Systematic Pharmacology and GEO Database Mining Revealed the Mechanism of XFZYD Therapy for ASCVD

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Abstract
Background: Xuefu Zhuyu decoration (XFZYD), as a traditional Chinese compound recipe, has been used to treat atherosclerosis cardiovascular disease (ASCVD) for thousands of years in China, but its effective compounds and underlying treatment molecular mechanism remains promiscuous, which severely limits its clinical application.

Methods: The effective components and its targets of XFZYD were predicted and screened based on the TCMSP database. The candidate therapeutic targets of ASCVD were screened by Pharmacogenomics Knowledgebase (PharmGKB) and Comparative Toxicogenomics Database (CTD). Kyoto Encyclopedia of Genes and Genomes (KEGG) pathway analyses for target proteins were performed using DAVID database. Subsequently, protein-protein interaction (PPI) analyses were conducted using the STRING database. Differentially expressed genes in GSE71226 were identified using GEO2R. Finally, molecular docking was performed by Schrodinger software.

Results: A total of 108 effective compounds and 137 candidate therapeutic targets were screened. Analyzing the relationships among effective compounds, candidate therapeutic targets, and signaling pathways, the therapy mechanism of XFZYD for ASCVD were mainly reflected in the protection of vascular endothelium, anti-inflammatory, antioxidant stress, etc. Moreover, the expression profile in GSE71226 supported our findings, while molecular simulation docking results also demonstrated the reliability of the predicted results.

Conclusions: This study demonstrates the therapeutic potential of XFZYD for the treatment of ASCVD based on systemic pharmacology, which could provide a guiding principle for its clinical application as well as valuable insights for further drug discovery. Key words: Systematic pharmacology; atherosclerosis; Xuefu Zhuyu decoration; ASCVD

1. Background
Atherosclerosis cardiovascular disease (ASCVD) was a systemic disease based on atherosclerosis, becoming a leading killer worldwide due to the high morbidity and mortality (1). Atherosclerosis is a pathological status characterized by fibrogenesis, chronic inflammation, lipid accumulation and vascular wall immunity disorders (2, 3). As atherosclerotic plaques develop into advanced stages,
brittle plaques tend to rupture(4), leading to acute cardiovascular events such as ischemic stroke and myocardial infarction.

At present, clinical medications ideas for ASCVD mainly focus on correcting of atherogenic dyslipidemia and platelet aggregation. The clinical efficacy of statins and aspirin have been well established(5, 6). Although current atherosclerosis medications can partially relieve the symptoms of ASCVD patients, gastrointestinal reactions or liver and kidney damage etc. were occur constantly, due to individual differences, adverse effects of medicine or usage without doctor's prescription, which greatly disturb the therapeutic efficacy.

Traditional Chinese Medicine (TCM) plays an important role in the adjuvant treatment of ASCVD(7). Compared with CMM of simple prescription, TCM compound recipe has the characteristics of multi-component, multi-target and multi-pathway interaction. Xuefu Zhuyu Decoration (XFZYD), a famous herbal remedy, has been used to relieve symptoms in patients with ASCVD for thousand years in China with few adverse events(8). XFZYD was composed of 11 herbs: Radix rehmanniae, Angelica sinensis, Rhizoma Chuanxiong, Carthamus tinctorius, Radix paeoniae rubra, Fructus Aurantii, Bupleunum, Platycodon grandiflorum, Achyranthes bidentata, Licorice, Semen Pruni Persicae. The formula has been proven reliable and effective for curing ASCVD(8, 9) and its risk factors hyperlipidemia(10) and hypertension(11).

Experimental studies have shown that XFZYD could control inflammatory response(12, 13), increase coronary blood flow, improve the cardiac microcirculation, accommodate blood lipids(10), and prevent platelet aggregation, maintaining vessel growth in physiological or repair range to avoid angiogenesis in the atheromatous plaque(14).

However, due to the complexity of components of XFZYD, it is difficult to systematically explain the molecular therapy mechanism of XFZYD using conventional methods. Network pharmacology provides a new idea for fundamental studying the multi-targeted mechanism for multiple ingredients in this prescription(15). In the present study, through exploration of the complex network of XFZYD-therapeutic targets-biological processes-pathways, the therapy mechanism of XFZYD for atherosclerosis was elaborated from the perspective of multi-component, multi-target and multi-
2. Methods
2.1. Identification of effective compounds of XFZYD
The effective components in XFZYD were identified from Traditional Chinese Medicine System Pharmacology (TCMSP, http://lsp.nwu.edu.cn/browse.php) database. The database provides comprehensive information about ingredients in herbs including chemical structure, oral bioavailability (OB), half-life (HL), drug likeness (DL), drug targets, etc. The pharmacokinetic properties including absorption, distribution, metabolism, and excretion (ADME) are important contributors for bioactivities of drugs. In this study, three ADME-related parameters including oral bioavailability (OB) ≥ 30%, half-life (HL) ≥ 4, and drug likeness (DL) ≥ 0.18 were employed to identify the potential effective compounds in XFZYD. As recommended by TCMSP, the compounds with OB ≥ 30% and HL ≥ 4 have good absorption and slow metabolism after oral administration, while the compounds with DL ≥ 0.18 were chemically suitable for drug development.

2.2. Prediction of compound-related targets
The compound-related targets were predicted depending on chemical similarities and pharmacophore models via TCMSP databases. TCMSP compound data were obtained from databases such as DrugBank, HIT, TTD, PharmGKB, etc. All the targets obtained above were standardized as gene names and UniProt IDs by searching from UniprotKB database with “Homo sapiens” species.

