

# *Study on the Mechanism of "Radix Pseudostellariae—Ligusticum Chuanxiong—Cassia Twig - Rehmannia" in Treating Chronic Heart Failure*

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**Abstract:** Objective: To explore the mechanism of "Radix pseudostellariae - Ligusticum Chuanxiong - cassia zhi - Rehmannia" in the treatment of cardiogenic heart failure through network pharmacology. Methods The drug targets of "Radix pseudostellariae - Ligusticum Chuanxiong - cassia zhi - Rehmannia" were screened based on TCMSP database and TCMID database, and the disease-related targets of heart failure were screened by GeneCard database. The intersection targets of active ingredients and disease-related targets were obtained, that is, the potential targets for drug treatment of chronic heart failure. The "drug-active ingredient-Potential target" network was constructed using Cytoscape 3.7.2 software, and the core active ingredients in the network were analyzed. The intersection targets were imported into STRING database for protein interaction (PPI) network analysis, and potential core targets were screened out. DAVID database was used to analyze the GO function and KEGG pathway enrichment of potential core targets. Results A total of 24 active ingredients and 193 potential targets were screened. The core active components such as luteolin, ursolic acid, sterol, stigmasterol and myricone were obtained. STAT3, JUN, TP53, MAPK1, HSP90AA1, RELA, AKT1, ESR1, MAPK14, FOS, CCND1, IL6, RB1, CDKN1A, VEGFA, NR3C1, MAPK8, TNF, RXRA, AR core targets; A total of 173 signaling pathways were selected, including IL-17 signaling pathway, PI3K-Akt signaling pathway, TNF signaling pathway and calcium signaling pathway. Conclusion "Radix pseudostellariae - ligusticum Chuanxiong - cassia twidder-Rehmannia" plays a role in the treatment of chronic heart failure through the action of luteolin, ursolic acid, sterol, phytosterol, myricones and other active components in IL-17 signaling pathway, PI3K-Akt signaling pathway, TNF signaling pathway, calcium signaling pathway and other pathways.

## 1. Introduction

Heart failure (referred to as heart failure) is a group of complex clinical syndrome caused by various causes of cardiac structure and/or function abnormalities, with ventricular systolic and/or diastolic dysfunction as the main manifestation, and is the outcome of all cardiovascular diseases in

the late stage<sup>[1]</sup>. By 2020, the estimated number of cardiovascular diseases in China is 330 million, of which 8.9 million are heart failure. The prevalence of heart failure was 1.38% in people aged 35 years and above, 3.09% in people aged 60-79 years and 7.55% in people aged 80 years and above<sup>[2]</sup>. Therefore, controlling heart failure, effectively improving the quality of life of patients and reducing the incidence of heart failure is one of the focuses of cardiovascular disease prevention and treatment.

Modern medicine believes that the pathogenesis of heart failure is mainly due to activation of Frank-Starling mechanism, neurohumoral mechanism (including RAAS activation, etc.) and ventricular remodeling for various reasons, so that the heart and other important organs of the body have corresponding functional and structural changes, such as chest tightness, asthma, decreased activity tolerance, edema, etc. At present, the main treatment of heart failure is drug therapy. It is represented by the combination of  $\beta$ blockers, diuretics, ACEI/ARB/ARNI, SGLP2 inhibitors and other drugs. When necessary, digoxin, dopamine and other positive inotropic drugs of digitalis or non-Digitalis are used. Non-drug therapies include cardiac resynchronization therapy (CRT), implantable cardioverter defibrillator (ICD), left ventricular assist device (LVAD), and heart transplantation<sup>[3]</sup>. At present, there are still some deficiencies in these treatments, and new treatments are still being explored globally.

