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Exploring the mechanism of Epimedii folium and notoginseng radix against vascular dementia based on network pharmacology and molecular docking analysis

pharmacological mechanisms of EH-PN for VD

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Abstract

To explore the mechanism of Epimedii Folium (HF) and Notoginseng Radix (NR) intervention in vascular dementia (VD). This study used the Traditional Chinese Medicine Systems Pharmacology (TCMSP) database to collect the active ingredients and potential drug targets of HF and NR, the Uniprot database to convert drug target names into gene names, GeneCards, Drugbank, Therapeutic Target Database, and Online Mendelian Inheritance in Man database to collect the potential disease targets of VD, and then combined them with the drug targets to construct the HF-NR-VD protein-protein interaction (PPI) network by Search Tool for the Retrieval of Interacting (STRING). Cytoscape (version 3.7.1) was used to perform cluster analysis of the PPI network. Metascape database was used for Gene Ontology (GO) and Kyoto Encyclopedia of Genes and Genomes (KEGG) enrichment analysis. The potential interaction of the main components of the HF-NR couplet medicine with core disease targets was revealed by molecular docking simulations. There were 23 predicted active ingredients in HF and NR, and 109 common drug targets that may be involved in the treatment of VD. Through PPI network analysis, 30 proteins were identified as core proteins owing to their topological importance. GO functional analysis revealed that the primary biological processes were mainly related to inflammation, apoptosis, and the response to oxidative stress. KEGG pathway enrichment analysis revealed that TNF and PI3K/Akt signaling pathways may occupy the core status in the anti-VD system. Molecular docking results confirmed that the core targets of VD had a high affinity for the main compounds of the HF-NR couplet medicine. We demonstrated the multi-component, multi-target, and multi-pathway characteristics of HF-NR couplet medicine for the treatment of VD and provided a foundation for further clinical application and experimental research.

Abbreviations: AD = Alzheimer's disease, Bcl-2 = B-cell lymphoma 2, CASP3 = caspase 3, EF = Epimedii Folium, EGFR = epidermal growth factor receptor, GO = gene ontology, IL-1β = interleukin 1 beta, IL-6 = interleukin 6, iNOS = inducible nitric oxide synthase, KEGG = Kyoto encyclopedia of genes and genomes, MAPK8 = mitogen-activated protein kinase 8, NR = notoginseng radix, PPI = perform protein-protein interaction, STRING = search tool for the retrieval of interacting, TCM = traditional Chinese medicine, TCMSP = traditional Chinese medicine systems pharmacology database and analysis platform, TNF = tumor necrosis factor, VD = vascular dementia, VEGF = vascular endothelial growth factor, VEGF-A = vascular endothelial growth factor-A.

Keywords: Epimedii Folium, network pharmacology, notoginseng radix, vascular dementia

1. Introduction

Vascular dementia (VD) is a dementia syndrome characterized by brain nerve tissue damage caused by a series of cerebrovascular diseases, such as hemorrhagic stroke, ischemic stroke, chronic acute cerebral ischemia, and hypoxia.[1] The pathogenesis of VD is related to various factors, including aging, genetic predisposition, abnormal conditions, illiteracy,

TT and BC contributed equally to this work.

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The authors have no conflicts of interest to disclose.

The datasets generated during and/or analyzed during the current study are publicly available.

Ethical compliance is not necessary in this study.

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hypertension, coronary infarction, obesity, diabetes, atrial fibrillation, et al.[2] Recent research demonstrates that VD accounts for around 30% in Asia and other developing countries,[3–5]with somewhat lower estimates of approximately 15% to 20% of dementia cases in some developed countries such as North America and Europe.[6,7] VD is another common neurodegenerative disease, such as Alzheimer's disease (AD).[8] Currently, owing to the increasing aging of the Chinese population and the influence of contemporary lifestyles, the number of patients with VD is growing rapidly. It affects a patient's quality of life and gives rise to a serious economic burden on the patient's family. To date, no medication has been approved for the prevention or treatment of VD.

Traditional Chinese medicine (TCM) has a particular role in understanding VD as a neurodegenerative disease. TCM believed that the causes of VD are related to internal damage caused by the 7 emotions, persistent ailment, aging and physical weakness, et al lead to the dual deficiency of qi and blood, insufficiency of kidney essence, phlegm-blood stasis obstruction, and gradually make the brain marrow deprived

