Potential active compounds and molecular mechanism of Xuefu Zhuyu decoction for atherosclerosis, based on network pharmacology and molecular docking

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Abstract
To explore the potential active compounds and molecular mechanism of Xuefu Zhuyu decoction (XFZYD) in the treatment of atherosclerosis (AS) based on network pharmacology and molecular docking.

The effective components and action targets of XFZYD were screened by using TCMSP database. And then, the action targets of AS were collected by GeneCards database. The intersection targets between the effective components' targets of XFZYD and AS-related action targets were used to construct PPI networks. GO and Kyoto Encyclopedia of Genes and Genomes enrichment analysis were performed on these intersection targets. Finally, molecular docking software was used to excavate the active compounds of the core targets VEGFA and AKT1.

We detected 225 active components of XFZYD, and found that quercetin, kaempferol, luteolin, naringenin, β-sitosterol, isorhamnetin, stigmasterol, baicalein, nobiletin, and β-carotene are the potential active compounds of XFZYD; STAT3, IL6, JUN, VEGFA, MAPK14, and AKT1 are the core target proteins of the active compounds, among which VEGFA and AKT1 are the key target proteins. PPI network results showed that β-carotene, quercetin, kaempferol, luteolin, and naringenin had higher degree values and more corresponding targets than other 5 active compounds and had the stable binding ability to regulatory proteins VEGFA and AKT1. The core components β-carotene, quercetin, kaempferol, and luteolin exerted their therapeutic effects on AS by acting on the key target proteins VEGFA and AKT1 to regulate fluid shear stress and the AGE-RAGE signaling pathway and IL-17 signaling pathway of diabetic complications of AS. The molecular docking results showed that VEGFA and AKT1 had great docking ability with the targeted active compounds, and β-carotene is the best.

The active components of XFZYD, including β-carotene, quercetin, kamanol, and luteolin, can act on VEGFA and AKT1. These active ingredients play a role in alleviating and treating AS by regulating fluid shear stress and participating in signaling pathways such as AGE-RAGE of atherosclerosis and diabetes mellitus complicated with AS. β-carotene is a potential inhibitor of VEGFA and AKT1 and treats AS through antioxidant action.

Abbreviations: AS = atherosclerosis, GO = Gene Ontology, KEGG = Kyoto Encyclopedia of Genes and Genomes, OB = Oral absorption efficiency, TCM = Traditional Chinese Medicine, XFZYD = Xuefu Zhuyu decoction.

Key Words: AKT1, atherosclerosis, molecular docking, network pharmacology, VEGFA, Xuefu Zhuyu decoction, β-carotene

1. Introduction
Atherosclerosis (AS) is a critical cause of many cardiovascular and neurological diseases. (1) Statins are currently restricted in their use in the clinic due to liver and muscle damages, (2) so it is of practical significance to find more safe and effective drugs to prevent AS. In Traditional Chinese Medicine (TCM), AS belongs to the category of “pulse carbuncle” and “pulse paralysis,” which...
could affect the blood vessels. AS can result in cardiovascular disease such as strokes, myocardial infarction, and chest arthralgia. Blood stasis, according to ancient doctors, was astringent and stagnant, gathered heat and formed poison, and toxic evil was most likely to injure muscles and veins. A therapeutic treatment strategy involves improving circulation and reducing blood stasis.

The Xuefu Zhuyu decoction (XFZYD), created by Wang Qingren in the Qing Dynasty, is the therapy for blood stasis which includes Persicae semen (Taoren), Carthami flos (Honghua), Angelicae sinensis radix (Danggui), Rehmanniae radix (Dihuang), Achyranthes bidentatae radix (Niuxi), Chuanxiong rhizome (Chuanxiong), Platycodonis radix (Jiegeng), Paeoniae radix rubra (Chishao), Aurantii fructus (Zhiqiao), and Bupleuri radix (Chaihu). It can improve blood flow, reduce blood congestion, boosts energy, and relieves pain. Clinical research reported that XFZYD can significantly improve microcirculation, expand microvessels, increase tissue perfusion, alleviates clinical symptoms, and so on. Many diseases, including myocardial fibrosis, AS, hypertension, unstable angina pectoris, and myocardial ischemia-reperfusion injury, have been treated using XFZYD. However, more research is needed into the material basis and molecular targets of multicomponent, multichannel, and multitarget XFZYD in the prevention and treatment of AS to give an evidence-based foundation for clinical wide application and new drug development.

