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Exploring the potential mechanisms of Ferulic Acid for treating COVID-19 based on Network Pharmacology and Molecular Docking as well as experimental verification

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ABSTRACT: Using network pharmacology, we aimed to examine the underlying mechanisms of FA in treating COVID-19. GeneCards and OMIM were queried for information on FA treatment and COVID-19 targets. The PPI network was obtained using the software Cytoscape. GO and KEGG enrichment analyses were used to identify biological pathways associated with the target proteins. Using AutoDock Tools, simulate molecular docking and predict the extent of FA binding to key targets. In *in vitro* cell experiments, A549 served as the object, various concentrations of FA were used to prepare the cell model, and qRT-PCR was used to detect the expression of TLR4, ACE, and EGFR genes. Network pharmacology screened 18 FA and COVID-19 intersection targets, enriched 1594 GO entries, and targeted the regulation of the inflammatory response. 29 KEGG pathways involving COVID-19, NF-B, and Toll-like receptor signalling were significantly enriched. Cell experiments confirm that FA can effectively inhibit the expression of TLR4, ACE, and EGFR. This study aims to shed new light on the search for potential COVID-19 treatments.

1. INTRODUCTION

COVID-19 infection due to SARS-CoV-2, with fever and cough common symptoms in patients with high transmissibility, has a substantial negative impact on physical and mental health. Critically unwell patients quickly develop ARDS, MODS, and potentially life-threatening disorders (Taylor & Taylor, 2023). Many people have lost their lives because of this, so there is an urgent need to create and explore medications to treat COVID-19 (Antonio et al., 2020). Despite the trend toward synthetic chemistry for medication development, using plants to cure and prevent disease is still very important (Veeresham, 2012). In China, the therapeutic effect proves that the application of traditional Chinese medicine, for example, *Resina Ferulae*, *Angelica sinensis* (Oliv.) Diels, *Chuanxiong Rhizoma*, and *Cimicifugae Rhizoma* are essential in preventing and treating COVID-19 (Liu et al., 2019; Xing et al., 2020). Several natural products, especially small molecule compounds from those herbs, have already been described as active ingredients and virtually tested with success against viruses (Apaydin et al., 2021; Newman & Cragg, 2020). Hence, small-molecule drugs have been pinned on high hopes since the beginning of the COVID-19 epidemic (Ibitoye & Ajiboye, 2019). FA is a small molecule compound with the biological activity of phenols, which is widely present in natural plants. Many beneficial properties such as antiviral, antioxidant, antibacterial, anti-cancer, anti-diabetic, and other properties have aroused people's attention (Antonopoulou et al., 2021; Kaur et al., 2022; Nankar

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et al., 2017; G.W. Yang et al., 2015). FA has been demonstrated to inhibit a number of viruses, including the H1N1 flu virus, and also functions as an antiviral drug through several methods of action, HIV, and respiratory syncytial virus (Ibitoye & Ajiboye, 2018; Ling et al., 2015; Rehman et al., 2019; Sonar et al., 2017). Besides, FA is crucial for the anti-inflammatory actions (Ma et al., 2020; Mir et al., 2018; Wu et al., 2021; Yuan et al., 2016), and can reduce acute lung injury brought on by LPS by blocking the TLR4/NF-B signaling pathway (X. Wang et al., 2017). However, there are currently no studies focused on FA in treating COVID-19.

The investigation of disease and drug action mechanisms using network pharmacology studies has grown in popularity recently (Li et al., 2022). As a new promising approach and tool, network pharmacology can comprehensively analyze the interactions among network parameters and potential drugs by establishing a component-target-pathway-disease network (L. Wang et al., 2022). In addition, molecular docking is frequently utilized to forecast how tiny compounds attach to the corresponding target proteins or enzymes (Rudrapal et al., 2022). Both techniques have been successfully applied in drug discovery to treat diseases (Mu et al., 2021; Saikia & Bordoloi, 2019). To predict potential targets and pathways of FA in the treatment of COVID-19, this study used network pharmacology and molecular docking, along with experimental validation, to investigate possible mechanisms.

2. METHODS

2.1. Potential FA and COVID-19 targets

The molecular structure of FA is obtained by searching for Ferulic acid in PubChem (https://pubchem.ncbi.nlm.nih.gov/) (Daina et al., 2017). Swiss Target Prediction (http://www.swisstargetprediction.ch/) (Keiser et al., 2007) and the ensemble Approach (https://sea.bkslab.org/) (Rappaport et al., 2017) have been used to predict the performance of potential target genes of FA, delete duplicate target genes, and finally obtain the target gene set of FA. Using the Genecards database (https://www.genecards.org/) (Amberger et al., 2015) and OMIM database (https://omim.org/) (Szklarczyk et al., 2019) by searching the keywords "SRAS-COV-2", "COVID-19", then to establish the data set COVID-19 goals, to obtain genes associated with COVID-19.

2.2. Identification of key targets for FA Treatment of COVID-19

By using VENNY2.1 (https://bioinfogp.cnb.csic.es/tools/venny/index.html) used to find possible therapeutic targets for FA treatment of COVID-19, intersect the target genes of FA with the disease's target genes and create a map. The FA target gene sets and the COVID-19-related gene sets were intersected to produce the linked gene sets.

2.3. PPI network

Gene sets connected to FA, and COVID-19 were added to the STRING database (Zhou et al., 2019), to obtain connections between protein interactions for subsequent research. The species was limited to Homo sapiens. Results are stored in TSV format with a medium confidence level of 0.4. Cytoscape 3.7.2 software built-in betweenness network in CytoNca analysis, the higher the score, the more involved biological functions, to exclude the crucial COVID-19 targets for FA therapy, the more significant.

2.4. Gene Ontology (GO and KEGG pathway enrichment analyses

Metascape platform (http://metascape.org/GP/index.html) (Berman et al., 2003) was used to annotate and enrich the target genes of FA and COVID-19 through the GO database, including CC, MF, BP, and KEGG database. The main biological processes and metabolic pathways of its therapeutic effects were obtained, and the data results were saved.

2.5. Molecular docking

The PubChem database was used to find the molecular structure of FA, and the ChemBioOffice 2016 program's ChemBio3D mapping module was used to determine FA's 3D structure by force field optimization. Meanwhile, The PDB database was used to find the target protein's 3D structure (Berman et al., 2003). PyMOL (Version 2.1.1) is used for hydrogenation, dehydration, removal of solvent molecules, elimination of undesirable ligands, and retention of a single strand of the homodimer. The Autodock vina (Version 1.1.2)was used to calculate the root-mean-square deviation (RMSD).Then Autodock (Version 4.2.6) software docked the receptor protein with FA molecule ligand, and PyMOL was used to visualise the outcomes.

2.6. Drugs and chemical reagents

FA was Purchased from Macklin Biochemical Technology Co., Ltd Shanghai, ≥98 % HPLC grade. The solution was dissolved using DMSO and stored in a -80 refrigerator, and the CCK-8 kit was purchased from Topscience Co. Ltd. All rights reserved Shanghai.

2.7. Cell culture

Human pulmonary carcinoma cells (A549) (Shanghai Yaji Biotechnology Co., LTD). A549 cell cultures were kept in an incubator at 37°C with 5% CO_2, 10% foetal bovine serum, 2 mM glutamine, 100 U/mL penicillin, and 100 mg/ml streptomycin.

