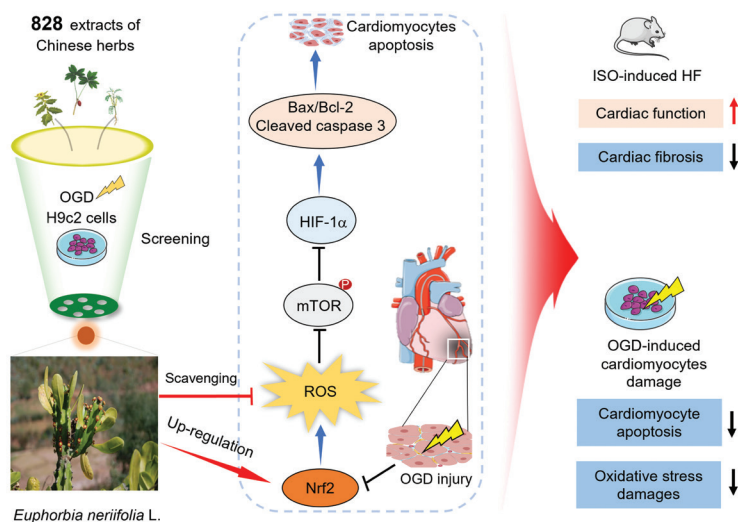


Euphorbia neriifolia L. extract attenuates heart failure by modulating the Nrf2/ROS/HIF-1 α signaling pathway

Graphical abstract



Authors

Taotao Lu, Manjong Wang, Yunyuan Huang, Donglei Shi, Xiao Li, Jiaqi Liu, Jian Li, Bei Jiang, Baoli Li and Yixiang Xu

Correspondence

jiangbei@dali.edu.cn (B. Jiang);
baolili@hainanu.edu.cn (B. Li);
yixxu@ecust.edu.cn (Y. Xu)

In brief

JM04, an extract from *Euphorbia neriifolia* L., protects cardiomyocytes against OGD injury and enhances cardiac function in mice with ISO-induced heart failure. This treatment decreases oxidative stress and restores Nrf2/ROS/HIF-1 α signaling, thereby alleviating myocardial damage and fibrosis.

Highlights

- *Euphorbia neriifolia* L. (JM04) was demonstrated to have cardiomyocyte protection effects, according to screening of 828 extracts of Chinese herbs.
- JM04 alleviated cardiac dysfunction and cardiac fibrosis in a mouse model of ISO-induced heart failure.
- JM04 suppressed oxidative stress and apoptosis, thereby protecting cardiomyocytes against OGD-induced damage through Nrf2/ROS/HIF-1 α pathway regulation.

Research Article

Euphorbia neriifolia L. extract attenuates heart failure by modulating the Nrf2/ROS/HIF-1 α signaling pathway

Taotao Lu^{a,b,1}, Manjiong Wang^{b,1}, Yunyuan Huang^{c,1}, Donglei Shi^a, Xiao Li^b, Jiaqi Liu^b, Jian Li^{a,b,d,e}, Bei Jiang^{d,*}, Baoli Li^{a,*} and Yixiang Xu^{b,*}

^aKey Laboratory of Tropical Biological Resources of Ministry of Education and Hainan Engineering Research Center for Drug Screening and Evaluation, School of Pharmaceutical Sciences, Hainan University, Haikou 570228, China

^bState Key Laboratory of Bioreactor Engineering, Shanghai Frontiers Science Center of Optogenetic Techniques for Cell Metabolism, Frontiers Science Center for Materiobiology and Dynamic Chemistry, Shanghai Key Laboratory of New Drug Design, School of Pharmacy, East China University of Science and Technology, Shanghai 200237, China

^cHubei Key Laboratory of Genetic Regulation and Integrative Biology, School of Life Sciences, Central China Normal University, Wuhan 430079, Hubei, China

^dYunnan Key Laboratory of Screening and Research on Anti-pathogenic Plant Resources from West Yunnan, College of Pharmacy, Dali University, Dali 671000, China

^eKey Laboratory of Xinjiang Phytomedicine Resource and Utilization, Ministry of Education, School of Pharmacy, Shihezi University, Shihezi 832003, China

¹These authors contributed equally to this work.

*Correspondence: jiangbei@dali.edu.cn (B. Jiang); baolili@hainanu.edu.cn (B. Li); yixxu@ecust.edu.cn (Y. Xu)

Received: 16 August 2025; Revised: 12 November 2025; Accepted: 20 November 2025

Published online: 27 December 2025

DOI 10.15212/AMM-2025-0056

ABSTRACT

Heart failure (HF) remains a major global health challenge with limited therapeutic options, thus prompting the search for novel cardioprotective agents among traditional Chinese medicines. In this study, screening of 828 herbal extracts in an oxygen glucose deprivation injury H9c2 cardiomyocyte model led to the identification of *Euphorbia neriifolia* L. extract (JM04) as a promising candidate. JM04 significantly increased cell viability while decreasing lactate dehydrogenase release and reactive oxygen species (ROS) accumulation in both H9c2 and primary cardiomyocytes. *In vivo*, JM04 ameliorated cardiac function and attenuated myocardial fibrosis in isoproterenol-induced HF mice, as evidenced by echocardiography and histological analysis. Network pharmacology and mechanistic studies revealed that JM04 modulated the Nrf2/ROS/HIF-1 α axis by exerting free radical scavenging activity, activating the Nrf2 antioxidant pathway, restoring mTOR phosphorylation, and enhancing HIF-1 α expression, thereby protecting against cardiomyocyte apoptosis. Furthermore, UPLC-MS/MS identified six active components consistent with network pharmacology predictions, thus highlighting its multicomponent and multitarget nature. Collectively, these findings demonstrated that JM04 exerts potent cardioprotective effects through integrated antioxidant and signaling modulation, and support its further development as a candidate botanical drug for HF intervention.

Keywords: *Euphorbia neriifolia* L., Heart failure, Network pharmacology, Nrf2/ROS/HIF-1 α pathway

1. INTRODUCTION

Heart failure (HF), a complex clinical syndrome resulting from structural and/or functional cardiac abnormalities initiated by diverse etiological factors, is characterized by substantial morbidity and mortality. The adult prevalence rates of HF are 1%–2%, and a trend of earlier onset in younger populations is emerging [1, 2]. Global estimates suggest that HF affects more than 64 million

individuals worldwide, including more than 13 million confirmed cases among Chinese adults [3]. Notably, despite substantial research investment in cardiovascular therapeutics and the implementation of current gold-standard therapies post-diagnosis, the 5-year survival rate persistently remains below 50% [4, 5].

Current pharmacological management of HF follows guideline-directed medical therapy encompassing angiotensin-converting enzyme inhibitors, angiotensin

receptor blockers, diuretics, and beta-blockers. However, extended administration of these therapeutic agents can lead to adverse events, including electrolyte depletion, fluid loss, and dry cough [6, 7]. Combining different drug classes has shown potential in improving hemodynamic parameters in patients with HF and is crucial in enhancing myocardial function. Nevertheless, determining the optimal ratio of drugs of different classes for individual patients remains a clinical treatment challenge [8]. Therefore, effective intervention measures must be explored to prevent HF development.

Traditional Chinese medicine (TCM) is a promising therapeutic strategy for the prevention and treatment of HF, because of its multitarget, multicomponent, and multipathway mechanisms [9]. Although the pharmacological effects and underlying mechanisms of certain active ingredients require further investigation, TCM's multitarget characteristics make it a viable option for the prevention and management of cardiovascular diseases (CVDs) [10, 11]. TCM encompasses a wide variety of active ingredients with distinctive pharmacological effects, including lipid modulation, antioxidant and anti-inflammatory activity, endothelial protection, autophagy enhancement, and blood pressure lowering [12]. These multifaceted effects are highly beneficial in CVDs prevention and treatment. Moreover, the synergistic actions among multiple constituents in TCM might contribute to balanced pharmacological modulation, thus potentially decreasing the frequent risk of adverse effects with single-target or high-dose chemical drugs. Furthermore, numerous TCM formulations exert therapeutic benefits, such as Shexiang Baixin pill (SBP), Tongxinluo capsule (TXLC), Dixin Xuegang capsule, and Xinmailong, all of which have been applied in CVDs management [13, 14].

Herein, we systematically screened 828 Chinese herbal extracts derived from ethnopharmacological resources in Yunnan Province, Southwest China,

to identify potential therapeutic candidates for HF. Notably, the ethanol extract from aerial parts of *E. neriifolia* L. (designated JM04) demonstrated superior cardioprotective effects in preliminary cardiomyocyte viability assays (Figure 1). Use of *E. neriifolia* L., a member of the spurge family (*Euphorbiaceae*), has been historically documented in managing diverse pathological conditions, including inflammatory disorders, chronic pain, gastrointestinal dysfunction, and parasitic infections [15–17]. Phytochemical investigations have revealed its composition rich in bioactive diterpenoids and triterpenoids, which exhibit pleiotropic pharmacological activities encompassing anti-neoplastic, anti-inflammatory, and antiviral efficacy against HIV and influenza strains [18–21]. However, the therapeutic potential and molecular mechanisms underlying its cardioprotective effects remain unexplored in the scientific literature. Therefore, a critical need exists to systematically investigate the pharmacological properties and delineate the molecular mechanisms underlying *E. neriifolia* L.'s therapeutic potential in HF management. Herein, we used an oxygen glucose deprivation (OGD)-induced cardiomyocyte injury model and an isoproterenol (ISO)-induced HF mouse model to explore the protective effects of JM04. Further network pharmacology analysis indicated that JM04 protects cardiomyocytes by regulating the HIF-1 signaling pathway.

