Original Article

Integration of network pharmacology and molecular docking technology reveals the mechanism of the herbal pairing of Codonopsis Pilosula (Franch.) Nannf and Astragalus Membranaceus (Fisch.) Bge on chronic heart failure

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Background: The herbal pairing of Dangshen (DS) [Codonopsis pilosula (Franch.) Nannf.] and Huangqi (HQ) [Astragalus membranaceus (Fisch.) Bge.] (DHP) is a traditional Chinese herbal medicine that is frequently used to treat chronic heart failure (CHF) in China. However, the pharmacological mechanism of DHP has not been fully elucidated. This is the first study aimed to reveal the active mechanism of DHP in the treatment of CHF by using network pharmacology methods.

Methods: The active ingredients of DHP were obtained from the TCMSP database, and the potential targets of DHP were predicted using the SwissTargetPrediction database. CHF-related targets were searched by the DisGeNET and GeneCards databases. The common targets between the disease and herbs were obtained using a Venn diagram. The STRING database was utilized to obtain the protein-protein interaction data. Next, we used Cytoscape 3.7.2 software to construct and analyze the herb-ingredient-potential targets-disease network. Topology analysis was used to identify the key ingredients and hub genes. Gene Ontology (GO) and Kyoto Encyclopedia of Genes and Genomes (KEGG) pathway enrichment analyses were performed using the Metascape database to reveal the mechanism. Furthermore, molecular docking simulation was performed using AutoDock Vina software to assess the affinity of the key ingredients and hub genes.

Results: Five key ingredients and six hub genes were screened. The six hub genes were closely related to PI3K /AKT or ERK1/2 pathways. The KEGG pathways mainly involved the TNF signaling pathway, calcium signaling pathway, and cancer-related pathways. The GO enrichment analysis results showed that DHP might act on biological processes including positive regulation of kinase activity and cellular response to nitrogen compound via the three above-mentioned pathways in the treatment of CHF. Finally, the molecular docking results showed that the five key ingredients exhibited strong affinities to the six hub genes.

Conclusions: This study revealed the molecular mechanism that the flavonoids in DHP may alleviate endothelial dysfunction and cardiac hypertrophy via regulation of the TNF pathway and its downstream PI3K/Akt or ERK1/2 signaling pathways, or improve excitation-contraction coupling by regulating calcium

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signaling pathway, thereby improving CHF. These results provide insights for further experimentation on its pharmacological effects.

Keywords: Codonopsis pilosula (Franch.) Nannf.; Astragalus membranaceus (Fisch.) Bge.; network pharmacology; molecular docking

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Introduction

Chronic heart failure (CHF) is an advanced stage of cardiac dysfunction secondary to many diseases, and acts as the primary cause of death. Patients with CHF have mixed causes, which are not occur independently, and more than two-thirds of all cases of CHF can be attributed to four underlying aetiologies: ischaemic heart disease, hypertensive heart disease, chronic obstructive pulmonary disease and rheumatic heart disease (1). Patients with CHF mainly present with dyspnea, fatigue, limited exercise tolerance, and fluid retention, which significantly diminishes their quality of life (2). Despite advances in CHF treatment, morbidity and mortality remain high. It is reported that the global incidence of CHF ranges from 100 to 900 cases per 100,000 person-years (3) and is estimated 64.3 million individuals worldwide (4). Owing to the aging population, the prevalence of CHF is projected to increase 46% over the next 10 years. The total percentage of the population with CHF is predicted to increase from 2.42% to 2.97% in 2030 (5). Furthermore, the total medical expenses for patients with heart failure (HF) in the USA are estimated to reach US$53.1 billion by 2030 (6). CHF imposes an enormous burden on public health systems worldwide. Therefore, exploration of more effective treatments for CHF with fewer side effects is urgently needed.

Chinese herbal medicine (CHM) is characterized by multiple components, targets, and pathways, and has been widely applied to treat CHF in China for more than 2,000 years (7,8). Compared to Western medicine (WM) alone, integration of CHM and WM can better alleviate symptoms, more significantly improve exercise load, enhance quality of life, and has fewer side effects (9,10). In the theory of traditional Chinese medicine (TCM), Qi is the most basic substance of the human body and possesses the function of promotion, domination, defense, and warm. Qi deficiency syndrome is one of the basic syndromes in TCM and is characterized by physical weakness, shortness of breath, sweating, pale, low voice etc. (11). Qi deficiency syndrome is the major pathogenesis of CHF, encompassing its occurrence, development, and outcomes. Qi-boosting (YiQi) can be understood as supplementing Qi, the most basic substance of the human body, and restoring the function of Qi to alleviate the Qi deficiency syndrome (12). So Qi-boosting is an essential principle to treat disease with Qi deficiency syndrome such as CHF. The herbal pairing of Dangshen (DS) [Codonopsis pilosula (Franch.) Nannf.] and Huangqi (HQ) [Astragalus membranaceus (Fisch.) Bge.] (DHP) is compatible and exerts a synergistic effect on Qi-boosting, and has been widely used to treat disease with Qi deficiency syndrome such as CHF in China. Moreover, DHP is also the major herbal pairing of some Chinese formulas for CHF treatment, such as Qili Qiangxin capsules, Shenqi Fuzheng injection, and Qishen granules. These formulas can reduce the levels of N-terminal signal peptide of pro-B-type natriuretic peptide (NT-proBNP) and improve heart function, and thus, are widely used in combined therapy for the treatment of CHF (9,13-15). Our previous study and other animal experiments have shown that DHP has effects on the treatment of CHF, which may relate to regulating myocardial energy metabolism, inhibiting inflammation, improving cardiac remodeling, and enhancing myocardial contractility (16). However, due to the complexity of its chemical compounds, the mechanisms of DHP in the treatment of CHF have not fully been elucidated.

Network pharmacology is an emerging and powerful tool. It can predict the direct targets of the potential active compounds of CHM and systematically reveal the underlying mechanisms (17). Molecular docking can examine the interaction between the receptor and drug molecules, and predict its binding mode and affinity, and is a critical approach for structural molecular biology and computer-aided drug design for new drugs (18). In this study, we integrated network pharmacology and molecular docking technology to identify the complex mechanisms of

