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Molecular mechanism of Herba Eupatorii in treating COVID-19 based on network pharmacology and molecular docking

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Competing interests
The authors declare no conflicts of interest.

Abbreviations
BP, biological processes; COVID-19, coronavirus disease 2019; TCMSP, Traditional Chinese Medicine Systems Pharmacology Database and Analysis Platform; TCMIP; ntegrative Pharmacology-based Research Platform of Traditional Chinese Medicine; PPI, protein-protein interaction; GO, Gene Ontology; IL-17, interleukin-17; PI3K-Akt, phosphatidylinositol 3 kinase-protein kinase B;TNF, tumor necrosis factor; AKT1, RAC-alpha serine/threonine-protein kinase; TP53, cellular tumor antigen p53; JUN, transcription factor AP-1; ACTB, actin beta; EGFR, epidermal growth factor receptor; KEGG, Kyoto Encyclopedia of Genes; SARS-CoV-2; severe acute respiratory syndrome coronavirus 2; TCM, traditional Chinese medicine; BP, biological processes; CC; cellular component; MF; molecular function; mTORC, mammalian target of rapamycin complex; NL63, human coronavirus NL63; TP53, cellular tumor antigen p53; EGFR, epidermal growth factor receptor; IL-1β, interleukin-1 beta; iNOS, inducible nitric oxide synthase; COX-2, cyclooxygenase-2.

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Abstract
Objective: To explore the therapeutic target and molecular mechanism of Herba Eupatorii in the intervention of COVID-19 (coronavirus disease 2019) by network pharmacology. **Methods:** TCMSP (Traditional Chinese Medicine Systems Pharmacology Database and Analysis Platform) and TCMIP V2.0 (Integrative Pharmacology-based Research Platform of Traditional Chinese Medicine) databases were used to search the active ingredients and corresponding drug targets of Herba Eupatorii. Related targets of COVID-19 were searched in Genecards, pharmGKB, CTD, Drugbank and TTD databases. After the intersection targets were selected using VENNY 2.1 online platform, the PPI (protein-protein interaction) network was downloaded into STRING database, and the data were analyzed and sorted out using Cytoscape software to obtain the potential key targets for the treatment of COVID-19 by Herba Eupatorii. At the same time, using the data of active ingredients and intersection targets, a network of "TCM - active ingredients - key targets" was constructed in Cytoscape software to screen out chemical molecules with potential therapeutic effects. GO (Gene Ontology) functional enrichment analysis and KEGG (Kyoto Encyclopedia of Genes) pathway enrichment analysis of key target proteins were performed by R software. AutoDock Vina program was used for molecular docking of the top 5 active ingredients and key targets to calculate the minimum binding energy. **Results:** There were 26 active ingredients, 160 targets, and 1969 pathogenic genes of COVID-19, among which 59 genes were intersection targets of drugs and diseases. After PPI network screening, the key target proteins were AKT1 (RAC-alpha serine/threonine-protein kinase), JUN (transcription factor AP-1), TP53 (cellular tumor antigen p53), ACTB (actin beta) and EGFR (epidermal growth factor receptor). Through the network of "TCM - Active Ingredients-Key Targets", Luteolin, Eupatolin, Stigmasterol, Eupatoriopicrin and Dammaradienyl acetate were identified as the active ingredients with potential therapeutic effects in the treatment of COVID-19. After R software was used for GO enrichment analysis, 1978 GO items were obtained ($P < 0.05$), including 1870 BP items, 26 CC items and 82 MF items. 149 pathways were obtained by KEGG enrichment analysis ($P < 0.05$). It mainly involves IL-17 (interleukin-17) signaling pathway, TNF (tumor necrosis factor) signaling pathway, C-type lectin receptor signaling pathway, PI3K-Akt (phosphatidylinositol 3 kinase-protein kinase B) signaling pathway, and T Cell receptor signaling pathway, etc. The molecular docking results showed that the active ingredients had good binding activity with key targets. **Conclusion:** Through the potential chemical constituents of Luteolin, Eupatolin, Stigmasterol, Eupatoriopicrin and Dammaradienyl acetate, Herba Eupatorii may act on AKT1, JUN, TP53, ACTB, EGFR and other targets. Involvement in IL-17 signaling pathway, TNF signaling pathway, C-Type Lectin receptor signaling pathway, PI3K-Akt signaling pathway, T Cell receptor signaling pathway and other pathways play an anti-inflammatory and antiviral roles in intervening in the occurrence and development of COVID-19.

