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DATABASE ANALYSIS

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A Network Pharmacological Approach to Investigate the Mechanism of Action of Active Ingredients of *Epimedii Herba* and Their Potential Targets in Treatment of Alzheimer's Disease

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Statistical Analysis C
Data Interpretation D
Manuscript Preparation E
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Background: *Epimedii Herba* is a traditional Chinese herbal medicine used to treat central nervous system diseases such as Alzheimer's disease in China. However, the pharmacological mechanism is unclear. To investigate the mechanisms of *Epimedii Herba* in the treatment of Alzheimer's disease, we assessed effective compounds, corresponding targets, and related pathways of *Epimedii Herba* in the treatment of Alzheimer's disease based on network pharmacology.

Material/Methods: The active components and targets of *Epimedii Herba* were obtained through the TCMSP database and the DrugBank database. The DisGeNET database and GeneCards database were used to search for Alzheimer's disease targets. The common targets of components and disease were obtained by Wayne diagram. Gene ontology (GO) analysis and enrichment analysis of the Kyoto Encyclopedia of Genes and Genomes (KEGG) were performed using the DAVID database. The component-target-pathway interaction network model was constructed using Cytoscape software. Auto Duck Vina software was used for molecular docking to analyze the affinity of the key ingredients and the main targets.

Results: We screened 17 active ingredients and 27 key targets of *Epimedii Herba* in the treatment of Alzheimer's disease, which were related to the HIF-1 signaling pathway, TNF signaling pathway, PI3K-Akt signaling pathway, NF-κB signaling pathway, VEGF signaling pathway, and sphingolipid signaling pathway.

Conclusions: Based on network pharmacology, the multi-component, multi-target, and multi-pathway characteristics of *Epimedii Herba* in the treatment of Alzheimer's disease were explored. Our results provide new ideas for future pharmacological and experimental research on *Epimedii Herba* in the treatment of Alzheimer's disease.

MeSH Keywords: Alzheimer Disease • Medicine, Chinese Traditional • Pharmacologic Actions

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Background

Epimedii Herba is a traditional Chinese herbal medicine derived from the genus *Epimedium* of the Berberidaceae family, and its main chemical component is flavonoids [1]. It has been reported in the literature that it has a wide range of activities, such as improving cerebral blood flow to treat central nervous system diseases, promoting blood circulation, reducing depression symptoms, improving memory ability, and preventing brain damage caused by inflammation of nerve cells. It has important clinical medicinal value [2–4].

Alzheimer's disease (AD) is a degenerative disease of the nervous system. Because progressive cognitive function declines and cognitive behavior changes often occur in clinical development [5], it imposes a huge burden on the family and society [6]. The cause of the disease is still unclear [7]. Age is the biggest risk factor for AD, and the likelihood of suffering from Alzheimer's disease increases with age [6]. The main pathological feature is the aggregation of β-amyloid protein (Aβ) and abnormal phosphorylation of tau protein [5]. Traditional Chinese medicine often describes its symptoms as "dementia" and "forgetfulness" [8]. In the theory of traditional Chinese medicine, the elderly often have physiological characteristics of kidney and spleen insufficiency, which are the pathological basis of AD [9,10]. The book "Shen Nong Bencao Jing" emphasizes that brain-nourishing medicine is mainly based on kidney-nourishing Chinese medicine [11]. Therefore, tonifying the kidneys and strengthening the spleen are common methods of traditional Chinese medicine used to treat AD. *Epimedii Herba* is classified as a kidney tonic in China. It is the most well-known plant with aphrodisiac effects used in Asian countries [12]. It is used to nourish the kidney and improve AD. *Epimedii Herba* can improve AD and has antidepressant and neuroprotective effects [13]. *Epimedii Herba* thus plays an important role in the treatment of Alzheimer's disease.

Network pharmacology is the integration of various informatics approaches, including bioinformatics, systems biology, and pharmacology. It can analyze the interaction from multiple perspectives of drug-component-target-disease. Network pharmacology can explore the mechanism of action of drugs on diseases systematically and holistically, which is consistent with the holistic and systematic approach of traditional Chinese medicine to disease treatment [14]. To explore the beneficial effect of *Epimedii Herba* on Alzheimer's disease, the present study used a network pharmacology approach to comprehensively study the active ingredients of *Epimedii Herba* and the potential mechanism of action in treatment of Alzheimer's disease. To provide a theoretical basis for the clinical application of *Epimedii Herba* in the treatment of Alzheimer's disease, we further explored the synergistic relationship between the components, targets, and pathways of *Epimedii Herba*.

Material and Methods

Component collection and screening of *Epimedii Herba*

In the traditional Chinese medicine system pharmacology database and analysis platform TCMSP (*http://tcmspw.com/*), we used "*Epimedii Herba*" as a keyword to search for chemical components. According to ADME (absorption, distribution, metabolism, and excretion) related to chemical properties, the oral route is the most important and convenient way to transport drugs through the systemic circulation, and oral bioavailability (OB) is usually used to objectively evaluate whether a drug molecule has good absorbability [15]. Drug-likeness (DL) is a qualitative concept used in drug design [16]. According to the drug screening standard recommended by TCMSP [17], the active ingredient of *Epimedii Herba* was obtained by using the drug screening standard 20 with OB ≥30% and DL ≥0.18 [18] (Table 1).

Collection of targets related to active ingredients

The TCMSP database was used to find corresponding relevant targets of the active ingredients of *Epimedii Herba*. Then, targets were selected from DrugBank (*https://www.drugbank.ca/*) [19]. Through the UniProt database (*https://www.uniprot.org/*) [20], we collected the gene names of the corresponding species.

Collection of AD-related disease targets

The target genes related to AD were collected from 2 databases: the DisGeNET database (*http://www.disenet.org/*) [21] and the GeneCards database (*https://www.genecards.org/*) [22]. We used "Alzheimer's disease" as a search term and set the species as Homo sapiens. We screened for disease targets based on the score size and deleted the repeated options in the search results to obtain the final AD target genes.

Network model construction

Network analysis provides a scientific explanation of the complex relationships among traditional Chinese medicines, compounds, genes, and diseases. In the present study, network construction was carried out using Cytoscape 3.7.2 software [23] with the following 3 approaches:

1) For the chemical components of *Epimedii Herba* and its related target genes, the active components and corresponding targets of *Epimedii Herba* were summarized and then introduced into the Cytoscape 3.7.2 software to construct the network.
2) For the common targets of *Epimedii Herba* component targets and AD-related targets, to analyze the interaction between the *Epimedii Herba*-related targets and the disease-related targets, we inputted the drug component targets and

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Table 1. Potential active components of *Epimedii Herba.*