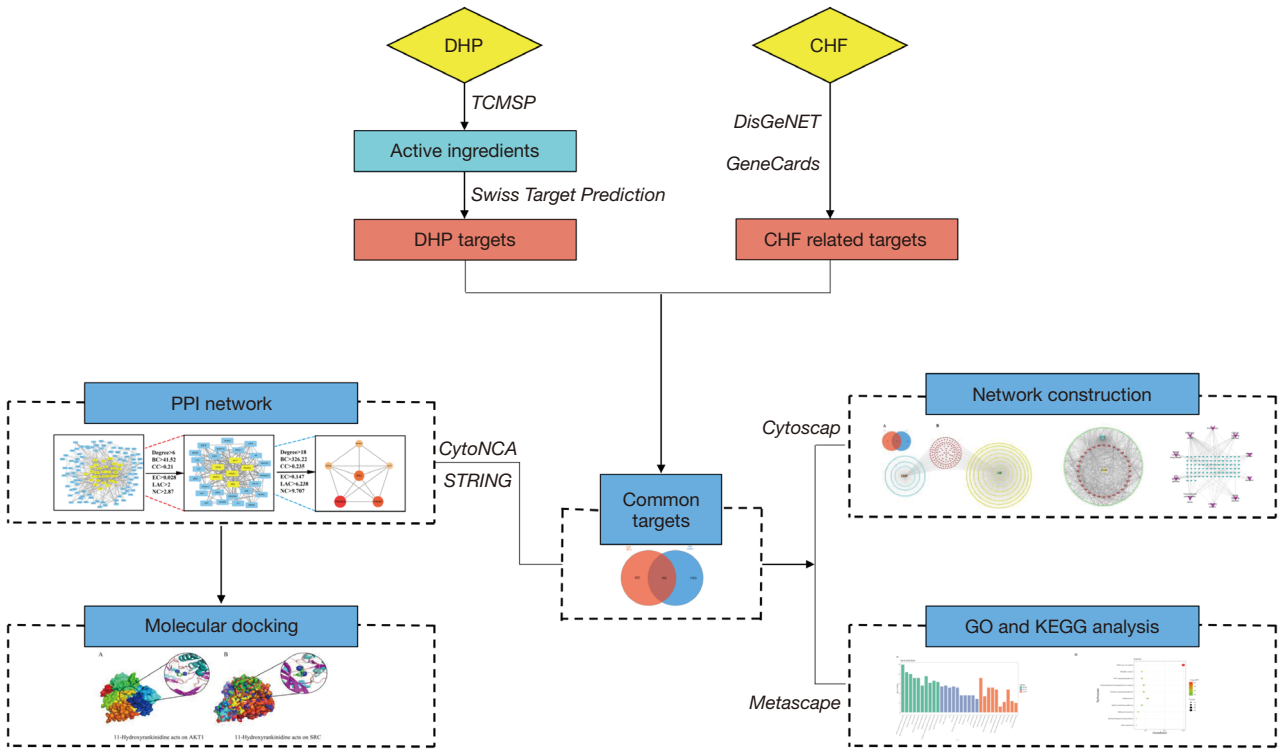


Figure 1 Flowchart of the present study. DHP, the herbal pairing of Codonopsis pilosula (Franch.) Nannf. (Dangshen, DS) and Astragalus membranaceus (Fisch.) Bge. (Huangqi, HQ); CHF, chronic heart failure; PPI, protein-protein interaction; GO, Gene Ontology; KEGG, Kyoto Encyclopedia of Genes and Genomes.

DHP on CHF. The study flowchart is shown in *Figure 1*. We present the following article in accordance with the MDAR reporting checklist (available at https://dx.doi.org/10.21037/apm-21-1469).

Methods

Active ingredients of DHP screening

The compounds of DS and HQ were collected from the Traditional Chinese Medicine Systems Pharmacology Database (TCMSP, https://tcmspw.com/tcmsp.php). This platform is designed for CHMs and includes pharmacochemistry, absorption, distribution, metabolism, and excretion (ADME) (19). Two ADME parameters, oral bioavailability (OB) and drug-likeness (DL) were used to screen the active ingredients of DHP.

Prediction of potential targets of DHP

The active ingredients of DHP were imported into the PubChem database (https://pubchem.ncbi.nlm.nih.gov/) (20) to obtain the two-dimensional (2D) structures of the compounds. Based on the SwissTargetPrediction database (https://www.swisstargetprediction.ch/) (21), the potential targets of DHP's active ingredients in Homo sapiens were predicted.

CHF related targets screening

CHF-related targets were retrieved from the DisGeNET database (https://www.disgenet.org/) (22) and the GeneCards database (https://www.genecards.org/) (23). "Chronic Heart Failure" was used as the keyword for screening in both databases. This study was conducted in accordance with the Declaration of Helsinki (as revised in 2013).

Common targets between the potential targets of DHP and CHF-related targets

The common targets were obtained after DHP potential

targets mapping onto CHF-related genes, and were displayed using a Venn diagram. These common targets play a vital role in medicinal treatment for disease and were used for subsequent analysis.

Protein-protein interaction (PPI) analysis

The common targets were imported into the Search Tool for the Retrieval of Interacting Genes/Proteins (STRING) database (https://string-db.org/) (24), which designs protein interaction analysis and PPI network construction. In our study, we set the interaction score as the highest confidence (>0.9). The PPI data were used for further topological properties analysis to identify the hub genes of DHP treatment of CHF.

Gene Ontology (GO) and Kyoto Encyclopedia of Genes and Genomes (KEGG) pathway enrichment analyses

To reveal the potential mechanisms of DHP in the treatment of CHF, we imported the common targets into the Metascape database (http://metascape.org/) (25) for GO and KEGG pathway enrichment analyses with Homo sapiens. GO constructs three different levels to describe genes: biological process (BP), molecular function (MF), and cellular component (CC) (26). KEGG analyzes the pathways involved in genes, which helps to better understand the relevant pathways (27). According to the corrected P value, the results of the GO and KEGG pathway enrichment analyses were sorted. R software (version 3.6.3) was used to visualize the results.

Network construction and analysis

Cytoscape 3.7.2 (https://cytoscape.org/) (28), a software platform used for visualizing molecular interaction networks and biological pathways, was utilized to draw the following networks: (I) common targets network; (II) Herb-Ingredient-Potential target-Disease network (H-I-P-D network); (III) PPI network; and (IV) Pathway-related targets network (P-R network). The Cytoscape plugins Network Analyzer and CytoNCA were used to analyze the topological properties of the networks (29,30). In the H-I-P-D network, ingredients with the top five degree values were identified as the key ingredients of DHP. After two rounds of screening the median using a series of parameters, including degree, betweenness centrality (BC), closeness centrality (CC), eigenvector centrality (EC), the local average connectivity-based method (LAC), and network centrality (NC), the hub genes of DHP in CHF treatment were filtered and used for subsequent molecular docking simulation.

Molecular docking validation

Molecular docking was performed with the key ingredients and hub genes to assess their binding affinities. The three-dimensional crystal structures of the hub genes were downloaded from the Research Collaboratory for Structural Bioinformatics Protein Data Bank (RCSB-PDB; https://www.rcsb.org) (31). The MOL2 format files of key compounds were retrieved from TCSMP. The downloaded proteins were processed by PyMol 2.4.0 to remove water molecules and extract ligands. All target proteins and compounds were saved as PDBQT format using AutoDock Tools (ADT) (32). AutoDock Vina software was then used to perform the molecular docking simulation, and PyMol 2.4.0 was used for visualization (33).

Statistical analysis

We used Cytoscape version 3.7.2 to analyse the topological data. GO and KEGG pathway enrichment analysis were conducted by Metascape database.

Results

Active ingredients and potential targets of DHP

According to the ADME parameters (OB ≥30% and DL ≥0.18) and invalid components exclusion, 33 active ingredients of DHP were obtained from the TCMSP database, 16 were obtained from DS, and 17 were obtained from HQ (*Table 1*). The 2D chemical structures of the 33 active ingredients were identified using PubChem. Based on these 2D structures, 795 targets were obtained from SwissTargetPrediction, including 385 targets from DS and 410 from HQ. Finally, we captured 561 targets as potential targets of DHP, after removing the duplicate values (https://cdn.amegroups.cn/static/public/apm-21-1469-1.pdf).

CHF- related targets

By screening the GeneCards and DisGeNET databases, 1,296 and 1,593 CHF-related genes were obtained, respectively. After removing duplications, 1,922 CHF-

Table 1 33 active ingredients of YQHX after ADME screening

Herb	Mol ID	Label	Molecule name	OB	DL
Dangshen	MOL005321	DS1	Frutinone A	65.9	0.34
Dangshen	MOL008400	DS2	Glycitein	50.48	0.24
Dangshen	MOL008407	DS3	Stigmasterone	45.4	0.76
Dangshen	MOL003036	DS4	ZINC03978781	43.83	0.76
Dangshen	MOL004355	DS5	Spinasterol	42.98	0.76
Dangshen	MOL001006	DS6	Chondrillasterol	42.98	0.76
Dangshen	MOL003896	DS7	7-methoxy-2-methyl isoflavone	42.56	0.2
Dangshen	MOL008411	DS8	11-hydroxyrankinidine	40	0.66
Dangshen	MOL008406	DS9	Spinoside A	39.97	0.4
Dangshen	MOL007514	DS10	Methyl icosa-11,14-dienoate	39.67	0.23
Dangshen	MOL006554	DS11	Taraxerol	38.4	0.77
Dangshen	MOL006774	DS12	Stigmast-7-enol	37.42	0.75
Dangshen	MOL008391	DS13	5alpha-Stigmastan-3,6-dione	33.12	0.79
Dangshen	MOL002879	DS14	Diop	43.59	0.39
Dangshen	MOL000449	DGS15	Stigmasterol	43.83	0.76
Dangshen	MOL000006	DGS16	Luteolin	36.16	0.25
Huangqi	MOL000378	HQ1	7-O-methylisomucronulatol	74.69	0.3
Huangqi	MOL000392	HQ2	Formononetin	69.67	0.21
Huangqi	MOL000433	HQ3	FA	68.96	0.71
Huangqi	MOL000438	HQ4	ZINC14758732	67.67	0.26
Huangqi	MOL000380	HQ5	Astrapterocarpan	64.26	0.42
Huangqi	MOL000211	HQ6	Mairin	55.38	0.78
Huangqi	MOL000371	HQ7	3,9-di-O-methylnissolin	53.74	0.48
Huangqi	MOL000239	HQ8	Jaranol	50.83	0.29
Huangqi	MOL000354	HQ9	Isorhamnetin	49.6	0.31
Huangqi	MOL000439	HQ10	Isomucronulatol-7,2'-di-O-glucosiole	49.28	0.62
Huangqi	MOL000417	HQ11	Calycosin	47.75	0.24
Huangqi	MOL000422	HQ12	Kaempferol	41.88	0.24
Huangqi	MOL000379	HQ13	Methylnissolin-3-O-Glucoside	36.74	0.92
Huangqi	MOL000033	HQ14	(24S)-24-Propylcholesta-5-Ene-3beta-Ol	36.23	0.78
Huangqi	MOL000387	HQ15	Bifendate	31.1	0.67
Huangqi	MOL000098	HQ16	quercetin	46.43	0.28
Huangqi	MOL000442	HQ17	1,7-Dihydroxy-3,9-dimethoxy pterocarpene	39.05	0.48