Traditional Chinese medicine has rich experience and obvious curative effect in preventing heart failure, delaying the course of heart failure and improving the quality of life of patients. The description of heart failure in ancient medical books, mostly in the records of "edema", "heart bi", "heart water" and other diseases. Its pathogenesis is the loss of Qi, blood and Yin and Yang of the heart, blood stasis, phlegm turbidness, water stopped accumulation. Deficiency of essence is the basic pathogenesis of heart failure, which determines the development trend of heart failure. Standard body is the variable factor of heart failure, affecting the disease changes of heart failure<sup>[4]</sup>. My supervisor Yuanlin Lei, chief physician, study "Theory of cold injury", "Synopsis of Golden Chamber" and other classics, combined with their own cognition and clinical experience of heart failure to Yixinfutingyin treatment of chronic heart failure, chronic heart failure in patients with obvious improvement effect. In the preliminary study, it was found that the tutor paid attention to the care of the spleen and stomach, warming the Yang and regulating the pulse, and the most frequently used drug combinations were Radix daustariae, Ligusticum Chuanxiong, cassia twig and Rehmannia. Therefore, this paper will further explore the effective active ingredients, core targets and potential mechanism of action of "Radix pseudostellariae - Ligusticum Chuanxiong - cassia twig - Rehmannia" in the treatment of chronic heart failure through network pharmacology, in order to provide certain basis for subsequent clinical and experimental studies.

## 2. Methods

### 2.1 Screening of active ingredients and targets of "Radix pseudostellariae - Ligusticum Chuanxiong - cassia twig -Rehmannia"

By retrieving TCMSP (<http://tcmospw.com/tcmosp.php>) and TCMIP (<http://www.tcmip.cn/TCMIP/index.PHP/Home/Login/LoginHTML>) database access pseudostellaria root, rhizoma ligustici wallichii - cassia twig - Rehmannia the Drug ingredients, and to satisfy both Oral bioavailability (OB) $\geq$ 30% and Drug-likeness(DL)  $\geq$ 0.18. Then, the corresponding targets of active ingredients were obtained through the TCMSP Database, and the results were imported into the UniProt Database platform (<http://www.Unitprot.org/>) and converted into a unified gene name, and the gene source was defined as "Homo sapiens".

## 2.2 Target acquisition of chronic heart failure disease

The search term "heart failure", through the GeneCard (<https://www.genecards.org/>) database retrieval is associated with chronic heart failure disease targets. And "pseudostellaria root, rhizoma ligustici wallichii - cassia twig - raw land", the active ingredient target matching, to obtain the intersection target (common target), that is, the potential target of "Radix pseudostellariae - Ligusticum Chuanxiong - cassia twig -Rehmannia" in the treatment of chronic heart failure. Using Venny 2.1 platform (<https://bioinfogp.cnb.csic.es/tools/venny/index.HTML>) map drug and disease crossover gene Wayne.

## 2.3 Construction of protein interaction (PPI) network

The intersection targets were imported into the STRING database (<http://string-db.org/cgi/input.pl>), and the species was set as "Homo sapiens", confidence score  $\geq 0.9$ , PPI network analysis was conducted, and the network data was saved as "tsv" format file. Import Cytoscape 3.7.2 software to construct the PPI network diagram. Topology analysis of PPI networks was carried out using the Network Analyzer plug-in function of Cytoscape software to obtain the network connectivity Degree, and potential core targets were selected based on the Degree value. A higher Degree indicates a stronger interaction between the target protein and other proteins.

## 2.4 Construction of "drug-active ingredients-potential targets" network

To introduce the drugs, active ingredients of "Radix pseudostellariae - Ligusticum Chuanxiong - Cassia twig - Rehmannia" and their potential targets (intersecting targets) in the treatment of chronic heart failure into Cytoscape 3.7.2 software, and build the network of "active ingredient-potential targets". In addition, Network Analyzer plug-in in Cytoscape software is used to analyze the topology property of the network, analyze parameters such as Nodes and Degree, and use the Degree value to reflect the importance of nodes in the network and analyze the core active components in the network.

## 2.5 GO function and KEGG pathway enrichment analysis

DAVID platform was used to analyze GO function and KEGG pathway enrichment of core targets. GO functional analysis mainly includes Biological Process (BP), Molecular Function (MF) and Cell Component (CC). Using microscopical platform (<http://www.bioinformatics.com.cn/>) to visualization of the data, to analyze "pseudostellaria root, rhizoma ligustici wallichii - cassia twig - Rehmannia" the way of regulation of heart failure.