Network pharmacology has become a brilliant way of studying traditional Chinese medicine's multicomponent and multitarget action mechanism. This method employs bioinformatics, system biology, and multidrug biology to integrate network analysis, which breaks down the multilevel and all-encompassing drug mechanism into its constituent parts. In this study, the network pharmacology method was combined with molecular docking technology to predict the therapeutic mechanism of multitarget and multilevel synergistic application of pharmacodynamic active components in XFZYD for the treatment of AS, which helps to clarify the molecular mechanism of XFZYD in the treatment of AS and improve the effectiveness of the drug.

2. Materials and Methods

2.1. Ethical issues and other conflicts of interest

GeneCards and PDH belong to public databases. The patients, animals, and protein structures involved in these databases have obtained ethical approval. These data have been maintained publicly available for any researchers to use. Thus, the relevant data could be downloaded gratuitously for users to do some research and publish relevant articles. Our study is based on the open-source data, so there are no ethical issues and other conflicts of interest.

2.2. Acquisition of XFZYD active ingredients

Using TCM System Pharmacology database and analysis platform (TCMSP, https://old.tcmsp-e.com/tcmsp.php), the compounds in XFZYD were obtained. The effective compounds were screened according to oral bioavailability ≥ 30% and drug-like ≥ 0.18. With the help of the UniProt database (https://www.uniprot.org), we converted the collected active components into gene names.

2.3. Acquisition of AS disease targets

AS disease targets were retrieved from the GeneCards database (https://www.genecards.org), search “AS.” We screened for AS disease targets to satisfy the Relevance score ≥ 3 of the disease set.

2.4. Construction and analysis of component-intersection target network

The related targets of XFZYD and the targets of AS were obtained. The intersection targets of the related targets of XFZYD and AS disease targets as well as the active compounds of XFZYD were introduced into Cytoscape 3.8.2 software, so as to construct “Components-AS-target” network, and then analyze and study the network.

2.5. PPI network building and key objective screening

Import the intersection target into the String database (https://string-db.org), choose Homo Sapiens as the race, and set the degree of confidence to 0.9 (the degree of confidence score represents the degree of protein interaction). We put the PPI network node data from the string database into Cytoscape 3.8.2 for network topology analysis and visualization, and the top 15 nodes are chosen as the core target of XFZYD for the treatment of AS, using degree as the screening criterion point.

2.6. Go and KEGG enrichment analysis

Set the threshold value to P ≤ .5 using the R language’s “clusterProfiler” computer package, choose 3 modules for enrichment analysis: biological process (BP), molecular function (MF), and cell composition (CC), then visually analyze the top 10 entries in each module. The intersection targets were analyzed using the KOBAS 3.0 database (http://kobas.cbi.pku.edu.cn/) for Kyoto Encyclopedia of Genes and Genomes (KEGG) enrichment, and we chose the first 15 signal pathways as significant enrichment pathways.

2.7. Molecular docking

The core target protein, whose species is human and the protein crystal resolution is <3Å, was obtained in the PDH database (http://www.rcsb.org/). The 3D structure of effective compounds and the position control’s target core protein was obtained from the PubChem database (https://pubchem.ncbi.nlm.nih.gov/). We download the 3D structure of active compounds in XFZYD targeting core protein and positive control in the PubChem database (https://pubchem.ncbi.nlm.nih.gov/). Utilize the Molecular Operating Environment (MOE v2019.0102 software) to optimize the target protein structure (dehydration and hydrogenation, energy optimization) and minimize the energy of the active compounds, as well as perform molecular docking of the processed target protein and small molecule compounds. To verify the binding of the target protein and the target active chemical, RSMD is utilized as the accuracy parameter of the molecular docking model (RSMD ≤ 4 Å is reliable, RSMD ≤ 2 Å is accurate), and S (E refine, the unit is kcal/mol) is used as the binding free energy parameter.