OB, oral bioavailability; DL, drug-likeness.

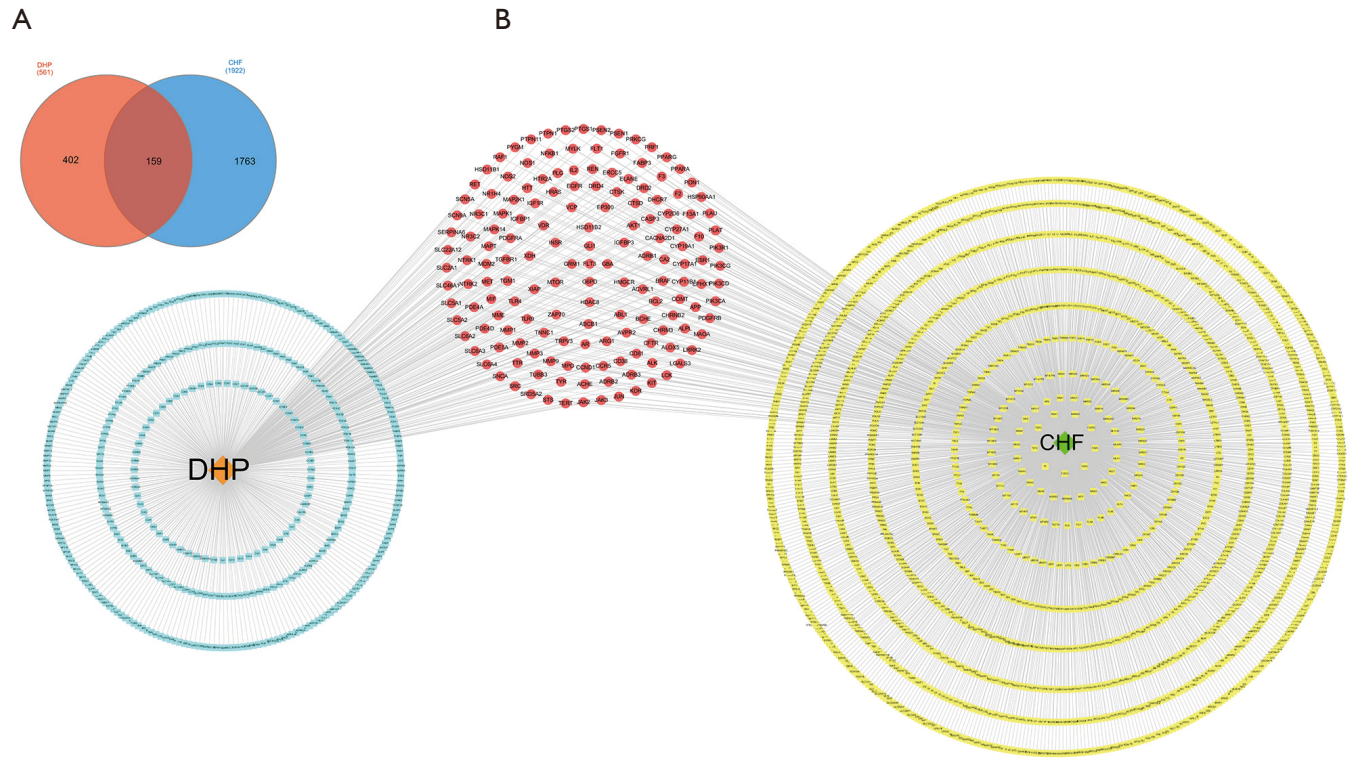


Figure 2 Common targets of DHP and CHF-related targets. (A) Venn diagram. There were 561 targets of DHP, 1,922 targets related to CHF, and 159 common targets shared by both. (B) Common targets network. The blue circle on the left represents the targets of DHP; the yellow circle on the right denotes CHF-related targets; and the red circle in the middle indicates the common targets of DHP in CHF treatment. DHP, the herbal pairing of Codonopsis pilosula (Franch.) Nannf. (Dangshen, DS) and Astragalus membranaceus (Fisch.) Bge. (Huangqi, HQ); CHF, chronic heart failure.

related genes were obtained (https://cdn.amegroups.cn/static/public/apm-21-1469-2.pdf).

Construction of the H-I-P-D network and screening out of key components

A total of 159 common targets between DHP and CHF were identified and displayed using a Venn diagram (*Figure 2A*, https://cdn.amegroups.cn/static/public/apm-21-1469-3.pdf). Next, we constructed the common targets network using Cytoscape 3.7.2 (*Figure 2B*). The common targets and the relevant active ingredients were input into Cytoscape 3.7.2 to construct the H-I-P-D network for visualization. The network comprised 193 nodes (including 32 active ingredients nodes, one disease node, one herb node, and 159 common targets nodes) and 853 edges (*Figure 3*). By using the Cytoscape Network Analyzer plug-in, the nodes were calculated based on the degree for topological analysis and sorted in descending order. The higher degree value, the more important corresponding ingredient was. Based on this principle, the top five nodes were screened out as key components, which included the following: 11-hydroxyrankinidine (degree =41), jaranol (degree =38), 7-methoxy-2-methylisoflavone (degree =37), astrapterocarpan (degree =36), and isorhamnetin (degree =36).

PPI network construction and hub genes screening

To reveal the mechanism of DHP in the treatment of CHF, a PPI network of the common targets was constructed using the STRING database, and was analyzed using the Cytoscape Network Analyzer and CytoNCA plug-ins for visualization (*Figure 4*). As shown in *Figure 4A*, the PPI network consisted of 119 nodes and 475 edges, which represented 119 interacting proteins and 475 interactions.

