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Exploring the Molecular Mechanisms of Eucommia Ulmoides Extract in Treating Atherosclerosis Based on Network Pharmacology and Molecular Docking Technology

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Keywords: Network pharmacology; eucommia ulmoides extract; atherosclerosis; molecular docking technology; targets; signaling pathway

Abstract: [Objective] This study aimed to investigate the mechanism of action of Eucommia ulmoides extract in treating atherosclerosis using network pharmacology and molecular docking technology. [Methods] The researchers screened the chemical active ingredients of Eucommia ulmoides using the TCMSP platform and collected the corresponding targets of these active ingredients using the Swiss Target Prediction platform. They searched for relevant targets of atherosclerosis in the DisGeNET, GeneCards, TTD, OMIM, Drug Bank, and Pharmgkb databases and obtained common targets between the two using the Venny platform. These common targets were then used to construct a protein interaction network and a core target network using Cytoscape 3.8.0, and topological parameters were obtained. GO and KEGG pathway enrichment analyses were performed using DAVID, and the results were visualized using bioinformatics and Omicshare platforms. The researchers also constructed and analyzed the "active ingredient-target-disease" network model using Cytoscape 3.8.0 software. Finally, AutoDock and Pymol software were used for molecular docking of important components and core targets to predict their binding ability. [Results] The researchers screened 27 active ingredients from Eucommia ulmoides, and found 206 common targets between atherosclerosis and active ingredients. These targets were mainly related to the Protestations in cancer, Prostate cancer, and Endocrine resistance pathways, among which AKT1 had the highest affinity for β-sitosterol. [Conclusion] This study suggests that multiple active ingredients in Eucommia ulmoides extract may exert various effects in the treatment of atherosclerosis. The findings provide a scientific basis for further development of clinical applications of Eucommia ulmoides extract.

1. Introduction

With the improvement of living standards and changes in lifestyle, atherosclerosis (AS) has become one of the main causes of cardiovascular disease, posing a serious threat to people's health. AS is an arterial disease based on endothelial injury and characterized by lipid deposition, and is one of the main causes of cardiovascular disease. The occurrence and development of atherosclerosis is a complex process involving multiple biochemical and molecular biology mechanisms. Currently, clinical methods for treating AS mainly include lifestyle changes, lipid-lowering drugs, antioxidants, and vasodilators [1], but these methods have problems such as significant side effects and poor efficacy. Therefore, finding a safe and effective treatment method is very important.

Eucommia ulmoides (Du Zhong in Chinese) is a precious Chinese herbal medicine with a long history of use and medicinal value. It is known as the "southern ginseng" and contains rich polyphenols and flavonoids, with effects such as strengthening tendons and bones, nourishing the liver and kidneys, invigorating the spleen and stomach, and anti-aging [2]. In recent years, studies have shown that Eucommia ulmoides extract has a certain therapeutic effect on atherosclerosis, but its specific molecular mechanism is not clear.

Network pharmacology can systematically analyze the interaction between drugs and biomolecules, predict the mechanism and indications of drugs, and analyze their side effects and interactions. Molecular docking technology can simulate the interaction between drug molecules and biomolecules, and predict the optimization direction of drug molecules. The combination of network pharmacology and molecular docking technology can provide comprehensive and accurate support for drug development. Therefore, this study will use network pharmacology and molecular docking technology to explore the molecular mechanism of Eucommia ulmoides extract in the treatment of atherosclerosis, providing new ideas for further research on the molecular mechanism of Eucommia ulmoides extract in the treatment of atherosclerosis.

2. Materials and methods

2.1 Screening of active ingredients and target proteins of Eucommia ulmoides

We searched the Traditional Chinese Medicine Systems Pharmacology Database and Analysis Platform (TCMSP) (https://old.Tcmsp-e.com/tcmspsearch. Php) for active ingredients of Eucommia ulmoides with an oral bioavailability (OB) greater than or equal to 30% and drug-likeness (DL) greater than or equal to 0.18. Then, we used PubChem database (http://pubchem.ncbi.nlm.nih.gov/) and Open Babel software to obtain the SMILES structure of these active ingredients and uploaded them to the Swiss Target Prediction platform (http://www.swisstargetprediction.ch/) to collect the corresponding target proteins of these active ingredients. We standardized the gene information of these active ingredients through UniProt database (https://www.uniprot.org/) and set the species as human to obtain the potential target proteins of these active ingredients.

