaling pathway. Previous studies have demonstrated that the MAPK signaling pathway contributes to the development of ARDS (40). Currently, the MAPK signaling pathway is considered to be deeply involved in the COVID-19 pathogenesis (41). Our analysis showed the hub genes of *JUN*, *MAPK14*, and *MAPK9* were involved in the MAPK signaling pathway. It was reported AS exhibited extraordinary anti-inflammatory activity by inhibiting the MAPK pathways (42). The results indicated that the synergistic therapy of AS inhibited COVID-19 partly by suppressing the MAPK pathways. The *STAT3* gene plays a significant part in inflammation control and immunity (43). In cells infected by SARS-CoV-2,

Table 3 The information for the core targets

Target	Degree
STAT3	24
JUN	22
MAPK14	18
MTOR	16
BDNF	15
CAT	15
MMP2	14
BCL2L1	13
HSP90AA1	11
NGF	11
MAPK9	10
BTK	8
IRF4	7
MMP1	7
NCOA3	7
NFATC1	7
CCNE1	5
CYP3A4	5
HBEGF	5
NR3C2	5
TH	5
F2	4
ITPKC	4
MUC1	4
PTAFR	4
PTPN1	4
NF2	3
PEBP1	3
TRIM21	3
VAV2	3
ACVR2B	2
CDK5R1	2
HSD11B1	2
PCNA	2
PDK2	2
POU2F1	2
RARB	2
SLC18A2	2
ZEB2	2
CYP2C19	1
GLRA1	1
HSD11B2	1
MSN	1
PDK3	1
RND1	1

increasing *STAT3* induced the secretion of pro-inflammatory cytokines and chemokines, which results in the infection of regional endothelial cells (44). It has been reported that AS attenuates neonatal hypoxic-ischemic brain damage by suppressing the STAT3 pathway (45), which indicates that AS could silence COVID-19 by attenuating the STAT3 signaling pathway. In the process of cytokine storm, the up-regulation of Th17 and Th17 cell cytokine IL-17A is mainly responsible for the immunopathology of COVID-19 and ARDS (36). Also, the activation of *STAT3* leads to the deposition of IL-17 (46). Growing evidence indicates that the host Th17 inflammatory responses contribute to the severe lung pathology and induce the mortality of lower airway infection of coronaviruses (47). Researchers have suggested targeting IL-17A signaling to manage COVID-19 patients by effectively inhibiting cytokine storm syndrome (48). Our network analysis demonstrated that AS could regulate Th17 and the IL-17A signaling pathway to exert a therapeutically beneficial effect on COVID-19. The PI3K/Akt signaling pathway regulates the release of inflammatory transmitters in inflammatory response in the lungs and airways (49). The latest studies demonstrated that during the SARS-CoV-2 infection, the PI3K/Akt/mTOR pathway was activated in a dose-dependent manner which activated cell apoptosis process (50). A previous study found that AS was beneficial to the treatment of diabetes-associated cognitive deficits through inhibiting the PI3K/Akt/NF-κB pathway (51). We conjectured that AS regulates the PI3K-Akt signaling pathway to potentiate the healing of COVID-19 (52). Severe oxidative stress triggered by

