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Exploring the mechanism of Erchen decoction in the treatment of atherosclerosis based on network pharmacology and molecular docking

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Abstract

Background: Atherosclerosis (AS) is the cause of most cardiovascular diseases and imposes a huge economic burden on society. Erchen decoction (ECD) is an effective formula for treating AS, but its therapeutic mechanism remains unclear. This study will explore the mechanism of ECD mechanism for treating AS using network pharmacology and molecular docking.

Methods: We searched ECD chemical composition information and related targets via Traditional Chinese Medicine Systems Pharmacology Database and Analysis Platform and SwissTargetPrediction databases, and gene names correction was performed using the UniProt database. AS-related targets were retrieved from OMIM, GeneCards, and DrugBank databases, and Venny 2.1 were used for intersection analysis. Protein-protein interaction network was constructed by the STRING database, and an interactive network of the drug-component-target-disease was drawn using the Cytoscape 3.9.0 software. Gene ontology and Kyoto Gene and Genome Encyclopedia enrichment analysis were performed by the DAVID database, and molecular docking validation of vital active ingredients and action targets of ECD was performed using AutoDock Vina software.

Results: The 127 active components of ECD act on AS by regulating 231 targets and 151 pathways. The 6 core components are quercetin, polyporenic acid C, 18α-hydroxyglycyrrhetic acid, glyuranolide, 3beta-hydroxychloroxy-24-methylene-8-lanostene-21-oic acid, and obacunone. They may regulate AS by regulating core target genes, such as JUN, SRC, AKT1, PTGS2, ESR1, AR, MAPK1, MAPK3, and RELA, and acting on multiple vital pathways, such as AGE-RAGE signaling pathway in diabetic complications, Lipid and AS, and Fluid shear stress and AS. Molecular docking showed that the selected target protein had good binding activity to the active ingredient.

Conclusions: ECD has the characteristics of multi-components, multi-targets and multi-pathways in the treatment of AS. The results provide a theoretical basis for the clinical application of ECD and its mechanism.

Abbreviations: AS = atherosclerosis, ATT = Arum ternatum Thunb, CER = Citri Exocarpium Rubrum, DL = drug-likeness, ECD = Erchen decoction, GO = gene ontology, KEGG = Kyoto Gene and Genome Encyclopedia, LDL = low density lipoprotein, NO = nitric oxide, PCW = Poria Cocos (Schw.) Wolf., PPI = protein-protein interaction, TCM = Traditional Chinese medicine, TCMSP = Traditional Chinese Medicine Systems Pharmacology Database and Analysis Platform.

Keywords: atherosclerosis, Erchen decoction, molecular docking, network pharmacology

1. Introduction

As a chronic inflammatory disease, atherosclerosis (AS) is mainly caused by endothelial injury, accumulation of lipids, smooth muscle cells, and necrotic cell debris. It is a progression from early endothelial dysfunction to the formation and destruction of late vulnerable plaques.[1,2] The growth of AS lesions can reduce blood flow in the lumen by > 50% and cause ischemia and hypoxia. The instability and rupture of lesions increase cardiovascular and cerebrovascular disease burden. Clinical complications can be seen in myocardial infarction and stroke, leading to high global mortality.[3] As China enters an aging society, the burden of cardiovascular disease is increasing, with 4 and 3 times as many absolute deaths from cardiovascular disease as in the United States and Western Europe, respectively,[4] and AS is the underlying pathologic cause of most acute and severe cardiovascular

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diseases.[5] The formation of AS is strongly associated with age, sex, obesity, smoking, inflammatory response, hypertension, diabetes, hyperlipidemia, intestinal microbiota, etc.[6–10] Current treatments include lifestyle changes, statins, and other therapies to treat and reduce low-density lipoprotein (LDL) cholesterol. However, due to the complex pathogenesis of AS, current drugs used for AS treatment are not fully effective in clinical practice. Long-term use of statins also causes statin-associated muscle symptoms, liver damage, insomnia, headaches, and other adverse reactions, as well as a greater residual risk of cardiovascular disease.[5,11–14] Traditional Chinese medicine (TCM) has advantages in AS therapy due to its multi-components, multi-targets, and multi-pathways properties.[15]

According to Chinese medicine, the main symptoms of AS are phlegm turbidity (Literally inflammation and lipid metabolism disorders) and blood stasis (Literally microcirculatory disruption, thrombosis, or obstruction). The occurrence of phlegm turbidity and blood stasis is strongly associated with the dysfunction of the spleen. The spleen is the source of phlegm and is responsible for transportation and transformation. When the transportation and transformation function of the spleen decreases, the transmission of qi, blood, fluid and water, and grain essence becomes obstructed, resulting in mutual stagnation of phlegm and stasis, which leads to AS over time. Erchen decoction (ECD) is from "Taiping Huimin Heji Jufang" of the Song Dynasty (around 960-1279 AD), which is a fundamental and representative formula for the clinical treatment of dampness and phlegm, with the effects of drying dampness, resolving phlegm, regulating qi and harmonizing the middle. Studies have shown that ECD has hypolipidemic, hypoglycemic, regulating lipid metabolism disorder, anti-inflammatory, etc.[16–19] ECD is composed of Arum ternatum Thunb (ATT, "Pinellia ternata" in English, "Banxia" in Chinese) 15 g, Citri Exocarpium Rubrum (CER, "Orange peel" in English, "Juhong" in Chinese) 15 g, Poria Cocos (Schw.) Wolf (PCW, "Poria cotta" in English, "Fuling" in Chinese) 9 g, and licorice ("Gancao" in Chinese) 4.5 g.

We all know that TCM complex composition and targets make it challenging to study clinical diseases because of its vague mechanism and unclear mechanism of action. However, the emergence of network pharmacology has broken the traditional drug development model of "one–disease, one–target, one–drug" and opened up a new research model of complex network relationships between multiple targets and multiple diseases.[20] Network pharmacology is an analytical approach based on system biology theory that allows the analysis of the interaction network of multiple factors such as drugs, protein targets, diseases, and genes at the systems level.[21] Molecular docking is a theoretical simulation method to study the interaction between small molecule ligands and protein receptors and predict the binding modes and affinities. Therefore, this study used network pharmacology to predict the chemical composition of ECD, the potential targets and key pathways of ECD in the treatment of AS, and used molecular docking technology to simulate the binding activity of active components and core targets, so as to provide a theoretical basis for exploring the mechanism of ECD in the treatment of AS. The detailed flowchart is shown in Figure 1.

2. Materials and methods

The data in the article come from public databases and do not involve human participants or animals or the personal data of any patient. Ethical approval and informed consent are not required.

2.1. ECD active ingredients and target genes database establishment

ECD is composed of 4 herbs including ATT, CER, PCW and licorice. The ingredients of these herbs were searched and collected from the Traditional Chinese Medicine Systems Pharmacology Database and Analysis Platform (TCMSP, https://tcmspw.com/index.php), and then the relevant active ingredients and corresponding target proteins were retrieved with the screening conditions of oral bioavailability ≥ 30% and drug-likeness (DL) ≥ 0.18. Corresponding target proteins for active ingredients not found in TCMSP were found in Canonical SMILES via PubChem (https://pubchem.ncbi.nlm.nih.gov/) and then to SwissTargetPrediction (http://www.swisstargetprediction.ch/) for predicted target protein download supplement. The UniProt database (https://www.uniprot.org/) was used for gene normalization, and the species was restricted to "Homo sapiens."