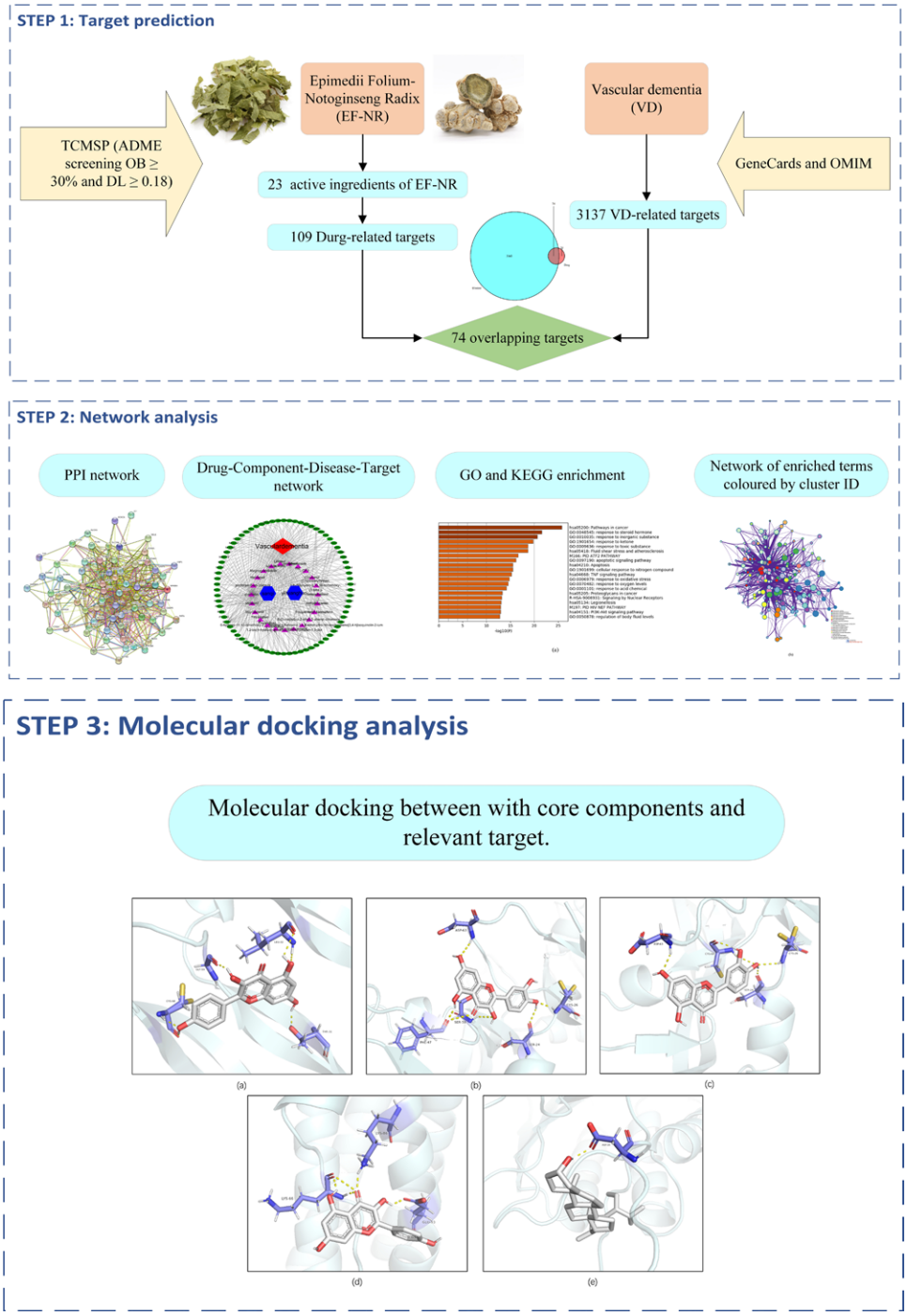

Figure 1. A schematic diagram based on the pharmacological network for revealing treatment mechanisms of HF-NR on VD. HF-NR = Epimedii Folium and Notoginseng Radix, VD = vascular dementia.

Table 1

Information of 23 potential effective components in HF-NR.

Molecule ID	Molecule name	OB(%)	DL
MOL001494	Mandenol	42.00	0.19
MOL001792	DFV	32.76	0.18
MOL000358	beta-sitosterol	36.91	0.75
MOL000449	Stigmasterol	43.83	0.76
MOL005344	ginsenoside rh2	36.32	0.56
MOL000098	quercetin	46.43	0.28
MOL001510	24-epicampesterol	37.58	0.71
MOL001645	Linoleyl acetate	42.10	0.20
MOL001771	poriferast-5-en-3beta-ol	36.91	0.75
MOL003044	Chryseriol	35.85	0.27
MOL003542	8-Isopentenyl-kaempferol	38.04	0.39
MOL000359	sitosterol	36.91	0.75
MOL000422	kaempferol	41.88	0.24
MOL004373	Anhydroicaritin	45.41	0.44
MOL004380	C-Homoerythrinan, 1,6-didehydro-3,15,16-trimethoxy-, (3.beta.)-	39.14	0.49
MOL004382	Yinyanghuo A	56.96	0.77
MOL004384	Yinyanghuo C	45.67	0.5
MOL004386	Yinyanghuo E	51.63	0.55
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
MOL004391	8-(3-methylbut-2-enyl)-2-phenyl-chromone	48.54	0.25
MOL004396	1,2-bis(4-hydroxy-3-methoxyphenyl)propan-1,3-diol	52.31	0.22
MOL000006	luteolin	36.16	0.25
MOL000622	Magnograndiolide	63.71	0.19

DL = drug-likeness, HF-NR = Epimedii Folium and Notoginseng Radix, OB = oral bioavailability.

of nourishment.[9] The syndrome types of VD mainly include the syndrome of kidney essence insufficiency, syndrome of blood stasis obstruction, syndrome of ascendant hyperactivity of liver yang, syndrome of dual deficiency of qi and blood, et al.[10] Studies have shown that kidney insufficiency and blood stasis obstruction are the most common syndromes of VD, accounting for 6.32% of the total number of patients with VD.[11] Therefore, research on VD with kidney deficiency and blood stasis obstruction syndrome is of great significance in the clinical treatment of VD. Because Chinese herbal medicine has the characteristics of mildness, multiple targets, multiple components, and fewer adverse reactions, TCM can effectively prevent and treat VD from a holistic perspective.[12] Epimedii Folium (Epimedium brevicornu Maxim. [Berberidaceae], Yinyanghuo, HF), and notoginseng radix (Panax notoginseng (Burk.) F. H. Chen. [Araliaceae], Sanqi, NR) is a couplet medicinal in TCM, which has the effects of warming and tonifying kidney-yang, activating blood, and resolving stasis. It is mainly used for the treatment of kidney deficiency and blood stasis in VD. However, due to the intricacy of the compositions and targets of HF and NR, it greatly restrains us to explore its mechanism of treatment by classical methods.[13]

With modern science and technology having entered the era of big data, network pharmacology has become a common method for researchers to explore complex biological systems from the perspective of integrated multi-compound networks.[14] Network pharmacology has become a unique advantage of TCM research, as it can precisely analyze the condition of "multi-component, multi-target, and multi-pathway" from a holistic perspective to reveal the complex molecular relationships among components and diseases.[12] The application of network pharmacology is of great help in exploring the chemical components and mechanisms of action of Chinese herbs.[15] Therefore, we aimed to explore the therapeutic mechanism of HF and NR couplet medicinals on VD by network pharmacology. This study shows a schematic diagram of research on the mechanism of EH-NR in the treatment of VD based on network pharmacology (Fig. 1).

