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Original Article

Mechanism of Gynostemmae Pentaphylli Herba in the treatment of ischemic stroke based on network pharmacology and molecular docking

Yonglei Liu[1*], Jishuai Zhao[2*], Jinjiao Song[2], Hongdie Mao[2], Guojing Tan[2], Yaling Zhang[2], Changyun Liu[1]

[1]Department of Neurology, Fujian Medical University Union Hospital, Fuzhou 350001, Fujian, China; [2]Department of Neurology, The First Affiliated Hospital of Dali University, Dali 671000, Yunnan, China. [*]Equal contributors.

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Abstract: Objectives: To analyze the mechanism of Gynostemmae Pentaphylli Herba in the treatment of ischemic stroke based on network pharmacology and molecular docking. Methods: We used various databases and software, including Cytoscape, Traditional Chinese Medicine Systems Pharmacology Database and Analysis Platform, Pubchem, Swiss Target Prediction, GenCards, String, and WebGestalt to identify the active components and targets of Gynostemmae Pentaphylli Herba, as well as the targets associated with ischemic stroke. The mechanism of Gynostemmae Pentaphylli Herba in treating ischemic stroke was analyzed from the perspective of protein-protein interaction (PPI) co-expression, Gene Ontology, and Kyoto Encyclopedia of Genes and Genomes (KEGG) analysis, and AutoDock was used for molecular docking. Results: A total of 12 active components were identified, and 276 potential targets of Gynostemmae Pentaphylli Herba were obtained. There were 3151 disease targets associated with ischemic stroke. The top 5 active components of Gynostemmae Pentaphylli Herba were Ruvoside_qt, quercetin, 3'-methyleriodictyol, Spinasterol, and Cholesterin (CLR) according to the node degree value. There were 186 common targets between the disease targets of cerebral ischemic stroke and drug targets of Gynostemmae Pentaphylli Herba, with 21 key targets obtained by PPI network analysis. KEGG analysis revealed enrichment in 45 signaling pathways. Biological process increased 139 biological processes. Molecular function enriched 17 cell functions. Cellular component enriched 20 cell components. Molecular docking found that the binding energy of other protein molecules to ligand small molecules was less than -5 kal·mol^{-1}, except that the binding energy of AKT1 to 3'-methyleriodictyol was greater than -5 kal·mol^{-1}. Conclusions: Gynostemmae Pentaphylli Herba may play a role in treating ischemic stroke by affecting various pathways through its active ingredients such as Ruvoside_qt, quercetin, 3'-methyleriodictyol, Spinasterol and CLR.

Keywords: Network pharmacology, molecular docking, Gynostemmae Pentaphylli Herba, ischemic stroke, mechanism

Introduction

Ischemic stroke is primarily caused by cerebral ischemia and hypoxia resulting from stenosis and blockage of the arterial lumen in the neck and brain [1]. It has a high incidence, accounting for 87% of all cerebrovascular diseases [2]. From the stroke prevention report, ischemic stroke has become the leading cause of disability and death among Chinese residents [3], thereby seriously threatening public health.

The leading cause of ischemic stroke injury is the sudden interruption of cerebral blood flow in specific brain areas, resulting in irreversible functional damage to the nerves [4]. Thrombolysis, anti-platelet aggregation, and anticoagulation are often used in treating ischemic stroke. Although related drugs can control the patients' physical symptoms and delay the progression of the disease, there are still significant deficiencies in improving neurological deficits and quality of life. Studies have shown that Gynostemmae Pentaphylli Herba can reduce the infarct volume of brain tissue in rats with middle cerebral artery occlusion and improve motor function during a stroke [5]. Another study [6] showed that Gynostemmae Pentaphylli Herba had significant sedative and hypnotic effects and was able to protect against isch-

emic brain damage. However, the protective effect of Gynostemmae Pentaphylli Herba on stroke has not been fully clarified. The studies on the mechanism of Gynostemmae Pentaphylli Herba in treating stroke are limited. Therefore, there is an urgent need to explore the therapeutic effect of Gynostemmae Pentaphylli Herba to provide better guidance for clinical treatment decision-making.

This study applied network pharmacology and molecular docking to explore the pharmacological mechanism of Gynostemmae Pentaphylli Herba in the treatment of ischemic stroke, through analyzing the effective active ingredients, action targets and critical pathways of Gynostemmae Pentaphylli Herba, hoping to provide guidance for drug development and clinical trials.

Data and methods

Identification of active components and drug targets

The active components of Gynostemmae Pentaphylli Herba were obtained by using the Traditional Chinese Medicine Systems Pharmacology Database and Analysis Platform (TCMSP) database (https://old.tcmsp-e.com/tcmsp.php) [7]. In the database, the screening conditions were set as oral bioavailability exceeding 30% and drug-likeness exceeding 0.18 [8]. The Structured Data Format (SDF) of each active ingredient was collected by PubChem database (https://pub-chem.ncbi.nlm.nih.gov/) [9]. Based on the active ingredient structural formula, the Swiss Target Prediction database (http://www.swisstargetprediction.ch/) [10] was applied, and the potential drug targets of Gynostemmae Pentaphylli Herba were identified.