2.8. Cell viability assay

Using the CCK-8 assay, cell viability was evaluated. The cells of the logarithmic growth cycle were subjected to trypsin digestion and centrifugation. The cell density was adjusted to 2104 cells/mL, and 200 L of cell suspension per well was inoculated into a 96-well plate. After the cells were attached to the wall, FA was added in various concentrations, and 24 hours were spent incubating the cells. An enzyme reader at 450 nm measures the absorbance value (OD value). Cell survival rate (%) = [A(drug)-A(blank)]/[A(control)-A(blank)] ×100%, The

experiment was done three times with three compound wells in each group.

2.9. Real-time quantitative reverse transcription PCR

Using a Tissue/cell RNA extraction Kit (Shanghai share-bio Biotechnology Co., Ltd), total RNA was extracted, and Using 2*Universal SYBR Green qPCR Premix (Shanghai share-bio Biotechnology Co., Ltd) for RT-qPCR. The primer sequences are listed in Table 1, and the target gene's amplification was compared to the level of the endogenous control gene GAPDH.

Primers employed inthis investigation

Name	Sequence 5'-3′
TLR4 F	AGACCTGTCCCTGAACCCTAT
TLR4 R	CGATGGACTTCTAAACCAGCCA
EGFR F	CCCACTCATGCTCTACAACCC
EGFR R	TCGCACTTCTTACACTTGCGG
ACE F	CCACGTCCCGGAAATATGAAG
ACE R	AGTCCCCTGCATCTACATAGC
MPO F	GATGTGCAACAACAGACGCA
MPO R	GAAGCCGTCCTCATACTCCG
F2 F	GCAAGCACCAGGACTTCAAC
F2 R	CCACGGCCTCCTCACAATAG
STAT3 F	GCAAGCACCAGGACTTCAAC
STAT3 R	CCACGGCCTCCTCACAATAG
GAPDH F	CATGTTCGTCATGGGTGTGAACCA
GAPDH R	ATGGCATGGACTGTGGTCATGAGT

3. RESULTS

3.1. Identify the Covid-19 and FA-related genes

Using the Genecard and OMIM databases, 467 COVID-19-related genes were analysed and identified, and 227 FA-inferred genes were identified using the Swiss Target Prediction and Similarity Ensemble method. By identifying the point where disease-related genes and compound target genes converge, we were able to map the interaction network using 18 FA (Figure 1).

3.2. Gene ontology analysis

Eighteen pertinent FA targets for COVID-19 treatment were found through the intersection of FA and COVID-19 targets. We carried out a GO enrichment analysis of these 18 gene targets and screened 1594 biological process items, comprising 1454 BP, 72 CC, and 68 MF entries, to clarify the biological roles of these genes. The findings demonstrate that treating COVID-19 with FA primarily involves related biological processes. Regulation of the metabolism of reactive oxygen species, production of interleukin-8, control of the inflammatory response, control of blood coagulation, and control of hemostasis are all positively regulated processes (Figure 2A). The primary biological elements linked to FA in the treatment of COVID-19 targets are the azurophil granule, secretory granule, cytoplasmic vesicle, external side of plasma membrane, endocytic vesicle, phagocytic vesicle, primary lysosome, and vesicle lumen (Figure 2B).

Additionally, serine-type peptidase activity, serine hydrolase activity, endopeptidase activity, serine-type endopeptidase activity, pattern recognition receptor activity, protease binding, lipopolysaccharide binding, heparin binding, and transcription cofactor binding are among the major molecular functions of FA in treating COVID-19 targets. (Figure 2C). We enhanced the biological process findings from GO and the pathway results from the Metascape database, and we found that the primary physiological and pathological process of COVID-19 was inflammation response (Figure 2D). According to to GO nomenclature, these target genes are essential for immunological and inflammatory responses. In addition, as indicated in the table, we identified 17 GO keywords from the enrichment analysis findings related to inflammation, showing that these target genes are crucial for the inflammatory response (Table 2).

3.3. KEGG pathway enrichment analysis

We performed a KEGG pathway analysis better to comprehend the potential processes underlying FA's anti-COVID-19 actions. The P value of less than 0.05 and an FDR of less than 0.25 were deemed statistically significant by the applicable objective. A total of 29 KEGG pathways were significantly enriched, mainly involving COVID-19, PD-L1 expression, and PD-1 checkpoint pathway in cancer, Chagas disease (American trypanosomiasis), Lipid and atherosclerosis, Toxoplasmosis, Renin-angiotensin system, HIF-1 signaling pathway, Measles, Bladder cancer, Hepatitis C (Figure 3A). The most significant 10 KEGG pathways and corresponding genes are shown in (Figure 3B). Meanwhile, based on the KEGG and GO analysis findings, we constructed a core target relation graph representing the correlation between GO and KEGG labelling (Figure 3C). In (Figure 3D), The change in color from yellow to red denotes the item's significant rise and fall in P-value. At the same time, the results also revealed that The biological processes and signaling pathways associated with inflammation and immunity were the most highly enriched in core target genes.

3.4. PPI network and core targets

The Cytoscape 3.7.2 software now includes FA and COVID-19 gene analysis. The core targets (betweenness greater than 10) comprised six target genomes, and the related gene betweenness results ranking in Table 3 were obtained through the built-in betweenness analysis network in CytoNca. These analyses were performed using the STRING database's PPI network (Figure 4).

3.5. Molecular Docking Analysis

Molecularly docking the top six targets (core targets) of betweenness in the PPI network with FA, the receptors are TLR4, MPO, EGFR, ACE, F2, STAT3, and the binding strength and activity are evaluated based on the binding energy and the volume of hydrogen bonds created. The binding activity of the receptor protein and the tiny ligand molecule FA are more stable when the binding energy is lower and more hydrogen

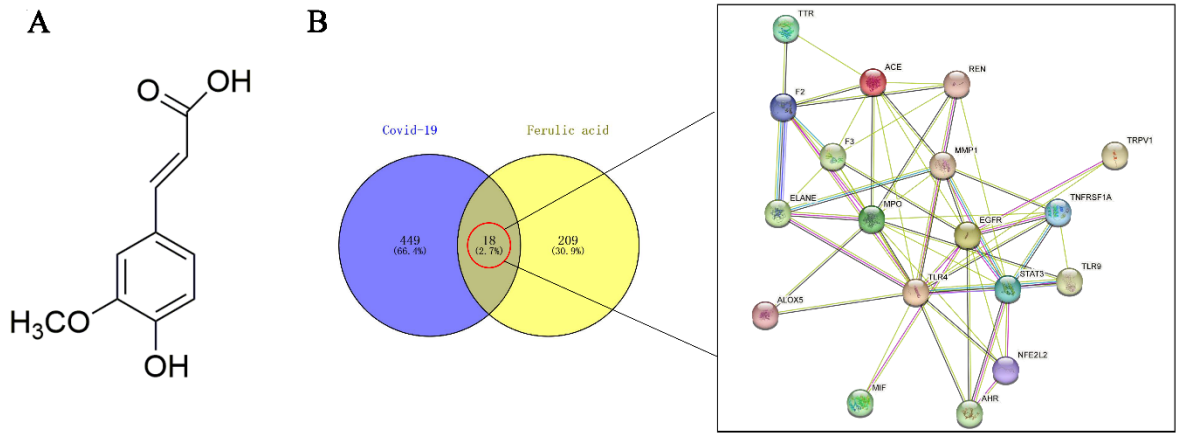

Figure 1. (A) Chemical composition of FA. (B) Identification of FA and COVID-19-associated genes.