	Molecule Name	OB (%)	DL
MOL000622	Magnograndiolide	63.71	0.19
MOL004367	olivil	62.23	0.41
MOL004388	6-hydroxy-11,12-dimethoxy-2,2-dimethyl-1,8-dioxo-2,3,4,8-tetrahydro-1H-isochromeno[3,4-h]isoquinolin-2-ium	60.64	0.66
MOL004382	Yinyanghuo A	56.96	0.77
MOL004396	1,2-bis(4-hydroxy-3-methoxyphenyl)propan-1,3-diol	52.31	0.22
MOL004386	Yinyanghuo E	51.63	0.55
MOL004391	8-(3-methylbut-2-enyl)-2-phenyl-chromone	48.54	0.25
MOL000098	quercetin	46.43	0.28
MOL004384	Yinyanghuo C	45.67	0.5
MOL004373	Anhydroicaritin	45.41	0.44
MOL001645	Linoleyl acetate	42.1	0.2
MOL000422	kaempferol	41.88	0.24
MOL004394	Anhydroicaritin-3-O-alpha-L-rhamnoside	41.58	0.61
MOL004425	Icariin	41.58	0.61
MOL004380	C-Homoerythrinan, 1,6-didehydro-3,15,16-trimethoxy-, (3.beta)	39.14	0.49
MOL003542	8-Isopentenyl-kaempferol	38.04	0.39
MOL001510	24-epicampesterol	37.58	0.71
MOL000359	sitosterol	36.91	0.75
MOL001771	poriferast-5-en-3beta-ol	36.91	0.75
MOL000006	luteolin	36.16	0.25
MOL003044	Chryseriol	35.85	0.27
MOL001792	DFV	32.76	0.18
MOL004427	Icariside A7	31.91	0.86

disease targets on the Bioinformatics website (*http://bioinformatics.psb.ugent.be/webtools/Venn/*) and drew a Venn diagram. We then obtained the intersection targets and used the STRING11.0 database (*http://string-db.org/*) to analyze the interaction between intersecting proteins (PPI) [24]. In STRING, the co-target genes were introduced, the organism was set as "homo sapiens", the confidence was set as "medium confidence", and the PPI results were derived as table text output (.tsv). Finally, the TSV file was imported into Cytoscape (version 3.7.2) for analysis. The function of "Network Analysis" was used to calculate the Degree and Betweenness of nodes in the Network. The larger the value is, the more critical the node is in the Network. We selected the target points whose parameters are greater than the average value to focus on analysis.

3) For the active components of *Epimedii Herba* AD-related genes and pathways, the pathway enrichment of the common target proteins was carried out using the KEGG database to obtain the relevant signal pathways. The effective components, target proteins, and pathway information of *Epimedii Herba* were imported into Cytoscape 3.7.2 to construct the "component-target-pathway" network model.

Enrichment analysis

Go enrichment analysis and KEGG path enrichment analysis of key target genes were carried out through the DAVID online database (*https://david.ncifcrf.gov/*) [25]. Then, we used OmicShare's online data analysis platform tool (*http://www.omicshare.com/tools*) to draw a bubble chart.

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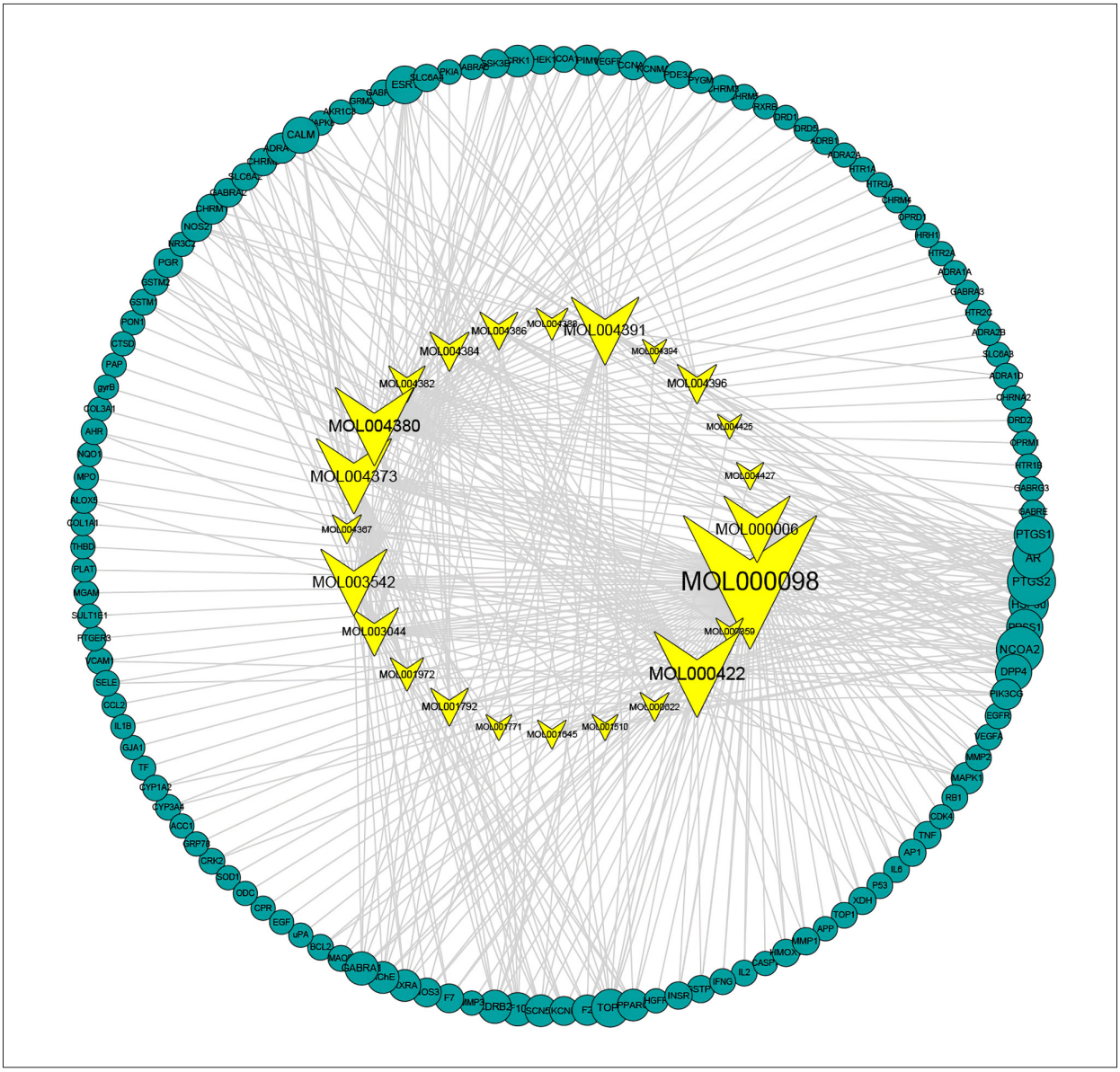

Figure 1. The active component-target network of *Epimedii Herba*. Yellow nodes represent active components, green nodes represent targets.

Molecular docking

We performed molecular docking of key components of *Epimedii Herba* in the network model with the targets and then obtained the protein crystal complexes of the main targets in the PDB database [26]. PyMOL software separated the original ligand from the target protein and removed the water molecule, phosphate, and other inactive related ligands of the target protein [27]. We imported the 2D structure of key small molecule compounds into ChemBio3D software and exported the format of mol2 to save its 3D structure (ChemBioOffice 2014). In Auto Dock vina software, we imported the 3D structures of small molecules and target proteins and exported the format of pdbqt. Then, we docked the molecules between target proteins to observe their affinity. Auto Dock vina is free software. Compared with the earlier product, Auto Dock, it has higher precision and faster calculation speed, and it can improve the speed and accuracy of docking [28].