2. Methods

2.1. Acquisition of effective components and targets of HF-NR

We collected the effective chemical components and drug targets of HF-NR couplet medicinal from the Traditional Chinese Medicine Systems Pharmacology database and analysis platform (TCMSP, https://tcmspw.com/tcmsp.php), which is the largest comprehensive and functional TCM molecular database in the world.[15] To collect the most comprehensive effective chemical components of HF-NR couplet medicinals, we set 2 basic conditions as the standards: oral bioavailability ≥ 30% and drug-likeness ≥ 0.18. These 2 basic conditions are the most essential indicators used to measure the absorption, distribution, metabolism, and excretion of each effective chemical component.[16] After combining the results acquired by the TCMSP, we input the drug-target names of the HF-NR couplet medicinal into the Uniprot (http://www.UniProt.org) database, and the "species selection was set as "Homo Sapiens."[17] We removed the non-human, reduplicative, and off-gauge drug target names of the HF-NR couplet medicinals, and the remaining were eventually converted to gene names.

2.2. Acquisition of disease targets of VD

Online Mendelian Inheritance in Man (https://omim.org/), GeneCards (https://www.genecards.org/), Drugbank (https://go.drugbank.com), and Therapeutic Target Database (http://db.idrblab.net/ttd/) databases were used to predict the disease targets relevant to VD.[18–21] The keywords "Vascular Dementia" were input into the database to comprehensively predict disease-target names of VD.

2.3. Acquisition of common targets of HF-NR and VD

The targets of HF-NR couplet medicine and VD were input into the Bioinformatics and Evolutionary Genomics Online Software Drawing Tool Platform (http://bioinformatics.psb.ugent.be/beg/),[22] and a Venn diagram was drawn to obtain the

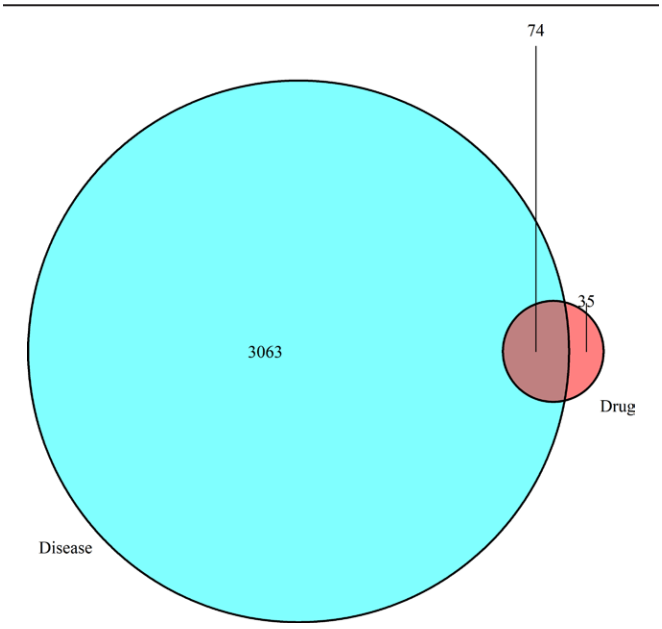

Figure 2. The Venn diagram.

common targets between HF-NR couplet medicine and VD, and the common targets were used as the core targets for subsequent analysis.

2.4. Construction of the protein-protein interaction (PPI) network

The common targets of HF-NR couplet medicine and VD were imported into the Search Tool for the Retrieval of Interacting (STRING, http://string-db.org/) to perform PPI network analysis.[23] The "species selection" of the target protein is set as "Homo sapiens," the confidence level is set as "greater than 0.4," and other parameters are set as default. Each edge of the PPI network represents the interaction relationship between proteins (the more lines in the figure, the greater the degree of association), and the ranking of the core proteins was sorted according to the degree of association.

The plugin molecular complex detection algorithm was used in the PPI interaction network to confirm the neighborhoods where core proteins are closely connected.[24]

2.5. Construction of the drug-component-disease-target network

Cytoscape (https://cytoscape.org/, version:3.7.1) is an authorized and free software commonly used by researchers to visualize molecular interaction networks and biological pathways that can combine these networks with gene expression profiles, annotations, and other data.[25] In this study, the potentially effective chemical components and common targets of HF-NR couplet medicine and VD were imported into Cytoscape 3.7.1 software, and a visual network was established to reflect the complex relationship between the couplet medicine and VD.

2.6. GO functional and KEGG pathway enrichment analysis

After completing the above series of studies, further analysis is necessary to explore the potential mechanism of HF-NR couplet medications in VD treatment. The common targets were input into the Metascape (http://metascape.org/) to complete Gene Ontology (GO) functional analysis and Kyoto Encyclopedia of Genes and Genomes (KEGG) pathways enrichment analysis,[26] the set was limited to"Homo sapiens," and the significance level was set to $P < .01$. GO analysis and KEGG pathway enrichment analysis of HF-NR couplet medicinals and VD were performed.

2.7. Molecular docking analysis

Molecular docking tools were used to determine whether the main components of the HF-NR couplet medicine have a binding capacity with core targets. We selected the top 5 main components of HF-NR and the core targets in the PPI analysis as potential receptors based on the degree value. The 3-dimensional structures of the main active components of HF-NR were obtained from the PubChem database, and the crystal structures of the core target genes were directly acquired from the RCSB PDB database (https://www.rcsb.org/).[27] The above-mentioned protein receptors and ligands were routinely processed using AutoDock Tools 1.5.7 software and saved as PDBQT format files. The AutoGrid program function was used to obtain the active docking sites, run the program for molecular docking, and obtain the associated binding energy. PyMOL 2.3.0 molecular graphics system showed a conformation with the best binding energy. Finally, molecular docking analysis revealed a visual interaction between the chemical components of the HF-NR-related genes.

3. Results

3.1. Acquisition of components and targets of HF-NR

According to the 2 screening criteria indexes (oral bioavailability ≥ 30% and drug-likeness ≥ 0.18), a total of 23 potential chemical components of HF-NR couplet medicinals were acquired from the TCMSP database, including 19 chemical components of HF, 6 in NR, and 2 duplicate components (Table 1). Based on the above results, we continued to screen 109 drug targets of HF-NR couplet medicinals using the TCMSP and Uniprot databases.

