

Study on the Mechanism of Gardenia Bean Soup for Gastroesophageal Reflux Disease Based on Network Pharmacology

Yunxiao Wu, Shuting Wang, Chenyue Xu, Xiaoming Wang*

School of Pharmacy, Guangdong Pharmaceutical University, Guangzhou, Guangdong, China

** Corresponding Author*

Abstract: Gastroesophageal reflux disease (GERD) is highly prevalent, and Gardenia bean soup is traditionally used to clear and disperse depressed heat; therefore, this study employs network pharmacology to explore its therapeutic molecular mechanisms and provide evidence for the modernization of traditional Chinese medicine. Drug and disease targets were obtained from TCMSP and other databases, and their intersection was used to construct a "drug-component-target" network, with a PPI network constructed using STRING and Cytoscape, GO and KEGG enrichment analysis performed with DAVID, and molecular docking carried out with Autodock. A total of 89 active components and 179 intersection targets were screened, with core targets including TP53, TNF, STAT3, AKT1, and others; GO and KEGG enrichment yielded numerous items and signaling pathways, and molecular docking showed that most components bound strongly to the targets. These findings suggest that Gardenia bean soup may regulate the PI3K-Akt/NF- κ B and other pathways through components such as quercetin, acting on targets including STAT3, AKT1, IL1B, BCL2, MMP2, MMP9, PTEN, and IGF1, thereby exerting anti-inflammatory and mucosal repair effects and reflecting the characteristics of multi-component, multi-target, and multi-pathway action.

Keywords: Network Pharmacology; Gardenia Bean Soup; Gastroesophageal Reflux; Core Targets; Molecular Docking

1. Introduction

Gastroesophageal Reflux Disease (GERD) is a common clinical disease. The incidence and recurrence rate are increasing year by year. The main manifestations are heartburn and acid

reflux caused by abnormal reflux of gastric contents. In severe cases, esophageal mucosal injury can be complicated. Modern medicine believes that its core mechanism lies in the imbalance between injury and defense factors: transient lower esophageal sphincter relaxation occurs spontaneously and frequently, esophageal hiatal hernia destroys the anti-reflux barrier, and increases the chance of gastric acid and bile reflux; the weakening of esophageal peristalsis, the decrease of saliva secretion and the damage of mucosal barrier delay acid clearance and reduce mucosal resistance. In addition, psychological factors such as anxiety and depression amplify the perception of symptoms, making the disease lingering and difficult to heal. According to its symptoms, traditional Chinese medicine classifies it into the category of 'vomiting acid' and 'esophageal hiccup'. The core pathogenesis is stomach disharmony and descending, and stomach qi ascending, which is closely related to liver qi invading stomach, damp-heat accumulation in middle energizer, spleen-stomach deficiency, transportation and transformation disorder, and lung-stomach ascending and descending disorder [1].

Gardenia bean soup is derived from 'Treatise on Febrile Diseases', which is composed of Gardenia jasminoides Ellis and Semen Sojae Praeparatum. It is simple and specialized, and has the function of 'clearing heat and dredging qi'. The "Treatise on Febrile Diseases" contains its indications such as "deficiency and boredom without sleep, heart chagrin" and "chest suffocation". The pathogenesis of heat stagnation in the chest and diaphragm and poor qi movement is highly consistent with the performance of substernal burning pain, annoyance and acid reflux in GERD patients. Therefore, modern doctors have expanded its application in the disease [2]. Rooted in systems biology and bioinformatics, network pharmacology transcends the conventional

single-target research paradigm. By integrating publicly available databases with multi-omics data, this approach establishes a hierarchical network encompassing drugs, components, targets, and pathways, thereby enabling a systematic elucidation of the synergistic mechanisms underlying traditional Chinese medicine compound formulas. The present study employs network pharmacology to identify the bioactive constituents and putative targets of Gardenia bean soup. By intersecting these targets with GERD-associated genes and integrating protein-protein interaction network topology analysis with molecular docking validation, we aimed to elucidate the molecular mechanisms underlying its multi-component, multi-target, and multi-pathway synergistic effects against GERD, thereby interpreting the connotation of "clearing and ventilating stagnated heat" (Qingxuan Yure) in modern scientific terms. It provides modern pharmacological basis for classical prescriptions in the treatment of digestive system diseases.

2. Materials and Methods

2.1 Active Components and Target Acquisition of Gardenia Bean Soup

On the TCMSP platform, the active ingredients were obtained under the screening conditions of oral bioavailability $\geq 18\%$ and drug-likeness ≥ 0.12 , and the ingredients reported in the literature with pharmacological activity but not up to this standard were included to ensure the integrity of the active ingredients of Gardenia jasminoides Ellis and Semen Sojae Praeparatum. The platform can obtain the relevant targets corresponding to each component. For components without identified targets, target prediction was performed using the SwissTargetPrediction database, and all predicted targets with a probability greater than 0 were retained for subsequent analysis.

2.2 Identification of Gastroesophageal Reflux Disease-Associated Targets

Using "gastroesophageal reflux disease (GERD)" as the query keyword, we systematically retrieved and screened GERD-associated molecular targets from the GeneCards repository and OMIM.