2.2. AS-related target genes database establishment

"Atherosclerosis" was used as the search term for disease targets in OMIM (https://omim.org/), GeneCards (https://www.genecards.org/), and DrugBank (https://go.drugbank.com/), and the target genes for AS were finally obtained by de-duplication.

2.3. Screening the common target genes of ECD and AS

The target genes of ECD and the target genes of AS were uploaded to the Venny 2.1 (https://bioinfogp.cnb.csic.es/tools/venny/) to obtain the intersection target genes, i.e., the potential target genes of ECD for AS.

2.4. Protein-protein interaction (PPI) network map of the ECD-AS-potential target genes

The intersection targets of ECD and AS were uploaded into the STRING database (https://string-db.org/), and the condition species was set to "Homo sapiens," the minimum required interaction score was set to 0.990, and the free nodes were cleared. These results were imported and visualized in Cytoscape version 3.9.0.[22]

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

The screened active ingredients of the 4 herbal medicines (ATT, CER, PCW, and licorice), the intersecting targets, and AS were used as targets. Cytoscape 3.9.0 software was used to construct a "drug-component-potential target gene-disease" network.

2.6. Gene ontology (GO) and Kyoto Gene and Genome Encyclopedia (KEGG) pathway enrichment analysis

The common targets of ECD and AS were imported into the DAVID database (https://david.ncifcrf.gov/) for enrichment analysis of GO and KEGG. The species-limited condition is "Homo sapiens." Visualizations were realized through the Bioinformatics platform (http://www.bioinformatics.com.cn/). And Cytoscape 3.9.0 software was applied to visualize the 20 most enriched pathways.

2.7. Molecular docking

The key active ingredients of ECD were loaded into SDF format files of their 2D structures via the PubChem database (https://pubchem.ncbi.nlm.nih.gov/), then imported into Chem3D for optimization and saved in mol2 format, and converted into pdbqt format using AutoDockTools 1.5.7 software. The crystal structures of the core target proteins were downloaded from the PDB database (https://www.rcsb.org/). Solvent and organic were removed using PyMol 2.5 software. AutoDockTools 1.5.7 software was used to add hydrogens and other modifications,

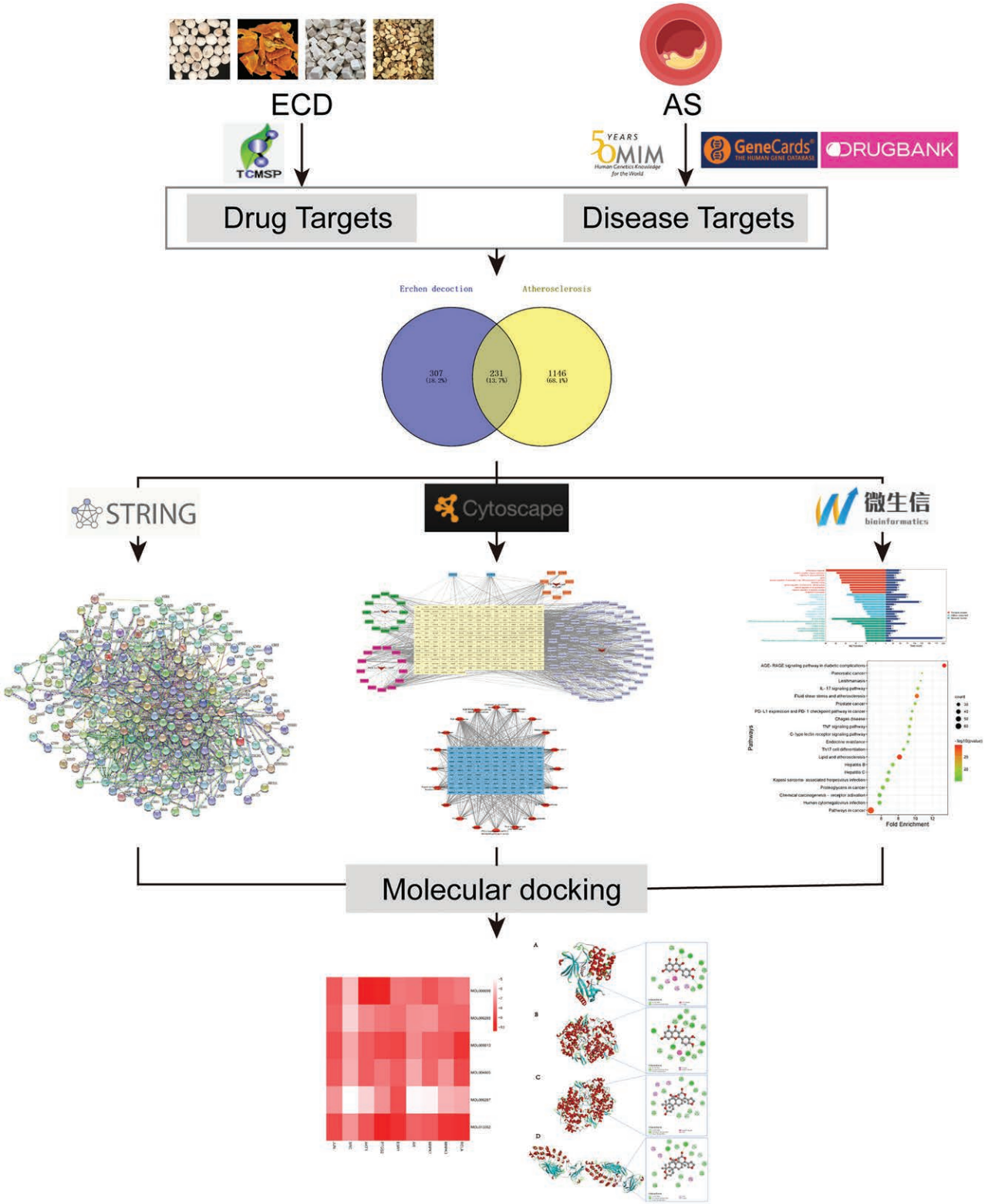

Figure 1. Network pharmacological study of ECD for the treatment of AS diagram. AS = atherosclerosis, ECD = Erchen decoction.

save proteins in pdbqt format, and adjust the grid box to include all protein structures. Save the parameter information of the box and use AutoDock Vina software to batch process the semi-flexible docking of ligand small molecules with receptor protein macromolecules. The calculated binding energy scores were used to evaluate the binding activity. We selected 6 key active ingredients and 9 core targets for molecular docking, and 20 binding energy sites were retained for each pair of docking. The conformation with the highest affinity was selected as the final docked conformation. Docking results were analyzed and visualized using Discovery Studio 2018 software for the first 4 models with the lowest binding energy.

3. Results

3.1. ECD active ingredients and target genes database establishment

The components of ECD were searched through the TCMSP database, and 127 active ingredients of ECD were obtained with oral bioavailability ≥ 30% and DL ≥ 0.18 as screening conditions. In descending order according to DL, these include (-)-Medicocarpin, Kanzonol F, Xambioona, Isoglycyrol and dehydroeburicoic acid, as shown in Table 1. The numbers of active ingredients were 13,9,15 and 92 for ATT, CER, PCW and Licorice, respectively. Among them, ATT and CER have a common component (beta-sitosterol), and CER and licorice have a common ingredient (naringenin). After removing duplicates of target genes corresponding to 127 active ingredients, a total of 538 drug targets were collected.