Results

Screening of active ingredients of Epimedium

TCMSP, a database of systems pharmacology for drug discovery from herbal medicines, includes 499 Chinese herbal medicines

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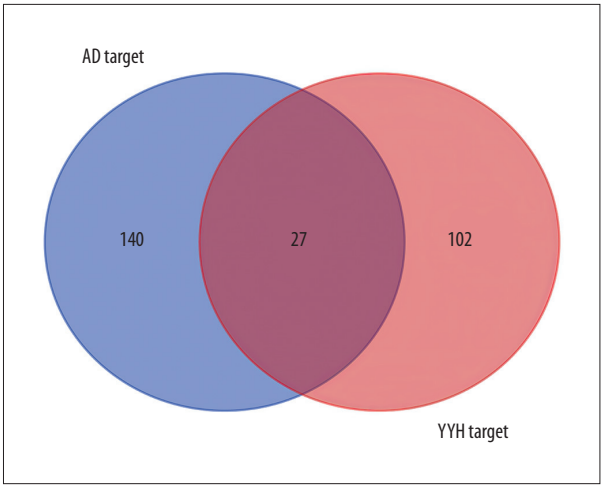

Figure 2. Common Gene Targets of *Epimedii Herba* and AD.

and 29 384 ingredients, and provides drug targets and diseases of each active compound, with unique ability to identify drug target networks and drug disease networks [19]. Searching for the chemical compositions of *Epimedii Herba* from the TCMSP database, we obtained 130 chemical ingredients. The screening conditions OB ≥30%, DL ≥0.18 were set and 23 active ingredients of *Epimedii Herba* were finally collected (Table 1).

Collection and screening of target information

Target proteins corresponding to 23 active components of *Epimedii Herba* were collected from the TCMSP database. We selected them from DrugBank and obtained 129 target proteins. The corresponding gene names were transformed through the UniProt database and the results were imported into Cytoscape 3.7.2 software to construct an active ingredient-target network of *Epimedii Herba* (Figure 1). In Figure 1, there are 153 nodes and 381 edges. The active ingredients of *Epimedii Herba* in the inner circle are represented by yellow arrow nodes and the gene targets in the outer circle are represented by green circular nodes. Compounds with larger degree values including: MOL000098 (quercetin) degree value=74, MOL000422 (kaempferol) degree value=42, MOL004380 (C-Homoerythrinan, 1,6-didehydro-3,15,16-trimethoxy-, (3.beta.)-) degree value=37, MOL004373 (Anhydroicaritin) degree value=35, MOL004391 (8-(3-methylbut-2-enyl)-2-phenyl-chromone) degree value=30, MOL000006 (luteolin) degree value=29, MOL003044 (Chryseriol)

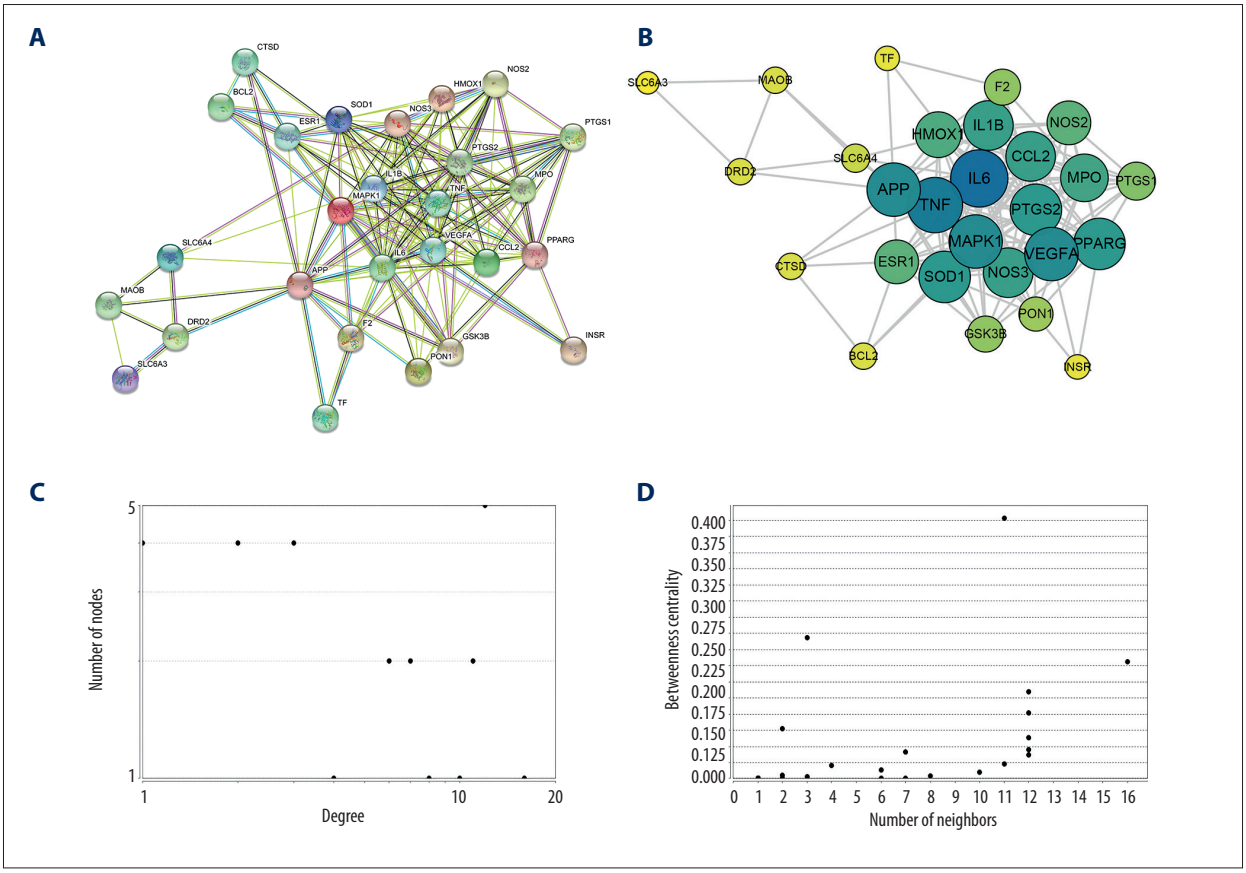

Figure 3. Analysis of key target genes of *Epimedii Herba* against AD. (**A**) PPI network diagram of Key Targets. (**B**) Interaction network of targets for *Epimedii Herba* against AD. (**C**) Distribution of degree. (**D**) Betweenness centrality. The size and color of the nodes are proportional to degree centrality by topology analysis.

