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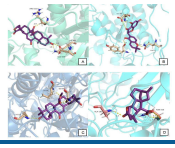
RESEARCH ARTICLE

Potential mechanism of Xuefu Zhuyu Decoction in Treating Lower Limb Venous Thrombosis based on Network Pharmacology and Molecular Docking Technology

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Abstract: Objective: This study aims to elucidate the mechanism of Xuefu Zhuyu decoction (XFZY) in treating lower limb venous thrombosis (LLVT) using network pharmacology and molecular docking technology. Methods: The active ingredients and corresponding targets of XFZY were determined through the Traditional Chinese Medicine Systems Pharmacology Database and Analysis Platform, PubChem, and SwissTargetPrediction databases. LLVT-related targets were searched from the Human Gene and Online Mendelian Inheritance in Man databases. Intersection targets were subjected to protein-protein interaction (PPI) network construction using the STRING database, with core targets identified based on their betweenness. Cytoscape was used to construct a "drug-component-target" network, and major compounds were screened out according to the betweenness. Gene Ontology (GO) and Kyoto Encyclopedia of Genes Genomes (KEGG) pathways enrichment analyses were conducted using the Metascape database. Molecular docking validation was carried out using AutoDock 1.5.7. Results: A total of 154 active constituents were identified, including major compounds such as baicalin, luteolin and flavanones. PPI, GO, and KEGG highlighted key targets for LLVT, including TP53, SRC and HSP90AA1, with major signaling pathways like PI3K-Akt, MAPK, and Rap1 pathways, involving various biological processes such as cancer, cell migration and phosphorylation. Stable complexes could be formed between the active constituents of XFZY and target proteins. Conclusion: This study provides preliminary insights into the multi-component, multi-target, and multi-pathway therapeutic mechanisms of XFZY for LLVT, laying a foundation for its clinical development.

Keywords: Network Pharmacology; Molecular Docking; Xuefu Zhuyu Decoction

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1. Introduction

LLVT is a condition characterized by abnormal coagulation of blood in the veins of the lower extremities, often associated with slow venous blood flow, vascular wall damage, and a hypercoagulable state of blood[1, 2]. Risk factors for LLVT include fractures, surgery, pregnancy, advanced age, physical inactivity, and genetic mutations[3, 4]. Symptoms typically include unilateral painful swelling in the lower limb[5], and if left untreated, can lead to complications such as pulmonary thromboembolism (PTE), which can result in hemodynamic instability, sudden death, and higher fatality and disability rates[6] . Current treatment options for lower extremity venous thrombosis include , surgical thrombectomy and interventional therapy. Anticoagulant therapy may entail the use of drugs including heparin, warfarin, dabigatran and edoxaban. Conversely, thrombolytic therapy may employ agents such as urokinase, alteplase, and reteplase[7, 8] . Heparin is a rapid-acting anticoagulant, but it can cause bleeding and thrombocytopenia in patients[9];warfarin, on the other hand, interacts extensively with food and other drugs and is not suitable for its efficacy[10] . Dabigatran and edoxaban are convenient for long-term anticoagulant treatment; however,they are expensive and require strict renal function monitoring[11] . The utilisation of urokinase in the treatment of lower limb venous thrombosis may potentially result in bleeding complications and requires close monitoring[12]. The high price of alteplase limits its widespread utilisation[13] . Reteplase poses a risk of recurrent thrombosis and bleeding[14]. Furthermore, these treatments should be carefully considered based on the patient's overall condition and risk of bleeding, as older patients may face a

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higher risk of complications and recurrence, as well as increased long-term treatment costs[15]. It is therefore imperative to investigate novel treatment modalities for LLVT. Traditional Chinese medicine embodies the characteristics of being multi-component, multi-target, and multi-pathway in the treatment of diseases. Xuefu Zhuyu Decoction, which has its origins in Wang Qingren's influential work "Medical Forest Reform," comprises 11 medicinal components including peach kernel, safflower, angelica, rehmannia, ox knee, chuanxiong, platycodon grandiflorum, red peony, fructus aurantii, licorice, and bupleurum. Its therapeutic effects primarily focus on enhancing blood circulation, eliminating stasis, promoting qi movement, and alleviating pain. Xuefu Zhuyu Decoction is also employed in the treatment of diverse cardiovascular pathologies such as coronary heart disease, myocardial infarction, cerebral infarction, and hypertension. The recent experimental studies conducted by Wang Yumiao on the Xuefu Zhuyu Decoction have demonstrated its efficacy in significantly increasing blood flow and reducing blood viscosity, thereby effectively preventing thrombosis[16] . Xie Yisong and colleagues have demonstrated that the Xuefu Zhuyu Decoction is an effective intervention for reducing the risk of venous thrombosis in patients[17]. Furthermore, clinical investigations have provided evidence of its efficacy in the management of venous thrombosis[18-20] .Nevertheless, there is a lack of fundamental research on its application in lower limb venous thrombosis, and the key targets and mechanisms involved are still unclear. Network pharmacology is an interdisciplinary field that integrates systems biology, modern pharmacology, bioinformatics, and computer science. It employs biological information networks to explore drug-disease relationships. By analyzing the topological structures of these networks, it aims to elucidate the mechanisms of action of drugs[21]. The objective of this study is to elucidate the mechanism of action of XFZY in the treatment of lower limb venous thrombosis through the application of network pharmacology, thereby providing a theoretical foundation for further research in this area.