3.2. Acquisition of potential targets of VD

We used GeneCards, Online Mendelian Inheritance in Man, Drugbank, and therapeutic target databases to explore the disease targets of VD and imported the keywords "Vascular Dementia" into the 2 databases mentioned above. As a result, 3137 VD-related targets were acquired after deduplication.

3.3. Acquisition of key targets

We imported 109 drug targets and 3137 disease targets into the bioinformatics and evolutionary genomics online software drawing tool platform, and a Venn diagram was drawn to obtain the intersection targets between HF-NR couplet medicinals and VD. The compounds-targets-disease Venn diagram consisted of 109 drug targets, 3137 disease targets, and 74 key targets (Fig. 2).

3.4. Construction of the PPI network

We input the 74 key targets of HF-NR couplet medicine and VD into the STRING database to establish the PPI network (Fig. 3a). The result shows that The PPI network involved 74 nodes and 629 edges. The analysis results indicate that interleukin-6 (IL-6, degree = 48), vascular endothelial growth factor-A (VEGF-A, degree = 47), mitogen-activated protein kinase 8 (MAPK8, degree = 45), caspase 3 (CASP3, degree = 44), and epidermal growth factor receptor (EGFR, degree = 43), et al were the core proteins in this network. The top 30 core proteins are shown in the study (see Fig. 3b).

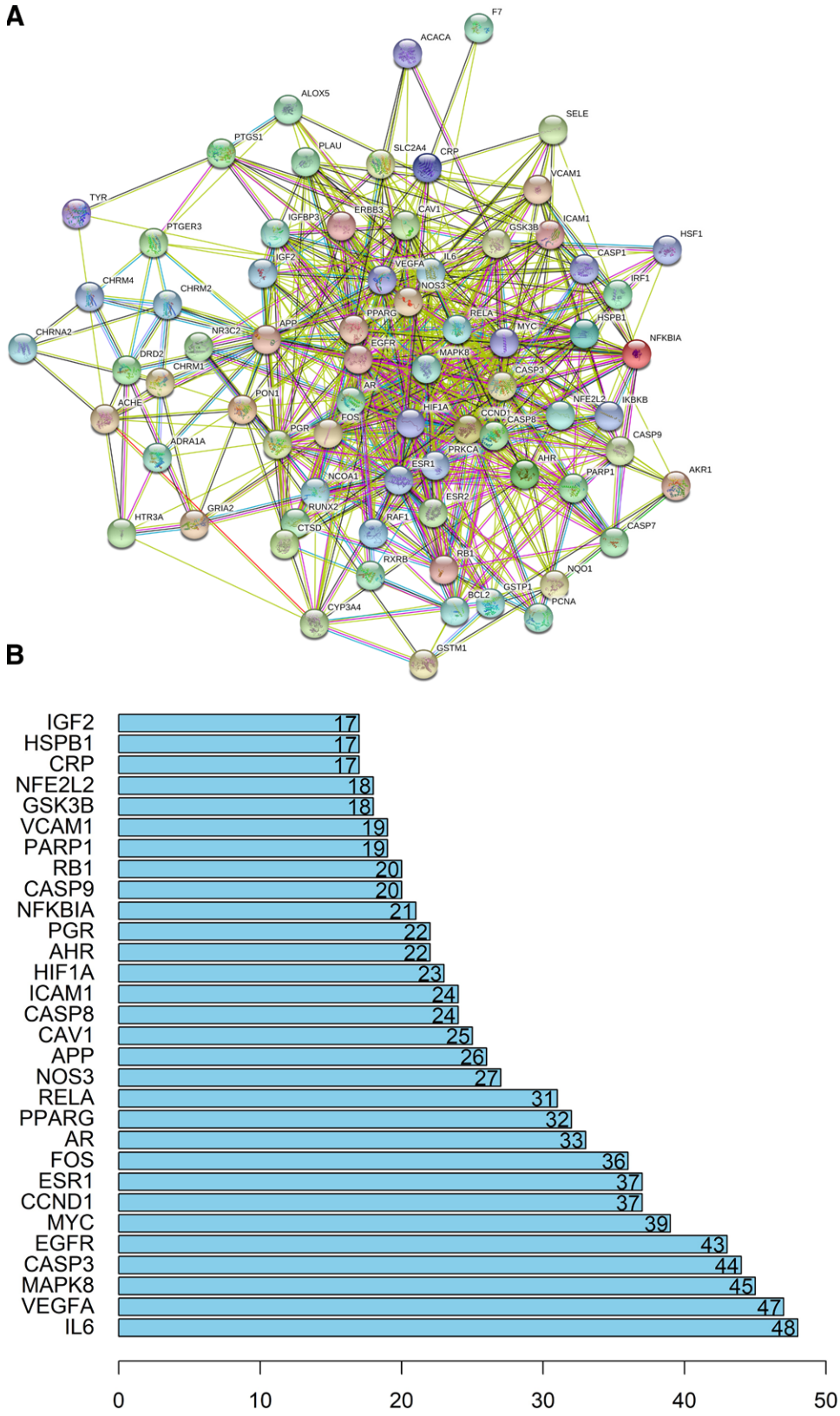

Figure 3. Construction of the PPI network and top 30 of the core targets. (a) The PPI network construction. (b) The top 30 of core proteins (the *x*-axis shows the count of interconnected targets, and the *y*-axis shows the top 30 core proteins). PPI = perform protein-protein interaction.

