Mechanism of *Gastrodia elata* Bl. in the Treatment of Chronic Heart Failure Based on Network Pharmacology and Molecular Docking

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Abstract [**Objectives**] To screen the active components and core targets of *Gastrodia elata* Bl. based on network pharmacology, and further explore its potential molecular mechanism in preventing and treating chronic heart failure (CHF). [**Methods**] The candidate active constituents of *G. elata* Bl. were screened by HERB, ETCM (Encyclopedia of Traditional Chinese Medicine) database, and bioinformatics analysis tool BATMAN-TCM platform, Swiss Target Prediction platform was used to predict compound targets, and CHF disease targets were searched in GeneCards and OMIM databases; the intersection targets were taken, and the String database and Cytoscape 3.10.0 software were used for protein-protein interaction (PPI) and topological analysis to obtain key active constituents and core target genes; the online tool bioinformatics platform was used for gene ontology (GO) function and Kyoto Encyclopedia of Genes and Genomes (KEGG) pathway enrichment analysis of common targets. Using the active constituent of *G. elata* Bl. as ligand and its core target as receptor, molecular docking visualization was carried out. [**Results**] 136 common targets of active constituents of *G. elata* Bl. and CHF were screened out, which mainly involved AGE-RAGE signaling pathway, lipid and atherosclerosis, interleukin-17 signaling pathway, non-alcoholic fatty liver, diabetic cardiomyopathy, cancer and other signaling pathways. The core target proteins mainly included albumin, catalytic subunit α activated by protein kinase CAMP, insulin gene, interleukin-1β, tumor necrosis factor, estrogen receptor 1, interferon γ, *etc.* The results of molecular docking showed that the ligand of the compound had good affinity with the target receptor. [**Conclusions**] *G. elata* Bl. prevents and treats CHF through multiple components, multiple targets, and multiple pathways. The potential mechanism of *G. elata* Bl. in treating CHF was preliminarily explored, providing a certain theoretical basis for subsequent research on its pharmacological material basis in treating CHF.

Key words Network pharmacology, *Gastrodia elata* Bl., Chronic heart failure, Molecular docking

1 Introduction

Chronic heart failure (CHF) is a cardiovascular clinical syndrome with structural destruction or abnormal function of the heart caused by various reasons. It can lead to impaired systolic or diastolic function of the heart, and decreased blood output of the heart. It is mainly manifested by congestion in the pulmonary circulation and (or) systemic circulation, and obvious insufficient blood reflux and perfusion of organs in the tissue[1]. It is the terminal stage of many cardiovascular diseases, and its prevalence, recurrence rate and mortality rate are increasing year by year[2]. Western medicine has certain limitations in the treatment of heart failure, and traditional Chinese medicine has become a hot spot in the treatment of diseases because of its advantages of multi-components, multi-targets and multi-pathways. *Gastrodia elata* Bl. is a perennial herb of Orchidaceae, which was first recorded in *Shen Nong's Materia Medica*[3]. Its long medicinal history and remarkable clinical efficacy have attracted many scholars to explore. It is a traditional precious Chinese medicine, widely distributed in southwest, east, central, northeast and other regions of China[4]. It is sweet in taste, neutral in nature, and attributive to the liver meridian. It has the effects of lowering blood pressure, lowering lipids, protecting blood vessels, and improving blood perfusion. The chemical components in *G. elata* Bl. are mainly gastrodin, vanillin, palmitic acid, p-hydroxybenzyl alcohol, p-hydroxybenzaldehyde, *etc.*[5], which are usually considered to be the main components of *G. elata* Bl. to exert pharmacological effects, and their strong antioxidant properties have attracted much attention in the treatment of vascular diseases. Network pharmacology has established a new model of complex network relationships between multiple targets and diseases, which provides a reference for disease pathogenesis and its therapeutic targets, and plays a guiding role in discovering molecular mechanisms of drug efficacy and developing new drugs[6]. In this study, the active constituents and potential mechanism of *G. elata* Bl. in the treatment of CHF were explored at molecular level by using network pharmacology method, so as to provide a theoretical basis for *G. elata* Bl. in the treatment of CHF.