2. Materials and methods

2.1 Screening of active ingredients and targets of Xuefu Zhuyu Decoction

Firstly, through a comprehensive literature search and the Traditional Chinese Medicine Systems Pharmacology Database and Analysis Platform (TCMSP), the active ingredients in Xuefu Zhuyu Decoction were identified. Subsequently, based on the criteria of Oral bioavailability (OB) ≥ 30% and Drug likeness (DL) ≥ 0.18[22] , these active ingredients were screened. The chemical structures of the selected active ingredients were verified using the PubChem. Additionally, the SwissTargetPrediction database was employed to predict the targets of these components, and the prediction results were integrated while eliminating any duplicated information.

2.2 Screening for lower limb venous thrombosis-related targets

Upon conducting a search in the Online Mendelian Inheritance in Man databases (OMIM) database[23] and Human Gene (Gene Cards) database[24] using the keyword "lower limb venous thrombosis", we preliminary screened related targets. Subsequent integration of this information, coupled with elimination of duplicates, allowed us to accurately identify specific targets closely associated with LLVT.

2.3 Construction of the “drug-component-target” network

The intersection of the component target and the disease target was obtained using the Venn diagram, and the “drug-component-target” network was constructed using Cytoscape software. Circles in the network graph are active ingredients and drugs; light blue diamonds are targets; edges represent the relationship of the three. The topological properties of the corresponding networks were analyzed using the CytoNCA plugin in Cytoscape, the network style was set according to the number centrality, and the size of circles and diamonds reflected the size of the betweenness value.

2.4 Construction of PPI about common targets and screening of core targets

The target intersection obtained was incorporated into the String online interaction database, with the species designated as Homo sapiens and other parameters maintained at their default settings. Without interact genes were eliminated, and a PPI network for the target intersection was established using a confidence level of ≥ 0.9 as the selection criterion. Subsequently, the corresponding PNG and TSV format files were downloaded, and the TSV files were imported into Cytoscape 3.8.0 software for network analysis. Utilizing the "CytoNCA" plug-in, the color and size of nodes in the graph were adjusted based on their betweenness value, while the thickness of edges was determined by the Combine Score. Through these procedures, a precise PPI network graph centered on core targets was ultimately created.

2.5 Analysis of GO enrichment and KEGG enrichment

The co-action targets of diseases and drugs identified in the preliminary screening should be imported into the Metascape platform. Homo sapiens should then be selected, and GO enrichment analyses and KEGG pathway analyses should be performed based on $P<0.05$. GO enrichment analysis focuses on three core dimensions: biological processes, molecular functions, and cellular composition. Subsequently, high-priority entries and signalling pathways were identified based on P values, and pathway integration and visualisation were performed using a microbial platform.

2.6 Simulations of molecular docking

In the constructed "drug-component-target" network diagram, sort according to the value of betweenness, and select the top four active components in numerical order.

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In the PPI network diagram, select the top five targets with betweenness values. The molecular structures of these active components and 3D structure in SDF format file were downloaded from TCMSP and PubChem databases. Open Babel software was used to convert and save the SDF file to PDB format. Core proteins of PPI network construction were screened in the PDB protein database, selecting only entries for Homo sapiens with X-ray crystallography as the analytical method and a resolution better than 3 angstroms. The confirmed proteins were downloaded in PDB format, imported into PyMOL software, and processed to remove water molecules, metal ions, and ligands, and finally saved as PDBQT files. These protein and small molecule files were subsequently processed in AutoDock 1.5.7, subjected to a hydrogenation treatment and saved in PDBQT format. The receptor and ligand PDBQT files were used in AutoDock1.5.7 to prepare docking boxes and determine the minimum binding energy. Finally, the ligand file with the lowest binding energy and the protein receptor were imported into PyMOL 2.4.0 to visualize the molecular docking.

3. Results

3.1 Active ingredients and targets of Xuefu Zhuyu Decoction

The main active ingredients of Xuefu Zhuyu Decoction were retrieved and screened using the TCMSP database and relevant literature. Ingredients with an oral bioavailability (OB) of ≥30% and a drug-likeness (DL) of ≥0.18 were considered as key active ingredients. A total of 260 active ingredients were identified, including 23 species of peach kernel, 20 species of Achyranthes bidentata, 92 species of Glycyrrhizae radix, 2 species of Angelica sinensis, 7 species of Ligusticum chuangxiong, 22 species of Carthapura, 7 species of platycodin platyflorum, 5 species of aurantii, 17 species of bupleurum, 29 species of red peony, and 36 species of raw rehmanniae. Upon a thorough analysis of 154 active ingredients in Xuefu Zhuyu Decoction, we identified 8 common compounds: stigmasterol, quercetin, kaempferol, baicalin, spinach sterol, luteolin, isorhamnosum and baicalin. After excluding duplicate terms and compounds lacking structural formulas, the formula was confirmed to contain a total of 154 active ingredients, some of which are detailed in Table 1. A total of 1039 potential drug targets were identified by PubChem and SwissTargetPrediction database.

Table 1. Some active components and ADEM parameters of Xuefu Zhuyu decoction.

