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**Original Research Article**

# Investigation of the potential pharmacological mechanism of action of Danhong injection against chronic heart failure using a network pharmacology-based approach

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## *Abstract*

***Purpose:*** *To investigate the mechanism of action of Danhong Injection (DHI) in chronic heart failure (CHF).*
***Methods:*** *Network pharmacology was employed to identify the bioactive compounds and targets of DHI in CHF. Bioinformatics analysis was used to examine the potential biological functions and pathways of the candidate targets, while molecular docking was conducted to evaluate the binding affinity of the ligand-protein complex.*
***Results:*** *Based on data mining from public databases, 65 bioactive ingredients and 246 potential targets of DHI were identified, along with 786 CHF-related genes. There were 48 common targets between DHI targets and CHF-related genes, and a protein-protein interaction (PPI) network of common targets containing 42 nodes and 204 edges was constructed. The 48 common targets were considered as effector proteins exerting anti-CHF effects, and they were shown to be involved in multiple signal-transduction pathways and disease-related pathways by bioinformatics analysis. Most of these targets had protein-binding capability and were located in plasma membrane as well as extracellular regions. The biological process of these proteins was primarily associated with gene regulation, response to hypoxia, and heart development. Binding capability between these active ingredients and proteins was further validated by molecular docking simulation.*
***Conclusion:*** *This study has shed new light on the pharmacological mechanism of action of DHI's effects on CHF, thus offering fresh leads for additional research into DHI's potential to cure CHF.*

***Keywords:*** *Danhong injection, Chronic heart failure, Molecular mechanism, Network pharmacology, Molecular docking*



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## INTRODUCTION

Chronic heart failure (CHF) is an advanced stage of most cardiovascular diseases, and causes increased risks of premature death. It is a complex clinical syndrome of cardiac insufficiency resulting in a variety of factors such as pressure and volume overload, loss of

muscles, primary muscle diseases and excessive peripheral demands. Dyspnea, tiredness, reduced exercise tolerance, and fluid retention are common symptoms of CHF in patients. CHF poses great economic pressure on the public medical system worldwide and significantly reduces the life quality of patients with CHF. The prevalence of CHF is rising due to increase in the aging population and the improved survival rate of cardiovascular diseases. The global incidence of CHF has been reported to range from 0.1 % to 0.9 % [1]. It is estimated that there are approximately 64.3 million CHF patients worldwide [2]. It is anticipated that the prevalence of CHF will increase by 46 % over the next 10 years, with the number of CHF patients increasing to 8 million by 2030 [3]. Current treatment modalities for CHF include drug therapy, valve replacement, and cardiac resynchronization therapy (CRT). However, there are still some defects regarding the clinical efficacy and safety of these treatments. Therefore, there is a need to develop better therapies for patients with CHF.

Traditional Chinese Medicine (TCM) contains a variety of bioactive substances that target several proteins and pathways. After thousands of years of practice, treatment for CHF using TCM has been demonstrated to significantly improve outcomes. A number of studies have suggested that the combined use of TCM and western medicine may achieve better therapeutic effects with fewer side effects in the treatment of CHF, when compared to using western medicine alone [4]. *Danhong* injection (DHI) contains two kinds of Chinese herbs: Danshen (dried root of *Salvia miltiorrhiza* Bunge) and Honghua (dried flower of *Carthamus tinctorius* L.), both of which can synergistically treat cardiovascular and cerebrovascular diseases, with reduced side effects. Since its debut, the clinical use of DHI in China has demonstrated that it has favorable therapeutic effects on patients with CHF [5]. However, due to the complex chemical constituents and targets of DHI, the pharmacological mechanism of DHI against CHF has not been fully explored yet.

Network pharmacology offers a way of investigating the extremely complex mechanisms of TCM in the treatment of diseases [6]. It includes the identification of TCM components and targets, network construction and topology analysis, as well as subsequent steps such as bioinformatics analysis and molecular docking. In the present study, the pharmacological mechanism of action of DHI against CHF was revealed by network pharmacology combined with bioinformatics analysis and molecular docking.

## EXPERIMENTAL

### Identification of the bioactive compounds in DHI

The Traditional Chinese Medicine Systems Pharmacology Database (TCMSP, https://tcmspw.com/tcmsp.php) is an online analysis platform designed for capturing the relationships among traditional Chinese herbs, compounds, targets, and diseases. It provides researchers with detailed pharmacokinetic properties of herbal compounds, so as to enable them to identify potential bioactive compounds for further studies. The chemical constituents of DHI with drug-likeness greater than or equal to 0.18 were used to identify the bioactive compounds.

### Identification of targets of bioactive compounds

From TCMSP database, the targets of the bioactive compounds of DHI were obtained. UniProt (http://www.uniprot.org/), which is a freely available comprehensive resource for protein sequence and annotation data. It was used in this study to collect the uniform symbols of human targets, with the exclusion of non-human targets and non-standard names of targets. Duplicates were also removed.