2.2 Acquisition of target genes related to atherosclerosis

We searched for target genes related to atherosclerosis using six databases, including DisGeNET, GeneCards, TTD, OMIM, Drug Bank, and Pharmgkb, which contain disease-related gene information. After merging and removing duplicate targets, we obtained the relevant targets for atherosclerosis.

2.3 Determination of the common targets of active components of Eucommia ulmoides and atherosclerosis

We used the above steps to obtain the potential target proteins of active components and disease-related target proteins of atherosclerosis. Then, we uploaded them to Venny2.1.0 (http://bioinformatics.psb.ugent.be/webtools/Venn/) to draw a Venn diagram to determine the common targets. The intersection of the obtained targets represents the potential targets of active components of Eucommia ulmoides that may act on the related targets of atherosclerosis.

2.4 Construction of protein interaction network and core target network

We imported the shared targets of Du-zhong active ingredients and atherosclerosis into the STRING platform Version 11.0, and constructed a Du-zhong protein interaction network for the treatment of atherosclerosis using Cytoscape software. We used its plugin MCODE to obtain the core target network.

2.5 GO functional enrichment analysis and KEGG pathway enrichment analysis

GO analysis includes three parts: biological process (BP), cellular component (CC), and molecular function (MF), which collectively describe the functions of gene products. We used the online tool DAVID to perform GO enrichment analysis on the 206 potential targets of Duzhong extract for the treatment of atherosclerosis, and obtained corresponding entries based on three plugins (GOTERM_BP_DIRECT, GOTERM_CC_DIRECT, GOTERM_MF_DIRECT). We imported the common target genes into the DAVID database (http://david.ncifcrf.gov/) for GO and KEGG pathway enrichment analysis, and selected the biological processes and pathways with $P<0.05$ based on three plugins (GOTERM_BP_DIRECT, GOTERM_CC_DIRECT, GOTERM_MF_DIRECT) and sorted them by the number of involved targets from high to low. We used R studio for image visualization processing, drew GO functional enrichment and KEGG pathway enrichment diagrams, and finally combined with the KEGG database to draw key pathway mechanism diagrams.

2.6 Construction and analysis of the "Active ingredient-Target-Disease" network model

We imported the information of traditional Chinese medicine, chemical components, their corresponding target proteins, and pathway information into Cytoscape 3.8.0 software to construct the "Active ingredient-Target-Disease" network.

2.7 "Active ingredient-core target" molecular docking

The core target protein was selected from the Uniprot database, and the best protein structure was downloaded from the PDB (http://www.pymol.org) database based on the protein's tertiary structure characteristics. The 2D structure of the active ingredient was obtained from PubChem and converted to a 3D structure mol2 file. Then, in the PyMOL (http://www.pymol.org) software, the ligands and non-protein molecules in the protein were removed, and the protein was processed by adding hydrogen, adding charge, and merging non-polar hydrogen, and saved as a PDBQT file. Finally, molecular docking was completed using AutoDock Tools 1.5.6 and AutoDock Vina software, and the results were visualized.

3. Results

3.1 Screening of common targets for active ingredients of Eucommia ulmoides and atherosclerosis

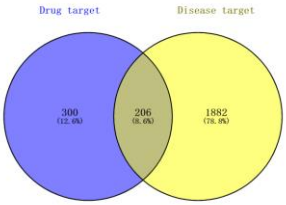


Figure 1: The intersection of the targets of action of Eucommia and the targets of atherosclerosis

Table 1: Basic information on the 28 active chemical constituents in Eucommia globulus