2.3 Protein-Protein Interaction Analysis and Screening of Hub Targets

After intersecting the predicted targets of bioactive constituents with GERD-associated genes, the shared targets were visualized using the Venn module on the bioinformatics platform, they were imported into the STRING database, and the 'Multiple proteins' module was selected. The species was limited to *Homo sapiens*, and the confidence level was set to the highest value of 0.900. After the network diagram is generated, the isolated nodes free from the network are deleted, and the results are saved as TSV format. Network topology analysis was then conducted with Cytoscape (version 3.10.3), where nodes were ranked by degree value, with the corresponding network map highlighting high-degree hubs as candidate core targets, and the network topology parameters were calculated with the help of CytoNCA plug-in. The six dimensions of BC, CC, DC, EC, LAC and NC were integrated for multi-index cross-validation. Finally, the key core targets of Gardenia bean soup in the treatment of GERD were screened out.

2.4 Establishment of the Component-Target Interaction Network and Screening of Key Constituents

The active components of the drug and the intersection targets obtained by the 2.3 steps were introduced into Cytoscape3.10.3 to construct a 'drug-component-target' ternary network model to visually present the correlation between the compound, chemical composition and molecular targets. Based on the degree value, the active components were ranked. The larger the degree value, the more the connected targets and the more core the network position. The top 10 were taken as the core compounds of Gardenia bean soup in the treatment of GERD.

2.5 Enrichment Analysis of GO Biological Functions and KEGG Pathways

The intersecting targets were then imported into the DAVID database (Database for Annotation, Visualization and Integrated Discovery) for functional annotation and pathway enrichment analysis, and the species was selected as '*Homo sapiens*'. The relevant data of BP, CC, MF and KEGG were exported by click analysis, and the histogram and enrichment bubble diagram were made with the help of WeChat platform.

2.6 'Pathway-Target' Network Diagram

Cytoscape3.10.3 software was used to visualize

the pathway and intersection target data obtained from 2.5 and 2.3 parts respectively, so as to present the interaction network between them.

2.7 Molecular Docking Verification of Core Active Ingredients and Core Targets

The SDF format of the active ingredient was obtained from PubChem, and then converted to mol2 by Open Babel. After hydrogenation in AutoDock Tools, the ligand was specified and the torsion bond was set, and the pdbqt was output. The core target protein was retrieved from the PDB database according to the UniProt number, and the high-resolution structure was downloaded. After removing water molecules and original ligands by PyMOL, it was also hydrogenated into a pdbqt receptor file in AutoDock Tools.

In the docking stage, the ligand and the receptor are paired in pairs, the docking box is set and the grid parameters are generated. AutoGrid is used to calculate the energy grid point, and AutoDock Docking is used to perform the docking simulation. Finally, the optimal conformation of binding energy was extracted by Analyze module and imported into PyMOL for three-dimensional visualization to show the binding mode and interaction relationship between core compounds and key targets.

3. Results and Analysis

3.1 Active Ingredients, Target Acquisition of Gardenia Bean Soup and Screening of Targets for GERD

Active constituents of Gardenia bean soup were collected from TCMSP, while those exhibiting pharmacological activity but not meeting the screening thresholds were additionally identified from published literature. A total of 89 active components were obtained, including 76 gardenia and 13 fermented soya beans. The targets with predictive possibility >0 were retained, and a total of 684 drug-related targets were obtained after combined deduplication.

The targets obtained by GeneCards were screened by three medians, and 928 high-correlation targets were obtained with the threshold of 'Relevance score ≥ 21.41 '. After merging with OMIM targets, a total of 1966 GERD-related targets were obtained. Using the WeChat platform to draw the Venn diagram for visual comparison, 684 active component targets of Gardenia bean soup intersected with 1966

disease targets of GERD, and 179 overlapping targets were finally screened as potential targets for the treatment of GERD by Gardenia bean soup (see Figure 1).

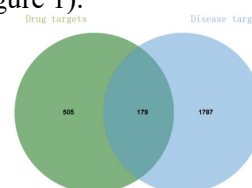


Figure 1. Drug Targets and Disease Targets Venn Diagram

3.2 Establishing the Protein-Protein Interaction Network and Extracting Key

Following construction of the preliminary network, 29 isolated nodes were removed; STRING analysis was then repeated for the 150 retained targets, yielding a refined PPI network (see Figure 2). The 179 shared targets were saved as a TSV file and loaded into Cytoscape 3.10.3 to generate the network map (see Figure 3). The nodes were divided into three groups according to the degree value: the darker the circle color, node size was positively correlated with degree value, with larger nodes representing more central targets in the network. CytoNCA plug-in was further used to screen the core targets. Firstly, the medians of six indicators including Betweenness, Closeness, Degree, Eigenvector, LAC and Network were calculated. The threshold was set as Betweenness ≥ 49.47215213 , Closeness ≥ 0.570512821 , Degree ≥ 50 , Eigenvector ≥ 0.066656038 , LAC ≥ 35.79310345 , Network ≥ 40.33578886 , and a total of 65 targets met the above conditions. Then these 65 targets were used to construct a sub-network for re-analysis and secondary screening. The thresholds were set to Betweenness ≥ 9.561139776 , Closeness ≥ 0.864864865 , Degree ≥ 54 , Eigenvector ≥ 0.126979873 , LAC ≥ 46.58181818 , Network ≥ 50.86897692 . Finally, 30 core targets of Gardenia bean soup in the treatment of gastroesophageal reflux disease were obtained. Based on this, the core target map (see Figure 4) and network parameter table were drawn (see Table 1).

