Antibacterial Mechanism of Common Cnidium Fruit Based on Network Pharmacology

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Abstract [**Objectives**] To investigate the antibacterial mechanism of Common Cnidium Fruit using network pharmacology. [**Methods**] Active components and targets of Common Cnidium Fruit were screened and obtained using the TCMSP database and HIT2.0 database. The collected targets were intersected with antibacterial/bacteriostatic targets obtained from the GeneCards database and OMIM database to identify the antibacterial/bacteriostatic targets of Common Cnidium Fruit. The active component-target network diagram of Common Cnidium Fruit was constructed using Cytoscape software and topological analysis was performed. GO enrichment analysis was performed on the target genes using the DAVID database. [**Results**] Screening yielded 25 active components of Common Cnidium Fruit, corresponding to 77 targets. Analysis identified 25 core antibacterial/bacteriostatic targets for Common Cnidium Fruit. Network analysis indicated that Common Cnidium Fruit may exert antibacterial/bacteriostatic effects through active components such as β-sitosterol, stigmasterol, and xanthoxylin, while activating the body's immune regulatory functions by acting on targets including CASP3, PTGS2, BCL2, JUN, and ESR1. [**Conclusions**] Common Cnidium Fruit may exert antibacterial/bacteriostatic effects through multiple pathways via a mechanism involving multiple components, multiple targets, and multiple pathways.

Key words Common Cnidium Fruit, Bacteriostatic activity, Mechanism of action, Network pharmacology

1 Introduction

Common Cnidium Fruit is the dried ripe fruit of *Cnidium monnieri* L. Cuss. in the Apiaceae family. It is warm in nature, pungent and bitter in taste, and has effects such as drying dampness and dispelling wind, killing parasites, and relieving itching. Clinically, it is primarily applied topically to treat skin diseases[1-2]. The plant is highly adaptable and widely distributed in East China and South Central China, including Heilongjiang, Shandong, Shanxi, Anhui, Zhejiang, and other regions[3]. Modern pharmacological studies have shown that Common Cnidium Fruit contains compounds such as coumarins, chromones, triterpenes, and volatile oils, and possesses pharmacological effects including anti-inflammatory, antibacterial/antipruritic, analgesic, anti-allergic, and immunomodulatory effects[4-5]. It has been reported that extracts of Common Cnidium Fruit have inhibitory effects against *S. aureus*, *E. coli*, *C. albicans*, *L. monocytogenes*, *etc.*[6-7]. However, the material basis and mechanism of action responsible for its antibacterial/bacteriostatic effects after topical application to the human body remain unclear. Therefore, this study utilized network pharmacology methods to explore the potential antibacterial/bacteriostatic mechanism of Common Cnidium Fruit, aiming to provide a theoretical basis and reference for the in-depth development and utilization of Common Cnidium Fruit as a plant-derived bacteriostatic agent.

2 Materials and methods

2.1 Screening of active components and targets Known compounds of Common Cnidium Fruit were collected using the Herbal components' Targets Platform (HIT2.0). Compounds were searched in the Traditional Chinese Medicine Systems Pharmacology Database and Analysis Platform (TCMSP) database. Active components with potential pharmacological activity (Drug-likeness, $DL \geqslant 0.18$) and likely ability to penetrate skin or mucosa (Topological Polar Surface Area, $TPSA \leqslant 140$) were screened, and their corresponding targets were obtained.

2.2 Screening of antibacterial/bacteriostatic targets and effective active components Using "Antibacterial" and "Bacteriostasis" as keywords, related targets were searched in the GeneCards database and the Online Mendelian Inheritance in Man (OMIM) database.

The collected antibacterial/bacteriostatic targets were summarized and deduplicated. They were then imported together with the target information of Common Cnidium Fruit into the Hiplot biomedical data online visualization tool (https://hiplot.cn/) to draw a Venn diagram, screening for potential targets through which Common Cnidium Fruit might exert antibacterial/bacteriostatic effects. Active components of Common Cnidium Fruit obtained in Section **2.1** that had no corresponding antibacterial/bacteriostatic targets were removed.