### Identification of CHF-related targets

The CHF-associated genes were identified from DisGeNET (https://www.disgenet.org/home/) and GeneCards (https://www.genecards.org/) databases. DisGeNET is a discovery platform that contains one of the largest publicly available collections of genes and mutant loci associated with human disease. It contains 1134942 gene-disease correlations and incorporates data from several sources. GeneCards is an integrated database that provides comprehensive data on all human genes, and offers a strong tool for screening relevant targets of human diseases. The keyword “chronic heart failure” were input into DisGeNET and GeneCards to identify CHF-related genes.

### Identification of common targets of DHI and CHF

The online tool E Venn (http://www.ehbio.com/test/venn) was used for the identification of common targets of CHF and DHI. The DHI

targets and CHF-related genes were submitted to the E Venn platform to obtain common targets.

**Analysis of protein-protein interaction (PPI)**

STRING is a database dedicated to physical and functional PPI. Currently, it contains 24,584,628 proteins from 5,090 organisms. The PPI data of the common targets of DHI and CHF were obtained through STRING 11.5 (https://string-db.org/) with a high confidence interaction score (0.7). Cytoscape is an open-source software platform for integrating, visualizing and analyzing networks. The PPI network of the common targets of DHI and CHF was constructed and analyzed using Cytoscape 3.9.1. Topological characteristics of the PPI network were generated using a Network Analyzer plugin, and the central network of hub genes was extracted based on the following criteria: betweenness centrality (BC) > 0.026, closeness centrality (CC) > 0.508, and degree centrality (DC) > 9.714.

**Bioinformatic analysis**

To understand the potential biological process and pathways that are involved in the anti-CHF effects of DHI, the common targets were submitted to the online tool DAVID (https://david.ncifcrf.gov/), to carry out gene ontology (GO), and Kyoto encyclopedia of genes and genomes (KEGG) pathway for enrichment analysis. GO annotation classifies genes into three categories: biological process (BP), molecular function (MF), and cellular component (CC). The KEGG facilitates analysis and improved comprehension of the gene regulatory circuits. For the purpose of sorting and visualizing GO keywords and KEGG pathways, the p-value logarithm was applied.

**Network construction and topological analysis**

Cytoscape 3.9.1 was used for network construction and topological analysis. A total of five networks were constructed, namely, herb-ingredient-target network, PPI network of the common targets of DHI and CHF, central network of hub genes extracted from PPI network, herb-ingredient-common targets network, and the pathway-targets network. Cytoscape plugin Network Analyzer was used to analyze topological properties of the network.

**Molecular docking**

Binding capability of the targets with active ingredients was evaluated by molecular docking. The corresponding 3D structure of 10 proteins, i.e., STAT3 (6NJS), AKT1 (6HHH), JUN (1JUN), VEGFA (6ZFL), MMP9 (4XCT), CASP3 (1NME), IL1B (1HIB), IL6 (1IL6), CAV1 (5IJP), and EDN1 (6DK5) were obtained from the UniProt-RCSB PDB database (https://www.rcsb.org/). The 3D chemical structures of the bioactive compounds each obtained from the PubChem database (https://pubchem.ncbi.nlm.nih.gov/). Each protein structure was prepared using PyMol to remove water molecules and irrelevant protein chains. Hydrogen was added and the gasteiger charge was calculated for the proteins and bioactive compounds via AutoDock Tools. Molecular docking was done using AutoDock, and the 2D and 3D diagrams of the most stable binding conformation were generated using LigPlus and PyMol software, respectively.

## RESULTS

**Bioactive components and targets of DHI**

The chemical ingredients of DHI, along with their drug-likeness value were downloaded from the TCMSP database. As a result, a total of 84 active ingredients including 65 in *Salvia miltiorrhiza* Bunge and 22 in *Carthamus tinctorius* L. were identified. Each of these chemical constituents has a total drug-likeness value greater than or equal to 0.18, and it was identified as bioactive compounds. The targets of these bioactive components were obtained from the TCMSP database. The unified symbol of human genes was obtained from the UniProt database. In effects, 136 targets of *Salvia miltiorrhiza* Bunge and 217 targets of *Carthamus tinctorius* L. were identified. In all, 246 targets of DHI were identified, with 107 overlapped targets between *Salvia miltiorrhiza* Bunge and *Carthamus tinctorius* L. The detailed characteristics of the bioactive components of DHI and their targets are shown in a herb-ingredient-target network (Figure 1).

**Results of topological analysis of PPI network of common targets**

To identify CHF-related genes, a search was conducted in the DisGeNET and GeneCards databases using the keywords: “chronic heart failure”. As a result, 786 CHF-related genes were identified, with 223 genes from the DisGeNET database, and 688 genes from the GeneCards database (**Table 1**). Subsequently, a total of 48 common targets were obtained by intersecting the CHF-related genes and DHI targets. To further identify the hub genes, the PPI information was retrieved from the STRING database, and the PPI network of the common targets was built. As a result, a PPI network with

42 proteins and 204 PPIs was generated (Figure 2 A), after the removal of 6 common targets due to the lack of relevant PPI data. Furthermore, based on the mean value of three topological features, a central network containing 10 hub genes was created (Figure 2 B). Table 1 shows detailed information on the 10 hub genes in the central network.