3.2. AS-related target genes database establishment

The GeneCards database was searched, and 4739 AS-related disease targets were obtained, sorted by Relevance score value from highest to lowest. Relevance scores > 1 were taken as a potential target for AS. Then we combined OMIM and DrugBank databases to add relevant targets and remove duplicate targets and finally obtained 1377 AS-related targets.

3.3. Screening the common target genes of ECD and AS

The 538 drug targets and 1377 disease targets were input into Venny 2.1 software to draw a Venn diagram, and 231 common targets were obtained after taking the intersection of the 2, i.e., 231 potential targets for ECD treatment of AS, as shown in Figure 2A.

3.4. PPI network map of the ECD-AS-potential target genes

Enter 231 common targets in the STRING database to obtain the PPI network diagram, as shown in Figure 2B. The top 25 genes were identified and listed by the R package, as shown in Figure 2C. These genes include JUN, SRC, AKT1, RELA, STAT3, TP53, MAPK3, MAPK1, RXRA, MAPK14, ESR1, PIK3CA, TNF, FOS, EGFR, IL6, CAV1, HDAC1, MAPK8, STAT1, NR3C1, AR, ITGB1, RB1 and VEGFA.

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

The drug-component-target-disease network was constructed by Cytoscape 3.9.0 software to reflect the complex relationship of ECD in the treatment of AS, as shown in Figure 3. Using Analyze Network, the top 6 target genes were PTGS2, ESR1, AR, NOS2, PPARG and ESR2 in descending order of node network degree, suggesting that these targets play an essential role in the regulatory network of ECD for AS. The first 6 compounds in order are MOL000098 (quercetin), MOL000285 (polyporenic acid C), MOL005013 (18α-hydroxyglycyrrhetic acid), MOL004905 (glyuranolide), MOL000287 (3beta-hydroxy-24-methylene-8-lanostene-21-oic acid) and MOL013352 (obacunone), suggesting that these compounds may be the main active ingredients in the treatment of AS.

3.6. GO and KEGG pathway enrichment analysis

A total of 559 biological processes, 101 cellular components, and 108 molecular functions were obtained from 231 common targets by GO enrichment analysis with $P < .01$ as the screening condition. The top 10 entries were selected separately for visualization through the Bioinformatics platform, as shown in Figure 4. Biological processes included inflammatory response, positive regulation of gene expression, response to lipopolysaccharide, aging, positive regulation of transcription from RNA polymerase II promoter, etc. cellular components included extracellular space, extracellular region, cell surface, plasma membrane, external side of plasma membrane, etc. molecular functions included enzyme binding, "RNA polymerase II transcription factor activity, ligand-activated sequence-specific DNA binding," identical protein binding, integrin binding, serine-type endopeptidase activity, etc.

By the KEGG pathway enrichment analysis, a total of 151 pathways were obtained that were significantly enriched for potential targets of ECD treatment for AS ($P < .01$). P value closer to 0, the more significant enrichment. The larger the Fold enrichment, the higher the gene enrichment. The top 20 pathways were graphed using the Bioinformatics platform according to the P value ranking, as shown in Figure 5. The top-ranked pathways include AGE-RAGE signaling pathway in diabetic complications, Lipid and AS, Pathways in cancer, Fluid shear stress and AS, and Kaposi sarcoma-associated herpesvirus infection. This suggests that the active components of ECD act

Table 1

The active ingredients in Erchen decoction. (Only the top 15 compounds of DL are shown, the rest are shown in Supplementary Table 1 http://links.lww.com/MD/K765).

Mol ID	Molecule name	OB (%)	DL	Drug
MOL004924	(-)-Medicocarpin	40.99	0.95	Licorice
MOL004988	Kanzonol F	32.47	0.89	Licorice
MOL005018	Xambioona	54.85	0.87	Licorice
MOL004948	Isoglycyrol	44.70	0.84	Licorice
MOL000300	Dehydroeburicoic acid	44.17	0.83	PCW
MOL000285	Polyporenic acid C	38.26	0.82	PCW
MOL000280	(2R)-2-[(3S,5R,10S,13R,14R,16R,17R)-3,16-dihydroxy-4,4,10,13,14-pentamethyl-2,3,5,6,12,15,16,17-octahydro-1H-cyclopenta[a]phenanthren-17-yl]-5-isopropyl-hex-5-enoic acid	31.07	0.82	PCW
MOL000283	Ergosterol peroxide	40.36	0.81	PCW
MOL000287	3Beta-hydroxy-24-methylene-8-lanostene-21-oic acid	38.70	0.81	PCW
MOL002670	Cavidine	35.64	0.81	ATT
MOL000276	7,9(11)-Dehydropachymic acid	35.11	0.81	PCW
MOL000289	Pachymic acid	33.63	0.81	PCW
MOL000273	(2R)-2-[(3S,5R,10S,13R,14R,16R,17R)-3,16-dihydroxy-4,4,10,13,14-pentamethyl-2,3,5,6,12,15,16,17-octahydro-1H-cyclopenta[a]phenanthren-17-yl]-6-methylhept-5-enoic acid	30.93	0.81	PCW
MOL000275	Trametenolic acid	38.71	0.80	PCW
MOL004917	Glycyroside	37.25	0.79	licorice

ATT = Arum ternatum Thunb, DL = drug likeness, OB = oral bioavailability, PCW = Poria Cocos (Schw.) Wolf.