2.3. Identification of ASCVD-related therapeutic targets
These ASCVD-related therapeutic targets were mined from two databases including Pharmacogenomics Knowledgebase (PharmGKB) and Comparative Toxicogenomics Database (CTD). The key words were “atherosclerosis” “coronary heart disease” “angina” “acute coronary syndrome” “stroke” “transient ischemic attacks” and “peripheral arterial disease”. All the targets in PharmGKB and the top 200 targets in CTD based on inference score were selected. All the targets obtained above were standardized as gene names and UniProt IDs by searching from UniprotKB database with “Homo sapiens” species.

2.4. Network construction and topological analysis
The compound-target network of XFZYD were constructed by Cytoscape v3.7.1 software which was a tool for analysis and visualization of the biological network(16). The topological analysis was
performed by the Network Analyzer module of Cytoscape software. According to the topology of network, degree centrality (DC), betweenness centrality (BC) and closeness centrality (CC) are the most important parameters for measuring the criticality of a node in the network, as well as the important index for new drug discovery and target prediction.

2.5. Protein-Protein Interaction (PPI) network construction and analysis

The PPI network was beneficial to explore the interaction of various proteins in complex diseases. The identified therapeutic targets were uploaded to the STRING database v11.0 (17) to obtain the protein-protein interaction information, including the physical and functional associations. Protein interactions with a confidence score of 0.9 or higher were obtained. The PPI network was visualized by Cytoscape v3.7.1(16). Network Analyzer was utilized for calculating the network topology parameters, in which the network was treated as undirected.

2.6. KEGG pathway enrichment analysis

The KEGG pathway enrichment analysis were carried out using DAVID database, which is an online biological knowledgebase and an analytic tool to extract biological information about gene functional classification, functional annotation, and enriched pathways(18). KEGG pathways with P value < 0.05 were considered to have significance.

2.7. GEO database validation

Peripheral blood RNA expression profiles of 3 atherosclerosis patients and 3 controls were obtained from GSE71226, the differentially expressed genes (DEGs) in GSE71226 were identified using GEO2R (https://www.ncbi.nlm.nih.gov/geo/geo2r/), p < 0.05 and | logFC |≥1.5 were the screening criteria.

2.8. Binding capacity between effective compounds and key targets by molecular docking

The X-ray crystal structures of the candidate therapeutic targets were taken from the RCSB PDB database, and all 3D structures of these components were obtained from the PubChem database. Molecular docking was performed by Schrodinger software. The compounds and target proteins were input and pretreated to perform the molecular docking command, and finally the docking score was obtained. The magnitude of the absolute value of docking score is proportional to the strength of bonding.

3. Results
3.1. Effective components of XFZYD and targets
A total of 129 compounds and 256 target proteins in 11 kinds of herbs were identified in XFZYD through TCMSP database with the criteria of OB ≥ 30%, HL ≥ 4 and DL ≥ 0.18 (Table S1).

3.2. ASCVD-related targets
ASCVD-related therapeutic targets were retrieved in two databases, including 73 in PharmGKB and 582 in CTD. After removing the repeated targets, a total of 620 ASCVD-related therapeutic targets were identified (Table S2), 137 of which were overlapped with targets of XFZYD (Fig. 1).

3.3. Compound-Target network of XFZYD for the treatment of ASCVD
The compound-targets network was constructed to elaborate the multiplex interplay between compounds and their related targets of XFZYD at a systematic perspective. The compound-target network consists of 245 nodes (108 active components and 137 candidate therapeutic targets) and 945 edges (Fig. 2). The compound quercetin has 90 targets, suggesting that it may be critical in the treatment of atherosclerosis (Table 1). Topological analysis was adopted to determine the core targets and compounds of XFZYD in the treatment of ASCVD with the screening criteria “DC ≥ 3, BC ≥ 0.000348 and CC ≥ 0.3754”. The topological network consists of 87 nodes (65 active components and 22 component targets) and 447 edges (Fig. 3). From this, it can be inferred that these high-degree compounds are likely to be the core pharmacodynamic substances in the XFZYD. Besides, the compound-targets network illustrated the multi-component, multi-target characteristics of XFZYD.

