

**Original Article**



# Study on the Mechanism of Herbal Medicines in Treating Myocardial Fibrosis Based on Data Mining and Network Pharmacology

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## Abstract:

**Objective:** Currently, myocardial fibrosis (MF) lacks effective pharmaceutical treatments, leading to a surge in patients seeking herbal remedies as supplementary and alternative options. However, the potential molecular mechanisms through which these herbal medicines exert their effects remain unclear and require systematic exploration. This study proposes a comprehensive method for systematically investigating the mechanism of herbal medicines in treating MF.

**Methods:** First, a comprehensive approach combining bibliometric analysis, pharmacokinetic evaluation, target prediction, and network construction was used to obtain MF-related herbal medicines, their corresponding candidate compounds, and potential targets. Subsequently, a bioinformatics analysis method was employed to utilize the overlapping genes between differentially expressed genes (DEGs) in MF patients and the target genes of MF-related herbal medicines. Additionally, the optimal herbal medicine combination with anti-MF properties was identified through association rule analysis.

**Results:** From ten herbal medicines targeting anti-MF, a total of 278 potential components and their corresponding 163 targets were identified. 19 overlapping genes were found between DEGs in myocardial fibrosis and the target genes of MF-related herbal medicines. Enrichment analysis revealed that the major signaling pathways involved were PI3K-Akt, MAPK, and PPAR. Sanqi (Radix Notoginseng) and Danshen (Radix Salviae Miltiorrhizae) were identified as the optimal combination for combating myocardial fibrosis.

**Conclusion:** This study provides insights into the mechanisms of MF treatment with herbal medicines at both the molecular and pathway levels. It identifies the optimal combination of herbs, offering new directions for developing new drugs treatment for complex diseases. The findings underscore the potential of integrating herbal medicines into therapeutic strategies for myocardial fibrosis and highlight the need for further research to validate these results and explore their clinical applications.

**Keywords:** Myocardial fibrosis; Mechanism; Herbal medicines; Traditional Chinese Medicine

## 1. Introduction

Myocardial fibrosis (MF) was characterized by the excessive accumulation of scar tissue due to an imbalance between the production and degradation of the extracellular matrix (ECM), was closely associated with cardiac and endocrine diseases<sup>[1]</sup>. MF can cause ventricular stiffness, reduced compliance, weakened contractility, and

conduction abnormalities. It has been verified to result in heart failure, myocardial infarction, hypertrophic cardiomyopathy<sup>[2]</sup>, and even severe cardiac insufficiency or sudden cardiac death<sup>[3]</sup>. Currently, there are no specific anti-MF drugs available for clinical practice. Most of the current medications used to address myocardial fibrosis were heart failure treatments, including

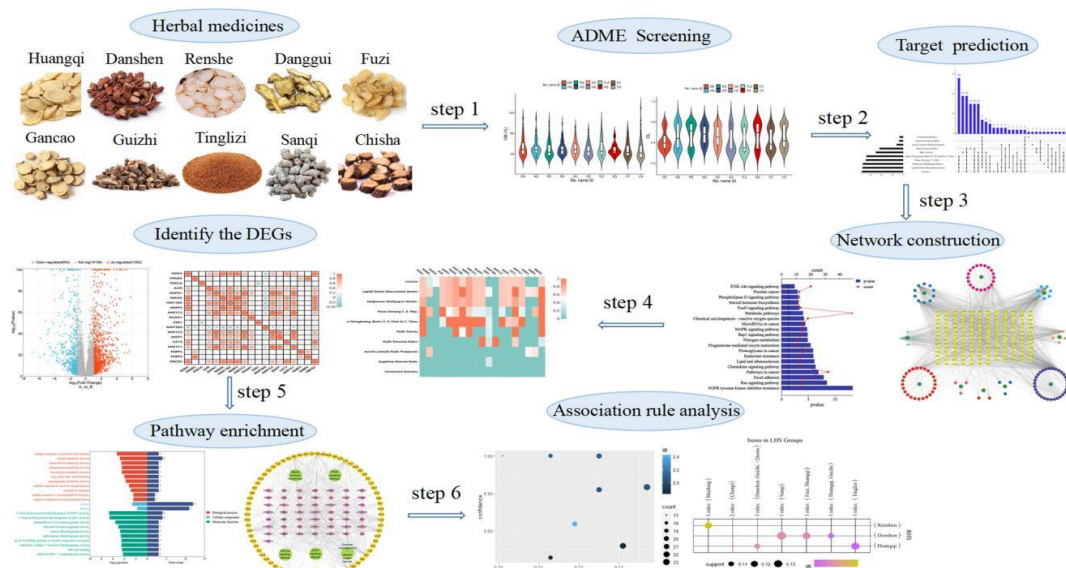
angiotensin receptor blockers (ARBs), diuretics, beta-blockers, and drugs that counteract aldosterone<sup>[4-5]</sup>. However, these drugs often have various unpleasant side effects such as nausea, insomnia, and skin rash, which can affect their clinical use. Therefore, more and more people are seeking complementary and alternative medical therapies.

Traditional herbal medicine is a comprehensive medical system with several thousand years of clinical experience<sup>[6-7]</sup>. Nowadays, an increasing number of patients with myocardial fibrosis are turning to herbal medicines for treatment. However, herbal medicines are a complex system that often contains dozens or even hundreds of structurally different chemical components. The multi-target and multi-biological pathways also make it extremely difficult to analyze the molecular mechanisms of herbal medicines. Relying solely on experimental methods makes it challenging to systematically and scientifically assess the mechanisms of Traditional Chinese Medicine (TCM). Consequently, the advancement and utilization of computational technology were essential for overcoming these hurdles.