Molecule ID	Chemical	MW	OB (%)	DL
MOL002058	40957-99-1	388.45	57.2	0.62
MOL000211	Mairin	456.78	55.38	0.78
MOL000358	beta-sitosterol	414.79	36.91	0.75
MOL000422	kaempferol	286.25	41.88	0.24
MOL004367	olivil	376.44	62.23	0.41
MOL000443	Erythraline	297.38	49.18	0.55
MOL005922	Acanthoside B	580.64	43.35	0.77
MOL006709	AIDS214634	374.42	92.43	0.55
MOL007059	3-beta-Hydroxymethyllenetanshiquinone	294.32	32.16	0.41
MOL000073	ent-Epicatechin	290.29	48.96	0.24
MOL007563	Yangambin	446.54	57.53	0.81
MOL009007	Eucommin A	550.61	30.51	0.85
MOL009009	(+)-medioresinol	388.45	87.19	0.62
MOL009015	(-)-Tabernemontanine	354.49	58.67	0.61
MOL009027	Cyclopamine	411.69	55.42	0.82
MOL009029	Dehydrodiconiferyl alcohol 4, gamma'-di-O-beta-D-glucopyanoside_qt	358.42	51.44	0.4
MOL009030	Dehydrodieugenol	326.42	30.1	0.24
MOL009031	Cinchonan-9-al,6'-methoxy-,(9R)-	324.46	68.22	0.4
MOL009038	GBGB	550.57	45.58	0.83
MOL009042	Helenalin	262.33	77.01	0.19
MOL009047	(+)-Eudesmin	386.48	33.29	0.62
MOL009053	4-[(2S,3R)-5-[(E)-3-hydroxyprop-1-enyl]-7-methoxy-3-methylol-2,3-dihydrobenzofuran-2- yl]-2-methoxy-phenol	358.42	50.76	0.39
MOL009055	hirsutin_qt	345.35	49.81	0.37
MOL009057	liriodendrin_qt	450.48	53.14	0.8
MOL000098	quercetin	302.25	46.43	0.28
MOL002773	beta-carotene	536.96	37.18	0.58
MOL008240	(E)-3-[4-[(1R,2R)-2-hydroxy-2-(4-hydroxy-3-methoxy-phenyl)-1-methylol-ethoxy]-3-methoxy-phenyl]acrolein	374.42	56.32	0.36
MOL011604	Syringetin	346.31	36.82	0.37

We collected 28 effective chemical active ingredients from the TCMSP database, as shown in Table 1. A total of 1,110 target proteins corresponding to the active ingredients were collected through the Swiss Target Prediction platform. The target genes were converted to standard gene names using the UniProt database, and genes without "homo sapiens" and "reviewed" UniProt IDs were removed, resulting in 506 potential target proteins for Eucommia ulmoides ingredients. Then, we screened 2,698 gene proteins through the DisGeNET, GeneCards, TTD, OMIM, DrugBank, and PharmGKB databases, and after removing duplicate target genes, 2,088 genes remained. We used Venny 2.1 to take the intersection and obtained 206 common targets, as shown in Figure 1.

3.2 Analysis of protein-protein interaction network and core target network of Du Zhong active ingredients and atherosclerosis

We imported the 206 shared gene targets into the STRING database and obtained 489 protein-protein interaction information (see Figure 2). Then, we imported the protein-protein interaction information into Cytoscape and used network topology analysis to select the targets whose node degree was greater than the average value of 6.62 as the core targets for Du Zhong treatment of atherosclerosis. We obtained 117 core targets and used the CytoNCA plugin to obtain the core target network (see Figure 3). The size and color of the nodes represent the degree value, with larger nodes indicating higher degree values and darker colors indicating stronger correlation with the mechanism of Du Zhong treatment for atherosclerosis. Among them, SRC, AKT1, HSP90AA1, MAPK3, PIK3CA, MAPK1, ESR1, PTPN11, EGFR, and others may be related to the treatment of atherosclerosis by Du Zhong extract.