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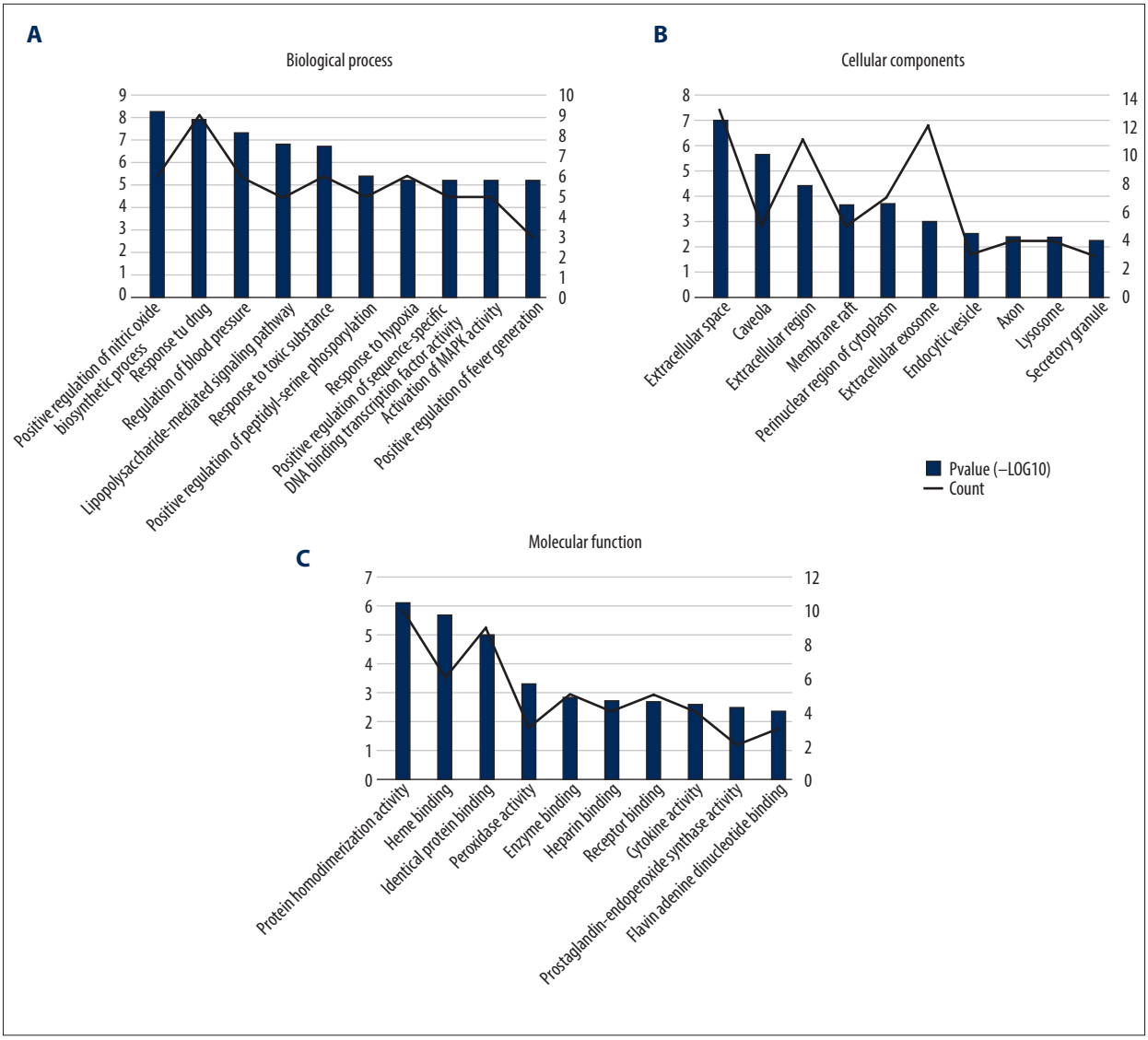

Figure 4. GO analysis for the key targets of *Epimedii Herba* against AD.

degree value=17, may be the main material basis of *Epimedii Herba* in the treatment of AD.

GeneCards is a comprehensive, authoritative compendium of annotative information on human genes, and DisGeNET is one of the largest available collections of genes and variants involved in human diseases. They can provide a variety of information such as genes and the relationship between genes and diseases. The GeneCards database was searched for genes related to Alzheimer's disease, and a total of 81 targets with scores ≥50 were screened. We retrieved 122 targets from the DisGeNET database and 167 disease targets were obtained after deleting the duplicate targets. A total of 129 drug targets were retrieved from the TCMSP database. We took the intersection of drug targets and disease targets. Then, we drew a Venn diagram to obtain 27 key target genes for *Epimedii Herba* in the treatment of Alzheimer's disease (Figure 2). The 27 targets were imported into the STRING 11.0 database to analyze protein-protein interactions (PPI) (Figure 3A). After that, the PPI results were exported as simple tabular text output (.tsv), and the TSV file was imported into Cytoscape (version 3.7.2) to draw the network diagram of target interaction. The size and color of the nodes are proportional to the degree. The larger the node and the darker the color, the more critical it is in the network. The results are shown in Figure 3B–3D. The average degree value of the PPI network is 6.30 and the average medium is 0.05. There are 5 targets whose degree value and medium were greater than the average. They are: APP (amyloid precursor protein, degree value=11; medium=0.4036), IL6 (interleukin 6, degree value=16; medium=0.181), VEGFA (vascular endothelial growth factor, degree value=12; medium=0.135), MAPK1 (mitogen-activated protein kinase 1, degree value=12;

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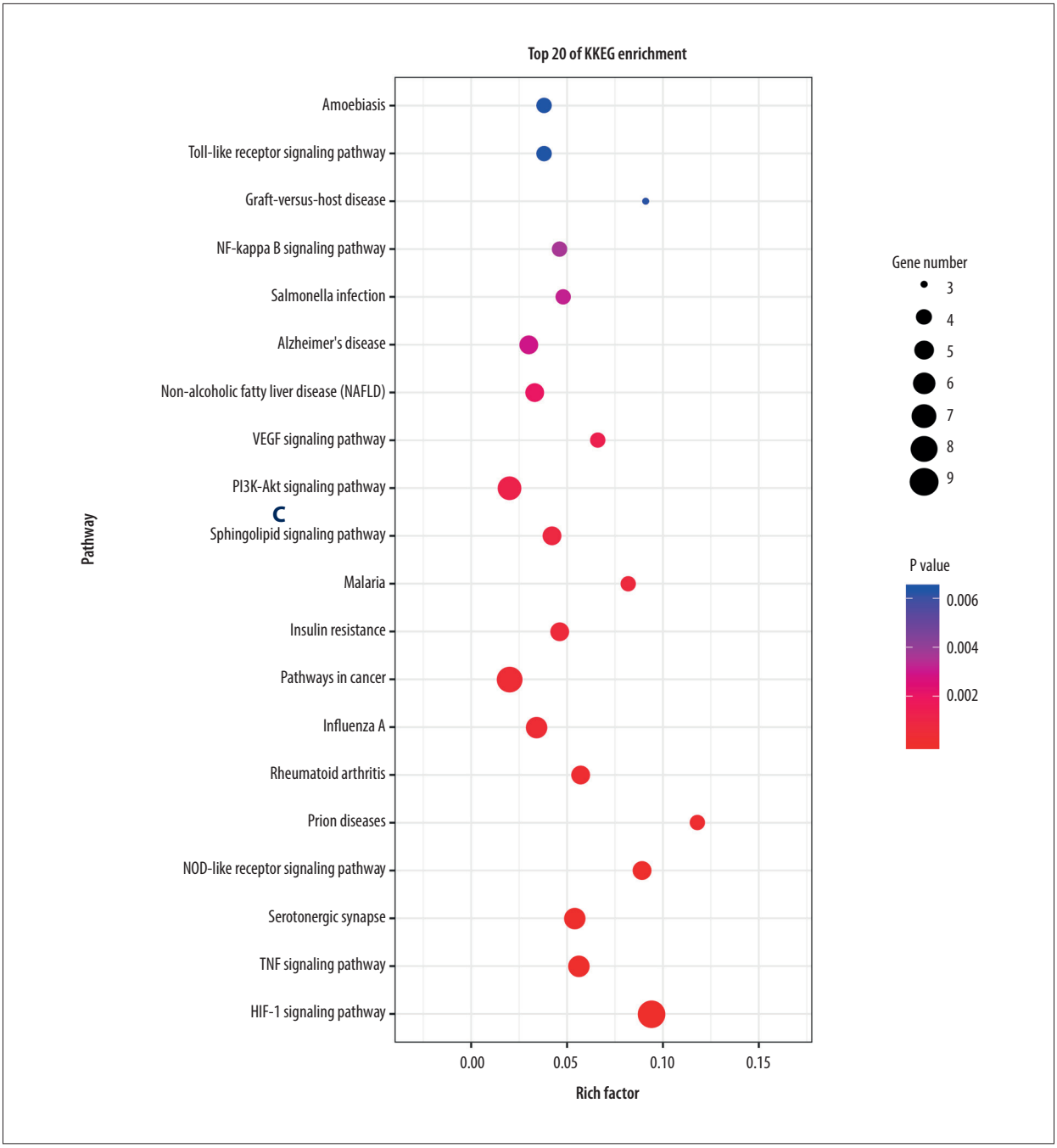

Figure 5. KEGG pathway enrichment of *Epimedii Herba* against AD. Top 20 pathways enriched based on target genes (the abscissa is the rich factor, the ordinate is pathway name, the size of the dot indicates the number of target genes, and the color represents the P-value).

medium=0.101), and TNF (tumor necrosis factor, degree value=12; medium=0.06).