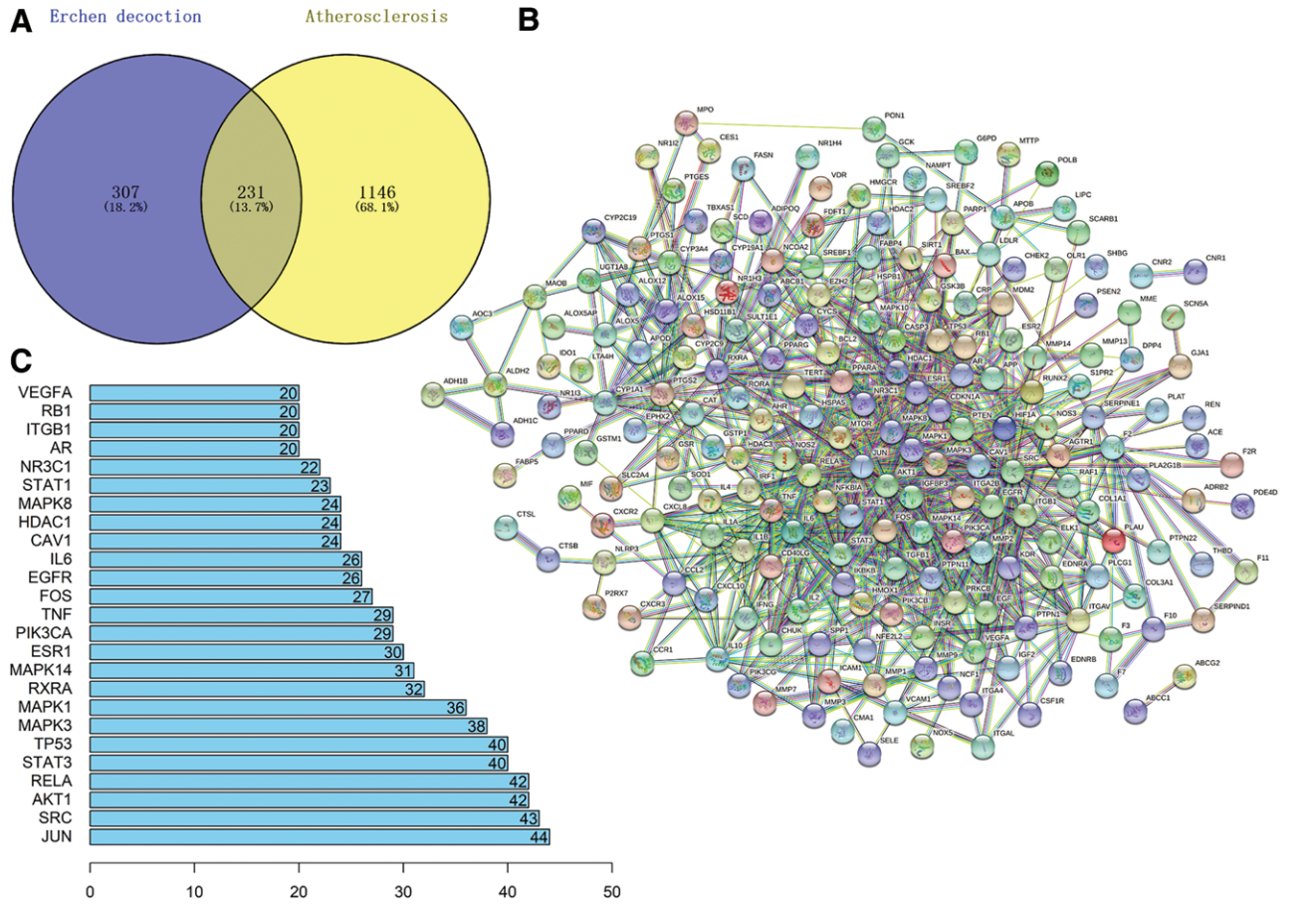

Figure 2. Potential target genes and PPI network map of ECD therapy for AS. (A) The Venn diagram of ECD and AS intersection targets. The overlap indicates potential target genes for ECD treatment of AS. (B) The PPI network map of ECD therapy for AS. (C) Bar plot of the number of top 25 key targets in PPI network map. AS = atherosclerosis, ECD = Erchen decoction, PPI = protein–protein interaction.

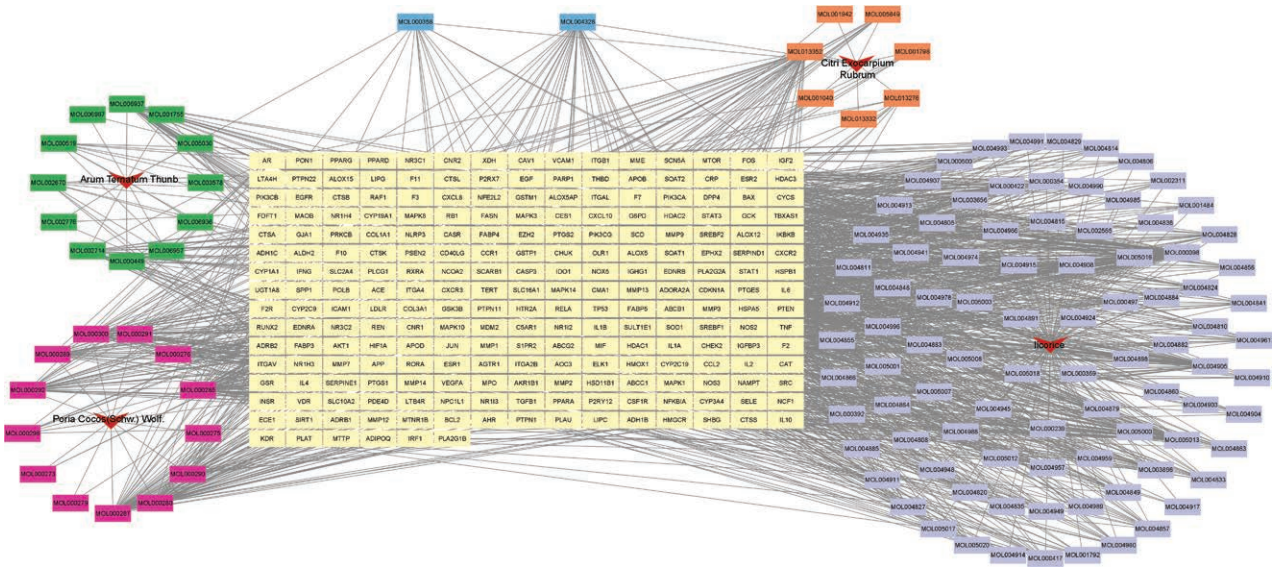

Figure 3. Drug-component-target-disease network diagram. Yellow represents the common target of drug-disease; red represents drugs; other colors represent the active ingredient of each drug.

primarily through these pathways in the antiatherogenic process. The top 20 pathways and their enriched genes were visualized using Cytoscape 3.9.0 to map the gene-pathway network, as shown in Figure 6. The top 6 high-frequency genes enriched in these pathways are MAPK1, MAPK3, RELA, AKT1, PIK3CA and PIK3CB, as shown in Table 2.

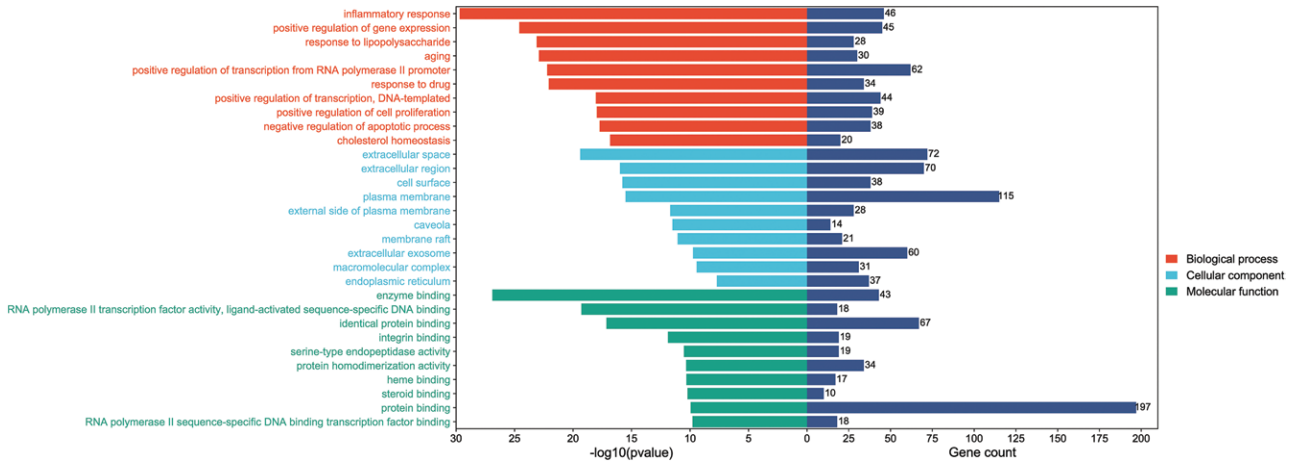

Figure 4. Left and right bilateral bar graph of the top 10 significantly enriched terms in the GO BP, CC, and MF. The y-axis shows the top 10 significantly enriched BP, CC, and MF categories, while the x-axis shows the -log10(*P* value) and the number of enriched genes for these terms. BP = biological processes, CC = cell component, GO = gene ontology, MF = molecular function.