Identification of ischemic stroke targets

In the GeneCards (https://www.genecards.org/) database was queried using the keyword 'cerebral ischemic stroke' to identify relevant disease targets of ischemic stroke.

Screening of shared and critical targets of Gynostemmae Pentaphylli Herba and ischemic stroke

The Venn diagram (https://bioinfogp.cnb.csic.es/tools/venny/) was used to obtain the common targets of Gynostemmae Pentaphylli Herba in the ischemic stroke treatment. The STRING (https://string-db.org/) database was used to analyze the potential targets. A "highest confidence" threshold of 0.700 was set, and protein-protein interaction (PPI)-related information (.tsv format file) was exported. The file was then imported into Cytoscape 3.9.1 software for visualization. Using the cytoHubba plug-in, the genes were ranked based on the maximal clique centrality (MCC) score and Degree, and the top 30 targets were identified as the key targets.

Pathway and functional analysis tools

We imported the key targets into the WebGestalt database (http://www.webgestalt.org/) [11]. Then we set over-representation, BH method, and false discovery rate (FDR) at less than 0.05 for Gene Ontology (GO) and Kyoto Encyclopedia of Genes and Genomes (KEGG) analysis. The results from KEGG analysis, biological process (BP), molecular function (MF), and cellular component (CC) were collected for weight analysis and histogram generation.

Ligand small molecules and receptor protein docking

AutoDock software was applied to dock the small ligand molecules with receptor proteins. The ligand used was the three-dimensional (3D) structure of the core active component (from the TCMSP database), and the receptor was the 3D structure of the core target (in the protein data bank database). The molecular docking mode was visualized using PyMOL software.

Results

Gynostemmae Pentaphylli Herba drug targets and ischemic stroke disease targets

A total of 24 active components of Gynostemmae Pentaphylli Herba were identified (**Table 1**). The SDF of 19 active ingredients were found and collected by the PubChem database. After that, the Swiss Target Prediction database was applied to predict potential drug targets, and the marks with a probability > 0.1 were considered for further analysis. The active ingredients with drug targets less than 0.1 were eliminated. Finally, the following

Table 1. 24 active components of Gynostemmae Pentaphylli Herba

MOL ID	Molecule name	OB (%)	DL
MOL000338	3'-methyleriodictyol	51.61	0.27
MOL000351	Rhamnazin	47.14	0.34
MOL000359	Sitosterol	36.91	0.75
MOL004350	Ruvoside_qt	36.12	0.76
MOL004355	Spinasterol	42.98	0.76
MOL005438	Campesterol	37.58	0.71
MOL005440	Isofucosterol	43.78	0.76
MOL007475	Ginsenoside f2	36.43	0.25
MOL000953	CLR	37.87	0.68
MOL000098	Quercetin	46.43	0.28
MOL009855	(24S)-ethylcholesta-5,22,25-trans-3beta-ol	46.91	0.76
MOL009867	4α,14α-dimethyl-5α-ergosta-7,9(11),24(28)-trien-3β-ol	46.29	0.76
MOL009877	Cucurbita-5,24-dienol	44.02	0.74
MOL009878	Cyclobuxine	84.48	0.70
MOL009888	Gypenoside XXXVI_qt	37.85	0.78
MOL009928	Gypenoside LXXIV	34.21	0.24
MOL009929	Gypenoside LXXIX	37.75	0.25
MOL009938	Gypenoside XII	36.43	0.25
MOL009943	Gypenoside XL	30.89	0.21
MOL009969	Gypenoside XXXV_qt	37.73	0.78
MOL009971	Gypenoside XXVII_qt	30.21	0.74
MOL009973	Gypenoside XXVIII_qt	32.08	0.74
MOL009976	Gypenoside XXXII	34.24	0.25
MOL009986	Gypentonoside A_qt	36.13	0.80

Note: OB: oral bioavailability, DL: drug-likeness, CLR: cholesterin.

12 active ingredients were screened: the (24S)-ethylcholesta-5,22,25-tra, the 3'-methyleriodictyol, the 4α,14α-dimethyl-5α-ergosta-7,9(11),24(28)-trien-3β-ol, the campesterol, the cholesterin (CLR), the cucurbita-5,24-dienol, the cyclobuxine, the isofucosterol, the quercetin, the ruvoside_qt, the sitosterol, and the spinasterol. After integrating the genes of the 12 active ingredients above, duplicate genes were removed. This resulted in a collection of 276 potential targets of Gynostemmae Pentaphylli Herba. Using the keyword 'cerebral ischemic stroke', GeneCards database was queried, and 3151 disease targets were obtained.