2. METHODS

2.1 Reagents

Nicorandil, 2,2-diphenyl-1-picrylhydrazyl, and LCZ696 were purchased from Bide Pharmatech, Ltd. (Shanghai, China). Fetal bovine serum (catalog number: 42F6590K), Dulbecco's modified Eagle's medium/F-12 (catalog number: 11330032), and Dulbecco's modified Eagle's medium (catalog number: 11995-065) were purchased

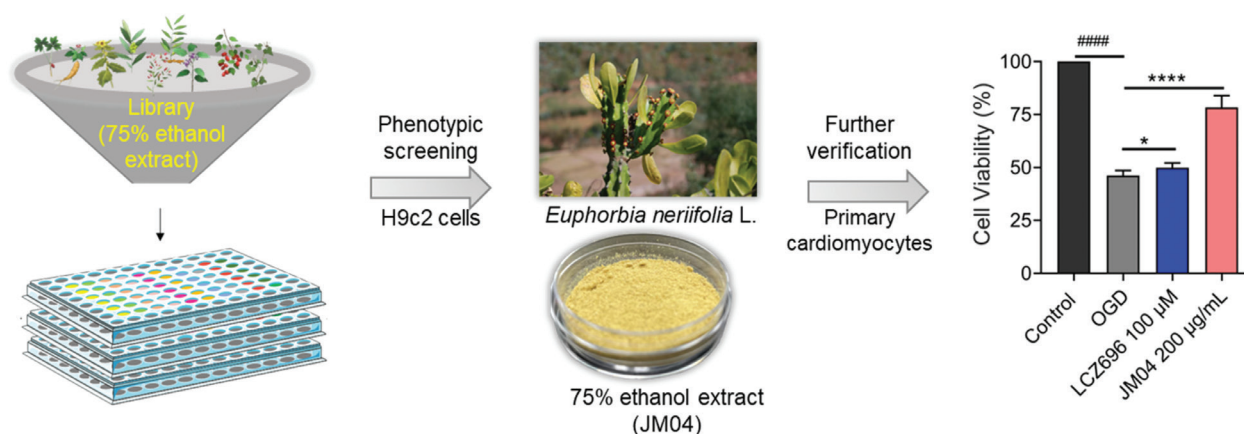


Figure 1 | The discovery of JM04 through phenotypic screening of a Chinese herbal medicine library.

Research Article

from Gibco (Grand Island, NY, United States). Phosphate buffered saline (PBS), penicillin-streptomycin (catalog number: 60162ES76), RIPA lysis buffer (catalog number: 20114ES60), a BCA Protein Quantification Kit (catalog number: 20201ES76), Super ECL Detection Reagent (catalog number: 36208ES60), and a Reactive Oxygen Species Assay Kit (catalog number: 50101ES01) were obtained from Yeasen (Shanghai, China). A Cell Counting Kit-8 (CCK-8) was procured from TargetMol (catalog number: C0005, Boston, United States). A CytoTox 96® Non-Radioactive Cytotoxicity Assay was procured from Promega Corporation (catalog number: G1780, Madison, United States).

2.2 Preparation of JM04

Aerial parts of *E. neriifolia* L., collected from western Yunnan Province, China, were provided by the College of Pharmacy at Dali University. The plant material was air-dried, ground to a fine powder, and subjected to triple extraction *via* reflux with 75% ethanol at 95°C for 2 hours. The resulting extracts were combined, filtered, and concentrated under reduced pressure, then lyophilized to obtain a stable powdered extract, designated JM04. A voucher specimen (20151209-2) of *E. neriifolia* L. has been deposited in the College of Pharmacy, Dali University.

2.3 Cell culture and isolation of neonatal rat cardiomyocytes

H9c2 cells were purchased from the Cell Bank of the Chinese Academy of Science (Shanghai, China); cultured in Dulbecco's modified Eagle's medium containing 10% fetal bovine serum and 1% penicillin-streptomycin solution; and incubated in a humidified atmosphere with 5% CO₂ at 37°C. Cardiomyocytes were isolated from neonatal Wistar rats (1–2 days) as described previously [22]. Briefly, neonatal rats were dissected, and their hearts were thoroughly washed in cold PBS solution and minced. Subsequently, tissue fragments were digested in a series of incubations at 37°C in pancreatin (1.2 mg/mL) and collagenase (0.14 mg/mL). Supernatants were collected and centrifuged at 200 × *g* for 5 min. After centrifugation, the cells were resuspended in Dulbecco's modified Eagle's medium/F-12 containing 5% heat-activated horse serum, ascorbate (0.1 mmol/L), insulin-transferring-sodium selenite medium supplement, penicillin (100 U/mL), streptomycin (100 mg/mL), and bromodeoxyuridine (0.1 mmol/L). The cell suspension was pre-plated at 37°C for 1 h to enrich cardiomyocytes in the cultures. The cells were then diluted to 1 × 10⁶ cells/mL and plated in laminin-coated (10 mg/mL) culture dishes according to the specific experimental requirements.

2.4 OGD model and drug treatment

H9c2 cells were seeded in 96-well plates at 8 × 10³ cells/well and cultured under normal conditions to 80%

confluence. JM04 (0, 10, 20, 50, 100, or 200 µg/mL) was added to the cells for 12 h under hypoxia. After incubation, the medium was removed, and the cells were exposed to hypoxia (oxygen deprivation, 0.5% O₂) and PBS solution for 6 h to induce cell injury. A control group not subjected to OGD was maintained in complete culture medium.

2.5 Cell viability assays

The protective effects of JM04 were evaluated in an OGD-induced H9c2 cell injury model. Cardiomyocyte viability was determined with CCK-8 assays according to the manufacturer's instructions. CCK-8 solution was added into the wells and incubated at 37°C for 1 h. The absorbance of the culture medium in each well was recorded at 450 nm with a Synergy H1 Hybrid Multi-Mode Reader (BioTek Instruments, Inc., Verm, United States). Three replicate experiments were conducted.

2.6 2,2-Diphenyl-1-picrylhydrazyl (DPPH) radical scavenging assays

We conducted 2,2-diphenyl-1-picrylhydrazyl radical scavenging assays as described previously [23]. Briefly, 100 µL JM04 at various concentrations was incubated with 100 µL DPPH solution (200 µM) in a 96-well plate at room temperature for 30 min, away from any source of light. Absorbance was measured at 517 nm. A sample containing methanol without JM04 served as a control. The DPPH radical scavenging ability (%) was calculated as $[(A_0 - A_1)/A_0] \times 100$, with A_0 and A_1 representing the absorbance of the control and the sample, respectively. Three replicate experiments were conducted.

2.7 Measurement of lactate dehydrogenase (LDH) levels

LDH levels were detected in H9c2 cells with a cytotoxicity assay kit. H9c2 cells were seeded in 12-well plates (8 × 10⁴ cells/mL), pretreated with JM04 (50, 100, or 200 µg/mL) for 12 h, and were followed by OGD treatment for 6 h. LDH levels were measured according to the manufacturer's instructions. Briefly, 50 µL supernatant from the OGD treatment was added to 50 µL working solution in a 96-well plate. After a 30 min reaction at room temperature under light-avoidant conditions, the reaction was stopped by addition of 50 µL stop solution. UV absorption values were measured at 490 nm with a Synergy H1 Hybrid Multi-Mode Reader.

2.8 Determination of intracellular reactive oxygen species (ROS)

H9c2 cells were cultured in 12-well plates (6 × 10⁴ cells/mL), then pretreated with JM04 (50, 100, and 200 µg/mL) for 12 h, and subjected to OGD treatment for 6 h. Next, 10 µM H2DCFDA (DCFH-DA) diluent was added into 12-well plates at 37°C in the dark for 30 min. The cells were washed three times with serum-free

culture medium after removal of DCFH-DA diluent. Subsequently, the cells were examined and imaged with a fluorescence microscope (Nikon, TS2RFL, Tokyo, Japan). Positive signals for ROS were quantified in ImageJ software.

2.9 Animal model

C57BL/6J mice (6–8 weeks, male) were obtained from Lingchang Biotechnology Co., Ltd. (Shanghai, China). After 1 week of acclimation, the mice were randomly divided into three groups ($n = 7$ –9): a control group, model group, and JM04-treated group. Mice in the model group and the JM04-treated group received subcutaneous injections of ISO twice daily. The dosage regimen was 40 mg/kg for days 1–2, 20 mg/kg for days 3–7, and 10 mg/kg for days 8–14. Control mice were subcutaneously injected with saline. Simultaneously, the JM04-treated groups were intragastrically administered 200 mg/kg once per day for 14 days, whereas the control group and model group were treated with water.