## 3. Results

### 3.1 Potential targets of "Radix pseudostellariae - Ligusticum Chuanxiong - cassia twig - Rehmannia" in the treatment of heart failure

Through TCMSP and TCMIP database retrieval, and according to OB and DL conditions screening, after deleting duplicate values, a total of 24 active ingredients were obtained. Target proteins corresponding to active ingredients were collected through TCMSP database, and the same targets were deleted to obtain 197 action targets. A total of 13223 heart failure related disease targets were obtained by GeneCards database retrieval and removal of duplicate genes. The target of disease related to heart failure was mapped with the active ingredient target of "Radix

pseudostellariae - ligusticum Chuanxiong - cassia twig - Rehmannia" and then the intersections were taken. Finally, 193 potential targets of "Radix pseudostellariae - ligusticum Chuanxiong - cassia twig - Rehmannia" were obtained (Figure 1).

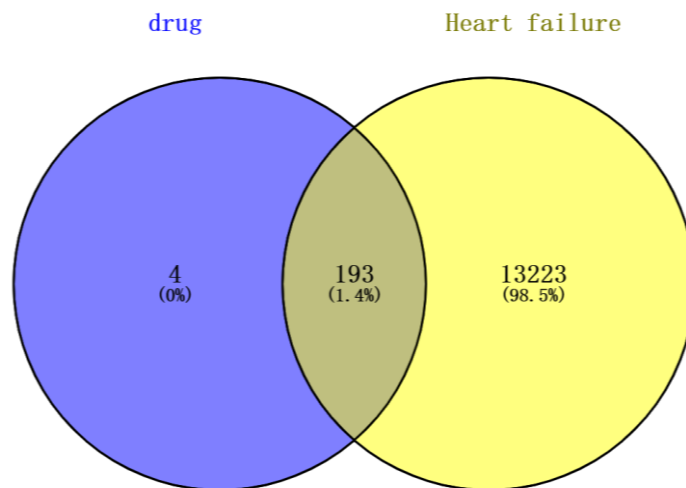


Figure 1: Venny diagram of drug target-disease target

### 3.2 PPI network of "Radix pseudostellariae - Ligusticum Chuanxiong - cassia twig - Rehmannia" in treating chronic heart failure

The PPI analysis of intersection targets was carried out using STRING 11.0 database, and the results were imported into Cytoscape 3.7.2 software to construct PPI Network. Network Analyzer plug-in was used to analyze the topological properties of the network, and the targets were screened according to the top 20 values of Degree. STAT3, JUN, TP53, MAPK1, HSP90AA1, RELA, AKT1, ESR1, MAPK14, FOS, CCND1, IL6, RB1, CDKN1A, VEGFA, NR3C1, MAPK8, TNF, RXRA, AR, It is suggested that the above genes may be the potential core targets of this drug combination in the treatment of chronic heart failure, and the results are shown in Figure 2.

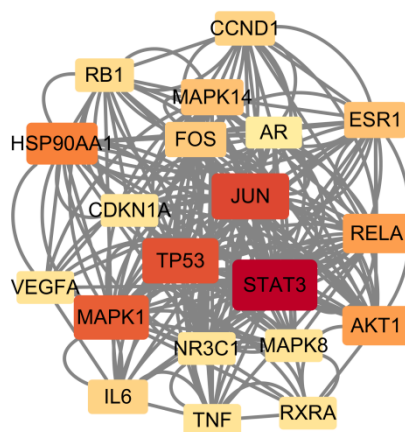


Figure 2: Top 20 targets of PPI network Degree

### 3.3 "Drug - active ingredient - potential target" network of "Radix pseudostellariae - Ligusticum Chuanxiong - Cassia twig - Rehmannia" in the treatment of chronic heart failure

