MINISTRY OF EDUCATION AND SCIENCE OF UKRAINE
KYIV NATIONAL UNIVERSITY OF TECHNOLOGIES AND DESIGN
Faculty of Chemical and Biopharmaceutical Technologies
Department of Biotechnology, Leather and Fur

QUALIFICATION THESIS

on the topic **The interaction mechanism between active ingredients of honeysuckle and uric acid metabolism genes**
First (Bachelor's) level of higher education
Specialty 162 "Biotechnology and Bioengineering"
Educational and professional program "Biotechnology"

Completed: student of group BEBT-21
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APPROVE

Head of Biotechnology, Leather and Fur Department, Professor,

Dr. Sc., Prof.

_____Olena MOKROUSOVA

«___»_____2025

ASSIGNMENTS

FOR THE QUALIFICATION THESIS

Ye Mingzhu

1. Thesis topic **The interaction mechanism between active ingredients of honeysuckle and uric acid metabolism genes**

Scientific supervisor Dr. Sc., Prof. Tetiana Shcherbatiuk

approved by the order of KNUTD “05” March 2025, № 50-уч

2. Initial data for work: assignments for qualification thesis, scientific literature on the topic of qualification thesis, materials of Pre-graduation practice

3. Content of the thesis (list of questions to be developed): literature review; object, purpose, and methods of the study; experimental part; conclusions

4. Date of issuance of the assignments 05.03.2025

WORK CALENDAR

№	The name of the stages of the qualification thesis	Terms of performance of stage	Note on performance
1	Introduction	until 11 April 2025	
2	Chapter 1. Literature review	until 20 April 2025	
3	Chapter 2. Object, purpose, and methods of the study	until 30 April 2025	
4	Chapter 3. Experimental part	until 11 May 2025	
5	Conclusions	until 15 May 2025	
6	Draw up a bachelor's thesis (final version)	until 25 May 2025	
7	Submission of qualification work to the supervisor for feedback	until 27 May 2025	
8	Submission of bachelor's thesis to the department for review (14 days before the defense)	28 May 2025	
9	Checking the bachelor's thesis for signs of plagiarism (10 days before the defense)	01 June 2025	Similarity coefficient ____% Citation rate ____%
10	Submission of bachelor's thesis for approval by the head of the department (from 7 days before the defense)	04 June 2025	

I am familiar with the task:

Student _____ Ye Mingzhu

Scientific supervisor _____ Tetiana SHCHERBATIUK

Abstract

Ye Mingzhu. The interaction mechanism between active ingredients of honeysuckle and uric acid metabolism genes. Manuscript.

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Objective: This study utilizes the integrated approach of network pharmacology combined with molecular docking to investigate the mechanism of action of honeysuckle in reducing hyperuricemia. Method: The active constituents and potential targets of honeysuckle were retrieved from the TCMSP, PubChem, SwissTargetPrediction, and UniProt databases. The hyperuricemia-associated targets were acquired from GeneCards and OMIM databases, and Draw Venn Diagram website was used to find common targets between the drug and the disease. Then use the STRING database to construct a PPI network of key targets and screen for core targets. GO and KEGG enrichment analyses were carried out through the David database and a component-target-pathway network was drawn using Cytoscape_v3.9.1 software. Finally, molecular docking was conducted using CB-Dock2 website and Discovery Studio software to verify the binding activity of the effective components with the core targets. Results: Upon screening, 23 active components of honeysuckle, 502 component targets, and 1375 disease targets were identified, with 125 common targets. GO and KEGG enrichment analysis results show that the active components of honeysuckle significantly participate in biological processes such as anti-inflammatory, anti-apoptotic, and gene expression regulation, involving molecular functions like enzyme binding and protein kinase activity. They also treat hyperuricemia through multiple signaling pathways, including the AGE-RAGE signaling pathway and cancer-related pathways. Molecular docking results show that the core targets IL6、TNF and TP53 have good binding activity with luteolin, quercetin, and kaempferol. Conclusion:

Multiple active components in honeysuckle can synergistically treat hyperuricemia through multi-component, multi-target, and multi-pathway approaches. The potential mechanism of action may involve influencing key targets such as IL6、TNF and TP53, thereby regulating multiple pathways to exert uric acid-lowering effects.

Key words: honeysuckle, hyperuricemia, network pharmacology, molecular docking, mechanism

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INTRODUCTION

In this study, we systematically analyzed the potential mechanism of action of honeysuckle in the treatment of hyperuricemia based on network pharmacology and molecular docking. Hyperuricemia (HUA) is a common metabolic disease caused by disorders in the purine metabolism or dysfunction in uric acid excretion in the body. Due to the high-purine diet of the public, its incidence rate is increasing. Although the current therapeutic drugs can effectively regulate the concentration of serum uric acid, long-term use will cause adverse reactions. Therefore, there is an urgent need for safer substitutes. Traditional Chinese medicines represented by honeysuckle have potential due to their natural safety and the synergistic effect of multiple components. Through the analysis of the "ingredient-target-pathway" network, this study was preliminarily confirmed that honeysuckle can exert its role in preventing and treating hyperuricemia through multiple active components (luteolin, quercetin, kaempferol, etc.), multiple targets (IL6, TNF, TP53, etc.) and multiple pathways (AGE-RAGE signaling pathway in diabetic complications, lipids and atherosclerosis, etc.), which provides a reference for drug development and utilization.

The relevance of the topic is network pharmacology and molecular docking analysis of honeysuckle in hyperuricemia treatment.