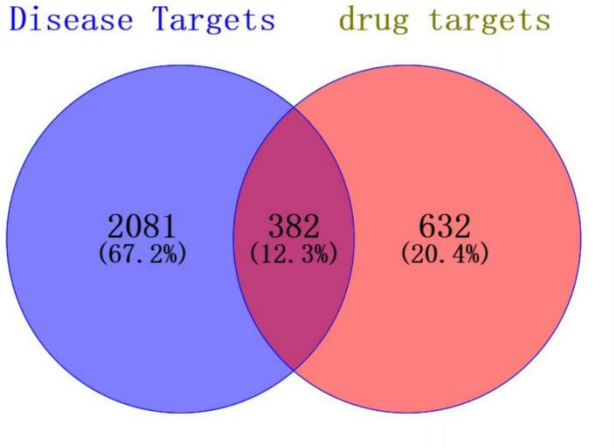
MOL ID	Molecule Name	OB/%	DL
MOL001323	Sitosterol alpha1	108.89	0.78
MOL001328	2,3-didehydro GA70	101.61	0.5
MOL001329	2,3-didehydro GA77	94.64	0.53
MOL001339	GA119	93.17	0.49
MOL001340	GA120	90.78	0.45
MOL001342	GA121-isolactone	88.6	0.54
MOL001343	GA122	88.11	0.5
MOL001344	GA122-isolactone	88.08	0.54
MOL001348	gibberellin 17	87.89	0.49
MOL001350	GA30	84.85	0.54
MOL001351	Gibberellin A44	83.71	0.54
MOL001352	GA54	80.36	0.53
MOL001353	GA60	80.3	0.53
MOL001355	GA63	79.91	0.54
MOL001358	gibberellin 7	78.77	0.5
MOL001360	GA77	76.76	0.53
MOL001361	GA87	76.36	0.57
MOL001368	3-O-p-coumaroylquinic acid	75.84	0.29
MOL001371	Populoside_qt	75.18	0.2
MOL000296	hederagenin	74.66	0.75
MOL000358	beta-sitosterol	73.8	0.75
MOL000493	campesterol	72.7	0.71
MOL001006	poriferasta-7,22E-dien-3beta-ol	72.67	0.76
MOL012461	28-norolean-17-en-3-ol	71.12	0.78

3.2 Lower limb venous thrombosis-related targets

In the Gene Cards and OMIM databases, 2463 and 534 targets related to LLVT were obtained by searching for the key words "lower limb venous thrombosis". After combining the disease targets in these two databases, duplicate terms were removed, and 2463 targets related to LLVT were finally identified.

3.3 Intersection targets and the "drug-component-target" network

Using the Venny 2.1 online software drawing platform, the disease-related targets of LLVT and the action targets of drug components of Xuefu Zhuyu decoction were processed, and the Venny diagram was generated accordingly. A total of 395 intersection targets were obtained, as shown in Figure 1. The constructed network and property files were imported into Cytoscape to draw the "drug-component-target" network diagram. Using the network analyzer plug-in topology analysis, according to the median filter key drug chemical composition. See Figure 2 and Table 2.



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Fig. (1). Intersection targets of Xuefu Zhuyu decoction and LLVT.

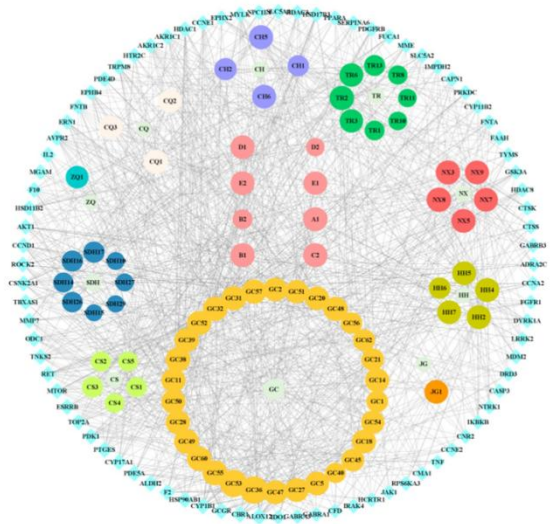


Fig. (2). The "drug-component-target" network.

Note: The pink circle represents the common chemical components of the drug; Purple circle, dark green circle, red circle, grass green circle, orange circle, yellow circle, light green circle, dark blue circle, blue circle, and flesh circle represent the chemical components of peach kernel, safflower, rehmannia, ox knee, chuanxiong, platycodon grandiflorum, red peony, fructus aurantii, licorice, and bupleurum, respectively; Light green circles represent drugs; Light blue diamonds represent targets; Edges represent connection relationships**.**

Table 2. Key chemical components of Xuefu Zhuyu Decoction.

Order Number	Medicine	Molecule Name	Betweenness
1	glycyrrhiza	3,22-Dihydroxy-11-oxo-delta(12)-oleanene-27-alpha-methoxycarbonyl-29-oic acid	0.067
2	glycyrrhiza	Phaseolinisoflavan	0.054
3	glycyrrhiza	licoisoflavanone	0.050
4	Peach kernel	GA120	0.039

3.4 PPI network and key targets

By comparing the betweenness of each target, we identified 5 core targets: TP53, SRC, PRKCD, PTGS2 and HSP90AA1. The core targets are shown in Table 3. The PPI network was constructed step by step based on these core targets and is shown in Figure 4. In this network, the color change of a node gradually changes from red with high betweenness values to blue with low betweenness values, indicating its influence in the network, that is, the higher the betweenness values, the more important the connection role of the target in the network.

Table 3. The key targets of Xuefu Zhuyu Decoction in the treatment of LLVT.