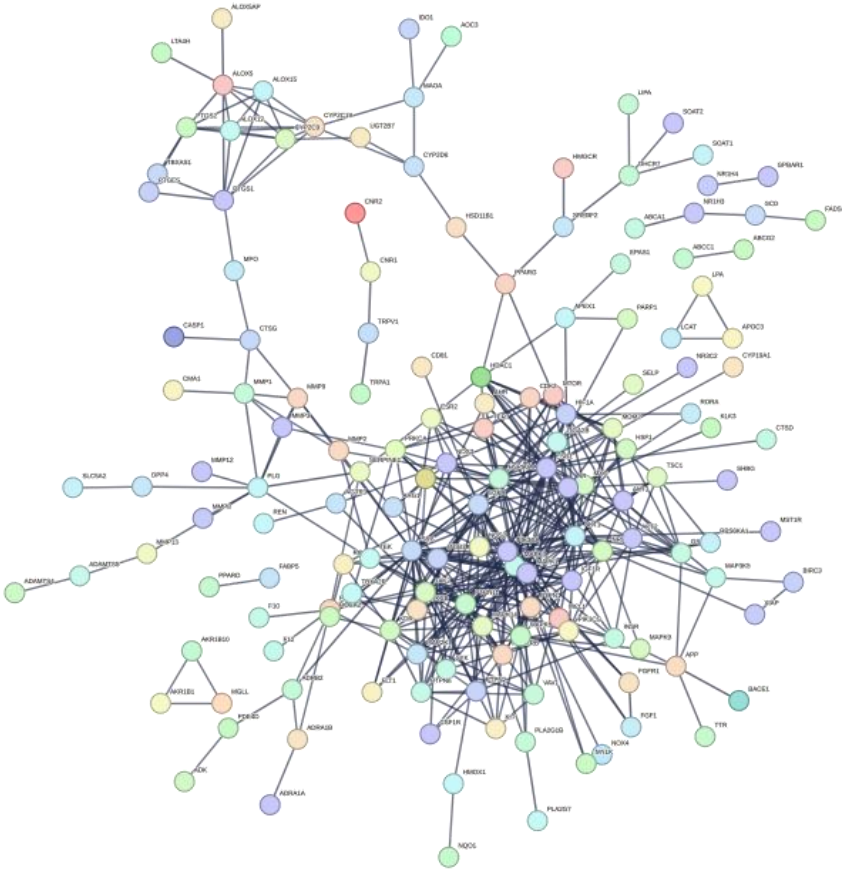


Figure 2: Protein PPI network between active constituents of Eucommia globulus - atherosclerosis shared target proteins

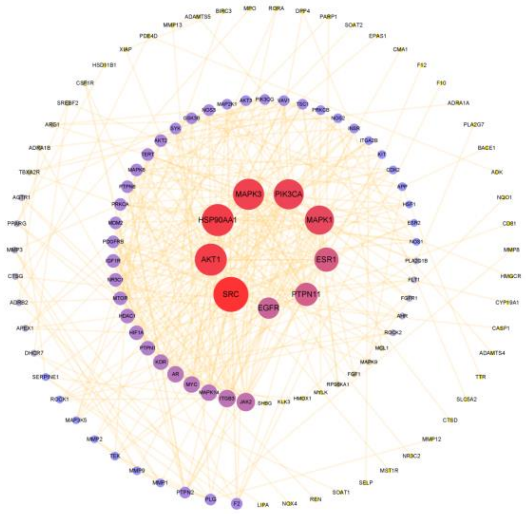


Figure 3: Core target network among the target proteins of Du Zhong active components and atherosclerosis.

3.3 GO functional enrichment analysis and KEGG pathway enrichment analysis

The GO analysis results show that the biological processes of Du Zhong extract in treating atherosclerosis mainly include regulation of inflammatory response, response to molecules of bacterial origin, response to lipopolysaccharides, vascular processes in the circulatory system, and cellular response to chemical stress. The cellular components mainly include membrane rafts, membrane microdomains, and membrane regions. The molecular functions mainly include steroid binding, protein tyrosine kinase activity, and nuclear receptor activity (see Figure 4 for details). The KEGG results indicate that Du Zhong extract mainly regulates signaling pathways such as Proteoglycans in cancer, Prostate cancer, and Endocrine resistance, acting on atherosclerosis (see Figure 5 for details).