Enrichment analysis of biological functions and pathways

To further explore the mechanism of *Epimedii Herba* on Alzheimer's disease, the 27 targets were imported into the DAVID database. The goals were: HMOX1 NOS2 TNF INSR SLC6A4 DRD2 MPO MAOB MAPK1 F2 PTGS2 IL1B SOD1 ESR1 PON1 PTGS1 BCL2 CTSD TF VEGFA PPARG GSK3B CCL2 NOS3 IL6 APP SLC6A3. Official gene symbols were selected in the Select identifier, gene List was selected in the List type item, homo sapiens was limited to species selection, homo sapiens was selected in the background, and the threshold value P<0.05 was

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Table 2. KEGG signal pathway information.

Term	Count	Percentage (%)	P value	Genes
HIF-1 signaling pathway	9	33.33	10^{-8}	MAPK1, TF, IL6, HMOX1, BCL2, VEGFA, NOS3, NOS2, INSR
TNF signaling pathway	6	22.22	3.44×10^{-5}	MAPK1, IL6, TNF, CCL2, PTGS2, IL1B
Serotonergic synapse	6	22.22	4.11×10^{-5}	MAPK1, APP, PTGS2, SLC6A4, PTGS1, MAOB
NOD-like receptor signaling pathway	5	18.52	4.39×10^{-5}	MAPK1, IL6, TNF, CCL2, IL1B
Prion diseases	4	14.81	2.36×10^{-4}	MAPK1, IL6, IL1B, SOD1
Rheumatoid arthritis	5	18.52	2.58×10^{-4}	IL6, TNF, CCL2, VEGFA, IL1B
Influenza A	6	22.22	3.44×10^{-4}	MAPK1, IL6, TNF, CCL2, GSK3B, IL1B
Pathways in cancer	8	29.63	3.69×10^{-4}	MAPK1, IL6, PTGS2, GSK3B, BCL2, VEGFA, PPARG, NOS2
Insulin resistance	5	18.52	5.64×10^{-4}	IL6, TNF, GSK3B, NOS3, INSR
Malaria	4	14.81	6.99×10^{-4}	IL6, TNF, CCL2, IL1B
Sphingolipid signaling pathway	5	18.52	8.39×10^{-4}	MAPK1, TNF, BCL2, CTSD, NOS3
PI3K-Akt signaling pathway	7	25.93	1.2×10^{-3}	MAPK1, IL6, GSK3B, BCL2, VEGFA, NOS3, INSR
VEGF signaling pathway	4	14.81	1.33×10^{-3}	MAPK1, PTGS2, VEGFA, NOS3
Non-alcoholic fatty liver disease (NAFLD)	5	18.52	1.97×10^{-3}	IL6, TNF, GSK3B, IL1B, INSR
Alzheimer's disease	5	18.52	2.91×10^{-3}	MAPK1, APP, TNF, GSK3B, IL1B
Salmonella infection	4	14.81	3.22×10^{-3}	MAPK1, IL6, IL1B, NOS2
NF-kappa B signaling pathway	4	14.81	3.68×10^{-3}	TNF, PTGS2, BCL2, IL1B
Graft-versus-host disease	3	11.11	6.25×10^{-3}	IL6, TNF, IL1B
Toll-like receptor signaling pathway	4	14.81	6.39×10^{-3}	MAPK1, IL6, TNF, IL1B
Amoebiasis	4	14.81	6.39×10^{-3}	IL6, TNF, IL1B, NOS2

set. GO enrichment (Figure 4) and biological pathway analysis of KEGG (Figure 5) were carried out on the targets of *Epimedii Herba* in the treatment of AD. Figure 4 shows that in the molecular function of GO enrichment analysis, *Epimedii Herba* has a greater influence on protein homodimerization activity, heme binding, identical protein binding, peroxidase activity, and cytokine activity. In the cellular components, *Epimedii Herba* has effects on extracellular space, caveola, axon, and lysosome. In the biological processes, *Epimedii Herba* has a great impact on drug response, positive regulation of peptidyl-serine phosphorylation, regulation of blood pressure, response to hypoxia and toxic substance, and LPS-mediated signaling pathways. Figure 5 shows that the top 20 pathways involved in the treatment of Alzheimer's disease by *Epimedii Herba* are HIF-1 signaling pathway, TNF signaling pathway, PI3K-Akt signaling pathway, NF-κB signaling pathway, VEGF signaling pathway, and sphingolipid signaling pathway. Specific parameters are shown in Table 2. The HIF-1 signaling pathway with the lowest FDR value and the lowest P-value was taken as an example to study the enrichment of the anti-AD targets of *Epimedii Herba*. The results are shown in Figure 6.

Component-target-pathway network model of *Epimedii Herba*

We used 27 target genes and their corresponding effective components and signaling pathways of *Epimedii Herba* to construct the "component-target-pathway" network model of *Epimedii Herba* (Figure 7). In Figure 7, there are a total of 59 nodes and 165 edges. Yellow, blue, and green represent the active components, targets, and regulatory pathways, respectively, of *Epimedii Herba*. Edges represent the interaction between these 3. The size of the node is proportional to the degree. The larger the node, the greater the effect in Alzheimer's disease. The results