Keywords: Herba Eupatorii; COVID-19; network pharmacology; inflammatory response; antiviral

Background

COVID-19 (coronavirus disease 2019) refers to acute pneumonia caused by human infection with SARS-CoV-2 (severe acute respiratory syndrome coronavirus 2). It is a highly contagious and epidemically prone respiratory disease [1]. The main transmission routes of SARS-CoV-2 are respiratory foam transmission and contact transmission, with a long incubation period [2]. The onset of the disease is usually accompanied by fever, cough, dyspnea and other symptoms [3]. According to data, this type of disease is usually associated with rapid viral replication, massive inflammatory cell infiltration and increased pro-inflammatory cytokine/chemokine response [4]. Antiviral drugs such as ribavirin and lopinavir or antimalarial drugs such as chloroquine and amodiaquine and other drugs with the potential to treat coronavirus infection are mainly used in clinical treatment. The "new use of old drugs" and the combination strategy are often used. For example, the broad-spectrum antiviral activity of chloroquine can be used in clinical practice as "new use of old drugs". The study has also confirmed that the combination of ribavirin and lopinavir can also play a significant therapeutic role [5].

TCM (traditional Chinese medicine) has a long history and has accumulated rich clinical experience. It is pointed out in the Novel Coronavirus Diagnosis and Treatment Protocol for Pneumonia (Trial Edition 3) that this disease belongs to the category of epidemic disease of TCM, which is the feeling of epidemic disease and the disease is located in the lung and the basic pathogenesis is characterized by "dampness, heat, poison and blood stasis," and traditional medicine including Chinese medicine has a good therapeutic effect on it [6]. Herba Eupatorii was first recorded in *Shennong Bencao Jing*. It has a flat nature and a spicy taste, and goes to the spleen, stomach and lung channels. It has the functions of relieving heat, aromatizing and dampening [7]. Modern pharmacological studies have shown that Herba Eupatorii has immunomodulatory, anti-inflammatory and antibacterial, anti-tumor and other pharmacological effects [8]. Herba Eupatorii is also used as a common drug in many basic prescriptions of COVID-19 because of the diseases and impurities, and is one of the basic combination drugs to drive away evil and remove impurities [9]. Network pharmacology can reflect the complex interaction between TCM compounds and targets, and observe TCM from the overall level, which is consistent with the characteristics of TCM treatment with multiple components, multiple targets and multiple pathways, providing a new idea for the study of the complex mechanism of TCM [10]. Due to the lack of specific drugs for COVID-19 at present, this study aims to obtain the action targets and pathways of Herba Eupatorii in the treatment of COVID-19 through the research method of network pharmacology, and to elaborate its potential mechanism of action, so as to provide a reference for further research of this drug in the future.

Methods

Collection of chemical constituents and related targets of Herba Eupatorii

In TCMIP V2.0 (http://www.tcmip.cn/TCMIP/index.php) and TCMSP platforms (http://tcmspw.com/), "Herba Eupatorii" was used as the key word, and similarity score > 0.8, OB ≥ 30% and DL ≥ 0.18 were used as the screening criteria for query results, respectively. The qualified targets and their corresponding chemical components were retrieved. The target was corrected according to Uniprot database (https://www.uniprot.org/), and the gene source was selected as "Homo sapiens", which was unified into the target format of Uniprot ID. The target set of TCM was constructed after the above targets were combined and weight removed.

Collection of COVID-19 related disease targets

With COVID-19 as the key word, In Genecards database (https://www.genecards.org/), pharmGKB database (https://www.pharmgkb.org/), Drugbank database (https://www.drugbank.ca/), CTD database (http://ctdbase.org//), providing database (http://db.idrblab.net/ttd/) to retrieve the related disease genes of COVID-19. With Relevance Score > 1 as the screening condition, the results of 5 databases were combined and duplicated items were deleted to construct the COVID-19 disease target set. Using R software, a Venn diagram of COVID-19 targets was drawn.

Intersection of disease and drug targets

VENNY 2.1 use online platform (https://bioinfogp.cnb.csic.es/tools/venny/) mapped Herba Eupatorii COVID - 19 targets the intersection of Wayne figure, and extract the intersection target data.

Construct protein interaction network to screen key targets

Import the above intersection targets into STRING database (https://string-db.org/), select the target type as Homo sapiens, and use interaction score > 0.700 as the filtering criterion to hide free nodes. Construct the protein interaction network of Herba Eupatorii for COVID-19 treatment and export node data. Open the data obtained in Cytoscape software, calculate the topology parameters of the network node through the plug-in CytoNCA, and conduct two consecutive screenings with the median of Degree (DC), Betweenness (BC) and Closeness (CC) as card value. The top five targets were selected as the key targets for Herba Eupatorii's treatment of COVID-19.

Build a network of "TCM - active ingredients - key targets"

The intersection targets and their corresponding active components were imported into Cytoscape software to construct a network of "TCM - active component - key target".

Key target protein GO enrichment analysis and KEGG pathway analysis

GO (Gene Ontology) functional enrichment analysis and KEGG (Kyoto Encyclopedia of Genes) pathway enrichment analysis were performed using R software, $P < 0.05$ being the screening condition, the top 10 biological processes (BP), cellular component (CC), molecular function (MF) and the top 20 KEGG pathways ranked by *P*-value were selected to draw the histogram. The KEGG pathway information was imported into Cytoscape software to construct a "KEGG pathway - key target" network.