The 24 active ingredients and 193 potential targets were selected through Cytoscape 3.7.2

software to construct the "drug-active ingredient-potential targets" network of Yixinhupingyin in the treatment of heart failure (FIG. 3). The network consisted of 220 nodes and 1669 edges, which included 4 traditional Chinese medicines, 24 active ingredients and 193 potential targets. Edges represented the interactions between drugs and compounds, compounds and targets, and targets and targets. Among them, Luteolin, ursolic acid, beta-sitosterol, stigmasterol, Myricanone and other corresponding targets were the most, which may be the core active components of the drug combination in the treatment of chronic heart failure. Studies have shown that luteolin has certain antihypertensive, vasodilating and anti-arteriosclerosis effects on the cardiovascular system. Zou Xiaoyan<sup>[5]</sup> et al. found that luteolin could reduce myocardial infarction area after MIRI, improve CF, reduce leakage of myocardial marker enzyme LDH, and improve left ventricular systolic function after perfusion. Luteino-7-diglucuronide<sup>[6]</sup> not only can prevent Stop iso-induced myocardial injury, fibrosis formation, but also significantly prevent anti-isoproterenol-induced myocardial dilation changes and cardiomyocyte hypertrophy. Ursolic acid can achieve lipid lowering by activating AMPK signaling pathway. Previous studies have shown that ursolic acid reduces the levels of Tumor necrosis factor (TNF- $\alpha$ ), Interleukin-1 $\beta$  (IL-1 $\beta$ ), IL-6, and IL-18 in diabetic nephropathy mice through TLR4-mediated inflammatory pathway. Reduce inflammation and prevent diabetic nephropathy; Ursolic acid can improve left ventricular shortening and left ventricular ejection fraction, increase nitric oxide (NO) level and inhibit reactive oxygen species production in mice. Reduce cardiac cell apoptosis<sup>[7]</sup>.

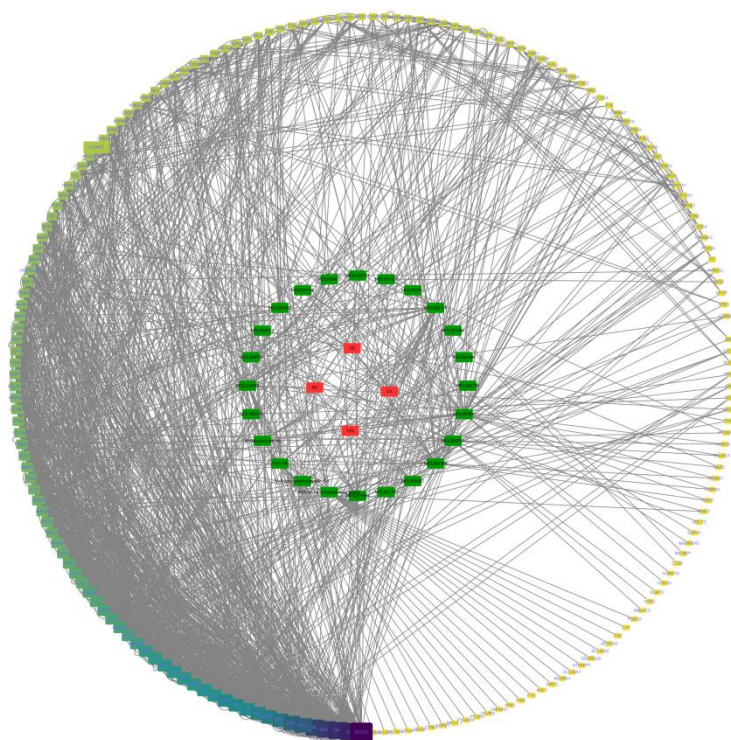


Figure 3: Network of "Drug -- Active ingredient -- potential target" (red represents drug, green represents ingredient, gradient color represents target)

### 3.4 KEGG signal pathway and GO functional enrichment analysis

Use DAVID platform to KEGG pathway enrichment analysis of potential core targets, a total of 173 signaling pathways were screened. The key pathways involved include IL-17 signaling pathway, PI3K-Akt signaling pathway, and TNF signaling pathway, Calcium signaling pathway, p53 signaling pathway, Apoptosis, HIF-1 signaling pathway, FoxO signaling pathway, cAMP signaling