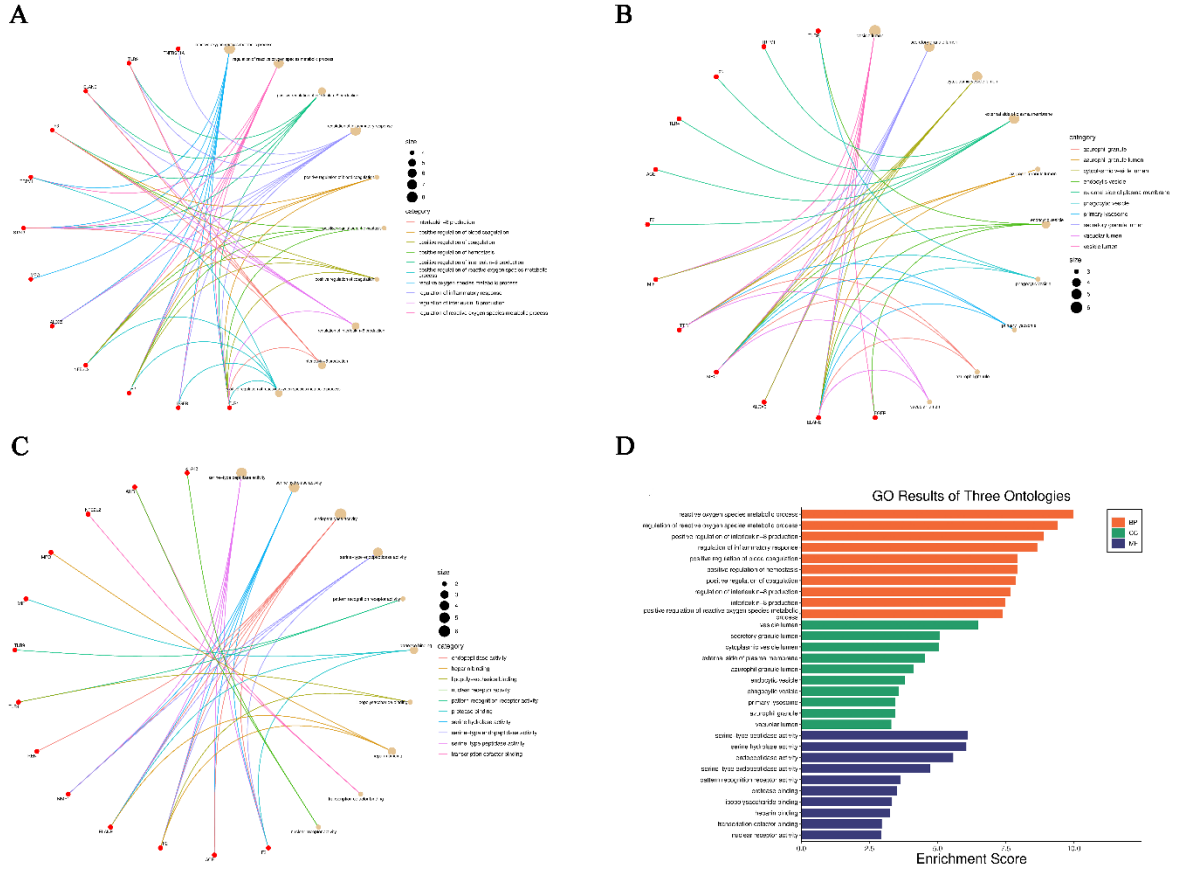

Figure 2. The examination of the core targets' enrichment. Top 10 of the analysis of the core targets by go enrichment BP (A), MF (B) and CC (C), (D)The enrichment analysis of the core targets, Terms and pathway are ordered according to the P value.

Table 1
Inflammatory-related GO terms enriched by the target genes.

ID	Description	P value	Gene ID	Count
GO:0050727	regulation of inflammatory response	2.23079E-09	TLR4/EGFR/F2/TNFRSF1A/ELANE/ALOX5/TLR9/STAT3	8
GO:0002526	acute inflammatory response	5.19004E-08	F3/F2/ELANE/STAT3/TRPV1	5
GO:0050729	positive regulation of inflammatory response	3.03378E-07	TLR4/EGFR/TNFRSF1A/TLR9/STAT3	5
GO:1900015	regulation of cytokine production involved in inflammatory response	4.28146E-07	TLR4/F2/ALOX5/STAT3	4
GO:0002534	cytokine production is involved in inflammatory response	5.11252E-07	TLR4/F2/ALOX5/STAT3	4
GO:0002532	production of molecular mediators involved in inflammatory response	1.6059E-06	TLR4/F2/ALOX5/STAT3	4
GO:0050728	negative regulation of inflammatory response	3.08441E-05	F2/TNFRSF1A/ELANE/ALOX5	4
GO:0002523	leukocyte migration involved in inflammatory response	0.000102359	ELANE/ALOX5	2
GO:1900017	positive regulation of cytokine production involved in inflammatory response	0.000214955	TLR4/STAT3	2
GO:0150076	neuroinflammatory response	0.002411334	EGFR/TRPV1	2
GO:0090594	inflammatory response to wounding	0.014221514	ALOX5	1
GO:1900225	regulation of NLRP3 inflammasome complex assembly	0.014221514	TLR4	1
GO:0044546	NLRP3 inflammasome complex assembly	0.01610322	TLR4	1
GO:0002438	acute inflammatory response to antigenic stimulus	0.022662508	ELANE	1
GO:1900016	negative regulation of cytokine production involved in inflammatory response	0.032886583	F2	1
GO:0002269	leukocyte activation is involved in the inflammatory response	0.046665802	TRPV1	1
GO:0002437	inflammatory response to antigenic stimulus	0.05935894	ELANE	1