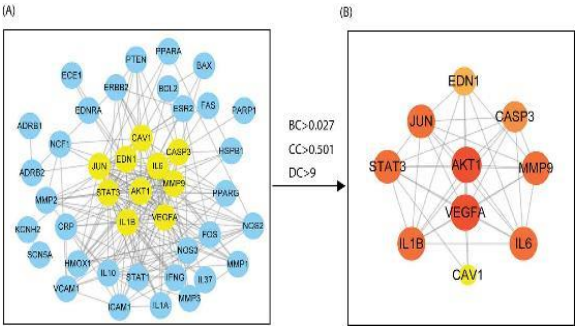


**Figure 2:** Topological analysis of the PPI network of 42 common targets. (A): PPI network containing 42 common targets shared by CHF and DHI; (B): Central network derived from the PPI network of the common targets based on three topological features. The deeper the orange, the higher the degree value

## Enrichment analysis

Data revealed that the common targets shared by DHI targets and CHF-related genes were significantly associated with 100 KEGG pathways ($p < 0.05$).

**Table 1:** Detailed information on the 10 hub genes in the central network

| Gene symbol | Gene name | Degree |
|---|---|---|
| AKT1 | AKT Serine/Threonine Kinase 1 | 23 |
| VEGFA | vascular endothelial growth factor A | 21 |
| STAT3 | Signal Transducer And Activator Of Transcription 3 | 25 |
| IL1B | Interleukin 1 Beta | 22 |
| CAV1 | Caveolin 1 | 12 |
| IL6R | Interleukin 6 | 25 |
| MMP9 | matrix metallopeptidase 9 | 17 |
| CASP3 | Caspase 3 | 16 |
| EDN1 | endothelin 1 | 14 |
| JUN | Jun Proto-Oncogene, AP-1 Transcription Factor Subunit | 20 |

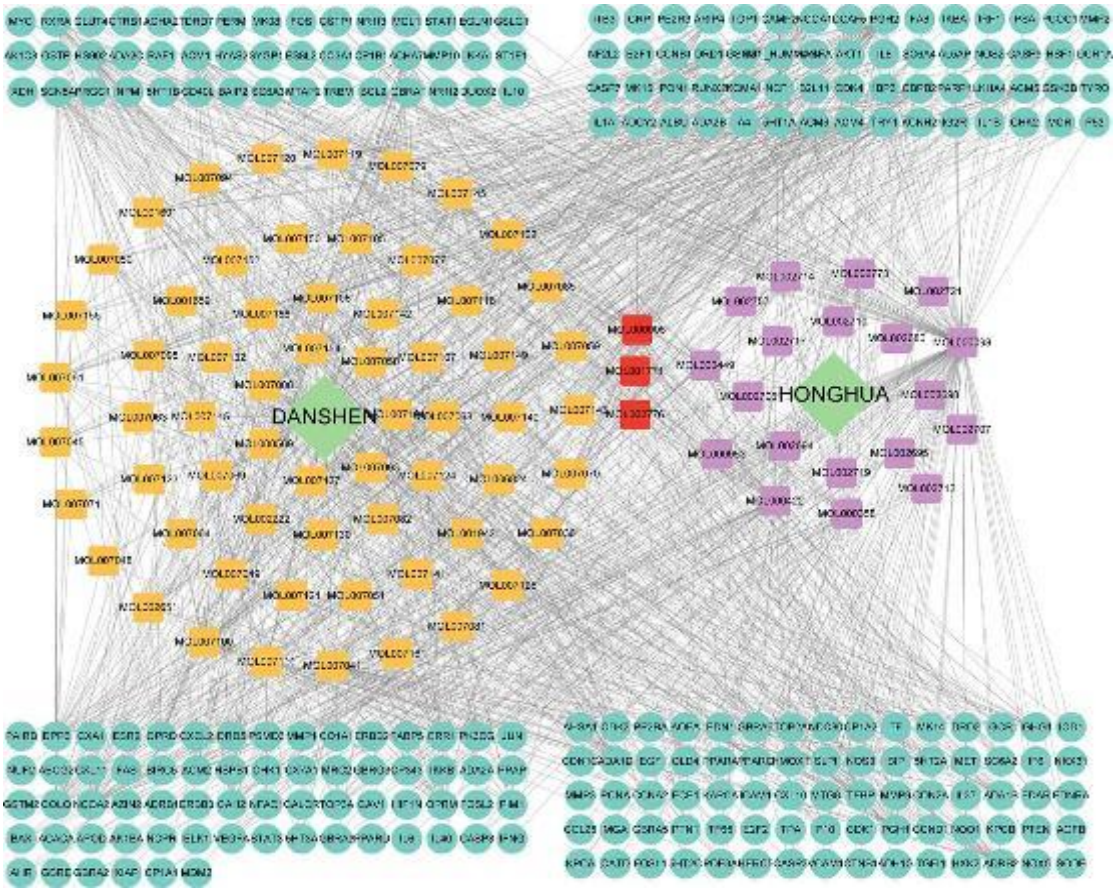


**Figure 1:** Herb-ingredient-target network of DHI. The squares represent bioactive compounds of DHI, and green diamonds represent herbs, while cycles represent targets of the active compounds