The purpose of the study is to explore the active components in honeysuckle with potential for anti-hyperuricemia based on network pharmacology and molecular docking, analyze their possible targets and mechanisms to provide theoretical references for drug development and clinical applications

The objectives of the study is to to screen the potential active components of honeysuckle and their corresponding targets; obtain the core targets related to hyperuricemia; construct the "component-target-disease" network and conduct GO and

KEGG enrichment analyses; the binding ability of key targets to active ingredients was verified by using the molecular docking; reveal the possible mechanism of action of honeysuckle against hyperuricemia.

The object of the study is the interaction mechanism between the active ingredients of honeysuckle and targets related to hyperuricemia.

Research methods include the network pharmacology and molecular docking.

The scientific novelty is that this study systematically combined network pharmacology with molecular docking technology to explore the potential mechanism of honeysuckle in treating hyperuricemia, revealing its multi-component, multi-target and multi-pathway action characteristics, filling the gap of systematic research in this field and having theoretical innovation significance.

The practical significance of the results obtained is that by analyzing the potential mechanism of honeysuckle in the treatment of hyperuricemia, it not only provides theoretical support for the clinical prevention and treatment of hyperuricemia, but also offers a new direction for drug development and the treatment of other metabolic diseases.

Chapter I
LITERATURE REVIEW

Hyperuricemia (HUA) is a common chronic metabolic disease in clinical practice. Its pathogenesis mainly stems from disorders in the purine metabolism or dysfunction in uric acid excretion in the body, leading to abnormally elevated serum uric acid levels[1]. According to the diagnostic criteria outlined in the "Guideline for the Diagnosis and Management of Hyperuricemia and Gout in China", an individual can be definitively diagnosed with the condition if their serum uric acid levels exceed 420 μmol/L on two non-consecutive days under fasting conditions[2]. In recent years, with the rapid development of the economy and society and changes of people's lifestyles, dietary structures of humans have shown a tendency towards high purine, which leads to abnormal increases in purine metabolism in the body and a continuous rise in uric acid content in the blood. Therefore, the incidence of hyperuricemia in the population has been increasing year by year and is constantly trending younger. Epidemiological data indicate that the overall prevalence of hyperuricemia in China has reached 13.3%, among which the proportion of male patients is as high as 19.4%, significantly higher than the 7.9% of female patients[3]. Currently, hyperuricemia has developed into the second most metabolic disease after diabetes, posing a potential threat to public health[4].

Hyperuricemia is not only the pathological basis for the occurrence of gout but also closely associated with kidney injury, cardiovascular diseases, etc[5]. In current clinical treatment, some commonly used uric acid-lowering drugs such as allopurinol, febuxostat, and benzbromarone can effectively regulate serum uric acid levels and achieve a rapid reduction in uric acid concentration. However, long-term use of these medications may be accompanied by adverse effects like hepatotoxicity, nephrotoxicity, and allergic reactions[6]. The treatment of hyperuricemia with traditional Chinese

medicine has a long history, offering advantages of few adverse reactions, safety, high efficiency and so on. However, according to the current situation, there is still a lack of corresponding medical theoretical support[7]. Therefore, the search for natural medicines or functional foods with the potential to lower uric acid and fewer side effects has become a hot direction in the fields of drug research and public health.

The concept of "Food and Medicine Homology" is an important idea in traditional Chinese medicine, which means that certain natural substances can not only be consumed as food but also have medicinal effects. These substances can be used to regulate the body or prevent and treat diseases. The origin of this theory can be traced back to the classic statement in the “Huang Di Nei Jing”, which states："Eating it on an empty stomach counts as food, while for patients, it counts as medicine." Compared with conventional Chinese medicinal materials, substances with both medicine and food have become key carriers for practicing the theory of "preventing diseases before they occur"(wei bing xian fang) in traditional Chinese medicine due to their natural safety[8]. At present, China has officially recognized 106 kinds of traditional Chinese medicines that are both food and medicine, including licorice, chrysanthemum, poria cocos, honeysuckle, perilla, etc. These have been deeply integrated into the national healthy diet system and gradually developed into indispensable important elements in modern health preservation and other fields.

Honeysuckle is the dried flower bud or the newly opened flower of *Lonicera japonica Thunb*, a plant of the *Caprifoliaceae* family. It is one of the traditionally used and commonly prescribed Chinese medicinal herbs[9]. According to the "Pharmacopoeia of the People's Republic of China", honeysuckle has the effects of clearing heat and detoxifying, anti-inflammation and reducing swelling, and dispelling wind-heat, and is widely used in the treatment of various heat-toxic diseases[10]. Modern pharmacological studies have revealed that honeysuckle contains luteolin, inositol, polysaccharides, and various flavonoids and organic acids, demonstrating significant biological activities

such as anti-inflammatory, antioxidant, antiviral, and immunomodulatory effects[11-12]. For instance, kaempferol not only effectively alleviates the inflammatory response in the liver microenvironment but also prevents cell apoptosis. It can also inhibit the further development of the stress response. Moreover, the flavonoids present in honeysuckle possess notable antioxidant efficacy, which can eliminate superoxide anions and hydroxyl radicals in the body, thereby alleviating joint and kidney damage caused by chronic inflammation and oxidative stress[13].