2.3 Construction and analysis of the antibacterial/bacteriostatic active component-target network Using Cytoscape 3.10.3 software, the effective active components of Common Cnidium Fruit related to antibacterial/bacteriostatic efficacy and their corresponding targets obtained in Section **2.2** were visualized and analyzed, establishing a medicinal plant-active component-target network diagram. Nodes represented the active compounds of Common Cnidium Fruit and their corresponding targets, while edges represented the interaction relationships between them. Topological analysis was performed based on the established network diagram. The importance of nodes within the network was evaluated using node degree value and betweenness centrality.

2.4 Construction and analysis of the antibacterial/bacteriostatic PPI network The antibacterial/bacteriostatic target genes of Common Cnidium Fruit obtained in Section **2.2** were input into the STRING database (https://string-db.org) to establish a pro-

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tein-protein interaction (PPI) network. Single protein nodes without interaction relationships in the network were removed, thereby exploring the pharmacological mechanism of Common Cnidium Fruit at the protein-protein interaction level. The established PPI network information was imported into Cytoscape software, and the PPI network was analyzed using the cytoHubba plugin. The MCC (Maximum Clique Centrality) algorithm was used to analyze the hub targets within the PPI network.

2.5 GO enrichment analysis for antibacterial/bacteriostatic effects The coding gene information for the antibacterial/bacteriostatic efficacy-related targets of Common Cnidium Fruit obtained in Section **2.2** was input into the DAVID database (https://david.ncifcrf.gov). Gene Ontology (GO) analysis was performed using "Homo sapiens" as the analysis species under the condition of $P<0.01$. The output analysis data was visualized using Hiplot to obtain GO terms highly correlated with the antibacterial/bacteriostatic effects produced by Common Cnidium Fruit.

3 Results and analysis

3.1 Active components and targets Based on the screening criteria, a total of 25 active compounds (Table 1) derived from Common Cnidium Fruit and 77 corresponding targets were screened from the TCMSP database and HIT2.0 database.

Table 1 Effective components of Common Cnidium Fruit

No.	Mol ID	Compound name	*DL*	*TPSA*
1	MOL001510	24-Epi-campesterol	0.71	20.23
2	MOL001771	poriferast-5-en-3beta-ol	0.75	20.23
3	MOL001941	Ammidin	0.22	52.58
4	MOL002881	Diosmetin	0.27	100.13
5	MOL002905	Zosimin	0.36	65.74
6	MOL000358	beta-sitosterol	0.75	20.23
7	MOL003584	Xanthoxylin N	0.21	48.67
8	MOL003588	Prangenidin	0.22	63.58
9	MOL003591	Ar-curcumene	92.04	0.65
10	MOL003597	cnidiadin	0.32	65.74
11	MOL003598	9-hydroxy-4-[(7-methoxy-2-oxochromen-8-yl)methyl]furo[3,2-g]chromen-7-one	0.84	103.02
12	MOL003600	cnidimol B	0.26	100.13
13	MOL003603	cnidimol E	0.20	111.13
14	MOL003604	cnidimol F	0.28	100.13
15	MOL003605	(*E*)-2,3-bis(2-keto-7-methoxychromen-8-yl)acrolein	0.71	95.95
16	MOL003606	cniforin A	0.47	92.04
17	MOL003607	cniforin B	0.6	92.04
18	MOL003608	*O*-Acetylcolumbianetin	0.26	65.74
19	MOL003610	Columbin	0.60	85.97
20	MOL003612	Edultin	0.52	92.04
21	MOL003616	Isobutyryl shikonin	0.32	100.90
22	MOL003617	isogosferol	0.25	72.81
23	MOL003619	[(1*R*)-1-(5,8-dihydroxy-1,4-dioxo-2-naphthyl)-4-methyl-pent-3-enyl] 3-methylbutanoate	0.35	100.90
24	MOL003624	O-Isovalerylcolum bianetin	0.36	65.74
25	MOL000449	Stigmasterol	0.76	20.23