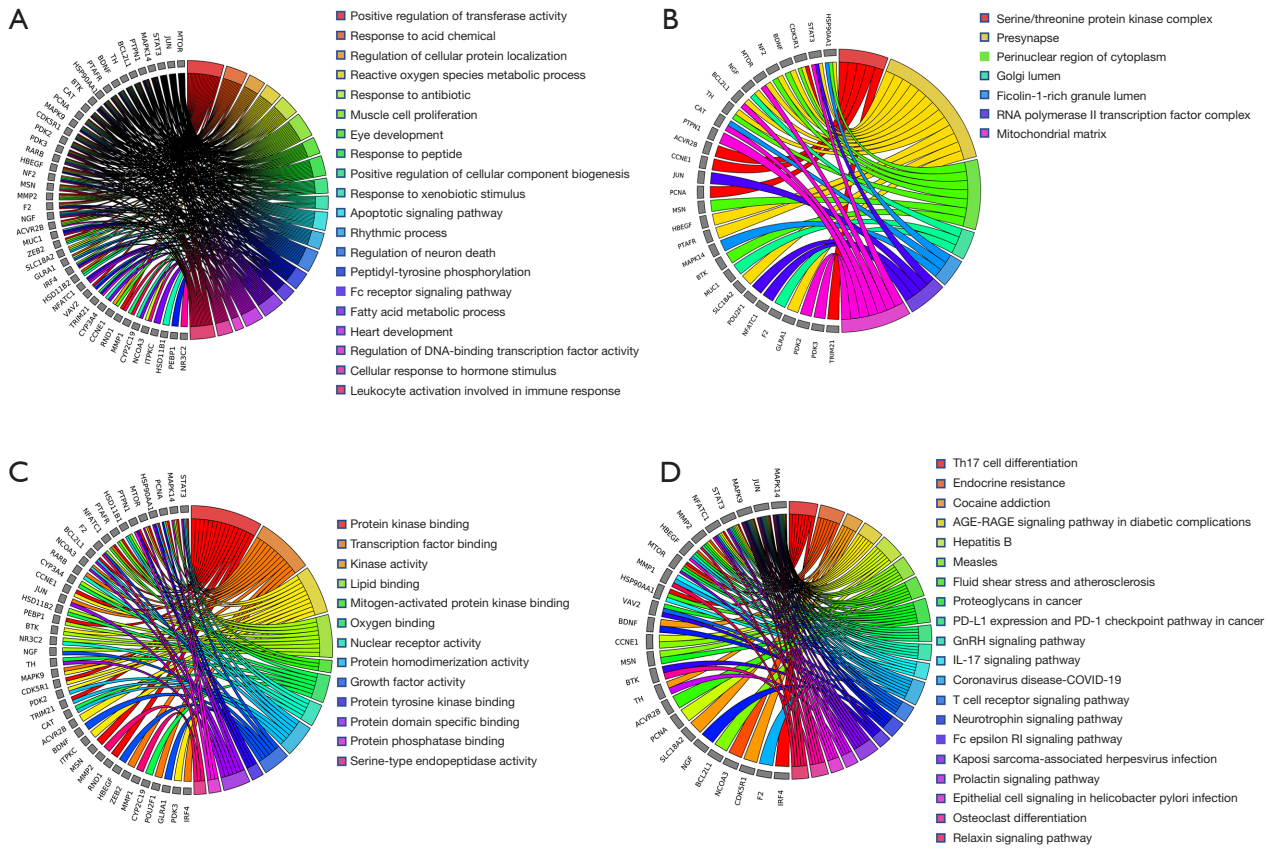

Figure 4 The enrichment analysis of the core targets. GO enrichment analysis of the core targets belonging to BP (A), MF (B) and CC (C). Term is on the right side of the chord plot circle, the gene is on the left side. The corresponding color of the gene ribbon is consistent with the color of Term, indicating that this gene is enriched in this term. (D) KEGG enrichment analysis of the core targets. Pathway is on the right side of the chord plot circle, the gene is on the left side. The corresponding color of the gene ribbon is consistent with the color of Term, indicating that this gene is enriched in this term. Terms and pathway are ordered according to the P value. GO, Gene Ontology; BP, biological process; MF, molecular function; CC, cellular component; KEGG, Kyoto Encyclopedia of Genes and Genomes.

SARS-CoV-2 can aggravate the severity of COVID-19 (53). Previous studies have demonstrated the anti-oxidation and anti-inflammatory effect of a homologous agent of AS by increasing the expression level of anti-oxidant factors and decreasing inflammatory factors IL-6 and IL-17 (54). Such findings might imply AS participates in the treatment of COVID-19 through its anti-inflammation and anti-oxidant abilities. The TNF signaling pathway is a critical pathway in the inflammatory response, which could also induce apoptosis and oxidative stress (55). Additionally, AS could promote cell growth and attenuate cell apoptosis by inhibiting the TNF signaling pathway (56).