Molecular docking verification

2D structures of active ingredients were retrieved and downloaded from PubChem database (http://zinc.docking.org/). Chem3D software was used to calculate the minimum free energy of active ingredients and saved in MOL2 format. In the PDB Protein Structure Database (http://www.rcsb.org/), the 3D structures of key targets in the protein interaction network were retrieved, and water molecules and small molecule ligands were deleted using PyMol software and saved in PDB format. The above macromolecule proteins and small molecule ligands were imported into AutoDockTools software for hydrotreating and format conversion, and the output was PDBQT format. AutoDock Vina was used for molecular docking, and the minimum binding energy between each active molecule and the key target was calculated.

Results

The active ingredients and related targets of peregrine

A total of 60 chemical components were retrieved from TCMSP database. Eleven active components were obtained by screening with OB ≥ 30% and DL ≥ 0.18, and 176 targets were obtained after combined weight removal. In the TCMIP V2.0 database, a total of 29 chemical components were searched, and 15 active components and 86 targets were obtained under the condition of similarity > 0.8. After Uniprot conversion and removal of repeated targets, a total of 160 drug targets were obtained. See Table 1 for the active ingredients of Herba Eupatorii.

Table 1 Active ingredients of Herba Eupatorii

Number	ID	Active ingredients	OB	DL	Counts
1	MOL000006	luteolin	36.16	0.25	55
2	NA	Eupatolin	NA	NA	44
3	MOL000449	Stigmasterol	43.83	0.76	30
4	NA	Thymolhydroquinone	NA	NA	20
5	NA	2-Isopropyl-5-Methylanisole	NA	NA	18
6	MOL000595	Eupatoriopicrin	76.78	0.36	10
7	NA	3Î’-Acetoxy-Dammara-20,24-Diene	NA	NA	7
8	NA	8,9-Dehydrothymol 3-O-Tiglate	NA	NA	7
9	MOL000604	Eupaformosanin	50.2	0.52	6
10	NA	9-Acetoxy-8,10-Dehydrothymol 3-O-Tiglate	NA	NA	6
11	NA	9-Acetoxythymo L3-O-Tiglate	NA	NA	6
12	NA	O-Coumaric Acid	NA	NA	6
13	MOL000584	7-acetoxy-8-hydroxy-9-isobutyryloxythymol	33.39	0.18	5
14	MOL000588	9-acetoxy-8,10-epoxy-6-hydroxythymol 3-O-angelate	61.44	0.21	5
15	MOL000605	taraxasteryl palmitate	33.84	0.31	5
16	NA	Taraxasteryl Palmitate	NA	NA	5
17	NA	Eupatoriopicrin	NA	NA	5
18	NA	Mesotrihydroxypiperidine	NA	NA	4
19	NA	3Î’,4Î’,5Î‘-Trihydroxypiperidine	NA	NA	4
20	MOL000359	sitosterol	36.91	0.75	3
21	NA	2-(1'-Hydroxy-2'-Oxopropyl)-5-Methylphenol	NA	NA	3
22	NA	1-Octacosanol,N-Octacosanol	NA	NA	2
23	NA	8-Methoxy-9-O-Isobutyrylthymol	NA	NA	1
24	MOL000363	amyrin Palmitate	32.68	0.3	0
25	MOL000592	Dammaradienyl acetate	46.52	0.82	0
26	MOL000596	[(3S,4aR,6aR,6aR,6bR,8aR,12S,12aR,14aR,14bR)-4,4,6a,6b,8a,12,14b-heptamethyl-11-methylene-1,2,3,4a,5,6,6a,7,8,9,10,12,12a,13,14,14a-hexadecahydropicen-3-yl] acetate	43.08	0.74	0

NA: This ingredient is listed in the TCMIP database

COVID-19 disease genes

By combining the filtering results of Genecards database, pharmGKB database, Drugbank database, CTD database and TTD database, 1969 genes related to COPD were obtained by deleting duplicates, as shown in Figure 1.

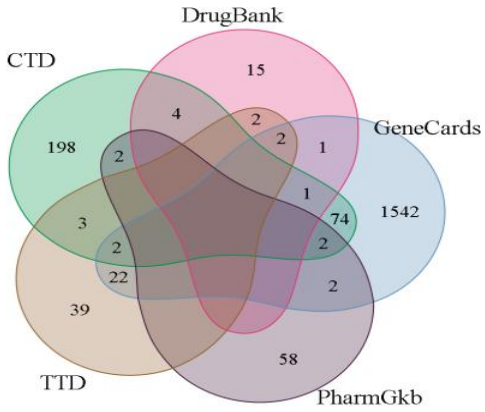

Figure 1 Venn diagram of genes associated with COVID-19

Get intersection targets

The 160 drug targets of Herba Eupatorii and 1969 disease-related genes of COVID-19 were imported into online software VENNY 2.1 to draw Venn diagrams (Figure 2), and 59 intersection targets were extracted.