3. Results

3.1. Obtain XFZYD and AS intersection target

Table 1 shows that there are 225 active components in total in XFZYD. Partial compounds are shown after comprehensive ranking according to oral bioavailability and drug-like. There were 239 XFZYD targets after the duplicate data were removed. There were 4681 action targets in the GeneCards database with “AS” as the keyword, of which 313 met the Relevance score ≥ 3 as AS’s action targets. Figure 1 demonstrates that XFZYD and AS have a total of 67 intersecting targets, including safflower, bupleurum, Achyranthes bidentata, and licorice, as indicated in Table 2.
| TCM                  | ID     | MOL ID    | Active ingredient                  | OB (%) | DL  |
|----------------------|--------|-----------|------------------------------------|--------|-----|
| Carthami flos        | HH1    | MOL002712 | 6-Hydroxykaempferol                | 62.13  | 0.27|
|                      | HH2    | MOL002680 | Flavoxanthin                       | 60.41  | 0.56|
|                      | HH3    | MOL002717 | 6-Hydroxykaempferol                | 60.41  | 0.56|
|                      | HH4    | MOL002710 | Pyrethrin II                       | 48.36  | 0.35|
|                      | HH5    | MOL000098 | Quercetin                          | 46.43  | 0.28|
|                      | HH6    | MOL002721 | Quercetin                          | 45.01  | 0.31|
|                      | HH7    | MOL000449 | Stigmasterol                       | 43.83  | 0.76|
|                      | HH8    | MOL002695 | Lignan                            | 43.32  | 0.66|
|                      | HH9    | MOL002707 | Phytolone                          | 43.18  | 0.50|
|                      | HH10   | MOL000422 | Kaempferol                         | 41.88  | 0.24|
|                      | HH11   | MOL002776 | Bacalain                           | 40.12  | 0.75|
|                      | HH12   | MOL002706 | Phytolaene                         | 39.56  | 0.50|
|                      | HH13   | MOL000953 | CLR                                | 37.87  | 0.68|
|                      | HH14   | MOL002773 | Beta-carotene                      | 37.18  | 0.58|
|                      | HH15   | MOL001771 | Poriferast-5-en-3beta-ol           | 36.91  | 0.75|
|                      | HH16   | MOL003358 | Beta-sitosterol                    | 36.91  | 0.75|
|                      | HH17   | MOL00006  | Luteolin                           | 36.16  | 0.25|
|                      | HH18   | MOL002698 | Lupeol-palmitate                   | 33.98  | 0.32|
|                      | HH19   | MOL002714 | Bacalain                           | 33.52  | 0.21|
|                      | HH20   | MOL002719 | 6-Hydroxyrafinarigenin             | 33.23  | 0.24|
| Persicae semen       | TR1    | MOL001371 | Populoside qt                      | 108.89 | 0.20|
|                      | TR2    | MOL001351 | Gibberellin A44                    | 101.61 | 0.54|
|                      | TR3    | MOL001348 | Gibberellin 17                     | 94.64  | 0.49|
|                      | TR4    | MOL001353 | GA60                               | 93.17  | 0.53|
|                      | TR5    | MOL001344 | GA122-Isolactone                   | 88.11  | 0.54|
|                      | TR6    | MOL001329 | 2,3-Dihydro GA77                   | 88.08  | 0.53|
|                      | TR7    | MOL001360 | GA77                               | 87.89  | 0.53|
|                      | TR8    | MOL001340 | GA120                              | 84.85  | 0.45|
|                      | TR9    | MOL001339 | GA119                              | 76.36  | 0.49|
|                      | TR10   | MOL001358 | Gibberellin 7                      | 73.80  | 0.50|
| Paeoniae radix rubra | CS1    | MOL001918 | Paeoniflorigenone                  | 87.59  | 0.37|
|                      | CS2    | MOL001925 | Paeoniflorine qt                   | 68.18  | 0.40|
|                      | CS3    | MOL007016 | Paeoniflorigenone                  | 65.33  | 0.37|
|                      | CS4    | MOL006996 | 1-α-beta-D-glucopyranosyl-paeoniflorone_qt | 65.08  | 0.35|
|                      | CS5    | MOL007022 | Evofolin B                         | 64.74  | 0.22|
|                      | CS6    | MOL007018 | 9-ethyl-neo-paeoniflorone_A_qt     | 64.42  | 0.30|
|                      | CS7    | MOL006992 | (2R,3R)-4-methyl-distylin          | 59.98  | 0.30|
|                      | CS8    | MOL007008 | 4-ethyl-paeoniflorine_qt           | 56.87  | 0.44|
|                      | CS9    | MOL000492 | (+)-Catechin                       | 54.83  | 0.24|
|                      | CS10   | MOL001924 | Paeoniflorine                      | 53.87  | 0.79|
| Chuanxiong rhizoma   | CQ1    | MOL000433 | FA                                 | 68.96  | 0.71|
|                      | CQ2    | MOL002140 | Perilolene                         | 65.95  | 0.27|
|                      | CQ3    | MOL002151 | Senkyunone                         | 47.66  | 0.24|
|                      | CQ4    | MOL002157 | Wallachide                         | 42.31  | 0.71|
|                      | CQ5    | MOL001494 | Mandelon                           | 42.00  | 0.19|
|                      | CQ6    | MOL002135 | Myricanone                         | 40.60  | 0.51|
|                      | CQ7    | MOL000359 | Sitosterol                         | 36.91  | 0.75|
| Aehyranthis bidentata radix | NX1 | MOL000785 | Palmitate                          | 64.60  | 0.65|
|                      | NX2    | MOL000098 | Quercetin                          | 46.43  | 0.28|
|                      | NX3    | MOL012542 | β-ecdysterone                      | 44.23  | 0.82|
|                      | NX4    | MOL000449 | Stigmasterol                       | 43.83  | 0.76|
|                      | NX5    | MOL002897 | Epiberberine                       | 43.09  | 0.78|
|                      | NX6    | MOL001006 | Poriferasta-7,22E-dien-3beta-ol    | 42.98  | 0.76|
|                      | NX7    | MOL004355 | Spinasterol                        | 42.98  | 0.76|
| Rehmanniae radix     | SDH1   | MOL000449 | Stigmasterol                       | 43.83  | 0.76|
|                      | SDH2   | MOL000359 | Sitosterol                         | 36.91  | 0.75|
| AngEelicae sinensis radix | DG1 | MOL000449 | Stigmasterol                       | 43.83  | 0.76|
|                      | DG2    | MOL000358 | Beta-sitosterol                    | 36.91  | 0.75|
| Platycodonis radix   | JG1    | MOL004580 | cis-Dihydroquercetin               | 66.44  | 0.27|
|                      | JG2    | MOL005996 | 2-O-methyl-3—O-β-D-glucopyranosyl Platycogenate A | 45.15  | 0.25|
|                      | JG3    | MOL004355 | Spinasterol                        | 42.98  | 0.76|
|                      | JG4    | MOL006070 | Robinin                           | 39.84  | 0.71|
|                      | JG5    | MOL000006 | Luteolin                           | 36.16  | 0.25|
|                      | JG6    | MOL001689 | Acacetin                           | 34.97  | 0.24|
|                      | JG7    | MOL001689 | Acacetin                           | 34.97  | 0.24|
| Aurantii fructus     | ZK1    | MOL002341 | Hesperetin                         | 70.31  | 0.27|
|                      | ZK2    | MOL005828 | Nobiletin                          | 61.67  | 0.52|
|                      | ZK3    | MOL004328 | Naringenin                         | 59.29  | 0.21|
|                      | ZK4    | MOL013381 | Marmin                             | 38.23  | 0.31|
|                      | ZK5    | MOL000358 | Beta-sitosterol                    | 36.91  | 0.75|