Meanwhile, the top 20 pathways with the lowest p-values were shown in Figure 3 A. These pathways mostly involve signal transduction and are linked to several diseases, as illustrated in Table 2. Four signal-transduction pathways, i.e., HIF-1 signaling pathway, TNF signaling pathway, relaxin signaling pathway, and IL-17 signaling pathway, provided novel clues for exploring the molecular mechanism of the action of DHI against CHF. A pathway-targets network was built in order to identify important targets influencing cellular pathways. As a result, a pathway-target network containing 55 nodes (20 pathway nodes and 35 target nodes) and 235 edges was constructed (Figure 3 B). In addition, the top 6 key targets with the highest degree value were JUN, IL6, FOS, AKT1, IL1B, and IFNG. The topological characteristics of the pathway-target network indicated more crucial roles of the six genes in regulating these pathways.

**Common target-involved GO terms**

The GO analysis revealed that the 48 common targets shared by CHF-related genes and DHI targets were significantly associated with 382 GO terms, which included 315 terms for BP, 26 terms for CC, as well as 41 MF terms. The top 10 terms with a higher gene count for each category were shown in Figure 4. It was found that most of common targets had protein binding capability, and were primarily located on the plasma membrane or in extracellular regions.

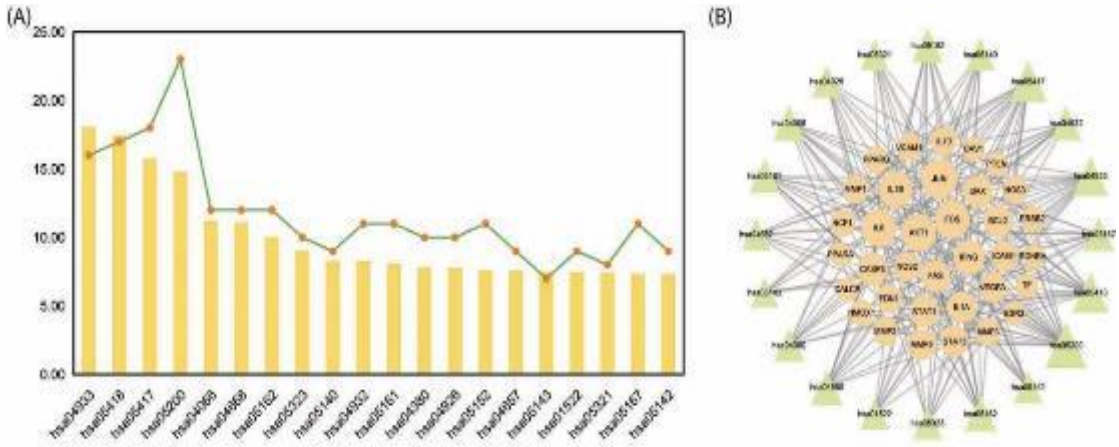


**Figure 3:** Analysis of KEGG pathways that are significantly associated with the common targets. (A): The top 20 enriched KEGG pathways ranked by gene count; (B): The pathway-targets network contains the top 20 enriched pathways and relevant genes.

**Table 2:** The characteristics of the top 20 pathways with the lowest p-value

| Term ID | Pathway description | $-Log10^{P}$ | Count |
|---|---|---|---|
| hsa04933 | AGE-RAGE signaling pathway in diabetic complications | 18.09 | 16 |
| hsa05418 | Fluid shear stress and atherosclerosis | 17.43 | 17 |
| hsa05417 | Lipid and atherosclerosis | 15.77 | 18 |
| hsa05200 | Pathways in cancer | 14.82 | 23 |
| hsa04066 | HIF-1 signaling pathway | 11.23 | 12 |
| hsa04668 | TNF signaling pathway | 11.10 | 12 |
| hsa05162 | Measles | 10.07 | 12 |
| hsa05323 | Rheumatoid arthritis | 9.04 | 10 |
| hsa05140 | Leishmaniasis | 8.32 | 9 |
| hsa04932 | Non-alcoholic fatty liver disease | 8.29 | 11 |
| hsa05161 | Hepatitis B | 8.10 | 11 |
| hsa04380 | Osteoclast differentiation | 7.81 | 10 |
| hsa04926 | Relaxin signaling pathway | 7.78 | 10 |
| hsa05152 | Tuberculosis | 7.66 | 11 |
| hsa04657 | IL-17 signaling pathway | 7.62 | 9 |
| hsa05143 | African trypanosomiasis | 7.51 | 7 |
| hsa01522 | Endocrine resistance | 7.48 | 9 |
| hsa05321 | Inflammatory bowel disease | 7.42 | 8 |
| hsa05167 | Kaposi sarcoma-associated herpesvirus infection | 7.35 | 11 |
| hsa05142 | Chagas disease | 7.34 | 9 |

Meanwhile, the common targets were highly connected with the gene expression regulation, response to drugs, hypoxia, heart development, etc. These findings offer fresh perspectives on the investigation of the molecular mechanism of the action of DHI on CHF.