Furthermore, clinical data indicate that SARS-CoV-2 infection is related to neurological and neuropsychiatric disease, including encephalopathies, encephalitis, acute disseminated encephalomyelitis, and inflammatory central nervous system (CNS) syndromes (57). It has been found that AS protects against neurotoxicity and accelerates nerve regeneration (58). It has been applied as a psychoactive drug for memory enhancement and in India for a long time (59). As shown in *Figure 4*, AS has a potential neuroprotective effect in COVID-19 by regulating the neurotrophin signaling pathway. Human coronaviruses could regulate numerous cellular processes such as apoptosis (60). In our research, the co-target genes enriched in the apoptosis pathway indicated that AS could exert a regulatory effect on COVID-19 through this pathway. Moreover, AS could even directedly act on the coronavirus disease-COVID-19 pathway. These features indicate that AS is an attractive potential drug for COVID-19 treatment.

Table 4 KEGG enrichment analysis of the core genes

ID	Description	P value	P.adjust	Gene	Count
hsa04659	Th17 cell differentiation	4.85E-08	8.19E-06	*JUN/MAPK14/HSP90AA1/STAT3/MTOR/NFATC1/MAPK9/IRF4*	8
hsa01522	Endocrine resistance	5.00E-07	4.23E-05	*JUN/MMP2/HBEGF/MAPK14/MTOR/MAPK9/NCOA3*	7
hsa05030	Cocaine addiction	4.24E-06	0.000238848	*BDNF/JUN/SLC18A2/TH/CDK5R1*	5
hsa04933	AGE-RAGE signaling pathway in diabetic complications	9.81E-06	0.000414381	*JUN/MMP2/MAPK14/STAT3/NFATC1/MAPK9*	6
hsa05161	Hepatitis B	1.45E-05	0.000491039	*CCNE1/JUN/PCNA/MAPK14/STAT3/NFATC1/MAPK9*	7
hsa05162	Measles	6.39E-05	0.0013971	*CCNE1/JUN/MSN/STAT3/BCL2L1/MAPK9*	6
hsa05418	Fluid shear stress and atherosclerosis	6.39E-05	0.0013971	*ACVR2B/JUN/MMP2/MAPK14/HSP90AA1/MAPK9*	6
hsa05205	Proteoglycans in cancer	6.61E-05	0.0013971	*MSN/MMP2/HBEGF/MAPK14/STAT3/MTOR/VAV2*	7
hsa05235	PD-L1 expression and PD-1 checkpoint pathway in cancer	7.95E-05	0.001493273	*JUN/MAPK14/STAT3/MTOR/NFATC1*	5
hsa04912	GnRH signaling pathway	9.81E-05	0.001585918	*JUN/MMP2/HBEGF/MAPK14/MAPK9*	5
hsa04657	IL-17 signaling pathway	0.000103225	0.001585918	*JUN/MMP1/MAPK14/HSP90AA1/MAPK9*	5
hsa05171	Coronavirus disease—COVID-19	0.000140101	0.001973088	*JUN/HBEGF/MMP1/MAPK14/STAT3/F2/MAPK9*	7
hsa04660	T cell receptor signaling pathway	0.000166623	0.002166098	*JUN/MAPK14/NFATC1/VAV2/MAPK9*	5
hsa04722	Neurotrophin signaling pathway	0.00031295	0.003777751	*BDNF/JUN/MAPK14/NGF/MAPK9*	5
hsa04664	Fc epsilon RI signaling pathway	0.000368061	0.003839622	*MAPK14/BTK/VAV2/MAPK9*	4
hsa05167	Kaposi sarcoma-associated herpesvirus infection	0.000385726	0.003839622	*JUN/MAPK14/STAT3/MTOR/NFATC1/MAPK9*	6
hsa04917	Prolactin signaling pathway	0.000411368	0.003839622	*MAPK14/STAT3/TH/MAPK9*	4
hsa05120	Epithelial cell signaling in Helicobacter pylori infection	0.000411368	0.003839622	*JUN/HBEGF/MAPK14/MAPK9*	4
hsa04380	Osteoclast differentiation	0.000438409	0.003839622	*JUN/MAPK14/BTK/NFATC1/MAPK9*	5
hsa04926	Relaxin signaling pathway	0.000454393	0.003839622	*JUN/MMP2/MMP1/MAPK14/MAPK9*	5
hsa05169	Epstein-Barr virus infection	0.00049199	0.00395935	*CCNE1/JUN/MAPK14/STAT3/BTK/MAPK9*	6
hsa05212	Pancreatic cancer	0.000563112	0.004296484	*STAT3/MTOR/BCL2L1/MAPK9*	4
hsa05135	Yersinia infection	0.000598652	0.004296484	*JUN/MAPK14/NFATC1/VAV2/MAPK9*	5
hsa04915	Estrogen signaling pathway	0.000618852	0.004296484	*JUN/MMP2/HBEGF/HSP90AA1/NCOA3*	5
hsa05170	Human immunodeficiency virus 1 infection	0.000635575	0.004296484	*JUN/MAPK14/MTOR/NFATC1/BCL2L1/MAPK9*	6

