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Network Pharmacology-Based Investigation of the Mechanisms of Zhenwu Tang in Treating Viral Myocarditis

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Abstract: *Background*: Zhenwu Tang is a typical Chinese medicinal preparation for the treatment of viral myocarditis (VMC) with remarkable clinical efficacy in alleviating symptoms such as palpitations, chest tightness, and fatigue in some patients, and reducing the frequency of episodes. Nonetheless, the exact mechanism by which it acts remains unclear. This research employed network pharmacology to investigate the therapeutic impact of Zhenwu Tang's relevant targets on VMC, along with molecular docking validation. *Methods*: The primary components of Zhenwu Tang and their respective targets were sourced from the Traditional Chinese Medicine Systems Pharmacology Database and Analysis Platform (TCMSP) and the UniProt database, GeneCards and the Online Mendelian Inheritance in Man (OMIM) databases were used to identify targets related to VMC. Disease Ontology (DO), Gene Ontology (GO), and Kyoto Encyclopedia of Genes and Genomes (KEGG) enrichment analyses were used to evaluate the common 'Zhenwu Tang-VMC' targets. Using Cytoscape software, the protein-protein interaction (PPI) network, 'compound-target-pathway' network, and 'target-organ' network were developed, and their network parameters were thoroughly examined. The interactions between core targets and active compounds were evaluated and validated by molecular docking. *Results*: A total of 93 targets of Zhenwu Tang active compounds and 2,243 VMC-related targets were screened, and 37 Zhenwu Tang-VMC common targets were obtained. DO analysis of these 37 common targets indicated that the "Zhenwu Tang-VMC" common targets showed high enrichment in respiratory system diseases. According to the molecular docking results, 60 VMC ligand-receptor pairs and 47 respiratory-associated VMC ligand-receptor pairs had binding energies lower than -7 kcal/mol. *Conclusion*: The present study suggests that Zhenwu Tang is a promising Chinese medicinal preparation for the treatment of viral myocarditis, especially respiratory-associated viral myocarditis. Our illustration of the particular molecular mechanism of Zhenwu Tang against VMC and its potent active compounds offers a theoretical foundation for further basic experimental validation, clinical translation, and application.

Keywords: Network pharmacology; Zhenwu Tang; Molecular docking; Viral myocarditis; Precision medicine

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1. Introduction

Viral myocarditis (VMC) is an inflammation of the heart muscle that can be either acute or chronic, either localized or diffuse, caused by viral infection. It is a type of infectious heart muscle disease, with common causative viruses including coxsackievirus group B and adenovirus. The virus can directly invade the myocardium or indirectly cause damage through an immune response. Common symptoms of VMC include fever, fatigue, palpitations, chest tightness, shortness of breath, and precordial pain. Certain patients might also have gastrointestinal issues like nausea and vomiting. In critical instances, individuals may encounter severe arrhythmias, heart failure, cardiogenic shock, or even unexpected death [1]. According to epidemiological surveys, the annual incidence of viral myocarditis worldwide is approximately 10–20 cases per 100,000 people, but the actual incidence may be higher, as some mild cases are asymptomatic or do not seek medical attention and are easily missed [2]. Approximately 1–5% of patients with viral infections (such as the common cold or diarrhea) may progress to myocarditis, based on the virus type and the person's immune condition [3].

The current research frontiers in viral myocarditis cover aspects such as pathogenesis, biomarkers, and treatment strategies. In terms of mechanism, the focus is on the direct invasion of the myocardium by viruses (such as coxsackievirus and influenza virus) and immune-mediated inflammatory damage, exploring the role of abnormal activation of immune cells (such as macrophages) and cytokine storms [4,5]. Specific RNAs have been found to be involved in viral replication [6]. In terms of treatment, in addition to traditional symptomatic support, mesenchymal stem cells (MSCs) are being explored to repair myocardial damage, as well as immunomodulatory and targeted therapies to reduce myocardial injury and improve cardiac function [7,8]. At the same time, attention is being paid to the specific differences in myocarditis caused by different viruses to provide a basis for precision treatment. The current clinical treatment principle for myocarditis is to reduce or control myocardial inflammation and effectively manage arrhythmias and heart failure. The principles of treatment are antiviral, myocardial protection, symptomatic support, and prevention and treatment of complications. General treatment includes strict bed rest during the acute phase and nutritional support. Drug treatment includes antiviral drugs, myocardial protective drugs (such as vitamin C, coenzyme Q10), and symptomatic treatment drugs (for arrhythmias, heart failure, etc.). Severe patients require life support techniques such as extracorporeal membrane oxygenation. After the condition stabilizes, it is necessary to gradually resume activities and have regular follow-up. Corresponding treatment is given for the cause to improve the prognosis, but there are shortcomings such as long treatment time, poor efficacy, and easy recurrence.

Traditional Chinese medicine (TCM) categorizes VMC as 'palpitations', 'chest impediment', and 'heart vexation'. Its etiology is mostly exogenous wind-heat or warm-heat pathogenic evils that invade the heart, consuming Qi and injuring Yin, leading to "heart and kidney Yang deficiency, and water-fluid retention overwhelming the heart." Typical manifestations include palpitations (irregular heartbeat), constriction in the chest and difficulty breathing, swelling in the legs, dislike of cold and cold hands and feet, reduced urination, a pale and swollen tongue with a white, slippery coating, and a deep, weak, or irregular pulse [9].

The ethanol extract of rhubarb can reduce the expression level of viral RNA in the serum and myocardial tissue of mice with VMC, accelerating the body's clearance of the virus. However, rhubarb contains anthraquinone components, and long-term or excessive use may cause nephrotoxicity, manifested as renal tubular damage and abnormal renal function indicators, especially for those with pre-existing renal insufficiency. At the same time, it may lead to electrolyte disturbances (such as hypokalemia) and intestinal dependence [10].

Puerarin, a flavonoid component in Pueraria lobata, can alleviate oxidative stress damage by improving the activity of superoxide dismutase (SOD) and regulating the content of malondialdehyde (MDA) in myocardial tissue, and can reduce the expression level of coxsackievirus B3 (CVB3) RNA in the myocardium. However, long-term, high-dose use of puerarin may increase the metabolic burden on the liver and kidneys. It has been reported that some patients experience elevated transaminases and a slight increase in serum creatinine, especially those with chronic kidney diseases should be cautious. A small number of people may experience allergic reactions such as skin rash and gastrointestinal discomfort [11].

Astragalus membranaceus, along with its active constituents, namely total flavonoids, total saponins, and the monomer compound astragaloside IV, exhibits inhibitory effects on the proliferation of the CVB3 virus. Furthermore, it confers cardioprotective benefits by activating the NF-κB signaling pathway and inducing the expression of inflammatory mediators, including interferon and tumor necrosis factor-α (TNF-α) [12]. However, excessive or long-term use of *Astragalus* may induce symptoms of "heatiness" (such as dry mouth and sore throat). Its total saponin components may irritate the renal tubules at high doses. When used in combination with other nephrotoxic drugs, it may increase the risk of renal function damage. Those with renal insufficiency need to reduce the dose.

Tanshinone IIA (TSA), as the main active component of *Salvia miltiorrhiza*, may alleviate myocardial damage in mice caused by CVB3 by inhibiting the inflammatory response and regulating the Th1/Th2 immune balance. However, long-term application of tanshinone IIA may affect coagulation function (prolong bleeding time), and animal experiments have suggested that high doses may lead to a decrease in the glomerular filtration rate, with potential effects on renal function. Some patients may experience gastrointestinal reactions such as nausea and diarrhea [13].

The combination of total alkaloids of *Sophora flavescens* and total saponins of *Panax quinquefolius* can reduce the serum levels of TNF-α, interleukin-2 (IL-2), interleukin-17 (IL-17), and interferon-gamma (IFN-γ) in rats with experimental autoimmune myocarditis (EAM), inhibit the activation of Th1 and Th17 cell subsets, and thus alleviate myocardial inflammatory damage. However, the total alkaloids of *Sophora flavescens* contain components such as oxymatrine, and long-term use may cause renal parenchymal damage, manifested as increased urinary protein [14]. Excessive total saponins of *Panax quinquefolius* may lead to excitement and insomnia. The combined use of the two may increase the metabolic burden on the kidneys, and those with renal insufficiency need to strictly monitor renal function indicators.