Node degree value of active components in Gynostemmae Pentaphylli Herba

The effective active ingredients, targets, and corresponding drug data network construction showed that the network had 12 nodes, including 6 drug nodes, 129 compound nodes, 137 intersection target nodes, and 616 interaction relationships (**Figure 1**; **Table 2**). On the basis of the node degree value, ruvoside_qt, quercetin, 3'-methyleriodictyol, spinasterol, and CLR were identified as the top 5 active ingredients.

Shared targets and PPI network construction

The intersection analysis of 3151 disease targets of cerebral ischemic stroke and 274 Gynostemmae Pentaphylli Herba drug targets was performed to obtain 186 Shared targets (**Figure 2**). PPI information was obtained by importing 186 common targets into the String database (**Figure 3A**). Its network has 171 nodes and 749 edges, and the average number of neighbors was 9.042. Cytoscape software was applied to visualize PPI information, and the top 30 key targets were identified according to MCC score and Degree (**Figure 3B**, **3C**). The score ranking of targets is shown in **Table 3**.

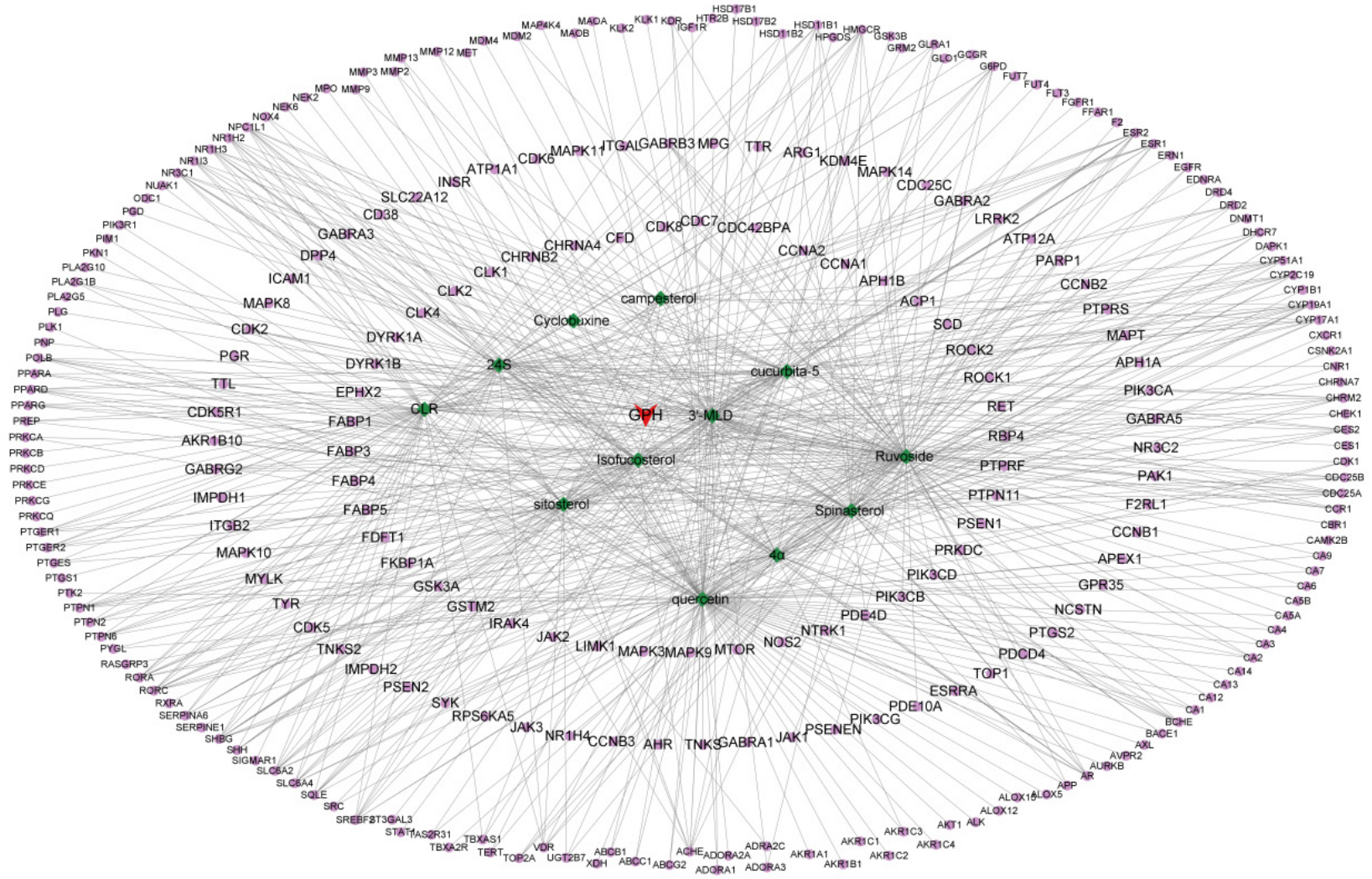

Figure 1. Drug-active ingredient-target network diagram. Note: GPH represents Gynostemmae Pentaphylli Herba; 24S represents (24S)-ethylcholesta-5,22,25-tra; 3'-MLD represents 3'-methyleriodictyol; 4α represents 4α,14α-dimethyl-5α-ergosta-7,9(11),24(28)-trien-3β-ol; cucurbita-5 represents cucurbita-5,24-dienol; Ruvoside represents Ruvoside_qt. The purple oval represents the active ingredient, and the yellow diamond represents the potential target of the drug.