Table 4 (*continued*)

Table 4 (*continued*)

ID	Description	P value	P.adjust	Gene	Count
hsa04662	B cell receptor signaling pathway	0.000751018	0.004881614	*JUN/BTK/NFATC1/VAV2*	4
hsa04012	ErbB signaling pathway	0.000859924	0.005382489	*JUN/HBEGF/MTOR/MAPK9*	4
hsa05219	Bladder cancer	0.001132067	0.006740745	*MMP2/HBEGF/MMP1*	3
hsa04658	Th1 and Th2 cell differentiation	0.001156696	0.006740745	*JUN/MAPK14/NFATC1/MAPK9*	4
hsa04530	Tight junction	0.001539769	0.008674033	*NF2/JUN/PCNA/MSN/MAPK9*	5
hsa04625	C-type lectin receptor signaling pathway	0.001821846	0.009931999	*JUN/MAPK14/NFATC1/MAPK9*	4
hsa04621	NOD-like receptor signaling pathway	0.002083434	0.01071649	*JUN/MAPK14/HSP90AA1/BCL2L1/MAPK9*	5
hsa04931	Insulin resistance	0.002092569	0.01071649	*PTPN1/STAT3/MTOR/MAPK9*	4
hsa05145	Toxoplasmosis	0.002389993	0.01187967	*MAPK14/STAT3/BCL2L1/MAPK9*	4
hsa04670	Leukocyte transendothelial migration	0.002549124	0.01230863	*MSN/MMP2/MAPK14/VAV2*	4
hsa04935	Growth hormone synthesis, secretion and action	0.002978636	0.01398304	*MAPK14/STAT3/MTOR/MAPK9*	4
hsa04010	MAPK signaling pathway	0.00339358	0.015500405	*BDNF/JUN/MAPK14/NFATC1/NGF/MAPK9*	6
hsa00140	Steroid hormone biosynthesis	0.003562811	0.015845134	*CYP3A4/HSD11B1/HSD11B2*	3
hsa04068	FoxO signaling pathway	0.004206855	0.01781729	*MAPK14/STAT3/MAPK9/CAT*	4
hsa05321	Inflammatory bowel disease	0.004263871	0.01781729	*JUN/STAT3/NFATC1*	3
hsa04728	Dopaminergic synapse	0.004322538	0.01781729	*MAPK14/SLC18A2/TH/MAPK9*	4
hsa04024	cAMP signaling pathway	0.004468315	0.017979648	*BDNF/JUN/NFATC1/VAV2/MAPK9*	5
hsa05166	Human T-cell leukemia virus 1 infection	0.004737377	0.018134522	*CCNE1/JUN/NFATC1/BCL2L1/MAPK9*	5
hsa04210	Apoptosis	0.004806845	0.018134522	*JUN/NGF/BCL2L1/MAPK9*	4
hsa04137	Mitophagy - animal	0.004840979	0.018134522	*JUN/BCL2L1/MAPK9*	3
hsa04920	Adipocytokine signaling pathway	0.005043329	0.018134522	*STAT3/MTOR/MAPK9*	3
hsa05031	Amphetamine addiction	0.005043329	0.018134522	*JUN/SLC18A2/TH*	3
hsa05133	Pertussis	0.006603137	0.023248544	*JUN/MAPK14/MAPK9*	3
hsa01521	EGFR tyrosine kinase inhibitor resistance	0.007350205	0.025350707	*STAT3/MTOR/BCL2L1*	3
hsa05131	Shigellosis	0.007703447	0.025786816	*JUN/MAPK14/MTOR/BCL2L1/MAPK9*	5
hsa04218	Cellular senescence	0.00778182	0.025786816	*CCNE1/MAPK14/MTOR/NFATC1*	4
hsa04151	PI3K-Akt signaling pathway	0.008333712	0.026853609	*BDNF/CCNE1/HSP90AA1/MTOR/NGF/BCL2L1*	6