| Mol ID     | compounds | OB (%) | DL  | HL    | herb                      | Target Number |
|------------|-----------|--------|-----|-------|---------------------------|---------------|
| MOL000006  | luteolin  | 36.16  | 0.25| 15.94 | Carthamus tinctorius      | 34            |
|            |           |        |     |       | Platyodon grandiflorum    |               |
| MOL000098  | quercetin | 46.43  | 0.28| 14.4  | Bupleunum Carthamus       | 83            |
|            |           |        |     |       | tinctorius Achyranthes    |               |
|            |           |        |     |       | bidentata Licorice        |               |
| MOL000173  | wogonin   | 30.68  | 0.23| 17.75 | Achyranthes bidentata     | 22            |
| MOL000239  | Jaranol   | 50.83  | 0.29| 15.5  | Licorice                  | 4             |
| MOL000296  | hederagenin| 36.91 | 0.75| 5.35  | Semen Pruni Persicae      | 2             |
| MOL000354  | isorhamnetin| 49.6  | 0.31| 14.34 | Bupleunum, Licorice       | 12            |
| MOL000358  | beta-sitostero| 36.91| 0.75| 5.36  | Radix paoniae rubra        | 10            |
|            |           |        |     |       | Angelica sinensis         |               |
| MOL000392 | formononetin | 69.67 | 0.21 | 17.04 | Licorice | 11 |
| MOL000417 | Calycosin | 47.75 | 0.24 | 17.1 | Licorice | 9 |
| MOL000422 | kaempferol | 41.88 | 0.24 | 14.74 | Bupleunum Carthamus tinctorius Achyranthes bidentata | 30 |
| MOL000433 | FA | 68.96 | 0.71 | 24.81 | Rhizoma Chuanxiong | 2 |
| MOL000449 | Stigmasterol | 43.83 | 0.76 | 5.57 | Bupleunum Radix paeoniae rubra, Angelica sinensis Radix rehmanniae Carthamus tinctorius Achyranthes bidentata | 3 |
| MOL000493 | campesterol | 37.58 | 0.71 | 4.71 | Semen Pruni Persicae | 2 |
| MOL000497 | licochalcone a | 40.79 | 0.29 | 16.2 | Licorice | 16 |
| MOL001323 | Sitosterol alpha1 | 43.28 | 0.78 | 5.64 | Semen Pruni Persicae | 1 |
| MOL001328 | 2,3-didehydro GA70 | 63.29 | 0.5 | 7.62 | Semen Pruni Persicae | 2 |
| MOL001329 | 2,3-didehydro GA77 | 88.08 | 0.53 | 7.6 | Semen Pruni Persicae | 1 |
| MOL001340 | GA120 | 84.85 | 0.45 | 8.4 | Semen Pruni Persicae | 1 |
| MOL001352 | GA54 | 64.21 | 0.53 | 10.19 | Semen Pruni Persicae | 1 |
| MOL001355 | GA63 | 65.54 | 0.54 | 9.85 | Semen Pruni Persicae | 1 |
| MOL001358 | gibberellin 7 | 73.8 | 0.5 | 9.79 | Semen Pruni Persicae | 1 |
| MOL001361 | GA87 | 68.85 | 0.57 | 8.76 | Semen Pruni Persicae | 1 |
| MOL001368 | 3-O-p-coumaroylquinic acid | 37.63 | 0.29 | 5.15 | Semen Pruni Persicae | 2 |
| MOL001454 | berberine | 36.86 | 0.78 | 6.57 | Achyranthes bidentata | 4 |
| MOL001458 | coptisine | 30.67 | 0.86 | 9.33 | Achyranthes bidentata | 4 |
| MOL001484 | Inermine | 75.18 | 0.54 | 11.72 | Licorice | 2 |
| MOL001494 | Mandenol | 42 | 0.19 | 5.39 | Rhizoma Chuanxiong | 2 |
| MOL001645 | Linoleyl acetate | 42.1 | 0.2 | 7.48 | Bupleunum | 2 |
| MOL001689 | acacetin | 34.97 | 0.24 | 17.25 | Platycodon grandiflorum | 12 |
| MOL001792 | DFV | 32.76 | 0.18 | 17.89 | Licorice | 3 |
| MOL001924 | paeoniflorin | 53.87 | 0.79 | 13.88 | Radix paeoniae rubra | 2 |
| MOL002135 | 3,5,6,7-tetramethoxy-2-(3,4,5-trimethoxyphenyl) chromone | 40.6 | 0.51 | 4.39 | Rhizoma Chuanxiong | 10 |
| MOL002140 | Perlolyrine | 65.95 | 0.27 | 12.62 | Rhizoma | 1 |
| MOL002157 | wallichilide | 42.31 | 0.71 | 6.85 | Chuanxiong | Rhizoma Chuanxiong | 2 |
| MOL002311 | Glycyrol | 90.78 | 0.67 | 9.85 | Licorice | Licorice | 8 |
| MOL002341 | Hesperetin | 70.31 | 0.27 | 15.78 | Fructus Aurantii | Fructus Aurantii | 2 |
| MOL002565 | lignan | 43.32 | 0.65 | 14.88 | Carthamus tinctorius | Carthamus tinctorius | 2 |
| MOL002695 | 6-Hydroxykaempherol | 62.13 | 0.27 | 14.29 | Carthamus tinctorius | Carthamus tinctorius | 4 |
| MOL002714 | baicalein | 33.52 | 0.21 | 16.25 | Achyranthes bidentata | Achyranthes bidentata | 18 |
| MOL002721 | quercetagenin | 45.01 | 0.31 | 13.82 | Carthamus tinctorius | Carthamus tinctorius | 2 |
| MOL002773 | beta-carotene | 37.18 | 0.58 | 4.36 | Carthamus tinctorius | Carthamus tinctorius | 20 |
| MOL002897 | epiberberine | 43.09 | 0.78 | 6.1 | Achyranthes bidentata | Licorice | 3 |
| MOL003656 | Lupiwhiteonne | 51.64 | 0.37 | 15.63 | Licorice | Licorice | 8 |
| MOL003847 | Inophyllum E | 38.81 | 0.85 | 15.51 | Licorice | Licorice | 4 |
| MOL003896 | 7-Methoxy-2-methylisoflavone | 42.56 | 0.2 | 16.89 | Licorice | Licorice | 9 |
| MOL004328 | naringenin | 59.29 | 0.21 | 16.98 | Licorice | Licorice | 22 |
| MOL004580 | cis-Dihydroquercetin | 66.44 | 0.27 | 14.51 | Platycodon grandiflorum | Platycodon grandiflorum | 2 |
| MOL004598 | 3,5,6,7-tetramethoxy-2-(3,4,5-trimethoxyphenyl)chromone | 31.97 | 0.59 | 15.54 | Bupleunum | Bupleunum | 2 |
| MOL004609 | Areapillin | 48.96 | 0.41 | 16.52 | Bupleunum | Bupleunum | 2 |