3.2 Antibacterial/bacteriostatic targets Using "Antibacterial" and "Bacteriostasis" as keywords, relevant targets were retrieved from the GeneCards database and OMIM database. After summarizing and deduplicating these targets, intersection analysis was performed with the target information of Common Cnidium Fruit, screening out 25 potential targets through which Common Cnidium Fruit might exert antibacterial/bacteriostatic effects (Fig. 1). All the active compounds of Common Cnidium Fruit listed in Table 1 have corresponding antibacterial/bacteriostatic targets.

3.3 Analysis results of the antibacterial/bacteriostatic active component-target network The effective active components of Common Cnidium Fruit related to antibacterial/bacteriostatic efficacy and their corresponding targets were visualized and analyzed using Cytoscape software, establishing a plant-active component-target network diagram (Fig. 2).

Topological analysis results showed that the active component-target network of Common Cnidium Fruit consisted of 51 nodes and 157 edges. As shown in Fig. 2, the outermost circle represents the effective active components of Common Cnidium Fruit, while the central circle represents the target proteins. According to the network topological analysis results, the average degree value of the established network nodes was 6.16, and there were 20 nodes with a degree value greater than the average. The average betweenness centrality value was 0.030 2, and there were 8 nodes with a betweenness centrality greater than the average. Nodes meeting both screening criteria are shown in Table 2.

It can be inferred that beta-sitosterol, stigmasterol, and xanthoxylin N may be the core effective components of Common Cnidium Fruit in exerting antibacterial/bacteriostatic effects; PTGS2, DPP4, PTGS1, and HSP90AA1 may be the core target proteins through which Common Cnidium Fruit exerts antibacterial/bacteriostatic effects.

Studies have found that phytosterols can inhibit the function of bacterial cell surface proteins and induce changes in bacterial membrane composition[8]. Beta-sitosterol is a common phytosterol and exhibits antibacterial activity against *S. aureus*[9]. Stigmasterol is a steroid and a common secondary metabolite in medicinal plants[10]. Stigmasterol exhibits bacteriostatic or bactericidal activity against both Gram-positive and Gram-negative bacteria, and also has inhibitory effects against fungi such as *C. albicans*[8]. According to literature reports, xanthoxylin possesses effective antibacterial, antifungal, and algicidal effects[11]. Based on this, it is hypothesized that Common Cnidium Fruit may exert its antibacterial/bacteriostatic effects through these major active components.

Bacterial infections are associated with inflammation. Prostaglandin-endoperoxide synthase 2 (PTGS2), also known as cyclooxygenase-2 (COX-2), plays a role in regulating relevant immune factors during bacterial pathogen infection in the body by triggering pro-inflammatory immune-related responses[12]. Studies have found that the PTGS2 inhibitor celecoxib can increase bacterial susceptibility to other conventional antimicrobial agents and re-

verse drug resistance[13-14]. Dipeptidyl peptidase-4 (DPP4) is a potential anti-diabetic target, and the DPP4 inhibitor sitagliptin can be used to treat type II diabetes[15]. Recent studies have found that sitagliptin can inhibit the bacterial virulence of *S. marcescens*, a clinically common opportunistic pathogen. According to research reports, active compounds extracted from camellia seed oil were found to effectively bind with the core antibacterial target HSP90AA1 through molecular docking calculations, and also demonstrated excellent bacteriostatic effects in antibacterial experiments[16]. That is, the active components in Common Cnidium Fruit may act on these targets, activate the body's immune regulatory functions, inhibit bacterial activity, and thereby assist the body in clearing bacterial pathogen infections.