Table 4 (*continued*)

Table 4 (*continued*)

ID	Description	P value	P.adjust	Gene	Count
hsa05204	Chemical carcinogenesis	0.008421546	0.026853609	*CYP3A4/HSD11B1/CYP2C19*	3
hsa05022	Pathways of neurodegeneration - multiple diseases	0.009155285	0.028246247	*BDNF/MAPK14/MTOR/BCL2L1/MAPK9/CDK5R1/CAT*	7
hsa05210	Colorectal cancer	0.009282394	0.028246247	*JUN/MTOR/MAPK9*	3
hsa00591	Linoleic acid metabolism	0.009359703	0.028246247	*CYP3A4/CYP2C19*	2
hsa05222	Small cell lung cancer	0.011154207	0.032999848	*RARB/CCNE1/BCL2L1*	3
hsa04215	Apoptosis - multiple species	0.011325392	0.032999848	*BCL2L1/MAPK9*	2
hsa05215	Prostate cancer	0.012869454	0.036652662	*CCNE1/HSP90AA1/MTOR*	3
hsa04750	Inflammatory mediator regulation of TRP channels	0.013229659	0.036652662	*MAPK14/NGF/MAPK9*	3
hsa05231	Choline metabolism in cancer	0.013229659	0.036652662	*JUN/MTOR/MAPK9*	3
hsa04914	Progesterone-mediated oocyte maturation	0.013967343	0.038072273	*MAPK14/HSP90AA1/MAPK9*	3
hsa05142	Chagas disease	0.014728143	0.039508827	*JUN/MAPK14/MAPK9*	3
hsa04960	Aldosterone-regulated sodium reabsorption	0.014966286	0.039520349	*HSD11B2/NR3C2*	2
hsa04620	Toll-like receptor signaling pathway	0.015512154	0.040331601	*JUN/MAPK14/MAPK9*	3
hsa05130	Pathogenic Escherichia coli infection	0.017191602	0.044020921	*JUN/MAPK14/F2/MAPK9*	4
hsa04668	TNF signaling pathway	0.018881874	0.047627414	*JUN/MAPK14/MAPK9*	3

KEGG, Kyoto Encyclopedia of Genes and Genomes.