Table 1. Core targets of Gardenia Bean Soup in the Treatment of Gastroesophageal Reflux Disease

name	UniProt ID	Degree	Betweenness Centrality
INS	P01308	64	22.45164095
CASP3	P42574	64	22.45164095
TP53	Q12888	64	22.45164095

AKT1	P31749	64	22.45164095
PPARG	P37231	64	22.45164095
STAT3	P40763	64	22.45164095
TNF	O75888	64	22.45164095
IL6	P05231	64	22.45164095
BCL2	P10415	63	20.72226734
MMP9	P14780	63	20.60687494
HIF1A	Q16665	63	20.8631077
IL1B	P01584	62	18.94025995
TGFB1	P01137	62	18.74088614
EGFR	P00533	62	19.52896953
ESR1	P03372	62	20.06686814
PTGS2	P35354	61	16.87847488
PTEN	P60484	60	16.50940573
FN1	P02751	60	15.64679997
EGF	P01133	60	16.72360292
IGF1	P05019	59	15.91584335
IFNG	P01579	59	13.64112596
MYC	P01106	59	15.3872468
MMP2	P08253	58	13.1866629
CCL2	P13500	58	12.54075365
FGF2	P09038	57	12.66242301
ICAM1	P05362	56	9.561139776
CCND1	P24385	56	13.51124335
ERBB2	P04626	55	12.32438077
BDNF	P23560	55	11.15379199
IL10	P22301	55	10.19125276

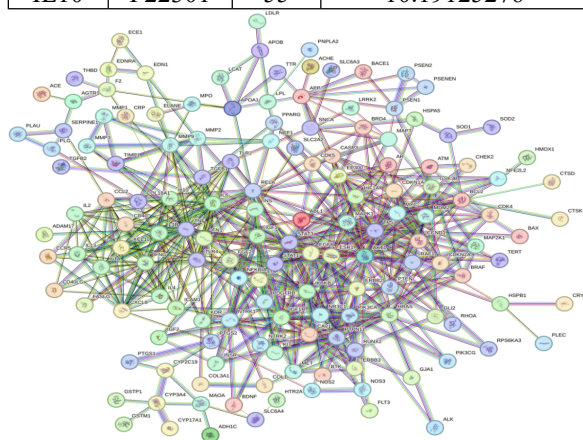


Figure 2. PPI Network Diagram

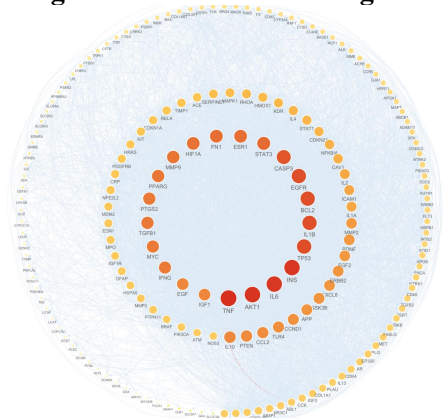


Figure 3. PPI Network Visualization Processing

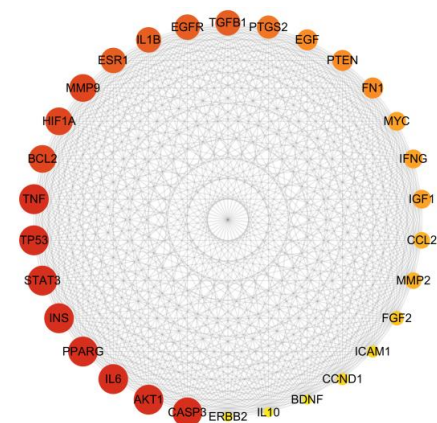


Figure 4. Visualization of 30 Core Targets

3.3 Construction and Analysis of 'Drug-Component-Target' Network

The active components, corresponding targets and identified intersection targets of *Gardenia jasminoides* Ellis and *Semen Sojae Praeparatum* were obtained. The three datasets were integrated in Cytoscape 3.10.3, generating a 'drug-compound-target' tripartite network for visualization (see Figure 5). The green inverted triangle in the network represents *Gardenia jasminoides* Ellis and *Semen Sojae Praeparatum*. The left node is the chemical composition of *Gardenia jasminoides* Ellis, the right node is the chemical composition of *Semen Sojae Praeparatum*, and the square node represents the intersection target. According to the degree value, the top 10 core compounds were taken as the pharmacodynamic material basis of *Gardenia* bean soup in the treatment of GERD, which were as follows: genistein, daidzein, 7,4'-Dihydroxyflavone, kaempferol, ursolic acid, ophthalmic acid, curdione, quercetin, 4,8,12,16-tetramethylheptadecan-4-olide, 3-Epioleanolic acid.