Table 2 Key nodes of antibacterial components-targets network of Common Cnidium Fruit and its topological features

Node name	Node type	Degree	Betweenness Centrality	Node name	Node type	Degree	Betweenness Centrality
Common Cnidium Fruit	Plant	25	0.292 0	PTGS1	Action Target	14	0.082 0
PTGS2	Action Target	22	0.192 9	HSP90AA1	Action Target	13	0.060 6
DPP4	Action Target	16	0.054 7	Stigmasterol	Compound	9	0.120 4
Beta-sitosterol	Compound	14	0.295 9	Xanthoxylin N	Compound	8	0.041 7

3.4 Analysis results of the antibacterial/bacteriostatic PPI network The PPI network for the antibacterial/bacteriostatic targets of Common Cnidium Fruit is shown in Fig. 3: this network contains 24 protein nodes and 96 edges. Each edge represents an interaction relationship between nodes. The more edges associated with a node, the more important the target protein corresponding to that node is. Hub targets were identified through topological analysis, and the results are shown in Fig. 4: the key target proteins for Common Cnidium Fruit's antibacterial/bacteriostatic effects are CASP3, PTGS2, BCL2, JUN, and ESR1.

Caspase-3 is a crucial downstream component in the caspase cascade activation and is a core protein that executes apoptosis[17]. Studies have found that chemical components acting on the CASP3 target and inhibiting the expression of the CASP3 protein can effectively suppress the physiological activity of *E. coli*[18]. BCL-2 family proteins control the intrinsic apoptosis pathway, and among them, the BAX and BAK proteins can drive cells into programmed cell death by controlling mitochondrial outer membrane permeabilization and subsequently initiating the caspase cascade[19]. Studies have shown that inhibiting BCL-2 can enhance the apoptosis and autophagy capabilities of macrophages, thereby modulating protective immune responses during *Mycobacterium tuberculosis* infection[20]. The JUN protein, together with Fos, Maf, ATF, and others, forms the transcription factor activator protein-1 (AP-1), which is involved in many physiological processes, such as cell proliferation, migration, and invasion[21]. Kikuchi *et al.*[22] found that bacterial extracts from *E. coli*, *P. aeruginosa*, and *H. pylori*, as well as lipopolysaccharide (LPS) from *E. coli*, can induce the production of the inflammatory cytokine interleukin-8 (IL-8). The macrolide antibiotic clarithromycin can alleviate inflammation caused by bacterial infection by acting on AP-1 and thereby inhibiting IL-8 production. Estrogen receptors modulate cells, pathways in the innate and adaptive immune systems, and the development of immune cells, leading to sex differences in human immunity to infection and autoimmunity[23]. Inhibiting the activity of estrogen receptor 1 (ESR1) can suppress mast cell infiltration and reduce the production of immunoglobulin E (IgE) and thymus and activation-regulated chemokine (CCL17), thereby alleviating inflammatory responses[24]. Based on this, the active compounds in Common Cnidium Fruit may exert antibacterial/bacteriostatic activity by acting on the above-mentioned target proteins.

3.5 Analysis results of the antibacterial/bacteriostatic GO enrichment analysis The obtained 25 targets associated with the antibacterial/bacteriostatic efficacy of Common Cnidium Fruit's active compounds were input into the DAVID database for GO analysis. Under the condition of $P < 0.01$, 167 GO terms were screened and obtained. Among them, there were 102 Biological Process (BP) terms, 39 Molecular Function (MF) terms, and 26 Cellular Component (CC) terms. The analysis results were imported into Hiplot. Filtering based on a combination of *P*-value, *Q*-value ($Q < 0.05$), and the number of enriched genes, the top 5 entries for BP, MF, and CC, respectively, were selected to draw bar charts (Fig. 5).

The GO analysis results indicate that the effective active compounds of Common Cnidium Fruit may exert antibacterial/bacteriostatic effects by regulating biological processes such as response to steroid hormone, response to hypoxia, response to decreased oxygen levels, response to oxygen levels, and response to exogenous stimulus; participating in biological processes at locations such as organelle outer membrane, outer membrane, mitochondrial outer membrane, myelin sheath, and BCL-2 family protein complex; and thereby influencing molecular functions such as estrogen response element binding, nuclear receptor activity, ligand-activated transcription factor activity, nuclear steroid receptor activity, and transcription coregulator activity. Studies have found that tissue sites with inflammation or infection caused by exogenous stimuli such as bacteria often exhibit hypoxia or decreased oxygen levels[25]. Furthermore, local hypoxic environments can induce transcriptional responses that further affect the function of

immune cells[26]. That is, the active components in Common Cnidium Fruit may help defend against the invasion of foreign pathogenic microorganisms by participating in the regulation of the human inflammatory response and immune response.