Zhenwu Tang is a traditional Chinese medicine formula that includes Fuzi, Baizhu, Fuling, Shengjiang, and Shaoyao. Its core efficacy is to "warm Yang and promote urination," and it is mainly used for the syndrome pattern of Yang deficiency and water-fluid retention, and has achieved good clinical efficacy [15]. The "Research Progress of Traditional Chinese Medicine in the Treatment of Viral Myocarditis in Children" mentions that modern Chinese medicine is commonly utilized for addressing edema, palpitations, asthma, and various ailments linked to Yang deficiency in the heart, kidney, spleen, and other organs. In modern pharmacological research, Fuzi warms and tonifies kidney Yang to assist heart Yang, and is the core of "warming Yang"; Baizhu strengthens the spleen and removes moisture, while Fuling aids in urination and eliminates dampness. The combination of the two "cultivates earth to control water"; Shengjiang supports Fuzi by warming yang and removing cold, but also warms and disperses water-Qi, promoting the discharge of water-fluid from the body surface or urine, and enhancing the power of promoting urination; Shaoyao on the one hand softens the liver and relieves spasm, alleviating the constriction of the heart vessels, and on the other hand, it restrains the warm and dry nature of Fuzi and Shengjiang, avoiding excessive consumption of Yin fluid, and at the same time promotes urination to assist in draining water [16]. This combination allows the entire formula to "warm Yang" while also "promoting urination," clearing the retained water-fluid on the basis of correcting Yang deficiency. These conditions correspond to VMC, so Zhenwu Tang may help to improve the symptoms of palpitations, edema, and shortness of breath in patients with viral myocarditis caused by "heart and kidney Yang deficiency and internal retention of water-fluid" [17,18].

Traditional Chinese medicine compound preparations have the characteristics of "multiple active molecules, multiple therapeutic targets, and unknown interaction mechanisms," which makes it impossible for researchers to directly design cell or animal experiments for drug verification. In recent years, computational biology, network pharmacology, and molecular simulation technology have developed rapidly. Computational biology is an interdisciplinary field that analyzes the laws of biological systems through mathematical modeling, algorithm development, and computer simulation, helping to analyze gene function, metabolic networks, etc. Network pharmacology utilizes systems biology to create a 'drug-target-disease' interaction network for analyzing how multi-component drugs work (such as Chinese medicine) from a holistic perspective, emphasizing the overall regulation of biological networks. Molecular simulation technology uses computer programs to simulate the structure, interaction, and dynamic changes of biological molecules at the atomic or molecular level, and can predict molecular function and binding modes. All three are crucial instruments for contemporary life science research and are vital in drug research and development, disease mechanism analysis, and other fields. Therefore, this article utilizes the advantages of computers in processing big data to integrate, preliminarily process, and analyze existing public databases of traditional Chinese medicine and diseases. By analyzing the interaction between drugs and multiple disease targets, it provides a theoretical basis for subsequent basic experiments and a new perspective for the clinical transformation of drugs. Therefore, this article aims to investigate the possible therapeutic mechanisms of Zhenwu Tang in VMC using network pharmacology, bioinformatics, and molecular docking techniques (**Figure 1**).

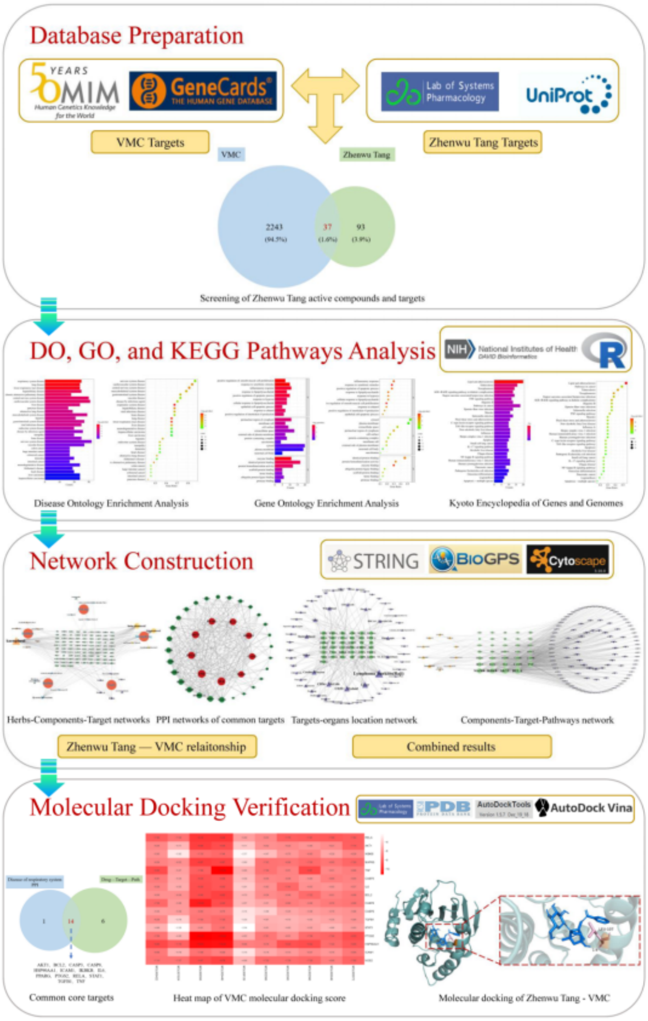


Figure 1. A detailed workflow of the network pharmacological investigation strategy for Zhenwu Tang in the treatment of VMC. Four parts include database preparation, DO, GO and KEGG pathway analysis, network construction, and molecular docking verification.

2. Materials and methods

2.1. Screening of active compounds and collection of potential targets of Zhenwu Tang

The TCMSP database (http://tcmspw.com/tcmsp.php) was used in this research to find the active compounds in Zhenwu Tang and their associated proteins. The selection criteria were established on two key pharmacokinetic parameters: oral bioavailability (OB) and drug-likeness (DL), with minimum values set at OB $\geq$ 30% and DL $\geq$ 0.18. Twenty-four active ingredients and their target proteins were identified. The UniProt database (https://www.uniprot.org) was utilized to transform the names of target proteins into their official gene symbols, resulting in a target library for the active ingredients of Zhenwu Tang [19].

2.2. Collection of VMC-related targets and identification of common targets

In the OMIM (https://omim.org/) and GeneCards (https://www.genecards.org/) databases, VMC-related genes were retrieved using the keywords "Acute Viral Myocarditis", "Chronic Viral Myocarditis", "Myocarditis of viral origin", and "Viral myocarditis". After removing duplicates, a list of VMC-related genes was obtained. A Venn diagram was used to visualize the overlap between the target genes of Zhenwu Tang's active ingredients and the VMC-related targets.

2.3. Construction of protein-protein interaction (PPI) network

Interacting genes and proteins were identified by importing the common "Zhenwu Tang-VMC" targets into the STRING database (http://string-db.org). The database was set to the "*Homo sapiens*" (human) species, and the PPI confidence score was set to 0.4 (medium confidence). The saved data results were imported into Cytoscape (v_3.10.0) software to construct a protein-protein interaction (PPI) network. The Degree value was calculated and the node size was adjusted to reflect the Degree value, in order to distinguish the importance of the targets.

2.4. DO, GO, and KEGG enrichment analyses

Using the "clusterProfiler", "org.hss.egg.db", "enrichment", "ggplot2", "GSEABase", and "DOSE" packages in R (v_4.5.0) software, The "Zhenwu Tang-VMC" targets commonly underwent enrichment analyses with Disease Ontology (DO), Gene Ontology (GO), and KEGG, with FDR < 0.05 and $P < 0.05$ as the screening criteria. The results were organized according to the *P*-value, and the corresponding bar charts and bubble charts were drawn to visualize the results.