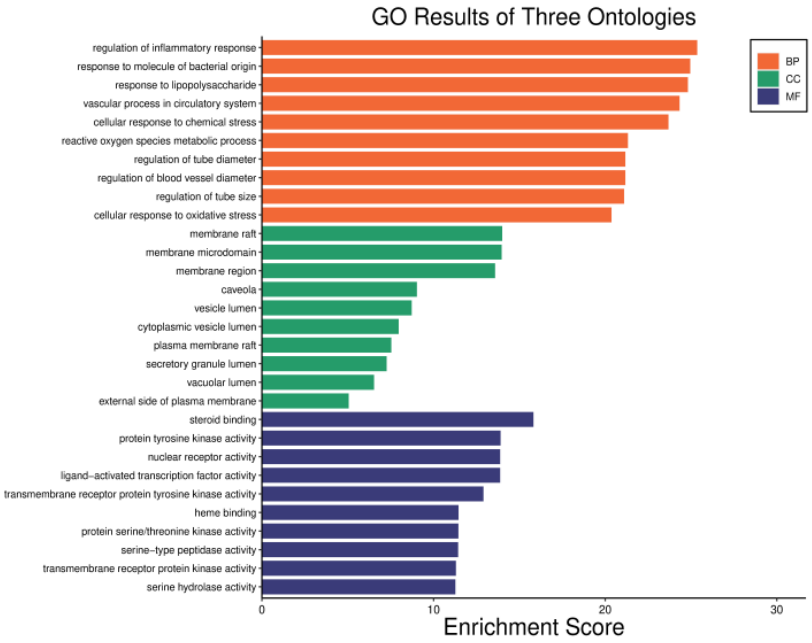


Figure 4: GO function enrichment chart

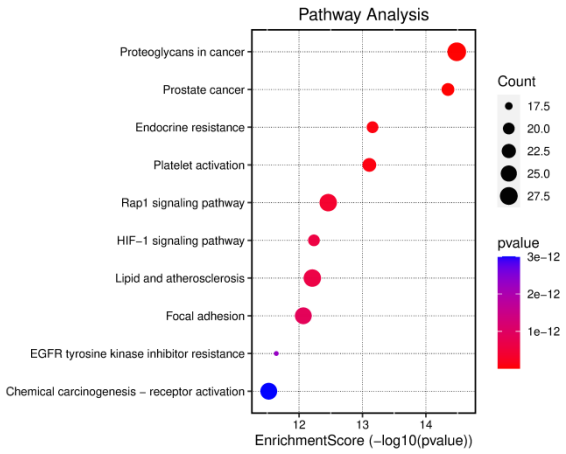


Figure 5: Enrichment analysis of KEGG pathways.

3.4 "Active ingredient-target-disease" network model construction

The results of the model show that active ingredients can act on multiple targets. For example, in Eucommia ulmoides, both chlorogenic acid and quercetin, as well as β-sitosterol, can act on atherosclerosis through multiple targets (see Figure 6 for details).