Gene name	Betweenness	Gene name	Betweenness
TP53	15894.70927	AKT1	5232.171856
SRC	10471.98564	CYP1A2	5104.610094
HSP90AA1	8235.087042	PLG	4928.748448
GAPDH	8062.42445	ALB	4774.710975
PTGS2	7708.393183	HIF1A	4700.888284
CXCR4	7200.494763	ITGB1	4519.941872
IL6	6998.750577	EP300	4424.029832
STAT3	6154.11366	DPP4	4405.794303
PRKCD	5361.625288	ESR1	4353.028452
EGFR	5300.504746	ADCY1	3997.867044

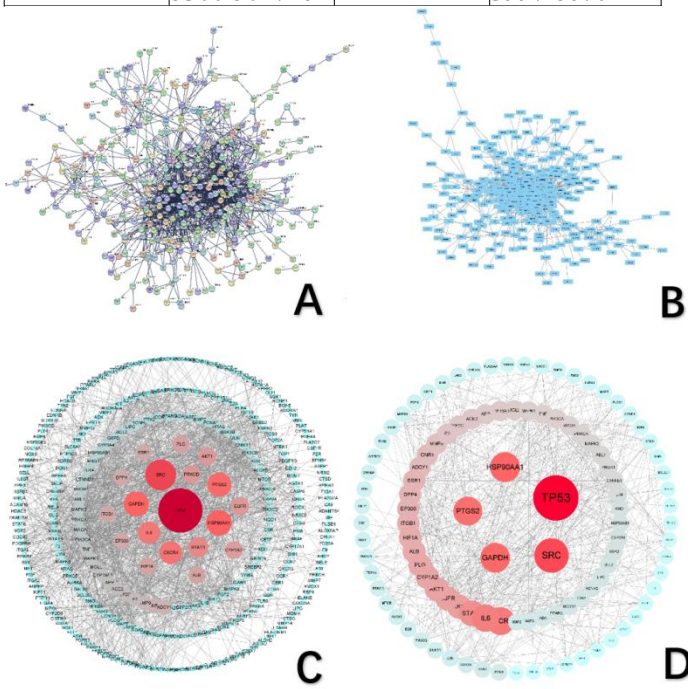


Fig. (3). PPI network of common targets of Xuefu Zhuyu decoction and LLVT.

Note: A is the protein-protein interaction diagram of the core targets generated by String database, B is the protein-protein interaction diagram of the core targets formed by deleting unrelated targets with Cytoscape 3.8.0, C is the protein-protein interaction network diagram of the core targets further screened. D is the protein-protein interaction network generated by screening the core five targets according to betweenness.

3.5 GO enrichment analysis

The obtained intersection targets were imported into the Matescape database for GO enrichment analysis. Results showed that a total of 3713 significant enrichment of GO entries ($P<0.05$), It covers 3,173 Biological Processes (BP), 181 Cellular Components (CC) and 359 Molecular Functions (MF). The top ten items were selected according to the ascending order of P values, and the corresponding bar charts were drawn with the help of

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the microbiology platform, as shown in Figure 4. It is concluded that the cell composition mainly includes cellular structures such as membrane rafts, membrane microdomains and receptor complexes. These structures participate in the positive regulation of movement, hormone response and phosphorylation through a range of biological functions, including kinase activity, phosphotransferase activity, kinase binding, growth factor binding and so forth. These findings revealed that Xuefu Zhuyu decoction may regulate the pathological process of LLVT by interfering with multiple biological pathways. Detailed entry information

related to GO enrichment analysis is shown in Table 4.

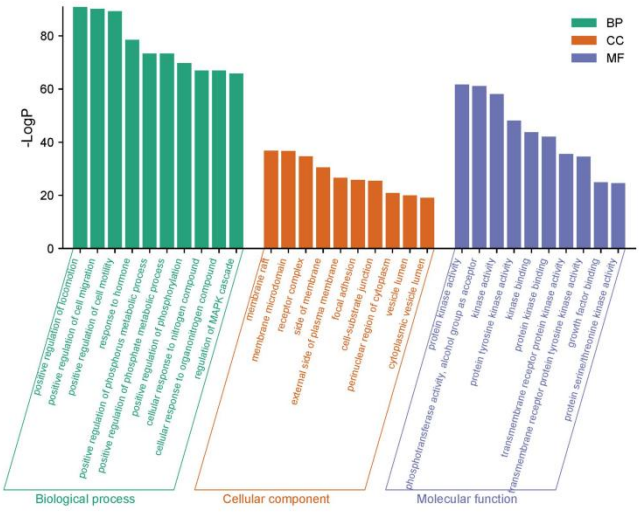


Fig. (4). GO Enrichment Analysis Bar Chart.

Table 4. The information pertaining to entries associated with GO enrichment.