2.5. Construction of topological networks

To scientifically clarify the connection between the active ingredients of Zhenwu Tang and the "Zhenwu Tang-VMC" targets, Cytoscape (v_3.10.0) software was used to establish a "drug-active compound-target" network for topological analysis and visualization. At the same time, A "compound-target-pathway" network was developed to illustrate the intricate interactions among compounds, targets, and pathways, to enhance our understanding of how the compounds function and discover new potential drug targets and pathways. The exact in vivo metabolism of Zhenwu Tang remains unclear, and its healing properties could affect multiple organs and tissues. The BioGPS database (https://biogps.org) was used to obtain mRNA data of the common "Zhenwu Tang-VMC" targets in different organs and tissues. Cytoscape (v_3.10.0) software was used to construct a "target-organ" network, and the size of each node was changed depending on its Degree value.

2.6. Molecular docking validation

To test the binding mode and fitness of the key active ingredients of Zhenwu Tang and the important targets, the active ingredients of Zhenwu Tang and the key action targets were subjected to molecular docking validation in turn. The core ligand structure files (mol2 format) were first retrieved from the TCMSP database (http://tcmspw.com/tcmsp.php), and then altered into PDB files by utilizing PyMol software. The target's crystal structure in three dimensions was acquired from the RCSB PDB database (https://www.pdb.org/), and then imported into PyMol for ligand separation. The ligand and receptor were then processed in AutoDockTools software for dehydration, hydrogen addition, and charge calculation, and stored as PDBQT files. After that, a docking grid box was generated in AutoDockTools to encompass the whole target protein, and the settings were saved as a txt file. Finally, AutoDock Vina software was used for molecular docking analysis. The visualization of free binding energy as a heat map was achieved using the "ggplot2" and "pheatmap" packages in R (v_4.5.0), while PyMol software was used to display molecular docking and its interactions.

3. Candidate targets and their corresponding compounds

3.1. Screening of active compounds and collection of potential targets of Zhenwu Tang

From the TCMSP database, we collected information on the active ingredients and targets of the five herbs in Zhenwu Tang: Fuling, Baishao, Shengjiang, Fuzi, and Baizhu. A total of 24 confirmed molecular compounds were discovered (**Table 1**). By searching the target module in TCMSP, 93 validated targets related to the compounds of Zhenwu Tang and their respective gene symbols were obtained. A Sankey diagram illustrated the connection between the herbal monomers and active compounds in Zhenwu Tang (**Figure 2**).

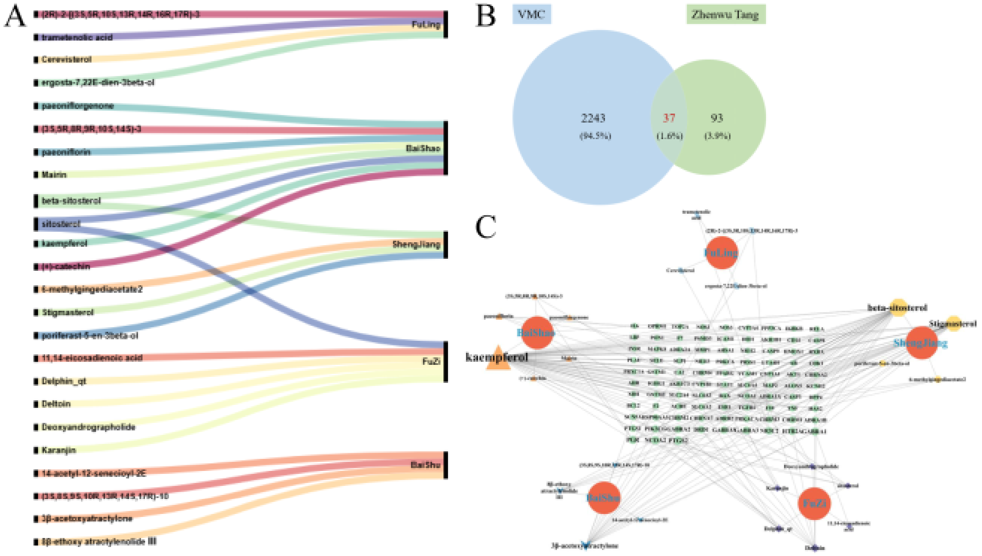


Figure 2. Screening of Zhenwu Tang active compounds and targets in VMC. (A) Sankey diagram of associations between Chinese herbal compounds and ingredients in Zhenwu Tang. (B) Venn diagram of 37 common targets between active compound targets of Zhenwu Tang and VMC disease targets. (C) Herb-compound-target network.

Table 1. Molecular compounds of Zhenwu Tang

MOL ID	Molecule name	OB (%)	DL	Herbal source
MOL000273	(2R)-2-[(3S,5R,10S,13R,14R,16R,17R)-3,16-dihydroxy-4,4,10,13,14-pentamethyl-2,3,5,6,12,15,16,17-octahydro-1H-cyclopenta[a]phenanthren-17-yl]-6-methylhept-5-enoic acid	30.93	0.81	Fuling
MOL000275	trametenolic acid	38.71	0.8	Fuling
MOL000279	Cerevisterol	37.96	0.77	Fuling
MOL000282	ergosta-7,22E-dien-3beta-ol	43.5	0.71	Fuling
MOL001918	paeoniflorgenone	87.59	0.36	Baishao
MOL001919	(3S,5R,8R,9R,10S,14S)-3,17-dihydroxy-4,4,8,10,14-pentamethyl-2,3,5,6,7,9-hexahydro-1H-cyclopenta[a]phenanthrene-15,16-dione	43.55	0.53	Baishao
MOL001924	paeoniflorin	53.87	0.78	Baishao
MOL000211	Mairin	55.37	0.77	Baishao
MOL000358	beta-sitosterol	36.91	0.75	Baishao, Shengjiang
MOL000359	sitosterol	36.91	0.75	Baishao, Fuzi
MOL000422	kaempferol	41.88	0.24	Baishao
MOL000492	(+)-catechin	54.82	0.24	Baishao
MOL006129	6-methylgingediacetate2	48.73	0.32	Shengjiang
MOL000449	Stigmasterol	43.82	0.75	Shengjiang
MOL001771	poriferast-5-en-3beta-ol	36.91	0.75	Shengjiang
MOL002211	11,14-eicosadienoic acid	39.99	0.2	Fuzi
MOL002388	Delphin_qt	57.76	0.27	Fuzi
MOL002392	Deltoin	46.69	0.36	Fuzi
MOL002395	Deoxyandrographolide	56.3	0.31	Fuzi
MOL002398	Karanjin	69.55	0.33	Fuzi
MOL000022	14-acetyl-12-senecioyl-2E,8Z,10E-atractylentriol	63.37	0.29	Baizhu
MOL000033	(3S,8S,9S,10R,13R,14S,17R)-10,13-dimethyl-17-[(2R,5S)-5-propan-2-yloctan-2-yl]-2,3,4,7,8,9,11,12,14,15,16,17-dodecahydro-1H-cyclopenta[a]phenanthren-3-ol	36.22	0.78	Baizhu
MOL000049	3β-acetoxyatractylone	54.06	0.21	Baizhu
MOL000072	8β-ethoxy atractylenolide III	35.95	0.21	Baizhu

3.2. Screening of VMC-related targets and targets of Zhenwu Tang

By integrating 1,322 VMC targets collected from GeneCards and 983 VMC targets from OMIM, we obtained 2,243 VMC-related disease targets after removing duplicates. In the Venn diagram (**Figure 2**, **Table 2**), 37 common targets of the active compounds of Zhenwu Tang and VMC disease targets were identified. These targets are candidate targets for the anti-viral myocarditis effect of Zhenwu Tang. A "drug-compound-target" network was constructed using Cytoscape, consisting of 5 herbal monomers, 24 active compounds, and 93 targets of Zhenwu Tang (**Figure 2**). The connections in the network illustrate the interactions between compounds and targets, and the size of the nodes reflects the strength of these interactions. Kaempferol (MOL000422), β-sitosterol (MOL000358), stigmasterol (MOL000449), and 3β-acetoxyatractylone (MOL000049) exhibited more interaction with the targets, implying that the therapeutic action of Zhenwu Tang against VMC includes several compounds working on different targets.