pathway, etc. The bubble diagrams of the first 10 signaling pathways related to chronic heart failure are shown in Figure 4. The 10 potential core targets of this drug combination in the treatment of chronic heart failure are basically directly related to the first 10 signaling pathways. GO function rich set analysis can obtain 1045 GO items, including 782 BP items, 107 CC items and 156 MF items, as shown in Figure 5. BP mainly focuses on response to drug, response to xenobiotic stimulus, and positive regulation of cell proliferation in aging cells proliferation, positive regulation of gene expression, and negative regulation of apoptotic process process, positive regulation of protein phosphorylation and other biological processes; CC mainly involves the integral component of plasma membrane, plasma membrane and integral component of presynaptic membrane membrane, postsynaptic membrane, presynaptic membrane and so on; MF mainly involves the activity of enzyme binding sites and RNA polymerase II transcription factors. RNA polymerase II transcription factor activity, ligand-activated sequence-specific DNA binding, identical protein binding, identical protein binding, protein homodimerization activity, etc. The biological processes closely related to chronic heart failure were selected for further enrichment analysis. It was found that the potential core targets of this drug combination for chronic heart failure were mainly involved in drug response, response to foreign biological stimuli, positive regulation of aging, cell proliferation, positive regulation of gene expression and other biological processes.