Order Number	Category	Description	LogP
1	Biological Processes	positive regulation of locomotion	-90.80
2	Biological Processes	positive regulation of cell migration	-90.10
3	Biological Processes	positive regulation of cell motility	-89.24
4	Biological Processes	response to hormone	-78.44
5	Biological Processes	positive regulation of phosphorus metabolic process	-73.37
6	Biological Processes	positive regulation of phosphate metabolic process	-73.37
7	Biological Processes	positive regulation of phosphorylation	-69.70
8	Biological Processes	cellular response to nitrogen compound	-66.94
9	Biological Processes	cellular response to organonitrogen compound	-66.88
10	Biological Processes	regulation of MAPK cascade	-65.87
11	Cellular Components	membrane raft	-36.76
12	Cellular Components	membrane microdomain	-36.69
13	Cellular Components	receptor complex	-34.67
14	Cellular Components	side of membrane	-30.47
15	Cellular Components	external side of plasma membrane	-26.54
16	Cellular Components	focal adhesion	-25.84
17	Cellular Components	cell-substrate junction	-25.42
18	Cellular Components	perinuclear region of cytoplasm	-20.89
19	Cellular Components	vesicle lumen	-19.99
20	Cellular Components	cytoplasmic vesicle lumen	-19.05
21	Molecular Functions	protein kinase activity	-61.70
22	Molecular Functions	phosphotransferase activity, alcohol group as acceptor	-61.11
23	Molecular Functions	kinase activity	-58.13
24	Molecular Functions	protein tyrosine kinase activity	-48.08
25	Molecular Functions	kinase binding	-43.71
26	Molecular Functions	protein kinase binding	-42.08
27	Molecular Functions	transmembrane receptor protein kinase activity	-35.53
28	Molecular Functions	transmembrane receptor protein tyrosine kinase activity	-34.50
29	Molecular Functions	growth factor binding	-24.88
30	Molecular Functions	protein serine/threonine kinase activity	-24.60

3.6 KEGG pathway enrichment analysis

The KEGG pathway enrichment analysis indicated that 230 pathways might be implicated in the treatment of LLVT using Xuefuzhuyu decoction. These include the cancer pathway, PI3K-Akt signaling pathway, MAPK signaling pathway, proteoglycan in cancer, Rap1 signaling pathway, and Ras signaling pathway, among others. The enrichment analysis suggested that these aforementioned signaling pathways could be the crucial pathways for Xuefu Zhuyu decoction in treating LLVT. Through literature search, it was found that PI3K Akt, MAPK, and Rap1 signaling pathways are key pathways for the treatment of LLVT. Based on the p-value magnitude, the top 20 KEGG signaling pathways were chosen to create the bubble diagram, illustrated in Figure 5. For additional pathway and gene information, refer to Table 5.

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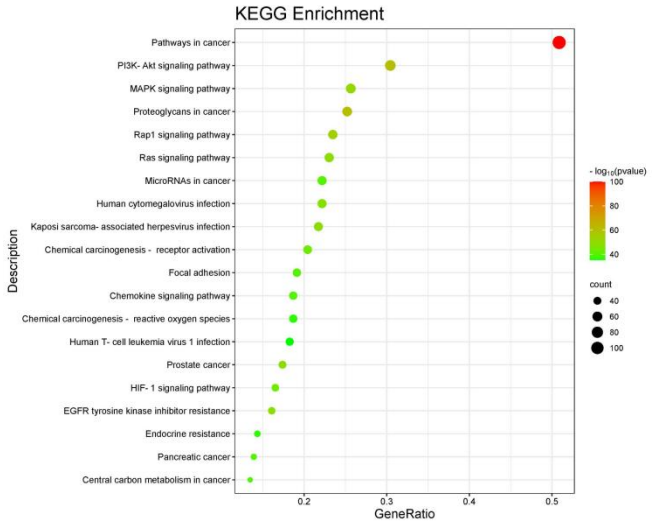


Fig. (5). Bubble plot of KEGG enrichment analysis.

Table 5. KEGG enriched pathway entry information.

Order Number	Pathway	LogP	Number of Genes
1	Pathways in cancer	-100	117
2	PI3K-Akt signaling pathway	-60.42	70
3	MAPK signaling pathway	-50.87	59
4	Proteoglycans in cancer	-60.08	58
5	Rap1 signaling pathway	-53.37	54
6	Ras signaling pathway	-48.96	53
7	MicroRNAs in cancer	-39.86	51
8	Human cytomegalovirus infection	-47.32	51
9	Kaposi sarcoma-associated herpesvirus infection	-49.44	50
10	Chemical carcinogenesis - receptor activation	-43.09	47
11	Focal adhesion	-39.87	44
12	Chemokine signaling pathway	-39.61	43
13	Chemical carcinogenesis - reactive oxygen species	-36.64	43
14	Human T-cell leukemia virus 1 infection	-35.43	42
15	Prostate cancer	-49.09	40
16	HIF-1 signaling pathway	-43.25	38
17	EGFR tyrosine kinase inhibitor resistance	-48.03	37
18	Endocrine resistance	-37.00	33
19	Pancreatic cancer	-39.66	32
20	Central carbon metabolism in cancer	-39.30	31