Table 2. 37 Common targets of active compounds of Zhenwu Tang and VMC disease targets

No.	Target
1	ACHE
2	AKT1
3	ALOX5
4	BAX
5	BCL2
6	CASP3
7	CASP8
8	CASP9
9	CD14
10	CHRM2
11	CYP1A1
12	CYP3A4
13	F2
14	HMOX1
15	HSP90AA1
16	ICAM1
17	IKBKB
18	IL6
19	KCNH2
20	LBP
21	MAPK8
22	NOS2
23	NR3C2
24	PIK3CG
25	PON1
26	PPARG
27	PPP3CA
28	PTGS2
29	RELA
30	SCN5A
31	SELE
32	SLC2A4
33	SLC6A4
34	STAT1
35	TGFB1
36	TNF
37	VCAM1

3.3. Construction of PPI of common targets

We built a PPI network using STRING and Cytoscape to explore how Zhenwu Tang treats VMC, focusing on 37 shared targets. In network analysis, the three main centrality measures are Degree Centrality (DC), Closeness Centrality (CC), and Betweenness Centrality (BC). In this study, we used DC, the most direct measure of node centrality, as the standard. The size of the nodes changes with the Degree value of the node. Based on the screening criteria of topological analysis, 19 targets with DC ≥ the median DC (16) were screened out, and these 19 core targets are displayed in the form of a bar chart, among which IL6 and TNF have a DC ≥ 2 times the median DC. Additionally, a cluster analysis was carried out with the MCODE plugin in Cytoscape to develop a well-connected sub-network (**Figure 3**).

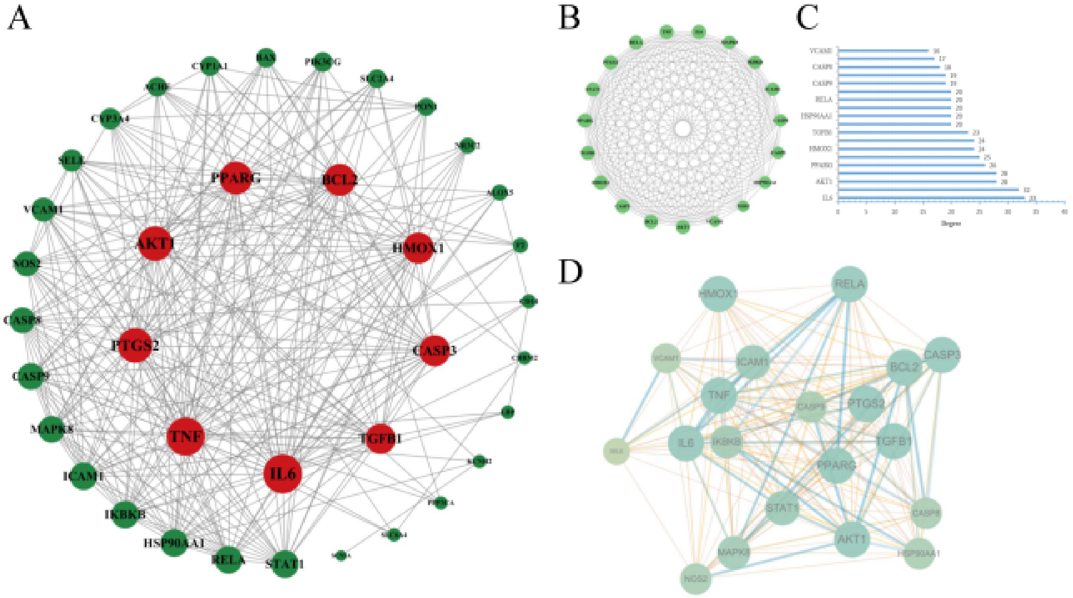


Figure 3. Identification of candidate targets through Protein-Protein Interaction (PPI) analysis. (A) PPI analysis. (B) Identification of top 19 core targets based on DC ≥ the median DC. (C) Bar chart of the top 19 core targets. (D) PPI network based on cluster analysis using the MCODE plugin.

3.4. DO, GO, and KEGG enrichment analyses

With FDR < 0.05 and $P < 0.05$ as the screening criteria, DO, GO, and KEGG enrichment analyses were performed on the 37 common "Zhenwu Tang-VMC" targets. The DO analysis identified enrichment in 760 disease categories, with the top 30 visualized using bar and bubble charts. The results showed that the highest-ranking respiratory diseases may induce the occurrence of viral myocarditis. A total of 106 GO terms were obtained from the GO analysis, of which 88 belonged to biological processes (BP), 11 to cellular components (CC), and 7 to molecular functions (MF). The most enriched BP term, inflammatory response, is mainly related to infections and diseases caused by tissue damage from pathogenic microorganisms, infectious agents, chemical stimuli, and other trauma. The CC terms mainly focus on the cytosol and cytoplasmic membrane. The MF terms that are highly enriched include identical protein binding, protein homodimerization activity, and enzyme binding. KEGG pathway enrichment analysis revealed 105 signaling pathways in total. The top 30 highly enriched KEGG pathways are closely related to lipids and atherosclerosis, cancer pathways, tuberculosis, toxoplasmosis, and Kaposi's sarcoma-associated herpesvirus infection (**Figure 4**).