The HF-NR couplet medicinal and VD PPI network was analyzed using plugin molecular complex detection and returned to 6 clusters (see Fig. 4). Cluster 1 was related to pathways in cancer, the PID CERAMIDE pathway, and the cAMP signaling pathway. Cluster 2 is associated with response to steroid hormones, gonadal development, and development receptor signaling pathway, and the MyD88 cascade is initiated on the plasma membrane. Cluster 4 was related to the PID caspase pathway, legionellosis, and the execution phase of apoptosis. Cluster 5 was related to the PID P53 downstream pathway, epithelial cell

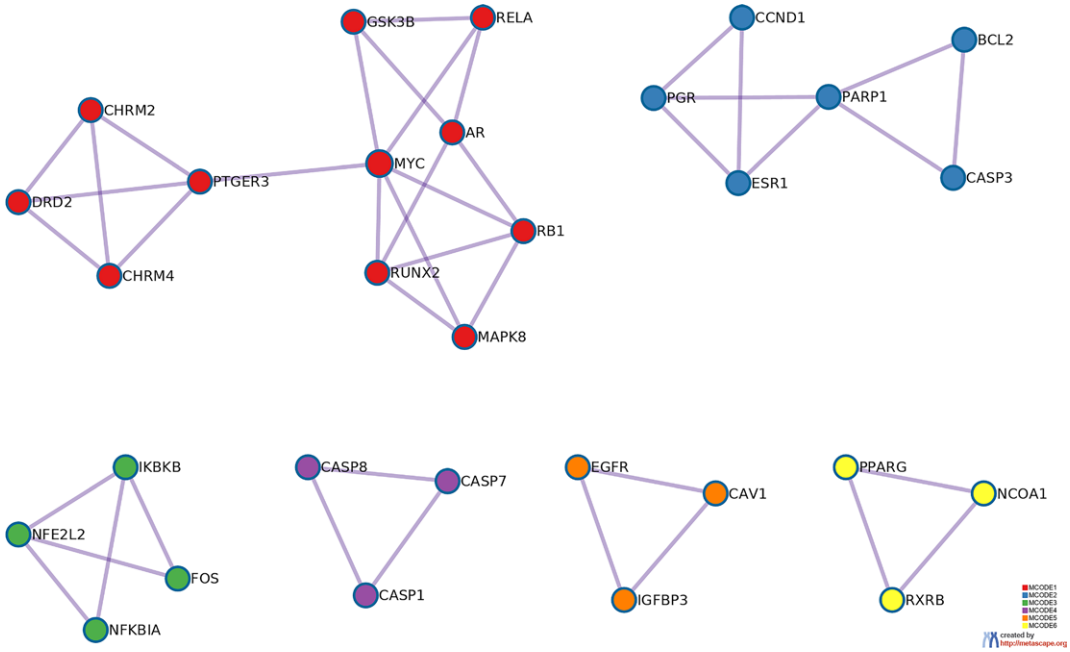

Figure 4. Clusters of PPI network. PPI = perform protein-protein interaction.

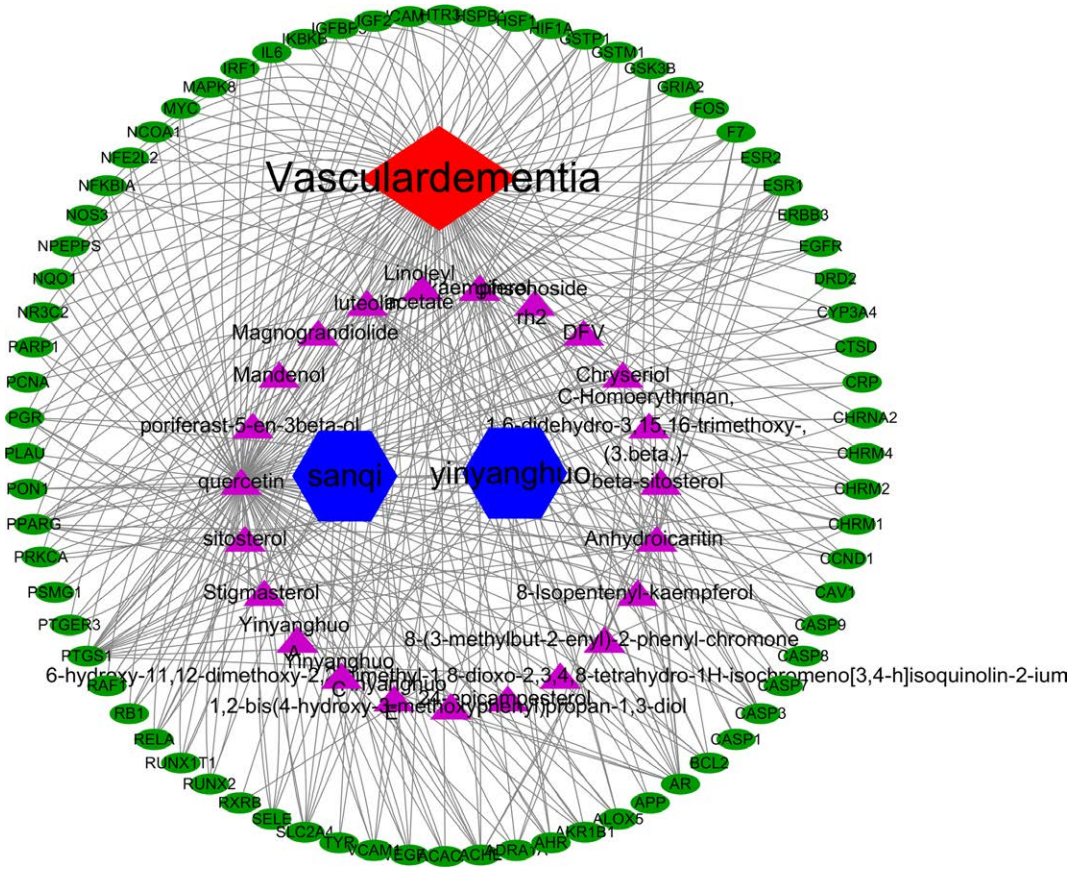

Figure 5. The drug-component-disease-target network. The red rhombus represents disease. The blue hexagons represent drugs. The purple triangles represent 23 drug compounds. The green ovals represent 74 key targets between drugs and disease.