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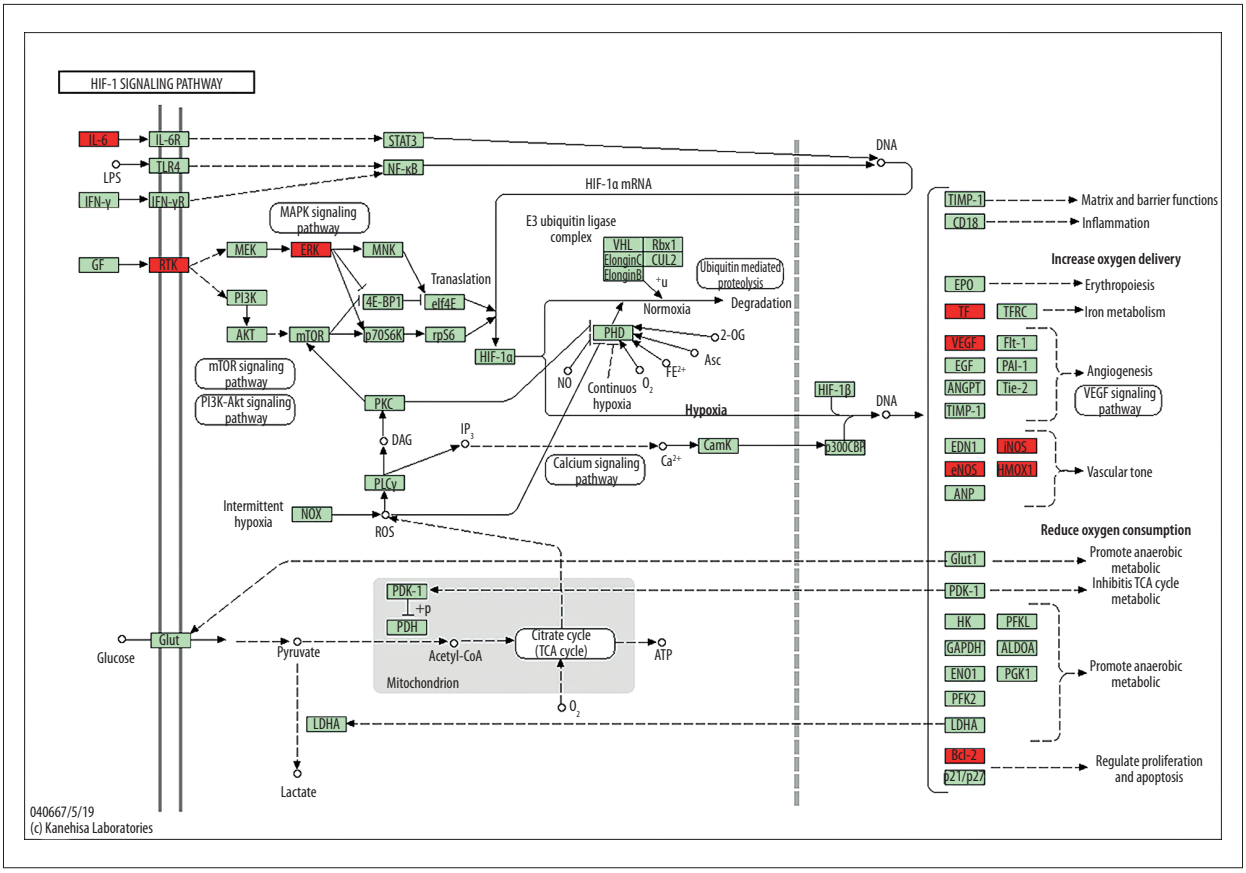

Figure 6. The analysis of HIF-1 signaling pathway. Red color represents targets of *Epimedii Herba* of enrichment in the HIF-1 pathway.

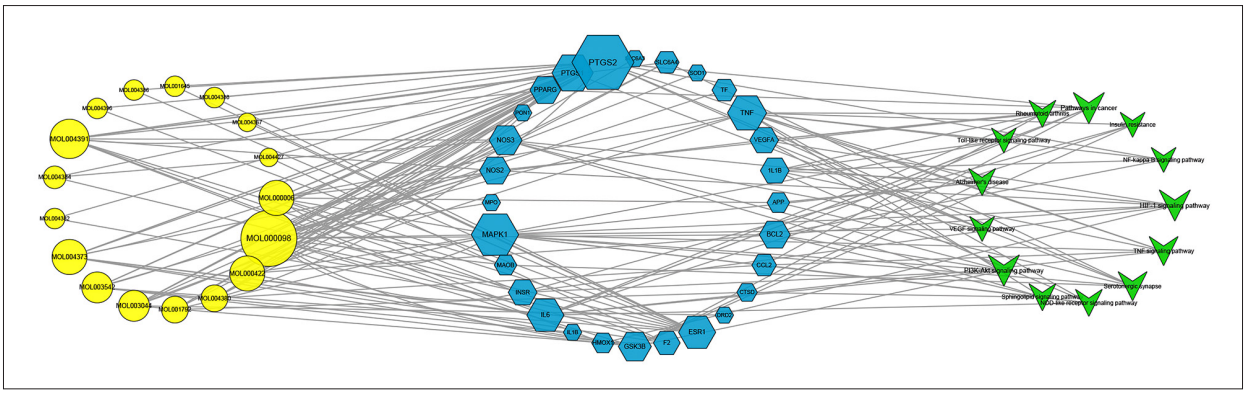

Figure 7. The active component-target-pathway network of *Epimedii Herba* against AD. Yellow nodes represent active ingredients; blue nodes represent key targets; green arrows represent pathways; edges represent interactions among them.

showed that prostaglandin-peroxide synthase 2 (PTGS2), mitogen-activated protein kinase 1 (MAPK1), prostaglandin-peroxide synthase 1 (PTGS1), tumor necrosis factor (TNF), estrogen receptor Body alpha (ESR1), and interleukin 6 (IL-6) are key nodes in the network, indicating that they are likely to be the core targets of *Epimedii Herba* treatment for Alzheimer's disease. The "component-target-pathway" network shows that multiple components, multiple targets, and multiple pathways of Epimedium cooperate with each other in Alzheimer's disease.

Docking of key molecules with the major target proteins

Quercetin with the largest degree of compound value was docked with the key target proteins prostaglandin-peroxide synthase 2 (PTGS2) in the network and the binding energy was -9.6 kcal/mol. Quercetin was docked with mitogen-activated protein kinase 1 (MAPK1) and the binding energy was –8 kcal/mol. The docking model is shown in Figure 8A, 8B. The docking binding energy of luteolin and tumor necrosis factor