Table 2. Top 5 active ingredients with node degree values

MOLID	Compound	Maximal clique centrality	Degree
MOL004350	Ruvoside_qt	100	109
MOL000098	Quercetin	104	105
MOL000338	3'-methyleriodictyol	67	67
MOL004355	Spinasterol	59	59
MOL000953	CLR	57	57

Note: CLR: cholesterin.

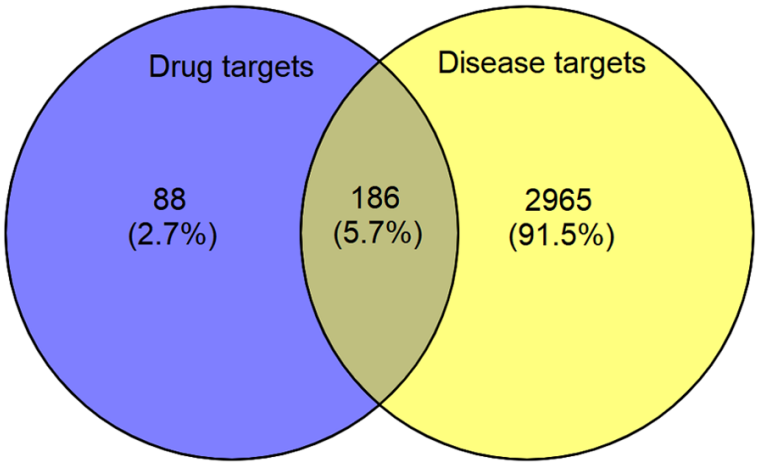

Figure 2. Wenn diagram. Note: There are 186 intersections between 3151 ischemic stroke disease targets and 274 Gynostemmae Pentaphylli Herba drug targets.

Finality, 21 key targets were obtained by integrating MCC and Degree screening results.

KEGG and GO analysis of key targets

The enrichment significance of FDR was set at less than 0.05, and KEGG enriched 45 signaling pathways, including EGFR tyrosine kinase inhibitor resistance, prolactin signaling pathway, VEGF signaling pathway, pancreatic cancer, endocrine resistance, melanoma, etc. BP enriched 139 biological processes, including regulation of phosphatidylinositol 3-kinase signaling, phosphatidylinositol 3-kinase signaling, phosphatidylinositol-mediated signaling, inositol lipid-mediated signaling, etc. MF enhanced 17 cell functions, including insulin receptor substrate binding, phosphatidylinositol-4,5-bisphosphate 3-kinase activity, phosphatidylinositol bisphosphate kinase activity, phosphatidylinositol 3-kinase activity, etc. CC enriched 20 cell components, mainly including phosphatidylinositol 3-kinase complex, class I, sorting endosome, phosphatidylinositol 3-kinase complex, etc. (**Figure 4**).

Molecular docking

The essential condition for a good binding ability of protein molecules to ligand small molecules is that the binding energy is lower than -5 kal·mol^{-1}. This study selected five critical active ingredients and five core targets with the most considerable Degree for molecular docking. After docking, we found that most of the binding energies were reliable, and only the critical point of AKT1 and 3'-methyleriodictyol were not in the above range (**Table 4**; **Figure 5**).

Discussion

Traditional Chinese medicine believes that ischemic stroke is caused by kidney deficiency, brain marrow dystrophy, emotional disorders, fatigue, and internal injuries resulting from disorders of qi and blood disorders as well as imbalance of yin and yang, which can lead to the formation of pathological products such as wind, fire, phlegm and blood stasis, thus inducing the obstruction of brain collaterals [12]. During the onset of ischemic stroke, the affected central nervous system can produce lesions that impair local nerve function. Gynostemmae Pentaphylli Herba could reduce the brain tissue infarction volume and protect against ischemic brain tissue damage. However, the precise mechanism of action remains to be discovered. Therefore, understanding the mechanism by which Gynostemmae Pentaphylli Herba works in ischemic stroke is of great significance.