| MOL004805 | (2S)-2-[4-(2,4-dihydroxy-3-(3-methylbut-2-enyl)phenyl]-8,8-dimethyl-2,3-dihydropyrano[2,3-f]chromen-4-one | 31.79 | 0.72 | 14.82 | Licorice | Licorice | 6 |
| MOL004806 | euchrenone | 30.29 | 0.57 | 15.89 | Licorice | Licorice | 3 |
| MOL004808 | glyasperin B | 65.22 | 0.44 | 16.1 | Licorice | Licorice | 8 |
| MOL004810 | glyasperin F | 75.84 | 0.54 | 15.64 | Licorice | Licorice | 9 |
| MOL004814 | Isotrifoliol | 31.94 | 0.42 | 7.91 | Licorice | Licorice | 7 |
| MOL004815 | (E)-1-(2,4-dihydroxyphe nyl)-3-(2,2-dimethylchromen-6-yl)prop-2-en-1-one | 39.62 | 0.35 | 16.16 | Licorice | Licorice | 9 |
| MOL004824 | (2S)-6-(2,4-dihydroxyphenyl)-2-(2-hydroxypropa n-2-yl)-4 | 60.25 | 0.63 | 4.31 | Licorice | Licorice | 9 |
| MOL004827 | Semilicoisoflavone B | 48.78 | 0.55 | 17.02 | Licorice | 6 |
| MOL004828 | Glepidotin A | 44.72 | 0.35 | 16.09 | Licorice | 10 |
| MOL004835 | Glypallichalcone | 61.6 | 0.19 | 17.01 | Licorice | 9 |
| MOL004838 | 8-(6-hydroxy-2-benzofuranyl)-2,2-dimethyl-5-chromenol | 58.44 | 0.38 | 8.71 | Licorice | 3 |
| MOL004841 | Licochalcone B | 76.76 | 0.19 | 17.02 | Licorice | 9 |
| MOL004848 | licochalcone G | 49.25 | 0.32 | 15.75 | Licorice | 9 |
| MOL004855 | Licoricone | 63.58 | 0.47 | 16.37 | Licorice | 5 |
| MOL004856 | Gancaonin A | 51.08 | 0.4 | 16.82 | Licorice | 6 |
| MOL004857 | Gancaonin B | 48.79 | 0.45 | 16.49 | Licorice | 7 |
| MOL004863 | 3-(3,4-dihydroxyphenyl)-5,7-dihydroxy-8-(3-methylbut-2-enyl)chromone | 66.37 | 0.41 | 15.81 | Licorice | 8 |
| MOL004864 | 5,7-dihydroxy-3-(4-methoxyphenyl)-8-(3-methylbut-2-enyl)chromone | 30.49 | 0.41 | 14.99 | Licorice | 8 |
| MOL004866 | 2-(3,4-dihydroxyphenyl)-5,7-dihydroxy-6-(3-methylbut-2-enyl)chromone | 44.15 | 0.41 | 16.77 | Licorice | 4 |
| MOL004882 | Licocoumarone | 33.21 | 0.36 | 9.66 | Licorice | 4 |
| MOL004883 | Licoisoflavone | 41.61 | 0.42 | 16.09 | Licorice | 8 |
| MOL004884 | Licoisoflavone B | 38.93 | 0.55 | 15.73 | Licorice | 7 |
| MOL004885 | licoisoflavone | 52.47 | 0.54 | 15.67 | Licorice | 8 |
| MOL004891 | shinpterocarpin | 80.3 | 0.73 | 6.5 | Licorice | 9 |
| MOL004898 | (E)-3-[3,4-dihydroxy-5-(3-methylbut-2-enyl)phenyl]-1-(2,4-dihydroxyphenyl)prop-2-en-1-one | 46.27 | 0.31 | 15.24 | Licorice | 7 |
| MOL004903 | liquiritin | 65.69 | 0.28 | 17.96 | Licorice | 3 |
| MOL004907 | Glyzaglabrin | 61.07 | 0.35 | 21.2 | Licorice | 9 |
| MOL004910 | Glabranin | 52.9 | 0.31 | 16.24 | Licorice | 4 |
| MOL004912 | Glabrone | 52.51 | 0.5 | 16.09 | Licorice | 9 |
| MOL004913 | 1,3-dihydroxy-9-methoxy-6-benzofuran[3,2-c]chromenone | 48.14 | 0.43 | 8.87 | Licorice | 6 |
| MOL004914 | 1,3-dihydroxy-8,9-dimethoxy-6-benzofuran[3,2-c]chromenone | 62.9 | 0.53 | 9.32 | Licorice | 5 |
| MOL004915 | 2-Chromenone | Eurycarpin A | 43.28 | 0.37 | 17.1 | Licorice | 8 |
| MOL004924 | (-)-Medicarpin | | 40.99 | 0.95 | 13.2 | Licorice | 1 |
| MOL004935 | Sigmoidin-B | | 34.88 | 0.41 | 14.49 | Licorice | 3 |
| MOL004941 | (2R)-7-hydroxy-2-(4-hydroxyphenyl)-chroman-4-one | | 71.12 | 0.18 | 18.09 | Licorice | 3 |
| MOL004945 | (2S)-7-hydroxy-2-(4-hydroxyphenyl)-8-(3-methylbut-2-enyl)chroman-4-one | | 36.57 | 0.32 | 17.95 | Licorice | 4 |
| MOL004948 | Isoglycyrol | | 44.7 | 0.84 | 6.69 | Licorice | 4 |
| MOL004949 | Isonicoflavone | | 45.17 | 0.42 | 15.55 | Licorice | 7 |
| MOL004957 | HMO | | 38.37 | 0.21 | 16.56 | Licorice | 9 |
| MOL004959 | 1-Methoxyphasellidin | | 69.98 | 0.64 | 9.53 | Licorice | 10 |
| MOL004961 | Quercetin der. | | 46.45 | 0.33 | 16.61 | Licorice | 8 |
| MOL004988 | Kanzonol F | | 32.47 | 0.89 | 9.98 | Licorice | 2 |
| MOL004989 | 6-prenylated eriodictyol | | 39.22 | 0.41 | 16.52 | Licorice | 3 |
| MOL004991 | 7-Acetoxy-2-methylisoflavone | | 38.92 | 0.26 | 17.49 | Licorice | 8 |
| MOL004993 | 8-prenylated eriodictyol | | 53.79 | 0.4 | 15.7 | Licorice | 2 |
| MOL005000 | Gancaonin G | | 60.44 | 0.39 | 16.13 | Licorice | 7 |
| MOL005001 | Gancaonin H | | 50.1 | 0.78 | 16.64 | Licorice | 4 |
| MOL005003 | Licoagrocarpin | | 58.81 | 0.58 | 9.45 | Licorice | 9 |
| MOL005007 | Glyasperins M | | 72.67 | 0.59 | 15.57 | Licorice | 9 |
| MOL005008 | Glycyrrhiza flavonol A | | 41.28 | 0.6 | 13.71 | Licorice | 6 |
| MOL005012 | Licoagroisoflavone | | 57.28 | 0.49 | 19.64 | Licorice | 8 |
| MOL005016 | Odoratin | | 49.95 | 0.3 | 16.35 | Licorice | 9 |
| MOL005017 | Phaseol | | 78.77 | 0.58 | 9.64 | Licorice | 8 |
| MOL005018 | Xambioona 4-one | | 54.85 | 0.87 | 14.5 | Licorice | 3 |
| MOL005828 | nobiletin | | 61.67 | 0.52 | 16.2 | Fructus Aurantii | 15 |
| MOL006992 | (2R,3R)-4-methoxyl-distylin | | 59.98 | 0.3 | 15.08 | Radix paoniae rubra | 4 |
| MOL013187 | Cubebin | | 57.13 | 0.64 | 12.4 | Bupleunum | 2 |
| MOL013381 | Marlin | | 38.23 | 0.31 | 4.68 | Fructus Aurantii | 1 |