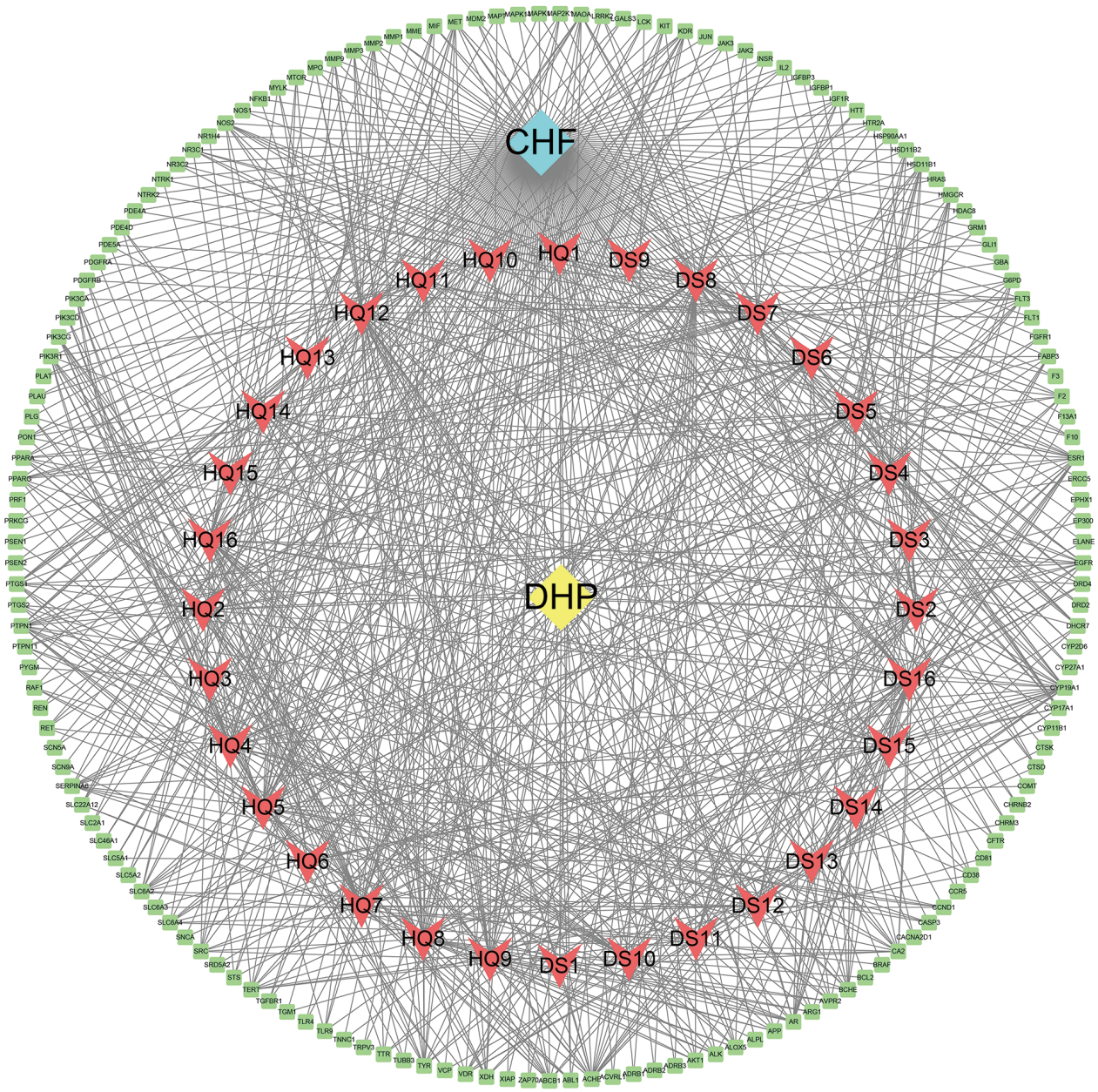


Figure 3 The Herb-Ingredient-Potential target-Disease network (H-I-P-D network). This network consists of 193 nodes and 853 edges. The yellow and blue diamond nodes represent the herb and disease, respectively. The red arrow-like nodes respectively represent the 32 active ingredients of DS and HQ corresponding to the common targets. The green nodes represent 159 common targets of DHP on CHF. The edges represent the interactions between the ingredients and targets. DHP, the herbal pairing of Codonopsis pilosula (Franch.) Nannf. (Dangshen, DS) and Astragalus membranaceus (Fisch.) Bge. (Huangqi, HQ); CHF, chronic heart failure.

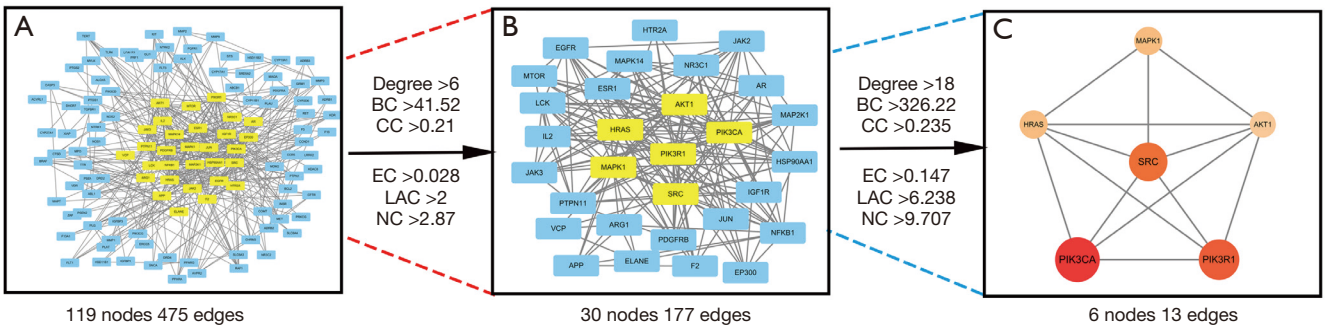


Figure 4 The entire screening process for the PPI network. (A) The original PPI network of common targets. (B) PPI network with nodes of degree >6, BC >41.52, CC >0.21, EC >0.028, NC >2.87, and LAC >2. (C) The six hub genes extracted from the above network with the nodes of degree >18, BC >326.22, CC >0.235, EC >0.147, NC >9.707, and LAC >6.238. Larger sizes and darker colors represent higher degree values. BC, betweenness centrality; CC, closeness centrality; EC, eigenvector centrality; LAC, local average connectivity-based method; NC, network centrality.

Table 2 Information of the six hub genes

Gene symbol	Gene name	Degree
PIK3CA	Phosphatidylinositol 4,5-bisphosphate 3-kinase catalytic subunit alpha isoform	40
PIK3R1	Phosphatidylinositol 3-kinase regulatory subunit alpha	38
SRC	Proto-oncogene tyrosine-protein kinase Src	37
HRAS	GTPase Hras	32
MAPK1	Mitogen-activated protein kinase 1	32
AKT1	RAC-alpha serine/threonine-protein kinase	31

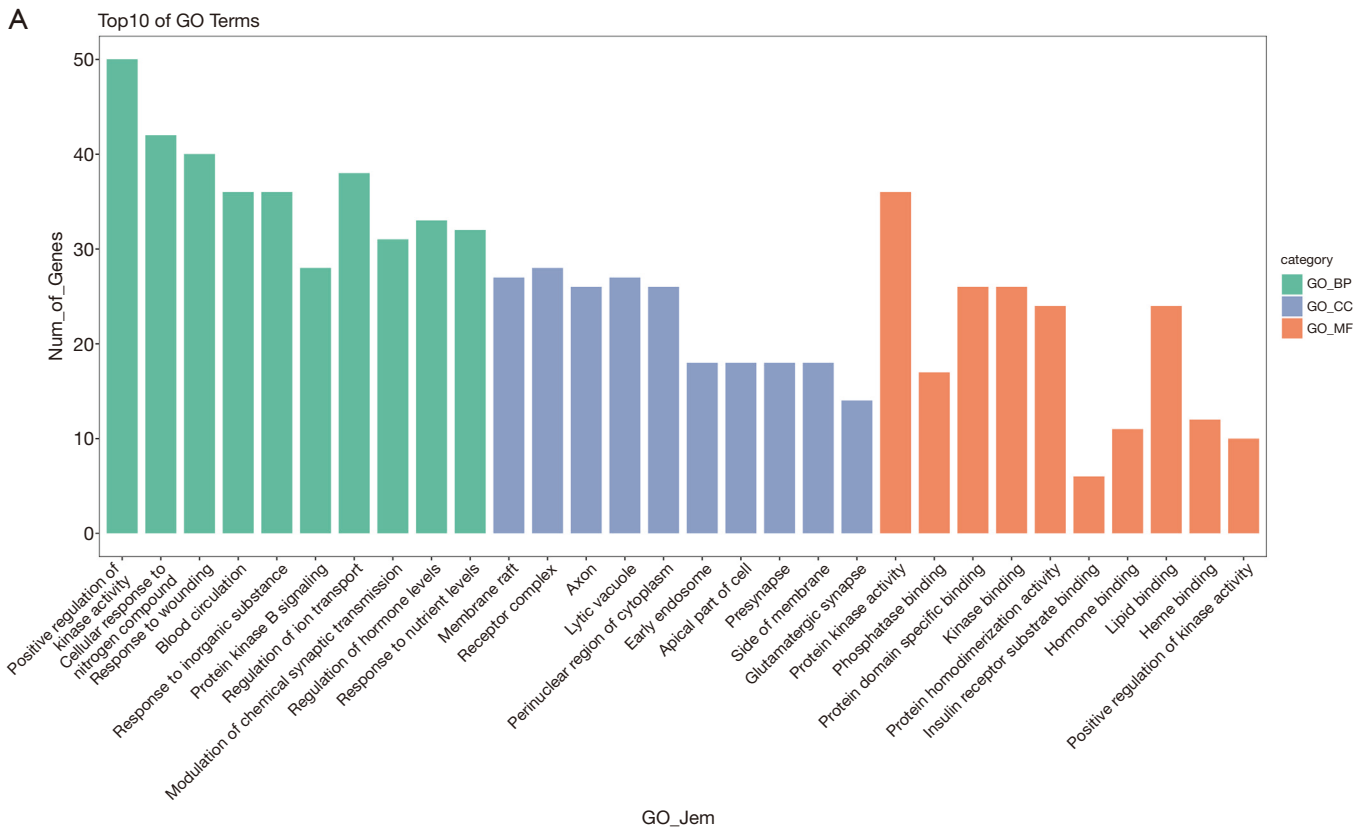
Six topological parameters ("degree", "BC", "CC", "EC", "NC", and "LAC") were used as filters to screen the hub genes. The first threshold was degree >6, BC >41.52, CC >0.21, EC >0.028, NC >2.87, and LAC >2, which developed 30 nodes and 177 edges. These 30 key nodes were then further selected with the second threshold of degree >18, BC >326.22, CC >0.235, EC >0.147, NC >9.707, and LAC >6.238, and finally a total of six nodes and 13 edges were involved in the cluster network. These six nodes were identified as hub genes, which likely exert principal effects on therapeutic mechanisms, and were used in the subsequent molecular docking. The top six hub genes were PIK3CA (degree =40), PIK3R1 (degree =38), SRC (degree =37), HRAS (degree =32), MAPK1 (degree =32), and AKT1 (degree =31) (*Table 2*).