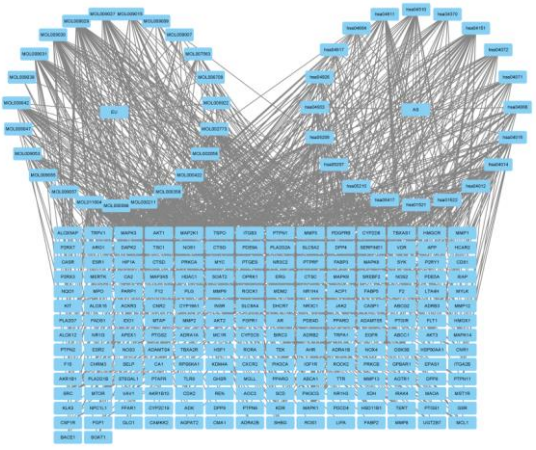


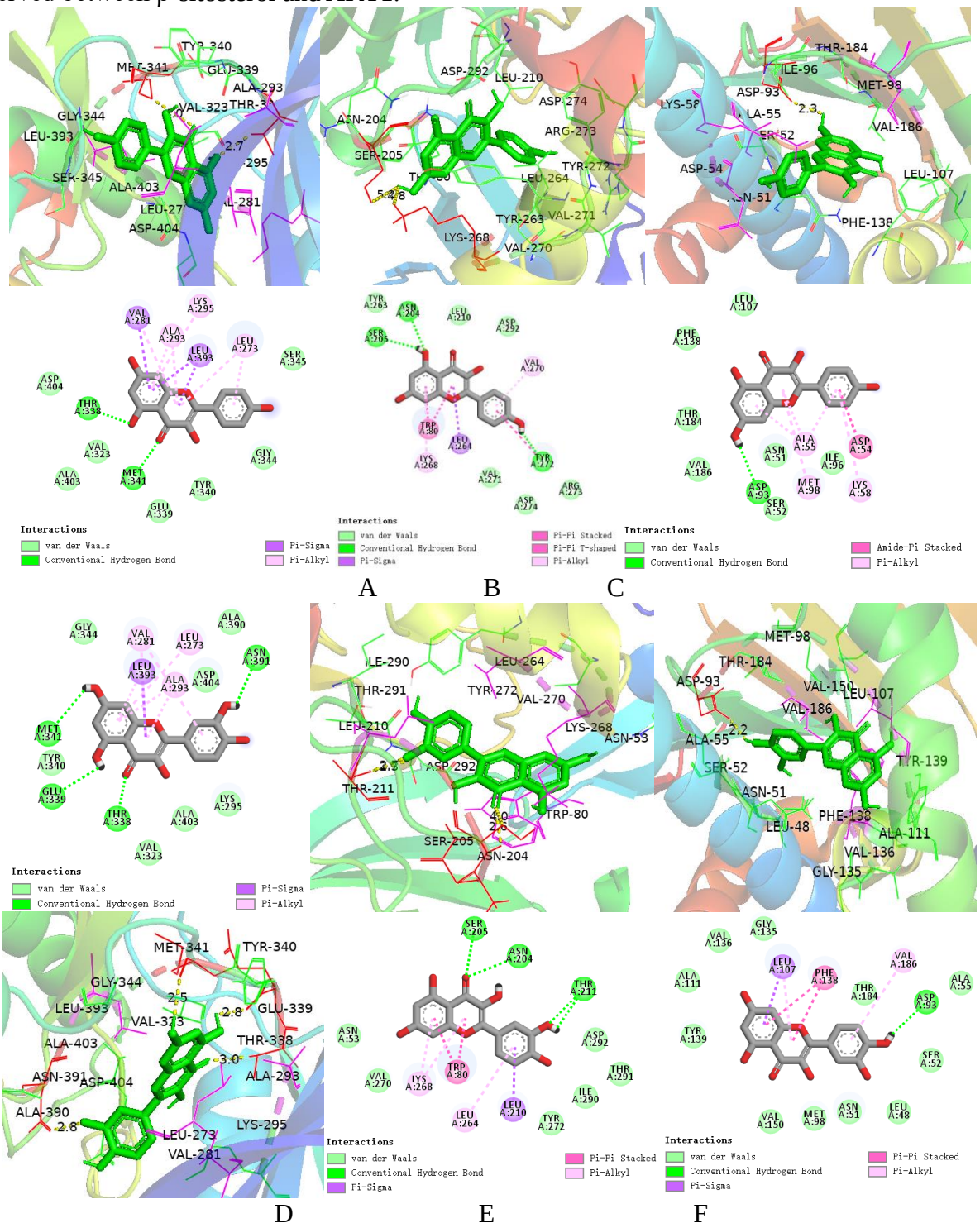
Figure 6: Network model of "active ingredients-targets-diseases"