At present, the mechanism of action of honeysuckle in treating hyperuricemia remains unclear. Most existing studies focus on in vitro experiments of honeysuckle extracts or single components, lacking systematic and multi-target exploration of the mechanism of action. Network pharmacology, an emerging approach that integrates systems biology and pharmacology, utilizes bioinformatics and computational biology tools to analyze the complex interactions between drug components and disease-related targets on a macro level. This enables the prediction of potential targets and the elucidation of key signaling pathways, thereby enhancing therapeutic efficacy and reducing adverse effects. It offers a novel strategy for the modernization of traditional Chinese medicine and the development of precision medicine[14-15].Molecular docking technology is based on the principle of computational simulation. By predicting the binding mode and affinity between small molecules and target proteins, it enables efficient screening of potential active components. It can efficiently identify lead compounds, and assist in revealing the mechanism of action of multiple components and multiple targets in traditional Chinese medicine, providing theoretical support and technical means for the research and development of new drugs, which is of great significance in the modernization research of traditional Chinese medicine[16-17.] The combination of two bioinformatics methods, namely network pharmacology and molecular docking technology, can enable the screening of potential targets at the system level and the verification of the binding ability of components to targets at the structural level. This can significantly improve the efficiency and scientific rigor of drug

research and development, and is conducive to the study of the mechanism of traditional Chinese medicine and the discovery of new drugs.

This study is dedicated to exploring the molecular mechanism of honeysuckle in treating hyperuricemia. By employing network pharmacology and molecular docking techniques, it systematically investigates the multi-component synergistic mechanism of honeysuckle in anti-hyperuricemia, reveals that the effective active components of the drug have significant binding activities with the key target proteins of disease-related signaling pathways and comprehensively clarifies the material basis of the efficacy of honeysuckle. This study aims to provide certain academic support and scientific reference for the prevention and treatment of hyperuricemia in clinical practice, and to create new ideas for drug development and the treatment of other metabolic diseases.

Summary of chapter I

1.Definition and epidemiology of hyperuricemia: Hyperuricemia is a metabolic disease caused by purine metabolism disorders or uric acid excretion dysfunction, with its incidence increasing, especially among younger people due to high-purine diets.

2.Limitations of Current HUA Therapies: Although conventional drugs (e.g., allopurinol, febuxostat) can be effective in lowering uric acid, long-term use is associated with adverse effects such as hepatotoxicity and allergy risk. Traditional Chinese medicine can be a safer therapeutic alternative, but it still lacks the corresponding medical theoretical support.

3.The functions of honeysuckle: As the traditional Chinese medicine that is both food and medicine, honeysuckle exhibits anti-inflammatory and antioxidant properties. It contains flavonoids and organic acids that reduce oxidative stress and inflammation, and has the potential to treat hyperuricemia.

4.Research methods and significance: The combination of network pharmacology and molecular docking can reveal the multi-target synergistic mechanism of honeysuckle,

fill the theoretical gap of its anti-HUA mechanism, and provide new ideas for drug development.

5.Aim and significance of this study: This study aims to utilize network pharmacology and molecular docking techniques to explore the molecular mechanism of honeysuckle in the treatment of hyperuricemia, providing a scientific basis for clinical application and new drug development.

Chapter II
OBJECT, PURPOSE, AND METHODS OF THE STUDY

2.1 SCREENING OF ACTIVE INGREDIENTS OF HONEYSUCKLE AND INFORMATION ON PREDICTED TARGETS OF ACTION

In the database of Traditional Chinese Medicine Systems Pharmacology Database (TCMSP, https://www.tcmsp-e.com/tcmsp.php)[18], the keyword "honeysuckle" was used for the search, and according to the pharmacokinetics (ADME) criteria, with thresholds set at oral bioavailability (OB)≥30% and drug-likeness (DL)≥0.18, so as to identify potential active ingredients. The SMILES structural formulae of the above active ingredients were obtained with the help of PubChem database (https://pubchem.ncbi.nlm.nih.gov/)[19], and then import these structures into the SwissTargetPrediction database(http://www.swisstargetprediction.ch/)[20] to find and predict potential drug targets. Select the targets with a prediction probability greater than zero as the potential candidate targets, integrate them with the component targets obtained from the TCMSP database, and remove duplicate target entries. Meanwhile, to ensure the accuracy and consistency of the target data, the obtained target data of active ingredients of honeysuckle need to be imported into the UniProt database (http://www.uniprot.org/)[21] for "Gene Symbol" standardization. Select the reviewed data and set the species screening condition to human (*Homo Sapiens*). Additionally, use the "VLOOKUP" formula in EXCEL software to match the gene names with the predicted protein targets acquired in the above steps, thereby gaining all the potential targets for honeysuckle.

2.2 ACQUISITION OF HYPERURICEMIA-RELATED TARGETS AND INTERSCTION TARGETS

Using "Hyperuricemia" as the search keyword, the gene targets associated with hyperuricemia were retrieved from the Human Gene Database (GeneCards, https://www.genecards.org/)[22] and the Online Mendelian Inheritance in Man (OMIM, https://www.omim.org/)[23]. Then, the collected target information is summarized and de-duplicated to obtain all the predicted disease targets.

Next, utilize the Draw Venn Diagram tool (https://bioinformatics.psb.ugent.be/webtools/Venn/) to perform the intersection analysis between the drug-active component targets and disease-related targets. This identified overlapping targets between the drug and the disease, and a Venn diagram was generated to visualize the number of intersection target genes. These intersection targets would serve as the basis for subsequent network analysis.

2.3 CONSTRUCTION OF INTERSECTION TARGETS PROTEIN INTERACTIONS PPI NETWORK AND SCREENING OF KEY TARGETS

Import the intersection targets between the drug ingredients and the disease into the STRING database (https://cn.string-db.org/)[24] for analysis. The human species range was selected and take 0.4 as the minimum required interaction score. Hide the free gene nodes and keep other settings unchanged to complete the construction of the protein-protein interaction network (PPI). The PPI network was visually processed and analyzed with the help of Cytoscape_v3.9.1 software[25-27], and the importance of each node was evaluated based on the degree value of each node to screen the core targets.