GO and KEGG pathway enrichment analysis

A total of 159 common targets were used to perform GO and KEGG pathway enrichment analyses using Metascape database and R software 3.6.3 for visualization.

According to the P value (P<0.05) and counts, the top 10 enriched BP terms, CC terms, and MF terms were displayed (*Figure 5A* and Table S1). The top 5 BP terms were as follows: positive regulation of kinase activity, cellular response to nitrogen compound, response to wounding, blood circulation, and response to inorganic substance. The top 5 CC terms were as follows: membrane raft, receptor complex, axon, lytic vacuole, and perinuclear region of cytoplasm. The top 5 MF terms were as follows: protein kinase activity, phosphatase binding, protein domain-specific binding, kinase binding, and protein homodimerization activity.

Furthermore, KEGG pathway analysis was also performed to identify pathways that exert a significant function on the therapeutic mechanism (*Figure 5B* and *Table 3*). The top 5 pathways included those in cancer, bladder cancer, the TNF signaling pathway, transcriptional



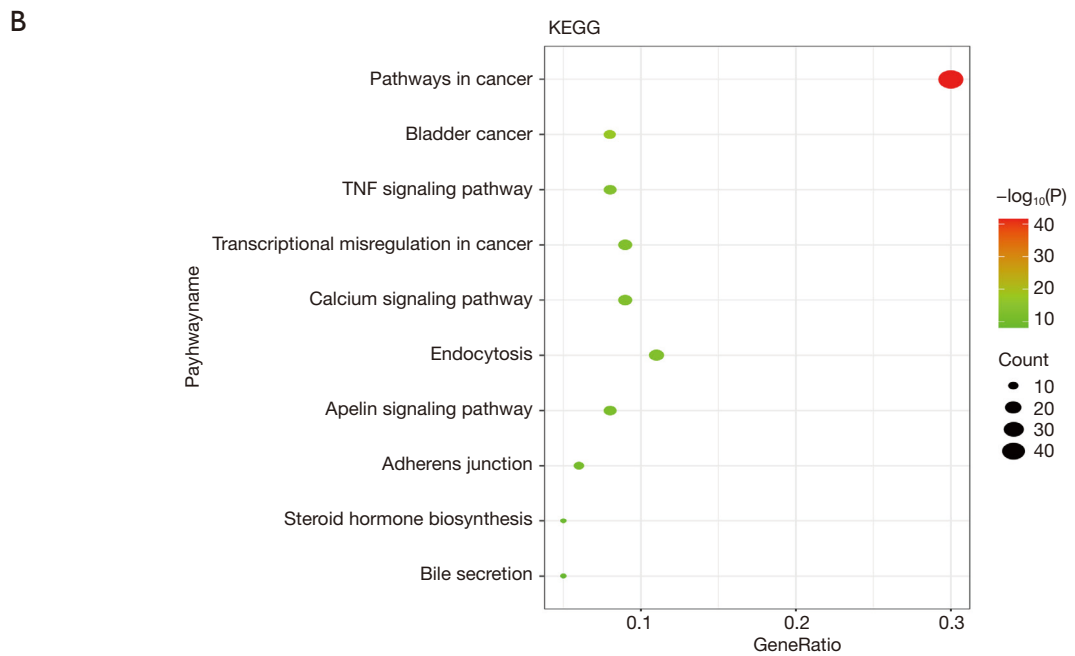


Figure 5 Enrichment analysis of common targets. (A) GO enrichment analysis. The top 10 terms of BP, CC, and MF. (B) Top 10 KEGG terms. The color represents the significance of the –log10(P) value, which is shown in a gradient from green to red, while bubble size represents the counts of the potential active targets involved in the pathways. GO, Gene Ontology; BP, biological process; CC, cellular component; MF, molecular function.

Table 3 Information of the top 10 pathways

Term ID	Description	$-Log_{10}(P)$	Count
hsa05200	Pathways in cancer	41.63	48
hsa05219	Bladder cancer	17.3	12
hsa04668	TNF signaling pathway	13.3	13
hsa05202	Transcriptional misregulation in cancer	12.87	15
hsa04020	Calcium signaling pathway	12.8	15
hsa04144	Endocytosis	12.8	17
hsa04371	Apelin signaling pathway	11.59	13
hsa04520	Adherens junction	10.98	10
hsa00140	Steroid hormone biosynthesis	8.86	8
hsa04976	Bile secretion	8.15	8