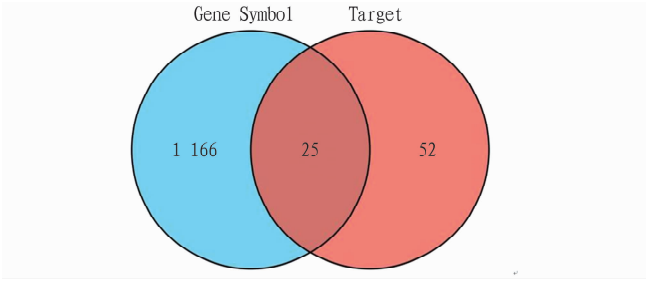


Fig. 1 Venn diagram of antibacterial/bacteriostatic gene targets and action targets of Common Cnidium Fruit

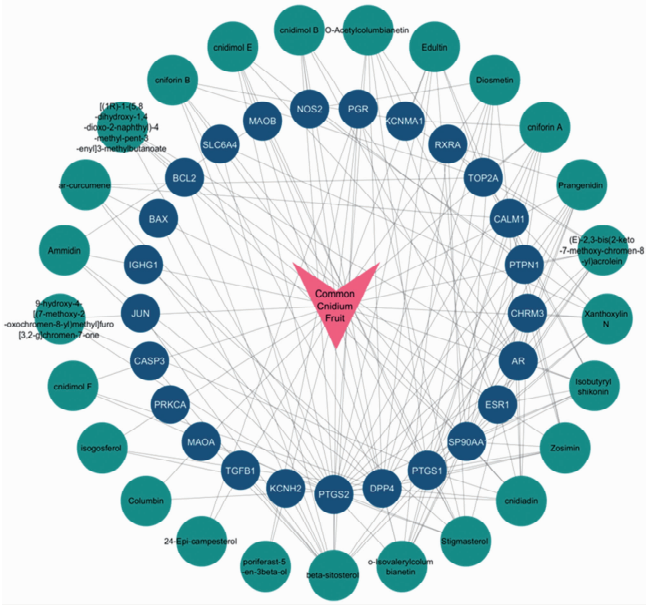


Fig. 2 Active components-targets network of Common Cnidium Fruit

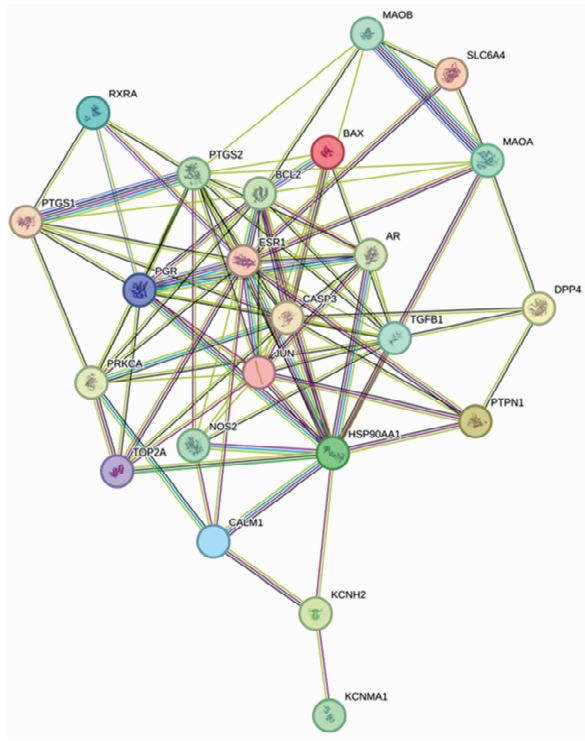


Fig. 3 PPI network of antibacterial/bacteriostatic targets of Common Cnidium Fruit