(Continued)
3.2. Construction and analysis of component-intersection target network

The network diagram of traditional Chinese medicine component target interaction was generated and mapped using Cytoscape 3.8.2 software based on the active components and intersection genes in XFZYD. The drug’s active ingredient is represented by the outer circle, the intersection gene target is represented by the middle rectangular node, and the correlation between the active ingredient and the intersection target is represented by the edge in Figure 2. By the “active compounds—intersection targets” network of active compounds in the degree of value ranking shows that the more the greater the value of the corresponding target, top compounds with quercetin, kaempferol, luteolin, naringenin, β-sitosterol, isorhamnetin, stigmasterol, baicalein, nobiletin, and β-carotene (Table 3).

3.3. Construction of the PPI network and key node screening

We imported the intersection targets into the string database for analysis and set the confidence level to ≥9. Then we used Cytoscape software to build the PPI network and analyze the network topology. The PPI network includes 67 nodes and 1076 edges, with a node degree of 32.1 on average (shown in Fig. 3). The darker the node color is, the larger the diameter is, representing the larger Degree value in Figure 3. Based on the PPI network’s degree values, the top 6-core target proteins were STAT3, IL6, Jun, VEGFA, Mapk14, and AKT1 (Fig. 4).

3.4. GO and KEGG enrichment analysis

The results of GO enrichment analysis revealed that GO functional enrichment analysis yielded 1761 GO entries, including 1636 BP entries, 43 CC entries, and 82 MF-related entries. BP is mainly related to lipopolysaccharide, nutrient level and oxidative stress, and oxidative stress. CC is mainly connected with membrane rafts, collagen-containing extracellular matrix, secretory granule cavities, platelet α particle cavities, and similar structures. Cytokine activity, RNA polymerase II transcription factor binding, and cytokine receptor binding are all associated with MF (Fig. 5).