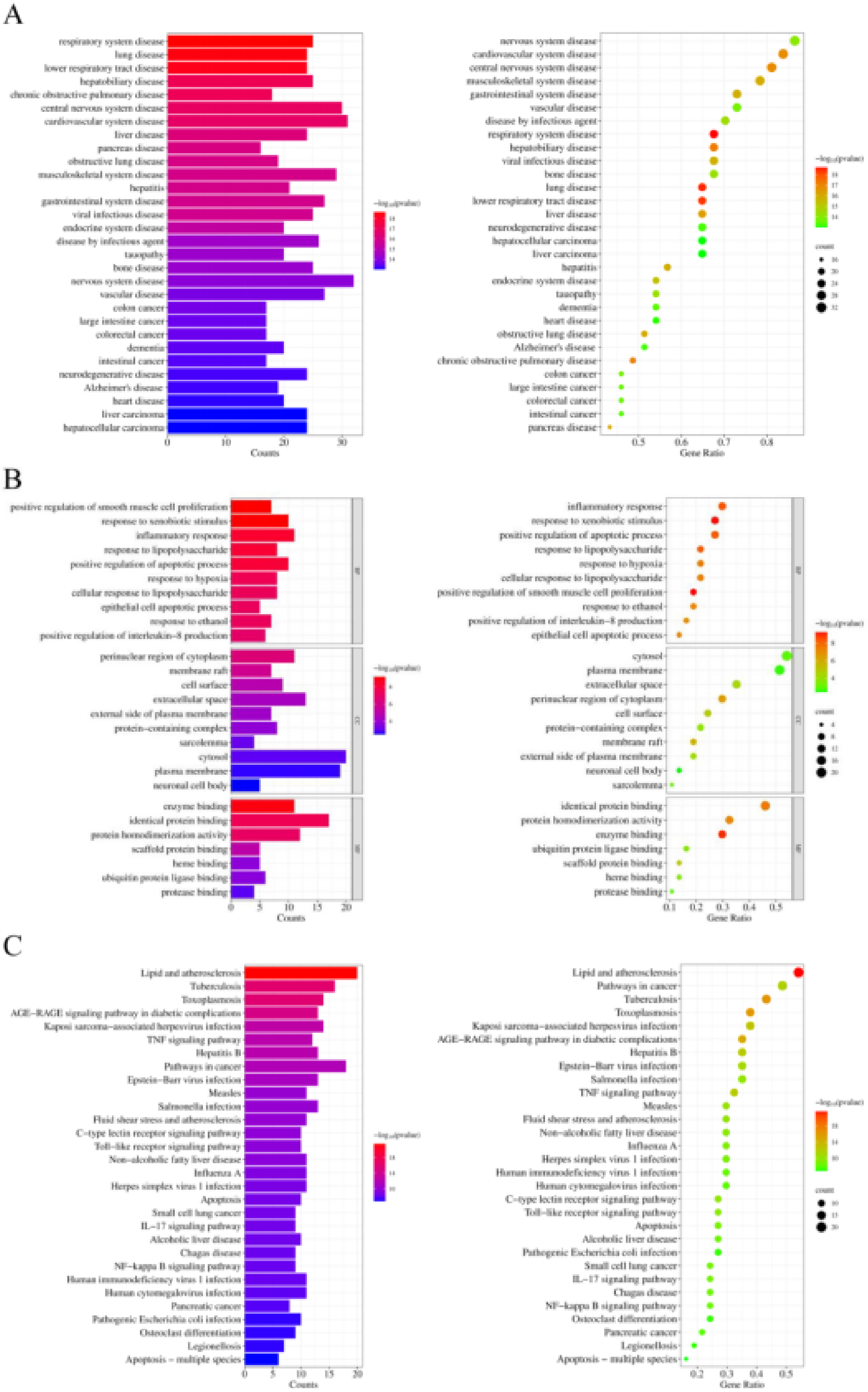


Figure 4. Results of DO, GO, and KEGG enrichment analysis. (A) Bar plot and bubble chart of the top 30 diseases based on DO enrichment analysis. (B) Bar plot and bubble chart of GO functional enrichment analysis. (C) Bar plot and bubble chart of the top 30 pathways based on KEGG enrichment analysis.

3.5. Compound-target-pathway network analysis and identification of core VMC targets

With the help of Cytoscape, a “compound-target-pathway” network was developed, utilizing all 105 KEGG pathways and their associated targets and compounds. **Table 3** shows the 20 targets with the highest Degree values in the network. Through a Venn diagram, the 16 common core targets between the 19 targets with DC ≥ the median DC in the “Zhenwu Tang-VMC” common target PPI and the top 20 targets with the highest Degree in the above network were visualized (**Figure 5**, **Table 4**). **Table 5** provides comprehensive details on the top 10 compounds within the network mentioned above.

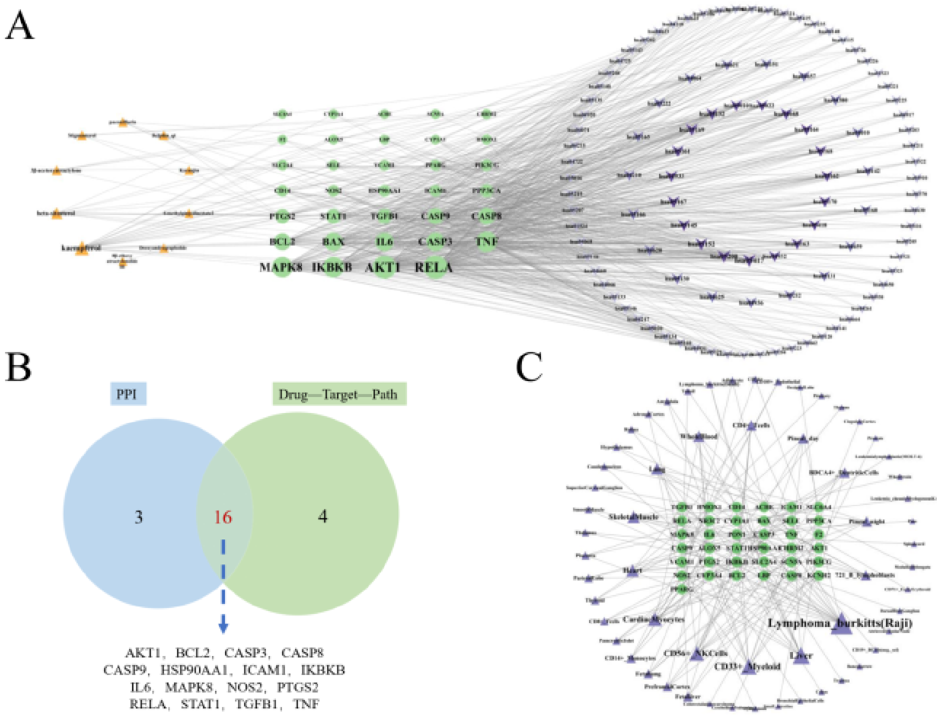


Figure 5. Construction of compound-target-pathway network, organ localization, and identification of core targets. (A) Compound-target-pathway network. (B) Venn diagram of common core targets from VMC-PPI and Drug-Target-Path analysis. (C) Target-organ network in VMC.

Table 3. Top 20 targets with the highest degree in the compound-target-pathway network

Gene name	Degree	Gene name	Degree
RELA	72	CASP9	34
AKT1	69	TGFB1	29
IKBKB	58	STAT1	28
MAPK8	57	PTGS2	27
TNF	56	PPP3CA	22
CASP3	47	HSP90AA1	15
IL6	46	ICAM1	15
BAX	46	CD14	14
BCL2	40	NOS2	14
CASP8	38	PIK3CG	12

Table 4. 16 core targets of Zhenwu Tang-VMC

Target	MOL ID
AKT1	MOL000422
BCL2	MOL000358, MOL000422
CASP3	MOL000358, MOL000422
CASP8	MOL000358
CASP9	MOL000358
HSP90AA1	MOL000358, MOL000422, MOL002388
ICAM1	MOL000422
IKBKB	MOL000422
IL6	MOL001924
MAPK8	MOL000422
NOS2	MOL000422
PTGS2	MOL000049, MOL000072, MOL000358, MOL000422, MOL000449, MOL002388, MOL002395, MOL002398, MOL006129
RELA	MOL000422
STAT1	MOL000422
TGFB1	MOL000358, MOL000449
TNF	MOL000422, MOL001924

Table 5. Detailed information of the Top 10 compounds in the compound-target-pathway network

Component ID	Component name	CAS	Degree	OB	DL
MOL000422	kaempferol	520-18-3	25	41.88	0.24
MOL000358	beta-sitosterol	83-46-5	12	36.91	0.75
MOL000049	3β-acetoxyatractylone	61206-10-8	5	54.06	0.21
MOL001924	paeoniflorin	23180-57-6	4	53.87	0.78
MOL000449	Stigmasterol	83-48-7	4	43.82	0.75
MOL002388	Delphin_qt	17670-06-3	3	57.76	0.27
MOL002398	Karanjin	521-88-0	2	69.55	0.33
MOL006129	6-methylgingediacetate2	-	1	48.73	0.32
MOL002395	Deoxyandrographolide	79233-15-1	1	56.3	0.31
MOL000072	8β-ethoxy atractylenolide III	113269-35-5	1	35.95	0.21

3.6. Composite target pathway analysis and core target identification of respiratory disease-related VMC

Many viruses that cause respiratory infections are also common pathogens of viral myocarditis, such as coxsackievirus, adenovirus, and influenza virus. The transmission route of these viruses is mostly through respiratory droplets. After infection, they first invade the respiratory mucosa. If the body's immunity is low or the virus is highly virulent, the virus can enter the blood circulation and then invade the myocardial cells, causing myocarditis [20]. DO analysis showed that the common "Zhenwu Tang-VMC" targets were highly enriched in respiratory diseases. Therefore, this study selected 25 targets of respiratory disease-related viral myocarditis from the DO analysis to construct a PPI network. The nodes depicted in the figure vary in size, with the circles expanding from small to large, signifying an increase in the DC value from low to high. Based on the screening criteria of topological analysis, 15 composite targets with DC ≥ the median DC (16) were screened out. The relationship between Zhenwu Tang and respiratory disease-related VMC was clarified by constructing a "compound-composite target-pathway" network based on 25 respiratory disease-related VMC targets, active compounds, and all pathways. **Table 5** shows the 20 targets with the highest Degree in the network mentioned above. Using a Venn diagram, researchers identified 14 common targets as the primary targets for respiratory disease-related viral myocarditis. (**Figure 6**, **Table 6**). Additionally, the network above lists the top 10 compounds with the highest Degree (**Table 7**).