2.9.1 Echocardiography. To evaluate the therapeutic effects of JM04 on ISO-induced HF, we assessed cardiac function with echocardiography. The cardiac function indices of ejection fraction (EF), fractional shortening (FS), left ventricular internal diastolic diameter (LVIDd), and left ventricular internal dimension systole (LVIDs) were measured with transthoracic echocardiography with a Vevo 3100 high-resolution imaging system (Visual Sonics, Vevo® 3100, Toronto, Canada).

2.9.2 Masson's trichrome staining. Myocardial fibrosis was assessed with Masson's trichrome staining. Briefly, mouse hearts were excised and fixed in 4% paraformaldehyde, embedded in paraffin, and cut into 4 μ m serial sections. The sections were stained with Masson's trichrome and observed under an ECLIPSE Ts2R microscope (Nikon, TS2RFL, Tokyo, Japan). ImageJ software was used to measure the fibrotic area.

2.10 Pharmacology network analysis

We initially obtained 157 chemical ingredients and their physicochemical properties from *E. neriifolia* L. from many prior studies [24, 25]. Criteria of oral bioavailability $\geq 30\%$ and drug-likeness activity ≥ 0.18 were used to screen candidate active compounds. We filtered HF-related targets from the GeneCards database (<https://www.genecards.org/>). The overlapping targets between compounds from *E. neriifolia* L. and those associated with HF were identified with a Venn diagram, thus yielding a set of common targets. The common targets were submitted to the STRING database version 11.0 (<https://string-db.org/>) for protein-protein interaction (PPI) analysis. Furthermore, direct targets were input into the DAVID 6.8 database (<https://davidbioinformatics.nih.gov/>) to obtain Kyoto Encyclopedia of Genes and

Genomes (KEGG) signaling pathway data. Statistical significance was determined using a threshold of 0.05.

2.11 Western blot analysis

H9c2 cells were cultured in 6 cm dishes, then pretreated with JM04 (50 or 100 μ g/mL) for 12 h and subjected to OGD treatment for 6 h. Next, the cells were lysed in RIPA lysis buffer containing 1% protease inhibitor. Subsequently, proteins were collected by centrifugation at $12,000 \times g$ for 10 min at 4°C. Protein concentrations were quantified with a BCA protein quantification kit. Equal amounts of protein from each sample cell were separated with SDS-PAGE and transferred to NC membranes. The membranes were then blocked with 5% milk powder in TBST for 1 h and incubated overnight with primary antibodies at 4°C. After being washed, the membranes were incubated with appropriate HRP-conjugated secondary antibodies for 1 h at room temperature. Finally, the membranes were photographed with imaging techniques (Tanon-4600SF), and ImageJ software was used to quantify the intensity. Primary antibodies to Bax (catalog number: AF1270, Beyotime Inc., Shanghai, China), Bcl-2 (catalog number: CY6717, Abways, Shanghai, China), cleaved caspase 3 (catalog number: AF1150, Beyotime Inc., Shanghai, China), mTOR and phosphorylated mTOR (P-mTOR) (catalog number: D198411 and D155324, respectively, Sangon Biotech Co., Ltd., Shanghai, China), HIF-1 α (catalog number: D222477, Sangon Biotech Co., Ltd., Shanghai, China), Nrf2 (catalog number: 16396-1-AP, Proteintech Group, Inc., United States), and β -actin (catalog number: 30102ES, Yeasen, Shanghai, China) were used. Secondary HRP-conjugated goat anti-rabbit antibodies were purchased from Yeasen, Shanghai, China.

2.12 UPLC-MS/MS analysis

The analysis of JM04 was conducted with a Waters Micromass Q-TOF system equipped with an ACQUITY UPLC HSS T3 (2.1 $\times$ 100 mm, 1.8 μ m) maintained at 35°C. The limit of detection was 1 ng/mL, and the flow rate was set at 0.3 mL/min. The column was eluted with a gradient of water/formic acid (100:0.1, v/v, A) and acetonitrile/formic acid (100:0.1, v/v, B): 0–55 min, 0–100% B. The temperature of the autosampler was kept at 4°C, and the injection volume was set at 10 μ L. Mass spectrometry was performed with a quadrupole-time-of-flight system (Waters, Massachusetts, United States) in negative and positive ion electrospray ionization (HESI-II) mode. The full MS scan ranged from 100 to 1,500 m/z. Nitrogen was used as the auxiliary and nebulizer gas.

2.13 Statistical analysis

Quantitative data are presented as mean $\pm$ SD. Statistical analysis was performed with *t*-test along with Sisak

Research Article

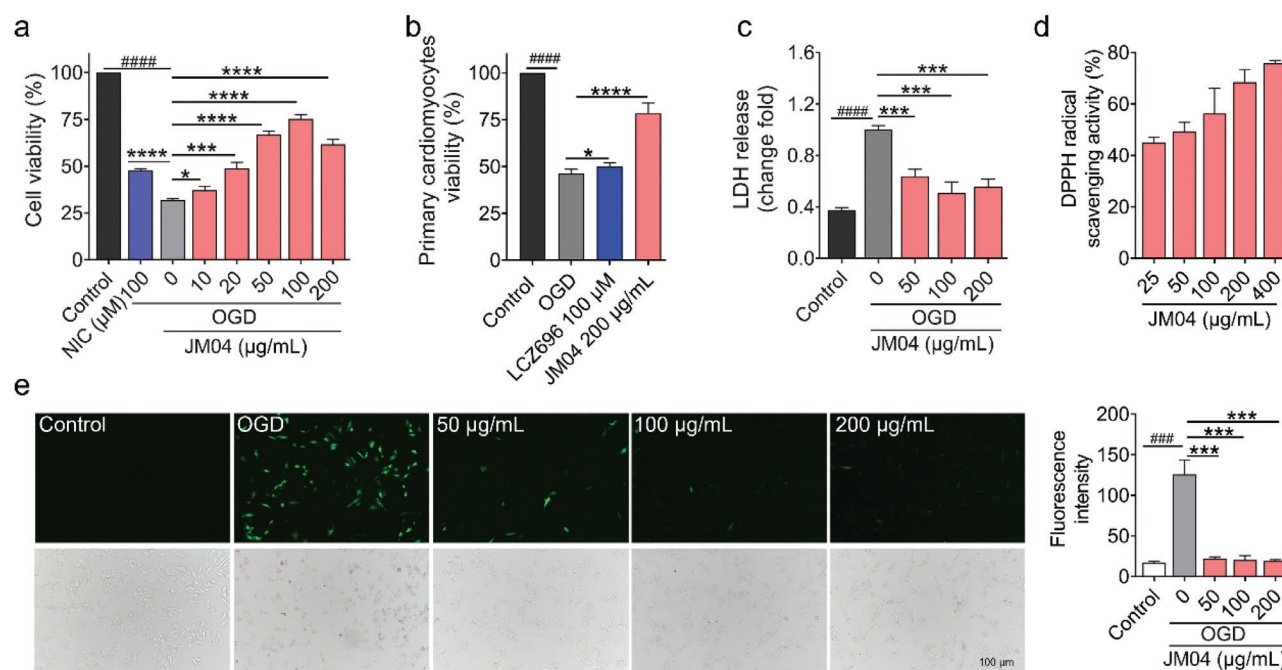


Figure 2 | JM04 treatment protects H9c2 cells and primary cardiomyocytes against OGD injury *in vitro*.

(a) Viability of H9c2 cells. (b) Viability of primary cardiomyocytes. (c) LDH assay in H9c2 cells. (d) DPPH radical scavenging ability. (e) Representative images of H9c2 cells and ROS fluorescence intensity (scale bars, 100 µm). The results are shown as mean $\pm$ SD ($n = 3$). #### $P < 0.0001$, #### $P < 0.0001$ vs. control group, * $P < 0.05$, *** $P < 0.001$, **** $P < 0.0001$ vs. OGD group.

multiple comparisons test. All analyses were performed in GraphPad Prism version 8.0.2 (GraphPad Software, United States), and $P < 0.05$ was considered to indicate statistical significance.

3. RESULTS

3.1 JM04 protects H9c2 cells and primary cardiomyocytes against OGD-induced injury

To determine the optimal concentration of JM04 for protecting H9c2 cells exposed to OGD treatment, we assessed cell viability with CCK-8 assays, in which nicorandil served as the positive control. OGD significantly decreased cell viability, whereas 10, 20, 50, or 100 µg/mL JM04 treatment, compared with OGD, markedly attenuated cell damage in a dose-dependent manner (Figure 2a). Furthermore, treatment with JM04 (200 µg/mL), compared with OGD, significantly increased primary cardiomyocyte viability (Figure 2b). LCZ696 served as the positive control [26]. In agreement with the cell viability results, LDH release was markedly greater in the OGD group than the control group (#### $P < 0.0001$, Figure 2c), thus indicating OGD-induced injury and subsequent cell membrane damage with LDH leakage. In contrast, JM04 treatment (50, 100, or 200 µg/mL) significantly protected cells from injury, thus resulting in less LDH release than observed in the OGD group (*** $P < 0.001$, Figure 2c).