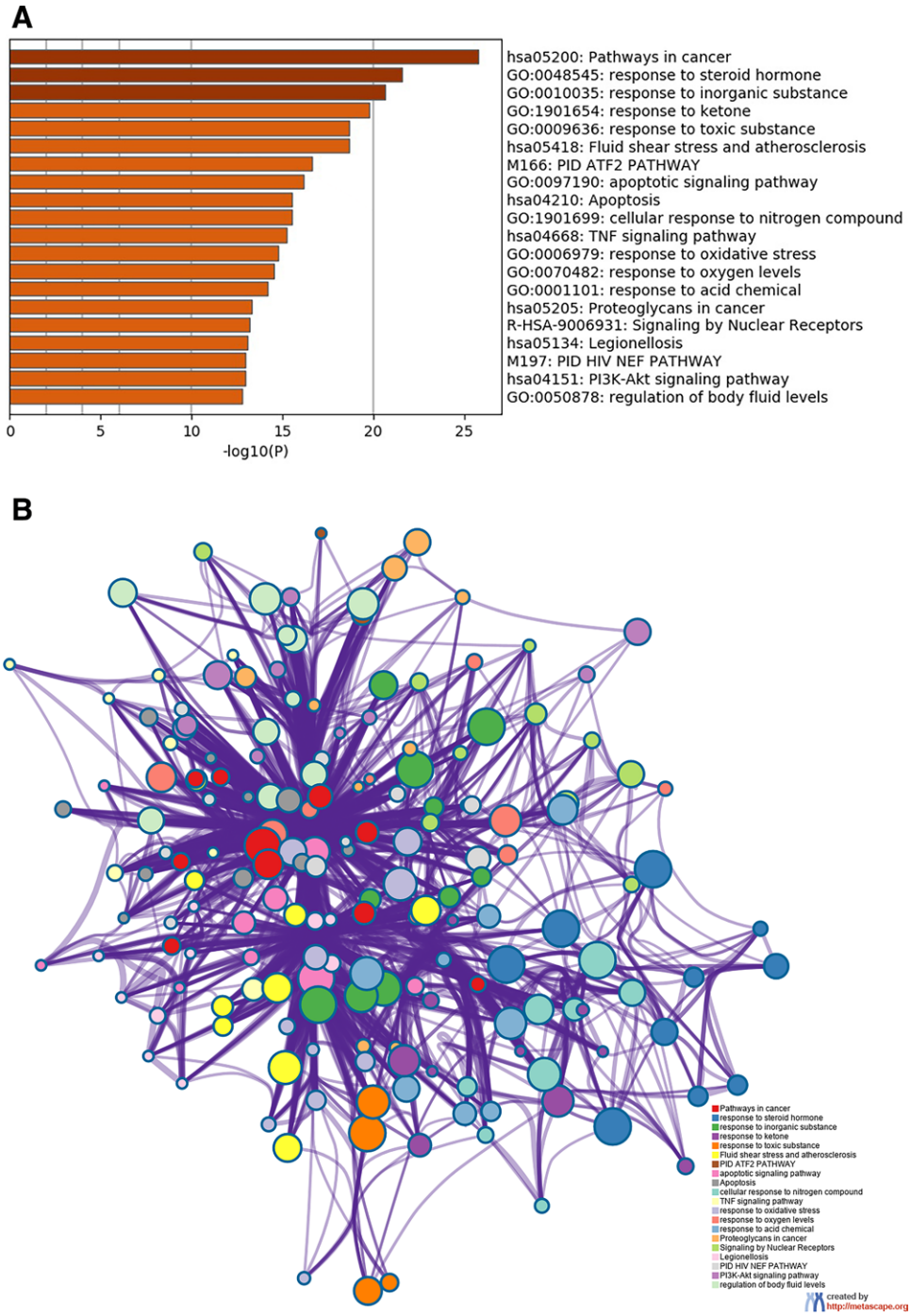

Figure 6. GO functional and KEGG pathway enrichment analysis. (a) Enriched Ontology Clusters. (b) Network of enriched terms colored by cluster ID. GO = gene ontology, KEGG = Kyoto encyclopedia of genes and genomes.

proliferation, and regulation of the MAPK cascade. Cluster 6 is related to the PID RXR VDR pathway, response to retinoic acid, and PPARA activation of gene expression.

3.5. Construction of the drug-component-disease-target network

There are 23 potential chemical components of HF-NR couplet medicinal and 74 key targets were input into Cytoscape 3.7.1 software on the computer. As a result, a "drug-compound-target-disease" interaction network was constructed (Fig. 5). In the network, edges represent interactions, red rhombus represents disease, blue hexagons represent drugs, purple triangles represent 23 drug compounds, and green ovals represent 74 key targets between HF-NR couplet medicinals and VD.

3.6. GO and KEGG analyses.

Metascape was used for GO and KEGG analyses to obtain enriched ontology clusters, which were showed in Figure 6: (a) PPI network construction. (b) Top 30 core proteins (the *x*-axis shows the count of interconnected targets and the *y*-axis shows the top 30 core proteins). The top 20 clusters with the representative enriched terms are listed in Table 2.

Table 2

Top 20 clusters with representative enriched terms (1 per cluster).

Term	Category	Description	LogP	Log(q-value)
hsa05200	KEGG pathway	Pathways in cancer	–25.76	–21.446
GO:0048545	GO biological processes	Response to steroid hormone	–21.57	–17.56
GO:0010035	GO biological processes	Response to inorganic substance	–20.64	–16.92
GO:1901654	GO biological processes	Response to ketone	–19.78	–16.31
GO:0009636	GO biological processes	Response to toxic substance	–18.66	–15.29
hsa05418	KEGG pathway	Fluid shear stress and atherosclerosis	–18.66	–15.29
M166	Canonical pathways	PID ATF2 PATHWAY	–16.59	–13.42
GO:0097190	GO biological processes	Apoptotic signaling pathway	–16.18	–13.06
hsa04210	KEGG pathway	Apoptosis	–15.52	–12.46
GO:1901699	GO biological processes	Cellular response to nitrogen compound	–15.48	–12.44
hsa04668	KEGG pathway	TNF signaling pathway	–15.23	–12.23
GO:0006979	GO biological processes	Response to oxidative stress	–14.77	–11.82
GO:0070482	GO biological processes	Response to oxygen levels	–14.54	–11.63
GO:0001101	GO biological processes	Response to acid chemical	–14.19	–11.32
hsa05205	KEGG pathway	Proteoglycans in cancer	–13.33	–10.58
R-HSA-9006931	Reactome gene sets	Signaling by nuclear receptors	–13.16	–10.42
hsa05134	KEGG pathway	Legionellosis	–13.09	–10.36
M197	Canonical pathways	PID HIV NEF PATHWAY	–12.94	–10.24
hsa04151	KEGG pathway	PI3K-Akt signaling pathway	–12.93	–10.24
GO:0050878	GO biological processes	Regulation of body fluid levels	–12.80	–10.15

GO = gene ontology, KEGG = Kyoto encyclopedia of genes and genomes.