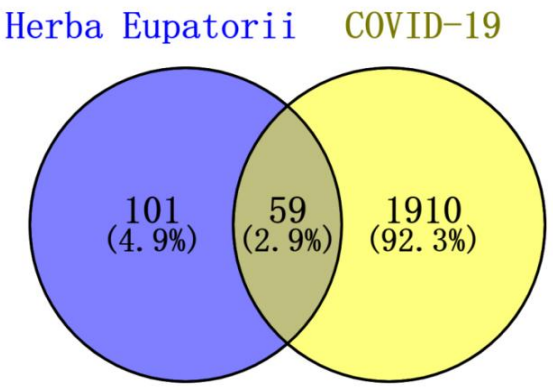

Figure 2 Venn diagram of intersection target between Herba Eupatorii and COVID-19

Build PPI network and acquire key targets

The 59 intersection targets were imported into STRING database, and scored with interaction score > 0.700 as the standard. After the dissociated nodes were hidden, the PPI network for the treatment of COVID-19 by Herba Eupatorii was constructed. There are 55 nodes (4 free nodes deleted) and 385 edges in the network. Nodes represent intersection targets, and edges represent aninteraction between targets, as shown in Figure 3. The data obtained from STRING were imported into Cytoscape software, and the median values of DC, BC and CC of the plug-in CytoNCA were 13, 10.635 and 0.534, respectively, to conduct the first screening and create a sub-network based on this criterion. The median values of DC, BC and CC of nodes in the subnetwork were calculated to be 15, 5.750 and 0.727, respectively. Based on this, the second screening was conducted to obtain the key targets of Herba Eupatorii for the treatment of COVID-19, as shown in Table 2. The screening process is shown in Figure 4. The top five key targets in DC ranking were selected as the core targets, which were AKT1, JUN, TP53, ACTB and EGFR.

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Table 2 Key targets of Herba Eupatorii for COVID-19

Key targets	DC	BC	CC
AKT1	22	27.390	0.923
JUN	21	20.504	0.888
TP53	20	22.067	0.857
ACTB	20	18.260	0.857
EGFR	19	17.450	0.827
IL6	19	13.021	0.827
CASP3	18	13.326	0.800
TNF	18	12.997	0.800
HSP90AA1	16	10.893	0.750

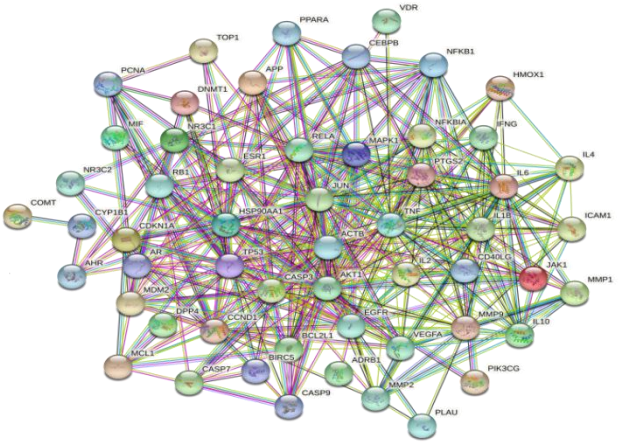

Figure 3 PPI network of Herba Eupatorii in treatment of COVID-19

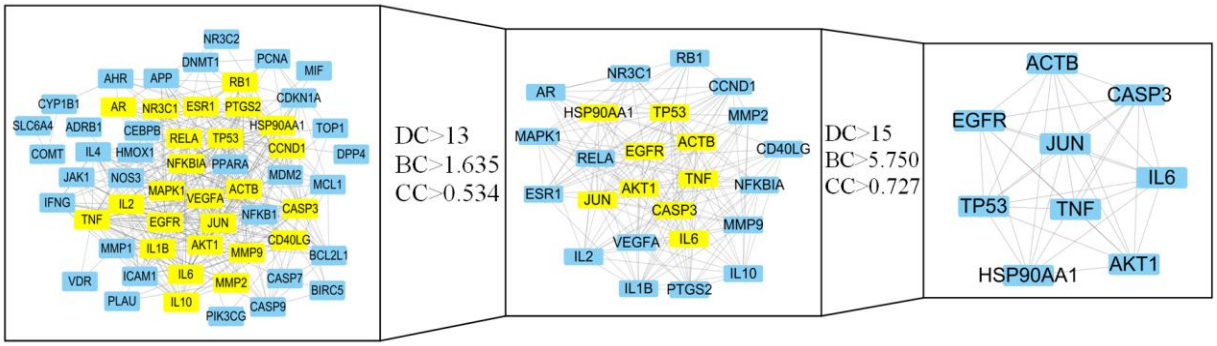

Figure 4 PPI screening process

"TCM - Active Ingredient - Target" network

Cytoscape software was used to construct a network of "TCM - active component - target" for the treatment of COVID-19 by Herba Eupatorii, as shown in Figure 5, with 73 nodes and 77 edges. Calculate the degree value of nodes in the network and export the data to excel format. In this study, the top five chemical constituents were selected according to degree value as the potential active ingredients for the treatment of COVID-19 by Herba Eupatorii, namely Luteolin, Eupatolin, Stigmasterol, Eupatoriopicrin, and Dammaradienyl acetate.