2 Materials and methods

2.1 Screening of effective active constituents and targets of *Gastrodia elata* Bl.

Using "*G. elata* Bl." as the key word, the effective active constituents of *G. elata* Bl. were searched in HERB (http://herb.ac.cn/), the encyclopedia database of traditional Chinese medicine ETCM (http://www.tcmip.cn/ETCM/), and the bioinformatics analysis tool BATMAN-TCM (http://bionet.ncpsb.org/batman-tcm/). Score cutoff = 10 was set in the BATMAN-TCM database, the corrected P value was 0.05, the reliability score of the target in ETCM ≥ 0.8. The Swiss Target Prediction platform (https://www.SwissTargetPrediction.cn/) was further utilized to obtain the targets of the active constituents of *G. elata* Bl. After sorting and deleting the duplicate targets, the gene names of the targets were normalized using the Uniprot database (https://www.uniprot.org/).

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2.2 Screening of disease targets for chronic heart failure In human gene database (GeneCards, https://www.genecards.org) and online human Mendelian genetic database (OMIM, http://omim.org/), "chronic heart failure" was entered into each database as a search term, and disease targets in each database were obtained, and the obtained targets were merged to delete duplicate values.

2.3 Screening of common targets of *Gastrodia elata* Bl. and chronic heart failure The online tool bioinformatics platform (https://www.bioinformatics.com.cn/) was used to obtain the intersection of *G. elata* Bl. target genes and CHF target genes, and a Wayne diagram was drawn for visual display.

2.4 Construction and analysis of protein interaction network The construction of the PPI network can be used to predict the core targets in the interaction network. The common targets of *G. elata* Bl. for CHF treatment were uploaded to the STRING database (https://string-db.org/), "Homo sapiens" was selected as the species, the minimum required interaction score was set to medium confidence (0.400), and independent nodes in the network were hidden, to obtain PPI relationships, and export data files. Then, Cytoscape 3.10.0 software was used to visualize the PPI network and CytoNCA plug-in was used to screen the central targets. According to the Degree value in the analysis results, the top 10 core targets of *G. elata* Bl. in the treatment of CHF were screened, and the molecular mechanism of *G. elata* Bl. in the treatment of CHF was further explained from the perspective of core targets.

2.5 GO function and KEGG pathway enrichment analysis The intersection genes of *G. elata* Bl. and CHF were imported into the David database[7] (https://david.ncifcrf.gov/), and "OFFICIAL GENE SYMBOL" was used as the identifier and "Homo sapiens" was used as the species category to perform gene ontology (GO) functional enrichment analysis (biological process, cell composition, molecular function) and Kyoto encyclopedia of genes and genomes (KEGG) pathway enrichment analysis to reveal the biological mechanism by which drugs regulate disease symptoms. The online tool bioinformatics platform (https://www.bioinformatics.com.cn/) was used for plot analysis, and the results were presented as bubble chart.

2.6 Molecular docking validation method

2.6.1 Preparation of small molecule ligand and protein receptor files. The 2D structure of the main active ingredient of the drug was obtained through the PubChem database (https://pubchem.ncbi.nlm.nih.gov/), and the minimum energy value was output by Chem3D software and the format was converted to mol2. The uniprot database (https://www.uniprot.org/) and the PDB database (https://www.rcsb.org/) were used to obtain the PDB format file of the 3D structure of the core target, and the obtained proteins and small molecules were imported into Auto Duck 1.5.7 software one by one, water molecules were removed, they were hydrogenated, and finally saved as PDBQT format files.

2.6.2 Preparation of docking active pockets. The PDBQT file obtained in the previous step was imported into Auto Dock 1.5.7 software, the molecular docking range was set to the maximum value of 1.000, and the central coordinate value (x/y/z) was input into the vina script to further obtain the minimum binding energy.