2.4 GO FUNTION AND KEGG PATHWAY ENRICHMENT ANALYSIS

The DAVID database (https://davidbioinformatics.nih.gov/) was employed to perform Gene Ontology (GO) functional enrichment analysis[28] and Kyoto Encyclopedia of Genes and Genomes (KEGG) enrichment analysis[29-30] on the intersection targets. Set the species as "*Homo sapiens*", and a significance threshold of P-value < 0.05 was applied to identify enriched terms. And the top 10 GO terms based on P-value in the categories of Biological Process (BP), Cellular Component (CC), and Molecular Function (MF), as well as the top 15 significantly enriched KEGG pathways, were selected for further analysis. A GO enrichment bar chart and KEGG pathway bubble chart were generated using the "Wei Sheng Xin" online visualization platform (http://www.bioinformatics.com.cn/).

2.5 CONSTRUCTION OF "ACTIVE INGREDIENT-TARGET-SIGNALING PATHWAY" NETWORK

Select the top 20 pathways ranked by P-value from the KEGG pathway enrichment analysis results, correspond the relationships of active ingredients, intersection targets, signaling pathways, drugs and diseases, and import them into the Cytoscape_v3.9.1 software to construct the "active ingredients-target-signaling pathway" network. This network clearly demonstrates the relationship among active ingredients of drugs, core targets and related pathways to investigate the potential mechanism of action of honeysuckle in the treatment of hyperuricemia.

2.6 MOLECULAR DOCKING VERIFICATION

Obtain the structural files of the key ingredients of honeysuckle in the PubChem database and save them in the "sdf" format, using them as small molecule ligands. Then

use the UniProt database to retrieve the gene names of the key protein targets and the corresponding names were entered into the RCSB PDB database (https://www.rcsb.org/)[31] to download the 3D protein structure of the macromolecule receptor, which was saved in "pdb" format. Next, perform molecular docking using the CB-Dock2 online website(https://cadd.labshare.cn/cb-dock2/php/blinddock.php)[32-33] to obtain docking scores. Finally, the Discovery Studio software is utilized to complete the visual analysis of 2D and 3D structures for the docking results.

Summary of chapter II

1.This chapter expounds the database, analysis software and experimental methods used in this study. Through multi-database integration, multi-level verification, visual analysis and standardized processing, a foundation has been provided for subsequent research.

2.Active ingredients screening and targets prediction: Based on the TCMSP database, the active ingredients of honeysuckle were screened (ADME standard), potential targets were predicted through SwissTargetPrediction and TCMSP databases, and use UniProt database to complete the gene standardization.

3.Disease targets acquisition and intersection targets analysis: Integrate the hyperuricemia-related targets from the GeneCards and OMIM databases, and determine the drug-disease intersection targets through venn diagram.

4.Construction of core target network: A PPI network was constructed using STRING databases and Cytoscape_v3.9.1 software, and core targets were screened based on degree.

5.GO and KEGG enrichment analysis to elucidate mechanisms: Conduct GO and KEGG enrichment analysis through the DAVID database to resolve the biological processes, molecular functions and signaling pathways related to the targets.

6.Network construction to visualize multi-target interactions: Use Cytoscape_v3.9.1 software to construct the "ingredients-targets-pathways" interaction network to clarify the mechanism of multi-target synergy.

7.Molecular docking for structural validation: CB-Dock2 and Discovery Studio were used to conduct molecular docking verification between core components and targets to gain an in-depth understanding of the binding affinity between small molecules and protein targets.

Chapter III
EXPERIMENTAL PART

3.1 ACTIVE INGREDIENTS AND CORRESPONDING TARGETS OF HONEYSUCKLE

A total of 236 effective components of honeysuckle were retrieved from the TCMSP TCMSP database. After screening under the set conditions of OB≥30% and DL≥0.18, 23 active ingredients that met the requirements were obtained, including quercetin, luteolin, kaempferol, etc. The basic information is shown in

Table 3.1. After obtaining the targets through the TCMSP database and SwissTargetPrediction database, then the acquired targets corresponding to each active ingredient were subjected to protein matching and gene annotation in the UniProt database, and by integrating and de-duplicating the targets, a total of 502 drug-related targets were ultimately gained. These results suggest that the active components of honeysuckle may exert their effects through multi-target synergy, laying the foundation for subsequent network analysis.

Table 3.1 Active ingredients of honeysuckle

Name of traditional Chinese medicine	ID	Molecule name	OB (%)	DL
honeysuckle	MOL001494	mandenol	42	0.19
	MOL001495	ethyl linolenate	46.1	0.2
	MOL002914	eriodyctiol (flavanone)	41.35	0.24
	MOL003006	(-)-(3R,8S,9R,9aS,10aS)-9-ethenyl-8-(beta-D-glucopyranosyloxy)-2,3,9,9a,10,10a-hexahydro-5-oxo-5H,8H-pyrano[4,3-d]oxazolo[3,2-a] pyridine-3-carboxylic acid_qt	87.47	0.23
	MOL003014	secologanic dibutylacetal_qt	53.65	0.29
	MOL002773	beta-carotene	37.18	0.58
	MOL003036	ZINC03978781	43.83	0.76
	MOL003044	chryseriol	35.85	0.27
	MOL003095	5-hydroxy-7-methoxy-2-(3,4,5-trimethoxyphenyl)chromone	51.96	0.41
	MOL003111	centauroside_qt	55.79	0.5
	MOL003117	ioniceracetalides B_qt	61.19	0.19
	MOL003128	dinethylsecologanoside	48.46	0.48
	MOL000358	beta-sitosterol	36.91	0.75