### 3.4. PPI network construction and topology analysis

In order to elucidate the systemic and pharmacological therapy mechanism of XFZYD for ASCVD, PPI network with 137 candidate therapeutic targets were constructed and visualized. Totally, 137 nodes and 598 edges were obtained in this network (Fig. 4A). Then, topological analysis was adopted to determine the core targets of XFZYD in the treatment of ASCVD with the screening criteria “DC ≥ 14, BC ≥ 0.00382 and CC ≥ 0.3598”. Finally, we identified 27 core targets of XFZYD (Fig. 4B), indicating that they might serve as vital targets of XFZYD in treating ASCVD.
3.5. KEGG enrichment analysis
KEGG pathway enrichment analysis was performed to elucidate 137 candidate therapeutic targets of XFZYD for ASCVD. The representative top 20 pathways based on the number of enriched genes as well as P value were shown in Fig. 5. These key targets were closely related to TNF signaling pathway, PI3K-Akt signaling pathway, VEGF signaling pathway, Toll-like receptor signaling pathway, etc., participating in the process of atherosclerotic plaque formation, such as oxidative stress, inflammatory response, angiogenesis etc.

3.6. Clinical validation based on GEO database
In accordance with the analysis of GSE71226 microarray data of atherosclerosis, 673 differentially expressed genes were identified, among which 133 were up-regulated and 540 were down-regulated in the atherosclerosis group (Table S3). There were 6 targets in the intersection with 137 candidate therapeutic targets of XFZYD, which included up-regulated PTGS2, MMP9 and BCL2L1 and downregulated JUN, VEGFA and CXCL2 in atherosclerosis group (Table 2). It was indicated that the prediction of candidate therapeutic targets of XFZYD for ASCVD was relatively reliable.

| Group               | Gene symbol | Gene title                          | Pvalue     | logFC |
|---------------------|-------------|-------------------------------------|------------|-------|
| up-regulated genes  | BCL2L1      | BCL2 like 1                         | 0.0163396  | 2.38  |
|                     | MMP9        | matrix metallopeptidase 9           | 0.0187166  | 2.27  |
|                     | PTGS2       | prostaglandin-endoperoxide synthase 2 | 0.0374968  | 1.75  |
| down-regulated genes| CXCL2       | C-X-C motif chemokine ligand 2      | 0.00806    | -1.59 |
|                     | JUN         | Jun proto-oncogene, AP-1 transcription factor subunit | 0.0008752  | -1.99 |
|                     | VEGFA       | vascular endothelial growth factor A | 0.0011307  | -1.69 |