2.6.3 Preparation of visual docking diagram. The minimum binding energy ligand file and protein receptor file in pdbqt format were selected and imported into PyMOL software together. 20 molecular docking maps can be obtained at one binding, and the clearest one was selected as the final display map.

3 Results and analysis

3.1 Screening results of active constituents and drug targets of *Gastrodia elata* Bl. 43 active constituents and 103 corresponding drug target genes were found in the HERB database; 18 active constituents and 257 corresponding drug target genes were found in Batmen-tcm database; 21 active constituents and 245 corresponding drug target genes were found in ETCM database; after removing duplicate and target-free components, 28 active constituents and corresponding 553 target genes were finally selected.

3.2 Screening of chronic heart failure disease targets 12 462 gene targets were screened in the Genecard database, and the median Score value was repeatedly taken. Finally, 1 561 gene targets were obtained by setting Score≥13.8; 618 gene targets were obtained by searching in OMIM database, and a total of 2 057 gene targets were obtained after removing duplicate targets.

3.3 Screening of common targets of *Gastrodia elata* Bl.—chronic heart failure Using online mapping tool bioinformatics, 553 *G. elata* Bl. drug targets and 2 057 chronic heart failure disease targets were imported, and Wayne diagram was drawn. After taking the intersection of the two, 136 drug-disease common targets were obtained (Fig. 1).

3.4 Construction and analysis of protein interaction network 136 intersection targets were imported into the STRING database to obtain the PPI network relationship diagram, as shown in Fig. 2 and Fig. 3). The diagram includes 134 nodes and 1 369 edges. The targets are represented by network nodes, network nodes of different colors represent different interactions, and the number of lines represents the intensity of interactions between targets. The greater the number, the stronger the effect and the greater the Degree.

The top 10 core target proteins in the figure are: ALB (albumin), PRKACA (protein kinase CAMP-activated catalytic subunit α), INS (insulin gene), IL1β (interleukin-1β), TNF (tumor necrosis factor), ESR1 (estrogen receptor 1), PPARG (peroxisome proliferator-activated receptor γ recombinant protein), IFNG (interferon γ), HIF1A (hypoxia-inducible factor 1 subunit α), SRC (non-receptor tyrosine kinase).

3.5 GO function and KEGG pathway enrichment analysis GO enrichment analysis and KEGG pathway enrichment analysis were performed on common targets using DAVID database. GO enrichment analysis included biological process (BP), cellular component (CC) and molecular function (MF). A total of 529 biologi-

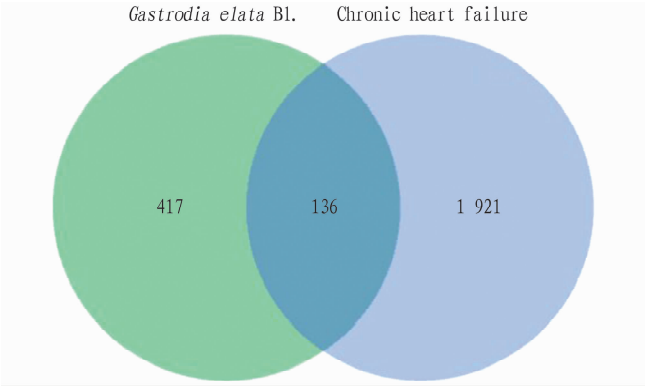


Fig. 1 Venn diagram of the intersection target of effective active constituents of *Gastrodia elata* Bl. and chronic heart failure