	MOL000006	luteolin	36.16	0.25
	MOL000098	quercetin	46.43	0.28
	MOL000449	stigmasterol	43.83	0.76
	MOL002707	phytofluene	43.18	0.5
	MOL003059	kryptoxanthin	47.25	0.57
	MOL003062	4,5'-Retro-.beta.,.beta.-Carotene-3,3'-dione, 4',5'-didehydro-	31.22	0.55
	MOL003101	7-epi-Vogeloside	46.13	0.58
	MOL003108	Caeruloside C	55.64	0.73
	MOL003124	XYLOSTOSIDINE	43.17	0.64
	MOL000422	kaempferol	41.88	0.24

3.2 ACQUISITION OF HYPERURICEMIA-RELATED TARGETS AND INTERSCTION TARGETS

By retrieving disease genes in the GeneCards and OMIM databases and summarizing and de-duplicating the collected targets, a total of 1,375 disease targets were gained. The drug and disease targets obtained from the above steps were then uploaded to the Draw Venn Diagram website to construct the Venn diagram and conduct intersection analysis. The results showed that there were 125 intersection targets, which may represent potential therapeutic targets of honeysuckle in the treatment of hyperuricemia. The Venn diagram of the disease-drug intersection genes is shown in Figure 3.1.

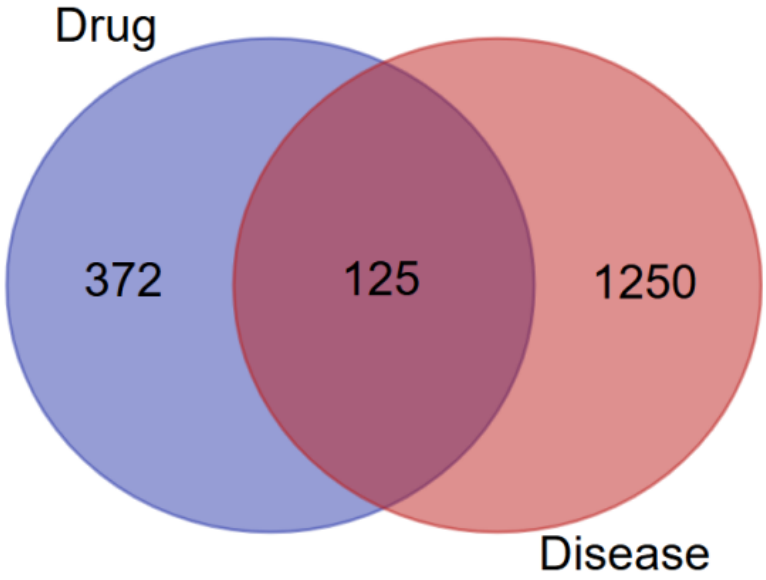

Figure 3.1 Disease-drug intersection gene Venn diagram

3.3 CONSTRUCTION OF INTERSECTION TARGETS PROTEIN INTERACTION PPI NETWORK AND SCREENING OF KEY TARGETS

The intersection targets were analyzed using the STRING database to construct the PPI network, as shown in Figure 3.2. Upon observation and analysis, it was found that the network had 125 nodes and 1,913 edges, indicating that there are interactions among the intersection targets, and 1,913 interaction paths have been generated. Moreover, there is exactly one single node in the network. After exporting the PPI network, visual analysis of the data was conducted in the Cytoscape_v3.9.1 software. According to the ranking based on the degree value, the top ten genes identified were IL6, TNF, TP53, PPARG, ESR1, BCL2, MYC, IL1B, CASP3, and IFNG respectively. These results suggest that the above-mentioned targets might be the core targets of honeysuckle in the treatment of hyperuricemia.

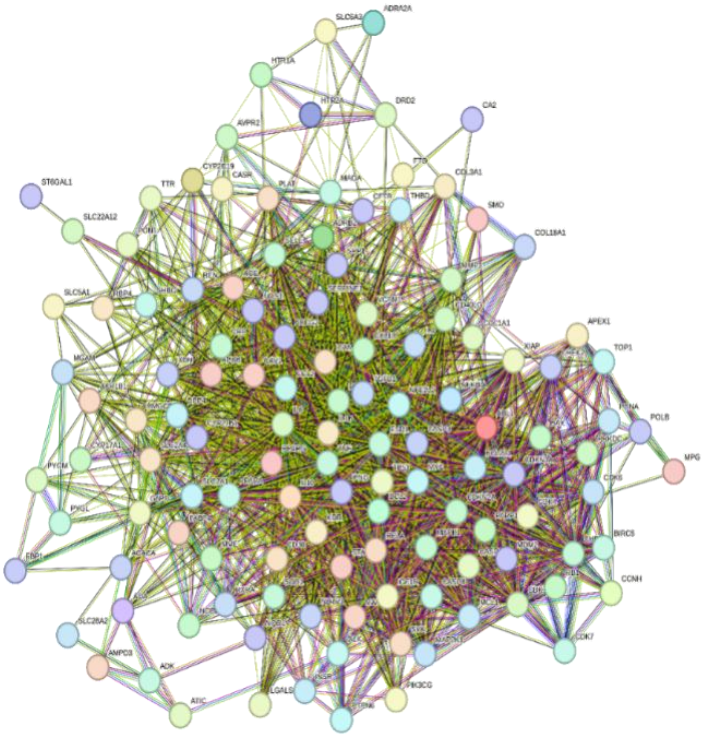

Figure 3.2 Disease-drug intersection gene PPI network

3.4 RESULTS OF GO FUNCTION AND KEGG PATHWAY ENRICHMENT ANALYSIS

3.4.1 GO FUNCTION ENRICHMENT ANALYSIS

GO enrichment analysis usually divides gene functions into three aspects: biological processes (BP), cellular components (CC), and molecular functions (MF). And in the bar chart, the higher the bar, the more significant the enrichment of the item. By setting the P-value < 0.05, the results showed that a total of 677 significantly enriched items were obtained through GO functional enrichment analysis, including 519 BP terms, 54 CC terms, and 104 MF terms.