3.2 JM04 has significant radical scavenging activity and inhibits OGD-induced oxidative damage in H9c2 cells

Prior investigations have demonstrated the substantial antioxidant activity of methanolic extracts from *E. neriifolia* L. leaves [27]. Oxidative stress, defined as excess production of ROS relative to antioxidant defense, is critical in HF pathophysiology and potentially results in cardiomyocyte damage [28]. To investigate whether JM04 might have antioxidative capabilities, we conducted DPPH radical scavenging assays. JM04 showed concentration-dependent DPPH radical scavenging ability (Figure 2d) and therefore has antioxidative potential. To further explore whether the *in vitro* protective effect of JM04 might be associated with oxidative stress, we conducted DCFH-DA assays to detect intracellular ROS production during OGD treatment. Intracellular ROS in H9c2 cells was significantly elevated in the OGD group but decreased with JM04 treatment (Figure 2e). These findings demonstrated that the cardioprotective effect of JM04 against OGD-induced injury in H9c2 cells is associated with its antioxidative stress properties.

3.3 JM04 alleviates cardiac dysfunction and myocardial fibrosis in mice with ISO-induced HF

To assess the cardioprotective efficacy of JM04 in HF, we established an *in vivo* mouse model of HF via

subcutaneous injection of ISO (Figure 3a) and assessed cardiac function through echocardiography. ISO administration markedly impaired both systolic and diastolic function in the left ventricle (Figure 3b). In contrast, treatment with JM04 (200 mg/kg) significantly increased cardiac performance, as evidenced by increased EF and FS (** $P < 0.01$, Figure 3c, d). Whereas no significant difference in LVIDd was observed between the JM04-treated and model groups (Figure 3e), the LVID was modestly but significantly lower in the JM04 group than the model group (* $P < 0.05$, Figure 3f). Additionally, although the heart weight-to-body weight (HW/BW) ratio appeared lower in JM04-treated mice than model mice, the difference was not statistically significant (Figure 3g). Histological analysis with Masson's trichrome staining revealed that ISO exposure led to a substantial increase in myocardial interstitial fibrosis, a hallmark of adverse cardiac remodeling. JM04 administration resulted in a visibly attenuated collagen deposition and fibrosis area with respect to that in the model group. However, the difference was not statistically significant (Figure 3h–i). Together, these results indicated that JM04 exerts cardioprotective effects in a mouse model of HF.

3.4 Network pharmacology analysis reveals potential mechanisms of JM04 against HF

To elucidate the molecular mechanisms underlying the cardioprotective effects of JM04, we used a network pharmacology approach. Through extensive literature mining, we retrieved 157 phytochemicals from *E. neriifolia* L., including alkaloids, glycosides, phenols, terpenoids, flavonoids, and saponins. On the basis of criteria of oral bioavailability $\geq 30\%$ and drug likeness ≥ 0.18 , we identified 12 compounds as candidate bioactive components (Table S1), thus suggesting their potential as therapeutic agents in HF. Prediction of molecular targets associated with these compounds with the Traditional Chinese Medicine Systems Pharmacology Database yielded 217 unique protein targets after removal of duplicates.

3.4.1 HF-related targets and overlapping targets of JM04 and HF. To explore potential therapeutic targets of JM04 for HF, we compiled HF-associated genes from GeneCards, the Therapeutic Target Database, Online Mendelian Inheritance in Man, and DrugBank. After eliminating redundancies, we

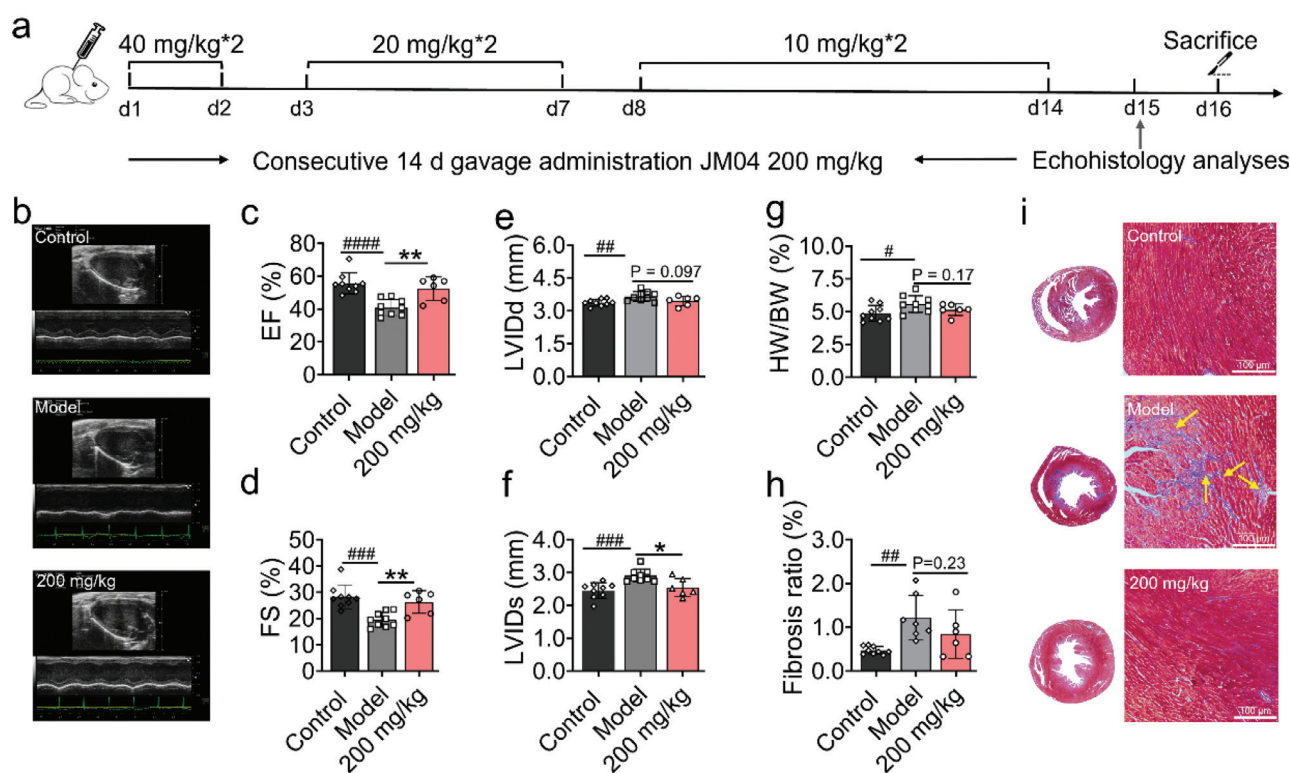


Figure 3 | JM04 treatment enhances cardiac function in ISO-induced HF.

(a) Schematic of the experimental design. (b) Representative M mode echocardiographic tracings. (c) Ejection fraction (EF%). (d) Fractional shortening (FS%). (e) Left ventricular internal diameter at diastole (LVIDd, mm). (f) Left ventricular internal diameter at systole (LVIDs, mm). (g) Ratios of heart to body weight of mice across groups. (h) Quantification of fibrosis area. (i) Representative Masson staining (scale bars, 100 μ m). Data are expressed as mean $\pm$ SD ($n = 7-9$). ## $P < 0.01$, ### $P < 0.001$, #### $P < 0.0001$ vs. control group; * $P < 0.05$, ** $P < 0.01$ vs. model group.