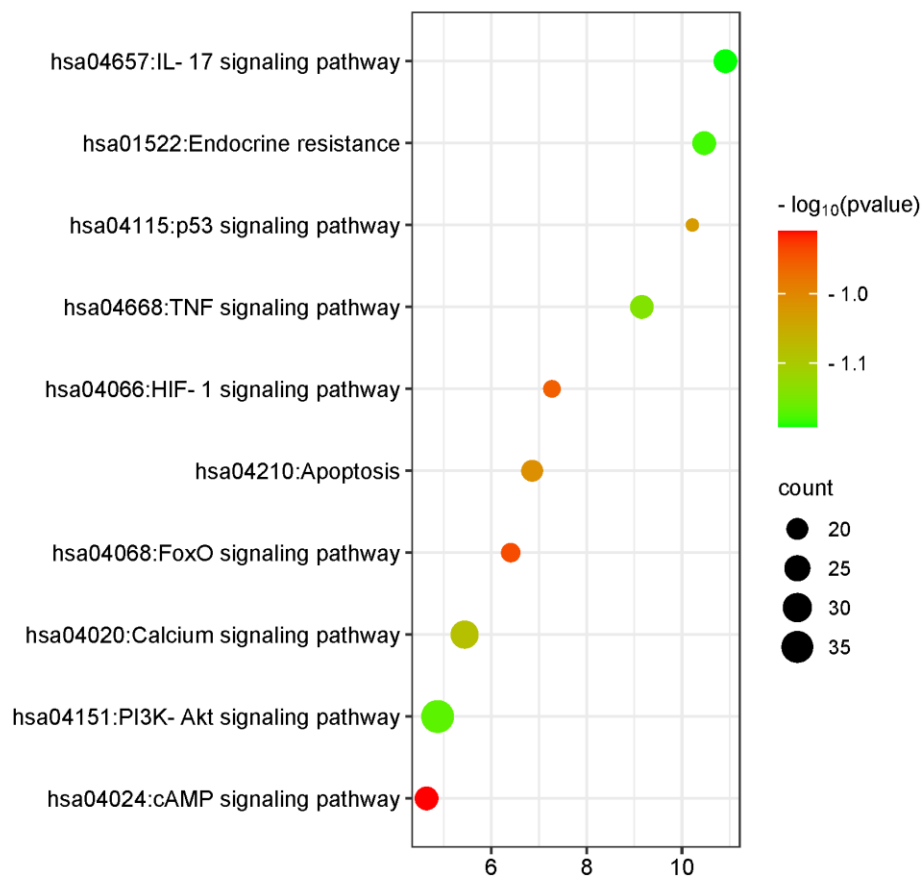


Figure 4: KEGG bubble map of the first ten signaling pathways

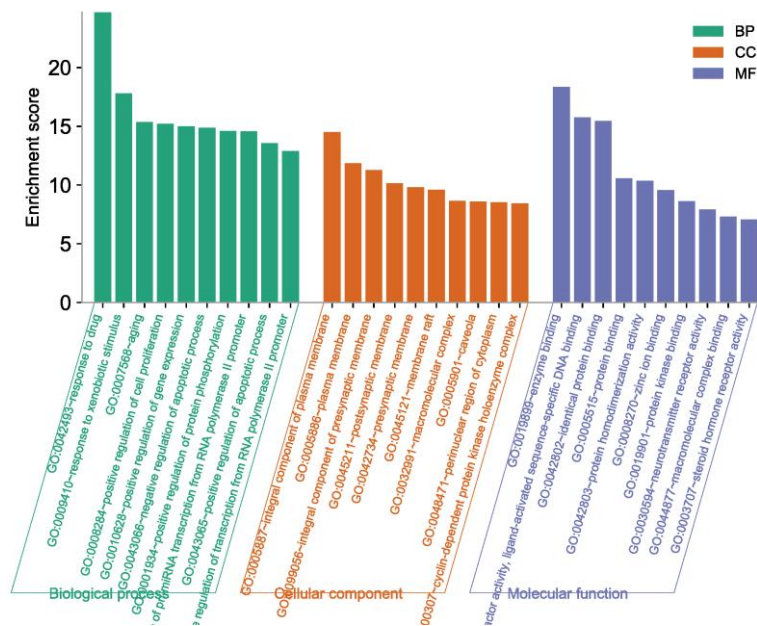


Figure 5: GO analysis diagram (three-in-one diagram of BP, CC and MF)

## 4. Discussion

The drug combination of *Radix pseudostellariae* - *Ligusticum Chuanxiong* - *cassia twig* - *Rehmanniae Radix* was derived from *Yixinfutingyin*, a common prescription used by tutors in treating patients with chronic heart failure. The prescription is used to treat the syndrome of deficiency of heart and kidney Yang in heart failure. The tutor believes that patients with heart failure have deficiency of qi and blood for a long period of illness, which causes blood stasis and damages qi and jin. They often give cassia twigs warm channels and regulate qi to help Yang, *Taizi Radix* to strengthen spleen and Qi and moisten lung to generate jin, *Chuanxiong* to promote qi and blood, and *Rehmannia* to promote Yin. Modern pharmacological studies have found that *Cassia twig* expansion of blood vessels, such as anti-inflammatory effects<sup>[8]</sup>, *Radix pseudostellariae* has the effects of reducing myocardial damage, anti-inflammatory and antioxidant<sup>[9]</sup>, *Ligusticum Chuanxiong* has the effects of anti-atherosclerosis, dilating blood vessels and protecting vascular endothelium<sup>[10]</sup>, *Rehmanni Huang* regulates angiogenesis through Ras/Raf/MEK/ERK signaling pathway and PI3K/AKT signaling pathway<sup>[11]</sup>.

The potential core targets are concentrated in the PI3K signaling pathway and its upstream and downstream pathways. It was found that PI3K family played an important role in cardiac physiological and pathological processes such as myocardial fibrosis, ventricular remodeling, cardiac autophagy, myocardial ischemia injury, and so on. By regulating TGF- $\beta$  and miR-21, PI3K $\alpha$  was increased to achieve anti-myocardial fibrosis effect. When PI3K $\gamma$  is activated in pathological condition, the cardiac function of mice is impaired and the contractile force of the heart is reduced. When stimulated (such as adrenaline), the level of cAMP of the heart is increased in mice with no catalytic activity of PI3K $\gamma$ . Further showing better contractility<sup>[12]</sup>. The downstream factors of PI3K signal pathway participate in the work of P53 signal pathway and cAMP signal pathway. The P53-mediated apoptotic signaling pathway can induce stress in heart failure. Accumulated p53 forms a tetramer, activates the transcription of various molecules, induces apoptosis and cell growth arrest, inhibition of angiogenesis, DNA repair, aging, etc<sup>[13]</sup>. Studies have shown that cAMP and PDE can regulate cardiac contractility<sup>[14]</sup>.

To sum up, Through the network pharmaceutical analysis of "*Radix pseudostellariae* - *ligusticum*

Chuanxiushi - cassia twig - Rehmannia" and chronic heart failure, it was found that its main mechanism of action was in anti-myocardial fibrosis, inhibition of ventricular remodeling, vasodilation, improvement of vascular endothelial function, etc., and this mechanism had multiple synergistic effects on coronary heart disease, cardiomyopathy and other cardiovascular diseases and endocrine system diseases. This result is consistent with the current clinical application law, clinical efficacy and research results. Some of the targets and molecular mechanisms have been verified by a number of experimental studies and used for clinical drug development. This study focused on the molecular mechanism level to study the effect of "Radix pseudostellariae - Ligusticum Chuanxiong - cassia branch - Rehmanniae" on chronic heart failure, and explored a number of potential therapeutic targets, which laid a good theoretical foundation for the next experimental verification, and opened up the direction for further revealing its molecular mechanism.