As shown in the GO enrichment analysis results (Figure 3.3), the BP terms are primarily involved in response to xenobiotic stimulus, positive regulation of gene expression, positive regulation of gene expression, negative regulation of apoptotic

process, positive regulation of apoptotic process, response to estradiol, response to hypoxia, positive regulation of cell population proliferation, intrinsic apoptotic signaling pathway in response to DNA damage and positive regulation of transcription by RNA polymerase II, etc. For CC terms, the enriched gene products were mainly located in protein-containing complex, caveola, cytosol, extracellular space, neuronal cell body, extracellular exosome, cyclin-dependent protein kinase holoenzyme complex, extracellular region, cytoplasm and membrane raft, etc. The MF terms included identical protein binding, enzyme binding, protein binding, protease binding, protein-containing complex binding, protein homodimerization activity, protein kinase activity, cytokine activity, BH3 domain binding and integrin binding, etc.

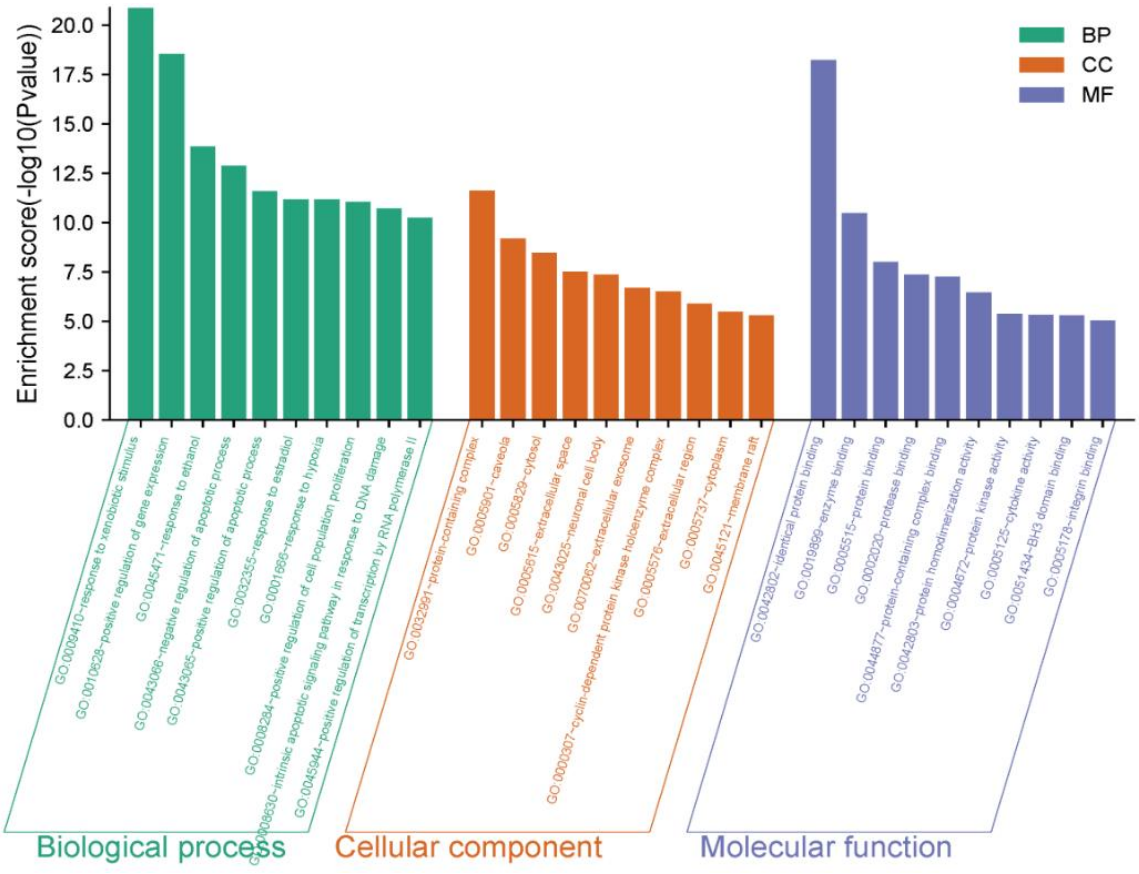

Figure 3.3 GO enrichment analysis

3.4.2 KEGG PATHWAY ANALYSIS

KEGG pathway enrichment analysis revealed that the active components of honeysuckle were significantly enriched in several important pathways related to metabolic regulation, inflammatory response, tumor progression and viral infection. As illustrated in the figure, the size of the circle is positively correlated with the number of genes enriched in the pathway, which means the larger the circle, the more genes are enriched. And a smaller P-value indicates a lower influence of random factors, and a higher the possibility of gene enrichment in this pathway is. After conditional screening, the results of KEGG pathway enrichment analysis showed that a total of 135 pathways were obtained. Among them, the top 15 pathways are respectively the AGE-RAGE signaling pathway in diabetic complications, pathways in cancer, lipid and atherosclerosis, Kaposi sarcoma-associated herpesvirus infection, hepatitis B, p53 signaling pathway, chronic myeloid leukemia, pancreatic cancer, human cytomegalovirus infection, fluid shear stress and atherosclerosis, small cell lung cancer, toxoplasmosis, human T-cell leukemia virus 1 infection, hepatitis C and TNF signaling pathway. The enrichment analysis of the KEGG pathway is shown in Figure 3.4. Furthermore, two of the most highly enriched pathways included the AGE-RAGE signaling pathway in diabetic complications and pathways in cancer were visualized and the results are presented in Figure 3.5 and Figure 3.6