Research Article

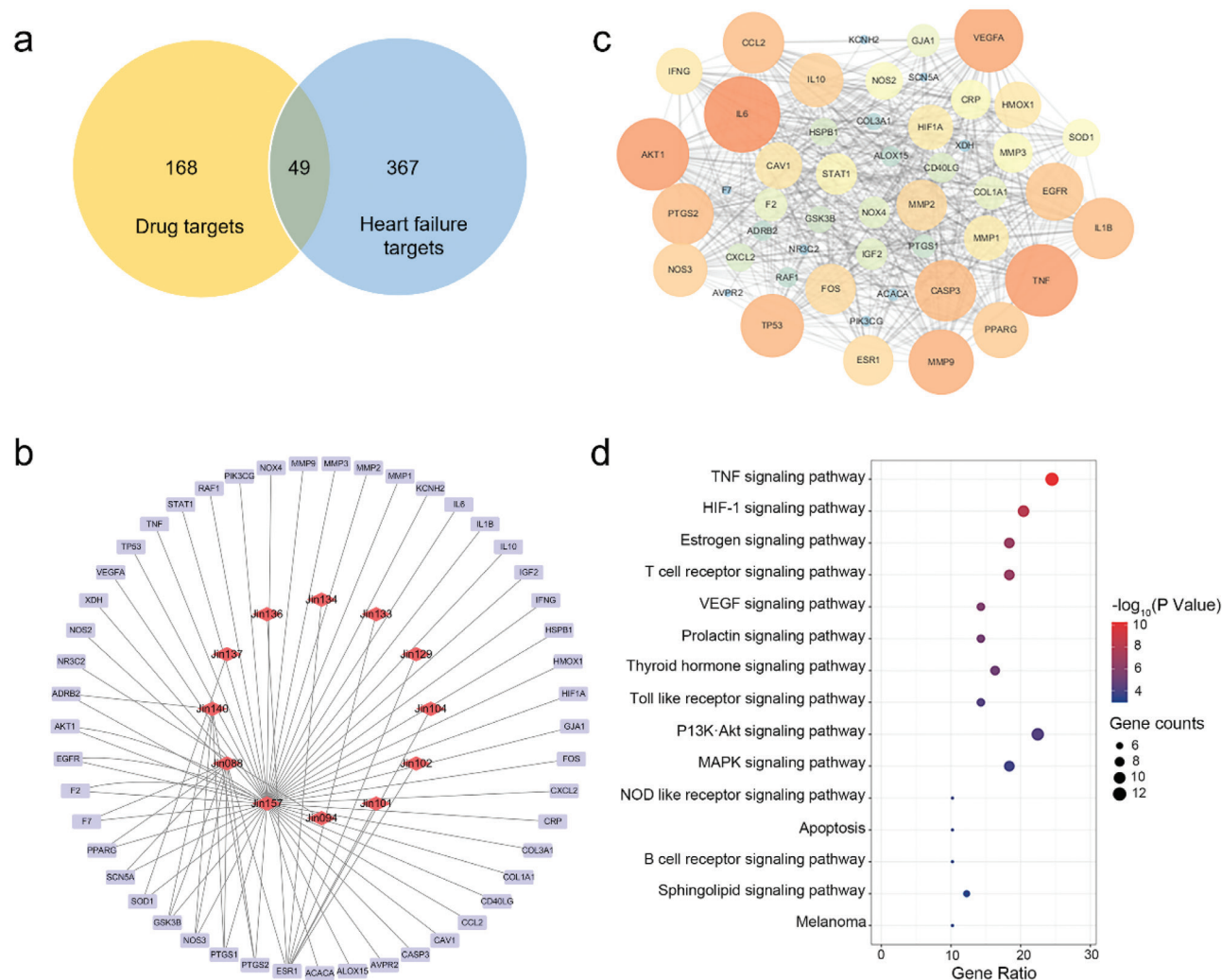


Figure 4 | Network pharmacology analysis profile of the ingredient-target-pathway relationships of *E neriifolia* L.

(a) Venn diagram illustrating that 12 bioactive ingredients share 49 potential targets with known pathological targets of HF. (b) Ingredient-target-pathway network of *E neriifolia* L. The 12 bioactive ingredients are presented as red circles. The predicted direct targets are shown as purple diamonds. (c) PPI network of 49 overlapping genes. (d) Representative enriched clusters of the KEGG pathway.

identified 367 HF-related targets. Venn diagram analysis revealed 49 overlapping targets between JM04-predicted proteins and HF-associated genes (Figure 4a, Table S2). These targets were then used to construct an ingredient-target-pathway network diagram in Cytoscape 3.7.2, which illustrated the multifaceted benefits of JM04 in the treatment of HF, through multicomponent, multitarget, and multipathway effects (Figure 4b).

3.4.2 PPI network. To further clarify the interactions among overlapping targets, we imported the 49 shared targets into the STRING database and visualized the results in Cytoscape 3.7.2. The resulting PPI network comprised 59 nodes and 160 edges (Figure 4c). Among them, IL6, VEGFA, TNF, AKT1, and MAPK1 exhibited the

highest degree values, thus suggesting their potential roles in mediating the therapeutic effect of JM04 against HF. These targets are known to be critically involved in inflammation, angiogenesis, and cellular survival pathways relevant to cardiac remodeling and dysfunction.

3.4.3 KEGG pathway enrichment analysis. After KEGG enrichment analysis, 87 pathways were sorted according to the number of enriched genes. The top 15 pathways (Figure 4d) were associated predominantly with TNF and HIF-1 receptor signaling pathways, which regulate complex biological metabolic processes such as cell proliferation, apoptosis, and inflammation. Notably, the TNF signaling pathway and HIF-1 signaling pathway emerged as key cascades, both of which have been implicated in regulating cell apoptosis, oxidative stress, and

inflammatory responses, all of which are key processes in HF pathogenesis [29, 30]. Given the established role of HIF-1 α in promoting cardiomyocyte survival under hypoxic stress, we focused on validating the HIF-1 signaling pathway as a primary mechanism of JM04 action.

3.5 JM04 ameliorates OGD-induced oxidative stress and myocardial apoptosis through up-regulating expression of the antioxidant-related protein Nrf2 and regulating the ROS/P-mTOR/HIF-1 α pathway

Building on the network pharmacology findings, which highlighted the HIF-1 signaling pathway as a central mechanism potentially mediating the cardioprotective effects of JM04, we next sought to experimentally validate this prediction. Specifically, we investigated the regulatory roles of JM04 in oxidative stress, HIF-1 α expression, and apoptosis in H9c2 cardiomyocytes subjected to OGD.

First, JM04 was found to significantly decrease ROS generation by scavenging radicals. The levels of ROS within cells are influenced not only by the availability of ROS scavengers but also by the modulation of antioxidant enzymes and proteins. Nrf2, a key transcription factor, plays a central role in the cellular antioxidant defense system by upregulating the expression of various antioxidant-associated genes involved in cellular defense against oxidative stress [31]. Therefore, to determine whether JM04 might attenuate ROS generation by activating Nrf2, we assessed Nrf2 protein expression with western blotting. The decreased expression of Nrf2 in OGD-treated H9c2 cells indicated functional impairment of the antioxidant system after OGD treatment (Figure 5a). However, JM04 increased the expression of Nrf2 (Figure 5a, b), thus suggesting that JM04 not only directly scavenges ROS but also activates antioxidant enzymes by enhancing Nrf2 levels, thereby inhibiting ROS production.

Furthermore, we examined the total mTOR and P-mTOR in H9c2 cells, because high concentrations or prolonged ROS treatment has been shown to inhibit mTOR activation, decrease synthesis of HIF-1 α , and induce apoptosis [20]. In agreement with these expectations, OGD treatment significantly inhibited P-mTOR, whereas JM04 restored P-mTOR levels, thus indicating a potential role in counteracting the inhibitory effect of OGD on P-mTOR activation (Figure 5c). Next, we examined the effect of JM04 on HIF-1 α protein in OGD-treated H9c2 cells. OGD induced HIF-1 α production, whereas JM04 treatment further promoted the increase in HIF-1 α (Figure 5d), thus suggesting its involvement in activating the mTOR/HIF-1 α signaling pathway. Finally, the effects of JM04 on apoptosis-associated proteins in H9c2 cells under OGD stimulation were explored. JM04 treatment reversed the OGD-induced increase in the pro-apoptotic protein cleaved caspase 3, thus indicating that JM04 inhibited cardiomyocyte apoptosis in H9c2 cells, whereas the protein expression of Bax and Bcl-2

showed nonsignificant decreasing or increasing trends. Together, these results suggested that JM04 inhibits cardiomyocyte apoptosis by directly scavenging intracellular ROS, upregulating Nrf2, and activating the mTOR/HIF-1 α signaling pathway.

3.6 UPLC-MS/MS analysis of JM04

To further understand the key components involved in the anti-HF effect of JM04, we performed UPLC-MS/MS to identify the components of JM04. The total ion chromatogram for JM04 based on UPLC-MS/MS analysis in positive and negative electrospray ionization mode confirmed pachypodol, yucalexin P-21, p-hydroxybenzaldehyde, xanthoxylin, dibutyl phthalate, and quercetin as active ingredients in JM04 (Figure S1), in agreement with the network pharmacology analysis.

4. DISCUSSION

HF is a multifaceted clinical syndrome driven by a complex interplay among hemodynamic, neurohormonal, and cellular disturbances. Despite substantial progress in understanding the pathophysiology of HF, including the roles of oxidative stress, inflammation, apoptosis, and maladaptive remodeling, a translational gap persists. Current therapeutic strategies remain inadequate in addressing the multifactorial nature of disease progression [4, 5, 7]. Conventional pharmacologic agents, although essential, often target single pathways and are associated with considerable adverse effects that compromise long-term adherence and efficacy. Increasing attention has turned toward multitarget approaches, including natural-product-based therapies, to modulate the diverse pathological cascades implicated in HF. TCM provides a rich pharmacopeia with demonstrated clinical potential in cardiovascular disease management [12–14], particularly through its synergistic and system-wide effects. Notably, formulations such as SBP and TXLC have shown benefits in modulating vascular integrity, myocardial energy metabolism, and neurohormonal activity, thereby delaying HF progression [32]. However, the discovery of novel cardioprotective agents from underexplored TCM sources, particularly those derived from regions with large populations of minority ethnic groups, remains a promising but understudied research avenue. In this context, we used a high-throughput screening strategy based on cellular models of hypoxic injury to assess 828 herbal extracts. The extract of *E. neriifolia* L. (JM04) emerged as a promising candidate for cardioprotection. Although *E. neriifolia* L. has long been recognized as a folk herbal medicine with pharmacological benefits for treating diseases or enhancing health [33, 34], its role in cardiovascular protection has been largely undocumented.