**Topological characteristics of the herb-component-common targets network**

To further investigate the relevant bioactive components of the common targets, a sub-network, consisting of 48 common targets and corresponding bioactive components, as well as herbs were extracted, as shown in **Figure 5**. As a result, a total of 44 bioactive components were screened out, including 36 of *Salvia miltiorrhiza* Bunge and 9 of *Carthamus tinctorius* L. Luteolin (MOL000006) was the only shared bioactive compound between *Salvia miltiorrhiza* Bunge and *Carthamus tinctorius* L. In addition, it was found that *Carthamus tinctorius* L. had 40 of the common targets while *Salvia miltiorrhiza* Bunge had 31 of the common targets. Twenty-three common targets were shared by *Salvia miltiorrhiza* Bunge and *Carthamus tinctorius* L. Tanshinone IIA (MOL007154), quercetin (MOL000098), and luteolin (MOL00006) were the top three bioactive substances with the highest degree value. Therefore, these components might have more significant roles in mediating the anti-CHF effects of DHI.

**Compound-target docking**

To assess the binding capability between active ingredients and protein targets, molecular docking analysis was conducted using Vina software. The cryptotanshinone from *Salvia miltiorrhiza* Bunge, and quercetin and tanshinone IIA from *Carthamus tinctorius* L. were selected for docking. As a result, the 3D and 2D diagrams of the most stable ligand-protein complex conformations were generated, as shown in **Figure 6**. Table 3 shows the details of binding energy and hydrogen bonding. Generally, a lower docking affinity between protein and ligand indicated a more stable protein-ligand complex would be formed. It was found that the most stable ligand-protein complex was quercetin-MMP9 pair (-9.23 kcal/mol), followed by cryptotanshinone-STAT3 (-8.66 kcal/mol) and tanshinone IIA-EDN1 (-7.57 kcal/mol) pairs. In addition, ubiquitous hydrogen bonding was observed in the ligand-protein conformations, and this might provide additional alterations in the physical and chemical properties of proteins. Molecular docking revealed that quercetin-CASP3 complex had the highest number and pattern of hydrogen bonds, and the hydrogen bond-forming residues were Arg207, Gln161, Arg64, Ser120, Cys163, and Gly122.