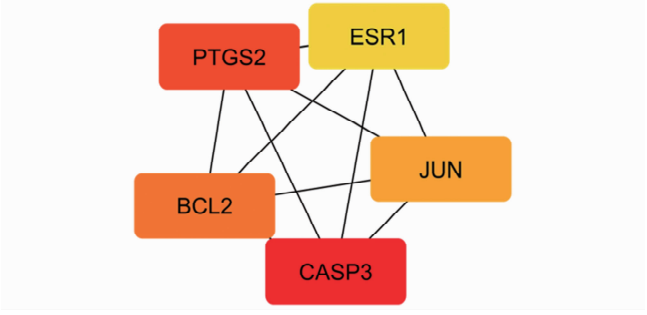
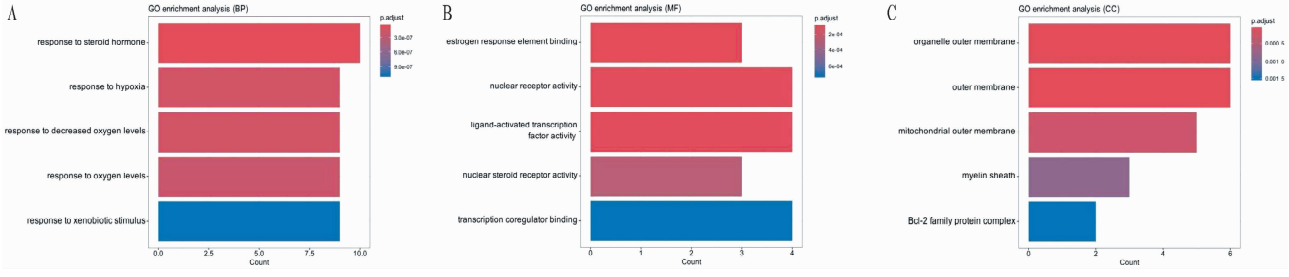


Fig. 4 PPI network of antibacterial/bacteriostatic Hub targets of Common Cnidium Fruit



NOTE A. BP enrichment results; B. MF enrichment results; C. CC enrichment results.

Fig. 5 GO functional enrichment analysis of antibacterial components of Common Cnidium Fruit

4 Conclusion and prospects

Screening through the HIT2.0 and TCMSP databases yielded a total of 25 active components of Common Cnidium Fruit. Searching and screening the GeneCards and OMIM databases identified 1 191 antibacterial/bacteriostatic-related targets. Intersection analysis with the targets of Common Cnidium Fruit resulted in 25 potential antibacterial/bacteriostatic targets for the main active components of Common Cnidium Fruit. Based on network pharmacology research and analysis, it is hypothesized that the main active compounds responsible for the antibacterial/bacteriostatic

effects of Common Cnidium Fruit are beta-sitosterol, stigmasterol, and xanthoxylin. Results from the PPI network and GO enrichment analysis suggest that Common Cnidium Fruit may exert antibacterial/bacteriostatic effects by acting on target proteins such as CASP3, PTGS2, BCL2, JUN, and ESR1, regulating the activity of receptors and transcription factors, and influencing the body's immune response to exogenous stimuli.