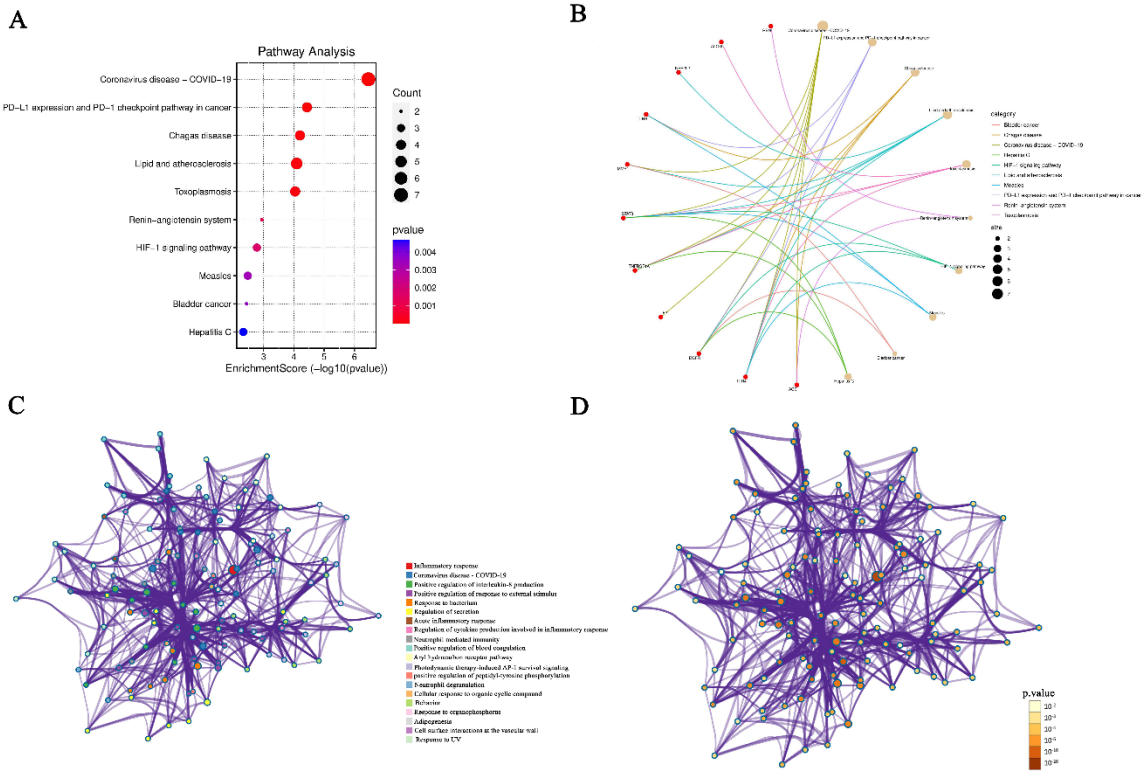

Figure 3. KEGG analysis of related genes. (A) The results of the ten top-ranking pathways. (B) The link between genes and pathways. (C) Association and P values (D) of GO and KEGG enrichment results.

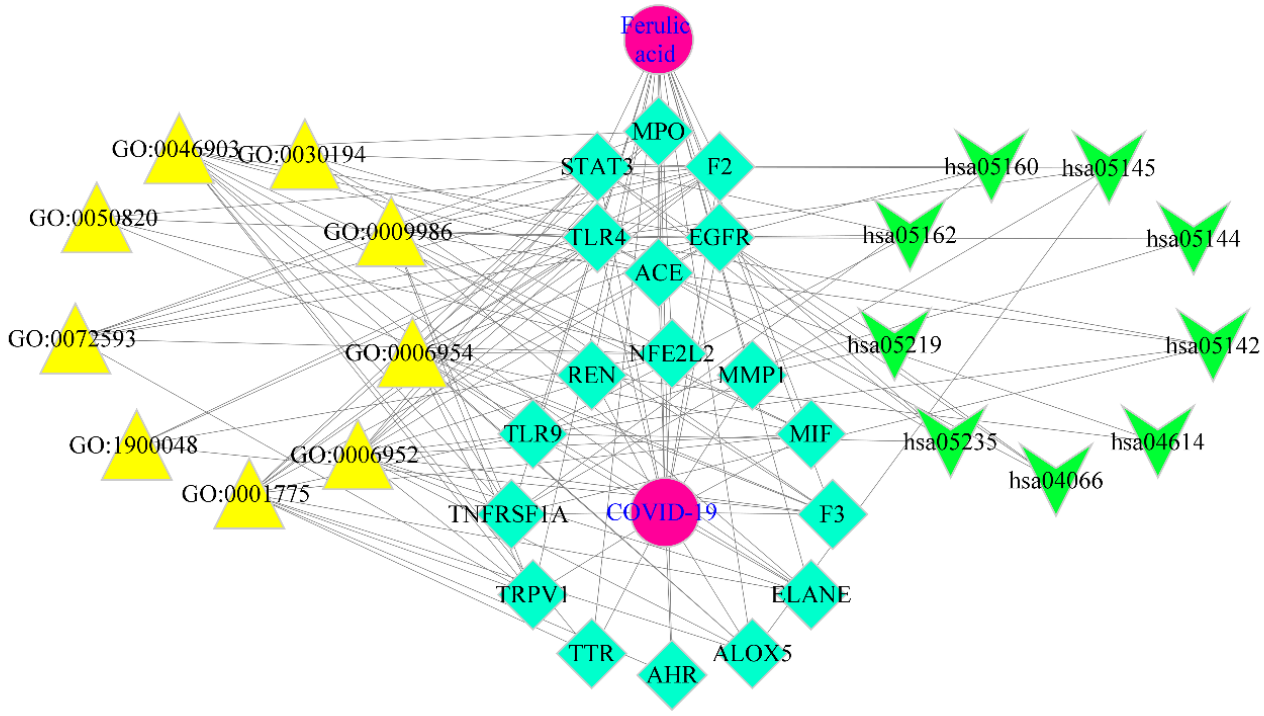

Figure 4. Detailing the intersection association of target-disease-function-pathway in FA to treat COVID-19, an integrated network map from key targets was plotted.

Table 2
Results ranked by Betweenness

Ranked	Gene name	Betweenness
1	TLR4	92.61905
2	MPO	32.833332
3	EGFR	26.116667
4	ACE	20.635714
5	F2	13.116667
6	STAT3	10.119047
7	MMP1	4.169048
8	F3	3.0190477
9	REN	2.0666666
10	TNFRSF1A	1.4523809
11	NFE2L2	0.95238096
12	ELANE	0.9
13	TLR9	0
14	TRPV1	0
15	MIF	0
16	ALOX5	0
17	AHR	0
18	TTR	0

bonds are present. Table 4 of the docking results revealed that FA's binding energy to the core target was less than or equal to 5 kcal/mol, indicating that the binding is meaningful (Barros et al., 2023; Gilson et al., 1997; Marcelino et al., 2022))and a docking is considered reliable if the RMSD of all ligand atoms is less than 2.0 Trott and Olson (2010).We selected the interaction of the binding conformation of hydrogen bond number $\geq$2 for

Table 3
Molecular docking score and RMSD

Target	Drug	Docking score (kcal/mol)	RMSD
TLR4 (PDB ID 2Z63)		-5.51	0.69
MPO (PDB ID 5MFA)		-5.96	1.37
EGFR (PDB ID 1A2V)	FA	-5.23	1.64
ACE (PDB ID 1O8A)		-5.37	1.42
F2 (PDB ID 1A2C)		-5.87	1.61
STAT3 (PDB ID 5AX3)		-5.49	1.96

visualization (Figure 5). The docking scores were recorded in Table 3.