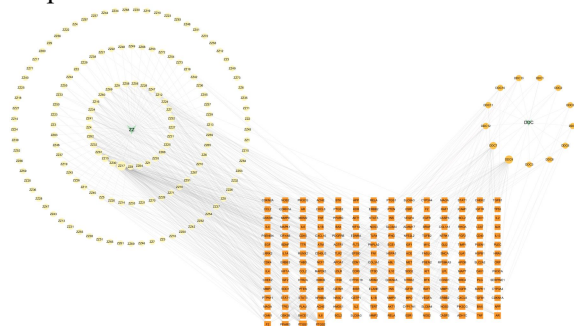


Figure 5 Drug-Component-Target Network Diagram

3.4 GO and KEGG Enrichment Analysis

A total of 1308 items were obtained by GO functional enrichment. Among them, 1006

biological processes are mainly involved in the positive regulation of gene and cell proliferation, the negative regulation of apoptosis process, etc. There were 124 cell components, which were mostly located in subcellular structures such as plasma membrane, protein complex and extracellular space. There are 154 molecular functions, mainly involving the same protein binding, enzyme binding, protein kinase activity and other biochemical functions. The top 20 most significant enrichments of each of the three categories were selected to draw a three-in-one visual map (see Figure 6). Pathway enrichment analysis based on the KEGG database revealed 185 significantly enriched signaling pathways. KEGG enrichment analysis revealed that the core pathways closely associated with the therapeutic effects of Gardenia bean soup against GERD primarily included the cancer-related pathways, proteoglycans in cancer, AGE-RAGE signaling pathway in diabetic complications, and PI3K-Akt signaling pathway. The top 20 signal pathways with the most significant enrichment were selected, and the enrichment bubble diagram was drawn with the Enrichment value as the abscissa and the pathway name as the ordinate (see Figure 7). The bubble size in the figure reflects the number of target genes involved in the pathway. The larger the bubble, the more genes involved and the higher the degree of enrichment. Greater color intensity is associated with reduced P-values, which in turn signifies more robust enrichment and increased confidence in the results.

3.5 'Target-Pathway' Network Construction

The 185 pathways obtained by KEGG enrichment analysis and 179 intersection targets

were imported and the square part of the 'target-pathway' network was constructed to represent the intersection target, and the circle represented the pathway (see Figure 8).