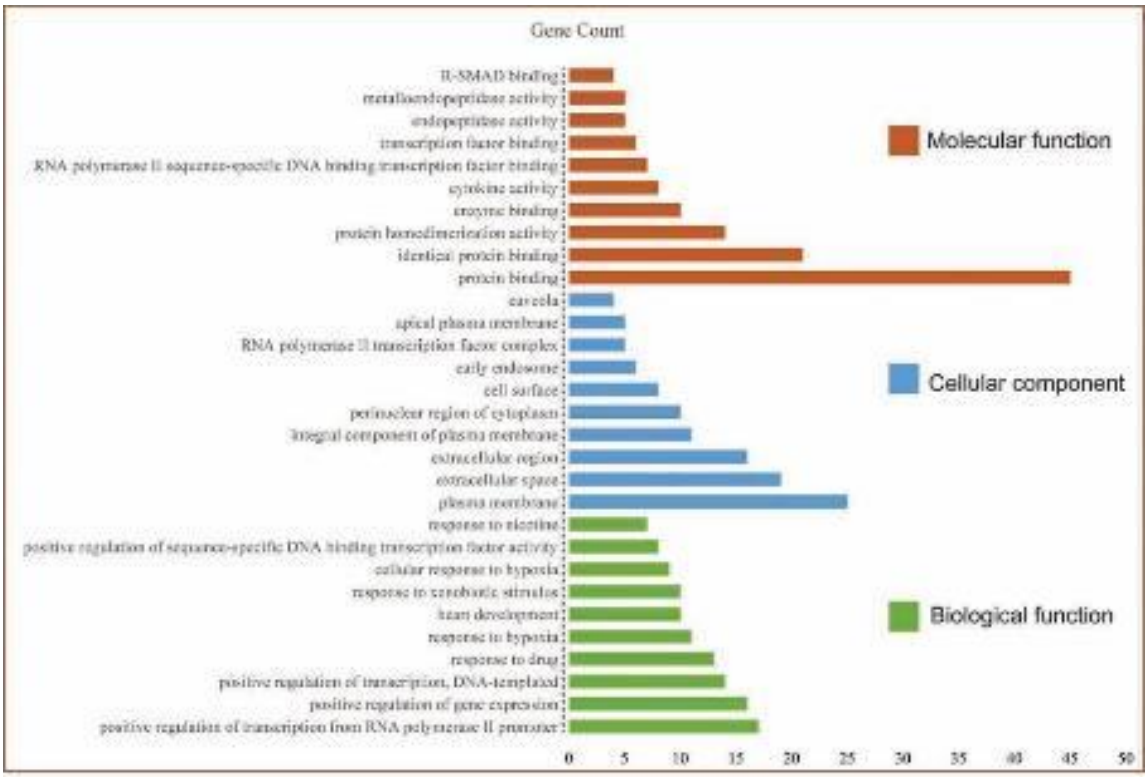


**Figure 4:** GO terms significantly associated with the common targets

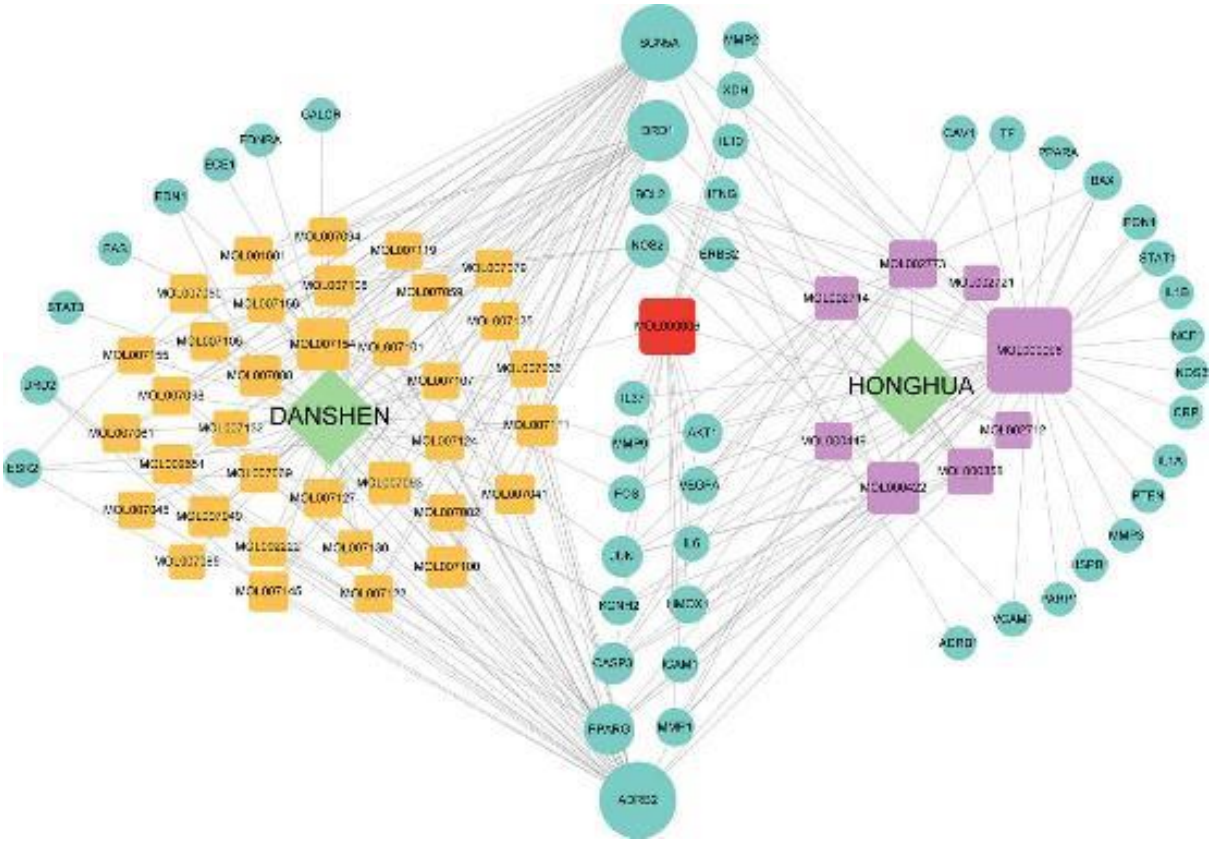


**Figure 5:** Herb-ingredient-common target network of the action of DHI on CHF. The green diamonds represent the herbs, the squares and cycles represent active compounds and target genes. The size of the node represents the degree value, and a bigger node size indicated a higher degree

**Table 3:** Detailed results of molecular docking

| Ligands | Hub gene | PDB ID | H-bonds residues | Docking affinity (kcal/mol) |
|---|---|---|---|---|
| Cryptotanshinone | STAT3 | 6NJS | Lys37, Asp369, Leu438 | -8.66 |
| | AKT1 | 6HHH | Thr87, Arg15 | -7.4 |
| | VEGFA | 6zfl | Glu38, Asn75, Leu97 | -6.86 |
| | JUN | 1-Jun | Arg276, Gly275, Gly274 | -4.51 |
| Quercetin | MMP9 | 4XCT | Ala189, Leu188 | -9.23 |
| | CASP3 | 1nme | Arg207, Gln161, Arg64, Ser120, Cys163, Gly122 | -6.87 |
| | IL1B | 1HIB | Leu80, Leu134 | -6.41 |
| | IL6 | 1IL6 | Phe174, Met68 | -6.16 |
| | CAV1 | 5IJP | Gln73, Arg163 | -5.91 |
| Tanshinone IIA | EDN1 | 6DK5 | Ile19 | -7.57 |

## DISCUSSION

Clinically, DHI administration could significantly ameliorate the clinical symptoms of patients with CHF, and better efficacy can be achieved by the integration of DHI with western medicine, compared to using Western medicine alone [5]. Although the clinical efficacy of DHI in CHF has been confirmed in extensive studies, its specific pharmacological mechanism of action remains to be fully elucidated. Here, an integrated strategy of network pharmacology and other downstream methods were employed to comprehensively determine the underlying mechanism of DHI against CHF. This study data provided insight into the key compounds, hub targets, and pathways that contribute to the anti-CHF effects of DHI.