### Table 1 (Continued)

| TCM                  | ID      | MOL ID     | Active ingredient    | OB (%) | DL  |
|----------------------|---------|------------|----------------------|--------|-----|
| Bupleuri radix       | CH1     | MOL004644  | Sainfuran            | 79.91  | 0.23|
|                      | CH2     | MOL013187  | Cubebin              | 57.13  | 0.64|
|                      | CH3     | MOL000354  | Isorhamnetin         | 49.60  | 0.31|
|                      | CH4     | MOL004609  | Areapillin           | 48.96  | 0.41|
|                      | CH5     | MOL004628  | Octulaquine          | 47.82  | 0.28|
|                      | CH6     | MOL004624  | Longikaurin A        | 47.72  | 0.53|
|                      | CH7     | MOL000998  | Quercetin            | 46.43  | 0.28|
|                      | CH8     | MOL004653  | (+)-Anomalain        | 46.06  | 0.66|
|                      | CH9     | MOL000449  | Stigmasterol         | 43.83  | 0.76|
| Glycyrrhizae radix et rhizoma | GC1     | MOL002311  | Glycyrol             | 90.78  | 0.67|
|                      | GC2     | MOL004904  | Licoppranocoumarin   | 80.36  | 0.65|
|                      | GC3     | MOL004891  | Shingyeuropearin      | 80.30  | 0.73|
|                      | GC4     | MOL003017  | Phaseol              | 76.77  | 0.58|
|                      | GC5     | MOL004841  | Licochalcone B       | 76.76  | 0.19|
|                      | GC6     | MOL004810  | Glyasperin F         | 75.84  | 0.54|
|                      | GC7     | MOL001484  | Inermine             | 75.18  | 0.54|
|                      | GC8     | MOL000500  | Vestitol             | 74.66  | 0.21|
|                      | GC9     | MOL000507  | Glyasperins M        | 72.67  | 0.59|

OB = oral absorption efficiency, TCM = traditional Chinese medicine, XFZYD = Xuefu Zhuyu decoction.

### Table 2

| Name of traditional Chinese medicine | Quantity of active ingredients | Number of predicted targets | Common target with AS |
|-------------------------------------|--------------------------------|-----------------------------|-----------------------|
| Carthami flos                        | 22                             | 186                         | 51                    |
| Persicae semen                      | 23                             | 35                          | 5                     |
| Glycyrrhizae radix et rhizoma        | 32                             | 199                         | 62                    |
| Angelicae sinensis radix            | 2                              | 40                          | 5                     |
| Achyranthis bidentatae radix         | 20                             | 363                         | 79                    |
| Chuanxiong rhizoma                  | 7                              | 22                          | 9                     |
| Platycodonis radix                  | 7                              | 63                          | 19                    |
| Paeoniae radix rubra                 | 29                             | 80                          | 18                    |
| Aurantii fructus                    | 5                              | 69                          | 26                    |
| Bupleuri radix                      | 17                             | 166                         | 50                    |
| Rehmanniae radix                    | 2                              | 26                          | 3                     |

AS = atherosclerosis.

### Figure 1.

Venny map of XFZYD targets and AS targets. AS = atherosclerosis, XFZYD = Xuefu Zhuyu decoction.

3.3. Construction of the PPI network and key node screening

We imported the intersection targets into the string database for analysis and set the confidence level to ≥9. Then we used Cytoscape software to build the PPI network and analyze the network topology. The PPI network includes 67 nodes and 1076 edges, with a node degree of 32.1 on average (shown in Fig. 3). The darker the node color is, the larger the diameter is, representing the larger Degree value in Figure 3. Based on the PPI network’s degree values, the top 6-core target proteins were STAT3, IL6, Jun, VEGFA, Mapk14, and AKT1 (Fig. 4).

3.4. GO and KEGG enrichment analysis

The results of GO enrichment analysis revealed that GO functional enrichment analysis yielded 1761 GO entries, including 1636 BP entries, 43 CC entries, and 82 MF-related entries. BP is mainly related to lipopolysaccharide, nutrient level and oxidative stress, and oxidative stress. CC is mainly connected with membrane rafts, collagen-containing extracellular matrix, secretory granule cavities, platelet α particle cavities, and similar structures. Cytokine activity, RNA polymerase II transcription factor binding, and cytokine receptor binding are all associated with MF (Fig. 5).

KEGG pathway enrichment analysis was performed on 67 intersection targets using KOBAS 3.0 database, then draw the bubble diagram by the top 15 signal pathways. The color of the bubble denotes the degree of enrichment, while the size of the bubble represents the number of enriched genes (Fig. 6). The results indicate that XFZYD may influence the occurrence and progression of AS via fluid shear stress, and signaling pathways such as AGE-RAGE, TNF, and IL-17. The results show...
that XFZYD might regulate fluid shear stress and atherosclerosis, AGE-RAGE signaling pathway, TNF signaling pathway, and IL-17 signaling pathway to influence the development of AS (Figs. 7 and 8).

### 3.5. Molecular docking

The docking results in Table 4 indicate that active drugs targeting the VEGFA and AKT1 proteins have a high affinity for their targets ($S < -6$ kcal/mol) and an accurate binding model.
We chose positive controls (AG-13958 for VEGFA and ipatasertib for AKT1) as a reference. The binding of β-carotene, quercetin, kaempferol, luteolin, and naringin to VEGFA and AKT1 protein targets was more stable than the positive control, and β-carotene binding was the strongest (S < 8 kcal/mol) (Figs. 9–12).