To systematically study the mechanism of herbal medicines in treating myocardial fibrosis, a

comprehensive method was proposed. This method includes knowledge graph construction, literature mining, pharmacokinetic evaluation, multi target identification, bioinformatics analysis, and association rule analysis. First, the 10 most commonly used herbal medicines for treating myocardial fibrosis and their corresponding active ingredients were identified through bibliometric analysis and text mining. Second, a computer-based pharmacokinetic model system was used to screen for potential active compounds in the anti-MF herbs, and the targets of these potential active ingredients were identified. Third, overlapping genes were identified between the differentially expressed genes (DEGs) in patients with myocardial fibrosis and the target genes of herbal medicines used for treating myocardial fibrosis.

Fourth, a comprehensive analysis identified key signaling pathways, including PI3K-Akt, MAPK, PPAR, and Steroid hormone biosynthesis pathways, indicating that herbal remedies exhibit therapeutic effects for treating myocardial fibrosis by acting on target genes within these pathways. Finally, association rule analysis was used to extract the optimal combination of herbal medicines for anti-MF. Figure 1 shows the framework of this study.



**Figure 1. The workflow of this study**

## Material and methods

### Data mining

### Data Sources

To extract relevant herbal medicines for

myocardial fibrosis, bibliometric analysis was conducted using the Web of Science and Pubmed databases. The keywords "myocardial fibrosis" and "herbal medicine" or "traditional Chinese medicine" were used to collect relevant literature from the establishment of the database to January

2025.

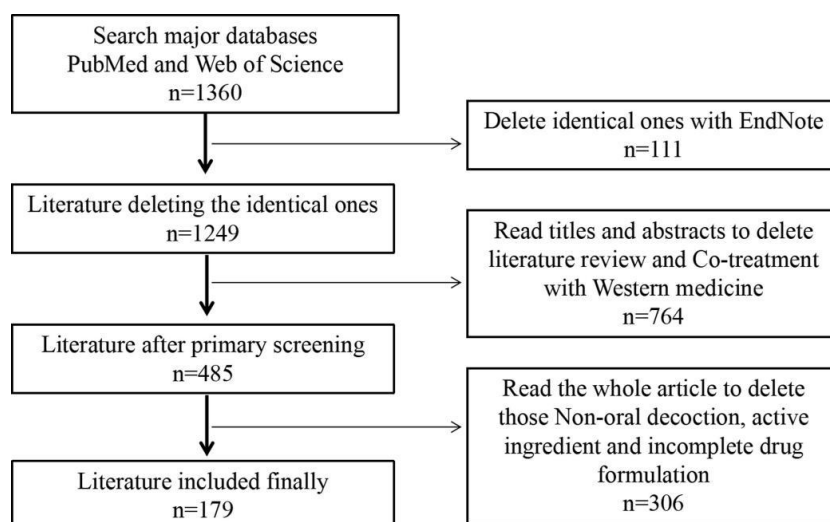
### Extraction of Effective Data

Inclusion criteria: (a) Diagnosed with "myocardial fibrosis"; (b) The full name of the herbal medicine appears in the prescription; (c) TCM treatment shows clear efficacy.

Exclusion criteria: (a) Non-clinical or animal studies, such as review articles; (b) Non-oral decoction treatments including acupuncture, nursing, local herbal application, and injection; (c) Addition or reduction of drugs or incomplete drug ingredients; (d) Co-treatment with Western

medicine.

Based on the inclusion and exclusion criteria outlined previously, a total of 211 articles were selected for inclusion. The approach utilized for conducting the literature search is illustrated in Figure 2. In our research, the herbal medicine names were standardized in accordance with the Pharmacopoeia of the People's Republic of China (published by the National Pharmacopoeia Commission in 2020) and the Chinese Materia Medica (edited by the Editorial Board of Chinese Materia in 1999).



**Figure 2** The flow chart of literature retrieval

### Data Preparation of Herbal Medicines

#### Collection of Active Compounds

The chemical ingredients in herbal medicines were sourced from the TCMSP (Traditional Chinese medicine systems pharmacology database and analysis platform, <https://therbsp-e.com/>) and the HERB database (a high through put experiment-and reference-guided database of traditional Chinese medicine, <http://herb.ac.cn/>). The structural drawings of these compounds in Canonical SMILES format were collected from the PubChem database (<https://testpubchem.ncbi.nlm.nih.gov/>).

Early assessment of absorption, distribution, metabolism, excretion, and toxicity (ADMET) of compounds in the discovery stage can significantly reduce the failure rate related to pharmacokinetics in clinical stages<sup>[8-9]</sup>. Therefore, we utilized two computational models, prediction of oral bioavailability (PreOB) and prediction of

drug-likeness (PreDL), to evaluate the pharmacokinetic properties of the collected compounds. This approach enabled us to screen for active ingredients in herbal medicines for the treatment of myocardial fibrosis.

Oral bioavailability (OB) is a critical pharmacokinetic parameter in the ADME process, indicating the efficiency of oral drug absorption into the systemic circulation<sup>[10]</sup>. Due to the lack of appropriate pharmacological properties, particularly in terms of OB, many components in traditional Chinese medicine fail to reach specific protein target sites within cells<sup>[11]</sup>. Therefore, in this study, a reliable computational model was used to predict the components of herbal medicines related to myocardial fibrosis<sup>[12]</sup>. The formula was shown in (1).

$$f(x) = \sum_{i=1}^n (\alpha_i - \alpha_i^*) K(x, x_i) + b \quad (1)$$

Where  $\alpha_i$  and  $\alpha_i^*$  are the Lagrange multipliers,  $b$  is the regression parameter  $K$  and  $(x, x_i)$  is the kernel

function, which uses the radial basis function, as shown in formula (2).

$$K(x, x_i) = \exp\left(-\frac{\|x-x_i\|^2}{r^2}\right) \quad (2)$$

Where  $\gamma$  is the nuclear parameter.

Drug-likeness (DL) represents the similarity between a compound's structure and known drug structures<sup>[13]</sup>. Evaluating DL in the early stages of drug development helps screen for promising compounds and increases the hit rate of candidate drugs<sup>[14]</sup>. Currently, the PreDL model is used to calculate the DL index based on the following formula.

$$F(x, y) = \frac{x*y}{(|x|^2+|y|^2-x*y)} \quad (3)$$

Where  $x$  is the descriptors of the herbal ingredients associated with MF, and  $y$  denotes the mean of the total molecular descriptors of all drugs in the Drugbank database.