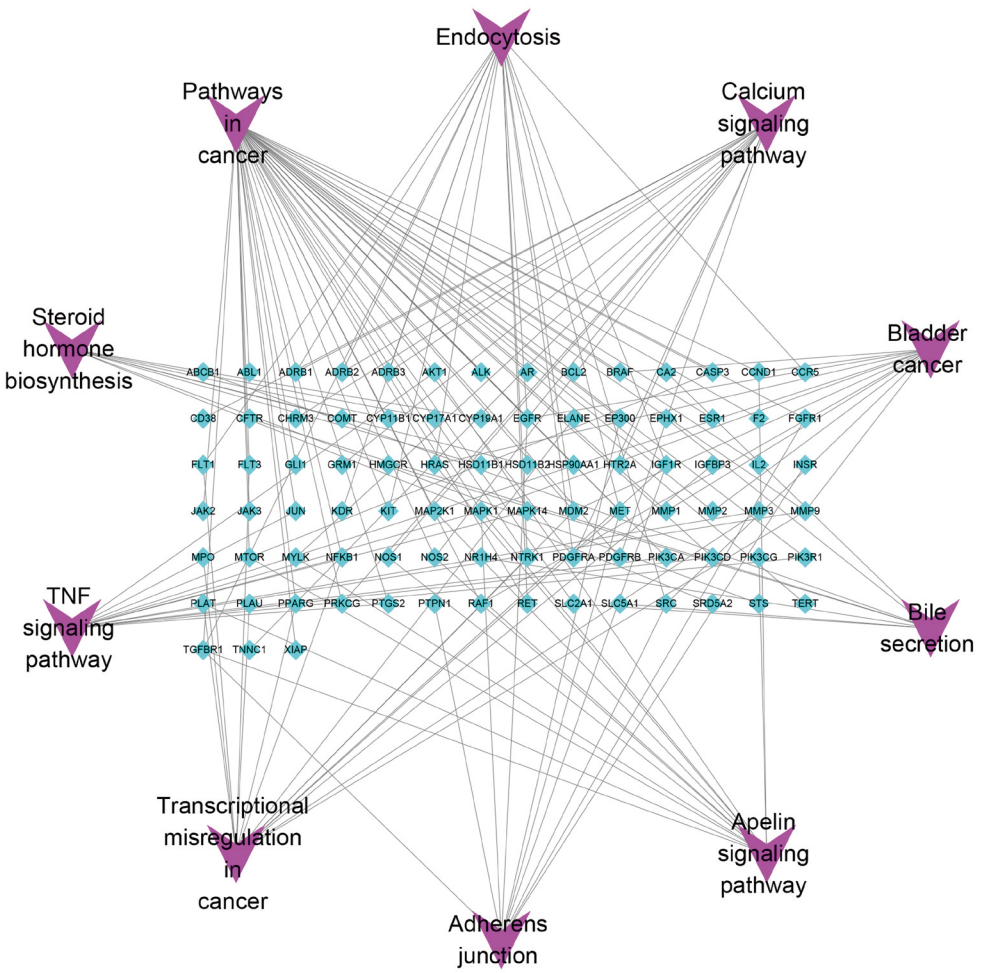


Figure 6 The pathway-related targets network (P-R network). This network comprised 97 nodes and 159 edges. The nodes represented top 10 pathways (marked in purple), and the targets related to these pathways are marked in blue.

Table 4 Positive control group molecular docking information

Key ingredients	Hub gene	PDB ID	Docking affinity (kcal/mol)
Buparlisib	*PIK3CA*	6OAC	−4.4
LY294002	*PIK3R1*	5UBT	−8.3
Dasatinib	*SRC*	1KSW	−8.6
FTI-277	*HRAS*	2C5L	−5.3
Camptothecin	*MAPK1*	6G9J	−9.1
3,3'-diindolylmethane	*AKT1*	6HHH	−8.6

misregulation in cancer, and the calcium signaling pathway. Based on the counts of targets involved in each pathway, a P-R network was established and analyzed using Cytoscape 3.7.2 (*Figure 6*). The P-R network comprised 97 nodes and 159 edges. The purple nodes represented the top 10 pathways, and the blue nodes represented the related-targets.

Molecular docking analysis

In the present study, we simulated the docking of six hub genes (*PIK3CA*, *PIK3R1*, *SRC*, *HRAS*, *MAPK1*, and *AKT1*) and five key ingredients of DHP (11-hydroxyrankinidine, jaranol, 7-methoxy-2-methylisoflavone, astrapterocarpan, and isorhamnetin) to assess the protein-ligand binding potential. The related proteins of PIK3CA, PIK3R1, SRC, HRAS, MAPK1, and AKT1 were obtained from PDB, and the PDB IDs were 6OAC, 5UBT, 1KSW, 2C5L, 6G9J, and 6HHH, respectively. Furthermore, given that previous experiments had shown that drugs such as buparlisib (34,35), LY294002 (36,37), dasatinib (38,39), FTI-277 (40), camptothecin (41,42), and 3,3'-diindolylmethane (43,44) could interact with these six hub genes, they were selected as the positive control group. Similar to the positive group (*Table 4*), the five key ingredients exhibited strong affinity with the six hub genes, and their average docking affinity was −7.37 kcal/mol, indicating strong binding energy (Table S2). For the targets PIK3CA, SRC, and HRAS, their affinity with most of the key components was higher than the corresponding positive drugs, and AKT1-11-hydroxyrankinidine exhibited the best binding activity. Taking this pair as an example, a small molecule ligand of 11-hydroxyrankinidine could potentially fit into the interface pocket of AKT1, and the details of the docking are displayed in *Figure 7*. The binding modes of the six hub genes with the key ingredients are displayed in *Figure 7* and *Table 5*.

Discussion

As the major herb pairing for Qi-boosting in TCM, DHP plays an important role in the treatment of CHF. Clinical studies have shown that formulas with DHP as the main herb pairing exert significant therapeutic effects on CHF (9,13-15). Our previous experiment demonstrated that DHP improves heart function in a rat model of HF (after myocardial infarction caused by coronary artery ligation) through regulation of myocardial energy metabolism (16). However, the complex mechanism of DHP in the treatment of CHF has not been fully elucidated. In this study, we aimed to explore the molecular mechanism of DHP in CHF using network pharmacology and molecular docking technology.

In this study, five key ingredients of DHP were screened from the H-I-P-D network, including 11-hydroxyrankinidine, jaranol, 7-methoxy-2-methyl isoflavone, astrapterocarpan, and isorhamnetin. All of these belong to flavonoids, except for 11-hydroxyrankinidine, which is a kind of Gelsemium alkaloids (45), indicating that flavonoids are the main compounds of DHP in CHF treatment. Flavonoids can protect the cardiovascular system through anti-inflammatory, antioxidant, and anti-platelet aggregation effects (46,47). They can also reduce the downregulation of endothelial nitric oxide synthase (eNOS) and the level of reactive oxygen species (ROS), and increase the bioavailability of nitric oxide (NO), thereby improving endothelial function (48). Sun *et al.* reported that flavonoids extracted from propolis could reduce pathological myocardial hypertrophy via the PI3K/AKT signaling pathway (49). Endothelial dysfunction and cardiac hypertrophy are two important pathophysiological mechanisms, which serve as the main risk factors of CHF (50). Astrapterocarpan may improve endothelial dysfunction by inhibiting the activation of the MAPK3/1