Our mechanistic investigations revealed that JM04 exerts cardioprotective effects by modulating oxidative stress and apoptosis, two hallmark processes in HF pathology [28, 35]. In the OGD-induced injury model,

Research Article

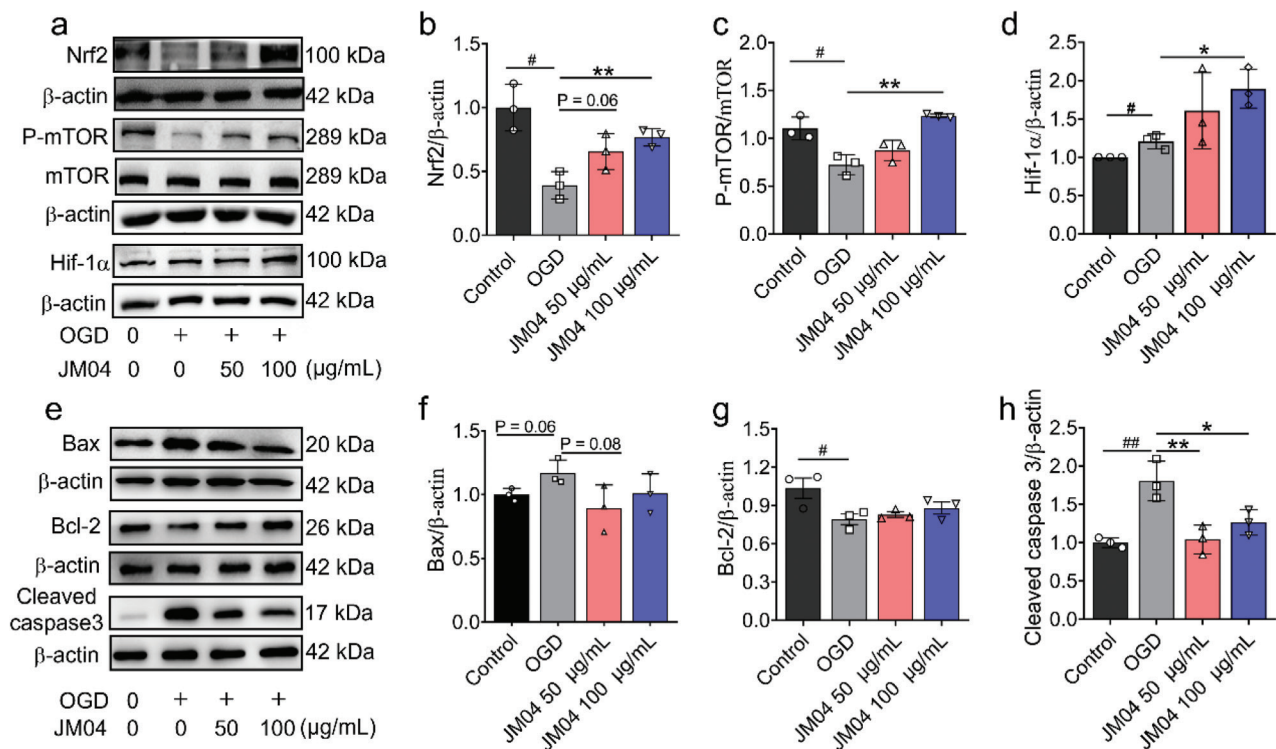


Figure 5 | JM04 modulates oxidative stress and apoptosis-related signaling pathways in OGD-injured H9c2 cells.

(a, e) Representative western blot bands for Nrf2, P-mTOR^{Ser2448}, total mTOR, and HIF-1α. (b–d) Quantitative analysis of Nrf2, P-mTOR/mTOR, and HIF-1α expression levels. (e) Representative western blot bands for Bax, Bcl-2, and cleaved caspase-3. (f–h) Quantitative analysis of Bax, Bcl-2, and cleaved caspase-3 expression levels. Data are presented as mean ± SD (n = 3). #P < 0.05, ##P < 0.01 compared with control group; *P < 0.05, **P < 0.01 compared with OGD group.

JM04 not only enhanced cell viability and decreased LDH release, but also significantly suppressed ROS generation. Further mechanistic investigations and network pharmacology analysis revealed that JM04's cardioprotective role involves sequential activation of the Nrf2 and HIF-1α pathways. In the OGD-induced injury model, JM04 significantly suppressed ROS generation. We propose that JM04 first activates the Nrf2 signaling pathway, which in turn upregulates a suite of antioxidant genes. This potent scavenging of excess ROS not only mitigates oxidative damage but also creates a more stable cellular redox environment permitting effective HIF-1α stabilization and function [36]. When the myocardium is subjected to stressors such as ischemia, hypoxia, or oxidative stress, HIF-1α, a master regulator of cellular adaptation, is activated, thereby promoting angiogenesis, energy metabolism, and anti-apoptotic signaling, facilitating cardiomyocyte adaptation to the hypoxic environment, and promoting the establishment of collateral circulation [37]. Furthermore, our data demonstrated that JM04 independently promotes HIF-1α synthesis by restoring the expression of P-mTOR, a key upstream regulator of HIF-1α [38]. This dual action of JM04, which creates a conducive environment for HIF-1α function via Nrf2 but also directly enhances HIF-1α synthesis via

P-mTOR, ensures robust upregulation of HIF-1α levels in OGD-injured cardiomyocytes [39, 40]. Consequently, the Nrf2-mediated antioxidant response and P-mTOR-mediated HIF-1α synthesis collectively constitute a synergistic network that enhances cardiomyocyte survival and resists stress-induced damage, thus exerting powerful anti-HF effects through multiple targets.

Numerous studies have shown that TCM therapies typically exert therapeutic effects through multicomponent, multipathway, and multitarget mechanisms, which might confer distinct advantages over conventional Western medicine in certain contexts [41]. These properties have led to the clinical approval and widespread use of TCM preparations such as SBP and TXLC in CVDs management [32]. Both SBP and TXLC are complex multiherbal formulations containing bioactive constituents such as cholic acid, deoxycholic acid, cinobufagin, ginsenoside Rb1, paeoniflorin, hirudin, and jujuboside A, many of which are terpenoid or terpenoid-derived chemicals [42, 43]. These compounds have been reported to exert cardioprotective effects by modulating oxidative stress, inflammatory signaling, and mitochondrial integrity. Similarly, JM04 is enriched in diterpenoid and triterpenoid constituents, such as pachypodol and quercetin, which have potent antioxidant and anti-inflammatory

activities. Given this compositional resemblance, JM04 might be hypothesized to share common mechanistic pathways with SBP and TXLC, particularly through activation of the Nrf2 antioxidant system, inhibition of excessive ROS accumulation, and modulation of inflammatory mediators involved in HF progression. Further comparative studies are warranted to quantitatively correlate the terpenoid profiles of JM04 with those of SBP and TXLC, and to potentially provide a basis for identifying conserved cardioprotective scaffolds.

To deconvolve the complex composition of JM04, we conducted network pharmacology analysis. This approach identified 12 candidate bioactive ingredients in JM04, with 49 overlapping targets mapped to HF-associated genes. KEGG pathway enrichment revealed the HIF-1 signaling cascades as central regulatory axes. Subsequent UPLC-MS/MS analysis confirmed the presence of several of bioactive ingredients predicted by network pharmacology, including known Nrf2 activators such as quercetin and pachypodol [44-47]. Although six components were identified in JM04,

their relative quantitative contributions to the overall pharmacological activity remain to be determined. This limitation should be addressed by establishing a quantitative chemical profile and performing correlation analysis between component concentration and biological activity. Future work will follow a bioactivity-guided fractionation strategy, together with combination assays and experiments using key monomeric compounds, to isolate, quantify, and confirm the primary active constituents responsible for the cardioprotective effects of JM04.