Therefore, the screening thresholds for the two models were set as  $OB \geq 30\%$  and  $DL \geq 0.18$ . Compounds in herbal remedies that meet both of these screening criteria are selected as active molecules for further research.

### Collection of Herb Targets

We employed TargetNet, SwissTarget Prediction, and the SysDT model (based on Support Vector Machine, SVM, and Random Forest, RF) to forecast potential targets. In the SysDT method, compound-target interactions meeting both  $PF \geq 0.7$  and  $SVM \geq 0.8$  criteria were considered valid targets. Furthermore, simplified molecular linear input of compounds was utilized in SwissTarget Prediction and TargetNet databases to enhance retrieval of target gene information for active ingredients. Subsequently, targets were mapped to the Therapeutic Target Database (TTD, <http://database.idrb.cqu.edu.cn/TTD/>) to identify targets associated with their respective diseases.

### Network Construction and Analysis

The compound-target (C-T) network was constructed using active ingredients and potential targets. The networks were established and analyzed using Cytoscape 3.7.2<sup>[15]</sup>. In these networks, nodes represented components, targets, or signaling pathways, while edges depicted relationship between nodes. Two key topological parameters "degree" and "betweenness" were calculated using the Network Analyzer and

CentiScaPe 1.2 plugins within Cytoscape.

### GO/KEGG Function Enrichment Analysis

To elucidate the significantly enriched biological processes and molecular functions of the obtained gene set, gene ontology (GO) analysis and Kyoto Encyclopedia of Genes and Genomes (KEGG) pathway enrichment analysis were performed using the R package clusterProfiler<sup>[16]</sup>. GO analysis systematically described biological processes, while KEGG analysis highlighted the importance of various signaling pathways within protein interaction networks, elucidating the systemic effects of compounds in herbal medicines for disease treatment. Visualization of GO terms and KEGG pathways, along with assembly of pathways related to MF, was performed using the ggplot2 package.

### Identification Of Differentially Expressed Genes In MF Patients

To ascertain differentially expressed genes (DEGs) in myocardial fibrosis patients, we downloaded gene expression profiles from the Gene Expression Omnibus (GEO) database, specifically for the dataset GSE225336<sup>[17]</sup>. DEG analysis was performed using the Limma package in R version 4.2.0, and the cutoff criteria for selecting DEGs were set as a false discovery rate (FDR)  $< 0.05$  and  $|\log_2\text{-fold change (FC)}| > 1$ .

### Association Rule Analysis

Based on the top ten frequently prescribed herbal medicines for the prevention and treatment of MF, we analyzed the association relationship between 168 anti-MF herbal formulas, the association relationships among the core herbal medicines, and the best herb group in association rules according to the optimal combination of medicinal types.

Association rule learning algorithm was the most commonly used method for identifying and analyzing relationships in transactional data<sup>[18-19]</sup>. Many studies have utilized association rule learning algorithms to uncover hidden relationships in the medical field<sup>[20-21]</sup>. The Apriori algorithm, a commonly used data mining method for association rule analysis, identifies co-occurrence patterns rather than causal relationships. It has been applied in various medical research contexts to predict variable features<sup>[22-23]</sup>.

Support, confidence, and lift were the main metrics used to evaluate association relationships in the context of the Apriori algorithm. Support was a measure used to evaluate the usefulness of association rules and represents the proportion of prescriptions that contain the combination of herbal medicines A and B out of the total number of prescriptions. Confidence represents the proportion of prescriptions that contain herbal medicine B when herbal medicine A was already present, indicating the ratio of prescriptions where both herbal medicines A and B co-occur to the total number of prescriptions containing herbal medicine A. Lift reflected the correlation between herbal medicines A and B in association rules. A lift value exceeding 1 signifies a stronger positive relationship, whereas a lift value below 1 indicates a stronger negative relationship. A lift value of 1 indicated no correlation. In this study, association rule analysis was performed on 168 anti-MF herbal formulas, setting minimum thresholds of  $\geq 10\%$  for support and  $\geq 80\%$  for confidence<sup>[24]</sup>.

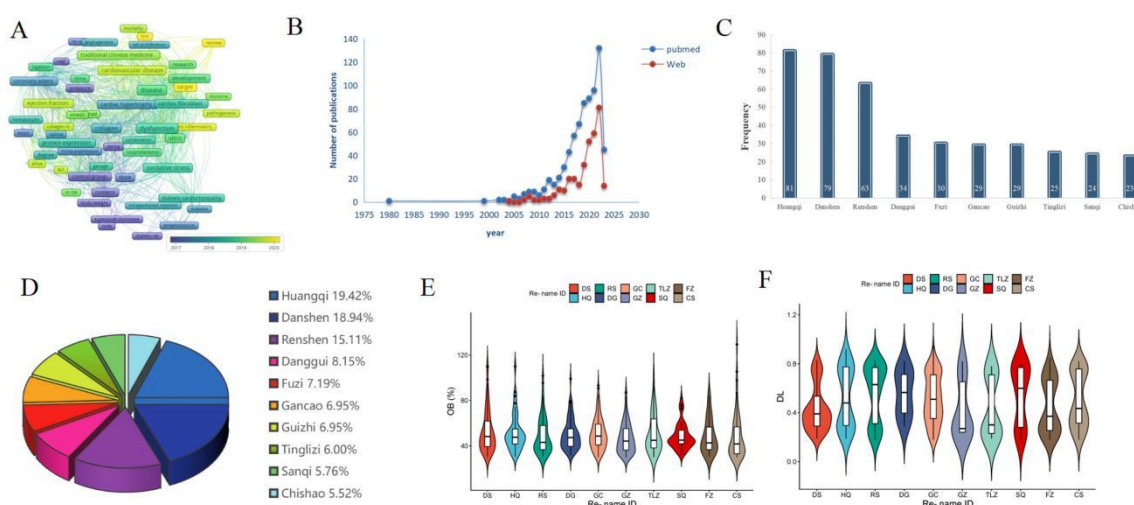
## Results

### ADME Screening

The bibliometric analysis revealed a significant association between myocardial fibrosis and cardiovascular diseases, with a noticeable increase in publications related to traditional Chinese medicine (TCM) for its prevention and treatment (Figure 3A and 3B). Based on literature mining and prescription data analysis, 161 herbal medicines were identified for preventing and treating myocardial fibrosis. Among these, Huangqi (*Radix Astragali Mongolici*), Danshen