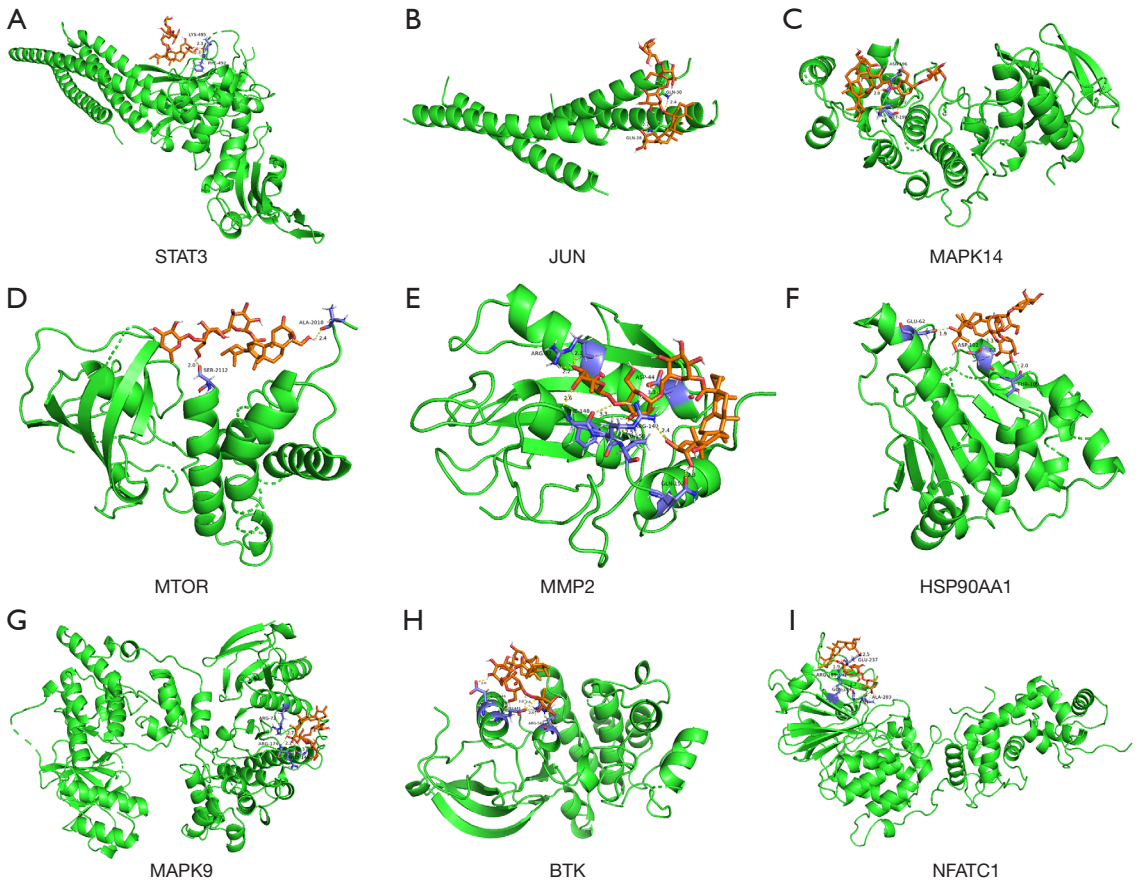

Figure 5 AS and COVID-19 co-targeted proteins molecular docking by the AutoDock server. Molecular docking simulation for STAT3 (A), JUN (B), MAPK14 (C), MTOR (D), MMP2 (E), HSP90AA1 (F), MAPK9 (G), BTK (H), NFATC1 (I) protein with AS. AS, asiaticoside; COVID-19, coronavirus disease 2019.

In molecular docking, AS showed a strong affinity with these co-core proteins, indicating that AS might indeed exert a potent role in treating SARS-CoV-2 by effectively targeting the core proteins and the corresponding pathway involved. This study has presented a comprehensive and systematic understanding of the potential curative mechanism of AS in the treatment of COVID-19.

Conclusions

This study systematically explored the potential mechanism of AS and found it has the possibility to treat COVID-19 through numerous targets and pathways, mainly associated with immune regulation and the inflammatory response. However, there are limitations of network pharmacology concerning its prediction. These potential targets and pathways predicted by network pharmacology tools and bioinformatic techniques need to be confirmed by further experimental evidence.

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Footnote

Conflicts of Interest: All authors have completed the ICMJE uniform disclosure form (available at https://atm.amegroups.com/article/view/10.21037/atm-22-51/coif). The authors have no conflicts of interest to declare.

Ethical Statement: The authors are accountable for all aspects of the work in ensuring that questions related to the accuracy or integrity of any part of the work are appropriately investigated and resolved. The study was conducted in accordance with the Declaration of Helsinki (as revised in 2013).

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