4. Discussion

AS is a risk factor in the occurrence and development of cardiovascular and cerebrovascular diseases. So far, researchers have not been able to fully understand AS because its mechanism is related to multiple factors and the activation of many genes.\(^{12}\) With changes in nutrition and living standards, the incidence of AS is increasing year after year and is trending younger. Therefore, controlling the occurrence and progression of atherosclerotic is an urgent problem to be solved.

The prospective mechanism of XFZYD in the treatment of AS was explored using network pharmacology and molecular docking technologies in this study. Based on the active compounds-intersection targets network of XFZYD, we screened out quercetin, kaempferol, luteolin, naringin, β-sitosterol, isorhamnetin, stigmasterol, baicalein, nobiletin, and β-carotene, all of which could bind to key regulatory proteins. Studies have
Figure 6. The top 15 of KEGG enrichment analysis of AS of XFZYD. KEGG = Kyoto Encyclopedia of Genes and Genomes, XFZYD = Xuefu Zhuyu decoction.

Figure 7. Fluid shear stress and AS of XFZYD. AS = atherosclerosis, XFZYD = Xuefu Zhuyu decoction.
shown that quercetin can scavenge superoxide free radicals and reduce oxidative stress damage. It also shows anti-inflammatory and antiapoptotic activities. In addition, quercetin has cardiovascular protection. Luteolin, a compound in various plants, has many pharmacological actions, including anti-inflammatory, antitumor, antiviral, uric acid lowering, and cardiovascular disease prevention and treatment. It has been shown that luteolin, which works by modulating the phenotypic transition of macrophages, can block angiotensin-induced apoptosis. Kaempferol could be beneficial in the treatment of AS by lowering cholesterol levels, promoting antioxidant defenses, and improving vasculitis. β-carotene is the primary precursor of vitamin A and its metabolite retinoic acid. Studies have shown that the conversion of β-carotene to vitamin A delays the development of atherosclerosis by reducing hepatic lipid secretion in mice. β-carotene also can improve atherosclerosis caused by vitamin A deficiency. The results showed that XFZYD contained many active compounds and played a synergistic role in the treatment of AS.

STAT3, IL6, Jun, VEGFA, Mapk14, and Akt are the primary target proteins in the PPI network (Fig. 4). STAT3 is a member of the STAT family of proteins, which are involved in cell growth, apoptosis, carcinogenesis, and other life processes. Monte et al. reported that inhibiting vascular endothelial growth factor (VEGF) expression in human umbilical vein endothelial cells in vitro by decreasing STAT3 phosphorylation; hence, boosting VEGF-induced endothelial cell migration and proliferation and delaying the formation of atherosclerotic plaque. It is reported that STAT3 can bind to the promoter region of the VEGF gene and regulate its transcription. When the inflammatory inducible factor IL6 binds to relevant receptors, it can facilitate signal transmission in cells, activate the JAK2/STAT3 signal pathway, and boost vascular smooth muscle cell proliferation. Inactivation of the c-Jun/AP-1 signaling pathway can effectively relieve AS in vivo and reduce the proliferation and migration of atherosclerotic vascular smooth muscle cells (VSMCs), according to Rongjing Ji. The findings show that the major active chemicals operate on a variety of target proteins to serve an overall regulatory effect.

The KEGG pathway enrichment analysis revealed that XFZYD inhibited AS via modulating the fluid shear stress, the AGE-RAGE signal pathway, the TNF signal pathway, and the IL-17 signal pathway (Fig. 6). AS and fluid shear stress are intricably linked. The balance of shear force is strongly correlated with the integrity of the vascular intima, as evidenced by research. Atherosclerotic plaque development is positively correlated with low shear force in blood flow. Atherosclerotic plaque development and dispersion are mostly determined by the presence of low blood flow shear force. Through oxidative stress, the AGE-RAGE signaling pathway can exacerbate vascular damage. Vascular damage is closely relative to plaque development in AS. Further investigation showed the pathways involved in the treatment of AS by XFZYD are related to the VEGFA signaling pathway, AKT1 signaling pathway, and MAPK14 signaling pathway. Meanwhile, the pathways involved in the treatment of diabetes complicated by AS by XFZYD are related to the VEGFA signaling pathway, AKT1 signaling pathway, and CCL2 signaling pathway. VEGFA is a critical angiogenesis growth factor, promoting macrophage infiltration, endothelial cell proliferation and differentiation, and foam cell formation. AKT1 is a serine/threonine-protein kinase that, among other things, helps regulate vascular endothelium and VSMCs and plays a role in protecting the cardiovascular system.