(*Radix Salviae Miltiorrhizae*), Renshen (*Radix Ginseng*), Gancao (*Radix Glycyrrhizae*), Guizhi (*Ramulus Cinnamomi*), Tinglizi (*Semen Lepidii Apetali*), Sanqi (*Radix Notoginseng*), Fuzi (*Radix Aconiti Lateralis Preparata*), Danggui (*Radix Angelica sinensis*), and Chishao (*Radix Paeoniae Rubra*) were identified as the top ten frequently used herbal medicines for myocardial fibrosis treatment (Figure 3C and Figure 3D).

The PreOB pre-screening model was applied to calculate the OB values of the components of these herbs, resulting in 736 compounds meeting the query requirement ( $OB \geq 30\%$ ), a substantial proportion among all herbs analyzed (Figure 3E). For instance, tanshinone IIA, derived from Danshen, is widely recognized in herbal medicine for inhibiting myocardial fibrosis through the TGF- $\beta 1$ /Smad2/3 signaling pathway, alongside anti-inflammatory and antioxidant effects<sup>[25-26]</sup>. Additionally, using the PreDL model, 682 candidate compounds with satisfactory DL values were identified from the selected herbal medicines (Figure 3F). Many of these compounds have been reported in the literature for their anti-MF activity. For example, 20(S)-ginsenoside Rg3 can alleviate myocardial fibrosis by increasing ACY1 expression and inhibiting the TGF- $\beta 1$ /Smad3 pathway<sup>[27]</sup>. The effects of the main active components of the selected herbs on H9c2 cell viability were shown in Supplementary Material 1. Ultimately, after simultaneous screening using both models, a total of 278 chemical substances meeting the criteria of  $OB \geq 30\%$  and  $DL \geq 0.18$  were identified, demonstrating favorable pharmacokinetic properties.



**Figure 3.** (A) Keywords' network of literature about herbal medicines for treating MF. (B) Number of publications on the treatment of MF with related herbal medicines. (C) The top 10 herbal medicines

for treating MF. (D) The percentage of top ten herbal medicines in the treatment of MF. (E, F) Violin diagram of OB and DL content.

### Target Identification

Through a multi-target prediction approach, a total of 163 potential targets were identified for the 278 candidate compounds. The distribution of gene targets across various herbs is illustrated in Figure 4A. The results reveal that a majority of active chemical substances target multiple proteins, with most targets associated with varying numbers of compounds, highlighting the synergistic effects of herbal ingredients and the multi-component nature of traditional Chinese medicine (Figure 4B). For instance, quercetin targets 93 proteins, and resveratrol targets 90 proteins. Moreover, tannic acid acts functions not only as an EGFR inhibitor but also as a MAPK antagonist<sup>[28-29]</sup>.

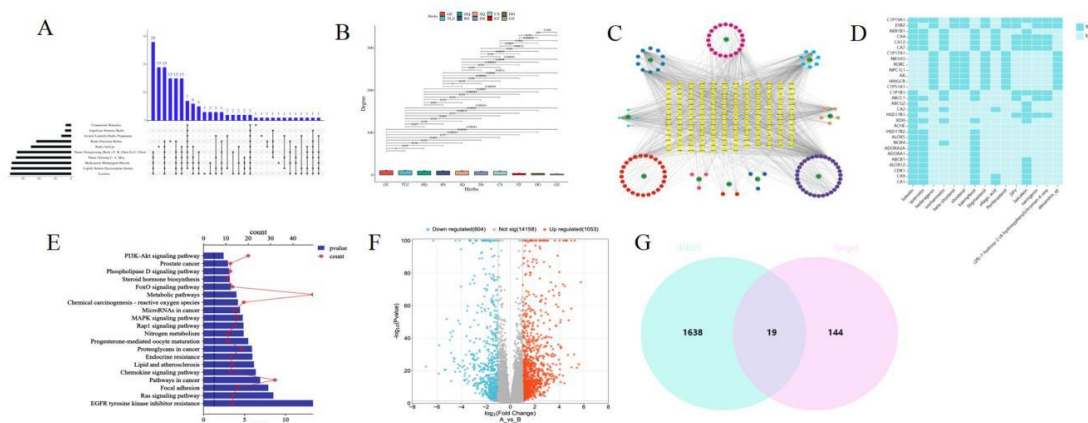
### Network Construction and Analysis

In recent years, network pharmacology has emerged as a leading and focal area in traditional Chinese medicine research, with network construction playing a pivotal role in such studies. Typically, herbal medicines exert their therapeutic effects by targeting specific protein targets, and the drug response (including therapeutic effects and side effects) was influenced by the topological properties of the network. Therefore, we employed network pharmacology to explore how multi-component therapeutic systems, such as the ten aforementioned herbal medicines, exert their therapeutic effects on MF.

Figure 4C illustrates the compound-target (C-T) network constructed using 278 active ingredients from these herbal medicines and their

corresponding 163 target proteins. In this network, active ingredients and their targets were represented by hexagons and diamonds, respectively. A key quantitative indicator in the C-T network was the degree, which represented the count of edges attached to each node. Network analysis revealed that among the active ingredients, quercetin (GC1/HQ2) exhibited the highest degree (degree=94), demonstrating the multi-target characteristics of compounds in traditional Chinese medicine. Additionally, network analysis focused on the top 30 targets and active ingredients based on their degree in the C-T network, revealing their interrelationships (Figure 4D).