Together, our results suggest that JM04 might act through a coordinated Nrf2/ROS/mTOR/HIF-1 α axis to preserve myocardial function under stress conditions (Figure 6). Importantly, this integrated approach, combining phenotypic screening, network-based prediction, *in vitro* validation, and chemical profiling, offers a robust paradigm for elucidating the therapeutic potential of traditional medicinal plants in HF management. Nonetheless, further studies are warranted to clarify the pharmacokinetics, bioavailability, and synergistic

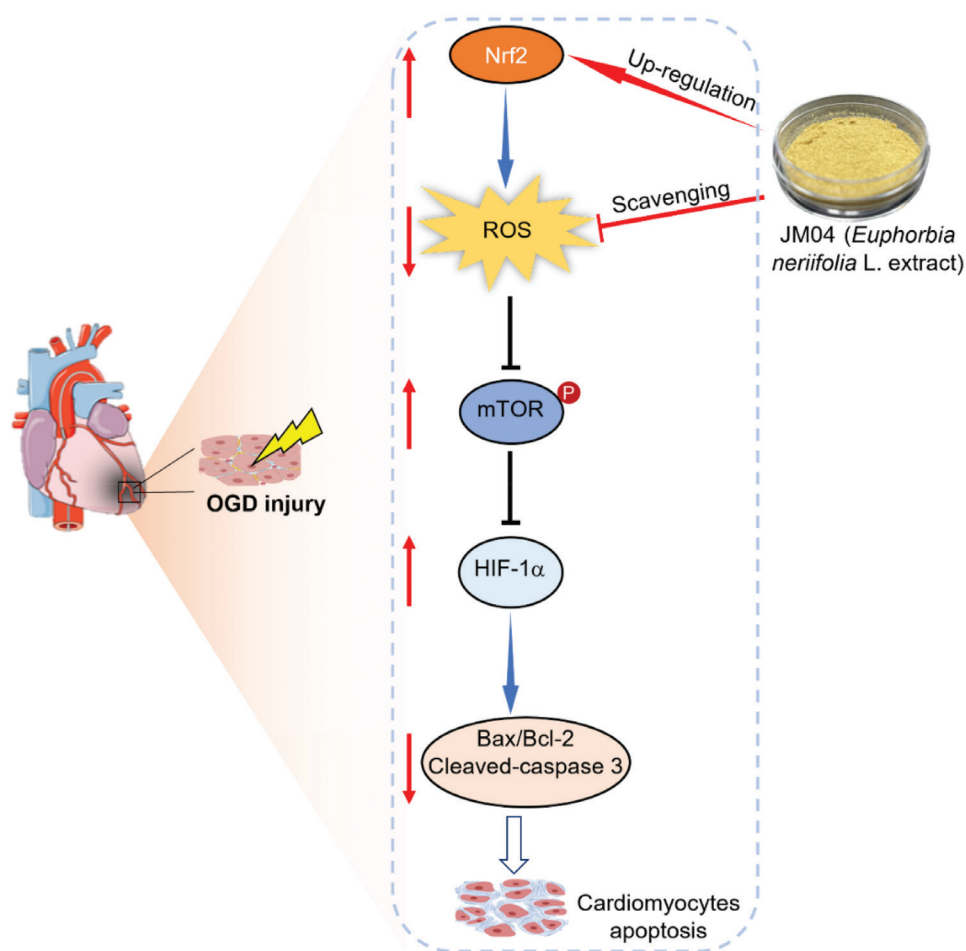


Figure 6 | JM04 prevents OGD-induced H9c2 cell damage via regulation of a signaling pathway involving the antioxidant-associated protein Nrf2 and ROS/mTOR/HIF-1 α .

Research Article

interactions among JM04 constituents. Additionally, translation into clinical application will require the development of standardized extraction methods, safety evaluations, and large-animal efficacy models.

DATA AVAILABILITY STATEMENT

The data used and analyzed in this study are available from the corresponding author upon reasonable request.

ETHICS STATEMENT

All animal experimental procedures complied with the Guide for the Care and Use of Laboratory Animals (National Institutes of Health Publication, revised 1996, No. 86-23, Bethesda, MD, USA) and were approved by the Animal Research Committee of Hainan University (approval No. SYXK2023-0031).

AUTHOR CONTRIBUTIONS

Yixiang Xu, Baoli Li, and Jian Li designed the study. Bei Jiang provided the Chinese herbal medicine extract library. Yixiang Xu and Manjiong Wang screened 828 herbal extracts. Yixiang Xu and Baoli Li supervised the experimental work. Taotao Lu performed the in vitro and in vivo experiments. Yunyuan Huang conducted the network pharmacology analysis. Xiao Li assisted in establishing the animal model and conducting animal experiments. Taotao Lu and Jiaqi Liu analyzed the data. Yixiang Xu, Manjiong Wang, and Yunyuan Huang contributed to data interpretation. Taotao Lu wrote the manuscript. Yixiang Xu, Baoli Li, Manjiong Wang, and Yunyuan Huang revised the draft. Donglei Shi provided technical support and advice throughout the study.

ACKNOWLEDGMENTS

This work was supported by the National Natural Science Foundation of China (Grant Nos. 22477025, 32121005, 22307038, and 22467010), the National Key Research and Development Program of China (Grant No. 2021YFA0804904), the Shanghai Frontier Science Center of Optogenetic Techniques for Cell Metabolism (Grant No. 2021 Sci & Tech 03-28), the Innovative Research Team of High-level Local Universities in Shanghai (grant No. SHSMU-ZDCX20212702), the Chinese Special Fund for State Key Laboratory of Bioreactor Engineering (Grant No. 2060204), and Fundamental Research Funds for Hainan University (KYQD(ZR)-22010).

CONFLICT OF INTEREST

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported herein.

SUPPLEMENTARY MATERIAL

Supplementary Material can be downloaded from https://amm-journal.org/wp-content/uploads/2025/12/amm20250056_Suppl.pdf.

REFERENCES

- [1] Savarese G, Becher PM, Lund LH, Lund LH, Seferovic P, Rosano GMC, et al.: Global Burden of Heart Failure: A Comprehensive and Updated Review of Epidemiology. *Cardiovascular Research* 2023, 118:3272–3287.
- [2] McDonagh TA, Metra M, Adamo M, Gardner RS, Baumbach A, Böhm M, et al.: 2021 ESC Guidelines for the Diagnosis and Treatment of Acute and Chronic Heart Failure: Developed by the Task Force for the Diagnosis and Treatment of Acute and Chronic Heart Failure of the European Society of Cardiology (ESC). With the Special Contribution of the Heart Failure Association (HFA) of the ESC. *European Journal of Heart Failure* 2022, 24:4–131.
- [3] Hao G, Wang X, Chen Z, Zhang L, Zhang Y, Wei B, et al.: Prevalence of Heart Failure and Left Ventricular Dysfunction in China: The China Hypertension Survey, 2012–2015. *European Journal of Heart Failure* 2019, 21:1329–1337.
- [4] Cahill TJ, Choudhury RP, Riley PR: Heart Regeneration and Repair after Myocardial Infarction: Translational Opportunities for Novel Therapeutics. *Nature Reviews Drug Discovery* 2017, 16:699–717.
- [5] Vogel B, Acevedo M, Appelman Y, Bairey Merz CN, Chieffo A, Figtree GA, et al.: The Lancet Women and Cardiovascular Disease Commission: Reducing the Global Burden by 2030. *The Lancet* 2021, 397:2385–2438.
- [6] Bots SH, Groepenhoff F, Eikendal ALM, Tannenbaum C, Rochon PA, Regitz-Zagrosek V, et al.: Adverse Drug Reactions to Guideline-Recommended Heart Failure Drugs in Women: A Systematic Review of the Literature. *JACC: Heart Failure* 2019, 7:258–266.
- [7] Heidenreich PA, Bozkurt B, Aguilar D, Allen LA, Byun JJ, Colvin MM, et al.: 2022 AHA/ACC/HFSA Guideline for the Management of Heart Failure: A Report of the American College of Cardiology/American Heart Association Joint Committee on Clinical Practice Guidelines. *Circulation* 2022, 145:e895–e1032.
- [8] Cui H, Han S, Dai Y, Xie W, Zheng R, Sun Y, et al.: Gut Microbiota and Integrative Traditional Chinese and Western Medicine in Prevention and Treatment of Heart Failure. *Phytomedicine* 2023, 117:154885.
- [9] Hao P, Jiang F, Chen Y, Yang J, Zhang K, Zhang M, et al.: Traditional Chinese Medication for Cardiovascular Disease. *Nature Reviews Cardiology* 2014, 12:115–122.
- [10] Hao P, Jiang F, Cheng J, Ma L, Zhang Y, Zhao Y: Traditional Chinese Medicine for Cardiovascular Disease: Evidence and Potential Mechanisms. *Journal of the American College of Cardiology* 2017, 69:2952–2966.
- [11] Leung EL, Xu S: Traditional Chinese Medicine in Cardiovascular Drug Discovery. *Pharmacological Research* 2020, 160:105168.
- [12] Xu Q, Bauer R, Hendry BM, Fan T, Zhao Z, Duez T-P, et al.: The Quest for Modernisation of Traditional Chinese Medicine. *BMC Complementary and Alternative Medicine* 2013, 13:132.
- [13] Jiang Y, Zhao Q, Li L, Huang S, Yi S, Hu Z: Effect of Traditional Chinese Medicine on the Cardiovascular Diseases. *Frontiers in Pharmacology* 2022, 13:806300.
- [14] Li L, Zhou X, Li N, Sun M, Lv J, Xu Z: Herbal Drugs Against Cardiovascular Disease: Traditional Medicine and Modern Development. *Drug Discovery Today* 2015, 20:1074–1086.
- [15] Bigoniya P, Rana AC: Radioprotective and In-vitro Cytotoxic Sapogenin from *Euphorbia neriifolia* (Euphorbiaceae)