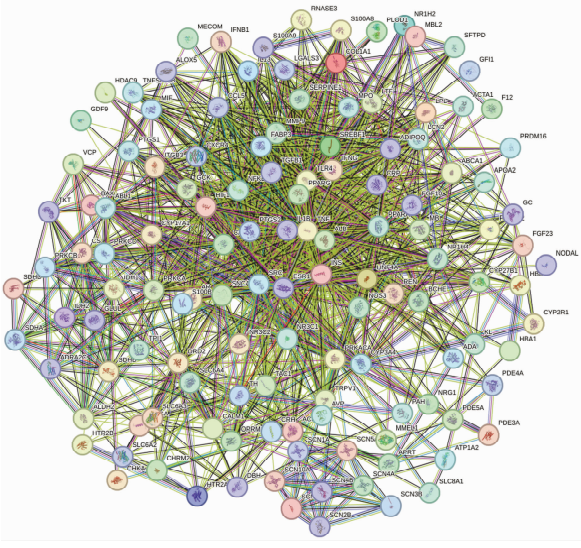


Fig. 2 PPI network diagram of *Gastrodia elata* Bl. in the treatment of CHF

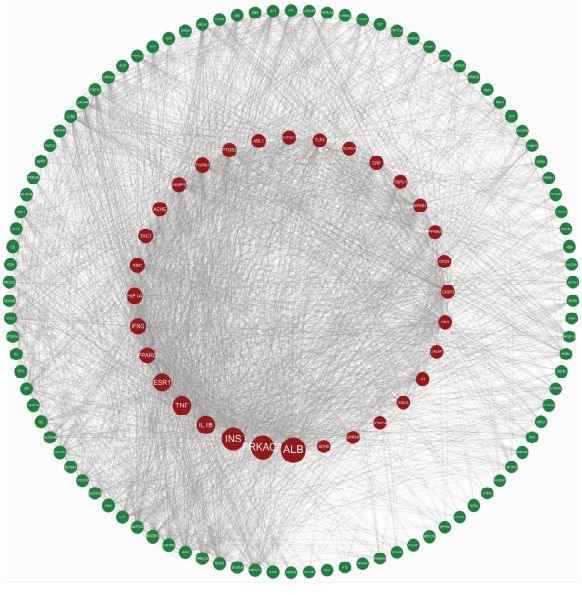
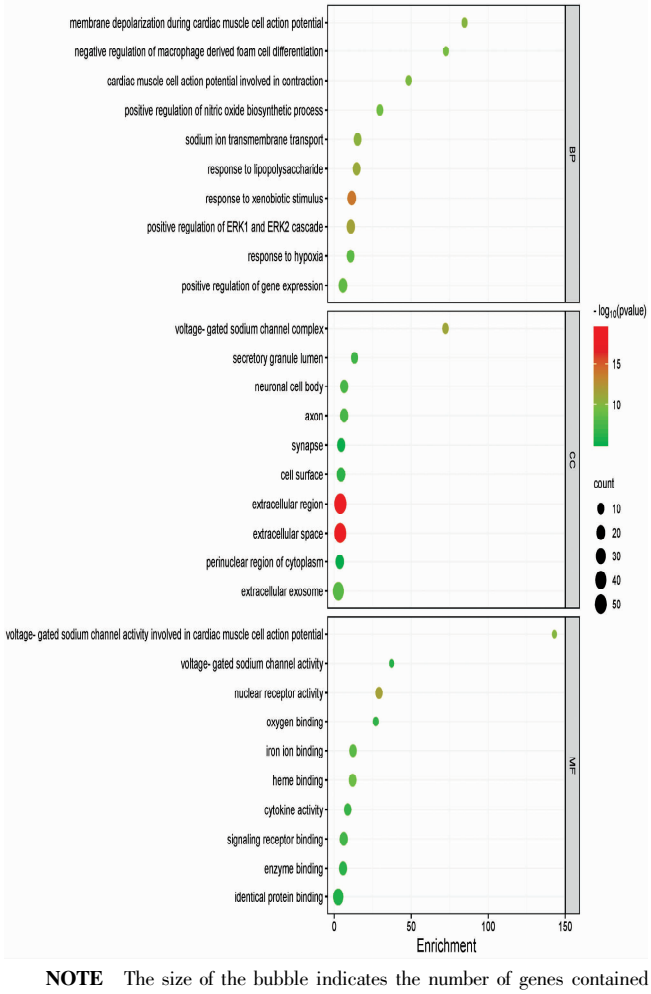