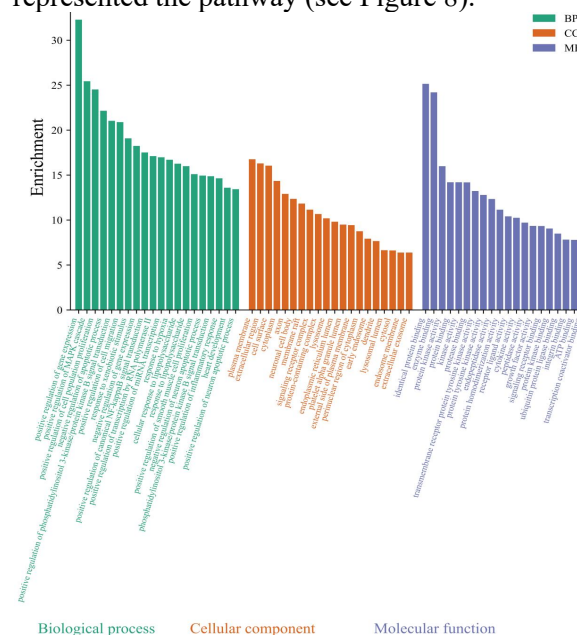


Figure 6. BP, CC, MF Three-in-one Histogram

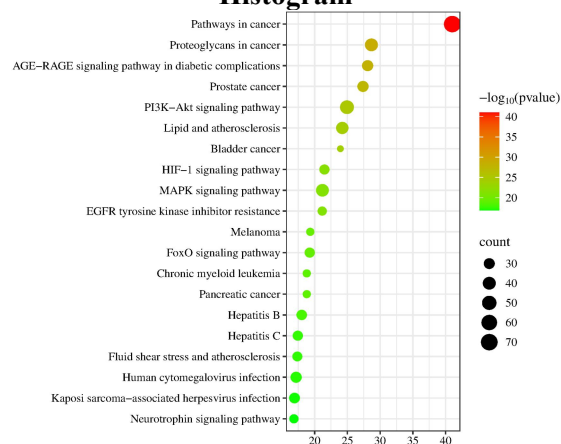


Figure 7. Enriched Bubble Diagram

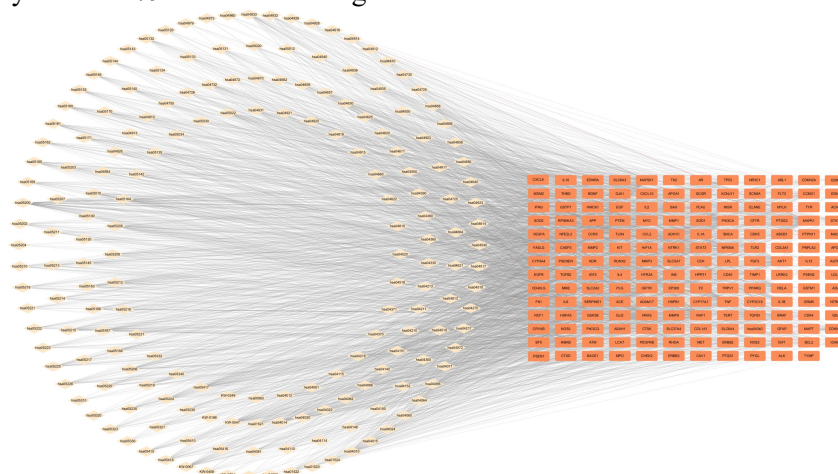


Figure 8. 'Pathway-Target' Network Diagram

3.6 Molecular Docking

The 10 key active components obtained in the 3.4 step were verified by molecular docking with 30 core targets (see Figure 9). The binding energy <-5.0 kcal/mol indicates that the binding activity is good [3], and <-7.0 kcal/mol indicates that the binding activity is very good. The deeper

the red in the heat map, the higher the affinity. Complexes with docking affinity lower than -5.0 kcal/mol were loaded into PyMOL 3.1.8 for three-dimensional visualization. Take the top 20 with the lowest binding energy as an example for illustration (see Figure 10).



Figure 9. Matrix heat Map of Molecular Docking Results

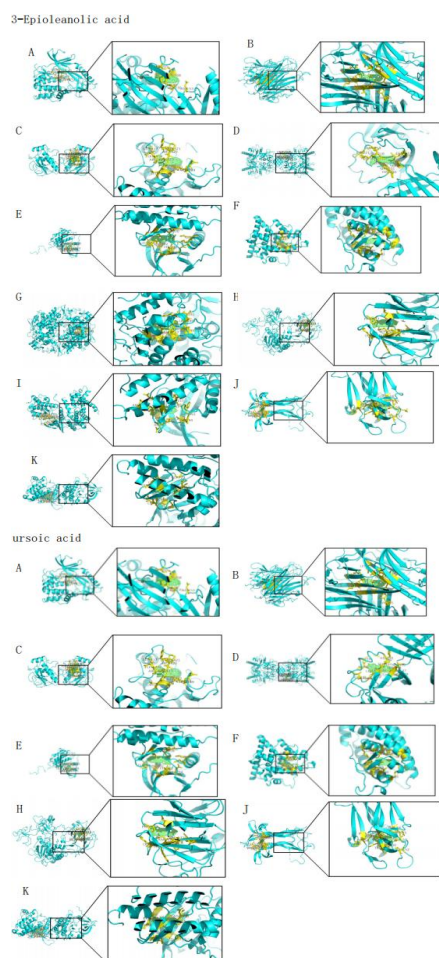


Figure 10. The Docking Diagrams of the 20 Molecules with the Lowest Binding Energy. (A) AKT1 (B) TNF (C) MMP9 (D) TGFB1 (E) EGFR (F)ESR1 (G) PTGS2 (H) MMP2 (I)CCND1 (J) BDNF (K) ERBB2

4. Discussion

4.1 Mechanism of Gardenia Bean Soup in the Treatment of Gastroesophageal Reflux Disease

Chinese herbal compound can regulate multiple signaling pathways through multiple components and multiple targets, inhibit esophageal inflammation in multiple ways, prevent reflux aggravation, and promote mucosal repair. It has obvious advantages in reducing adverse reactions, reducing recurrence rate, and improving clinical total effective rate [4-6]. The pathogenesis of GERD is mainly depression, acid, heat and reverse, and the syndrome of 'depression and heat' is formed due to the burning of the context by the real fire in the stomach and the transformation of liver depression into fire [7]. Excessive activation of NF- κ B-driven inflammatory signaling, together with increased production of downstream pro-inflammatory mediators (IL-1 β and TNF- α), constitutes one of the key molecular underpinnings of the "stagnated heat" syndrome [8]. Gardenia bean soup's function of clearing stagnated heat and dredging qi movement can play an anti-inflammatory role by inhibiting this pathway, so that stagnated heat can be dispersed, qi movement can be smoothed, and reflux, heartburn and other symptoms can be alleviated. Experiments have confirmed that Gardenia bean soup can significantly reduce the content of

MDA and NO in esophageal tissue and plasma of reflux esophagitis model rats, increase SOD activity, and reduce the damage of oxygen free radicals to esophageal mucosa [9]. At the same time, it also increased plasma gastrin and motilin levels, decreased vasoactive intestinal peptide levels, relatively increased lower esophageal sphincter pressure, and reduced reflux [10].

4.2 Active components of Gardenia Bean Soup in The Treatment of Gastroesophageal Reflux Disease

Ten core active components of Gardenia bean soup in the treatment of GERD were screened by network pharmacology. They were quercetin, daidzein, genistein, 7,4'-Dihydroxyflavone, kaempferol, ursolic acid, acid, curdione, 4,8,12,16-tetramethylheptadecan-4-olide, 3-Epioleanolic acid. Among them, quercetin, genistein, daidzein, 7,4'-dihydroxyflavone and kaempferol are flavonoids, which inhibits macrophage-driven inflammation by regulating the NF- κ B pathway, thus producing anti-inflammatory activity. [11]. Quercetin can inhibit the expression of RAGE receptor, block the combination of AGEs and RAGE, inhibit the AGE-RAGE axis, block the excessive activation of PI3K-Akt signaling pathway, and reduce esophageal inflammation. Zheng Guo et al. [12] confirmed that quercetin can play a role in the treatment of GERD by inhibiting the production of IL-1 β and IL-6, reducing esophageal mucosal inflammation and promoting mucosal repair. Daidzein has a gastrointestinal protective effect, which can prevent the increase of hydrogen peroxide, calcium ion and free iron levels in tissues during reflux, improve the expression of pro-inflammatory cytokines, and have a protective barrier effect on esophageal injury [13]. Flavonoids have a variety of pharmacological activities, which can reduce the secretion of gastric digestive juice, repair damaged cells and gastric mucosal tissues, and have antioxidant activity [14].