GO and KEGG enrichment analysis

In R software, $P < 0.05$ was used as the screening standard for GO enrichment analysis of key targets. There were 1,978 GO items, as shown in Figure 6. The abscissa represents the number of genes, the ordinate represents the GO items, and the color represents the enrichment significance. There are 1,870 of them in BP. It mainly involves regulation of steroid response, response to a steroid hormone, response to radiation, and cellular response to chemical biological processes such as stress and negative regulation of apoptotic signaling pathway; there are 26 CC, mainly involving raft, microdomain, transcription regulator complex, Vesicle Lumen, Caveola and so on.

There are 82 MFS. It mainly involves cytokine receptor binding, RNA polymerase II-specific DNA-binding transcription factor binding and nuclear receptor activity, ligand-activated transcription factor activity, ubiquitin-like protein ligase binding and other processes. There are 149 KEGG items, as shown in Figure 7. The abscissa represents the number of genes, the ordinate represents KEGG pathway, the color represents enrichment significance, and the redder the color represents the more significant gene enrichment. Among them, IL-17 signaling pathway, TNF signaling pathway, C-Type Lectin receptor signaling pathway and PI3K-Akt signaling are mainly involved pathway, T cell receptor signaling pathway, etc.

According to the above KEGG data, a "KEGG pathway-gene" network was constructed, as shown in Figure 8, in which there were 67 nodes (47 target genes and 20 KEGG pathways) and 303 edges. This network indicates that the 20 KEGG pathways obtained by enrichment are regulated by these 47 target genes. The data were exported to excel and sorted according to DC values. The top five genes were RELA, NFKB1, MAPK1, AKT1 and NFKBIA, and the number of regulated pathways were 19, 19, 17, 16 and 15, respectively.