Table 6. 14 core targets of respiratory-related "Zhenwu Tang-VMC"

Target	MOL ID
TNF	MOL001924, MOL000422
TGFB1	MOL000358, MOL000449
STAT1	MOL000422
RELA	MOL000422
PTGS2	MOL000358, MOL000422, MOL006129, MOL000449, MOL002388, MOL002395, MOL002398, MOL000049, MOL000072
PPARG	MOL000422
IL6	MOL001924
IKBKB	MOL000422
ICAM1	MOL000422
HSP90AA1	MOL000358, MOL000422, MOL002388
CASP8	MOL000358
CASP3	MOL000358, MOL000422
BCL2	MOL000358, MOL000422
AKT1	MOL000422

Table 7. Detailed information of the Top 10 compounds in the respiratory-related "Zhenwu Tang-VMC"

Component ID	Component name	CAS	Degree	OB	DL
MOL000422	kaempferol	520-18-3	19	41.88	0.24
MOL000358	beta-sitosterol	83-46-5	9	36.91	0.75
MOL001924	paeoniflorin	23180-57-6	4	53.87	0.78
MOL002388	Delphin_qt	17670-06-3	3	57.76	0.27
MOL000049	3β-acetoxyatractylone	61206-10-8	3	54.06	0.21
MOL000449	Stigmasterol	83-48-7	2	43.82	0.75
MOL002398	Karanjin	521-88-0	2	69.55	0.33
MOL006129	6-methylgingediacetate2	-	1	48.73	0.32
MOL002395	Deoxyandrographolide	79233-15-1	1	56.3	0.31
MOL000072	8β-ethoxy atractylenolide III	113269-35-5	1	35.95	0.21

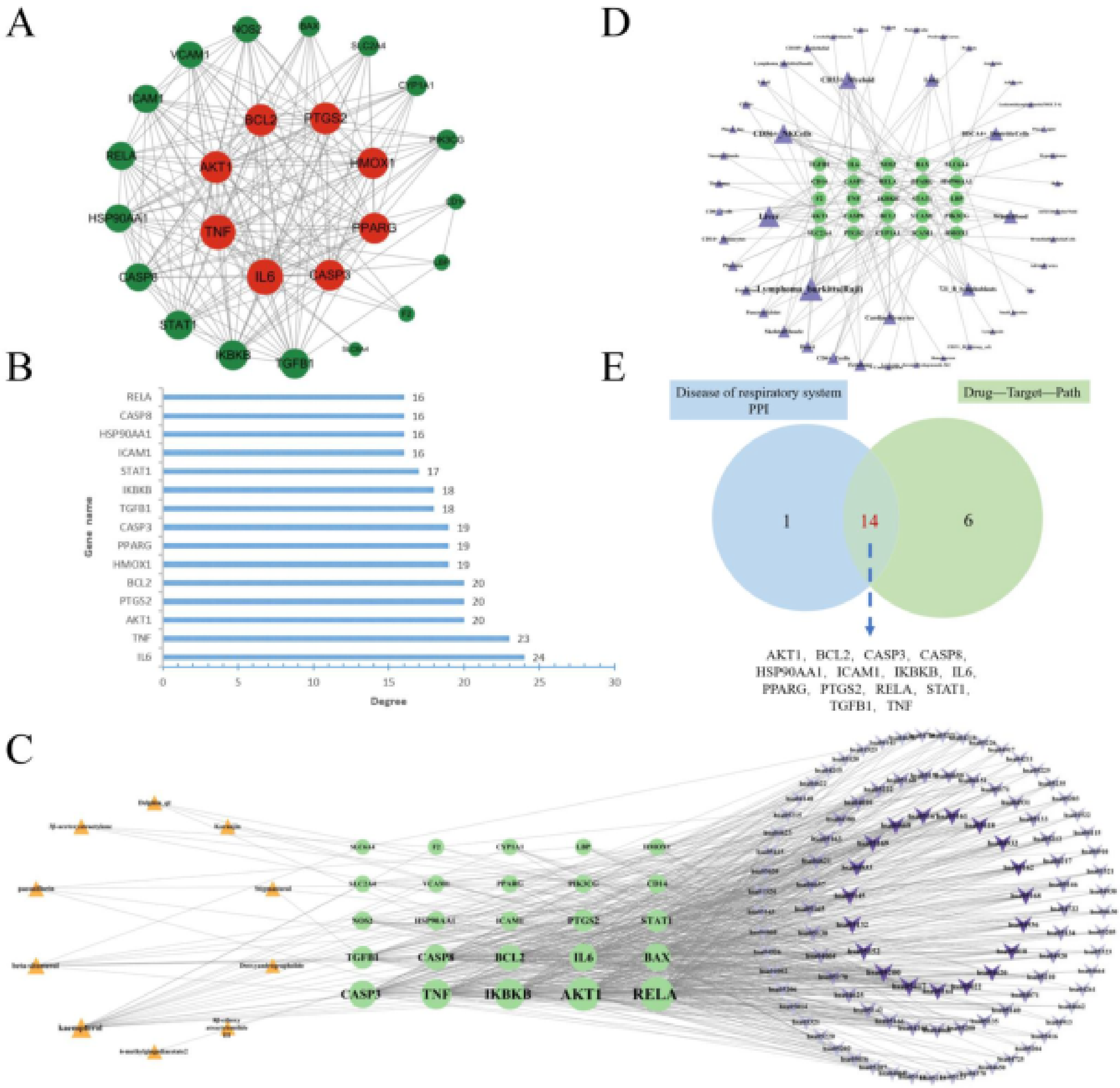


Figure 6. Core target identification in Disease of respiratory system via network pharmacology. (A) PPI analysis. (B) Bar chart of the top 15 core targets. (C) Compound-target- pathway network. (D) Target-organ network. (E) Venn diagram of 14 common targets between Disease of respiratory system PPI and Drug-target-pathway network.

3.7. Target-organ analysis

Using the BioGPS database, the mRNA levels of all 37 common "Zhenwu Tang-VMC" targets were evaluated to analyze the in vivo metabolic process of Zhenwu Tang against VMC. Using Cytoscape, the "target-organ" network revealed that the organs associated with the "Zhenwu Tang-VMC" targets include Burkitt's lymphoma. (Raji) (16 targets), liver (11 targets), CD33+ myeloid (10 targets), CD56+ NK cells (9 targets), cardiomyocytes (8 targets), heart and skeletal muscle (both 7 targets), lung and whole blood (both 6 targets). These 9 organs are closely related to the immune system, digestive system, circulatory system, respiratory system, and cardiovascular system, indicating that the treatment of VMC by Zhenwu Tang includes systemic immune activation. In addition, the "target-organ" network of the 25 respiratory disease-related VMC targets includes the following organs: Burkitt's lymphoma (Raji) (10 targets), liver (9 targets), CD56+ NK cells (8 targets), CD33+ myeloid (7 targets), 721_B lymphoblastoid, BDCA4+ dendritic cells, lung, whole blood, and cardiomyocytes (all 5 targets), which is similar to the organ localization ranking of the "Zhenwu Tang-VMC" related targets.

3.8. Validation and visualization of molecular docking results

To validate the results of the network pharmacology analysis, we obtained receptor proteins for molecular docking from the RCSB PDB database and used AutoDock for molecular docking studies to explore the interaction of screened molecular compounds as ligands with core target proteins. If the binding energy is well below -7 kcal/mol, it suggests a strong affinity between the compound and the target protein. The binding energy results shown in the heat map indicate that 60 ligand-receptor pairs in VMC and 47 ligand-receptor pairs in respiratory-related VMC had binding energies below -7 kcal/mol (**Figure 7**). It can be seen that the docking scores of RELA, HSP90AA1, NOS2, PTGS2, MAPK8, and TNF with the compounds are lower than those of other proteins, which indicates that these proteins may be potential binding targets for the treatment of VMC by Zhenwu Tang. In the respiratory system-related "Zhenwu Tang-VMC" targets, RELA, HSP90AA1, PTGS2, and TNF showed stronger binding energy than other target proteins. For the lowest binding energy score in each compound, that is, the compound and target with the highest free binding energy score, Pymol was used to visualize their interaction and binding mode (**Figure 8**). The interactions consist of hydrogen bonds, hydrophobic forces, and stacking interactions, which help maintain a stable structure.