3.6. FA inhibited the expression of TLR4, ACE and EGFR

The activity of A549 cells after FA treatment was firstly measured by CCK-8 assay to exclude false positive results caused by the cytotoxicity of FA. No cytotoxic effect on cell viability was evident with increased FA concentration (Figure 6A). Consequently, FA in non-toxic concentrations (10, 20, and 40 μM) were used for the following experiments. Research has demonstrated that COVID-19 infection can lead to a deadly inflammatory response in the host's body. To investigate the impact of FA on the main target during this inflammatory state, we examined the expression of core target mRNA in A549 cells when exposed to TNF-α stimulation. As depicted in (Figure 6B-G), TLR4, ACE, EGFR, F2, MPO, and STAT3 expression increased significantly under TNF-α stimulation. However, FA effectively inhibited this increase in a dose-

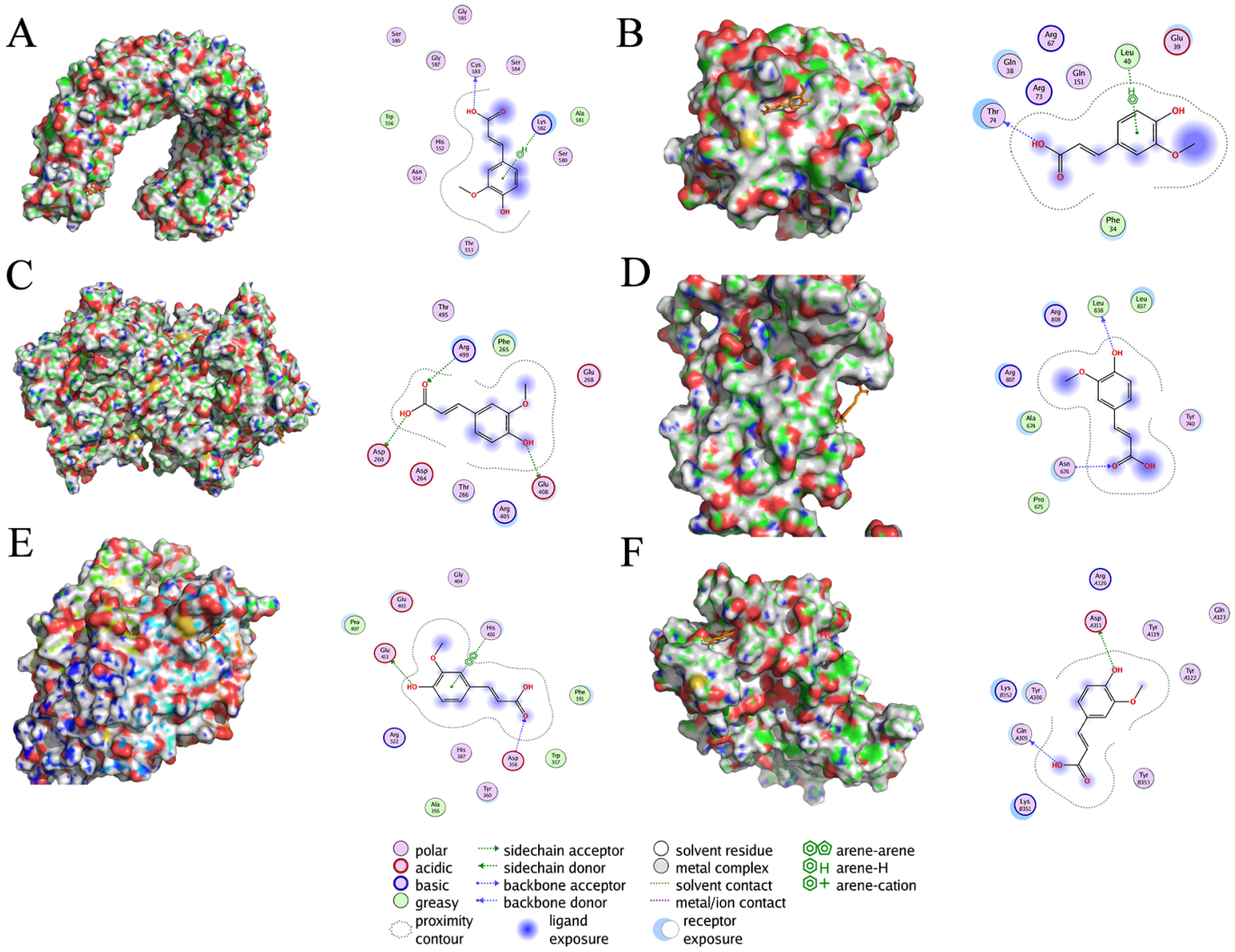

Figure 5. Structural interactions of FA and key target receptors. (A) TLR4 protein - FA; (B) F2 protein - FA; (C) MPO protein - FA; (D) EGFR protein - FA; (E) ACE protein - FA; (F) STAT3 protein - FA.

dependent manner. These findings align with the results of our molecular docking study. These findings suggest that FA may play a key role in controlling immune responses by influencing the expression of these central targets.

4. DISCUSSION

Through network pharmacological methods and molecular docking analysis, we screened the existing database and discovered 227 potential targets of FA. The results showed the pharmacological targets, functions, and signalling pathways of FA's anti-COVID-19 effects. First, all relevant genetic targets for FA therapy for COVID-19 were identified by bioinformatics assays, including F3, ACE, TLR4, EGFR, F2, TNFRSF1A, ELANE, NFE2L2, ALOX5, MPO, TLR9, STAT3, TTR, REN, MMP1, AHR, TRPV1. TLR4 is a cell-indicated immune receptor that binds particularly strongly to that spike protein of SARS-CoV-2 and activates TLR4 signal transduction, thereby increasing the expression of ACE2, high expression of ACE2 is beneficial to the infection of SARS-CoV-2 (Aboudounya & Heads, 2021), leading to ARDS and inflammation. According to the existing literature reports, The activation and abnormal expression of TLR4 may be linked to excessive inflammation in COVID-19 patients, so TLR4 antagonists may help treat COVID-19 (Bank et al., 2021; Tatematsu et al., 2016). MPO, a crucial mediator of the innate immune system, can mediate the proteolytic cleavage of alpha-1-microglobulin to form t-alpha-1-microglobulin, which potently inhibits oxidation of low-density lipoprotein particles and limits vascular damage., inhibits MPO to reduce the damage of inflammation to body tissues (Davies & Hawkins, 2020; Lazarević-Pasti et al., 2015; Rehring et al., 2021). In COVID-19 patients, overexpression of EGFR has been found to lead to pulmonary fibrosis, as high expression of EGFR will abnormally activate the NF-κB and STAT3 signaling pathways, thus exacerbating the inflammatory response of patients (Cuza et al., 2022; Xu et al., 2021). ACE Catalyzing