3.7 The analysis of the molecular docking

The findings of this study demonstrate the existence of robust binding interactions between the active constituents of the Xuefu Zhuyu Decoction and pivotal target proteins, suggesting the existence of potential therapeutic effects. Table 6 reveals that the minimum binding energies for the active molecule and target protein are all less than 0, indicating spontaneous binding between the ligand and receptor. In this study, a binding energy of < -5.0 KJ/mol was used as the screening criterion[25]. The docking binding energies for the SRC target protein and the four active ingredients are all below -5 kcal/mol, with values of -6.76, -5.03, -5.40, and -5.24 kcal/mol, respectively, indicating strong binding activity between the core protein target and active ingredients. The docking binding energy between 3,22-dihydroxy-11-oxo-delta(12)-oleanene-27-alpha-methoxycarbonyl-29-oic acid (3,22-Dihydroxy-11-oxo-delta(12)-oleanene-27-alpha-methoxycarbonyl-29-oic acid) and GAPDH protein is the lowest at -6.89 kcal/mol. The four components with the lowest binding energies are selected for the molecular docking diagram. Analyzing Figure 6, it is evident that in Figure 6A, at the amino acid positions of ARG-318, GLU-339, and LYS-401, 3,22-dihydroxy-11-oxo-di(12)-oleic acid-27-α-methoxycarbonyl-29-acid forms three hydrogen bonds with SRC, with bond lengths of 2.9A, 1.8A, and 2.3A, respectively; In Figure 6B, at the amino acid positions of TYR-340 and GLN-351, a flavonone forms four hydrogen bonds with SRC, with bond lengths of 2.4A, 2.1A, 3.0A, and 2.5A, respectively. In Figure 6C, at the amino acid positions of CYS-152, SER-98, and ILE-14, 3,22-dihydroxy-11-oxo-di(12)-oleicacid-27-α-methoxycarbonyl-29-acid forms three hydrogen bonds with GAPDH, with bond lengths of 2.3A, 2.9A, and 2.1A, respectively. In Figure 6D, a hydrogen bond is formed between GA120 and GAPDH at the amino acid positions of ALA-183 and ASN-316, with bond lengths of 2.2A, 1.8A, 2.1A, and 3.3A, respectively. The protein ID numbers of TP53, SRC, HSP90AA1, GAPDH and PTGS2 are P04637, P12931, P07900, P04406 and P35354. This indicates that there is an interaction between the active ingredients of Xuefu Zhuyu Decoction and key target proteins, and the more hydrogen bonds there are, the shorter the hydrogen bond length, and the stronger the interaction force[26]. These interactions can lead to the formation of stable complexes between compounds and target proteins.

Table 6. Docking results of the active components with the core target proteins.

Order number	Active ingredient	Binding energy(kcal/mol)				
		TP53	SRC	HSP90AA1	GAPDH	PTGS2
1	3,22-Dihydroxy-11-oxo-delta(12)-oleanene-27-alpha-methoxycarbonyl-29-oic acid	-4.33	-6.76	-5.08	-6.89	-5.16
2	Phaseolinisoflavan	-4.13	-5.03	-4.60	-4.80	-3.47
3	licoisoflavanone	-4.51	-5.40	-4.58	-4.98	-3.69
4	GA120	-4.51	-5.24	-5.05	-5.77	-4.93

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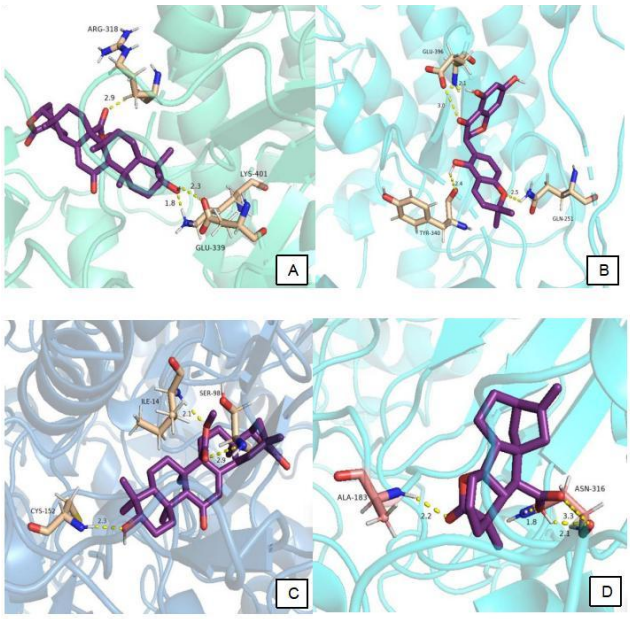


Fig. (6). Molecular docking diagram.

Fig. (6). Molecular docking diagram. Note: A is the molecular docking diagram of 3, 22-dihydroxy-11-oxo-bis (12) -oleic acid-27- α -methoxycarbonyl-29-acid and SRC. B shows the molecular docking diagram offlavanone and SRC; C shows the molecular docking diagram of 3, 22-dihydroxy-11-oxo-bis(12)-oleic acid-27- α -methoxycarbonyl-29-acid and GAPDH; D shows the molecular docking diagram ofGA120 and GAPDH.

4. Discussion

At present, the exact pathogenesis of LLVT is not clear. Fracture, trauma, long-term lie in bed, age, gender, and other factors are likely to induce the occurrence of venous thrombosis of lower limbs[27, 28]. In the theory of traditional Chinese medicine, LLVT is classified as malignant vein, pulse obstruction, haemoma, tenoma, blood stasis and other diseases. Its clinical manifestations include the superficial veins of the limb are cord-like, red, like magbots, hard and painful. The Neijing Suwen is one of the earliest documents to record venous thrombosis, which first proposed the location and nature of vascular diseases. Ge Hong, a renowned medicinal scientist of the Eastern Jin Dynasty, described "evil pulse disease" in his work "Emergency Prescription for Elbow Reserve" and coined the term "evil pulse". Thereafter, medical scholars in various dynasties referred to deep vein thrombosis as " evil pulse", "pulse obstruction", "blood tumor", "tendon tumor", "blood stasis", etc[29]. The primary cause of lower limb venous thrombosis is blood stasis obstructing the meridians, leading to impaired blood flow. Its pathogenesis is associated with poor blood circulation and stagnation of qi and blood[30]. Similar accounts are found in texts such as "Blood Syndrome Theory", "Thirteen Medical Treatises" and "Qian Jin Yao Fang". The treatment methods vary based on the doctor's approach, primarily including promoting blood circulation, removing blood stasis, unblocking meridians, promoting dampness and blood circulation, clearing away heat and blood stasis, invigorating qi and warming, and softening and resolving nodules[31]. Clinical efficacy observations and pharmacological studies have shown that Xuefu Zhuyu Decoction enhances the permeability of capillary networks, improves local microcirculatory blood flow at the fracture site, and accelerates the clearance of blood clots and metabolites[32-36]. Research further indicates that the combination of Xuefu Zhuyu Decoction with standard medical treatment significantly ameliorates patients' hypercoagulable states. Post-treatment, there are significant improvements in blood rheology and lipid profiles compared to pre-treatment levels[37]. Multiple studies have shown that Xuefu Zhuyu Decoction is a leading prescription for treating lower limb venous thrombosis primarily due to blood stasis[38-40].