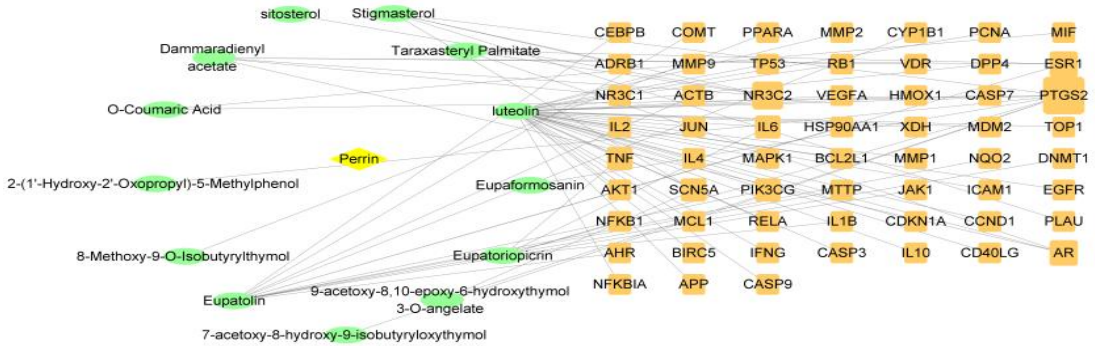

Figure 5 "TCM - Active Ingredient - Target" network

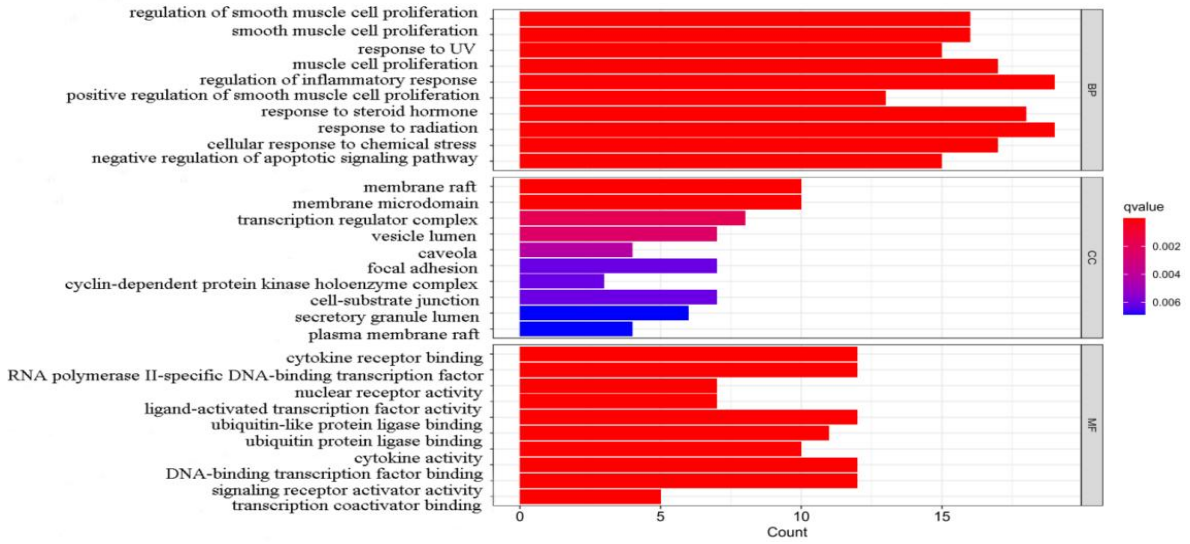

Figure 6 Column diagram of GO enrichment analysis

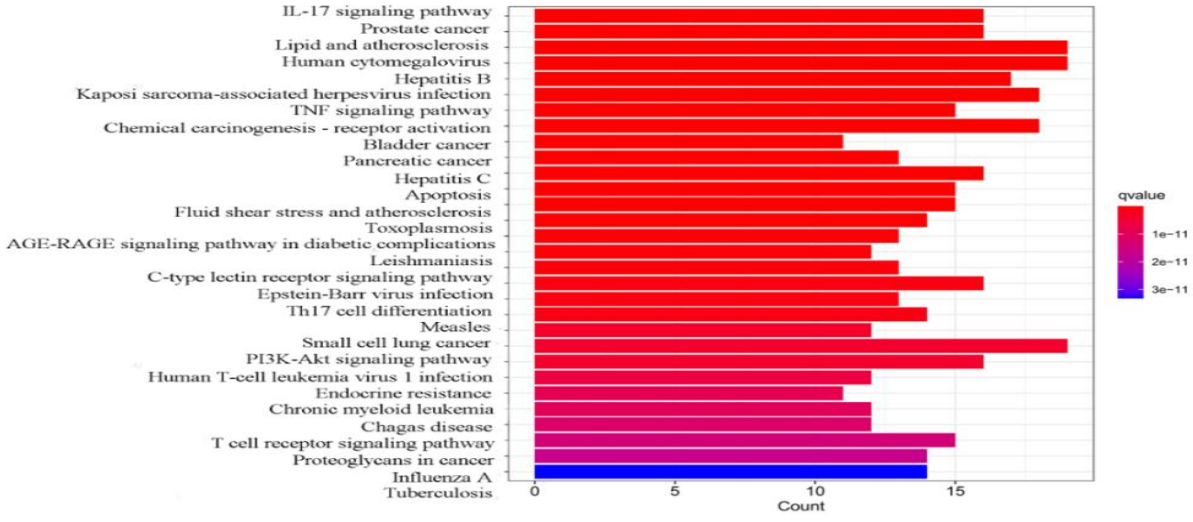

Figure 7 Column diagram of KEGG pathway enrichment analysis

Molecular docking verification

AutoDock Vina program was used to dock the top 5 chemical components in the network of "TCM - active ingredients - target" with the core target, and calculate the minimum binding energy. The lower the binding energy, the stronger the binding force between the chemical composition and the target. It is generally believed that binding energy less than -5.0 kJ/mol represents good binding activity, and less than -7.0 kJ/mol represents strong binding activity [11]. In this study, the minimum binding energy between the active ingredients and the core target molecule was ≤ -5.0 kJ/mol, and the binding energy of 20 molecules was ≤ -7.0 kJ/mol. The specific results were shown in Table 3. The three results with the smallest molecular docking binding energy were visualized, as shown in Figure 9.

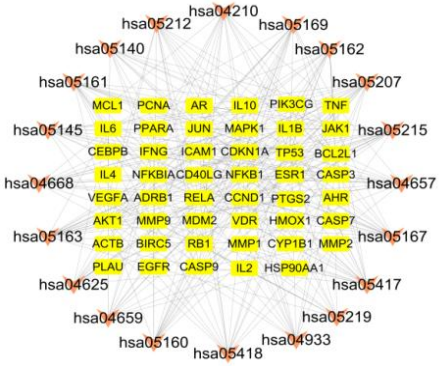

Figure 8 "KEGG pathway - gene" network diagram

Table 3 Molecular docking results

Compound	Binding energy with ACTB (kcal/mol)	Binding energy with AKT1 (kcal/mol)	Binding energy with EGFR (kcal/mol)	Binding energy with JUN (kcal/mol)	Binding energy with TP53 (kcal/mol)
Dammaradienyl acetate	-9.2	-8.6	-7.7	-5.5	-7.5
Eupatolin	-8.7	-10.1	-7.8	-5.8	-7.9
Eupatoriopicrin	-7.9	-8.6	-7.3	-5.2	-7..0
luteolin	-8.0	-10.1	-7.6	-5.6	-7.3
Stigmasterol	-8.7	-11.6	-8.1	-5.5	-7.6