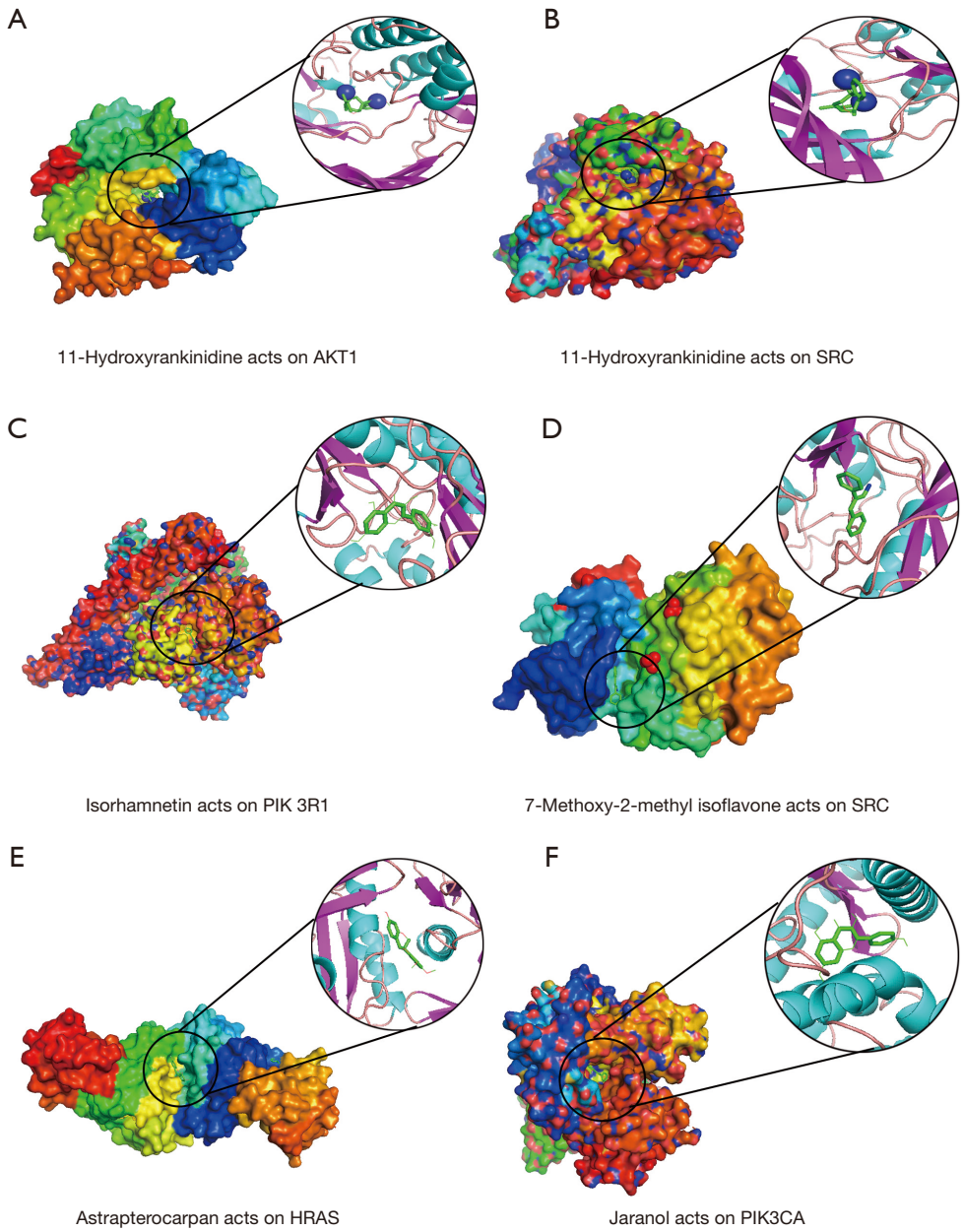


Figure 7 Molecular docking modes of the six hub genes and five key ingredients. The colored irregular complex on the left represents proteins, and the green stick on the right represents ingredients.

Table 5 Molecular docking information

Key ingredients	Hub gene	PDB ID	Docking affinity (kcal/mol)
11-hydroxyrankinidine	*AKT1*	6HHH	–10.0
11-hydroxyrankinidine	*SRC*	1KSW	–9.1
Isorhamnetin	*PIK3R1*	5UBT	–8.0
7-methoxy-2-methyl isoflavone	*MAPK1*	6G9J	–7.7
Astrapterocarpan	*HRAS*	2C5L	–6.0

signaling pathway (51). Isorhamnetin protects against endothelial dysfunction and cardiac hypertrophy via the PI3K/AKT pathway (52,53). Based on the results of topological analysis and literature retrieval, flavonoids may be the main compounds of DHP in the treatment of CHF. Numerous studies also shown that isorhamnetin may play an important role in CHF treatment.

After screening of the PPI network, six hub genes were identified, including PIK3CA, PIK3R1, SRC, HRAS, MAPK1, and AKT1. Phosphatidylinositol 3-kinases (PI3Ks) are involved in cellular functions such as cell proliferation, survival, growth, differentiation, and apoptosis (54). PIK3CA and PIK3R1 are the class I PIK3s. Inhibition of PIK3s can prevent many age-related changes in the heart and protect the heart function of aged mice (55). PIK3CA, PIK3R1, and AKT1 are associated with PI3K/AKT pathways. As mentioned above, isorhamnetin can improve endothelial dysfunction and cardiac hypertrophy via the PI3K/AKT pathway (52,53). The rat sarcoma (RAS) protein exists in cardiac myocytes as well as in cancer cells, and its high level of expression relates to numerous growth responses, such as promotion of cardiac hypertrophy, and is an important risk factor for CHF (56). HRAS, an isoform of Ras proteins, can regulate the PI3K-AKT signaling pathway and may be an important modulator of cardiac growth (57,58). It has been reported that the expression of the MAPK1 [also known as extracellular regulated protein kinase 2 (ERK2)] gene and protein increases rapidly in CHF rats, and the process of cardiac remodeling is delayed by inhibiting the expression of ERK2 (59). The ERK1/2 pathway is also closely related to myocardial hypertrophy and endothelial dysfunction (60,61). SRC is a non-receptor protein tyrosine kinase, and plays a key role in many cellular processes, including cell growth, proliferation, differentiation, and neuronal signal (62,63). SRC is involved in the PI3K/Akt and ERK1/2 signaling pathways, and plays an important role in the occurrence and development of endothelial dysfunction and cardiac hypertrophy (64-68). Therefore, DHP may alleviate endothelial dysfunction and myocardial hypertrophy via the PI3K/Akt or ERK1/2 signaling pathways, thereby improving CHF. PIK3CA, PIK3R1, SRC, HRAS, MAPK1, and AKT1 may also play critical roles in this process.

Meanwhile, the six hub genes and five key ingredients of DHP were investigated by molecular docking simulation. Compared to the positive group, the five key ingredients exhibited strong affinities to the six hub genes. Therefore, they can be explored as the main components of new natural medicines of DHP in the treatment of CHF in the future.

The top five pathways after KEGG enrichment analysis included pathways in cancer, bladder cancer, the TNF signaling pathway, transcriptional misregulation in cancer, and the calcium signaling pathway, which mainly relates to cancer, inflammation, and calcium regulation. Due to critical roles of SRC, HRAS, and the PI3K/Akt or ERK1/2 signaling pathway in the occurrence and development of tumors, this may explain why the enrichment results were closely related to cancer pathways (69-72). The TNF signaling pathway is associated with inflammation and is mainly involved in regulating immune cells. TNF can also mediate many downstream pathways, such as the PI3K/Akt or ERK1/2 signaling pathways, resulting in cardiac hypertrophy and endothelial dysfunction (73-76). A previous study showed that restricting the TNF-α can inhibit the process of CHF, which may become a novel way to treat CHF (77). The excitation-contraction coupling of cardiomyocytes is closely related to calcium ion (Ca^{2+}) regulation. Abnormal Ca^{2+} handling results in impaired Ca^{2+} cycling that affect both systolic and diastolic functions, and is considered as an important mechanism of CHF (78,79). Our previous study found that Astragalus granules could improve cardiac function in HF mice caused by thoracic aortic constriction (TAC) by reversing Ca^{2+}/calmodulin-dependent protein kinase II (CaMKII) overexpression-induced Ca^{2+} handling disorder (80).

The results of BP in the GO enrichment analysis were primarily associated with the positive regulation of kinase activity and the cellular response to nitrogen compound. Many kinases are involved in PI3K/Akt, ERK1/2, and calcium signaling pathways, such as AKT1, MAPK1, and CaMKII. Abnormal regulation of these kinases plays an important role in the development of CHF (81-83). Flavonoids can improve endothelial function by increasing the bioavailability of NO (48).

Above all, our network pharmacology results indicate that flavonoids may be the main compounds of DHP in the treatment of CHF. The role of isorhamnetin in CHF warrants further study. Firstly, the flavonoids in DHP may regulate the TNF pathway and act on hub genes to regulate TNF-mediated downstream PI3K/Akt or ERK1/2 signaling pathways, thereby alleviating endothelial dysfunction and cardiac hypertrophy and improving CHF. They may also improve excitation-contraction coupling by regulating the calcium signaling pathway (*Figure 8*). These