This study retrieved data using various methods, with screening criteria of OB ≥ 30% and DL ≥ 0.18, yielding a total of 154 active components in Xuefu Zhuyu Decoction, including corn sterol alpha 1, gibberellin 17, 3-O-coumaroyl quinic acid, poplar glycoside, saponin, β-sitosterol, and camphor sterol. Through the drug-active component-target network diagram, four core active components were identified: 3,22-Dihydroxy-11-oxo-delta(12)-oleanene-27-alpha-methoxycarbonyl-29-oic acid, bean isoflavone, flavanone and gibberellin derivatives. These four active components may constitute the key pharmacological factors in Xuefu Zhuyu Decoction's therapeutic effect on LLVT[41]. Animal experiments have shown that flavanones can increase blood flow and promote blood circulation[42]. Dong Xiaowu's research demonstrated that natural flavonoid derivatives exhibit cardiovascular protective effects such as inhibiting the oxidation of low-density lipoprotein, anti-platelet aggregation, and vasodilation[43]. The aforementioned four compounds are primarily derived from the medicinal herb licorice. The compound DL in licorice is generally high, with Shinpterocarpin being the most abundant[44]. Shinpterocarpin belongs to the isoflavone class, and multiple studies have shown that this component can increase aortic and coronary flow, inhibit thrombin induced platelet aggregation, and resist experimental thrombosis through calcium antagonism which plays an important role in the prevention and treatment of LLVT[45-47]. Licorice, as a common medicine in traditional Chinese medicine, is usually used as an adjunct in the entire formula, playing a role in inducing meridians, tonifying, and harmonizing various medicines. At present, there are over 280 known components of licorice, mainly including flavonoids, triterpenoid saponins, alkaloids, and glycyrrhizic glycosides. Some of them have been proven to have a positive effect on blood clots[48]. An in-depth analysis was conducted on 154 active ingredients of Xuefu Zhuyu Decoction, and a total of 8 common compounds were obtained, namely stigmasterol, quercetin, kaempferol, baicalin, resveratrol, luteolin, isorhamnosum, and baicalin. Through in vitro studies on high blood viscosity syndrome models, it was found that quercetin can reduce the aggregation of red blood cells by improving the variability of red blood cells and to some extent reducing the content of fibrinogen in plasma, thereby

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reducing blood viscosity. Quercetin can also inhibit collagen activated platelet tyrosine protein phosphorylation, inhibit tyrosine kinase Syk and phosphatidic acid C-y2 tyrosine phosphorylation in the platelet glycoprotein VI collagen receptor signaling pathway, and then reducing platelet aggregation and collagen activated thrombosis[49-51]. Researchers have found that the antithrombotic effect of baicalin is mainly achieved by inhibiting both intrinsic and extrinsic coagulation pathways, thereby reducing platelet aggregation[52, 53]. Recent studies have shown that baicalin can exert anti thrombotic effects by inhibiting the production of heparin (FXa) and thrombin (Thrombin) in umbilical vein endothelial cells. In addition, baicalin also exerts corresponding anti thrombotic effects by inhibiting the production of plasminogen activator inhibitor-1 (PAI-1) induced by tumor necrosis factor-a (TNF-a)[54]. Numerous studies have shown that luteolin, icariin, and baicalin can also effectively alleviate thrombosis[54-59]. It can be seen that the ingredients in Xuefu Zhuyu Decoction are complex and diverse. Some compounds such as baicalin, luteolin, isoflavones, and flavanones can treat LLVT by reducing blood viscosity, reducing platelet aggregation, reducing blood flow, and inhibiting thrombin. However, there are also some compounds (such as kidney bean isoflavones) that lack relevant experimental evidence to prove their effects. It is relatively little research on the ingredients of Xuefu Zhuyu Decoction, and it cannot be ruled out that there may be new compounds generated that have not been discovered.

Based on PPI network analysis of the common targets of Xuefu Zhuyu Decoction and LLVT, we found that TP53 SRC, PRKCD, PTGS2, and HSP90AA1 are target genes with high betweenness values. Research has shown that TP53 is related to the formation of thrombosis in combination with ET[60]. After incubation with human platelets, artificially synthesized tetradecanoyl peptides can inhibit thrombus formation under pathological high shear rates by dissociating Src/ β 3 interactions[61]. In addition, multiple studies have shown that inhibiting the function of Src can alleviate the occurrence of LLVT[62-64]. The PRKCD mediated neutrophil extracellular trap pathway may lead to the occurrence of thrombosis in patients with polycythemia vera[65]. Therefore, it can be inferred that the active ingredients of Xuefu Zhuyu Decoction mainly act on TP53 key genes such as Src and PRKCD regulate the occurrence and development of LLVT.