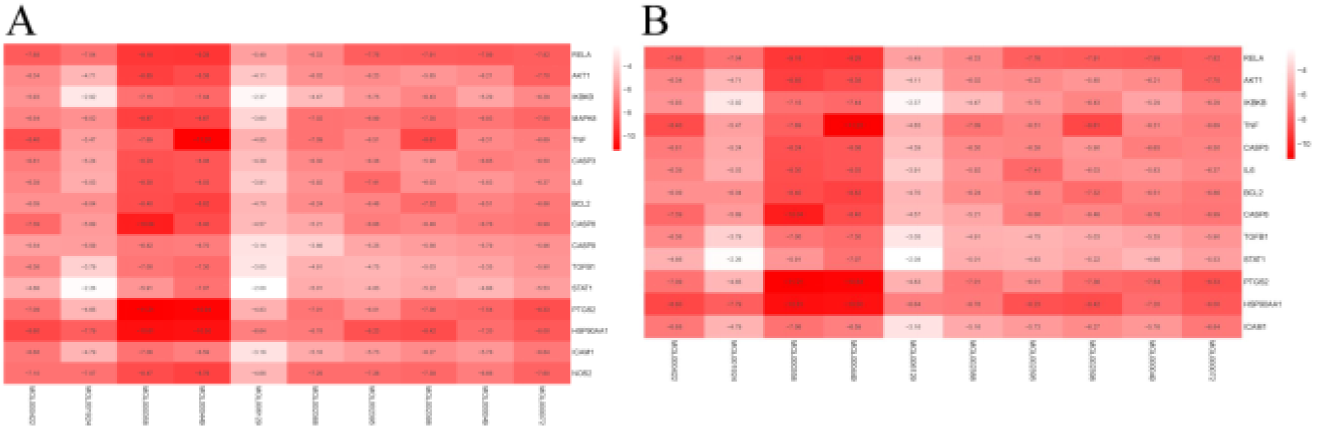
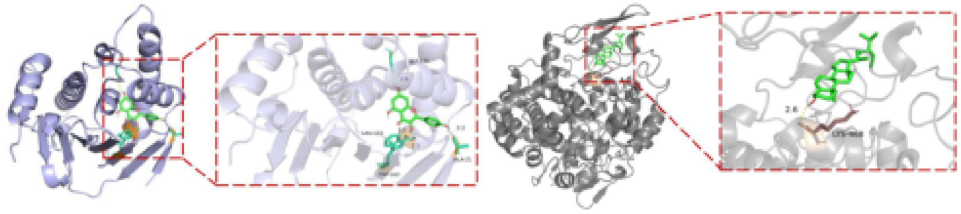
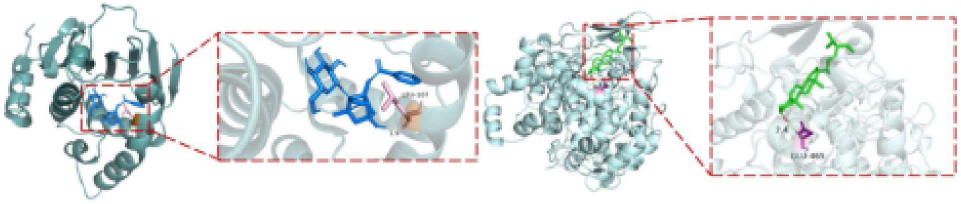


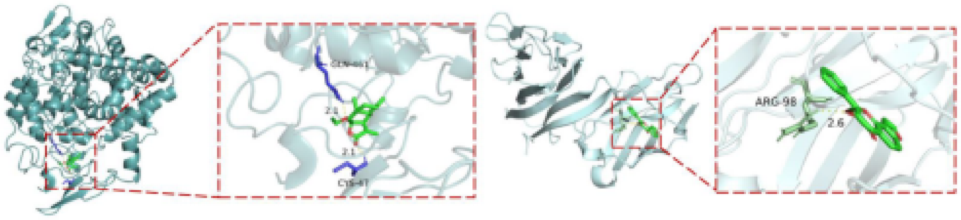
Figure 7. Validation and screening of molecular docking. (A) Heatmap of VMC molecular docking scores. (B) Heatmap of Disease of respiratory system molecular docking scores. Binding energies (kcal/mol) of core targets and active compounds of Zhenwu Tang.



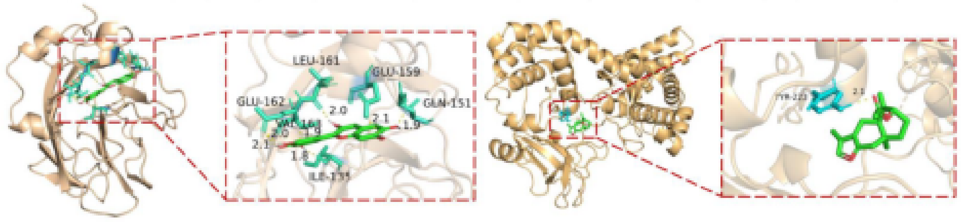
HSP90AA1 (PDB ID:1UY6) – kaempferol (-8.60kcal/mol) PTGS2 (PDB ID:5IKR) – beta-sitosterol (-11.21kcal/mol)



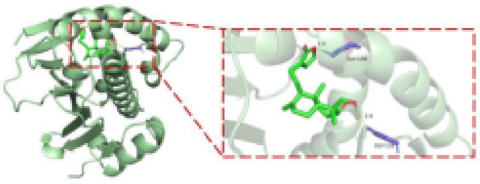
HSP90AA1 (PDB ID:1UY6) – paeoniflorin (-7.79kcal/mol) PTGS2 (PDB ID:5IKR) – Stigmasterol (-10.94kcal/mol)



PTGS2 (PDB ID:5IKR) –8β-ethoxy atractylenolide III (-8.33kcal/mol) TNF (PDB ID:5M2J) – Karanjin (-8.61kcal/mol)



NOS2 (PDB ID:6JWM) – Delphin_qt (-7.25kcal/mol) RELA (PDB ID:3QXY) – 3β-acetoxyatractylone (-7.99kcal/mol)



HSP90AA1 (PDB ID:1UY6) – Deoxyandrographolide (-8.23kcal/mol)

Figure 8. Docking patterns of core targets and active compounds of VMC.

4. Discussion

This study first obtained 24 active compounds and target information from 5 herbs (Fuling, Baishao, Shengjiang, Fuzi, and Baizhu) related to Zhenwu Tang from TCMSP. Combined with the UniProt database, we obtained 96 drug targets related to Zhenwu Tang. At the same time, we introduced the GeneCards and OMIM databases to obtain 2,243 disease targets related to VMC. After taking the intersection of the above-mentioned drug targets of Zhenwu Tang and the disease targets of VMC, we obtained 37 common "Zhenwu Tang-VMC" targets, which are candidate targets for the anti-viral myocarditis effect of Zhenwu Tang. Following that, we introduced DO, GO, and KEGG enrichment analyses to evaluate the distribution of the 37 common "Zhenwu Tang-VMC" targets in biological processes (BP), cellular components (CC), molecular functions (MF), and signaling pathways. To find the core targets of Zhenwu Tang against viral myocarditis, we introduced the STRING database to construct a PPI network. Based on the screening criteria of topological analysis, we screened out 19 targets with a DC greater than or equal to the median DC. At the same time, we constructed a "compound-target-pathway" network and selected the top 20 targets with the highest Degree. The intersection of these two sets of targets yielded 16 core targets. In addition, we proposed a "composite target-pathway" topological network model for the mechanism of Zhenwu Tang against viral myocarditis and a "target-organ" network model for predicting the metabolic process of Zhenwu Tang in VMC treatment. Ultimately, we employed molecular docking to assess how core targets interact with top-tier compounds.