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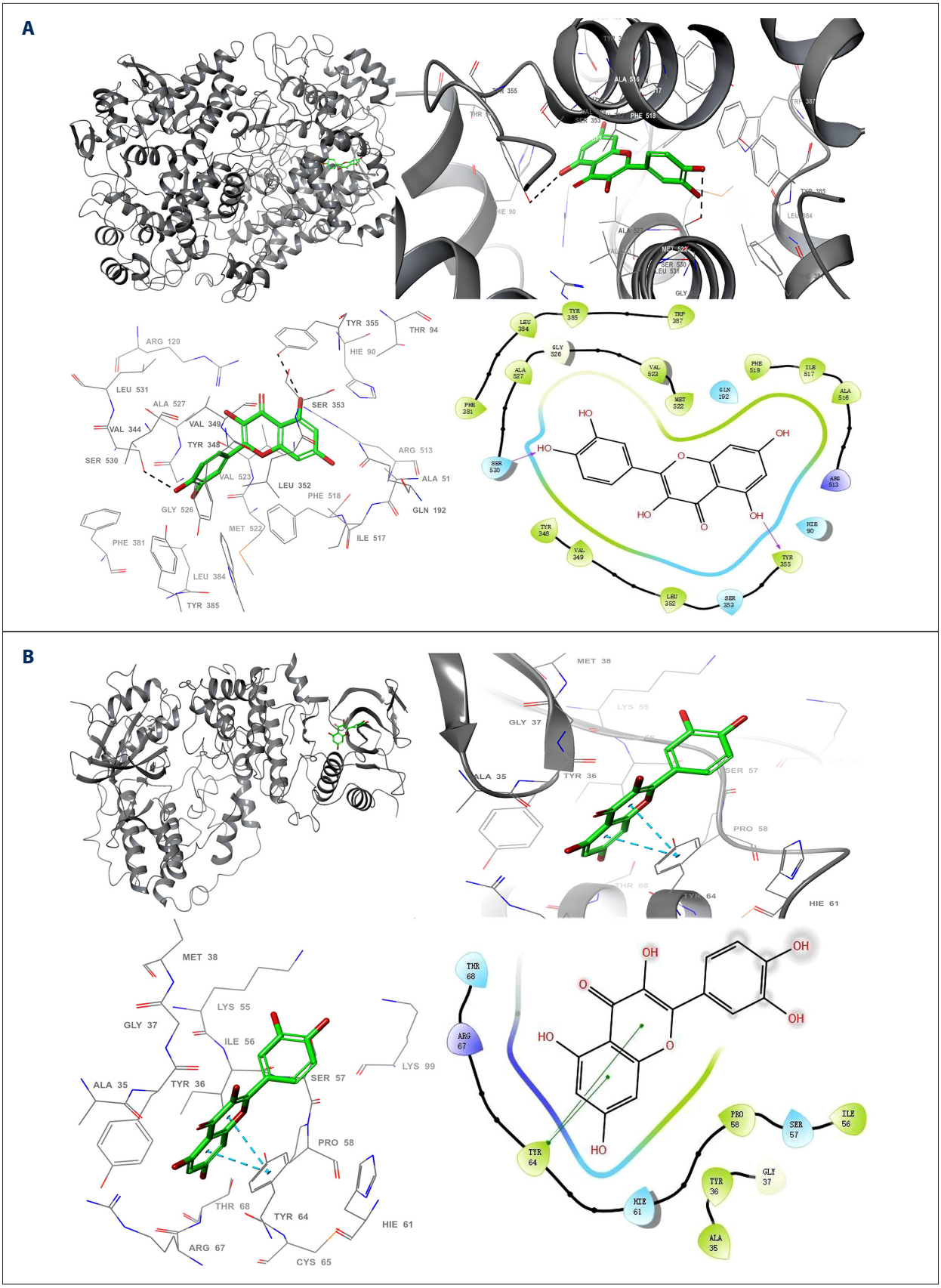
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VAL 344
VAL 349
TYR 348
SER 530
VAL 523
LEU 352
PHE 518
GLY 526
MET 522
PHE 381
LEU 384
TYR 385
TYR 355
THR 94
HIE 90
SER 353
ARG 513
ALA 516
GLN 192
ILE 517
B
MET 38
GLY 37
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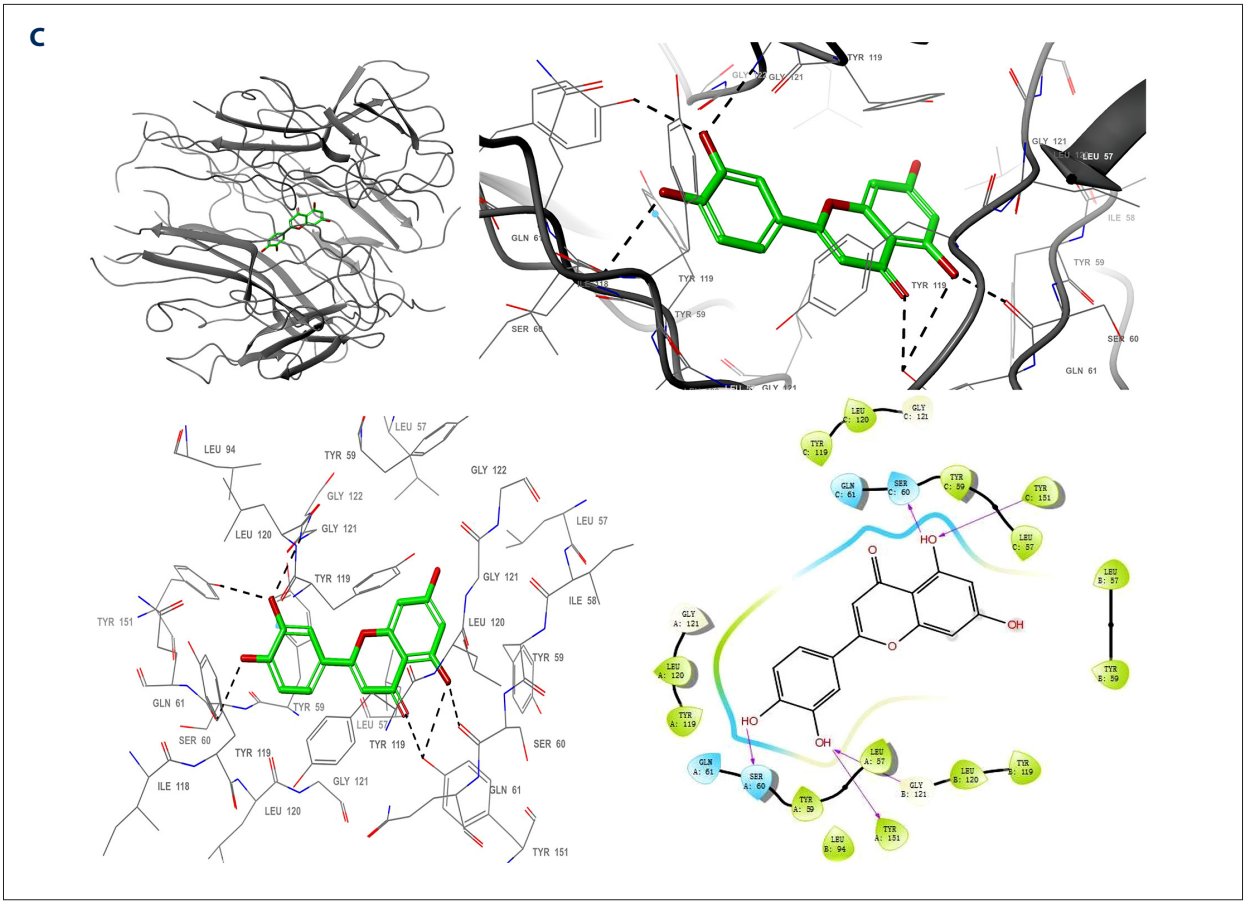

Figure 8. Docking of key molecules with the major target proteins. (**A**) Molecular docking model of the Quercetin and Prostaglandin-endoperoxide synthase 2. (**B**) Molecular docking model of the Quercetin and Mitogen-activated protein kinase 1. (**C**) Molecular docking model of the Luteolin and Tumor necrosis factor alpha. The black dotted line and purple arrow in the figure represent hydrogen bonds and the blue dotted line and green arrow represent pi-pi stacking.

Table 3. Docking of molecules with major target proteins.

Target	PDB ID	Ligand	Three dimensional coordinates of the active site	Molecule	Affinity (kcal/mol)
PTGS2	5KIR	NAG	x=23.59; y=2.418; z=34.258	Quercetin	–9.6
MAPK1	4QP4	36O	x=32.637; y=46.133; z=42.3	Quercetin	–8
TNF	6OOY	A7M	x=–11.496; y=1.548; z=–18.373	Luteolin	–10.6

was –10.6 kcal/mol. The docking model is shown in Figure 8C. The black dotted line and purple arrow in the figure represent hydrogen bonds and the blue dotted line and green arrow represent pi-pi stacking. The results show that the above key molecules are well docked with important target proteins and have a good affinity, suggesting that *Epimedii Herba* has high accuracy in the treatment of Alzheimer's disease (Table 3).