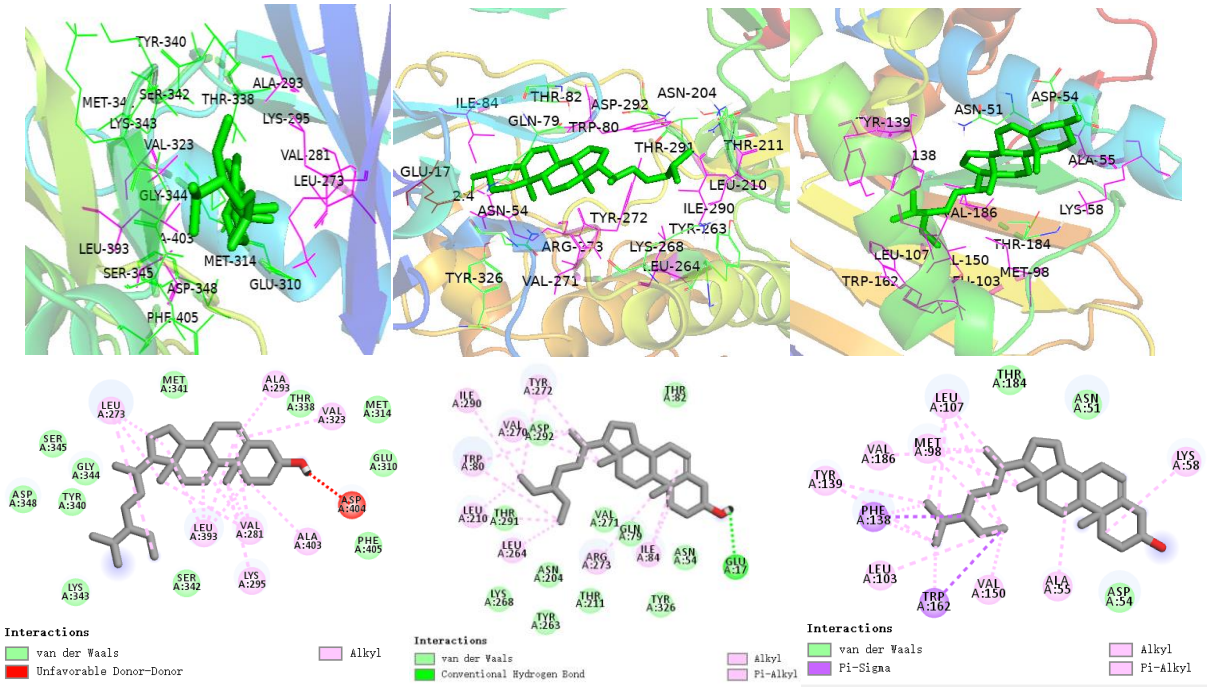
3.5 Molecular Docking of Active Ingredients and Core Targets

Table 2: Combined capability component analysis

Number	Key targets	PDB ID	Active ingredients ingredientsingredients	Binding energy(kcal/mol)
A	SRC	4u5j	kaempferol	-8.4
B	AKT1	7nh5	kaempferol	-9.3
C	HSP90AA1	5h22	kaempferol	-7.0
D	SRC	4u5j	quercetin	-8.0
E	AKT1	7nh5	quercetin	-10.4
F	HSP90AA1	5h22	quercetin	-8.7
G	SRC	4u5j	beta-sitosterol	-8.7
H	AKT1	7nh5	beta-sitosterol	-11.2
I	HSP90AA1	5h22	beta-sitosterol	-9.2

The core targets SRC and AKT1 were docked with their corresponding active ingredients to predict their binding ability. The smaller the binding free energy, the greater the affinity between the receptor and the ligand, and the more stable the conformation (see Table 2). The results were visualized using PyMOL software (see Figure 7). The results showed that all docking binding energies were less than 0, indicating that the molecular docking between all core targets and active ingredients had relatively small binding free energies and good affinity, with the highest affinity observed between β-sitosterol and AKT1.





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Note: A: Docking of piceatannol with SRC; B: Docking of piceatannol with AKT1; C: Docking of piceatannol with HSP90AA1;D: Docking of Quercetin with SRC; E: Docking of Quercetin with AKT1; F: Docking of Quercetin with HSP90AA1;G: Docking diagram of β-sitosterol with SRC; H: Docking diagram of β-sitosterol with AKT1; I: Docking diagram of β-sitosterol with HSP90AA1.