Furthermore, KEGG functional enrichment analysis of the 163 target proteins identified significant enrichment in pathways such as the EGFR signaling pathway, MAPK signaling pathway, PI3K-AKT signaling pathway, and some cancer-related pathways (Figure 4E). These pathways, particularly EGFR, MAPK, and PI3K/AKT, play crucial roles in MF by reducing the pathological accumulation of ECM. Cancer-related pathways suggest potential involvement in cell death pathways implicated in clinical myocardial fibrosis development. For instance, research by Zheng Ming's team highlighted programmed cell death 5 as a apoptosis-accelerating protein, that could regulate the process of cardiac fibrosis by transmitting negative feedback<sup>[30]</sup>. Gaining insight into how these signaling pathways contribute to MF could lead to the identification of effective preventive targets for the condition.



**Figure 4.** (A) Upset Wayne analysis for predicting common targets. (B) The degree value of target

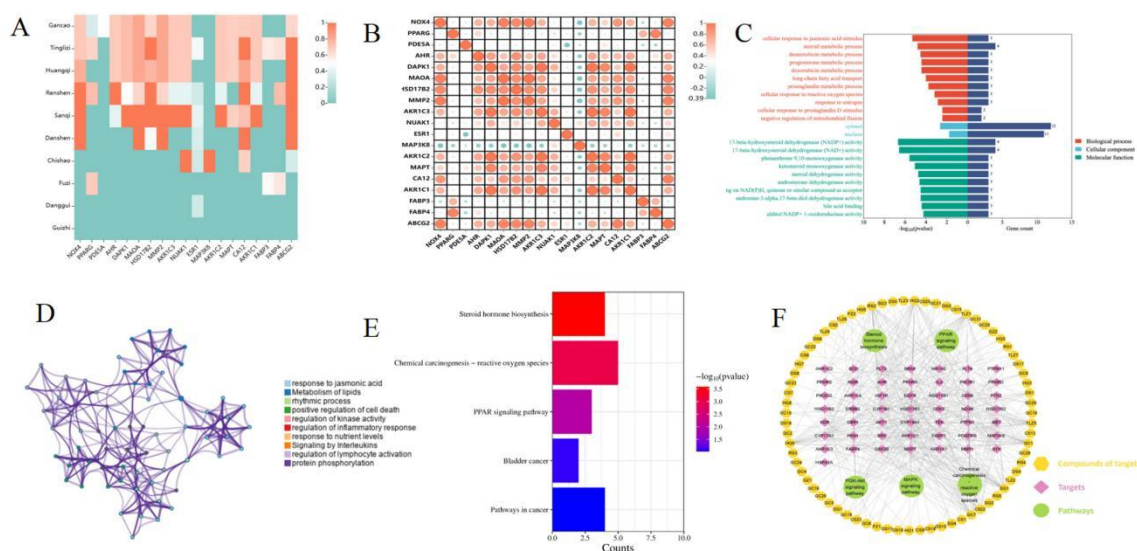
genes in herbal medicines treatment of MF. (C) The C-T network. Hexagons and diamonds represent the chemicals from ten herbal medicines and all their potential targets, respectively. (D) The heatmap of C-T interaction analysis. (E) The top 20 pathways for herbal medicines treatment of MF. (F) The volcano plots for the 1657 DEGs in the GSE225336 dataset. (G) The overlapped genes between DEGs in MF and the target genes of the MF-related herbs.

### Identification of MF DEGs

To find the intersection of DEGs in MF and target genes of the MF-related herbal medicines, we first identified 1657 DEGs (1053 up-regulated and 604 down-regulated) in GSE225336 (Figure 4F). Subsequently, a Venn diagram analysis revealed 19 overlapping genes between these DEGs and the target genes of the MF-related herbs (Figure 4G). The correlation heatmap of these 19 overlapping target genes with herbal associations is depicted in Figure 5A, highlighting Tinglizi and Sanqi as having high correlations with these overlapping target genes, suggesting their pronounced effects on MF. Furthermore, based on the topological overlap matrix, a heatmap illustrating the correlation between these overlapping genes is shown in Figure 5B, with darker colors indicating stronger gene interactions.

Figures 5C and 5D present the GO functional enrichment analysis results of the 19 overlapping target genes, revealing their involvement in key

biological processes associated with MF. For instance, recent research has demonstrated that the estrogen receptor G protein-coupled receptor 30-specific agonist G-1 reduces myocardial fibrosis and inhibits cardiac fibroblast proliferation in animal models of myocardial infarction<sup>[31]</sup>. Moreover, KEGG functional enrichment analysis of these 19 overlapping target genes indicated significant enrichment in pathways such as Steroid hormone biosynthesis, Chemical carcinogenesis - reactive oxygen species, and PPAR-related signaling pathway (Figure 5E). Integrating this analysis with KEGG pathways related to potential targets of the ten herbal medicines synthesized five major biological pathways relevant to MF. Using these targets and pathways, the Compound-Target-Pathway (C-T-P) network was constructed (Figure 5F), where yellow hexagons represent compounds, purple diamonds represent targets, and green circles denote pathways. Understanding the roles of these signaling pathways in MF pathogenesis may reveal effective targets for MF prevention.