We applied network pharmacology to collect the active components of Gynostemmae Pentaphylli Herba and their targets as well as the disease targets of ischemic stroke in the relevant database. There were 186 common targets between Gynostemmae Pentaphylli Herba and ischemic stroke, and the primary molecular targets of Gynostemmae Pentaphylli Herba for treating ischemic stroke were identi-

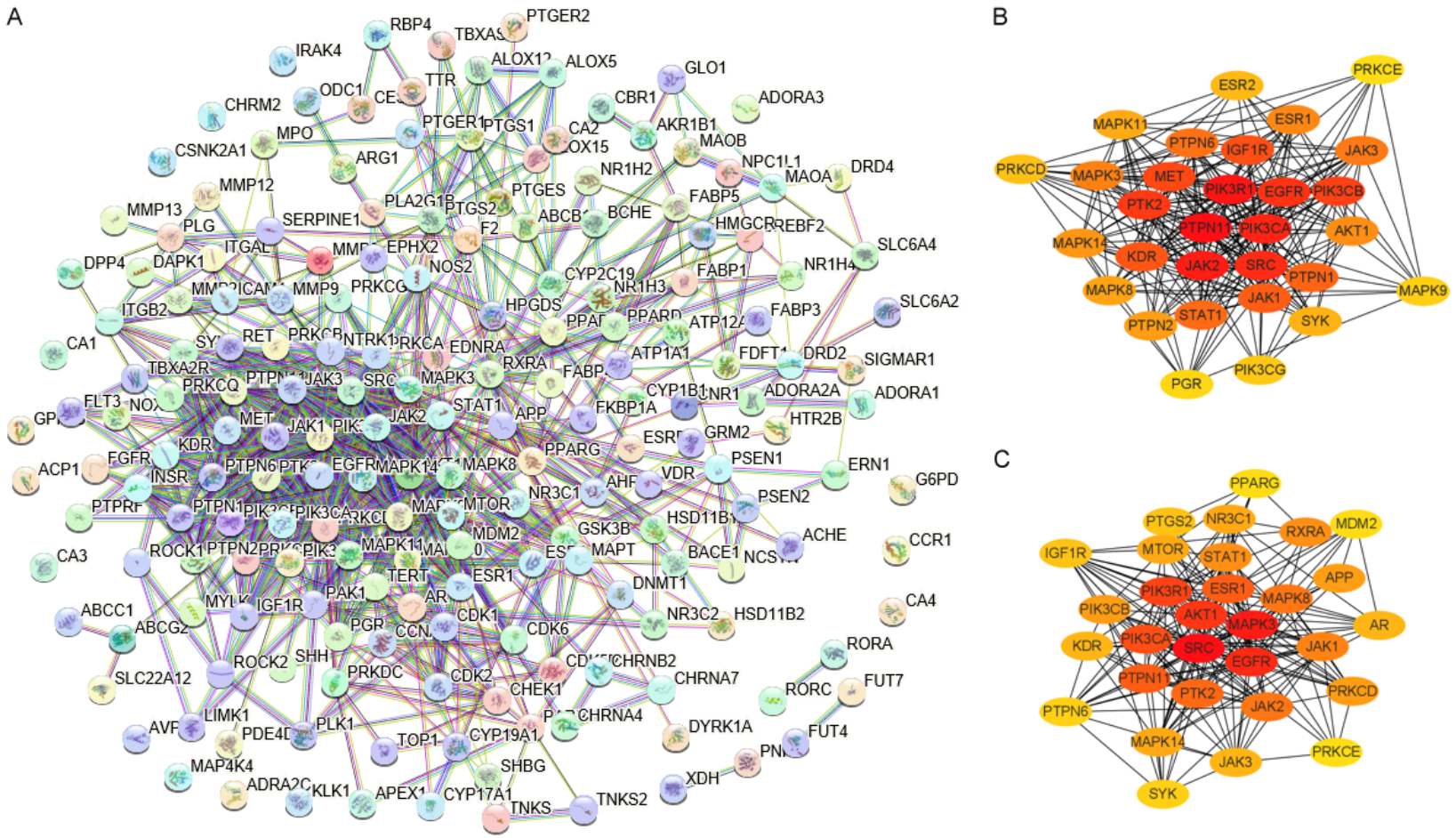

Figure 3. PPI network diagram. Note: (A) is all genes' protein-protein interaction (PPI) information, (B) is the PPI information of the top 30 genes screened according to the Kyoto Encyclopedia of Genes and Genomes (KEGG) score, and (C) is the top 30 gene information screened according to Degree. The more bright the color in (B and C), the higher the score.

Table 3. Topological analysis results of MCC and Degree algorithms (top 30)

Gene symbol	MCC	Gene symbol	Degree
PTPN11	196510	SRC	52
PIK3R1	183006	MAPK3	45
JAK2	181261	EGFR	44
PIK3CA	173838	AKT1	36
SRC	167235	PIK3R1	32
EGFR	149707	PIK3CA	29
PTK2	136120	PTPN11	28
PIK3CB	125808	ESR1	28
MET	102216	PTK2	27
IGF1R	58782	JAK2	26
KDR	52378	MAPK8	26
JAK1	42492	JAK1	23
PTPN1	31954	RXRA	22
STAT1	28052	STAT1	22
PTPN6	25668	PRKCD	20
PTPN11	196510	SRC	52
PIK3R1	183006	MAPK3	45
JAK2	181261	EGFR	44
PIK3CA	173838	AKT1	36
SRC	167235	PIK3R1	32
EGFR	149707	PIK3CA	29
PTK2	136120	PTPN11	28
PIK3CB	125808	ESR1	28
MET	102216	PTK2	27
IGF1R	58782	JAK2	26
KDR	52378	MAPK8	26
JAK1	42492	JAK1	23
PTPN1	31954	RXRA	22
STAT1	28052	STAT1	22
PTPN6	25668	PRKCD	20