Viral myocarditis is an acute or chronic inflammation of the heart muscle, either localized or widespread, resulting from a viral infection. Common viruses include coxsackievirus group B and adenovirus. The virus can directly invade the myocardium or indirectly damage the myocardium through immune reactions [21]. Zhenwu Tang is a classic formula in traditional Chinese medicine, derived from "Treatise on Cold Damage," composed of five herbs: Fuling, Shaoyao, Baizhu, Shengjiang, and Fuzi. It has the effect of warming Yang and promoting urination, and is commonly used to treat yang deficiency and water retention syndrome. Symptoms include aversion to cold and cold limbs, scanty urination, limb edema, abdominal pain and diarrhea, palpitations and dizziness. Its compatibility feature is to combine warming Yang with promoting urination, so that Yang Qi is restored and water dampness is naturally transformed. It is widely used clinically for chronic nephritis, heart failure, endocrine disorders, and other conditions belonging to Yang deficiency and water retention [22,23]. Furthermore, Zhenwu Tang has been applied in the clinical treatment of viral myocarditis and has shown significant efficacy. However, there is still a lack of in-depth understanding of its bioactive components and specific mechanisms of action.

In this study, GO enrichment analysis identified several biological processes of Zhenwu Tang against VMC, including infections and diseases related to tissue damage caused by pathogenic microorganisms, infectious agents, chemical stimuli, and other trauma. KEGG analysis results showed that the common "Zhenwu Tang-VMC" targets are closely related to pathways associated with VMC, such as lipids and atherosclerosis, cancer pathways, tuberculosis, toxoplasmosis, and Kaposi's sarcoma-associated herpesvirus infection. In a chronic heart failure (CHF) model, Zhenwu Tang regulated the Th1/Th2 balance, reducing the pro-inflammatory factor IFN-γ by 35% and increasing the anti-inflammatory factor IL-10 by 40%. Its component Fuling polysaccharide can activate the AMPK pathway, inhibit NLRP3 inflammasome assembly, reduce ASC speck formation by 60%, and decrease caspase-1 activity by 45% [24]. In a myocardial ischemia-reperfusion injury model, after Zhenwu Tang treatment, the malondialdehyde (MDA) content in myocardial tissue decreased by 30%, and the activities of serum creatine phosphokinase (CK) and creatine kinase isoenzyme (CK-MB) decreased by 40% and 35%, respectively, suggesting that it reduces oxidative damage by improving lipid metabolism [25]. In a myocardial fibrosis model, after treatment with Ershen Zhenwu Tang (containing Zhenwu Tang components), the protein expression of α-smooth muscle actin (α-SMA), type I collagen (Col-I), and type III collagen (Col-III) decreased by 45%, 50%, and 40%, respectively, while the phosphorylation levels of p-PI3K, p-AKT, and p-mTOR were significantly downregulated [26]. The inhibition of this pathway may reduce fibroblast activation and collagen deposition, thereby improving myocardial remodeling. DO analysis showed that the common "Zhenwu Tang-VMC" targets showed high enrichment in respiratory diseases. Respiratory disease-related viral myocarditis often first presents with obvious respiratory symptoms, which persist for days to weeks, and then gradually develop myocardial damage. The severity of respiratory symptoms is somewhat related to the onset of myocarditis, and some patients only show myocardial problems after respiratory symptoms resolve [27]. Most of the VMC targets of Zhenwu Tang are directed at respiratory diseases, indicating that it may show better therapeutic effects in respiratory disease-related viral myocarditis, but further research is needed. This study developed a ‘compound-target-pathway’ network to pinpoint key proteins and compounds, followed by molecular docking for validation, aiding in understanding how Zhenwu Tang treats VMC. The specific steps were to evaluate how the 16 core “Zhenwu Tang-VMC” targets (AKT1, BCL2, CASP3, CASP8, CASP9, HSP90AA1, ICAM1, IKBKB, IL6, MAPK8, NOS2, PTGS2, RELA, STAT1, TGFB1, TNF) interact with their corresponding 10 active molecular compounds (kaempferol, delphinidin, karanjin, paeoniflorin, stigmasterol, deoxyandrographolide, β-sitosterol, 6-methylgingediacetate2, 3β-acetoxyatractylone, 8β-ethoxy atractylenolide III).

It is obvious that RELA, HSP90AA1, NOS2, PTGS2, MAPK8, and TNF have lower binding affinity on all active compounds than other core targets. Previous studies have shown that TNF and NF-κB (RELA) activate downstream signaling networks by forming a positive feedback loop, where TNF can induce nuclear translocation of NF-κB p65 (RELA), while activated NF-κB further promotes TNF gene transcription [28]. The two synergistically upregulate the PTGS2/PGE2 pathway and NOS2-mediated NO generation, promoting VMC progression by exacerbating oxidative stress reactions and recruiting immune cell infiltration into myocardial tissue. As an early warning biomarker for infectious myocarditis, TNF expression levels are significantly elevated in myocardial tissue and serum of VMC mouse models, and its concentration changes are significantly correlated with myocardial damage markers (such as CK-MB) activity and cardiac function indicators (such as left ventricular ejection fraction, LVEF), suggesting that it can serve as a potential biomarker for assessing disease activity. In addition, drugs targeting the NF-κB pathway (such as curcumin) have been proven to effectively inhibit RELA/p65 nuclear translocation, reduce inflammatory factor release in myocardial tissue, and thereby alleviate myocardial inflammatory damage [29]. PTGS2 (cyclooxygenase-2), as a primary enzyme that regulates the conversion of arachidonic acid into prostaglandins, has its metabolite PGE2 with dual regulatory effects of pro-inflammation and anti-inflammation. Preclinical studies have shown that PTGS2 inhibitors (such as celecoxib) can reduce myocardial inflammatory damage in VMC models by reducing PGE2 [30]. In the process of myocardial fibrosis, HSP90AA1 activates the TGFβ/Smad pathway by stabilizing the TGFβ receptor (TGFβRI), promoting collagen deposition [31]. PGE2 can further amplify the fibrotic cascade reaction through secondary activation of NF-κB signaling. In addition, MAPK8 (JNK) promotes apoptosis by phosphorylating the pro-apoptotic protein Bax and inhibiting the anti-apoptotic protein Bcl-2, working synergistically with NO mediated by NOS2 - NO exacerbates oxidative stress by damaging mitochondrial function, and the two together destroy the structural integrity of cardiomyocytes [32].

This research investigated how Zhenwu Tang might treat VMC using network pharmacology and molecular docking techniques, providing a theoretical reference for its clinical application, but there are still several shortcomings. For instance, database dependence leads to bias in target and pathway analysis. The study relies on databases such as TCMSP, GeneCards, and OMIM to obtain compound targets and VMC-related genes, but the databases themselves have limitations, where TCMSP’s collection of traditional Chinese medicine components may be incomplete (such as triterpene components in Fuling and polysaccharide components in Baishao may not be fully included), leading to the omission of some potential targets. The results of enrichment analysis (DO, GO, KEGG) depend on database annotations, and the annotations of some pathways (such as myocardial energy metabolism-related pathways) may be incomplete, leading to incomplete interpretation of Zhenwu Tang’s regulatory mechanism. Other than that, network pharmacology and molecular docking are only big data processing and analysis based on algorithms. The current results provide an exploration of the mechanism and potential therapeutic effects of Zhenwu Tang against VMC from a computer technology perspective. Future research still needs further verification through basic research such as cell or animal experiments.

5. Conclusion

To sum up, this study used network pharmacology, molecular docking, and other bioinformatics methods to discover that the pharmacological mechanism and molecular basis of Zhenwu Tang in treating VMC have the characteristics of multiple components, multiple targets, and multiple pathways. Kaempferol, delphinidin, karanjin, paeoniflorin, stigmasterol, deoxyandrographolide, β-sitosterol, 6-methylgingediacetate2, 3β-acetoxyatractylone, and 8β-ethoxy atractylenolide III may be the main bioactive components of Zhenwu Tang in treating viral myocarditis. It can prevent and treat VMC by intervening in inflammatory regulation, cell apoptosis, immune response, and signal transduction during the VMC process. This study reveals the potential mechanism of Zhenwu Tang in treating VMC, and these findings are expected to provide a theoretical basis for the clinical utilization and future translation of Zhenwu Tang in VMC.

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Disclosure statement

The authors declare no conflict of interest.

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