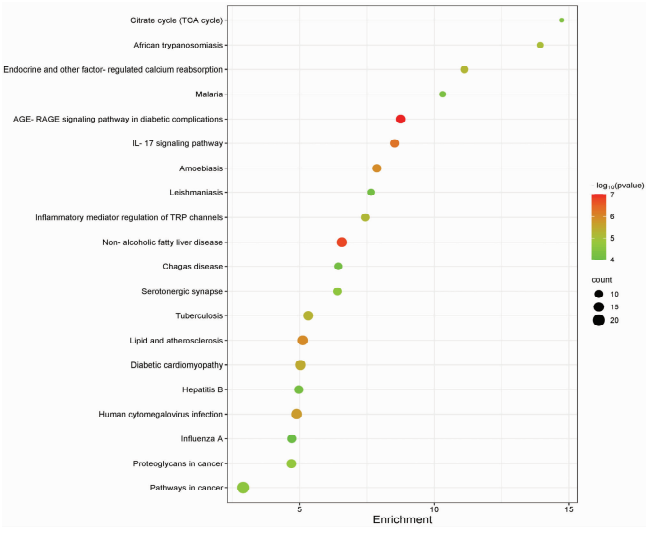
Fig. 3 Visual analysis of the protein interaction target of CHF treated with *Gastrodia elata* Bl. by Cytoscape

cal processes, 59 cellular components, 115 molecular functions and 125 KEGG items were collected. Then, according to the *P* value, the top 10 items of GO enrichment analysis and the top 20 items of KEGG pathway enrichment were listed and uploaded to the bioinformatics platform for bubble chart visualization analysis (Fig. 4 and Fig. 5). BP is mainly enriched in the response to exogenous stimuli, the positive regulation of ERK1 and ERK2 cascade, the response to hypoxia, the positive regulation of gene expression, the membrane depolarization during the action potential of cardiomyocytes, and the contraction of cardiomyocytes. CC is mainly concentrated in extracellular space, extracellular domain, cell surface, neuronal cell body, axon, synapse, sodium channel complex, *etc.*; MF is mainly enriched in exactly the same protein binding, enzyme binding, signal receptor binding, nuclear receptor activity, sodium channel activity, cytokine activity, *etc.* KEGG pathway is mainly enriched in AGE-RAGE signaling pathway in diabetic complications, lipid and atherosclerosis, interleukin-17 (IL-17) signaling pathway, non-alcoholic fatty liver disease, diabetic cardiomyopathy, cancer pathway and other aspects.



NOTE The size of the bubble indicates the number of genes contained in the enrichment analysis, and the color depth of the bubble indicates the *P* value (the smaller the *P* value, the redder the color).

Fig. 4 Bubble diagram of GO functional enrichment analysis



NOTE The size of the bubble indicates the number of genes of the corresponding signal pathway, and the color depth of the bubble indicates the *P* value (the redder the color, the smaller the *P* value).

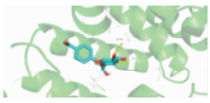
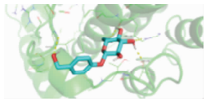
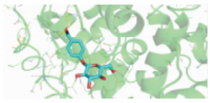
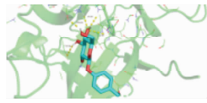
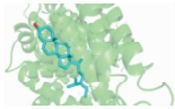
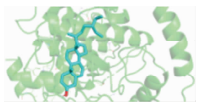
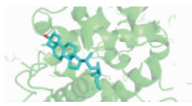
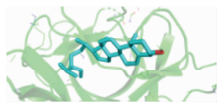
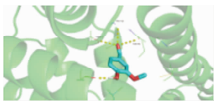
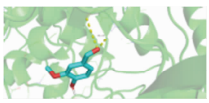
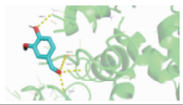
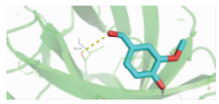
Fig. 5 Bubble diagram of KEGG pathway enrichment analysis