Note: CLR: cholesterin, MCC: maximal clique centrality.

fied to be Ruvoside_qt, quercetin, 3'-methyleriodictyol, Spinasterol, and CLR. Studies [13] found that flavonoids, phytosterols, and saponins were the active ingredients of Gynostemmae Pentaphylli Herba in treating coronary heart disease, including quercetin and spinasterol. In addition, another study [14] explored the mechanism of Gynostemmae Pentaphylli Herba in treating atherosclerosis based on network pharmacology. It was revealed that quercetin had the most effective targets among the many effective active ingredients of Gynostemmae Pentaphylli Herba. This is consistent with the results of this study. Furthermore, PPI network analysis in this study proved that 21 targets, such as SRC, MAPK3, EGFR, AKT1, and PIK3R1, were vital targets, indicating that these targets had the most potent interaction. Kratimenos et al. [15] showed that hypothermia and SRC kinase could inhibit apoptotic cell death and regulate the hypoxic nerves. When compared to low temperature alone in the pig brain, the inhibition of SRC kinase further reduced the activation of CaM kinase IV during a short period of low temperature after hypoxia. Another study [16] showed that SRC kinases could inhibit multiple signaling pathways that trigger focal adhesions and nuclei under the membrane, regulate calcium signaling, and prevent cell death. It has been found in a systematic study [17] that SRC kinase is an effective neuroprotective target in acute hypoxic injury.

MAPK3, as a member of MAPK family, can regulate cellular activities, such as proliferation, differentiation, and growth [18]. As a signaling pathway, MAPK regulates cell activity and inflammation, which can transmit signals on the cell membrane to the nucleus in response to various stimuli. Existing evidence has confirmed that MAPK has a regulatory function on cerebrovascular diseases. In ischemic or hemorrhagic cerebrovascular diseases, MAPK can induce the production of numerous pro-inflammatory mediators that can compromise the integrity of the blood-brain barrier and aggravate neuroinflammation, causing neurovascular damage [19]. Studies [20] have shown that the activation of the EGFR/MAPK signaling pathway can stimulate the proliferation of neural stem cells and enhance the recovery of neurological function after transient cerebral ischemic injury. The ligand of EGFR, transforming growth factor-α (TGF-α), is involved in neurogenesis and angiogenesis in the injured brain. The expression of the TGFα-EGFR receptor in glial cells increases after ischemia, which can improve neurological recovery after stroke [21]. Research has indicated that induction of AKT and ERK pathways and their transcriptome profiles can prevent severe hypoxia-induced neuronal damage [22]. The activated AKT1-CREB pathway can rapidly increase the expression of RNF146 to prevent further neuronal death mediated by PARP1 [23]. Activation of the TrkA-Akt1/2-CREB-Jmjd3 pathway inhibits inflammation caused by changes in microglia phenotype and the release of inflammatory