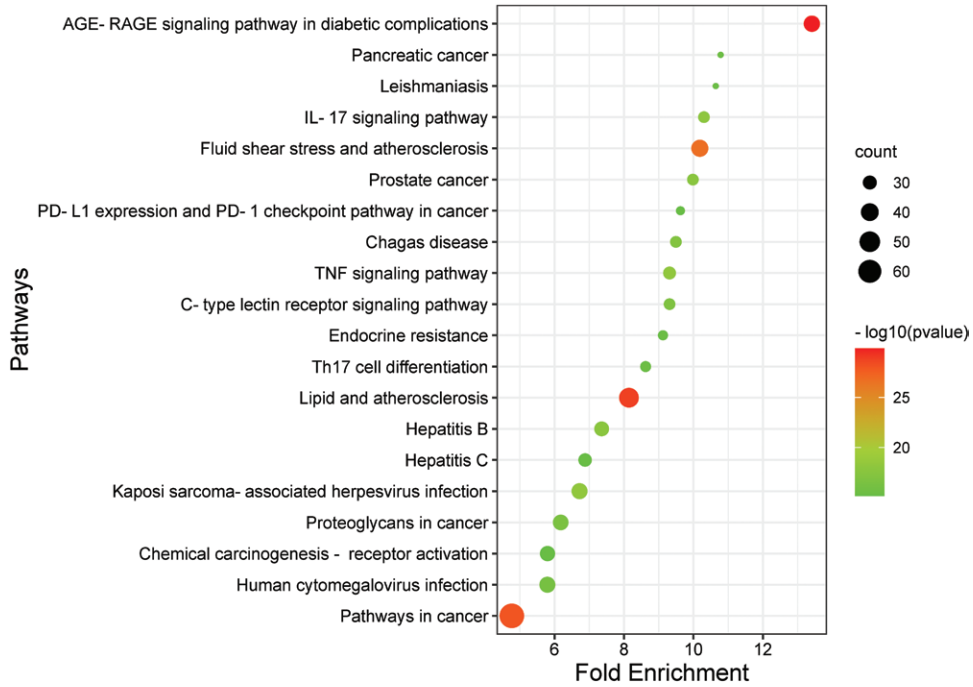

Figure 5. Bubble chart of the top 20 significantly enriched terms in KEGG pathways. The y-axis shows the top 20 significantly enriched KEGG pathways, while the x-axis shows Fold enrichment. The color indicates a range of *P* values; the redder it is, the more significant the enrichment. The size indicates the number of different genes; the larger it is, the greater the number of genes. KEGG = Kyoto encyclopedia of genes and genomes.

3.7. Molecular docking

The top 3 target proteins of the PPI network result, the top 3 target proteins of the drug-component-target-disease Network, and the top 3 target proteins of the KEGG enrichment pathway were verified by molecular docking with the top 6 core components of the drug-component-target-disease Network. The final results of 54 sets of receptor-ligand docking were obtained, as shown in Table 3 and Figure 7. The results of the first 4 lowest binding energies showed: the binding energy of AKT1(PDB ID: 7nh5) and quercetin was −10.4 kcal/mol, and the binding energy of PTGS2 (PDB ID: 5ikq) binding capacity of quercetin −9.9 kcal/mol, PTGS2 (PDB ID: 5ikq) binding capacity of obacunone −9.9 kcal/mol, RELA (PDB ID: 1nfi) binding capacity of obacunone was −9.5 kcal/mol, and the visualization results were shown in Figure 8.

We found that the ligand molecule quercetin establishes relatively stable hydrogen bonding connections with amino acid residues ASN A:204, SER A:205, ILE A:290, and THR A:291 of the receptor AKT1, of which amino acid residue ASN A:204 produces 2 hydrogen bonding connections with quercetin. We speculate that hydrogen bonding is the main force that dominates the binding of AKT1 to quercetin. In addition, the binding of AKT1 and quercetin was also affected by Van der Waals forces and Pi-Pi Stacked (Fig. 8A); Quercetin bound to amino acid residues ARG B:377, GLN A:374, TYR B:374, GLY B:534, ASN B:538, and ASN B:376 of PTGS2 by hydrogen bonding with high overall binding strength. Extensive Van der Waals forces were also generated with numerous amino acid residues such as GLN B:375, PRO A:127, LYS B:533, and VAL B:229, which may be related to the binding pocket conformation of the

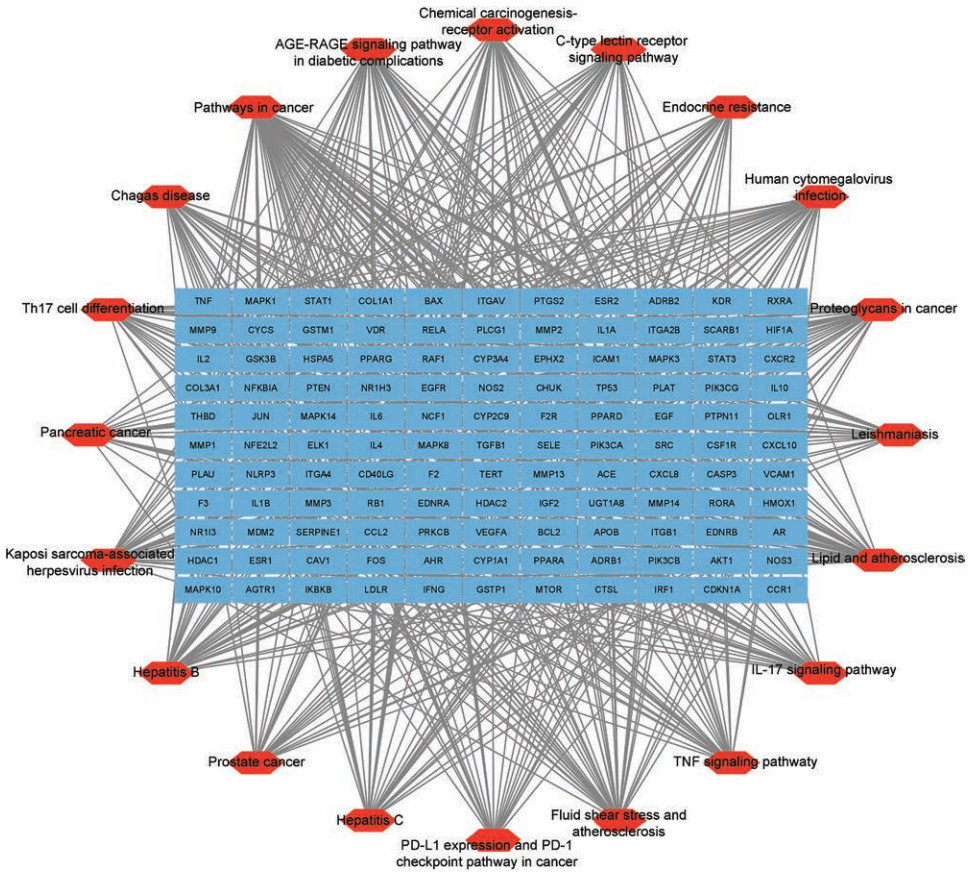

Figure 6. Gene-pathway. Red indicates the pathway; Blue indicates the targets rich in the pathway.