4.3 Identification of Core Targets in the Treatment of GERD by Gardenia-Soybean Decoction

With the help of PPI network and CytoNCA plug-in screening, 30 core targets of Gardenia bean soup in the treatment of GERD were finally obtained. IL-6 is a cytokine that promotes inflammation and inflammation-cancer transformation. STAT3 is involved in the

IL-6/STAT3 inflammatory signaling pathway. Activation can up-regulate the mRNA and protein expression levels of pathway-related molecules and aggravate esophageal tissue inflammation [15]. AKT1 belongs to the Akt family. As a serine/threonine protein kinase, AKT1 is a key regulator involved in cell survival, proliferation and growth signaling pathways. It plays an important role in the PI3K/Akt pathway and participates in the inflammatory response [16]. TP53 is an important tumor suppressor factor, and its downstream protein p53 is associated with esophageal mucosal exposure and accelerated cell proliferation. Excessive acid-base stimulation will increase the expression of p53. Inhibition of TP53 can effectively reduce the expression of downstream proteins and alleviate abnormal cell proliferation [17]. Studies have shown that esophageal reflux stimulants can increase the level of TNF- α and lead to esophagitis. Therefore, the improvement of symptoms in patients with reflux esophagitis can be evaluated by reducing the expression of plasma IL-6 and TNF- α [18]. EGF is an epidermal growth factor. As a cytoprotective peptide, EGF not only enhances cell proliferation and differentiation, but also acts as a gastrointestinal mucosal protective factor, which is related to intestinal maturation and intestinal epithelial cell homeostasis [19]. EGF can participate in the occurrence and development of GERD.

4.4 The Pathway of Gardenia Bean Soup in The Treatment of Gastroesophageal Reflux Disease

KEGG enrichment analysis indicated that the enriched pathways underlying the therapeutic effects of Gardenia bean soup against GERD primarily comprised Pathways in cancer, Proteoglycans in cancer, the AGE-RAGE cascade in diabetic complications, and the PI3K-Akt cascade. PI3K/Akt is closely related to the proliferation and apoptosis of esophageal epithelial cells. Excessive proliferation and insufficient apoptosis may lead to esophageal cancer [20]. PI3K/Akt pathway is involved in most of the body's inflammatory response. PI3K activation activates downstream Akt to phosphorylate it, p-Akt activates downstream nuclear transcription factor NF- κ B, increases gene transcription of cellular inflammatory factors, and finally initiates the inflammatory response. NF- κ B is inhibited in the cytoplasm by

I κ B protein in the resting state, and acid and bile-like substances in the reflux can also activate NF- κ B and increase the expression of pro-inflammatory factors [21]. Studies have confirmed that exogenous anti-inflammatory substances can negatively regulate the progression of inflammatory response through the PI3K/Akt-NF- κ B pathway [22]. The occurrence of GERD begins with the deep tissue inflammatory immune response mediated by inflammatory factors. Inflammation can lead to esophageal injury. Therefore, controlling inflammation is the key to the treatment of GERD. Zhuang Jiayuan reported that n-3PUFAs markedly attenuated esophageal inflammation in a GERD rat model via modulation of the PI3K/Akt-NF- κ B axis. The receptor of AGE, RAGE, has a high affinity with AGEs, and the expression of RAGE increases during inflammatory response [23-24]. AGE-RAGE signaling further activates the MAPK cascade as well as the PI3K/Akt-NF- κ B axis, promote the inflammatory response, and participate in the inflammatory process of GERD [25]. Studies have shown that MyD88 and TRAF-6 are key proteins in the TLR4/NF- κ B pathway. Treatment with quercetin substantially lowers the protein levels of key components in this pathway and blocks the nuclear entry of p65 [26], which subsequently suppresses the secretion of inflammatory mediators such as TNF- α , IL-1 β , and IL-6, ultimately mitigating esophageal mucosal inflammation and tissue damage.

4.5 Innovations and Limitations

The innovation of this study is mainly reflected in two aspects: one is the expansion of the research perspective. In the past, the research of Gardenia bean soup mostly focused on the field of anti-depression, anti-anxiety and other mental and psychological fields. To our knowledge, this is the first study to apply this prescription to digestive system diseases, in which a network pharmacology strategy was employed to systematically reveal the mechanisms underlying its therapeutic effects against GERD, which filled the gap of classical prescriptions in the study of digestive diseases. The second is the integration of theoretical level. In the present study, the TCM pathogenesis of "stagnated heat" was correlated with modern medical mechanisms, including inflammatory responses, oxidative stress injury, and esophageal mucosal barrier disruption. This approach seeks to bridge

TCM theory and Western medicine at the molecular level, offering novel insights into the biological underpinnings of TCM syndromes.