3.6 Molecular docking results Molecular docking was carried out using the drug-disease core targets ALB, PRKACA, INS, and IL1B as receptors, and the key active components of *G. elata* Bl., β-sitosterol, and vanillin as ligands. The results showed that the binding energy of gastrodin, β-sitosterol, and vanillin with the drug-disease core targets ALB, PRKACA, INS, and IL1B was lower than −4.0 kcal/mol (see Table 1 for binding energy, and see Table 2 for docking diagram). After visualization, it can combine with the help of intermolecular forces to form a stable spatial conformation.

Table 1 Molecular docking binding energy (kcal/mol) required for docking of 3 effective active constituents and 4 target proteins of *Gastrodia elata* Bl.

	ALB	RKACA	INS	IL1B
Gastrodia elata Bl.	−6.7	−5.9	−6.9	−5.6
β-sitosterol	−5.2	−5.8	−5.6	−4.5
Vanillin	−5.4	−5.8	−4.4	−4.0

Table 2 Molecular docking results of 3 effective active constituents and 4 target proteins of *Gastrodia elata* Bl.

	ALB	RKACA	INS	IL1B
Gastrodia elata Bl.				
β-sitosterol				
Vanillin				

4 Discussion

CHF is a chronic, refractory and spontaneously progressive disease with complex etiology[8], and it is closely related to myocardial remodeling, inflammatory stimulation, oxidative stress and ion channels. *G. elata* Bl. is widely planted in Guangxi, Yunnan, Guizhou and other places. Because of humid climate, fertile soil and abundant land resources, it is very suitable for the growth of *G. elata* Bl. It has been used as a prescription for the treatment of cardiovascular diseases and food resource for thousands of years[9]. Relevant studies have shown that the active constituents and targets of *G. elata* Bl. have certain effects on the treatment of CHF.

Gastrodin has been shown to regulate multiple intracellular signaling pathways and reduce the inflammatory stimulation effects on functional cells such as cardiomyocytes and endothelial cells[10-11]. In rat ischemia-reperfusion experiments, the level of anti-inflammatory factor IL-10 was increased in gastrodin-treated rats, while the levels of pro-inflammatory factors IL-6, IL-1 and tumor necrosis factor (TNF)-α were all decreased, suggesting that gastrodin can alleviate the inflammatory response and exert cardioprotective effects[12]. Differential gene aggregation analysis was performed on pharmacological target of gastrodin's cardioprotective effects. The results showed that it was interrelated with multiple signaling pathways such as glucose stabilization, insulin response, and regulation of glycolipid metabolism[13-14]. The results of metabolomics, RNA sequencing and transcriptome gene sequencing experiments show that gastrodin is closely related to cell cycle, DNA replication ability, DNA damage and repair, and gene regulation[15-16]. β-sitosterol can act on epidermal cells in inflammasomes and macrophages, and protect cardiomyocytes and vascular endothelial cells by inhibiting the activation of MAPK signaling pathway and reducing the production of TNF-α and IL-6 in cells[17]. According to *in vitro* experiments[18], lipopolysaccharide (LPS) leads to increased expression of inflammatory cytokines such as tumor necrosis factor-α (TNF-α), cyclooxygenase-2 (Cox-2), and interleukin-6 (IL-6) in blood vessels, and β-sitosterol inhibits LPS induction through MAPK and NF-κB signaling pathways, which can reduce the expression and secretion of these cytokines, helping to improve atherosclerosis. It also inhibits angiotensin II (Ang II)-induced cell proliferation by preventing aortic smooth muscle cell cycle progression, promoting apoptosis, in-