The results of fluid shear stress and atherosclerosis enrichment pathway (Fig. 7), AGE-RAGE signaling pathway (Fig. 8), and PPI core protein ranking (Fig. 4) showed that
VEGFA and AKT1 are closely related to the development of AS, and maybe the key regulatory proteins of XFZYD in the treatment of AS. We screened several main active compounds in XFZYD, such as luteolin, naringin, β-sitosterol, isorhamnetin, stigmasterol, baicalin, and β-carotene (Table 4), based on the TCMSP database and “active compounds-intersection targets network.” These screened compounds may act on VEGFA and AKT1 protein, then we do molecular docking. The findings reveal that active drugs that target the proteins VEGFA and AKT1 have a strong bond ability (S < −6 kcal/mol) and binding model accuracy (RMSD < 2). β-carotene, quercetin, kaempferol, luteolin, and naringenin not only have higher degree values and correspond to more targets, but also have good binding ability to key regulatory proteins VEGFA and AKT1, they may be active chemicals with the potential to treat AS. The above results reflected that XFZYD has the biological characteristics of multicomponents, multitargets, and multipathways. These components, targets, and pathways are woven into a network to treat atherosclerosis at a holistic level.

In summary, we studied the enrichment analysis of action targets of XFZYD in treating atherosclerosis, then carried out the network topology analysis of the TCM component-action target network diagram and protein interaction network diagram. We found that β-carotene, quercetin, kaempferol, and luteolin in Safflower can act on VEGFA and AKT1 and other targets. By regulating the shear stress of vascular fluid and participating in the regulation of AGE-RAGE and other signaling pathways in atherosclerosis and diabetes mellitus complicated with AS, it can play a role in alleviating and treating AS. The active compounds that target VEGFA and AKT1 show strong docking ability and could be potential inhibitors of VEGFA and AKT1. β-carotene has the best docking ability of the bunch, and our study has shown that it can help prevent AS through antioxidants. In short, XFZYD treats AS with multiple components, multiple targets, and multiple pathways, providing ideas and references for AS and the theoretical basis for subsequent experimental studies. But there are also some limitations in our study, there are no further experiments to verify the results. Next, we will verify the results through animal or cells experiments.

### Table 4

| Core target | PDBID | Targeted active compound | Source | S (kcal/mol) | RMSD |
|-------------|-------|--------------------------|--------|--------------|------|
| VEGFA       | 3QTK  | β-Carotene               | Carthami flos | –8.5126  | 1.7990 |
|             |       | Quercetin                | Carthami flos | –6.4146  | 2.7384 |
|             |       | luteolin                 | Bupleuri radix | Glycyrrhizae radix et rhizoma | –6.1290  | 0.6845 |
|             |       | Baicalein                | Bupleuri radix | Glycyrrhizae radix et rhizoma | –6.1284  | 1.0777 |
|             |       | Ellagic acid             | Paeoniae radix rubra | Achyranthis bidentatae radix | –6.1038  | 1.2106 |
|             |       | AG-13958                 | positive control | –7.6979  | 1.8274 |
|             |       | PTC-299                  | –7.1679  | 1.4264 |
|             |       | NM-3                     | –6.0531  | 1.8093 |
| AKT1        | 6HHF  | β-Carotene               | Carthami flos | –9.6154  | 1.4225 |
|             |       | Quercetin                | Carthami flos | –6.5852  | 1.8190 |
|             |       | Kaempferol               | Bupleuri radix | Glycyrrhizae radix et rhizoma | –6.4700  | 1.2972 |
|             |       | Baicalein                | Bupleuri radix | Glycyrrhizae radix et rhizoma | –6.4625  | 0.7620 |
|             |       | Naringenin               | Aurantii fructus | –6.3506  | 1.0781 |
|             |       | luteolin                 | Glycyrrhizae radix et rhizoma | –6.3010  | 1.1852 |
|             |       | Ipatasertib              | positive control | –8.4336  | 1.0894 |
|             |       | Capivasertib             | –8.1244  | 1.3326 |
|             |       | Afuresertib              | –7.7206  | 1.2655 |
Figure 9. Docking pattern of β-carotene and VEGFA.

Figure 10. Docking pattern of AG-13958 and VEGFA.

Figure 11. Docking pattern of β-carotene and AKT1.
Author contributions

Jingshan Zhao, Lin Liu, Yingyun Li, Boyu Liu performed main analysis and drafted the article. Qing Xu, Quan Shen, Weikang Li helped in the introduction sections. All authors wrote, read, and approved the article.