This study explored the material basis and potential mechanism of action of Common Cnidium Fruit in antibacterial/bacteriostatic activity using network pharmacology methods, indicating the development potential of Common Cnidium Fruit as a plant-derived bacteriostatic agent. It should be noted that natural plant-derived bacteriostatic agents generally have slightly weaker bactericidal effects, and the bacteriostatic activity and spectrum of inhibition vary among agents from different plant sources. Research on compound disinfectants is still in its early stages and requires more experimental studies for exploration and validation[27]. Li *et al.* [28] investigated the *in vitro* antifungal effect of osthole, an active chemical component in Common Cnidium Fruit, using the microdilution checkerboard method. They demonstrated that when osthole was combined with fluconazole, endogenous reactive oxygen species (ROS) significantly increased, resulting in a significant synergistic inhibitory effect against fluconazole-resistant *C. albicans*[28]. Zhang Dandan *et al.* [29] studied the bacteriostatic activity of the combination of Common Cnidium Fruit and *S. flavescens* extracts by determining the minimum inhibitory concentration (MIC). They found that when Common Cnidium Fruit and *S. flavescens* extracts, each at concentrations below their individual MICs, were used in combination, the antibacterial efficacy against *S. aureus* and *E. coli* was enhanced, effectively inhibiting bacterial growth and reproduction. Furthermore, Zhou *et al.* [30] found that osthole can restore the bactericidal activity of polymyxin against plasmid-mediated colistin-resistant mcr-1-positive bacteria, including *E. coli* and *K. pneumoniae*, thereby preventing severe infections caused by polymyxin-resistant bacteria. Current research reports on the synergistic antibacterial effects of Common Cnidium Fruit extracts combined with other active components suggest that combining the multi-target bacteriostatic ability of Common Cnidium Fruit with other plant-derived bacteriostatic agents or common antibiotics is beneficial for reducing the dosage of bacteriostatic agents and helps in combating infections caused by novel drug-resistant bacteria.

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VEGF, HO-1, and GLUT1, thereby promoting angiogenesis, adaptation of energy metabolism, and antioxidant defense. On the other hand, its sustained or excessive activation may contribute to pathological processes[12]. The PI3K/AKT signaling pathway serves as a crucial upstream approach regulating the stability and activity of HIF-1α, with its phosphorylation playing a vital role in ischemic myocardium protection[13]. In this study, the expression levels of HIF-1α, P-PI3K, and P-AKT proteins were significantly decreased in the cardiac tissue of the model group mice. This reduction corresponded with the pathological features observed in myocardium subjected to severe hypoxic and oxidative stress conditions, reflecting a compromised adaptive protective mechanism. Y01 intervention, particularly at high doses, significantly upregulated the expression of these three key proteins in a dose-dependent manner. This finding suggests that Y01 exerts myocardial protective effects primarily through activation of the PI3K/AKT pathway, which in turn stabilizes and activates HIF-1α. Based on the finding that Y01 significantly enhances oxidative stress markers, it is hypothesized that Y01 serves as a critical molecular approach in mitigating myocardial oxidative damage through activation of the PI3K/AKT/HIF-1α signaling pathway. This hypothesis aligns closely with the central role of the HIF-1α pathway as predicted by network pharmacology analysis.

This study integrated network pharmacology predictions with *in vivo* pharmacodynamic assessments and molecular biology experiments to systematically elucidate the mechanisms by which agarwood essential oil (Y01) inhibits MI. This approach establishes a robust foundation for the development of novel myocardial protection strategies grounded in traditional Chinese medicine. Furthermore, it was demonstrated that Y01 enhanced myocardial antioxidant defense capacity through the activation of the PI3K/AKT/HIF-1α signaling pathway (significantly reducing MDA level and LDH activity while increasing SOD activity), representing the primary mechanism by which Y01 improves MI injury. This study has several limitations. First, although the ISO-induced acute myocardial injury model employed is well-established, it does not fully replicate the chronic MI caused by coronary atherosclerosis commonly observed in clinical settings. Second, the investigation primarily focused on the HIF-1α pathway, while other potential targets and pathways identified through network pharmacology, such as MAPK14, were not extensively validated. Third, the necessity of further confirming the involvement of this pathway using pathway-specific inhibitors (such as PI3K inhibitor LY294002) or gene intervention techniques (such as siRNA-mediated knockdown of HIF-1α) remains unaddressed. In future studies, we will employ coronary ligation models that more closely align with clinical pathogenesis to further validate the efficacy of Y01. Additionally, we will conduct an in-depth investigation into the specific roles of downstream effector molecules of HIF-1α in mediating the protective effects of Y01.

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