Table 2

Top 6 genes on the KEGG pathway enrichment.

Gene	Count	Pathways
MAPK1	19	AGE-RAGE signaling pathway in diabetic complications/Lipid and atherosclerosis/Pathways in cancer/Kaposi sarcoma-associated herpesvirus infection/TNF signaling pathway/IL-17 signaling pathway/Hepatitis B/Prostate cancer/Chagas disease/C-type lectin receptor signaling pathway/Proteoglycans in cancer/Human cytomegalovirus infection/Pancreatic cancer/Leishmaniasis/Th17 cell differentiation/Endocrine resistance/Hepatitis C/PD-L1 expression and PD-1 checkpoint pathway in cancer/Chemical carcinogenesis—receptor activation
MAPK3	19	AGE-RAGE signaling pathway in diabetic complications/Lipid and atherosclerosis/Pathways in cancer/Kaposi sarcoma-associated herpesvirus infection/TNF signaling pathway/IL-17 signaling pathway/Hepatitis B/Prostate cancer/Chagas disease/C-type lectin receptor signaling pathway/Proteoglycans in cancer/Human cytomegalovirus infection/Pancreatic cancer/Leishmaniasis/Th17 cell differentiation/Endocrine resistance/Hepatitis C/PD-L1 expression and PD-1 checkpoint pathway in cancer/Chemical carcinogenesis—receptor activation
RELA	18	AGE-RAGE signaling pathway in diabetic complications/Lipid and atherosclerosis/Pathways in cancer/Fluid shear stress and atherosclerosis/Kaposi sarcoma-associated herpesvirus infection/TNF signaling pathway/IL-17 signaling pathway/Hepatitis B/Prostate cancer/Chagas disease/C-type lectin receptor signaling pathway/Human cytomegalovirus infection/Pancreatic cancer/Leishmaniasis/Th17 cell differentiation/Hepatitis C/PD-L1 expression and PD-1 checkpoint pathway in cancer/Chemical carcinogenesis—receptor activation
AKT1	17	AGE-RAGE signaling pathway in diabetic complications/Lipid and atherosclerosis/Pathways in cancer/Fluid shear stress and atherosclerosis/Kaposi sarcoma-associated herpesvirus infection/TNF signaling pathway/Hepatitis B/Prostate cancer/Chagas disease/C-type lectin receptor signaling pathway/Proteoglycans in cancer/Human cytomegalovirus infection/Pancreatic cancer/Endocrine resistance/Hepatitis C/PD-L1 expression and PD-1 checkpoint pathway in cancer/Chemical carcinogenesis—receptor activation
PIK3CA	17	AGE-RAGE signaling pathway in diabetic complications/Lipid and atherosclerosis/Pathways in cancer/Fluid shear stress and atherosclerosis/Kaposi sarcoma-associated herpesvirus infection/TNF signaling pathway/Hepatitis B/Prostate cancer/Chagas disease/C-type lectin receptor signaling pathway/Proteoglycans in cancer/Human cytomegalovirus infection/Pancreatic cancer/Endocrine resistance/Hepatitis C/PD-L1 expression and PD-1 checkpoint pathway in cancer/Chemical carcinogenesis—receptor activation
PIK3CB	17	AGE-RAGE signaling pathway in diabetic complications/Lipid and atherosclerosis/Pathways in cancer/Fluid shear stress and atherosclerosis/Kaposi sarcoma-associated herpesvirus infection/TNF signaling pathway/Hepatitis B/Prostate cancer/Chagas disease/C-type lectin receptor signaling pathway/Proteoglycans in cancer/Human cytomegalovirus infection/Pancreatic cancer/Endocrine resistance/Hepatitis C/PD-L1 expression and PD-1 checkpoint pathway in cancer/Chemical carcinogenesis—receptor activation

KEGG = Kyoto Gene and Genome Encyclopedia.

Table 3

Molecular docking results of key active ingredients and the core targets of ECD.

Mol ID	Active ingredient	Binding affinity (kcal/mol)								
		JUN	SRC	AKT1	PTGS2	ESR1	AR	MAPK1	MAPK3	RELA
MOL000098	Quercetin	−8.4	−6.2	−10.4	−9.9	−7.7	−7.8	−8.5	−7.9	−7.6
MOL000285	Polyporenic acid C	−8.2	−5.8	−7.3	−7.7	−7.9	−7.2	−7.1	−8.0	−8.1
MOL005013	18α-Hydroxyglycyrrhetic acid	−8.7	−6.7	−8.3	−8.8	−8.9	−7.3	−8.2	−8.3	−9.3
MOL004905	Glyuranolide	−8.4	−6.5	−8.3	−7.7	−8.3	−6.6	−8.1	−7.1	−8.8
MOL000287	3Beta-hydroxy-24-methylene-8-lanostene-21-oic acid	−7.1	−4.8	−5.8	−7.2	−8.7	−5.0	−5.1	−6.4	−6.6
MOL013352	Obacunone	−8.8	−7.1	−8.5	−9.9	−9.4	−8.2	−8.4	−9.3	−9.5

ECD = Erchen decoction.

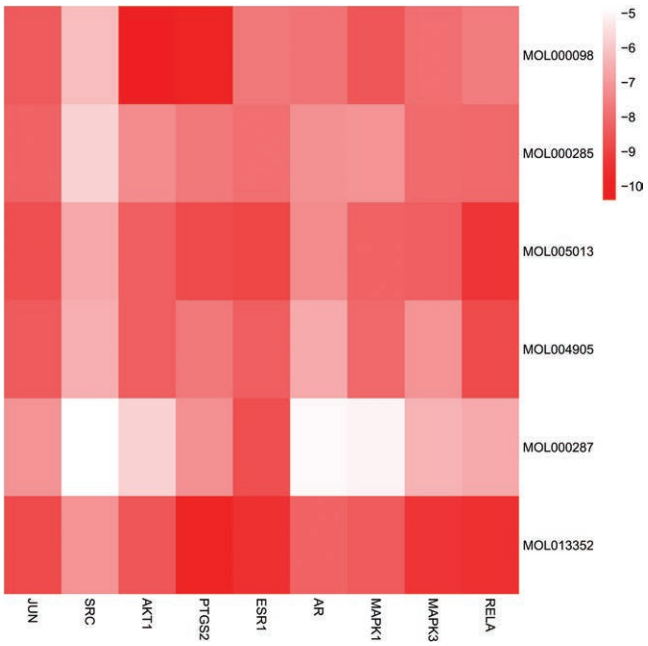

Figure 7. Binding energy heatmap of molecular docking. The redder the color represents, the lower the binding energy.

protein (Fig. 8B); obacunone produced only one hydrogen-bonding interaction with GLN B:373 of PTGS2. Still, multiple Van der Waals interaction forces were also present, from which we concluded that Van der Waals forces dominated the molecular docking process in this group, which was influenced by both hydrogen bonding and hydrophobic interactions (Fig. 8C); The binding of obacunone to RELA exhibits similar characteristics to obacunone-PTGS2, with widespread Van der Waals forces dominating the binding of both ligand and receptor. At the same time, hydrogen bonding and hydrophobic interactions are secondary auxiliary binding elements (Fig. 8D).