Table 3: The docking score of XFZYD on atherosclerosis

| Target | PDB ID | MOL ID       | Docking score |
|--------|--------|--------------|---------------|
| BCL2L1 | 3SP7   | MOL000006    | -6.145        |
| BCL2L1 | 3SP7   | MOL000098    | -5.739        |
| CXCL2  | 5OB5   | MOL000098    | -7.022        |
| IL-6   | 5FUC   | MOL000006    | -6.559        |
| IL-6   | 5FUC   | MOL000098    | -6.587        |
| IL-6   | 5FUC   | MOL000173    | -5.028        |
| JUN    | 5T01   | MOL000006    | -5.814        |
| JUN    | 5T01   | MOL000098    | -5.495        |
| JUN    | 5T01   | MOL000173    | -4.631        |
| JUN    | 5T01   | MOL000358    | -1.212        |
| JUN    | 5T01   | MOL000392    | -4.504        |
| JUN    | 5T01   | MOL000422    | -4.760        |
| JUN  | ST01 | MOL002773 | -1.504 |
|------|------|-----------|--------|
| MAPK1 | 6RQ4 | MOL000006 | -2.709 |
| MAPK1 | 6RQ4 | MOL000098 | -5.611 |
| MAPK1 | 6RQ4 | MOL000497 | -5.500 |
| MAPK14 | 6SFI | MOL000173 | -2.728 |
| MAPK14 | 6SFI | MOL000417 | -6.768 |
| MAPK14 | 6SFI | MOL000497 | -5.611 |
| MAPK14 | 6SFI | MOL002311 | -8.496 |
| MAPK14 | 6SFI | MOL003656 | -8.328 |
| MAPK14 | 6SFI | MOL004175 | -7.033 |
| MAPK14 | 6SFI | MOL004815 | -7.033 |
| MAPK14 | 6SFI | MOL004824 | -7.093 |
| MAPK14 | 6SFI | MOL004828 | -7.745 |
| MAPK14 | 6SFI | MOL004835 | -8.043 |
| MAPK14 | 6SFI | MOL004891 | -8.093 |
| MAPK14 | 6SFI | MOL004898 | -6.097 |
| MAPK14 | 6SFI | MOL005000 | -7.018 |
| MAPK14 | 6SFI | MOL005012 | -7.403 |
| MAPK14 | 6SFI | MOL005016 | -7.159 |
| MAPK14 | 6SFI | MOL005017 | -7.018 |
| MAPK3 | 6GES | MOL004328 | -5.671 |
| MMP9  | 6ESM | MOL000006 | -5.700 |
| MMP9  | 6ESM | MOL000098 | -6.312 |
| MMP9  | 6ESM | MOL000214 | -4.905 |
| MMP9  | 6ESM | MOL0005828 | -2.798 |
| PTGS2 | 5F19 | MOL000006 | -7.716 |
| PTGS2 | 5F19 | MOL000098 | -7.902 |
| PTGS2 | 5F19 | MOL000173 | -7.521 |
| PTGS2 | 5F19 | MOL000239 | -7.429 |
| PTGS2 | 5F19 | MOL000354 | -7.474 |
| PTGS2 | 5F19 | MOL000358 | -7.453 |
| PTGS2 | 5F19 | MOL000392 | -7.170 |
| PTGS2 | 5F19 | MOL000417 | -7.579 |
| PTGS2 | 5F19 | MOL000422 | -7.507 |
| PTGS2 | 5F19 | MOL000449 | -7.565 |
| PTGS2 | 5F19 | MOL000493 | -7.117 |
| PTGS2 | 5F19 | MOL000497 | -7.415 |
| PTGS2 | 5F19 | MOL0001323 | -6.811 |
| PTGS2 | 5F19 | MOL001340 | -5.115 |
| PTGS2 | 5F19 | MOL001352 | -5.830 |
| PTGS2 | 5F19 | MOL001355 | -6.828 |
| PTGS2 | 5F19 | MOL001361 | -5.160 |
| PTGS2 | 5F19 | MOL001454 | -7.544 |
| PTGS2 | 5F19 | MOL001458 | -7.601 |
| PTGS2 | 5F19 | MOL001484 | -6.701 |
| PTGS2 | 5F19 | MOL001944 | -5.521 |
| PTGS2 | 5F19 | MOL001645 | -4.539 |
| PTGS2 | 5F19 | MOL001689 | -7.464 |
| PTGS2 | 5F19 | MOL001792 | -6.831 |
| PTGS2 | 5F19 | MOL002135 | -5.624 |
| PTGS2 | 5F19 | MOL002140 | -6.557 |
| PTGS2 | 5F19 | MOL002157 | -4.973 |
| PTGS2 | 5F19 | MOL002311 | -8.494 |
| PTGS2 | 5F19 | MOL002341 | -7.550 |
| PTGS2 | 5F19 | MOL002565 | -6.253 |
| PTGS2 | 5F19 | MOL002712 | -7.368 |
| PTGS2 | 5F19 | MOL002714 | -6.836 |
| Gene   | Chromosome | Accession  | Log2 Fold Change |
|--------|------------|------------|-----------------|
| PTGS2  | 5F19       | MOL002721  | -7.576          |
| PTGS2  | 5F19       | MOL002773  | -7.722          |
| PTGS2  | 5F19       | MOL002897  | -6.881          |
| PTGS2  | 5F19       | MOL003656  | -8.177          |
| PTGS2  | 5F19       | MOL003847  | -6.824          |
| PTGS2  | 5F19       | MOL004328  | -7.913          |
| PTGS2  | 5F19       | MOL004580  | -7.975          |
| PTGS2  | 5F19       | MOL004805  | -7.974          |
| PTGS2  | 5F19       | MOL004808  | -7.335          |
| PTGS2  | 5F19       | MOL004810  | -8.041          |
| PTGS2  | 5F19       | MOL004814  | -7.258          |
| PTGS2  | 5F19       | MOL004815  | -8.037          |
| PTGS2  | 5F19       | MOL004824  | -8.075          |
| PTGS2  | 5F19       | MOL004827  | -7.706          |
| PTGS2  | 5F19       | MOL004828  | -6.869          |
| PTGS2  | 5F19       | MOL004835  | -6.103          |
| PTGS2  | 5F19       | MOL004841  | -6.607          |
| PTGS2  | 5F19       | MOL004848  | -7.360          |
| PTGS2  | 5F19       | MOL004855  | -7.877          |
| PTGS2  | 5F19       | MOL004856  | -8.120          |
| PTGS2  | 5F19       | MOL004857  | -9.344          |
| PTGS2  | 5F19       | MOL004884  | -8.878          |
| PTGS2  | 5F19       | MOL004885  | -7.210          |
| PTGS2  | 5F19       | MOL004891  | -7.345          |
| PTGS2  | 5F19       | MOL004898  | -7.917          |
| PTGS2  | 5F19       | MOL004903  | -7.345          |
| PTGS2  | 5F19       | MOL004907  | -7.725          |
| PTGS2  | 5F19       | MOL004910  | -7.343          |
| PTGS2  | 5F19       | MOL004912  | -7.642          |
| PTGS2  | 5F19       | MOL004915  | -7.446          |
| PTGS2  | 5F19       | MOL004924  | -4.804          |
| PTGS2  | 5F19       | MOL004935  | -7.894          |
| PTGS2  | 5F19       | MOL004948  | -8.152          |
| PTGS2  | 5F19       | MOL004949  | -8.086          |
| PTGS2  | 5F19       | MOL004959  | -7.705          |
| PTGS2  | 5F19       | MOL005000  | -8.757          |
| PTGS2  | 5F19       | MOL005001  | -7.045          |
| PTGS2  | 5F19       | MOL005003  | -6.510          |
| PTGS2  | 5F19       | MOL005008  | -8.224          |
| PTGS2  | 5F19       | MOL005012  | -7.780          |
| PTGS2  | 5F19       | MOL005016  | -7.258          |
| PTGS2  | 5F19       | MOL005017  | -7.847          |
| PTGS2  | 5F19       | MOL005018  | -6.991          |
| PTGS2  | 5F19       | MOL005828  | -7.270          |
| PTGS2  | 5F19       | MOL013187  | -7.200          |
| PTGS2  | 5F19       | MOL013381  | -6.758          |
| RELA   | 3QXY       | MOL000006  | -5.254          |
| RELA   | 3QXY       | MOL000098  | -6.271          |
| RELA   | 3QXY       | MOL000173  | -4.963          |
| RELA   | 3QXY       | MOL000354  | -5.728          |
| RELA   | 3QXY       | MOL000422  | -5.817          |
| RELA   | 3QXY       | MOL000497  | -5.488          |
| RELA   | 3QXY       | MOL0001689 | -5.400          |
| RELA   | 3QXY       | MOL002714  | -5.166          |
| RELA   | 3QXY       | MOL004328  | -5.864          |
| STAT3  | 6NS        | MOL000497  | -4.473          |
| VEGFA  | 4KZN       | MOL000006  | -5.593          |
| VEGFA  | 4KZN       | MOL000098  | -5.617          |
| VEGFA  | 4KZN       | MOL002714  | -4.422          |
| HSP90AA1| 6U99       | MOL000006  | -5.964          |
| HSP90AA1| 6U99       | MOL000098  | -5.850          |
| HSP90AA1| 6U99       | MOL04915   | -5.759          |
| HSP90AA1| 6U99       | MOL004883  | -5.597          |
| HSP90AA1| 6U99       | MOL004949  | -5.482          |
| HSP90AA1| 6U99       | MOL000422  | -5.394          |
| HSP90AA1| 6U99       | MOL002721  | -5.246          |
3.7. Molecular docking