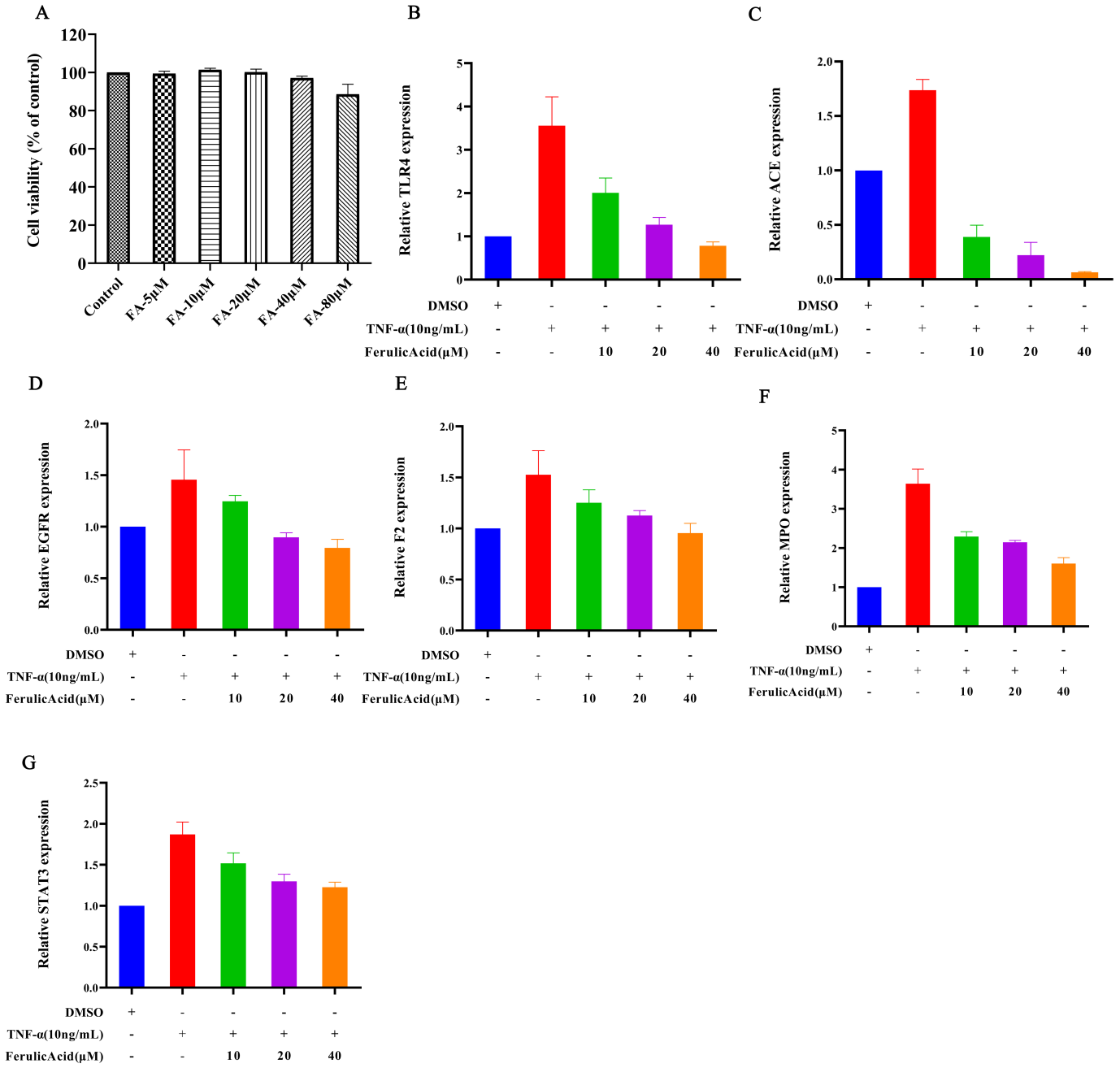

Figure 6. (A) FA reduced the expression of target genes in A549 cells. A FA was incubated for 24 hours, and a CCK-8 assay was used to detect it. A549 cells were treated with DMSO or 10,20, 40 μM FA, and 6 h later. As indicated, cells were treated with either TNF-α (10 ng/ml) or PBS for 30 min. mRNAs expression for the (B), TLR4, (C), ACE, (D), EGFR, (E), F2, (F), MPO and (G), STAT3 was analyzed quantitatively by real-time PCR. Data were expressed as mean ± sd from three independent experiments.

the conversion of angiotensin I into the physiologically active peptide angiotensin II, the regulation of the homologous ACE2 gene may be involved in the progression of several human coronavirus-induced diseases, including SARS-CoV and SARS-CoV-2 (Scialo et al., 2020).

Moreover, ACE2 has been proposed as a coronavirus host cell receptor. Hence, for the therapy of COVID-19, it is possible to decrease viral infection and pathogenic inflammation by reducing ACE (Junior et al., 2021; Khurana & Goswami, 2022; G. Yang et al., 2020). The transcription regulator STAT3 is essential for managing inflammation and immune function. Pro-inflammatory cytokines and chemokines are released when STAT3 is overactive and unbalanced during COVID-19 infection. By inhibiting STAT3 function, COVID-19 can be treated (Matsuyama et al., 2020).

The results of the GO and KEGG analyses of target gene enrichment are intriguing. Initially, it was discovered that the target gene is enriched in the cell's immunity and defense mechanisms, including positive regulation of interleukin-8 production, regulation of the metabolic process for reactive oxygen species, and regulation of the inflammatory response. Therefore, we speculate that this may directly affect the outcome of viral infection. At the same time, The HIF-1 signaling pathway, the NF-B signaling pathway, and the Toll-like receptor signaling pathway are primarily involved in COVID-19. Also, the molecular docking results show that FA can bind to the COVID-19 core targets, confirming FA's potential and pharmacological action in the treatment of COVID-19. Characterized by inhibition of cell necrosis and inflammation-related, we speculate that FA treatment for COVID-19 reduces cytokine expression levels and alleviates excessive inflammation in COVID-19 by inhibiting activation of inflammatory pathways while improving the body's immunity.

5. CONCLUSION

Network pharmacology and bioinformatics gene analysis support our findings that FA participates in antiviral, anti-inflammatory, and immunomodulatory actions via various biological processes and cell signalling pathways. More research and experimental validation are required, but COVID-19 therapy may also be an option.

ABBREVIATIONS

The list of abbreviations associated with this article provided as supplementary document S1.

CONFLICTS OF INTEREST

The authors declare that they have no competing interests.

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ETHICAL APPROVAL

Ethical approval not required.

REFERENCES

Aboudounya, M.M., Heads, R.J., 2021. SARS-CoV-2 May Bind and Activate TLR4 to Increase ACE2 Expression, Facilitating Entry and Causing Hyperinflammation. Mediators of Inflammation. 4, 8874339. https://doi.org/10.1155/2021/8874339

Amberger, J.S., Bocchini, C.A., Schiettecatte, F., Scott, A.F., Hamosh, A., Omim, Org., 2015. Online Mendelian Inheritance in Man (OMIM(R)), an online catalog of human genes and genetic disorders. Nucleic Acids Research. 43, 789–798. https://doi.org/10.1093/nar/gku1205

Antonio, A., Wiedemann, L., Vf, V.-J., 2020. Natural products' role against COVID-19. RSC Advances. 10, 23379–23393. https://doi.org/10.1039/D0RA03774E

Antonopoulou, I., Sapountzaki, E., Rova, U., Christakopoulos, P., 2021. Ferulic Acid From Plant Biomass: A Phytochemical With Promising Antiviral Properties. Frontiers in Nutrition. 8, 777576. https://doi.org/10.3389/fnut.2021.777576

Apaydin, C.B., Cinar, G., Cihan-Ustundag, G., 2021. Small-molecule Antiviral Agents in Ongoing Clinical Trials for COVID-19. Current Drug Targets. 22, 1986–2005. https://doi.org/10.2174/1389450122666210215112150

Bank, S., De, S.K., Bankura, B., Maiti, S., Das, M., Ak, G., 2021. ACE/ACE2 balance might be instrumental to explain the certain comorbidities leading to severe COVID-19 cases. Bioscience Reports. 41, 41. https://doi.org/10.1042/BSR20202014