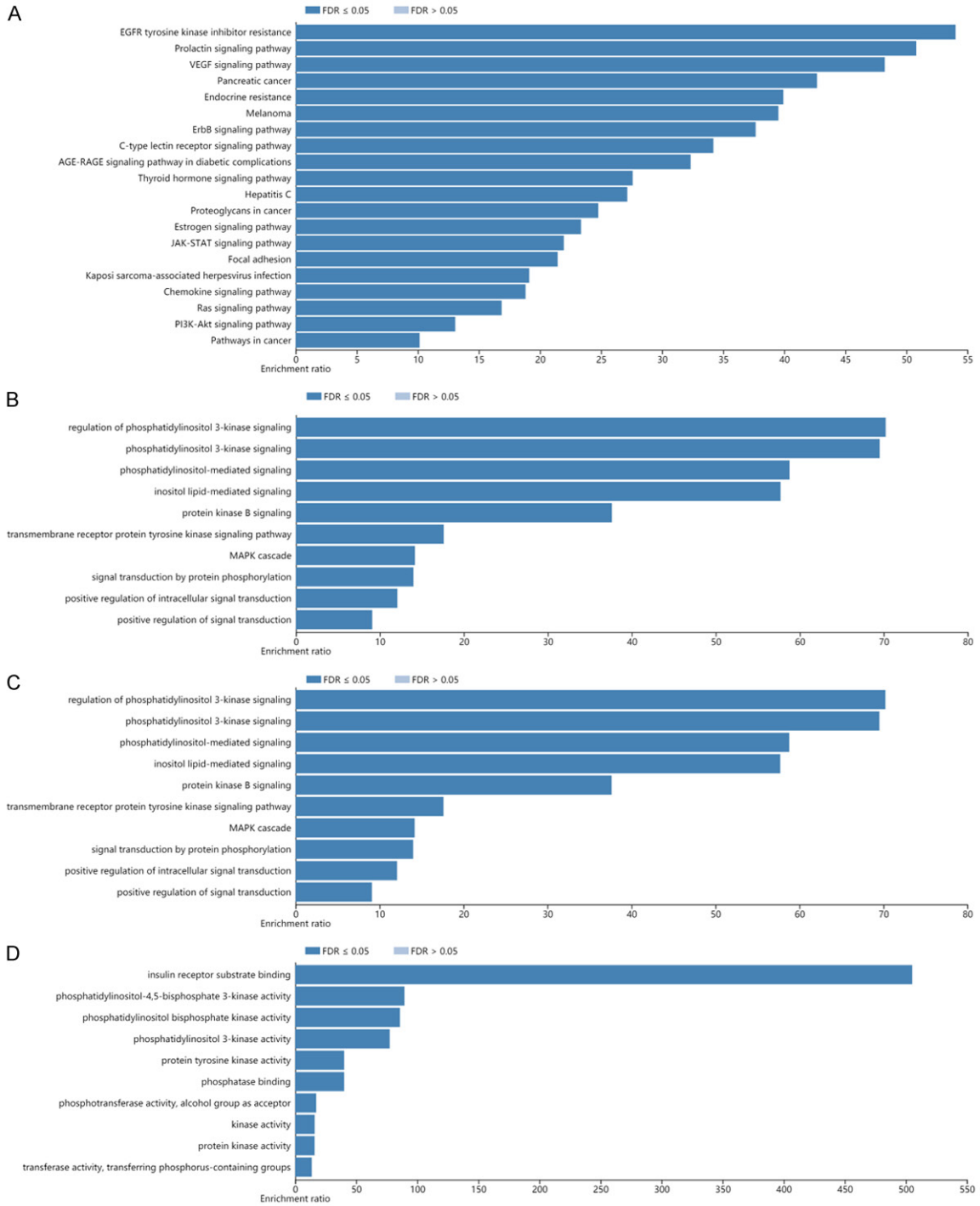

Figure 4. Functional enrichment analysis. Note: (A) is Kyoto Encyclopedia of Genes and Genomes (KEGG) analysis (top 20), (B) is the biological process (BP) enrichment analysis (top 10), (C) is molecular function (MF) enrichment analysis (top 10), and (D) is cellular component (CC) enrichment analysis (top 10).

mediators, thereby exerting a neuroprotective effect [24]. Zhao et al. [25] suggested that reducing the regulation of PIK3R1 by miR-221-p3 and activating the TGF-β pathway could improve ischemic stroke. Another study [26] believed that PIK3R1 was one of the key targets for treating anti-cerebral ischemia, and changes in the activation of signaling pathways,

Table 4. Molecular docking binding energy of key active components and core targets (kcal·mol^{-1})

MOLID	Compound	SRC	MAPK3	EGFR	AKT1	PIK3R1
MOL004350	Ruvoside_qt	-8.95	-7.53	-6.83	-8.14	-6.88
MOL000098	quercetin	-7.90	-7.87	-4.20	-6.87	-6.31
MOL000338	3'-methyleriodictyol	-8.17	-5.21	-5.98	-4.39	-6.42
MOL004355	Spinasterol	-8.07	-8.26	-7.11	-7.05	-7.62
MOL000953	CLR	-9.61	-7.90	-6.82	-7.69	-7.20

Note: CLR: cholesterin.