Discussion

In this study, 23 active components of *Epimedii Herba* were screened from the TCMSP database, among which 17 active components were associated with Alzheimer's disease. A variety of compounds have been reported in the literature. The main components of *Epimedii Herba* are flavonoids, and icariin is considered to be one of the main pharmacologically active components. Icariin can inhibit β-secretase activity, reduce the content of β-amyloid protein in the hippocampus and the mRNA level of β-secretase [29], improve cognitive function by

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inhibiting the loss of neurons and the formation of Aβ [30], inhibit nerve inflammation [31], inhibit atrophy of axons and dendrites [32], increase the content of neurotrophic factors, and promote the proliferation and differentiation of neural stem cells [33]. The cholinergic hypothesis is considered an important hypothesis in the process of AD. *Epimedii Herba* has been proved to inhibit AChE [34]. Studies have shown that long-term use of icariin can effectively improve cognitive dysfunction [30], indicating that icariin is important for the treatment of AD. The present study explored the potential mechanism of active ingredients in Epimedium for the treatment of AD, and provides a theoretical basis for the development of effective drugs in treatment of AD. Oxidative stress, inflammatory response, accumulation of β-amyloid protein, and hyperphosphorylation of tau protein play a crucial role in the progression of AD, leading to neurodegenerative changes [35–37].

Quercetin, one of the active ingredients in *Epimedii Herba*, can significantly reduce the expression of COX-2, iNOS, IL-1β, IL-6, and TNF-α, reduce inflammation [36], and can inhibit amyloid plaques and neurofibrillary tangles [38,39]. These data suggest that *Epimedii Herba* can effectively prevent and treat Alzheimer's disease. Quercetin can improve the learning and memory levels in dementia rats and significantly reduce the level of hippocampal cell apoptosis [40]. Icariin can reduce Aβ accumulation, reduce the expression of related inflammatory mediators, improve learning and memory abilities, and maintain the ultrastructure hippocampal neurons [41]. Some active ingredients, such as kaempferol, chlorophenol, 8-(3-methylbutan-2-alkenyl)-2-phenylchromone, and linoleic acetate, have not been reported or are rarely reported in the literature, and they may become potential directions for future research on *Epimedii Herba* in the treatment of Alzheimer's disease. It has been reported that kaempferol reduces glutamine-induced oxidative stress in mouse hippocampal neuron HT22 cells by regulating B cell lymphoma 2 (Bcl-2), apoptosis-inducing factor (AIF), and mitogen-activated protein kinase (MAPK) [42]. Kaempferol can protect PC12 and T47D cells from β-amyloid-induced toxic effects [43], and protects PC12 cells from oxidative stress-induced cytotoxicity [44].

This study predicted 27 key targets for treating Alzheimer's disease with PTGS2, 1L1B, MAPK1, GSK3B, TNF, PPARG, and VEGF. Among them, PTGS2 has been shown to play an important role in the progression of AD [45]. PTGS2 mediates the signal transduction pathway between glial cells and neuronal cells that regulates the interaction between IL-1β and Aβ, thereby inducing the aggravation of AD [46]. In contrast, PTGS2 inhibitors can reverse cognitive decline in AD by inducing neurogenesis and reducing apoptosis in transgenic mice [47]. IL1B can stimulate the synthesis of PTGS2. The metabolites of PTGS2 accelerate the phosphorylation of tau protein and the tangled deposition of nerve fibers, which can accelerate the AD process [48]. MAPK is a mitogen-activated protein kinase that can regulate cell functions, including cell proliferation, differentiation, survival, and apoptosis [49]. In late AD, MAPK1 is elevated. Hyperphosphorylation of tau in the progression of AD disease is a key factor in the generation of neurofibrillary tangles (NFT). Mitogen-activated protein kinase 1 (MAPK1) is thought to play a role in hyperphosphorylation [50]. Glycogen synthase kinase 3β (GSK3B) is involved in energy metabolism and neuronal cell development. The present study found that activation of GSK3B can lead to abnormal tau regulation and abnormal protein phosphorylation [51]. Tumor necrosis factor (TNF) is a pro-inflammatory factor that plays an important role in rheumatoid arthritis (RA) and many inflammatory diseases (such as Parkinson's disease and AD) [52]. Inhibition of TNFR1 can reduce brain inflammation, so TNFR1 is a promising therapeutic target for AD [53]. Peroxisome proliferator-activated receptor γ (PPARG) is a functional effector that regulates metabolism and is related to the pathology of neurodegenerative diseases. Early PPARG targeted therapy in AD can suppress disease progression [54]. Pathological angiogenesis is considered to be a key factor in the development of AD. There is increasing evidence that neurodegenerative diseases are related to insufficiency of the brain [55–57], which can cause hypoxia in the brain and damage the parenchymal cells and the blood-brain barrier (BBB) [58]. VEGF is a key factor in angiogenesis, which can affect AD disease progression [59,60].

The quercetin with the highest degree was molecularly docked with the key target proteins, such as prostaglandin-peroxide synthase 2 (PTGS2) and mitogen-activated protein kinase 1 (MAPK1). The results showed that there was a strong affinity. Studies have shown that quercetin inhibits OA-induced hyperphosphorylation of tau protein and oxidative stress in HT22 hippocampal neurons through MAPK and PI3K/Akt/GSK3β signaling pathways, and has the potential to treat AD [61]. Quercetin also inhibits lipopolysaccharide-induced nitric oxide synthase (iNOS) and COX-2 gene expression, blocks the activation of nuclear factor κβ [62], and plays a role in various inflammatory responses [63]. Quercetin has enhanced HO-1 protein expression, increased HO-1 mRNA expression, and reduced TNF-α levels. It can reduce inflammation [64]. Luteolin, one of the ingredients of Epimedium, can reduce cyclooxygenase 2 (COX-2), tumor necrosis factor alpha (TNF-α), interleukin 1 beta (IL-1 beta), interleukin 6 (IL-6) interleukin 8 (IL-8), and other inflammatory mediators, and increase the expression of cellular antioxidants Nrf2 and HO-1 [65]. Studies have found that luteolin can downregulate the phosphorylation of p38 MAPK [66]. Drug inhibitors of both P38 MAPK and ERk1/2 reduce the transcriptional activity of NF-κB and eliminate the subsequent COX-2 expression induced by Aβ [67]. *In vitro* studies also demonstrated that luteolin can inhibit β-secretase and reduce hyperphosphorylation of tau protein [68].

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Key targets were enriched by KEGG to obtain pathways closely related to Alzheimer's disease, such as the PI3K-Akt signaling pathway, HIF-1 signaling pathway, TNF signaling pathway, NF-κB signaling pathway, Toll-like receptor signaling pathway, and VEGF signaling pathway. This study shows the characteristics of multiple active ingredients, multi-targets, and multiple pathways of *Epimedii Herba* in the treatment of Alzheimer's disease, which provides a theory for the clinical application of and further research on *Epimedii Herba* in the treatment of Alzheimer's disease.

Conclusions

In summary, the potential targets and molecular mechanisms of *Epimedii Herba* in the treatment of Alzheimer's disease were investigated through the network pharmacology system. Nine of the 27 key targets of *Epimedii Herba* against AD act on the HIF-1 signaling pathway. The HIF-1 signaling pathway is the key pathway of *Epimedii Herba* against Alzheimer's disease. Studies have shown that M30, a new multifunctional iron chelator, activates the hypoxia-inducible factor (HIF)-1alpha signaling pathway and regulates the levels of the amyloid precursor protein, which has a potential neuroprotective effect [69].

Neurotropin® shows an effective neuroprotective effect by inhibiting Aβ-induced oxidative damage and reducing Aβ deposition in the hippocampus by regulating the HIF-1α/MAPK signaling pathway [70]. In conclusion, the present results may inspire and guide the next step in pharmacological and experimental research on *Epimedii Herba* in the treatment of Alzheimer's disease.

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Conflicts of interest

None.

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