To further verify the binding capacity between active compounds and key targets, molecular docking through Schrodinger was performed. The degree ranked the top 10 target proteins of PPI topology analysis network and 6 DEGs that overlapped with candidate therapeutic targets were selected as docking objects. Since the PDB structures of FOS have no original ligands, further analysis has to pause. The docking results were listed in Table 3, where PTGS2 and Inophyllum E exhibited the tightest binding (Fig. 6). There were 9 targets that bind to the compound luteolin and 10 targets binding to the quercetin in these 13 docking targets, indicating a major role of luteolin and quercetin for XFZYD in the treatment of ASCVD.
4. Discussion

ASCVD is a kind of complex and multifactorial disease and remains the leading cause of death worldwide (19). TCM has characteristics of multi-component and multi-target, which can affect different biological processes to control symptoms and solve the fundamental problems. In the present study, the effective compounds and candidate therapeutic targets in XFZYD for the treatment of ASCVD were 109 and 137, respectively. Moreover, 56.2% of 137 candidate therapeutic targets of XFZYD could be overlapped by at least 2 effective compounds, which demonstrated the effective compounds in XFZYD worked against ASCVD through a multitarget synergistic way. In addition, 94.44% of 108 effective compounds acted on at least 2 candidate therapeutic targets. Besides, luteolin was contained in 2 herbs (Carthamus tinctorius and Platycodon grandiflorum), quercetin was contained in 4 herbs (Bupleunum, Carthamus tinctorius, Achyranthes bidentate and Licorice), and kaempferol was contained in 4 herbs (Bupleunum, Licorice, Carthamus tinctorius and Achyranthes bidentate) acted on 35, 90 and 33 candidate therapeutic targets against ASCVD, respectively, suggesting that XFZYD is a combination of multiple herbs, multiple compounds and multiple targets in the treatment of ASCVD. Quercetin could effectively regulate the inflammatory process of atherosclerosis by attenuating TLR-NF-κB signaling pathway in vascular endothelial cells to inhibit the adhesion of leukocyte (20). Luteolin significantly reduced atherosclerosis induced by high-fat diet in ApoE mice and reduced ox-LDL-induced inflammatory response in vitro by inhibiting transcriptional activator 3 (STAT3)(21). Kaempferol could alleviates ox-LDL-induced apoptosis in HAECs by inhibiting the TLR4/NF-κB signaling pathway(22), and mediated lipid accumulation reduction and cholesterol efflux increase from THP-1-derived macrophages and inhibited the formation of ox-LDL-induced macrophage foam cell(23). KEGG pathway analysis showed that these candidate therapeutic targets were highly correlated with atherosclerosis, mainly involving inflammatory response, oxidative stress, angiogenesis and apoptosis. It was suggested that XFZYD may influence the inflammatory response in atherosclerosis by affecting TNF signaling pathway and Toll-like receptor (TLR) signaling pathway.