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References

[1] Herrington W, Lacey B, Sherliker P, et al. Epidemiology of atherosclerosis and the potential to reduce the global burden of atherothrombotic disease. Circ Res. 2016;118:355–46.
[2] Working Group on Safety Evaluation of Statins. Expert consensus on safety evaluation of statins (Chinese). Chin J Cardiol, 2014;42:890–4.
[3] Yang P, Mingxue Z, Jiajuan G. The use of traditional Chinese medicine to supplement Qi and activate blood circulation in the prevention and treatment of atherosclerosis (Chinese). Chin Med J, 2021;7:362–6.
[4] Yuanyuan Z, Jiekun L, et al. Exploring pharmacological mechanisms of Xuefu Zhuyu decoction in the treatment of traumatic brain injury via a network pharmacology approach. Evid Based Complement Alternat Med. 2018;2018:8916938.
[5] Sikui Y. Clinical effect of modified Xuefu Zhuyu decoction on unstable angina pectoris of coronary heart disease (Chinese). Chin J Clin Ration Drug Use. 2020;13:133–7.
[6] Na Q. Effect of Xuefu Zhuyu decoction on patients with coronary heart disease and angina pectoris (Chinese). Chin J Mod Drug Appl. 2020;14:220–1.
[7] Jinfeng L, Huafeng L, Guiling Z. A Meta-analysis of efficacy of the Xuefu Zhuyu decoction on coronary angiography from heart disease with the Xinzhu Yuzu symptom (Chinese). Chin J Clin Med. 2020;12:141–3.
[8] Kui F, Gu W, Gao F, et al. Research on effect and mechanism of Xuefu Zhuyu decoction on CHD based on meta-analysis and network pharmacology. Evid Based Complement Alternat Med. 2020;2021:1–15.
[9] Shi X, Zhu H, Zhang Y, et al. XuefuZhuyu decoction protected cardiomyocytes against hypoxia/reoxygenation injury by inhibiting autophagy. BMC Complement Altern Med. 2017;17:325.
[10] Sakle NS, More SA, Mokale S, et al. network pharmacology-based approach to explore potential targets of Caesalpinia pulcherrima: an updated prototype in drug discovery. Sci Rep. 2020;10:17217.
[11] Hopkins AL. Network pharmacology: the next paradigm in drug discovery. Nat Chem Biol. 2008;4:682–90.
[12] Xue X, Deng Y, Wang J, et al. Hydroxysafflor yellow A, a natural compound from Carthamus tinctorius L with good effect of alleviating atherosclerosis. Phytomedicine. 2021;191:153694.
[13] Zhang YM, Zhang ZY, Wang RX. Protective mechanisms of quercetin against myocardial ischemia reperfusion injury. Front Physiol. 2020;31:956.
[14] Oboh G, Ademusoy AO, Ogumuyi OB. Quercetin and its role in chronic diseases. Adv Exp Med Biol. 2016;929:377–87.
[15] Imran M, Rauf A, Abu-Emrid T, et al. Luteolin, a flavonoid, as an anti-cancer agent: A review. Biomed Pharmacother. 2019;112:109084.
[16] Feng Z, Wang C, Jin Y, et al. Kaempferol-induced GPER upregulation attenuates atherosclerosis via the PI3K/AKT/Nrf2 pathway. Pharm Biol. 2021;59:1106–16.
[17] ZhouF WX, Pino I, et al. β-Carotene conversion to vitamin a delays atherosclerosis progression by decreasing hepatic lipid secretion in mice. J Lipid Res. 2020;61:1491–503.
[18] Harari A, Melnikov N, Kandel Kfr M, et al. Dietary β-carotene resuces Vitamin A deficiency and inhibits atherogenesis in apolipoprotein e-deficient Mice. Nutrients. 2020;12:1625.
[19] Yali X, Huang H, Dan H, et al. Role of G protein signal regulator 5 in the development of atherosclerosis (Chinese). JMR. 2016;45:178–80.
[20] Dal Monte M, Martin J, Ristori C, et al. Hyposia effects on prosangio- genic factors in human umbilical vein endothelial cells: functional role of the peptide somatostatin. Naunyn Schmiedebergs Arch Pharmacol. 2011;383:593–612.
[21] Dutzmann J, Daniel JM, Bausachs J, et al. Emerging translational approaches to target STAT3 signalling and its impact on vascular disease. Cardiovasc Res. 2015;106:365–74.
[22] Ganta VC, Choi M, Kudatadez A, et al. VEGFR165b modulates endothe- lial VEGFR1-STAT3 Signaling pathway and angiogenesis in human and experimental peripheral arterial disease. Circ Res. 2017;120:282–95.
[23] Ji R, Gu Y, Zhang J, et al. TRIM7 promotes proliferation and migration of VSMCs and HUVECs in atherosclerosis by targeting AKT1. Eur Rev Med Pharmacol Sci. 2019;23:2223–33.