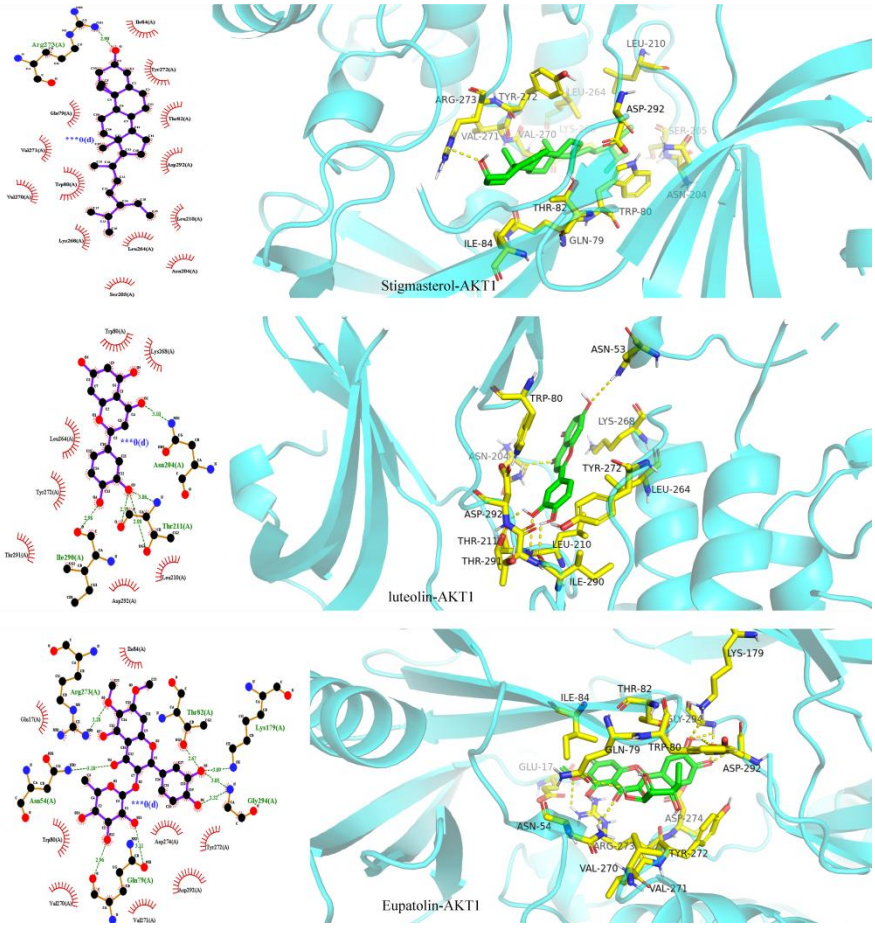

Figure 9 Diagram of molecular docking results

Discussion

After screening through the PPI network, the potential key targets for the treatment of COVID-19 by Herba Eupatorii were obtained, including AKT1, JUN, TP53, ACTB, EGFR, etc. AKT1 is a member of the AKT kinase family. The previous study has shown that overexpression of constitutively active AKT1 can promote viral protein synthesis, while dominant negative mutation of AKT1 can significantly inhibit viral RNA expression and further reduce viral capsid protein expression and viral release [12]. At the same time, the activation of AKT1 will aggravate inflammation and metabolism-related reactions, which can induce TSC1/TSC2 (tuberous sclerosis complex 1/ tuberous sclerosis complex 2) and mTORC (mammalian target of rapamycin complex) signal transduction after the stimulation of inflammatory factors, thereby regulating the growth of endothelial cells and restoring endothelial barrier function. It also plays an important role in the stimulation and migration of immune cells, and further deeply antagonizes the systemic or local inflammatory response of COVID-19 9 [12-14]. In addition, AKT1 is also associated with viral entry factors TMPRSS2 and DPP4 [15, 16], which is an ideal target with a broad spectrum of anti-inflammatory and antiviral properties. JUN is an integral part of AP1 transcription factor family, which can coordinate the transcription regulation of multiple genes in physiological conditions and is essential for cell differentiation, proliferation and apoptosis [17]. Because inflammatory cytokine markers are elevated in most patients with severe COVID-19, c-Jun can participate in a variety of cell biological processes, including inflammatory response, mediate cytokine induced inflammatory response, establish feedback loop, and thus enhance the inflammatory effect after viral infection [18]. Therefore, targeting c-Jun can be used to treat inflammatory diseases implicated in COVID-19. *TP53* gene encodes the p53 protein, which acts in response to DNA damage by triggering cell cycle arrest, apoptosis, and/or senescence. However, p53 is also a component of innate and adaptive antiviral immunity and can play a role in antiviral immunity. Study has found that the non-structural proteins encoded by coronaviruses can cause the destruction of endogenous p53, and the expression of p53 can inhibit the replication of infectious SARS-CoV-1 and human coronavirus NL63 (human coronavirus NL63), suggesting that p53 is an antagonist of coronaviruses [19]. In addition, TP53 (cellular tumor antigen p53) can inhibit inflammatory responses in a large number of human tissues [20]. ACTB, a highly conserved cytoskeletal structural protein, is considered to be a common housekeeping gene and has been used as an internal reference gene for related cancers [21]. Study has found that cancer patients are at higher risk of severe disease outcome and death due to SARS-CoV-2 infection. Because ACTB can play a key role in lung cancer, it is boldly predicted that ACTB may be one of the regulators of cancer and COVID-19 comorbidity, but it is still unclear [22]. EGFR (epidermal growth factor receptor) is a membrane glycoprotein with tyrosine kinase activity, and its physiological role is to regulate the development and homeostasis of epithelial tissues. In pathological state, EGFR signaling pathway remains active after SARS-CoV clearance and can lead to fibrosis. Therefore, blocking EGFR can be used as a method to reduce inflammation and prevent or restore fibrosis [23]. Experiment has proved that EGFR inhibitor has antiviral and anti-fibrosis effects [16].