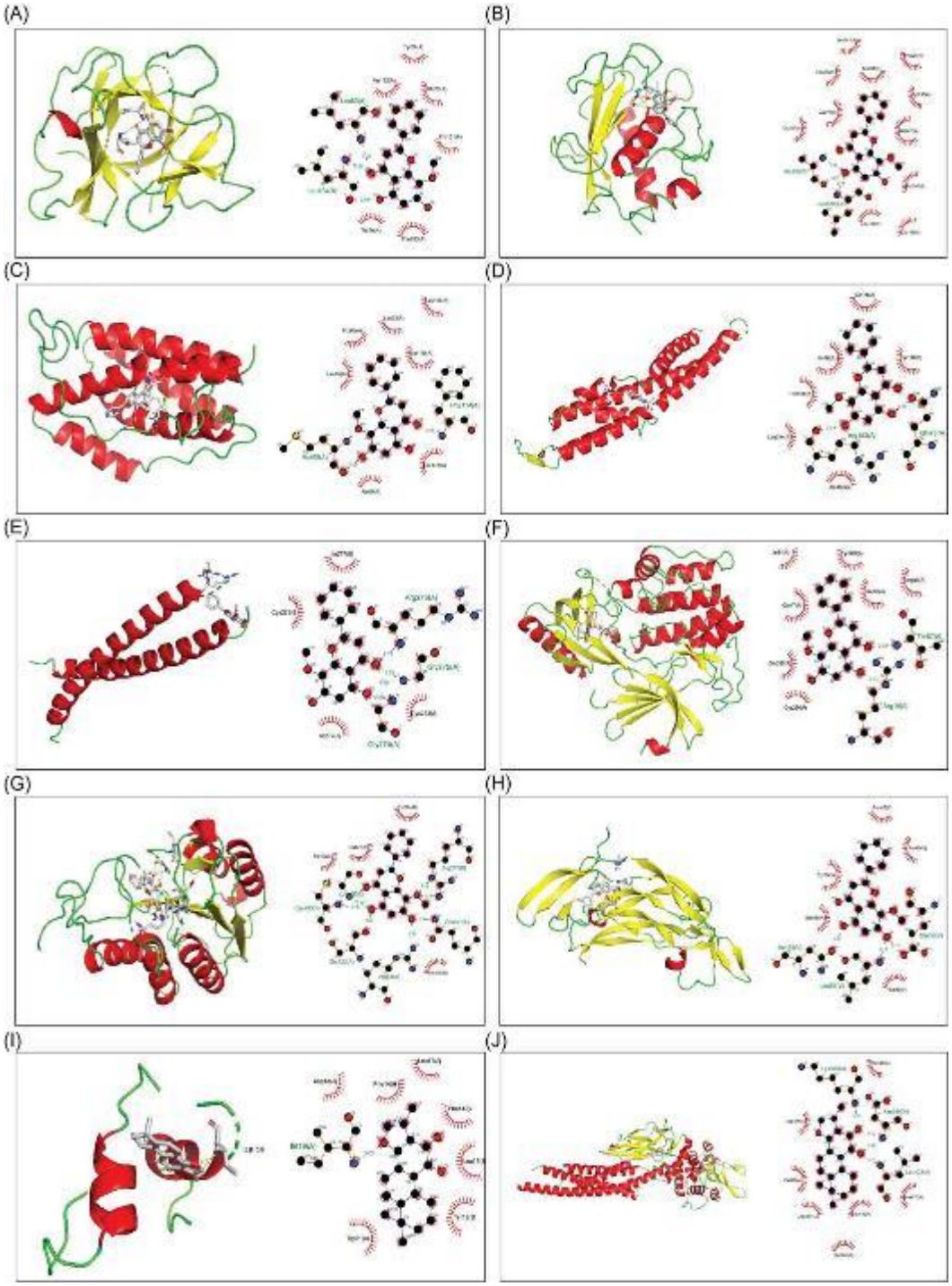


**Figure 6.** The 3D and 2D diagrams of the most stable ligand-protein complex conformations. (A): IL1B-quercetin complex; (B): MMP9-quercetin complex; (C): IL6-quercetin complex; (D): CAV1-quercetin complex; (E): JUN-quercetin complex; (F): AKT1-quercetin complex; (G): CASP3-quercetin complex; (H): VEGFA-quercetin complex; (I): EDN1-tanshinone IIA complex; (J): STAT3-cryptotanshinone complex.

The exploration of bioactive compounds is essential for understanding the pharmacological effects of TCM. Based on the value of drug-likeness, a total of 65 bioactive compounds of DHI which potentially have anti-CHF effects were screened. Herb-ingredient-common target network demonstrated that the four active ingredients: *viz*, quercetin, luteolin, kaempferol, and tanshinone IIA, had the most targets. It has been suggested that these compounds might exert broader cellular regulatory effects and contribute to the therapeutic effects on CHF [7,

8]. Quercetin is a plant pigment that exerts therapeutic effects on cardiovascular diseases. It was found that quercetin reduced the incidence of heart failure and maintain mitochondrial homeostasis [7]. In addition, quercetin increased the expression of nuclear factor erythroid-derived 2 -like 2 (NFR2), a regulator of cellular and impenetrable to oxidants, in order to ameliorate doxorubicin-induced irreversible heart failure [8].