**Figure 5. (A) The correlation between overlapped genes and herbs. (B) A heatmap of the correlation of overlapped genes. (C) GO enrichment of overlapped genes. (D) Visualized functional enrichment and gene interaction analysis results. (E) The KEGG functional enrichment analysis of the overlapping 19 target genes. (F) The C-T-P network. Yellow hexagons, purple diamonds, and green circles represent compounds, targets, and pathways, respectively.**

## Screening Core Combination of Herbal Medicines for Anti-MF

Combination herbal therapies offer a wide range of distinct advantages in treating complex diseases. Typically, TCM clinics utilize fixed combinations of two or three herbs, which simplify treatment and clarify herbal effects more effectively than formulations with more herbs. Building on our previous study, we identified 10 association rules from core herbal medicines used for treating MF, summarized in Table 1. To analyze these association rules further, we plotted a scatter plot illustrating the distribution of lift values, with support on the x-axis and confidence on the y-axis (Figure 6A). Notably, all association rules exhibited relatively high lift values, indicating a strong likelihood that the antecedent herb and consequent herb occur together in these

rules compared to when the consequent herb is used alone. A clustering matrix diagram depicted the general distribution of clustered association rules (Figure 6B). The horizontal axis represents 7 clusters, and the vertical axis represents items generated by these clusters (rules). The depth of color within each circle indicates the degree of lift, where darker colors denote higher lift values. Circle size reflects the support degree, with larger circles indicating higher support levels. The herb combinations  $\{\text{Guizhi, Huangqi}\} \geq \{\text{Danshen}\}$ ,  $\{\text{Danshen, Guizhi}\} \geq \{\text{Huangqi}\}$  were interactively selected and they revealed the association rules connecting from antecedent (LHS) to consequent (RHS) item sets. The results of these interactively selected association rules consistently corresponded with association rules #3  $\{\text{Danshen, Fuzi}\} \geq \{\text{Huangqi}\}$  and #4  $\{\text{Fuzi, Huangqi}\} \geq \{\text{Danshen}\}$ , as seen in Table 1.

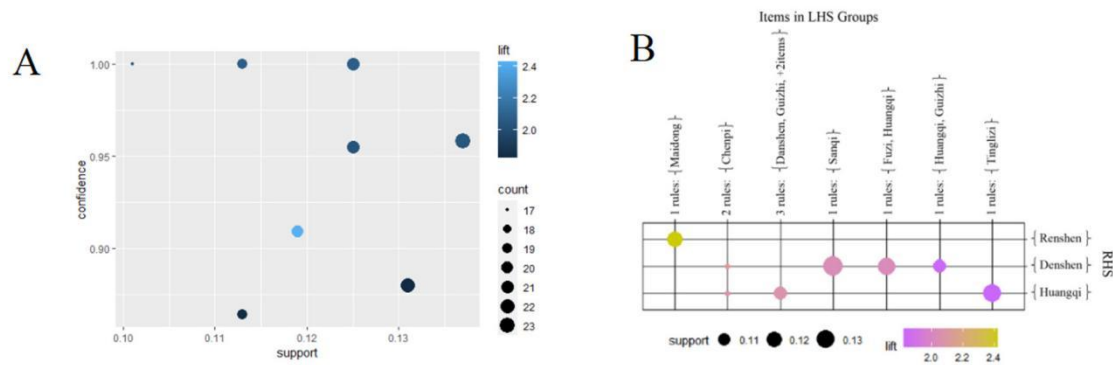


Figure 6. (A) Scatter plot for 10 rules. (B) Grouped matrix for 10 association rules.

Table 1. Association rules of herbal formulas for anti-MF.

No.	Association Rules	Support	Confidence	Lift
1	$\{\text{Sanqi}\} \Rightarrow \{\text{Danshen}\}$	0.137	0.958	2.038
2	$\{\text{Tinglizi}\} \Rightarrow \{\text{Huangqi}\}$	0.131	0.880	1.825
3	$\{\text{Danshen, Fuzi}\} \Rightarrow \{\text{Huangqi}\}$	0.125	1.000	2.074
4	$\{\text{Fuzi, Huangqi}\} \Rightarrow \{\text{Danshen}\}$	0.125	0.955	2.030
5	$\{\text{Maidong}\} \Rightarrow \{\text{Renshen}\}$	0.119	0.909	2.424
6	$\{\text{Danshen, Guizhi}\} \Rightarrow \{\text{Huangqi}\}$	0.113	1.000	2.074
7	$\{\text{Guizhi, Huangqi}\} \Rightarrow \{\text{Danshen}\}$	0.113	0.864	1.837
8	$\{\text{Chenpi}\} \Rightarrow \{\text{Danshen}\}$	0.101	1.000	2.074
9	$\{\text{Chenpi}\} \Rightarrow \{\text{Huangqi}\}$	0.101	1.000	2.074
10	$\{\text{Guizhi, Tinglizi}\} \Rightarrow \{\text{Huangqi}\}$	0.101	1.000	2.074

## Discussion

Myocardial fibrosis, marked by an abnormal accumulation of collagen, elevated collagen protein levels, and a notable rise in collagen volume, was a prevalent pathological feature seen

in various cardiovascular diseases at certain stages of their progression<sup>[32]</sup>. Recent trends indicate that the incidence of MF was continuously rising and showing a tendency to affect younger individuals, with characteristics of rapid onset and high mortality<sup>[33]</sup>. However, effective treatments to

inhibit or reverse MF were currently lacking in clinical practice.

Traditional Chinese Medicine, a cornerstone of medical practices spanning over 5000 years, holds a crucial position in treating human ailments, providing a wider range of therapeutic approaches than previously explored methods. Research has found that TCM holds significant potential as a therapeutic strategy for treating MF, owing to its notable efficacy and low side effects, drawing considerable attention from scholars globally. Therefore, in this study, by using knowledge mapping and literature mining methods, we counted 161 herbal medicines used in the treatment of MF, among which the commonly used ones include Danshen, Renshen, Danggui, and Gancao. Analysis of the top ten herbal medicines revealed 278 potential active ingredients and 163 corresponding target proteins. For instance, Tanshinone IIA, found in Danshen, has been shown to mitigate MF by suppressing MMP-2 and MMP-9 expression, aligning with previous research findings<sup>[34]</sup>. Furthermore, we explored the primary signaling pathways influenced by these herbal medicines in treating MF by identifying intersections between DEGs in MF and target genes of MF-related herbal medicines. Initially, 1657 DEGs were identified in GSE225336, and subsequently, 19 overlapping genes were obtained between the DEGs in MF and the target genes of MF-related herbal medicines. Finally, KEGG analysis highlighted five key biological pathways related to MF, including PI3K-Akt, MAPK, steroid hormone biosynthesis, Chemical carcinogenesis - reactive oxygen species, and PPAR signaling pathways (Figure 5E). Among these pathways, PI3K-Akt signaling emerged prominently, involving 20 out of 50 identified targets. Evidence suggests that the PI3K-Akt pathway contributes significantly to MF improvement by attenuating apoptosis and autophagy while promoting anti-inflammatory effects through increased M2 macrophage expression<sup>[35-36]</sup>. Additionally, the Chemical carcinogenesis - reactive oxygen species (ROS) pathway plays a critical role in oxidative stress-induced fibrosis, during myocardial ischemia, hypoxia, and tissue remodeling, ROS production increases in activated fibroblasts, potentially tightly regulating their phenotype and function and promoting the process of fibrosis<sup>[37]</sup>. In addition to its effects on collagen synthesis, ROS