4. Discussion

AS is a chronic inflammatory disease that occurs primarily in middle-aged and older adults over 40. In recent years, the age of onset has been getting younger. Western medicine is widely used in clinical practice but is not always effective and has specific side effects.[11–14] As a natural product, Chinese herbal medicine is an excellent supplement for reducing toxic side effects, reducing the dosage of western medicine and improving patients' quality of life, which has attracted more and more attention in recent years. There is no disease name "atherosclerosis" in TCM, which mainly belongs to the "mai bi" category according to its clinical manifestations. The location of the disease is in the pulse, and the pathogenesis is closely related to phlegm turbidity. Dan Xi Xin Fa (Zhu Danxi Experience in Practicing Medicine, written in 1347) said, "The method of treating phlegm is to strengthen the spleen soil and dry spleen dampness, which is the root of the treatment." Therefore, the treatment should strengthen the spleen, dry dampness and dissipate phlegm. The representative prescription is ECD. ECD has a significant clinical effect on treating AS after syndrome differentiation and treatment. However, due to TCM complex composition and targets, its therapeutic mechanism still needs to be clarified. Therefore, this study used network pharmacology and molecular docking to investigate ECD potential mechanism for treating AS.

When performing PPI network analysis of ECD potential targets for treating AS, we listed the top 25 targets and selected the top 3 targets for later molecular docking, which were JUN, SRC and AKT1. These 3 targets are mainly related to tumor formation, angiogenesis and cell growth. In the drug-component-target-disease Network, we obtained the top 6 targets and the top 6 active components of ECD in treating AS. The targets were PTGS2, ESR1, AR, NOS2, PPARG and ESR2. These targets are mainly related to cancer, inflammatory response, lipid metabolism and maintenance of the normal function of the cardiovascular systems, etc. We selected the first 3 targets as receptors for further molecular docking. The key active ingredients were quercetin, polyporenic acid C, 18α-hydroxyglycyrrhetic acid, glyuranolide, 3beta-hydroxy-24-methylene-8-lanostene-21-oic acid and obacunone. These may be the core components of ECD therapy for AS, and we treated them as ligands for late molecular docking.

We performed GO enrichment analysis for 231 common targets. It was found that ECD affected inflammatory response, response to lipopolysaccharide, aging, extracellular space, cell surface, plasma membrane, etc., to treat AS. In the KEGG enrichment analysis, according to the *P* value, the main pathways of ECD were AGE-RAGE signaling pathway in diabetic complications, Lipid and AS, and Pathways in cancer. According to the count of enriched genes, the main pathways of ECD were Pathways in cancer, Lipid and AS, and Fluid shear stress and AS. In order of Fold enrichment, the main pathways of ECD were AGE-RAGE signaling pathway in diabetic complications, Pancreatic cancer, and Leishmaniasis. As you can see, AGE-RAGE signaling pathway in diabetic complications, Lipid and AS, Pathways in cancer, Fluid shear stress and AS may be the core pathways for ECD to play a therapeutic role. AGE-RAGE signaling pathway plays a key role in AS, especially in diabetes. AGE binds to RAGE and activates a series of inflammatory and fibrotic pathways (pro-inflammatory and pro-fibrotic), causing tissue injury and resulting in the inflammatory lesions of many disorders, such as AS. Studies have shown that inhibiting the accumulation of AGE and the expression of RAGE in diabetes can play a vascular protective role and improve AS.[23,24] There are many studies on Lipid and AS pathways.[25–27] Under normal circumstances, the absorption, consumption and transformation of lipids in the body are relatively balanced, so the blood lipid content of the human body is stable. In lipid metabolism disorders, the accumulation of yellow atheromatous

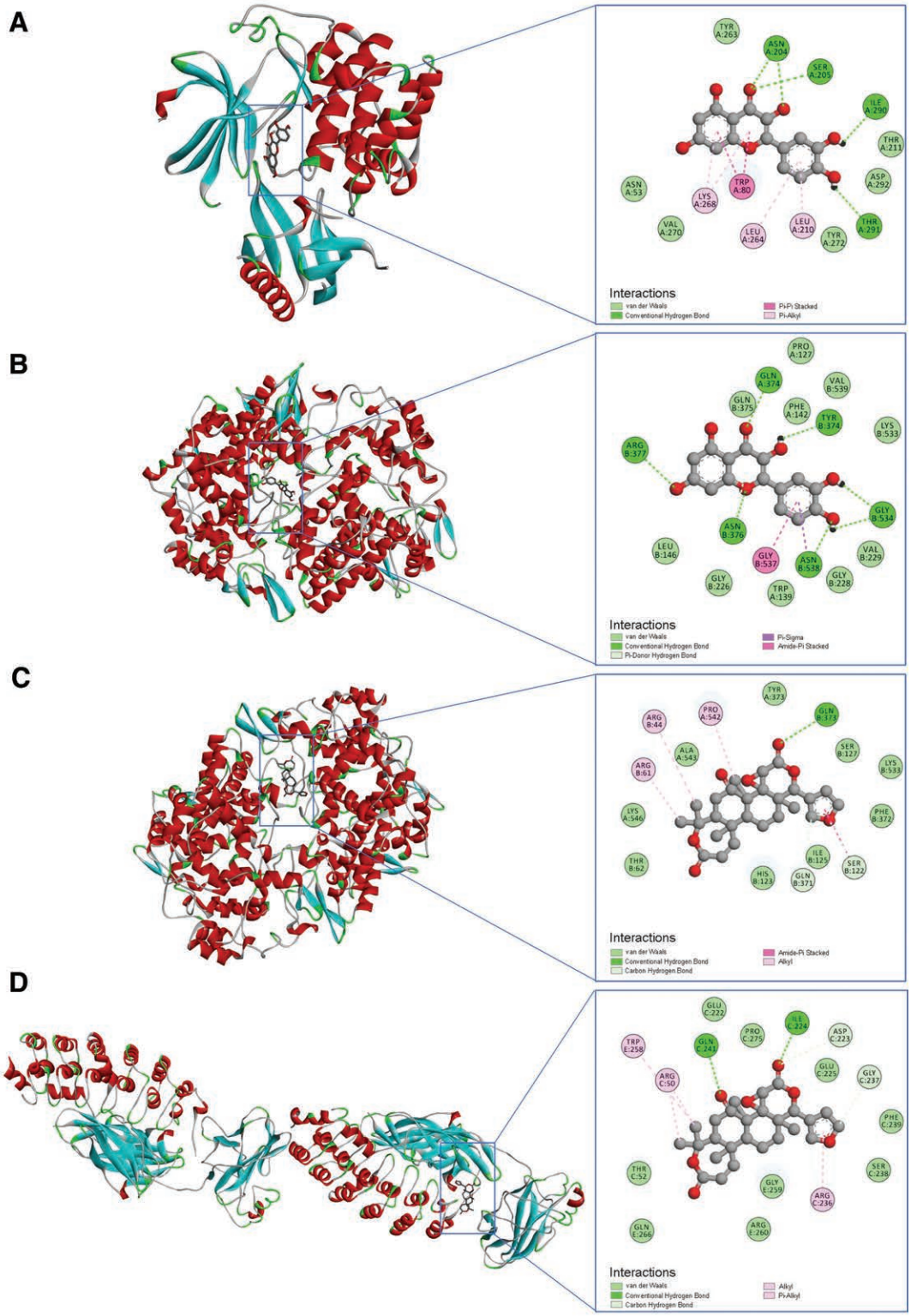

Figure 8. The top 4 significant molecular docking. (A) The docking diagram of AKT1 and quercetin (binding energy = −10.4 kcal/mol). (B) The docking diagram of PTGS2 and quercetin (binding energy = −9.9 kcal/mol). (C) The docking diagram of PTGS2 and obacunone (binding energy = −9.9 kcal/mol). (D) The docking diagram of RELA and obacunone (binding energy = −9.5 kcal/mol).