There are also some shortcomings in this study. Firstly, network pharmacology is essentially a prediction method based on database and algorithm. The interaction between the screened components and targets needs to be further verified by in vivo and in vitro experiments. Secondly, molecular docking is only the result of computer simulation, which reflects the theoretical binding ability of ligands and receptors. The real biological effects need to be confirmed by cell experiments and animal models. Finally, the network pharmacology method has a greater weight on the high content components, and may ignore some trace components with extremely low content but strong biological activity. The contribution of these components is easily underestimated, and the follow-up study can be supplemented by means of metabolomics.

5. Conclusion

In this study, network pharmacology was used to screen out the key active components that may play a role in gastroesophageal reflux disease in Gardenia bean soup, such as quercetin and daidzein. Then their targets were predicted, and then intersected with GERD-related disease targets, and gradually established a 'drug-component-target' ternary network and protein interaction network. From the analysis results, STAT3, AKT1 and IL6 are the core targets, and the pathway mainly involves PI3K-Akt signaling pathway and AGE-RAGE signaling pathway in diabetic complications.

From the perspective of the mechanism of action, it is speculated that Gardenia bean soup is likely to rely on flavonoids such as quercetin and daidzein. By targeting several key nodes such as STAT3, AKT1, and TNF, it interferes with the abnormal activation of PI3K-Akt/NF- κ B and AGE-RAGE pathways, thereby pressing down the inflammatory response of the esophageal mucosa, reducing the damage caused by oxidative stress, and promoting the repair of damaged mucosa. These results preliminarily reveal the synergistic effect of 'multi-component, multi-target and multi-pathway' in the treatment of GERD, and provide some theoretical basis for the modern mechanism explanation of Gardenia bean soup for digestive system diseases. It also points out the direction for cell experiments,

animal experiments and clinical transformation research.

Acknowledgements

This research was supported by Project of Administration of Traditional Chinese Medicine of Guangdong Province of China (20252016), Medical Scientific Research Foundation of Guangdong Province of China (B2026081), Open Program of Guangdong Provincial Key Laboratory for Research and Evaluation of Pharmaceutical Preparations (2026).

Reference

- [1] Sun, P. (2012). The treatment of gastro-oesophageal reflux disease with traditional Chinese medicine. *Journal of Chinese Medicine*, (140), 48.
- [2] Ren, L., Fan, Y., Luo, H., Zhang, Y., Wang, Y., & Li, X. (2024). PACAP/VIP in the prefrontal cortex mediates the rapid antidepressant effects of zhizichi decoction. *Journal of Ethnopharmacology*, 335, 118638.
- [3] Huang, J., Lin, S., Lin, L., Zheng, J., Chen, Z., Xie, J., & Liu, X. (2025). Multi-pathway mechanisms of Liu junzi decoction in promoting glioma apoptosis and reversing drug resistance: Network pharmacology and experimental validation. *Frontiers in Pharmacology*, 16, 1682550.
- [4] Zhang, R., Yang, Z., Pan, X., Liu, Y., Huang, Q., Song, Z., Yao, Q., Li, D., & Zhang, Y. (2025). The positive role of Chinese herbal medicine as an adjunctive therapy for refractory gastroesophageal reflux disease: A systematic review and network meta-analysis. *Medicine*, 104(21), e42565.
- [5] Liu, J., Huang, J., Zhang, B., Yin, X., Lv, M., Liu, Z., Wang, F., & Tang, X. (2022). Treatment of the gastroesophageal reflux disease with Chinese herbal medicine (Banxia Xiexin decoction): Evidence from meta-analysis. *Evidence-Based Complementary and Alternative Medicine*, 2022, 1500660.
- [6] Wang, F., Zhang, B., Yin, X., Liu, Z., Lv, M., & Tang, X. (2020). Efficacy of Chinese herbal formula Sini Zuojin decoction in treating gastroesophageal reflux disease: Clinical evidence and potential mechanisms. *Frontiers in Pharmacology*, 11, 76.
- [7] Jiang, X., Cheng, H., Zhang, N., Li, Y., Wang, Y., & Chen, J. (2022). Study on syndrome rules of stagnated heat in liver and stomach of non-erosive reflux disease based on microecology of tongue coating. *Medicine*, 101(44), e31618.
- [8] Lawrence, T. (2009). The nuclear factor NF- κ B pathway in inflammation. *Cold Spring Harbor Perspectives in Biology*, 1(6), a001651.
- [9] Kim, S. H., Shin, M. R., Lee, A. R., Kim, D. H., Park, H. J., & Roh, S. S. (2020). Improvement of inflammation through antioxidant pathway of Gardeniae Fructus 50% EtOH extract (GE) from acute reflux esophagitis rats. *BioMed Research International*, 2020, 4826176.
- [10] Liu, X., Ma, Y., He, L., Zhang, H., Li, J., & Wang, Y. (2016). Effects of Zhizi Ganchen Chi Decoction, Dahuang Huanglian Xiexin Decoction and their combined formula on gastrointestinal hormones in rats with reflux esophagitis. *Chinese Journal of Basic Medicine in Traditional Chinese Medicine*, 22(6), 768-770.
- [11] Hämäläinen, M., Nieminen, R., Vuorela, P., Heinonen, M., & Moilanen, E. (2007). Anti-inflammatory effects of flavonoids: Genistein, kaempferol, quercetin, and daidzein inhibit STAT-1 and NF- κ B activations, whereas flavone, isorhamnetin, naringenin, and pelargonidin inhibit only NF- κ B activation along with their inhibitory effect on iNOS expression and NO production in activated macrophages. *Mediators of Inflammation*, 2007, 45673.
- [12] Guo, Z., Tang, Y., Li, M., Yang, L., Liu, L., Li, P., & Liu, S. (2025). Integrated transcriptomic and metabolomic analyses reveal the mechanism by which quercetin inhibits reflux esophagitis in rats. *PLoS One*, 20(5), e0321959.
- [13] Deng, T., Zhang, N., Liu, Y., Li, J., & Wang, H. (2021). Daidzein ameliorates experimental acute reflux esophagitis in rats via regulation of cytokines. *Die Pharmazie—An International Journal of Pharmaceutical Sciences*, 76(2-3), 84-91.
- [14] Li, Y., Pham, V., Bui, M., Song, L., & Wu, J. (2020). Preventative and therapeutic potential of flavonoids in peptic ulcers. *Molecules*, 25(20), 4626.
- [15] Wang, W., & Ma, X. (2020). miR-210 promotes malignant progression in patients with recurrent reflux esophagitis by