also critically regulated extracellular matrix (ECM) degradation and remodeling by influencing the synthesis and activity of matrix metalloproteinases (MMPs)<sup>[38]</sup>. MAPK and PPAR signaling pathways also influence MF development<sup>[39-40]</sup>, these findings underscore the importance of these pathways in MF pathogenesis. Given their diverse roles in cell growth, proliferation, apoptosis modulation, and immune activation, we hypothesize that these ten herbal remedies may interfere with these processes, thereby exhibiting regulatory impacts on autophagy, immunotherapy, and anti-inflammatory approaches. The candidate compounds may exert synergistic effects by targeting proteins that mediate interactions and crosstalk between different pathways, indicating that the ten herbal medicines may exert synergistic effects in different pathways<sup>[41]</sup>. Moreover, several target proteins were found to participate in multiple signaling pathways, implying potential interactions that regulate MF progression.

Many studies have reported that herbal combination exhibit greater pharmacological efficacy than a single herbal in the treatment of complex diseases<sup>[42-43]</sup>. Typically, two or three herbs were combined together, which were the frequently fixed clinical forms of TCM. These formulations were more simplified compared to those with a larger number of herbs, and the effects of the herbs were clarified to a greater extent. Building on this premise, we extracted core herbal combinations for MF treatment, analyzing their interrelationships and identifying optimal combinations through association rules. Association rule analysis revealed that the combination of two herbal medicines with the highest support is Danshen and Sanqi. For three herbal medicines, the combination with the highest support is Danshen, Fuzi, and Huangqi. The clinical effectiveness of TCM in treating MF has been well-documented. Based on clinical symptoms and etiology, TCM categorizes MF under domains such as "palpitations," "chest obstruction," and "arrhythmia." The morphological manifestations of myocardial hypertrophy and stiffness due to myocardial fibrosis can also be classified as "micro-hypoplasia"<sup>[44]</sup>. MF was primarily located in the heart and was considered within the domain of the heart chakra<sup>[45]</sup>.

According to TCM, the heart governs the blood vessels, and chronic heart diseases inevitably affected blood circulation. Impaired blood circulation leads to stasis, and since body fluids and blood share a common origin, stasis could transform into phlegm and dampness. This results in a state of mutual entanglement between phlegm and stasis, which, if unresolved, could generate toxins that harm muscles and tissues. Thus, phlegm, stasis, and toxins were key pathological factors in MF, with their interactions forming a vicious cycle that leads to the condition<sup>[46-47]</sup>. Consequently, TCM scholars often approach the treatment of MF by addressing the pathological nature of "blood stasis," "phlegm turbidity," and "toxin resolution." They employ methods such as activating blood stasis, resolving phlegm, lowering turbidity, clearing heat, and detoxifying. This approach aligns with the research findings.

Based on existing literature, herbal remedies for MF have been summarized and categorized as follows: tonifying qi and nourishing yin, as well as enhancing blood circulation to disperse blood stagnation, clearing heat-toxin, inducing diuresis to remove edema, and suppressing hyperactive liver and subsiding yang. Next, take Danshen and Huangqi as examples to illustrate their effects on myocardial fibrosis. Danshen was a medicine that promotes blood circulation and removes blood stasis. It has the effects of nourishing and cooling blood, and it was commonly used in the treatment of cardiovascular diseases. The main components of Danshen were tanshinone I, IIA, and IIB. Tanshinones have pharmacological effects such as dilating coronary arteries, lowering blood lipids, and anti-thrombosis. Among these, tanshinone IIA was the most abundant and structurally representative tanshinone in Danshen<sup>[48]</sup>. Zhang's team found that tanshinone IIA could prevent the formation of cardiac fibrosis by inhibiting the expression of  $\beta$ -microtubulin, thus exerting cardioprotective effects<sup>[49]</sup>. Huangqi belongs to the group of qi tonic medicines. It benefits qi and warms yang, induces diuresis to remove edema, and is commonly used to treat various cardiovascular and respiratory diseases. Its main component, astragaloside, has cardiogenic, immune regulation, and anti-tumor effects. Wei's study demonstrated that astragaloside IV can prevent cardiac fibrosis by acting on the miR-135a-TRPM7 pathway, which subsequently modulates the TGF- $\beta$ /Smads signaling cascade<sup>[50]</sup>.

These examples illustrated the potential of traditional Chinese medicine in the treatment of MF, highlighting the diverse mechanisms through which these herbal medicines exert their therapeutic effects. The combination of herbal medicines can work synergistically, often exhibiting greater efficacy than single herbal treatments. For instance, the combination of Huangqi and Danshen was considered a model for benefiting qi and activating blood circulation. This combination addresses both qi deficiency and blood stasis, replenishing qi, transforming blood stasis, generating new blood, and ensuring smooth blood vessels.