## References

- [1] Heart failure Group, Cardiology Branch of Chinese Medical Association, Heart failure Professional Committee of Chinese Medical Doctor Association, Editorial Board of Chinese Journal of Cardiovascular Diseases. Chinese Guidelines for Diagnosis and Treatment of heart failure 2018[J]. Chinese Journal of Cardiovascular Diseases, 2018, 46(10): 760-789.
- [2] Chinese Cardiovascular Health and Disease Report Compilation Group. Chinese Journal of Circulation, 2020 Summary [J]. 2021, 36(6):521-545.
- [3] Wang H, Li Y Y. Comprehensive management of patients with aggravated chronic heart failure Chinese expert consensus 2022[J]. Chinese Journal of Circulation, 2022, 37(03):215-225.
- [4] Wu Mianhua. Internal Medicine of Traditional Chinese Medicine [M]. Beijing: China Traditional Chinese Medicine Press Co., LTD., 2021.
- [5] Zou Xiaoyan, Fan Xiangcheng, Tian Youqing etc. Luteolin against myocardial ischemia/reperfusion injury and inhibit lipid metabolism and apoptosis research [J]. Journal of nanjing university of Chinese medicine, 2020, 4 (3): 380-386. The DOI: 10.14148 / j.i SSN. 1672-0482.2020.0380.
- [6] Du Xiaoye, Gu Chenglin, Zhang Yongdeng. Luteolin—7-2 glucose aldehyde nucleoside resistance isopropyl adrenaline induced myocardial damage research [J]. Journal of Chinese medicine, 2019 (01): 86-89 + 98. DOI: 10.16368 / j.i SSN. 1674-8999.2019.01.020.
- [7] Li Jian et al. Ursolic acid alleviates inflammation and against diabetes-induced nephropathy through TLR4-mediated inflammatory pathway. [J]. Molecular medicine reports, 2018, 18(5): 4675-4681.
- [8] Xiao S, Song M, Chen C. The design of disengaging mechanism of radix pseudostellariae and soil[J]. IOP Conference Series Materials Science and Engineering, 2017, 274(1):012015.
- [9] Teng Liqing, Zhou Tao, Wang Xiao et al. Research progress of chemical constituents and pharmacological effects of Pseudostellariae pseudostellariae [J]. Food and Drug, 201, 23(01):73-79.
- [10] Zou C, Tan H, Zeng J, et al. Hepatitis C virus nonstructural protein 4B induces lipogenesis via the Hippo pathway[J]. Archives of Virology, 2023, 168(4).
- [11] Fang Z, Hu X, Chen Z, et al. Radix pseudostellariae of Danzhi Jiangtang capsule relieves oxidative stress of vascular endothelium in diabetic macroangiopathy[J]. Saudi Pharmaceutical Journal, 2020, 28(6).
- [12] Qin W, Cao L, Massey IY. Role of PI3K/Akt signaling pathway in cardiac fibrosis. Mol Cell Biochem. 2021 Nov;476(11):4045-4059. doi: 10.1007/s11010-021-04219-w. Epub 2021 Jul 10. PMID: 34244974.
- [13] Shi W L, Zhao J, Yuan R, et al. Combination of Ligusticum Chuanxiong and Radix Paeonia Promotes Angiogenesis in Ischemic Myocardium through Notch Signalling and Mobilization of Stem Cells[J]. Evidence-based Complementary and Alternative Medicine, 2019, 2019:1-12.
- [14] Calamera G, Moltzau LR, Levy FO, Andressen KW. Phosphodiesterases and Compartmentation of cAMP and cGMP Signaling in Regulation of Cardiac Contractility In Normal and Failing already. Int J Mol, Sci. 2022 Feb; 15 (23 (4): 2145. Doi: 10.3390 / ijms23042145 PMID: 35216259; PMCID: PMC8880502.