lipids in the intima of the artery leads to an abnormal increase in blood lipids and causes AS. Studies have found a molecular link between atherogenesis and tumorigenesis. Such as oxidized LDL (ox-LDL) and its receptor lectin-like oxidized LDL receptor-1 (LOX-1) is closely related to the development of AS and involved in a variety of mechanisms closely related to the development of cancers, which indicates that the pathophysiological mechanisms of AS and tumorigenesis overlap.[28,29] In addition, studies on Fluid shear stress and AS pathway are common. Atherosclerotic lesions are found primarily in curved arteries

and near side branches, where flow perturbations generate shear stress.[30,31] Different shear stresses can affect the gene expression of endothelial cells, and endothelial cell dysfunction plays an important role in AS. Many studies have shown that flow shear stress affects the structure and function of vascular endothelial cells by regulating kinase activity, inducing vascular inflammation, and regulating the genetic phenotype of endothelial cells, thereby affecting the occurrence and development of atherosclerotic lesions.[32,33] The results of targets enriched in pathways showed connections between MAPK1, MAPK3, RELA, AKT1, PIK3CA and PIK3CB and more pathways, suggesting that these genes play an important role in the mechanism of action of ECD in the treatment of AS. These genes are mainly related to inflammatory response, angiogenesis and tumor. The first 3 genes were selected as receptors for molecular docking.

Molecular docking technique was used to evaluate the binding activity of 9 core proteins (JUN, SRC, AKT1, PTGS2, ESR1, AR, MAPK1, MAPK3, and RELA) and 6 core components (quercetin, polyporenic acid C, 18α-hydroxyglycyrrhetic acid, glyuranolide, 3beta-hydroxy-24-methylene-8-lanostene-21-oic acid, and obacunone). The lower the binding energy is, the more stable the ligand binds to the receptor.[34] The results showed that all docking Vina scores were less than –4.25 kcal/mol, indicating that the core components of ECD in treating AS have a certain binding ability to each core target. The 4 groups with the best binding affinity were quercetin and AKT1, quercetin and PTGS2, obacunone and PTGS2, obacunone and RELA. Their binding energies were –10.4, –9.9, –9.9, and –9.5 kcal/mol, respectively, indicating a good docking effect between them. Quercetin and obacunone have anti-inflammatory, anti-oxidation, hypoglycemic and protective effects on vascular endothelial cells, which have positive significance for treating cardiovascular diseases such as AS. Luo et al showed that quercetin prevents atherogenesis by targeting KEAP1/NRF2 interactions to suppress macrophage pyroptosis.[35] Luo et al showed that quercetin alleviates AS by suppressing oxidized LDL-induced senescence in plaque macrophages by inhibiting the p38MAPK/p16 pathway.[36] Rey et al showed that quercetin can ameliorate AS by influencing gut microbes and dietary microbiota-accessible carbohydrates.[37] In addition, studies have shown that quercetin can reduce lipid deposition, reduce total cholesterol and LDL in the blood, improve antioxidant capacity and inhibit vascular inflammation from exerting anti-AS effects.[38–40] Obacunone is a limonin triterpenoid compound mainly distributed in citrus plants of the Rutaceae family. It has various pharmacological effects such as anti-inflammatory, anti-oxidation, anti-fibrosis, and prevention of obesity and hyperglycemia.[41–45] Studies have shown that obacunone can prevent cardiovascular diseases caused by endothelial dysfunction, such as Rosaceae, by inhibiting arginase activity and increasing nitric oxide production.[46] AKT, also known as PKB (protein kinase B), is a signal transduction molecule that regulates many processes, including metabolism, proliferation, cell survival, and angiogenesis.[47] It is a key node in the PI3K-Akt signaling pathway. Studies have shown that when the PI3K-Akt signaling pathway is dysregulated, the phosphorylation of AKT and endothelial nitric oxide synthase is inhibited, which will accelerate endothelial cell apoptosis and inflammation, and accelerate the formation of AS.[48] PTGS2, also known as COX-2 (cyclooxygenase-2), is a key enzyme in the biosynthesis of prostaglandin. PTGS induces inflammatory responses and is associated with cardiovascular diseases such as atherosclerotic thrombosis.[49,50] RELA is a Protein Coding gene. The RELA gene plays an extremely important role in the NF-κB signaling pathway, a cellular signaling system that regulates inflammation. Studies have shown that inhibiting NF-κB phosphorylation significantly reduces the inflammatory response and improves AS.[51,52]

However, there are some limitations to network pharmacology. When we use network pharmacology to study the efficacy of active ingredients in prescription decoctions, the method simply superimposes the chemical constituents of single herbs. However, complex chemical reactions occur during the decoction of Chinese medicines, which may damage the original active ingredients or produce new active ingredients. The present study did not take into account the complexity of the chemical reactions occurring during the boiling process of the herbal compound ingredients and the influence of the metabolites of the active ingredients in the human body, and further experimental validation is required to determine whether the chemical composition in the medicinal solution has changed and whether this change has had an impact on the therapeutic effects we have analyzed. In addition, network pharmacology is based on conclusions drawn from analyses of existing experimental results, and there may still be targets and pathways that have not yet been identified. Despite some unavoidable limitations in the study of network pharmacology, it still provides a multidimensional research strategy for complex herbal formulations.

5. Conclusions

In summary, this study initially explored the mechanism of ECD in treating AS based on network pharmacology and molecular docking. The main active ingredients of ECD are quercetin, polyporenic acid C, 18α-hydroxyglycyrrhetic acid, glyuranolide, 3beta-hydroxy-24-methylene-8-lanostene-21-oic acid, obacunone, etc. They may regulate AGE-RAGE signaling pathway in diabetic complications, Lipid and AS, Pathways in cancer, Fluid shear stress and AS, and other signaling pathways through multiple targets such as JUN, SRC, AKT1, PTGS2, ESR1, AR, MAPK1, MAPK3, RELA, etc. to interfere with inflammatory response, response to lipopolysaccharide, aging, extracellular space, cell surface, plasma membrane, and other processes or functions against AS. In addition, we have verified that the core components of ECD have good binding activity to the core targets of AS using molecular docking techniques. The results showed that ECD has multi-component, multi-target and multi-pathway characteristics for treating AS. The results of this study provide a theoretical basis for the mechanism of ECD in the treatment of AS and its clinical ap-plication. However, further validated experiments are still required.

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