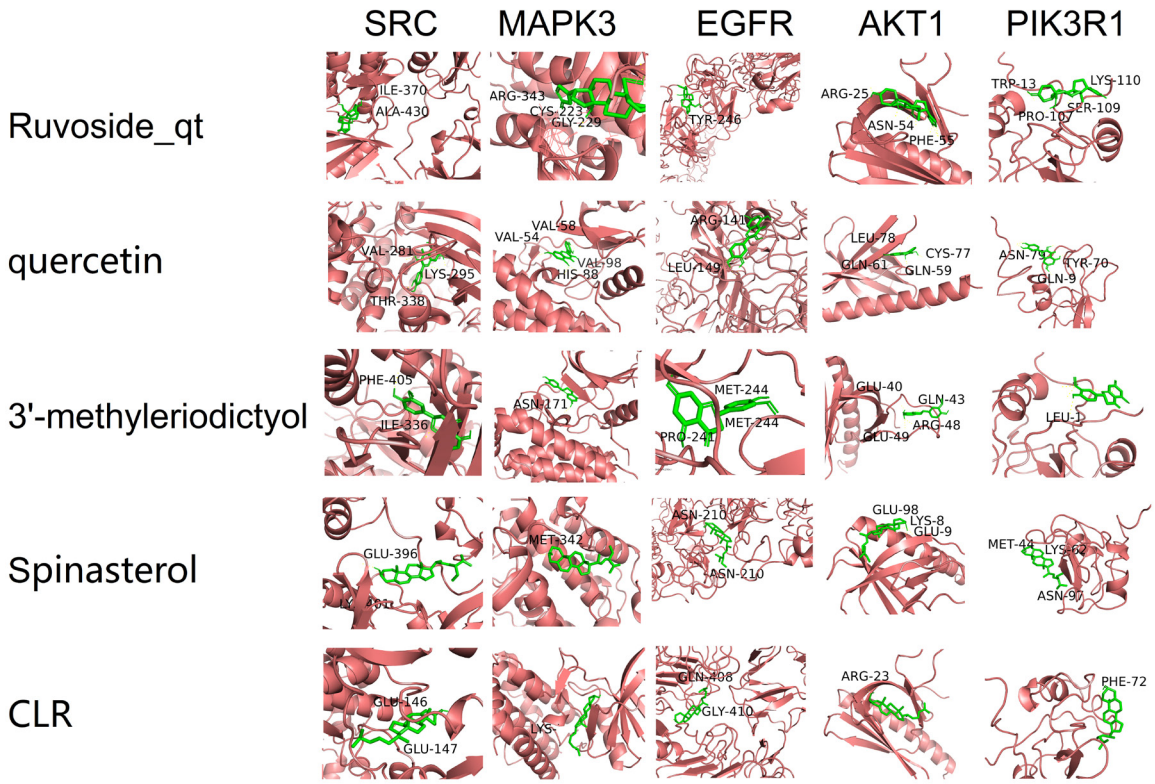

Figure 5. Molecular docking mode of key active components and core targets. Note: CLR: cholesterin.

including PIK3R1, may represent potential biological mechanisms for drugs targeting ischemic cerebrovascular disease. Our study demonstrated that Gynostemmae Pentaphylli Herba and its effective active ingredients may modulate the expression of ischemic stroke-related proteins (SRC, MAPK3, EGFR, AKT1, PIK3R1, etc.) through co-expression networks involving PPI, thus playing a neuroprotective role in the brain.

We further analyzed the pathways involved in these 21 targets. After GO and KEGG analysis, we found that the mechanism of action of Gynostemmae Pentaphylli Herba on ischemic stroke involves multiple targets, active components and pathways, with a focus on membrane-associated processes.

Gynostemmae Pentaphylli Herba regulates multiple biological processes, including phosphatidylinositol 3-kinase signaling, phosphatidylinositol-mediated signaling, and inositol lipid-mediated signaling. This mechanism is involved in various signaling pathways, such as EGFR tyrosine kinase inhibitor resistance, prolactin signaling pathway, VEGF signaling pathway, pancreatic cancer, endocrine resistance and melanoma to improve the symptoms of ischemic stroke. A study [27] found that the prolactin signaling pathway was an effective mechanism of musk in treating ischemic st-

roke. Additionally, a VEGF signaling pathway plays a critical role in angiogenesis. Mu et al. [28] demonstrated that CALM2 could enhance the polarization of macrophages, thereby mediating the proliferation and migration of vascular endothelial cells in tumor tissues through the regulation of VEGF and HFI-1 signaling pathways.

Overall, Gynostemmae Pentaphylli Herba may play a role in treating ischemic stroke through active components, such as Ruvoside_qt, quercetin, 3'-methyleriodictyol, Spinasterol, and CLR, targeting multiple pathways and affecting various biological processes. However, this study has the following limitations. The mechanism of Gynostemmae Pentaphylli Herba on ischemic stroke is predicted by data mining. The possible active components, targets, and pathways of Gynostemmae Pentaphylli Herba are only discussed theoretically, but the mechanism of active drug components on ischemic stroke cannot be entirely determined. Future research needs to involve basic experiments to verify the pharmacological agents. In addition, although the results of this study are supported by relevant literature, experimental verification has yet to be conducted. Relevant experimental studies on the mechanism of action of Gynostemmae Pentaphylli Herba in the treatment of ischemic stroke can be combined with the results of this study in the future to further elucidate the pharmacological effects of this herb. In addition, the data of this study were collected from various major databases or obtained using software. We only discussed the possible mechanism of action between the active ingredients of Gynostemmae Pentaphylli Herba and ischemic stroke. The intensity of movement between the two could not be accurately determined, nor could interference caused by factors such as the origin and quality of the drug be excluded. Upon reviewing classical literature, we found that most network pharmacology studies only applied the TCMSP database to search for active ingredients and molecular targets of traditional Chinese medicine. Some studies also combined the TCMSP and HERB databases. However, it is worth noting that new databases are continuously emerging over time.

Disclosure of conflict of interest

None.

Address correspondence to: Changyun Liu, Department of Neurology, Fujian Medical University Union Hospital, No. 29, Xinquan Road, Gulou District, Fuzhou 350001, Fujian, China. Tel: +86-0591-86218056; E-mail: changyunliu@fjmu.edu.cn

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