Luteolin is a flavonoid with multiple cardio-protective effects, and has been reported to ameliorate cardiac dysfunction in rats with heart failure by targeting SERCA2a SUMOylation [9]. As a well-known flavonoid, kaempferol has been proven to protect the heart of diabetic rats in isoproterenol-induced heart failure model by regulating Akt/GSK-3β signaling pathway [10]. Moreover, a number of studies have demonstrated that kaempferol prevents cardiac remodeling, and is involved in the pathogenesis of CHF. Tanshinone IIA is the main effective chemical constituent extracted from *Salvia miltiorrhiza,* and protects against heart failure in multiple ways such as preventing cardiomyocyte apoptosis, reducing the inducibility of atrial fibrillation, and alleviating ventricular remodeling. However, in order to fully understand the effective components of DHI with anti-CHF activity, further identification and metabolomic analysis of blood components exposed to DHI are required.

Our data showed that multiple targets, such as AKT1, STAT3, IL6, IL1B, and MMP9, might be pivotal in the action of DHI against CHF. For example, Kapustian *et al* revealed that Akt1 activity was dynamically controlled as heart failure progressed [11]. In addition, Akt1 mediates the attenuating effect of Apelin-13 on cardiac fibrosis in rats with heart failure. IL6/STAT3 signaling was activated in heart failure and it was reported to mediate the effects of miR-320 on accelerating CHF with cardiac fibrosis [12]. These findings suggest that IL6/STAT3 axis might be a participant in the pathogenesis of heart failure, and could serve as candidate targets for the drug development in CHF. The IL-1 cytokines play a crucial pathogenetic role in the progression of heart failure. Recently, Pascual-Figal *et al* found a close correlation was found between IL-1β concentrations and the 1-year mortality of CHF patients [13]. MMP9 is elevated after myocardial infarction and could exacerbate ischemia-induced CHF [14]. Although a number of targets were identified here, a precise assessment of the target expression at the gene or protein level should be conducted to investigate the synergistic effects of various bioactive compounds in DHI.

A variety of cellular phenotypes and functions are often mediated by numerous pathways and signals. To completely understand the pharmacological mechanism of DHI against CHF, it is therefore imperative to investigate any potential pathways or signals implicated in the treatment of CHF by DHI. In this study, it was discovered that the candidate targets against CHF were significantly enriched in a number of signaling pathways, including the TNF signaling pathway, the IL-17 signaling pathway, the relaxin signaling pathway, and the HIF-1 signaling pathway. These signaling pathways may be the means by which DHI exerts its anti-CHF effects. Cellular responses to hypoxia are mediated by hypoxia signaling pathways, one of which is HIF-1 signaling. The intracellular metabolic and inflammatory response processes, both of which have a profound impact on heart function, depend on the HIF-1 signaling pathway. Increasing evidence suggested that the HIF-1 signaling pathway could mediate a protective effect of drugs against heart failure [15]. TNF-α, a cytokine that promotes inflammation, has been extensively implicated in the pathogenesis of CHF. Despite the unfavorable clinical effects of TNF-α blockade in heart failure, Dittrich *et al* recently proposed a novel cardioprotective approach by targeting TNF-α converting enzyme to prevent the cleavage of the transmembrane TNF-α [16].

Relaxin is a pregnancy hormone that exerts cardioprotective effects during pathological events such as heart failure and myocardial infarction. According to clinical studies, relaxin's vasodilator properties make it a promising therapy option for those with heart failure [17]. IL-17 could regulate cardiac ventricular remodeling and cardiac fibrosis in heart failure and overexpression of IL-17 was involved in the pathogenesis of heart failure in mice [18]. On the basis of the present findings, further research is required to link the regulatory effects of DHI on these pathways to anti-CHF phenotype.
Above all, through the integrated approach of network pharmacology, bioinformatics analysis, and molecular docking, the bioactive compounds, potential targets, and pathways of DHI in the action of anti-CHF were fully elucidated.

**Limitations of the study**

However, this study has some limitations. Firstly, the spectrum of the active ingredients were obtained based on the parameter of drug-

likeness, which does not reflect the types and contents of the drug in real situations. Secondly, the targets and pathways associated with the anti-CHF effects of DHI were not validated by biological experiments.

## CONCLUSION

In this study, network pharmacology was used to identify the active substances as well as the anti-CHF prospective targets of DHI. The identified targets are associated with a variety of biological functions and KEGG pathways, offering fresh insights into the molecular mechanism of the action of DHI on CHF. These findings, however, still need to be confirmed by *in vivo* and i*n vitro* experiments.

## DECLARATIONS

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### *Funding*

None provided.

### *Ethical approval*

None provided.

### *Availability of data and materials*

The datasets used and/or analyzed during the current study are available from the corresponding author on reasonable request.

### *Conflict of Interest*

No conflict of interest associated with this work.

### *Contribution of Authors*

The authors declare that this work was done by the authors named in this article and all liabilities pertaining to claims relating to the content of this article will be borne by them.



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