hibiting autophagy, and inhibiting contractile-synthetic phenotype switching effects, and it can be used as a therapeutic strategy to prevent cardiovascular diseases[19]. In addition, combined with Western blotting and quantitative PCR at protein and gene level experiments[20], it was observed that β-sitosterol activated peroxisome proliferator-activated receptor γ (PPARG) and adenosine 5'-monophosphate-activated protein kinase (AMPK), and negatively regulated the expression of mammalian target of rapamycin (mTOR), which contributed to the inhibition of Ang II-induced vascular smooth muscle cell migration and reduced intracellular oxidative stress and inflammatory response. Vanillin has antioxidant, antiproliferative, antidepressant and antiglycation properties. Vanillin can reduce the levels of oxidative stress markers malondialdehyde, inflammatory cytokines TNF-α, IL-6 and IL-1β, and pro-apoptotic proteins Bax and caspase-3 in rat cardiomyocytes, enhance cardiac angiogenesis and regulate oxidative stress, inflammation and apoptosis through Akt/HIF-1α/VEGF signaling pathway, and effectively improve cardiomyocyte necrosis[21]. In doxorubicin (doxo)-induced H9c2 cell experiments[22], vanillin can inhibit the accumulation of reactive oxygen species (ROS) induced by doxorubicin oxidative stress and hinder ERK phosphorylation, and restore cardiomyocyte viability, with a potential protective effect on cardiotoxicity. Through network pharmacology and molecular docking technology, it was found that gastrodin, β-sitosterol, vanillin, *etc.* were the main active compounds of *G. elata* Bl. to exert therapeutic effects; ALB, PRKACA, INS, IL1B, *etc.* were the key targets of *G. elata* Bl., and may mainly exert therapeutic effects through signaling pathways such as HIF-1α, Akt, AMPK, and MAPK; *G. elata* Bl. may be the key mechanism in the treatment of heart failure by inhibiting oxidative stress, inflammatory response in vivo and anti-apoptosis.

To sum up, this study used network pharmacology and molecular docking technology to preliminarily explore the complex mechanism of multi-component, multi-target, and multi-pathway synergistic interaction in prevention and treatment of CHF by *G. elata* Bl., providing a reference for the related research in the future. However, there are certain limitations in predicting the mechanism of action of drugs and diseases based on public databases and bioinformatics analysis, and it is necessary to verify it from more molecular, cellular, animal and clinical experimental studies to provide a more credible scientific basis for *Gastrodia elata* Bl. to treat CHF.

References

[1] WANG H, LIANG YC. 2018 Chinese guidelines of heart failure[J]. Chinese Journal of Cardiology, 2018, 46(10): 760 – 789. (in Chinese).

[2] HU SS. Report on cardiovascular health and diseases in China 2021: An updated summary[J]. Chinese Circulation Journal, 2022, 37(6): 553 – 578. (in Chinese).

[3] WEI FQ, HUANG R, HE HY, *et al.* Research progress on pharmacological action and application of *Gastrodia elata* Bl.[J]. Chinese Journal of Ethnomedicine and Ethnopharmacy, 2021, 30(11): 72 – 76. (in Chinese).

[4] XU B, WU C, LI ZJ, *et al.* Resource distribution and post-harvest investigation of *Gastrodiae rhizoma*[J]. Chinese Journal of Information on Traditional Chinese Medicine, 2021, 28(7): 11 – 16. (in Chinese).

[5] CHEN C, LIN BB, SU PC, *et al.* Phenolic compositions and *in vitro* antioxidant activities of *Gastrodia elata* Bl. f. elata from different habitats in China[J]. Journal of Northwest A&F University (Natural Science Edition), 2021, 49(12): 144 – 154. (in Chinese).

[6] PINZI L, RASTELLI G. Molecular docking: Shifting paradigms in drug discovery[J]. International Journal of Molecular Sciences, 2019, 20(18): 4331.

[7] SHERMAN BT, HAO M, QIU J, *et al.* DAVID: A web server for functional enrichment analysis and functional annotation of gene lists (2021 update)[J]. Nucleic Acids Research, 2022, 50(W1): W216 – W221.