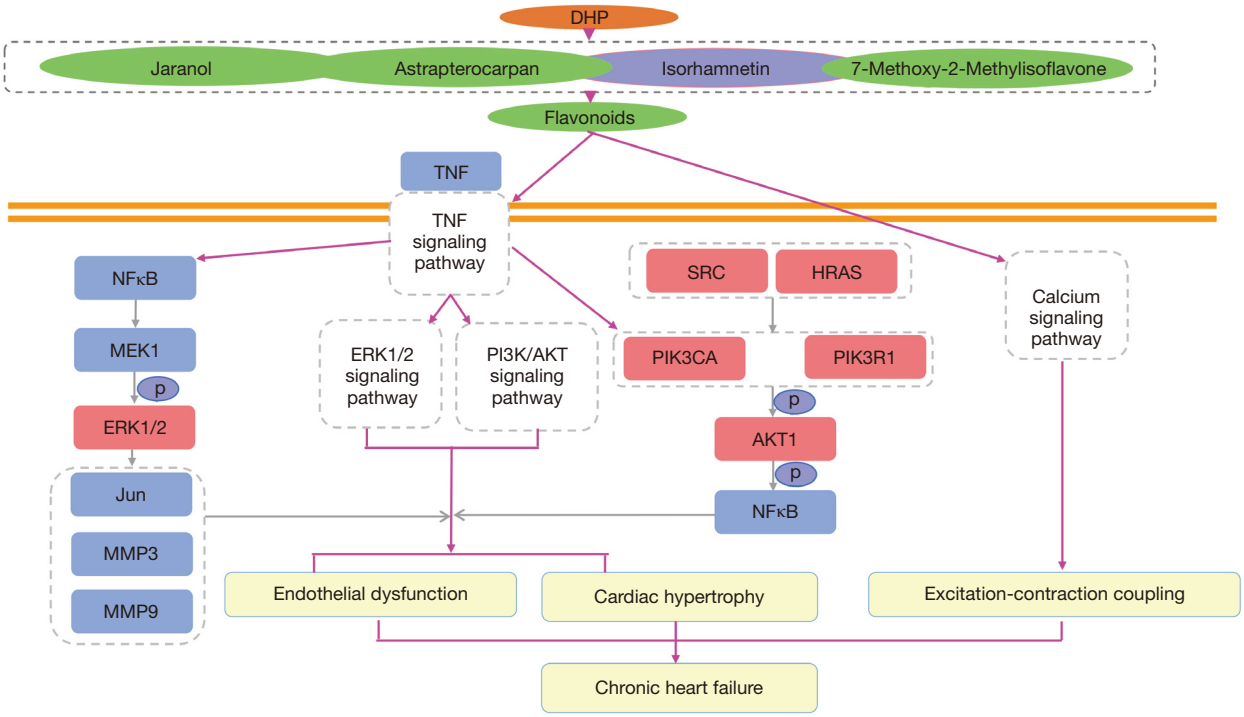


Figure 8 Potential molecular mechanism of DHP on CHF treatment. The red nodes represent hub genes or the most important ingredients; the green nodes represent the herbs and key ingredients; and the blue nodes represent the relevant targets in the relevant pathway. DHP, the herbal pairing of Codonopsis pilosula (Franch.) Nannf. (Dangshen, DS) and Astragalus membranaceus (Fisch.) Bge. (Huangqi, HQ); CHF, chronic heart failure.

pathways are all related to the BPs of positive regulation of kinase activity and cellular response to nitrogen compound. Furthermore, our molecular docking results showed that five key ingredients exhibited strong affinities to six hub genes.

Conclusions

This study revealed the molecular mechanism of DHP in the treatment of CHF by utilizing network pharmacology and molecular docking. However, further experiments are required to conform these findings and provide insights for future research.

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Footnote

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Conflicts of Interest: All authors have completed the ICMJE uniform disclosure form (available at https://dx.doi.org/10.21037/apm-21-1469). The authors have no conflicts of interest to declare.

Ethical Statement: The authors are accountable for all aspects of the work in ensuring that questions related to the accuracy or integrity of any part of the work are appropriately investigated and resolved. This study was conducted in accordance with the Declaration of Helsinki (as revised in 2013).

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Supplementary

Table S1 Information for molecular docking

Number	Hub gene	PDB ID	Mol ID	Compound	Docking affinity (kcal/mol)
1	PIK3CA	6OAC	MOL008411	11-Hydroxyrankinidine	-5.4
			MOL000239	Jaranol	-5.2
			MOL003896	7-Methoxy-2-methyl isoflavone	-5.6
			MOL000380	(6aR,11aR)-9,10-dimethoxy-6a,11a-dihydro-6H-benzofurano[3,2-c]chromen-3-ol	-4.9
			MOL000354	isorhamnetin	-3.9
2	PIK3R1	5UBT	MOL008411	11-Hydroxyrankinidine	-5.9
			MOL000239	Jaranol	-7.8
			MOL003896	7-Methoxy-2-methyl isoflavone	-7.8
			MOL000380	(6aR,11aR)-9,10-dimethoxy-6a,11a-dihydro-6H-benzofurano[3,2-c]chromen-3-ol	-7.8
			MOL000354	isorhamnetin	-8
3	SRC	1KSW	MOL008411	11-Hydroxyrankinidine	-9.1
			MOL000239	Jaranol	-8.7
			MOL003896	7-Methoxy-2-methyl isoflavone	-8.8
			MOL000380	(6aR,11aR)-9,10-dimethoxy-6a,11a-dihydro-6H-benzofurano[3,2-c]chromen-3-ol	-8.4
			MOL000354	isorhamnetin	-8.7
4	HRAS	2C5L	MOL008411	11-Hydroxyrankinidine	-6.7
			MOL000239	Jaranol	-6.1
			MOL003896	7-Methoxy-2-methyl isoflavone	-6.5
			MOL000380	(6aR,11aR)-9,10-dimethoxy-6a,11a-dihydro-6H-benzofurano[3,2-c]chromen-3-ol	-6
			MOL000354	isorhamnetin	-7.2
5	MAPK1	6G9J	MOL008411	11-Hydroxyrankinidine	-8.6
			MOL000239	Jaranol	-7.2
			MOL003896	7-Methoxy-2-methyl isoflavone	-7.7
			MOL000380	(6aR,11aR)-9,10-dimethoxy-6a,11a-dihydro-6H-benzofurano[3,2-c]chromen-3-ol	-8.3
			MOL000354	isorhamnetin	-8.2
6	AKT1	2C5L	MOL008411	11-Hydroxyrankinidine	-10
			MOL000239	Jaranol	-7.9
			MOL003896	7-Methoxy-2-methyl isoflavone	-8.3
			MOL000380	(6aR,11aR)-9,10-dimethoxy-6a,11a-dihydro-6H-benzofurano[3,2-c]chromen-3-ol	-8.3
			MOL000354	isorhamnetin	-8.2
					average docking affinity: -7.37333

https://dx.doi.org/10.21037/apm-21-1469

Table S2 Information of top 10 Gene Ontology (GO) analysis

Number	Catogary	Term ID	Description	$-Log_{10}(P)$	Count
1	Biological processes(BP)	GO:0033674	positive regulation of kinase activity	-42.9	50
2	BP	GO:1901699	cellular response to nitrogen compound	-31.15	42
3	BP	GO:0009611	response to wounding	-27.77	40
4	BP	GO:0008015	blood circulation	-27.15	36
5	BP	GO:0010035	response to inorganic substance	-26.61	36
6	BP	GO:0043491	protein kinase B signaling	-26.13	28
7	BP	GO:0043269	regulation of ion transport	-25.52	38
8	BP	GO:0050804	modulation of chemical synaptic transmission	-23.79	31
9	BP	GO:0010817	regulation of hormone levels	-23.57	33
10	BP	GO:0031667	response to nutrient levels	-23.31	32
1	Cell component(CC)	GO:0045121	membrane raft	-22.72	27
2	CC	GO:0043235	receptor complex	-18.75	28
3	CC	GO:0030424	axon	-14.36	26
4	CC	GO:0000323	lytic vacuole	-14.22	27
5	CC	GO:0048471	perinuclear region of cytoplasm	-13.12	26
6	CC	GO:0005769	early endosome	-11.08	18
7	CC	GO:0045177	apical part of cell	-10.19	18
8	CC	GO:0098793	presynapse	-8.88	18
9	CC	GO:0098552	side of membrane	-7.71	18
10	CC	GO:0098978	glutamatergic synapse	-7.67	14
1	Molecular function(MF)	GO:0004672	protein kinase activity	-26.37	36
2	MF	GO:0019902	phosphatase binding	-14.9	17
3	MF	GO:0019904	protein domain specific binding	-13.32	26
4	MF	GO:0019900	kinase binding	-12.77	26
5	MF	GO:0042803	protein homodimerization activity	-12.23	24
6	MF	GO:0043560	insulin receptor substrate binding	-11.2	6
7	MF	GO:0042562	hormone binding	-10.89	11
8	MF	GO:0008289	lipid binding	-10.67	24
9	MF	GO:0020037	heme binding	-10.61	12
10	MF	GO:0051219	phosphoprotein binding	-10.24	10

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