As for the TNF signaling pathway, its downstream genes were mostly involved in inflammatory
response, the pro-inflammatory cytokine IL-1β activates the nuclear factor-κB (NF-κB) signaling pathway(24), induces the production of various pro-inflammatory cytokines such as TNF-α and IL-6, and positively regulates the further activation of NF-κB, resulting in an inflammatory cascade amplification effect(25). Monocyte chemoattractant protein-1 (MCP-1) recruited monocytes to migrate to the damaged vascular endothelium, then monocytes differentiate into macrophages, ingesting lipid particles transform into foam cells, leading to local amplification of inflammatory effect(26). Matrix metalloproteinases-9 (MMP9), the downstream product of TNF signaling pathway, is an important member of the matrix metalloproteinase family, which could promote the degradation and remodeling of extracellular matrix in the pathological state caused by inflammatory mediators, leading to the instability and rupture of atherosclerotic plaques, thrombosis and other complications(27, 28).

Meanwhile, the expression of MMP9 in peripheral blood of atherosclerosis patients was significantly up-regulated compared with the control group, confirming MMP9 was a potential therapeutic target for atherosclerosis, consistent with our predicted therapeutic targets of XFZYD.

With reference to the TLRs pathway(29), it includes TLRs, the well-defined pattern recognition receptors of immune system, in the chronic inflammation and immune response in atherosclerosis(30). TLRs engagement with their ligands stimulates pro-inflammatory cytokine production and foam cell generation, mediating the occurrence and development of coronary atherosclerotic plaque by regulating inflammation and immune response(31). Activation of the TLR signaling pathway leads to the production of multiple pro-inflammatory cytokines (IL-6, TNF-α) and chemokines (IL-8, MIP2), leading to the emergence of inflammatory responses(32, 33), accelerating the pathological process of atherosclerosis.

Moreover, the accumulation of vascular smooth muscle cells (VSMCs) promotes plaque formation and development by migrating, proliferating, and secreting extracellular matrix to interact with other cellular components leading to the formation of plaques and thickening of the vascular wall, and finally a narrowing of the blood vessels(34–36). Studies have shown that the activation of the PI3K-Akt signaling pathway aggravates atherosclerotic injury in atherosclerosis mice(37), and has an inhibitory effect on the apoptosis of VSMCs(38). In view of the promotion effect of PI3K-Akt pathway on
atherosclerosis and the increased expression of BCL2L1 (product of PI3K-Akt pathway), PI3K-Akt signaling pathway may be a crucial pathway to inhibit the apoptosis of VSMCs as well as accelerate the accumulation of plaque atherosclerosis, suggesting that inhibiting PI3K-Akt signaling pathway may be one of the pathways for XFZYD to treat atherosclerosis.

In addition, vascular endothelial dysfunction was the initiating factor of atherosclerosis(39). Vascular endothelial growth factor-A (VEGFA), the most specific and prominent angiogenic factor of VEGF family, has been reported to promote the proliferation and differentiation of vascular endothelial cells, promotes vascular endothelial regeneration, regulates vascular endothelial permeability(40). It was reported that endothelial production of VEGFA may elicit a protective response to vascular injury(41).

In the present study, the expression of VEGFA in atherosclerosis group were significantly decreased and the VEGFA has a larger degree in PPI topological analysis of candidate therapeutic targets of XFZYD, suggesting that inhibition of VEGF signaling pathway may be a potential pathway for XFZYD to treat atherosclerosis.

However, there were still shortcomings in this research. Since the pathological development of atherosclerosis involves complex pathological processes, the mechanism predicted above of XFZYD in treating atherosclerosis still needs to be supplemented by in vivo and in vitro experiments.

5. Conclusions
In the present study, we identified effective compounds of XFZYD that could potentially antagonize atherosclerosis through drug targets prediction, gene microarray analysis and network construction with reliable methods, which hopefully could accelerate the clinical application of XFZYD to the whole world.

Abbreviations
XFZYD Xuefu Zhuyu decoration
ASCVD atherosclerosis cardiovascular disease
TCM Traditional Chinese Medicine
TCMSP Traditional Chinese Medicine System Pharmacology
OB oral bioavailability
HL half-life
DL drug likeness
ADME absorption, distribution, metabolism, and excretion
PharmGKB Pharmacogenomics Knowledgebase
CTD Comparative Toxicogenomics Database
DC degree centrality
BC betweenness centrality
CC closeness centrality
PPI Protein-Protein Interaction
DEGs differentially expressed genes
TLR Toll-like receptor
NF-κB nuclear factor-κB
MCP-1 Monocyte chemoattractant protein-1
MMP9 Matrix metalloproteinases-9
VSMCs vascular smooth muscle cells
VEGFA vascular endothelial growth factor-A

Declarations

Authors’ contributions
FZ designed the experiments. BL, YX, and XZ wrote the manuscript and conducted the pharmacology information analyses. BL, JR, and SH visualized the data. CW and JL helped to do some supplement. All authors read and approved the final manuscript.

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Availability of data and materials
The datasets used and/or analysed during the current study are available from the corresponding author on reasonable request.
Ethics approval and consent to participate

Not applicable.

Consent for publication

Not applicable.

Competing interests

The authors declare that they have no competing interests.

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Figures

(A) The ASCVD-related targets. (B) The overlapped targets of ASCVD and XFZYD.
Figure 2

Compounds-targets network of XFZYD in treating ASCVD. Orange ellipse nodes represent potential therapeutic targets, green triangle nodes represent effective compounds, red triangle nodes represent the effective compound originated from multiple herbs.
Figure 3

Topological compounds-targets network of XFZYD. Orange ellipse nodes represent potential therapeutic targets, green and red triangle nodes represent effective compounds. The size of node is proportional to the value of the degree centrality by topology analysis.
Figure 4

(A) Protein-protein interaction (PPI) network. (B) PPI core network after topology analysis.

The size of the circle represents the node degree of the target protein.
The top 20 KEGG pathway enrichment analyses of 137 target proteins (P value < 0.05).
Figure 6

Molecular docking diagram of PTGS2 and Inophyllum E.

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