[8] HE XJ. Research progress on pathogenesis, diagnosis and treatment of heart failure[J]. Practical Journal of Cardiac Cerebral Pneumal and Vascular Disease, 2018, 26(7): 1 – 8. (in Chinese).

[9] WANG XJ, JIANG F, LI YL. Analysis of effect of Tianma (*Gastrodiae rhizoma*)[J]. Journal of Liaoning University of Traditional Chinese Medicine, 2022, 24(4): 74 – 79. (in Chinese).

[10] JIANG J, HUANG D, LI Y, *et al.* Heart protection by herb formula BanXia BaiZhu TianMa Decoction in spontaneously hypertensive rats[J]. Evidence-Based Complementary and Alternative Medicine, 2019, 2019: 5612929.

[11] XING Y, LI L. RETRACTED: Gastrodin protects rat cardiomyocytes H9c2 from hypoxia-induced injury by up-regulation of microRNA-21[J]. International Journal of Biochemistry and Cell Biology, 2019, 109: 8 – 16.

[12] YANG W, TENG L, DING JW, *et al.* Protective effect of gastrodin pretreatment on myocardial ischemia reperfusion injury[J]. Journal of Clinical Cardiology, 2017, 33(4): 381 – 385. (in Chinese).

[13] LU J, MA X, GAO WC, *et al.* Gastrodin exerts cardioprotective action via inhibition of insulin-like growth factor type 2/insulin-like growth factor type 2 receptor expression in cardiac hypertrophy[J]. ACS Omega Journal, 2021, 6(26): 16763 – 16774.

[14] BAI Y, MO K, WANG G, *et al.* Intervention of gastrodin in type 2 diabetes mellitus and its mechanism[J]. Frontiers in Pharmacology, 2021, 12: 710722.

[15] GONG X, CHENG J, ZHANG K, *et al.* Transcriptome sequencing reveals *Gastrodia elata* Blume could increase the cell viability of eNPCs under hypoxic condition by improving DNA damage repair ability[J]. Journal of Ethnopharmacology, 2022, 282: 114646.

[16] XIA C, ZHOU H, XU X, *et al.* Identification and investigation of miRNAs from *Gastrodia elata* Blume and their potential function[J]. Frontiers in Pharmacology, 2020, 11: 542405.

[17] LIAO PC, LAI MH, HSU KP, *et al.* Identification of beta-sitosterol as *in vitro* anti-inflammatory constituent in *Moringa oleifera*[J]. Journal of Agricultural and Food Chemistry, 2018, 66(41): 10748 – 10759.

[18] BI Y, LIANG H, HAN X, *et al.* Beta-sitosterol suppresses LPS-induced cytokine production in human umbilical vein endothelial cells via MAPKs and NF-kappaB signaling pathway[J]. Evidence-Based Complementary and Alternative Medicine, 2023, 2023: 9241090.

[19] CHEN Y, HE S, ZENG A, *et al.* Inhibitory effect of beta-sitosterol on the Ang II-induced proliferation of A7r5 aortic smooth muscle cells[J]. Analytical Cell Pathology (Amsterdam), 2023, 2023: 2677020.

[20] HE SM, HE SQ, CHEN YK, *et al.* Beta-sitosterol modulates the migration of vascular smooth muscle cells via the PPARG/AMPK/mTOR pathway[J]. Pharmacology, 2022, 107(9 – 10): 495 – 509.

[21] ELSEWEIDY MM, ALI SI, SHAHEEN MA, *et al.* Vanillin and pentoxifylline ameliorate isoproterenol-induced myocardial injury in rats via the Akt/HIF-1alpha/VEGF signaling pathway[J]. Food & Function, 2023, 14(7): 3067 – 3082.

[22] SIRANGELO I, SAPIO L, RAGONE A, *et al.* Vanillin prevents doxorubicin-induced apoptosis and oxidative stress in rat H9c2 cardiomyocytes[J]. Nutrients, 2020, 12(8): 2317.