R software was used to conduct KEGG pathway enrichment analysis for the key targets screened by PPI network. The results showed that IL-17 signaling pathway, TNF signaling pathway, C-type lectin receptor signaling pathway and PI3K-Akt signaling were mainly enriched pathway, T cell receptor signaling pathway, etc. Among them, the increase of pro-inflammatory cytokines such as IL-17 and TNF-α is the main reason for the deterioration of the disease of COVID-19 patients, and the activation of IL-17 signaling pathway leads to the emergence of a variety of cytokines (such as TNF-α, IL-1β) and chemokines, triggering cytokine storm [24]. TNF signaling pathway is widely involved in systemic inflammatory response. Upon binding to receptors, it can induce the activation of multiple genes and produce a large number of inflammatory cytokines, but it also plays an important role in the regulation of immune cells. Because IL-6 is associated with viral load, severity, severity, and prognosis in COVID-19 patients. It can inhibit viral replication through IL-6 and IL-1 [25]. PI3K-Akt signaling pathway can control various biological processes and different aspects of cell survival. Study has found that SARS-COV-2 overactivation of PI3K-Akt signaling pathway can regulate platelet activation and thrombosis. The blood is in a hypercoagulable state during COVID-19 infection [26]. T cells mediate adaptive immune response and secrete a variety of cytokines to regulate the body's inflammatory response [27]. C-type lectin receptors (CLRs) have diverse functions and play an important role in phagocytosis, endocytosis, pathogen recognition and complement activation [28].

After the network was constructed, the potential therapeutic molecules including Luteolin, Eupatolin, Stigmasterol, Eupatoriopicrin and Dammaradienyl acetate were screened according to the DC values of the nodes. Luteolin is a naturally occurring flavonoid, which has anti-cancer, anti-inflammatory, nerve protection and metabolic improvement effects [29]. Luteolin has extensive antiviral properties against long-term COVID-19, inhibiting entry of coronaviruses and serine proteases required for spike protein processing. In addition, Luteolin has neuroprotective effects in preventing neuroinflammation in patients with long-term COVID-19 [30]. Stigmasterol is a phytosterol with anti-inflammatory and analgesic effects [31]. Stigmasterol significantly inhibited the expression of pro-inflammatory mediators TNF-α, IL-6, IL-1β (interleukin-1 beta), iNOS (inducible Nitric Oxide Synthase) and COX-2 (cyclooxygenase-2), and increased the expression of anti-inflammatory cytokine IL-10. Stigmasterol was also predicted to have a potential inhibitory effect on COVID-19 based on silicone methods [32]. Eupatolin is a flavonol [33]. Eupatoriopicrin is a sesquiterpene lactone with biological activity. It inhibits the growth of HepG2 and McF-7 human cancer cell lines by inhibiting the production of NO, showing strong anti-inflammatory and cytotoxic activities [34], and has the potential to target inflammation as a lead substance [35]. However, there are few studies on Eupatolin and Dammaradienyl acetate.

Conclusion

In this study, the therapeutic targets and molecular mechanisms of Herba Eupatorii in the treatment of COVID-19 were explored through network pharmacology. The results showed that Luteolin, Eupatolin, Stigmasterol, Eupatoriopicrin and Dammaradienyl acetate were the main active ingredients with potential therapeutic effects. Therapeutic targets for COVID-19 may include AKT1, JUN, TP53, ACTB, EGFR and other targets. KEGG enrichment analysis showed that Il-17 signaling pathway, TNF signaling pathway, C-Type Lectin receptor signaling pathway and PI3K-Akt were mainly enriched signaling pathway, T cell receptor signaling pathway and other signaling pathways jointly play anti-inflammatory and antiviral roles in regulating cell metabolism, apoptosis, proliferation and other biological functions. To sum up, Herba Eupatorii plays a role in the treatment of COVID-19 and its related symptoms from many aspects and perspectives, providing a new perspective for clinical treatment and the development of specific drugs. However, due to the prediction results have not been proved by experiments, and limited to the delay of network pharmacology database update, there are still shortcomings in this study, which need to be further supplemented by subsequent studies.

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