Barros, A.G., Lpd, A., Njfd, S., Santos, B.R., 2023. An in silico study involving Mangiferin, 7-Epiclusianone, Fukugetin, Lapachol, Plumbagin and Guttiferone-A against C. albicans proteins. Natural Resources for Human Health. 3, 228–236. https://doi.org/10.53365/nrfhh/158808

Berman, H., Henrick, K., Nakamura, H., 2003. Announcing the worldwide Protein Data Bank. Nature Structural & Molecular Biology. 10, 980. https://doi.org/10.1038/nsb1203-980

Cuza, A.A.A., Avila, J.P., Martinez, R.M., Gonzalez, J.J., Aspuro, G.P., Martinez, G., Suzarte, J.A., Hernandez, M.R., Ane-Kouri, D.S., Ramos, A.L., C, T., 2022. Nimotuzumab for COVID-19: case

series. Immunotherapy. 14, 185–193. https://doi.org/10.2217/imt-2021-0269

Daina, A., Michielin, O., Zoete, V., 2017. SwissADME: a free web tool to evaluate pharmacokinetics, drug-likeness and medicinal chemistry friendliness of small molecules. Scientific Reports. 7, 42717. https://doi.org/10.1038/srep42717

Davies, M.J., Hawkins, C.L., 2020. The Role of Myeloperoxidase in Biomolecule Modification, Chronic Inflammation, and Disease. Antioxidants & Redox Signaling. 32, 957–981. https://doi.org/10.1089/ars.2020.8030

Gilson, M.K., Given, J.A., Bush, B.L., Mccammon, J.A., 1997. The statistical-thermodynamic basis for computation of binding affinities: a critical review. Biophysical Journal. 72, 1047–1069. https://doi.org/10.1016/S0006-3495(97)78756-3

Ibitoye, O.B., Ajiboye, T.O., 2018. Dietary phenolic acids reverse insulin resistance, hyperglycaemia, dyslipidaemia, inflammation and oxidative stress in high-fructose diet-induced metabolic syndrome rats. Archives of Physiology and Biochemistry. 124, 410–417. https://doi.org/10.1080/13813455.2017.1415938

Ibitoye, O.B., Ajiboye, T.O., 2019. Ferulic acid potentiates the antibacterial activity of quinolone-based antibiotics against *Acinetobacter baumannii*. Microbial Pathogenesis. 126, 393–398. https://doi.org/10.1016/j.micpath.2018.11.033

Junior, A.G., Tolouei, S., Livero, D.R., Gasparotto, F.A., Boeing, F., De Souza, T., P., 2021. Natural Agents Modulating ACE-2: A Review of Compounds with Potential against SARS-CoV-2 Infections. Current Pharmaceutical Design. 27, 1588–1596. https://doi.org/10.2174/1381612827666210114150607

Kaur, R., Sood, A., Lang, D.K., Arora, R., Kumar, N., Diwan, V., Saini, B., 2022. Natural Products as Sources of Multitarget Compounds: Advances in the Development of Ferulic Acid as Multitarget Therapeutic. Current Topics in Medicinal Chemistry. 22, 347–365. https://doi.org/10.2174/1568026622666220117105740

Keiser, M.J., Roth, B.L., Armbruster, B.N., Ernsberger, P., Irwin, J.J., Shoichet, B.K., 2007. Relating protein pharmacology by ligand chemistry. Nature Biotechnology. 25, 197–206. https://doi.org/10.1038/nbt1284

Khurana, V., Goswami, B., 2022. Angiotensin converting enzyme (ACE). Clinica Chimica Acta. 524, 113–122. https://doi.org/10.1016/j.cca.2021.10.029

Lazarević-Pasti, T., Leskovac, A., Vasić, V., 2015. Myeloperoxidase Inhibitors as Potential Drugs. Current Drug Metabolism. 16, 168–190. https://doi.org/10.2174/1389200216031508121206 40

Li, X., Wei, S., Niu, S., Ma, X., Li, H., Jing, M., 2022. Network pharmacology prediction and molecular docking-based strategy to explore the potential mechanism of Huanglian Jiedu Decoction against sepsis. Computers in Biology and Medicine. 144, 105389. https://doi.org/10.1016/j.compbiomed.2022.105389

Ling, Y., Wang, Z., Wang, X., Li, X., Wang, X., Zhang, W., Dai, H., Chen, L., Zhang, Y., 2015. Hybrid molecule from Farnesylthiosalicylic acid-diamine and phenylpropenoic acid as Ras-related signaling inhibitor with potent antitumor activities. Chemical Biology & Drug Design. 85, 145–152. https://doi.org/10.1111/cbdd.12393

Liu, J., Jiang, Y., Liu, Y., Pu, L., Du, C., Li, Y., Wang, X., Ren, J., Liu, W., Yang, Z., 2019. Yindan Jiedu Granules, a Traditional Chinese Medicinal Formulation, as a Potential Treatment for Coronavirus Disease. Frontiers in Pharmacology. 11, 634266. https://doi.org/10.3389/fphar.2020.634266

Ma, X., Guo, Z., Zhang, Z., Li, X., Liu, Y., Zhao, L., Wang, X., 2020. Ferulic Acid Protects against Porcine Parvovirus Infection-Induced Apoptosis by Suppressing the Nuclear Factor-κB Inflammasome Axis and Toll-Like Receptor 4 via Nonstructural Protein 1. . Evidence-Based Complementary and Alternative Medicine. 2020, 3943672. https://doi.org/10.1155/2020/3943672

Marcelino, R.C., De Araújo, L.P., Borba, J.B.D.M., Silveira, N.D., 2022. Molecular docking study involving bioactive natural compounds against SARS-CoV-2 proteins. Natural Resources for Human Health. 2, 366–377. https://doi.org/10.53365/nrfhh/147375

Matsuyama, T., Kubli, S.P., Yoshinaga, S.K., Pfeffer, K., Mak, T.W., 2020. An aberrant STAT pathway is central to COVID-19. Cell Death & Differentiation. 27, 3209–3225. https://doi.org/10.1038/s41418-020-00633-7

Mir, S.M., Ravuri, H.G., Pradhan, R.K., Narra, S., Kumar, J.M., Kuncha, M., Kanjilal, S., Sistla, R., 2018. Ferulic acid protects lipopolysaccharide-induced acute kidney injury by suppressing inflammatory events and upregulating antioxidant defenses in Balb/c mice. Biomedicine & Pharmacotherapy. 100, 304–315. https://doi.org/10.1016/j.biopha.2018.01.169

Mu, C., Sheng, Y., Wang, Q., Amin, A., Li, X., Xie, Y., 2021. Potential compound from herbal food of *Rhizoma Polygonati* for treatment of COVID-19 analyzed by network pharmacology: Viral and cancer signaling mechanisms. Journal of Functional Foods. 77, 104149. https://doi.org/10.1016/j.jff.2020.104149

Nankar, R., Prabhakar, P.K., Doble, M., 2017. Hybrid drug combination: Combination of ferulic acid and metformin as anti-diabetic therapy. Phytomedicine. 37, 10–13. https://doi.org/10.1016/j.phymed.2017.10.015