- Leaf. *Tropical Journal of Pharmaceutical Research* 2009, 8:521–530.
- [16] Sultana A, Hossain MJ, Kuddus MR, Rashid MA, Zahan MS, Mitra S, et al.: Ethnobotanical Uses, Phytochemistry, Toxicology, and Pharmacological Properties of *Euphorbia neriifolia* Linn. Against Infectious Diseases: A Comprehensive Review. *Molecules* 2022, 27:4374.
 - [17] Li JC, Dai WF, Liu D, Jiang MY, Zhang ZJ, Chen XQ, et al.: Bioactive Ent-isopimarane Diterpenoids from *Euphorbia neriifolia*. *Phytochemistry* 2020, 175:112373.
 - [18] Chang FR, Yen CT, Ei-Shazly M, Lin WH, Yen MH, Lin KH, et al.: Anti-human Coronavirus (anti-HCoV) Triterpenoids from the Leaves of *Euphorbia neriifolia*. *Natural Product Communications* 2012, 7:1415–1417.
 - [19] Choodej S, Pudhom K: Cycloartane Triterpenoids from the Leaves of *Euphorbia neriifolia*. *Phytochemistry Letters* 2020, 35:1–5.
 - [20] Li M, Zhao L, Liu J, Liu A, Jia C, Ma D, et al.: Multi-mechanisms are Involved in Reactive Oxygen Species Regulation of mTORC1 Signaling. *Cellular Signalling* 2010, 22:1469–1476.
 - [21] Yan SL, Li YH, Chen XQ, Liu D, Chen CH, Li RT: Diterpenes from the Stem Bark of *Euphorbia neriifolia* and Their In Vitro Anti-HIV Activity. *Phytochemistry* 2018, 145:40–47.
 - [22] Jiang K, Xu Y, Wang D, Chen F, Tu Z, Qian J, et al.: Cardioprotective Mechanism of SGLT2 Inhibitor Against Myocardial Infarction is Through Reduction of Autosis. *Protein & Cell* 2022, 13:336–359.
 - [23] Thabit S, Handoussa H, Roxo M, Cestari de Azevedo B, El Sayed NSE, Wink M: *Styphnolobium japonicum* (L.) Schott Fruits Increase Stress Resistance and Exert Antioxidant Properties in *Caenorhabditis elegans* and Mouse Models. *Molecules* 2019, 24:2633.
 - [24] Gao Y, Zhou JS, Liu HC, Zhang Y, Yin WH, Liu QF, et al.: Phorneroids A-M, Diverse Types of Diterpenoids from *Euphorbia neriifolia*. *Phytochemistry* 2022, 198:113142.
 - [25] Liu JH, Latif A, Ali M, Zhang GP, Xiang WJ, Ma L, et al.: Diterpenoids from *Euphorbia neriifolia*. *Phytochemistry* 2012, 75:153–158.
 - [26] Singh JSS, Burrell LM, Charif M, Squire IB, Clark AL, Lang CC: Sacubitril/Valsartan: Beyond Natriuretic Peptides. *Heart* 2017, 103:1569–1577.
 - [27] Kumar A, Mahanty B, Goswami RCD, Barooah PK, Choudhury B: In Vitro Antidiabetic, Antioxidant Activities and GC-MS Analysis of *Rhynchostylis retusa* and *Euphorbia neriifolia* Leaf Extracts. *3 Biotech* 2021, 11:315.
 - [28] Tsutsui H, Kinugawa S, Matsushima S: Oxidative Stress and Heart Failure. *American Journal of Physiology. Heart and Circulatory Physiology* 2011, 301:H2181–H2190.
 - [29] Geng B, Wang X, Park KH, Lee KE, Kim J, Chen P, et al.: UCHL1 Protects Against Ischemic Heart Injury via Activating HIF-1 α Signal Pathway. *Redox Biology* 2022, 52:102295.
 - [30] Zheng J, Chen P, Zhong J, Cheng Y, Chen H, He Y, et al.: HIF-1 α in Myocardial Ischemia-Reperfusion Injury (Review). *Molecular Medicine Reports* 2021, 23:352.
 - [31] Bellezza I, Giambanco I, Minelli A, Donato R: Nrf2-Keap1 Signaling in Oxidative and Reductive Stress. *Biochimica et Biophysica Acta - Molecular Cell Research* 2018, 1865:721–733.
 - [32] Jia Q, Wang L, Zhang X, Ding Y, Li H, Yang Y, et al.: Prevention and Treatment of Chronic Heart Failure Through Traditional Chinese Medicine: Role of the Gut Microbiota. *Pharmacological Research* 2020, 151:104552.
 - [33] Chang SS, Huang HT, Lin YC, Chao CH, Liao GY, Lin ZH, et al.: Neritriterpenols A-G, Euphane and Tirucallane Triterpenes from *Euphorbia neriifolia* L. and Their Bioactivity. *Phytochemistry* 2022, 199:113199.
 - [34] Mallavadhani UV, Satyanarayana KV, Mahapatra A, Sudhakar AV: A New Tetracyclic Triterpene from the Latex of *Euphorbia nerifolia*. *Natural Product Research* 2004, 18:33–37.
 - [35] Dubois-Deruy E, Peugnet V, Turkieh A, et al.: Oxidative Stress in Cardiovascular Diseases. *Antioxidants* 2020, 9:864.
 - [36] Kasai S, Shimizu S, Tatara Y, Pinet F: Regulation of Nrf2 by mitochondrial reactive oxygen species in physiology and pathology. *Biomolecules* 2020, 10:32.
 - [37] Kumar S, Wang G, Liu W, Ding W, Dong M, Zheng N, et al.: Hypoxia-induced Mitogenic Factor Promotes Cardiac Hypertrophy via Calcium-dependent and Hypoxia-inducible Factor-1 α Mechanisms. *Hypertension* 2018, 72:331–342.
 - [38] Chen X, Xie Q, Zhu Y, Xu J, Lin G, Liu S, et al.: Cardio-protective Effect of Tetrahydrocurcumin, The Primary Hydrogenated Metabolite of Curcumin In Vivo and In Vitro: Induction of Apoptosis and Autophagy via PI3K/AKT/mTOR Pathways. *European Journal of Pharmacology* 2021, 911:174495.
 - [39] Wang Z, Zhang Z, Zhao J, Yong C, Mao Y: Polysaccharides from *Enteromorpha prolifera* Ameliorate Acute Myocardial Infarction In Vitro and In Vivo via Up-regulating HIF-1 α . *International Heart Journal* 2019, 60:964–973.
 - [40] Yu ZP, Yu HQ, Li J, Li C, Hua X, Sheng XS: Troxerutin Attenuates Oxygen-glucose Deprivation and Reoxygenation-induced Oxidative Stress and Inflammation by Enhancing the PI3K/AKT/HIF-1 α Signaling Pathway in H9c2 Cardiomyocytes. *Molecular Medicine Reports* 2020, 22:1351–1361.
 - [41] Li B, Nasser MI, Masood M, Adlat S, Huang Y, Yang B, et al.: Efficiency of Traditional Chinese Medicine Targeting the Nrf2/HO-1 Signaling Pathway. *Biomedicine & Pharmacotherapy*, 2020, 126:110074.
 - [42] Chen Q, Zou J, Shi Y, Zhang X, Guo D, Luan F, et al.: Chinese Patent Medicine Tongxinluo: A Review on Chemical Constituents, Pharmacological Activities, Quality Control, and Clinical Applications. *Phytomedicine*, 2024, 132:155674.
 - [43] Lu L, Sun X, Chen C, Qin Y, Guo X: Shexiang Baoxin Pill, Derived from the Traditional Chinese Medicine, Provides Protective Roles Against Cardiovascular Diseases. *Frontiers in Pharmacology*, 2018, 9:2018.
 - [44] Liu FC, Yang YH, Liao CC, Lee HC: Xanthoxylin Attenuates Lipopolysaccharide-induced Lung injury Through Modulation of Akt/HIF-1 α /NF- κ B and Nrf2 Pathways. *International Journal of Molecular Sciences* 2024, 25:8742.
 - [45] Kim EK, Kim JH, Jeong S, Choi YW, Choi HJ, Kim CY, et al.: Pachypodol, a Methoxyflavonoid Isolated from *Pogostemon cablin* Benthams Exerts Antioxidant and Cytoprotective Effects in HepG2 Cells: Possible Role of ERK-dependent Nrf2 Activation. *International Journal of Molecular Sciences* 2019, 20:4082.
 - [46] Liu M, Guan G, Wang Y, Lu X, Duan X, Xiaotian X: p-Hydroxy Benzaldehyde, a Phenolic Compound from *Nostoc commune*, Ameliorates DSS-induced Colitis Against Oxidative Stress via the Nrf2/HO-1/NQO-1/NF- κ B/AP-1 Pathway. *Phytomedicine* 2024, 133:155941.
 - [47] Wang SH, Tsai KL, Chou WC, Cheng HC, Huang YT, Ou HC, et al.: Quercetin Mitigates Cisplatin-induced Oxidative Damage and Apoptosis in Cardiomyocytes Through Nrf2/HO-1 Signaling Pathway. *The American Journal of Chinese Medicine* 2022, 50:1281–1298.