- activating IL-6/STAT3 inflammatory signaling pathway. *Chinese Journal of Gastroenterology and Hepatology*, 29(6), 660–664.
- [16] Song, M., Bode, A. M., Dong, Z., & Lee, M. H. (2019). AKT as a therapeutic target for cancer. *Cancer Research*, 79(6), 1019-1031.
- [17] Redston, M., Noffsinger, A., Kim, A., Akarca, F. G., Rara, M., Stapleton, D., Nowden, L., Lash, R., Bass, A. J., & Stachler, M. D. (2022). Abnormal TP53 predicts risk of progression in patients with Barrett's esophagus regardless of a diagnosis of dysplasia. *Gastroenterology*, 162(2), 468-481.
- [18] Zheng, J., & Tao, L. (2025). Multidimensional mechanisms and therapies underlying gastroesophageal reflux disease: Focus on immunity, signaling pathways, and the microbiota-gut-brain axis. *Frontiers in Immunology*, 16, 1629944.
- [19] Duh, G., Mouri, N., Warburton, D., & Thomas, D. W. (2000). EGF regulates early embryonic mouse gut development in chemically defined organ culture. *Pediatric Research*, 48(6), 794-802.
- [20] Luo, Q., Du, R., Liu, W., Zhang, H., Chen, Y., & Wang, J. (2022). PI3K/Akt/mTOR signaling pathway: Role in esophageal squamous cell carcinoma, regulatory mechanisms and opportunities for targeted therapy. *Frontiers in Oncology*, 12, 852383.
- [21] Dumitru, C. D., Ceci, J. D., Tsatsanis, C., Kontoyiannis, D., Stamatakis, K., Lin, J. H., Patriotis, C., Jenkins, N. A., Copeland, N. G., Kollias, G., & Tschlis, P. N. (2009). Akt-mediated regulation of NF κ B and the essentialness of NF κ B activity for oncogenic transformation induced by Akt. *International Journal of Cancer*, 125(12), 2863-2870.
- [22] Tamizhselvi, R., Sun, J., Koh, Y. H., Mochhala, S., & Bhatia, M. (2009). Effect of hydrogen sulfide on the phosphatidylinositol 3-kinase-protein kinase B pathway and on caerulein-induced cytokine production in isolated mouse pancreatic acinar cells. *Journal of Pharmacology and Experimental Therapeutics*, 329(3), 1166-1177.
- [23] Zhuang, J. Y., Chen, Z. Y., Zhang, T., Liu, J., Li, Y., & Wang, H. (2017). Effects of different ratio of n-6/n-3 polyunsaturated fatty acids on the PI3K/Akt pathway in rats with reflux esophagitis. *Medical Science Monitor*, 23, 421-430.
- [24] Schmidt, A. M., Yan, S. D., Yan, S. F., & Stern, D. M. (2001). The multiligand receptor RAGE as a progression factor amplifying immune and inflammatory responses. *Journal of Clinical Investigation*, 108(7), 949-955.
- [25] Farhadi, A., Fields, J., Banan, A., & Keshavarzian, A. (2002). Reactive oxygen species: Are they involved in the pathogenesis of GERD, Barrett's esophagus, and the latter's progression toward esophageal cancer? *American Journal of Gastroenterology*, 97(1), 22-26.
- [26] Zhou, S., Wang, G., & Zhang, W. (2018). Effect of TLR4/MyD88 signaling pathway on sepsis-associated acute respiratory distress syndrome in rats, via regulation of macrophage activation and inflammatory response. *Experimental and Therapeutic Medicine*, 15(4), 3376-3384.