Newman, D.J., Cragg, G.M., 2020. Natural Products as Sources of New Drugs over the Nearly Four Decades from 01/1981 to 09/2019. Journal of Natural Products. 83, 770–803. https://doi.org/10.1021/acs.jnatprod.9b01285

Rappaport, N., Twik, M., Plaschkes, I., Nudel, R., Stein, I., Levitt, T., Gershoni, J., Morrey, M., Safran, C.P., Lancet, M., D., 2017. MalaCards: an amalgamated human disease compendium with diverse clinical and genetic annotation and structured search. Nucleic Acids Research. 45, 877–887. https://doi.org/10.1093/nar/gkw1012

Rehman, S.U., Ali, T., Alam, S.I., Ullah, R., Lee, Z.A., Rutten, K.W., Bpf, Mo, K., 2019. Ferulic Acid Rescues LPS-Induced Neurotoxicity via Modulation of the TLR4 Receptor in the Mouse Hippocampus. Molecular Neurobiology. 56, 2774–2790. https://doi.org/10.1007/s12035-018-1280-9

Rehring, J.F., Bui, T.M., Galan-Enriquez, C.S., Urbanczyk, J.M., Ren, X., Wiesolek, H.L., Sullivan, D.P., Sumagin, R., 2021. Released Myeloperoxidase Attenuates Neutrophil Migration and Accumulation in Inflamed Tissue. Frontiers in Immunology. 12, 654259. https://doi.org/10.3389/fimmu.2021.654259

Rudrapal, M., Gogoi, N., Chetia, D., Khan, J., Banwas, S., Alshehri, B., 2022. Repurposing of phytomedicine-derived bioactive compounds with promising anti-SARS-CoV-2 potential: Molecular docking, MD simulation and drug-likeness/ADMET studies. Saudi Journal of Biological Sciences. 29, 2432–2446. https://doi.org/10.1016/j.sjbs.2021.12.018

Saikia, S., Bordoloi, M., 2019. Molecular Docking: Challenges, Advances and its Use in Drug Discovery Perspective. Current Drug Targets. 20, 501–521. https://doi.org/10.2174/1389450119666181022153016

Scialo, F., Daniele, A., Amato, F., Pastore, L., Matera, M.G., Cazzola, M., Castaldo, G., Bianco, A., 2020. ACE2: The Major Cell Entry Receptor for SARS-CoV-2. Lung. 198, 867–877. https://doi.org/10.1007/s00408-020-00408-4

Sonar, V.P., Corona, A., Distinto, S., Maccioni, E., Meleddu, R., Fois, B., Floris, C., Malpure, N.V., Alcaro, S., Tramontano, E., Cottiglia, F., 2017. Natural product-inspired esters and amides of ferulic and caffeic acid as dual inhibitors of HIV-1 reverse transcriptase. European

Journal of Medicinal Chemistry. 130, 248–260. https://doi.org/10.1016/j.ejmech.2017.02.054

Szklarczyk, D., Gable, A.L., Lyon, D., Junge, A., Wyder, S., Huerta-Cepas, J., Simonovic, M., Doncheva, N.T., Morris, J.H., Bork, P., 2019. STRING v11: protein-protein association networks with increased coverage, supporting functional discovery in genome-wide experimental datasets. Nucleic Acids Research. 47, 607–613. https://doi.org/10.1093/nar/gky1131

Tatematsu, M., Yoshida, R., Morioka, Y., Ishii, N., Funami, K., Watanabe, A., Saeki, K., Seya, T., Matsumoto, M., 2016. Raftlin Controls Lipopolysaccharide-Induced TLR4 Internalization and TICAM-1 Signaling in a Cell Type-Specific Manner. Journal of Immunology. 196, 3865–3876. https://doi.org/10.4049/jimmunol.1501734

Taylor, J.W., Taylor, K.S., 2023. Combining probabilistic forecasts of COVID-19 mortality in the United States. European Journal of Operational Research. 304, 25–41. https://doi.org/10.1016/j.ejor.2021.06.044

Trott, O., Olson, A.J., 2010. AutoDock Vina: improving the speed and accuracy of docking with a new scoring function, efficient optimization, and multithreading. Journal of Computational Chemistry. 31, 455–461. https://doi.org/10.1002/jcc.21334

Veeresham, C., 2012. Natural products derived from plants as a source of drugs. Journal of Advanced Pharmaceutical Technology & Research. 3, 200–201. https://doi.org/10.4103/2231-4040.104709

Wang, L., Xiong, F., Zhao, S., Yang, Y., Zhou, G., 2022. Network pharmacology combined with molecular docking to explore the potential mechanisms for the antioxidant activity of *Rheum tanguticum* seeds. BMC Complementary Medicine and Therapies. 22, 121. https://doi.org/10.1186/s12906-022-03611-3

Wang, X., Shen, Y., Wang, S., Li, S., Zhang, W., Liu, X., Lai, L., Li, P.J., H., 2017. PharmMapper 2017 update: a web server for potential drug target identification with a comprehensive target pharmacophore database. Nucleic Acids Research. 45, 356–360. https://doi.org/10.1093/nar/gkx374

Wu, X., Lin, L., Wu, H., 2021. Ferulic acid alleviates lipopolysaccharide-induced acute lung injury through inhibiting TLR4/NF-κB signaling pathway. Journal of Biochemical and Molecular Toxicology. 35, 22664. https://doi.org/10.1002/jbt.22664

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, 941–951. https://doi.org/10.1016/S1875-5364(20)60038-3

Xu, G., Li, Y., Zhang, S., Peng, H., Wang, Y., Li, D., He, J.T., Tong, Z., Qi, Y., C., 2021. SARS-CoV-2 promotes RIPK1 activation to facilitate viral propagation. Cell Research. 31, 1230–1243. https://doi.org/10.1038/s41422-021-00578-7

Yang, G., Tan, Z., Zhou, L., Yang, M., Peng, L., Liu, J., Cai, J., Yang, R., Han, J., Huang, Y., He, S., 2020. Effects of Angiotensin II Receptor Blockers and ACE (Angiotensin-Converting Enzyme) Inhibitors on Virus Infection, Inflammatory Status, and Clinical Outcomes in Patients With COVID-19 and Hypertension: A Single-Center Retrospective Study. Hypertension. 120, 51–58.

Yang, G.W., Jiang, J.S., Lu, W.Q., 2015. Ferulic Acid Exerts Anti-Angiogenic and Anti-Tumor Activity by Targeting Fibroblast Growth Factor Receptor 1-Mediated Angiogenesis. International Journal of Molecular Sciences. 16, 24011–24031. https://doi.org/10.3390/ijms161024011

Yuan, J., Ge, K., Mu, J., Rong, J., Zhang, L., Wang, B., Wan, J., Xia, G., 2016. Ferulic acid attenuated acetaminophen-induced hepatotoxicity though down-regulating the cytochrome P 2E1 and inhibiting toll-like receptor 4 signaling-mediated inflammation in mice. American Journal of Translational Research. 8, 4205–4214.

Zhou, Y., Zhou, B., Pache, L., Chang, M., Khodabakhshi, A.H., Tanaseichuk, O., Benner, C., Chanda, S.K., 2019. Metascape provides a biologist-oriented resource for the analysis of systems-level datasets. Nature Communications. 10, 1523. https://doi.org/10.1038/s41467-019-09234-6