Table 3

The binding energy of top 5 compounds of HF-NR with VD-related core targets.

			Core gene docking score (kcal/mol)				
	Molecule name	CID	IL6	VEGFA	CASP3	MAPK8	EGFR
Core components	Quercetin	5280343	–4.39	–5.73	–4.04	–3.74	–3.32
	Kaempferol	5280863	–5.45	–6.27	–4.00	–4.82	–4.58
	Luteolin	5280445	–5.11	–5.63	–4.58	–5.29	–4.83
	Anhydroicaritin	14583584	–4.71	–4.56	–3.58	–4.35	–4.03
	β-Sitosterol	521199	–5.15	–5.94	–5.39	–3.32	–5.15

CASP3 = caspase 3, EGFR = epidermal growth factor receptor, HF-NR = Epimedii Folium and Notoginseng Radix, IL-6 = interleukin 6, MAPK8 = mitogen-activated protein kinase 8, VEGF-A = vascular endothelial growth factor-A, VD = vascular dementia.

3.7. Molecular docking analysis

The smaller the binding energy, the stronger is the binding force between the HF-NR component and the relevant target. Binding energy <–4.25 kcal/mol means that ligands and receptors have the possibility of binding energies <–5.00 kcal/mol indicates good binding strength.[28] In total, 25 compound-target docking results were obtained from molecular docking analysis. The molecular docking results are shown in Table 3. We placed the best pairs of ligand-protein pairs into PyMOL software for visualization, and the specific ligand-protein interactions of docking are shown in Figure 7.

4. Discussion

Through preliminary screening of network pharmacology, 23 effective components, 109 drug targets, and 3137 VD-related targets were acquired from the TCMSP database. Quercetin, kaempferol, luteolin, anhydroicaritin, and β-sitosterol are highly connected potential therapeutic components of HF-NR, which can be defined as the decisive compounds in VD. Quercetin, a plant flavonol from the flavonoid group of polyphenols, has long been reported to have anti-inflammatory effects.[29] Previous studies have shown that quercetin can effectively reduce the mRNA expression of hippocampal inflammatory cytokines such as Interleukin 1 beta (IL-1β), Interleukin 6 (IL-6), Tumor necrosis factor-α (TNF-α), inducible Nitric oxide synthase (iNOS), and Cyclooxygenase-2 (COX-2), and also inhibit the expression of the TLR4/NF-κB signaling pathway.[30] Kaempferol, a natural flavonol widely found in various vegetables and fruits, exhibits different functions such as anti-inflammatory, anti-oxidative, and anti-apoptotic pharmacological effects.[31,32] Zheng et al showed that kaempferol can inhibit the expression of hippocampal NF-κB p65 and reduce the mRNA expression of TNF-α, IL-1β, and IL-6, which is one of the ways that kaempferol interferes with VD.[33] Therefore, regulation of inflammatory cytokines, oxidative stress, and cell damage may be the main mechanisms underlying the therapeutic effect of quercetin and kaempferol on VD.

After identifying the intersection of the targets between the HF-NR couplet medicinals and VD using a Venn diagram, 74 key targets were acquired, including IL6, VEGF-A, MAPK8, CASP3, EGFR, MYC, CCND1, ESR1, FOS, AR, PPARG, RELA, NOS3, APP, ICAM1, et al To narrow the scope, a topology analysis method was adopted, and the "Degree" and "Closeness" were used as the main references based on the established PPI network. Through analysis, 74 nodes and 629 edges were identified in the PPI network. IL6 and VEGF-A have been found to be pivotal in the development of VD. Previous studies have established that these targets play essential roles in the pathogenesis of VD by affecting oxidative stress, inflammatory cytokines, adhesion molecules, endothelial cells, and angiogenesis.[34–36] IL-6 is an interleukin that mediates inflammation (in some conditions, it also has anti-inflammatory effects). It is secreted by T cells, B cells, monocytes, fibroblasts, keratinocytes, endothelial