Modern pharmacological studies have shown that the Huangqi Danshen medicinal pair mainly contains components such as astragaloside, astragalus polysaccharides, salvianolic acid, and tanshinones. Numerous studies have found that in the process of MF, this combination and its effective components exert antioxidant and anti-inflammatory effects through multiple components and targets. This action prevents excessive deposition of ECM, delays myocardial fibrosis, and exerts cardioprotective effects. Moreover, the combination of Danshen and Huangqi has been widely used in the clinical treatment of myocardial ischemia and myocardial infarction. Examples of commonly used Chinese patent drugs include QiShenYiQiDiWan and DanQiJiaoNang. These formulations leverage the synergistic effects of Huangqi and Danshen to provide significant therapeutic benefits in managing cardiovascular conditions.

MF is commonly present in cardiovascular diseases, but currently, there are no effective drugs that can reverse it. However, recent research has found that various herbal medicines, active ingredients, or herbal combinations can alleviate MF and improve cardiac function through multiple pathways. This underscores the enormous potential of traditional Chinese medicine in the prevention and treatment of MF, providing abundant ideas for the development of novel drugs. For example, in both in vivo and ex vivo experiments, berberine significantly inhibited MF and the proliferation of cardiac fibroblasts, protecting cardiac function through CDK2-T160 activity dependence<sup>[51]</sup>. TCM could be utilized in treating various types of fibrosis and cardiovascular diseases at different stages, thanks

to its comprehensive approach involving multiple components, targets, and levels.

With a deepening understanding of the mechanisms of MF and ongoing research on the discovery of effective components and mechanisms of action of anti-MF herbal medicines, alongside the enrichment and improvement of TCM theory, the combination of Chinese and Western medicine holds great potential in preventing and treating MF. Recent studies have shown that BMSCs transplantation was a potential method for treating myocardial injury and inhibiting MF, and it has been found that combining TCM intervention significantly enhances its efficacy<sup>[52]</sup>. Traditional Chinese medicine thus holds great therapeutic potential for MF and provides new options for clinical use.

Although remarkable progress has been made in exploring the in-depth mechanisms of TCM against MF, research on the anti-myocardial fibrosis effects of TCM is still in its infancy and faces many challenges. Research on the anti-MF effects of herbal medicines primarily relies on animal experiments. Most of these experiments do not involve syndrome differentiation and treatment, but rather utilize methods such as promoting blood circulation, resolving blood stasis, clearing heat, and detoxifying. Although these methods have demonstrated promising therapeutic effects, they lack comparative effectiveness studies between different treatment methods, thus failing to reflect the characteristic of "Three Categories of Etiological Factors" in TCM. Additionally, there was a notable lack of clinical research, especially high-quality, multicenter, large-scale, and randomized parallel trials. Although some herbal medicines, such as Danshen and Huangqi, have been used in clinical practice, adverse reactions like gastrointestinal discomfort, nausea, and vomiting may occur during application, which could be related to the dosage and duration of the herbal medicines<sup>[53-54]</sup>.

From the perspective of clinical medication, subsequent studies should focus on standardizing and improving trial designs by using rigorous randomized, double-blind, large-scale clinical trials to better verify the exact therapeutic effects of TCM against MF. Additionally, the specific medicinal components and effective sites of some TCM formulas, extracts, and monomers are not clearly defined, and their target points remain

unclear. Therefore, there is still a long way to go to prove the safety and efficacy of TCM anti-MF using scientific and objective methods.

## Conclusion

This study employed a comprehensive approach combining bibliometric analysis, pharmacokinetic screening, target identification, network analysis and bioinformatics analysis to elucidate the mechanisms of herbal medicines in treating MF. Additionally, core combinations of herbal medicines for MF treatment were identified using association rule analysis, offering novel insights into clinical prevention and treatment strategies for MF. Despite conducting a comprehensive systematic analysis, this study still has some limitations. For instance, it is limited to the secondary analysis of existing literature data, and the research findings still need to be validated through clinical and pharmacological studies.

## Data Availability

All data are publicly available, they are included in the article and supplemental materials, and all data we used to support the results of this study can be obtained from the corresponding author upon request.

## Declaration of Interest statement

The authors acknowledge that the content of this article has no conflict of interest.

## Funding Statement

This work was supported by the National Natural Science Foundation of China (No.82274117).

## Ethics Statement

This study did not involve human participants and/or animals.

## Supplementary Material

Supplementary Material 1 shows the effect of the main active ingredients of the selected herbs on the survival of H9c2 cells, indicating that the selected herbs have a certain degree of safety and efficacy.

## Acknowledgments

Zhang Bing designed the study and supervised the whole analysis. Wang Yinfeng acquired the data and performed statistical analysis. Chu Mengzhen drafted the article. Huang Jinjian checked the statistical analysis and substantially revised the

draft. Lin Zhijian read the whole manuscript and revised it. Wang Yu provided some necessary statistical suggestions and improved the English writing. All authors gave final approval of the version to be submitted.

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### Funding Statement

This work was supported by the Basic Scientific Research of Central Universities (2024-JYB-JBZD-054)

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**Abbreviations:** MF, myocardial fibrosis; DEGs, differentially expressed genes; ADMET, absorption, distribution, metabolism, excretion, and toxicity; C-T-P, compound-target-pathway; ECM, extracellular matrix; ARBs, angiotensin receptor blockers; TCM, Traditional Chinese; TCMSP, Traditional Chinese medicine systems pharmacology database and analysis platform Medicine; HERB, a highthroughput experiment-and reference-guided database of traditional Chinese medicine; PreOB, prediction of oral bioavailability; PreDL, prediction of drug-likeness; OB, Oral bioavailability; DL, Drug-likeness; SysDT, Systemsdrugtargeting; SVM, Support Vector Machine; RF, Random Forest; TTD, the Therapeutic Target Database; GO, gene ontology; KEGG, Kyoto Encyclopedia of Genes and Genomes; GEO, the Gene

Expression Omnibus; FDR, false discovery rate; C-T, the compound-target; ROS, reactive oxygen species; ECM, extracellular matrix; MMPs, matrix metalloproteinases;