Figure 7: Molecular docking result

4. Discussion and analysis

Du Zhong, as a traditional Chinese medicine, has the effects of warming the kidney and assisting yang, activating blood circulation and dredging collaterals in the theory of traditional Chinese medicine. In recent years, more and more studies have shown that Du Zhong extract also has certain pharmacological effects in the treatment of atherosclerosis. However, the mechanism of Du Zhong extract in the treatment of atherosclerosis is still unclear. Network pharmacology and molecular docking technology provide powerful tools for exploring the molecular mechanism of Du Zhong extract in the treatment of atherosclerosis.

In this study, we explored the molecular mechanism of Eucommia ulmoides extract in treating atherosclerosis through network pharmacology and molecular docking techniques. Our results indicated that Eucommia ulmoides extract contains multiple active components, and network pharmacology is a research method based on systems biology, computer science, and network science. By constructing a drug-target-disease network, we investigated the multi-target effects and complex mechanisms of Eucommia ulmoides extract. We found that several active components of Eucommia ulmoides extract, such as naringenin, quercetin, and β-sitosterol, can act on multiple targets including SRC, AKT1, HSP90AA1, MAPK3, and PIK3CA to exert various biological effects. Among them, targets such as SRC, AKT1, and HSP90AA1 are closely related to the occurrence and development of atherosclerosis. SRC is a non-receptor tyrosine kinase that is involved in smooth muscle cell proliferation and migration[3]. In addition, SRC plays an important role in regulating macrophage function, particularly in mediating inflammatory and immune responses. The adhesion, migration, and inflammation of macrophages are important links in the

development of atherosclerosis. SRC is involved in regulating the inflammatory signaling pathway mediated by all members of the Toll-like receptor (TLR) family, indicating its potential role in macrophage inflammation in atherosclerosis[4]. AKT1 can control cell signal transduction, energy metabolism, immune function, and thrombosis formation by regulating multiple signaling pathways[5]. HSP90AA1 is a heat shock protein that is a member of the HSP90 family, and it is associated with the process of lipid metabolism and cholesterol transport, which also play important roles in the occurrence and development of atherosclerosis[6-7]. A study found[8] that inhibiting the expression of HSP90 can reduce lipid accumulation and the expression of cholesterol transport proteins, thereby reducing the degree of atherosclerosis. This provides a new explanation for the pharmacological activity of Eucommia ulmoides extract. Furthermore, we found through molecular docking that proteoglycans in cancer, prostate cancer, endocrine resistance, platelet activation, and the Rap signaling pathway may be related to the occurrence and development of atherosclerosis. Therefore, the multi-target effects of Eucommia ulmoides extract can regulate various biological effects and thus play a therapeutic role in the treatment of atherosclerosis.

It should be noted that these predictions and docking results only serve as a theoretical basis for guiding subsequent experimental research and must be further validated through experimental verification. Some researchers have attempted to extract active compounds from Eucommia ulmoides extract, such as flavonoids and glycosides, and conducted pharmacological studies [9]. One study showed that the Eucommia ulmoides glycoside in the extract can inhibit the expression of inflammatory mediators such as IL-6, TNF-α, and NF-κB [10]. In addition, studies have also shown that certain components in Eucommia ulmoides extract can affect the activity of enzymes such as cytochrome P450, thereby affecting drug metabolism and toxicity. This suggests that the active ingredients in Eucommia ulmoides extract may affect the pathogenesis of atherosclerosis through multiple pathways, including inhibiting cholesterol synthesis and TG degradation, and reducing inflammation [11-12].

Overall, Eucommia ulmoides extract may affect the pathogenesis of atherosclerosis through multiple pathways. The application of network pharmacology and molecular docking technology provides researchers with an effective screening tool to identify active compounds in Eucommia ulmoides extract and predict their role in the pathogenesis of atherosclerosis. However, the predicted results must be experimentally verified for feasibility. In addition, further in-depth studies are needed on the pharmacological properties and toxicity of active compounds in Eucommia ulmoides extract in order to better utilize them in the treatment of related diseases such as atherosclerosis.Dulate these pathways and contribute to the treatment of atherosclerosis.

Acknowledgement

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