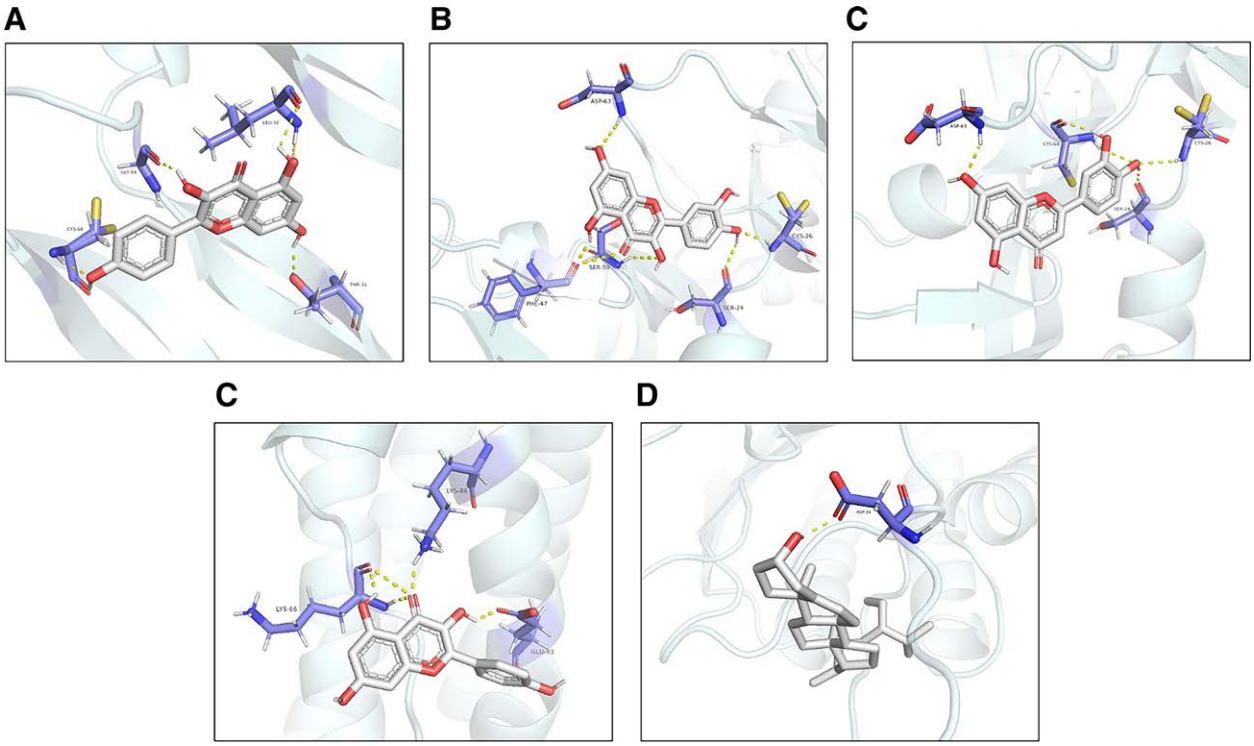

Figure 7. The result of molecular docking of the main components and core targets. (a) Kaempferol-VEGFA. (b) Quercetin-VEGFA. (c) Luteolin-VEGFA. (d) Kaempferol-SRC. (e) β-Sitosterol-CASP3. CASP3 = caspase 3, VEGF-A = vascular endothelial growth factor-A.

cells, mesangial cells, and some tumor cells.[37] Vascular endothelial growth factor (VEGF) and TNF-α are closely related to a variety of neural functions and are involved in changes in neural plasticity and nerve regeneration. Thus, VEGF and TNF-α have received considerable attention as potential candidate genes for VD therapy.[38] Previous studies showed that measurement results of serum markers in patients with VD showed that the degree of brain pathological injury after cerebral ischemia is directly proportional to the levels of pro-inflammatory cytokines such as IL-6, IL-1, and TNF-α (The greater the degree of brain pathological injury, the higher the level of pro-inflammatory cytokines).[39,40] Alfonso et al showed that TNF-α can promote the expression of VEGF at the mRNA and protein levels, and TNF-α has a synergistic effect with TGF-β1 or VEGF, which can promote the repair of nerve cells.[41] VEGF-A is encoded by the VEGF-A gene in humans.[42] Zhang et al have shown that VEGF-A not only affects vascular endothelial cells but also neurons.[43] Studies have shown that a decrease in VEGF-A can inhibit PI3K/Akt signaling pathway activation to exert an anti-angiogenic effect, thereby leading to the occurrence of diseases.[44] Wu et al demonstrated that VEGF-A can inhibit ischemic injury-induced potassium current enhancement, thereby protecting neurons through the PI3K signaling pathway.[45] In general, key proteins predicted by network pharmacology analysis play essential roles in anti-inflammation, anti-oxidation, anti-apoptosis, regulation of vascular endothelial function, and improvement of blood perfusion.

We inputted the 74 key targets into the Metascape database to identify the therapeutic mechanism of HF-NR couplet medicine against VD. GO functional enrichment analysis indicated that biological processes may be associated with the biological process of estrogen receptor binding and neurotransmitter receptor activity. KEGG pathway enrichment analysis predicted that tumor necrosis factor signaling pathways and PI3K/Akt signaling pathways may occupy the core status in the anti-VD system. TNF is a signaling pathway that combines with its receptors TNFR1 and TNFR2, which can trigger the activation of many genes in the cell membrane and activate the NF-Kappa B and MAPK signaling pathway.[46] TNF signaling mediates the inflammatory immune response and positively regulates the levels of inflammatory cytokines and mRNA expression of transcription factors.[47] Zhang et al have shown that downregulating the expression of the TNF signaling pathway can improve memory impairment in neurodegenerative diseases.[48] Feldman et al indicated that exposure to several cytokines, such as TNF-α, can activate the PI3K/Akt pathway.[49] Liu et al demonstrated that the expression of TNF-α was accompanied by the activation of P-Akt, which represents persistent activation of the PI3K/Akt pathway.[50] PI3K/Akt is a classic pathway that regulates cell proliferation, differentiation, apoptosis, and migration, and exists in various tissues and cells.[51] Previous studies have revealed that PI3K/Akt plays an essential role in upregulating the expression of B-cell lymphoma 2 (Bcl-2), inhibiting cell injury after ischemia and hypoxia, antagonizing cell apoptosis, and promoting cell survival.[52–54]

Molecular docking is the most common method for evaluating chemical composition-target interactions. Quercetin, kaempferol, luteolin, anhydroicartin, and β-sitosterol were the most stable chemical components for core target binding. The docking scores of IL6, VEGF-A, CASP3, MAPK8, and EGFR with the 5 core targets showed a good correlation. These results indicate that an interaction does not mediate HF-NR combination therapy for VD between a single active ingredient in HF-NR couplet medicine and a target protein in VD, but among multiple active ingredients in HF-NR couplet medicine and numerous target proteins in VD. Our data indicate that the efficacy and mechanisms of HF-NR combination therapy for VD are complicated and involve multicomponent interactions.

5. Conclusions

In conclusion, it is scientific and reliable to explore the mechanism of HF-NR combination therapy for VD from the perspective of network pharmacology. It is predicted that the active

ingredients of HF-NR couplet medicines may act on some key factors of the above signaling pathway and play an essential role in the treatment of VD. However, several factors affect the reliability of network pharmacology prediction results, including differences in the database, component screening indicators, and analysis tools. Therefore, experimental validation of the HF-NR combination therapy for VD is necessary for further research.

Author contributions

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