GO enrichment analysis of the target proteins of these genes revealed that their functions mainly focus on kinase activity, kinase binding, growth factor binding, protein kinase activity, participating in positive regulation of exercise, positive regulation of cell migration, positive regulation of cell motility, response to hormones, positive regulation of phosphorus metabolism, and other biological processes. The main signaling pathways identified by KEGG enrichment analysis include the cancer pathway PI3K Akt, MAPK and Rap1 signaling pathway, etc. Chen Song et al. confirmed through a mouse thrombus model induced by carrageenan and rabbit platelet aggregation stimulated by adenosine diphosphate that inhibiting the PI3K Akt pathway can exert a protective effect on thrombus formation[66]. The PI3K Akt pathway can mediate platelet activation, leading to the formation of coronary microthrombus[67]. Oxidative stress and inflammation play a crucial role in the progression of LLVT. The MAPK signaling pathway mediates inflammatory signaling, and inhibiting its expression can reduce the mRNA expression level of pro-inflammatory cytokines, thereby alleviating the formation of thrombosis[68, 69]. Meanwhile, inhibiting the MAPK signaling pathway can induce vascular endothelial growth factor, thereby promoting thrombus reperfusion[70]. The animal experiments found that Raf1 in the MAPK pathway controls the production of thromboxanes in platelets, inducing thrombosis[71]. The Rap1 signaling pathway can regulate the antagonistic balance between CalDAG-GEFI and RASA3, regulating platelet adhesion in circulation and at the site of vascular injury and inducing thrombus formation[72]. PI3K Akt, MAPK and Rap1 signaling pathways play a crucial role in the treatment of LLVT.

The molecular docking results indicate that the active ingredients of Xuefu Zhuyu Decoction have good affinity with TP53, SRC, HSP90AA1, GAPDH, and PTGS2, allowing the compound to form stable complexes with these target proteins. The binding between the compounds and proteins of the active ingredients of Xuefu Zhuyu Decoction is mainly through hydrogen bonding or pi stacking interactions, some of which are generated by van der Waals forces. The docking score between SRC and 3,22-Dihydroxy-11-oxo-delta (12) oleane-27-alpha methoxycarbonyl-29-oic acid was the highest, indicating that the binding of SRC with effective compounds may play an important role in the treatment of LLVT. In the docking diagram of these two substances, it can be observed that the interaction force between 3,22-Dihydroxy-11-oxo-delta (12)-oleane-27-alpha methoxycarbonyl-29-oic acid and GLU-339 and LYS-401 is the highest. Research has shown that mutations in GLU are frequently present in familial thrombotic diseases[73], and it has been demonstrated that mutations in GLU promote the production of thrombin activating protein, thereby inducing thrombosis[74]. Kim et al. found that Lys contains thrombus regulatory protein binding sites necessary for exerting anti fibrinolytic activity[75]. The above studies all indicate that the tight binding of 3,22-Dihydroxy-11-oxo-delta (12) - oleane-27-alpha methoxycarbonyl-29-oic acid with SRC can effectively alleviate the production of LLVT by acting on GLU-339 and LYS-401. Through analysis of other docking diagrams, it was found that the tight binding between flavanone and GLU can inhibit platelet aggregation[76]. The close combination of GA120 and ALA can enhance the thrombolytic activity of blood clots[77]. The molecular docking results strongly confirmed the alleviating effect of Xuefu Zhuyu Decoction on LLVT.

The therapeutic effect of Xuefu Zhuyu Decoction on LLVT has the characteristics of multiple components, targets, and pathways. This provides important theoretical basis for further clinical and experimental research, and also points out new directions for the treatment of lower limb venous thrombosis. This study is based on network data for prediction, but the results have certain limitations. There are still shortcomings such as limited search databases, incomplete literature collection in the database, and related compounds and targets not being included in the database. The effect of Xuefu Zhuyu

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Decoction on LLVT is complex, and further in vivo and in vitro experiments are needed to clarify the specific mechanism of action of Xuefu Zhuyu Decoction on LLVT.

CONCLUSION

In conclusion, our study employed a multi-faceted approach, integrating network pharmacology and molecular docking, to examine the components, targets, and mechanisms of XFZY in the context of LLVT treatment. The present study has identified the key components and targets of XFZY, including baicalin, luteolin, flavanones, TP53, SRC and HSP90AA1. The findings of this study indicate that the therapeutic effects of TMJT on DN are primarily mediated through the regulation of immune and inflammation-related pathways. The Gene Ontology (GO) and Kyoto Encyclopedia of Genes and Genomes (KEGG) databases identified several key signalling pathways, including the phosphatidylinositol 3-kinase (PI3K)-Akt, mitogen-activated protein kinase (MAPK), and Ras - related protein 1 (Rap1) pathways. These pathways are involved in a range of biological processes, such as cancer, cell migration, and phosphorylation. Molecular docking analysis indicates the potential for stable complexes to be formed between the active constituents of XFZY and target proteins.

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LIST OF ABBREVIATIONS

No.

AVAILABILITY OF DATA AND MATERIALS

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