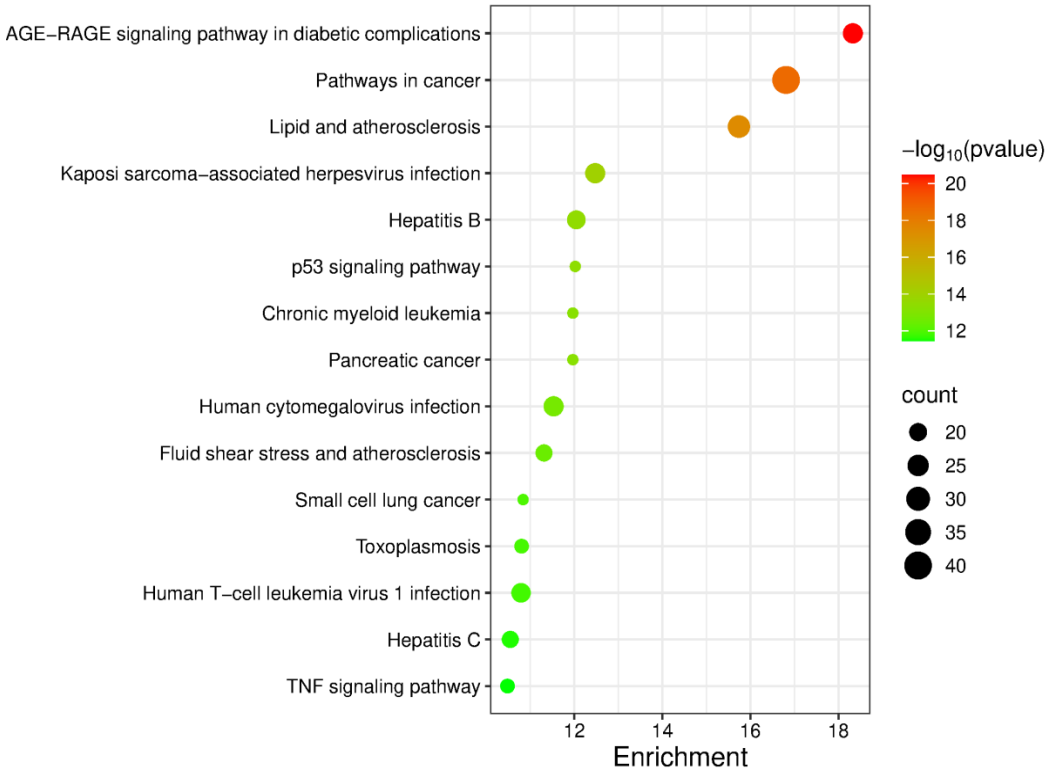

Figure 3.4 KEGG enrichment

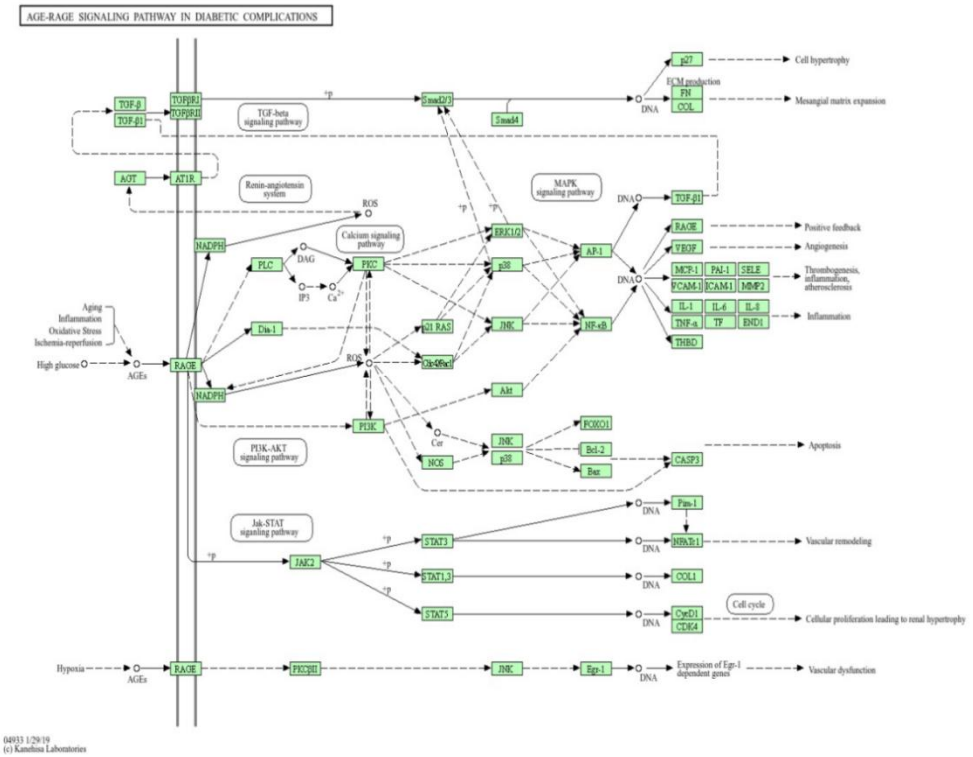

Figure 3.5 AGE-RAGE signaling pathway in diabetic complications

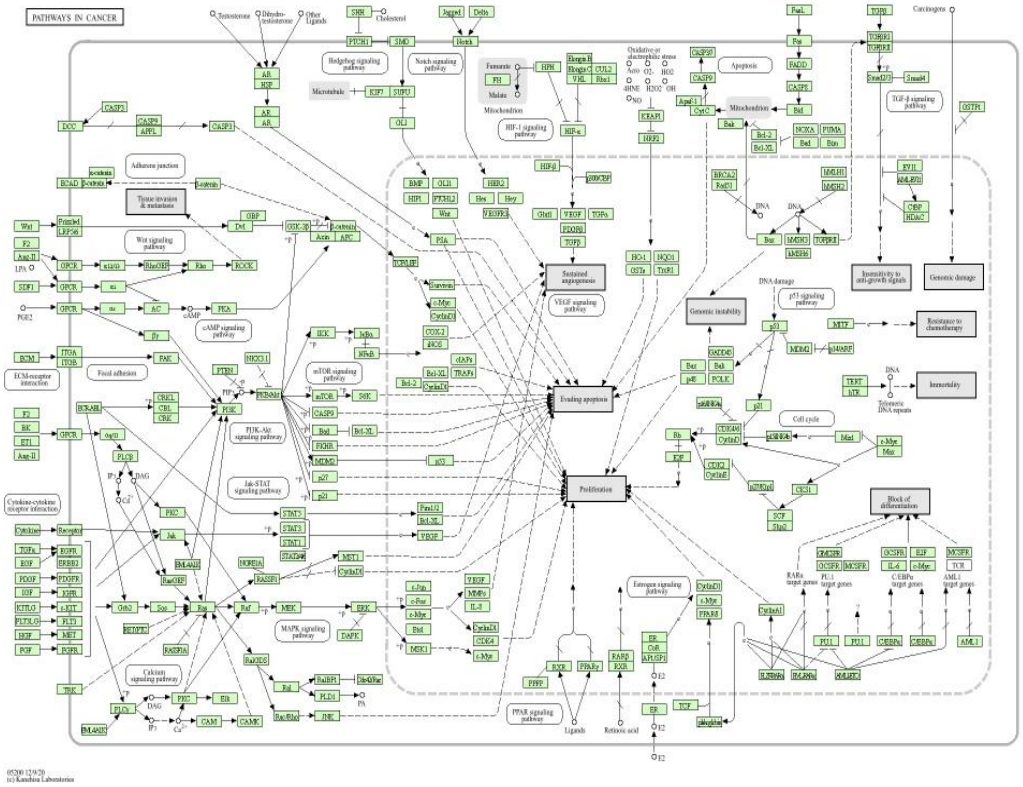

Figure 3.6 Pathways in cancer

3.5 CONSTRUCTION OF "ACTIVE INGREDIENT-TARGET-SIGNALING PATHWAY" NETWORK

By integrating data on the active ingredients, intersection targets, and signaling pathways, a “active ingredient-target-signaling pathway” network was constructed and visualized using Cytoscape_v3.9.1 software, as shown in Figure 3.7. The results indicate that ingredients such as luteolin and quercetin from honeysuckle are associated with multiple key targets, which are closely related to important biological pathways, including inflammatory pathways and purine metabolism pathways.

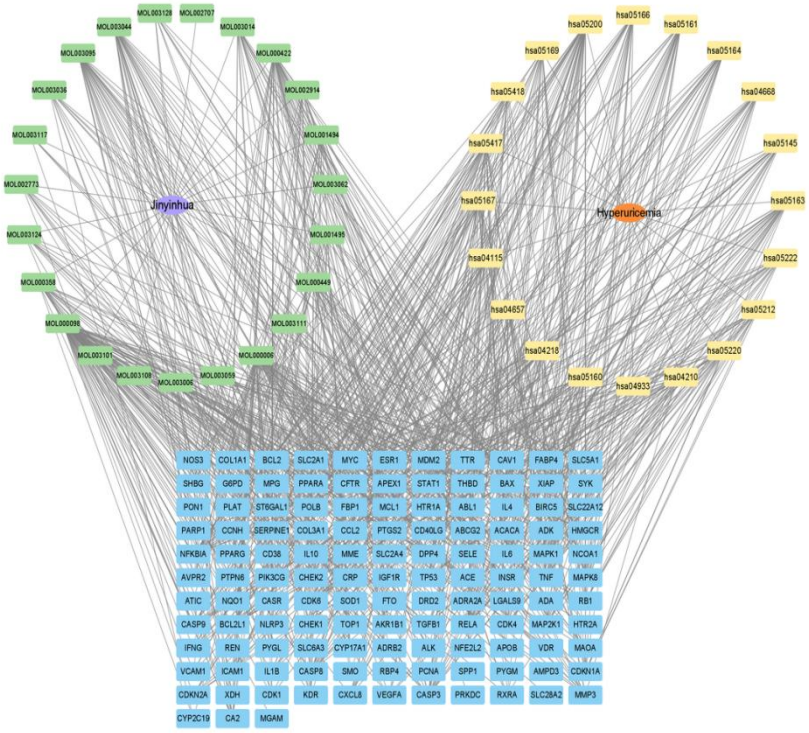

Figure 3.7 "Active ingredient-target-signaling pathway" network

3.6 MOLECULAR DOCKING VERIFICATION

ccording to the degree value, the top 3 core active ingredients (kaempferol, luteolin, quercetin) in the PPI network and the top 3 target genes (IL6, TNF, TP53) were selected for molecular docking verification using CB-Dock2 online website, and visual analysis was carried out through the Discovery Studio software. Through the docking scores, matching degrees and Binding activity of the docking results, we could find that the ingredients kaempferol, luteolin and quercetin all demonstrated good binding activities with the protein targets IL6, TNF and TP53, indicating that honeysuckle can exert therapeutic effects on hyperuricemia through key target sites. The two-dimensional planar diagrams of the ligand-receptor interaction are shown in Figure 3.8, and the three-dimensional stereoscopic diagrams are shown in Figure 3.9.

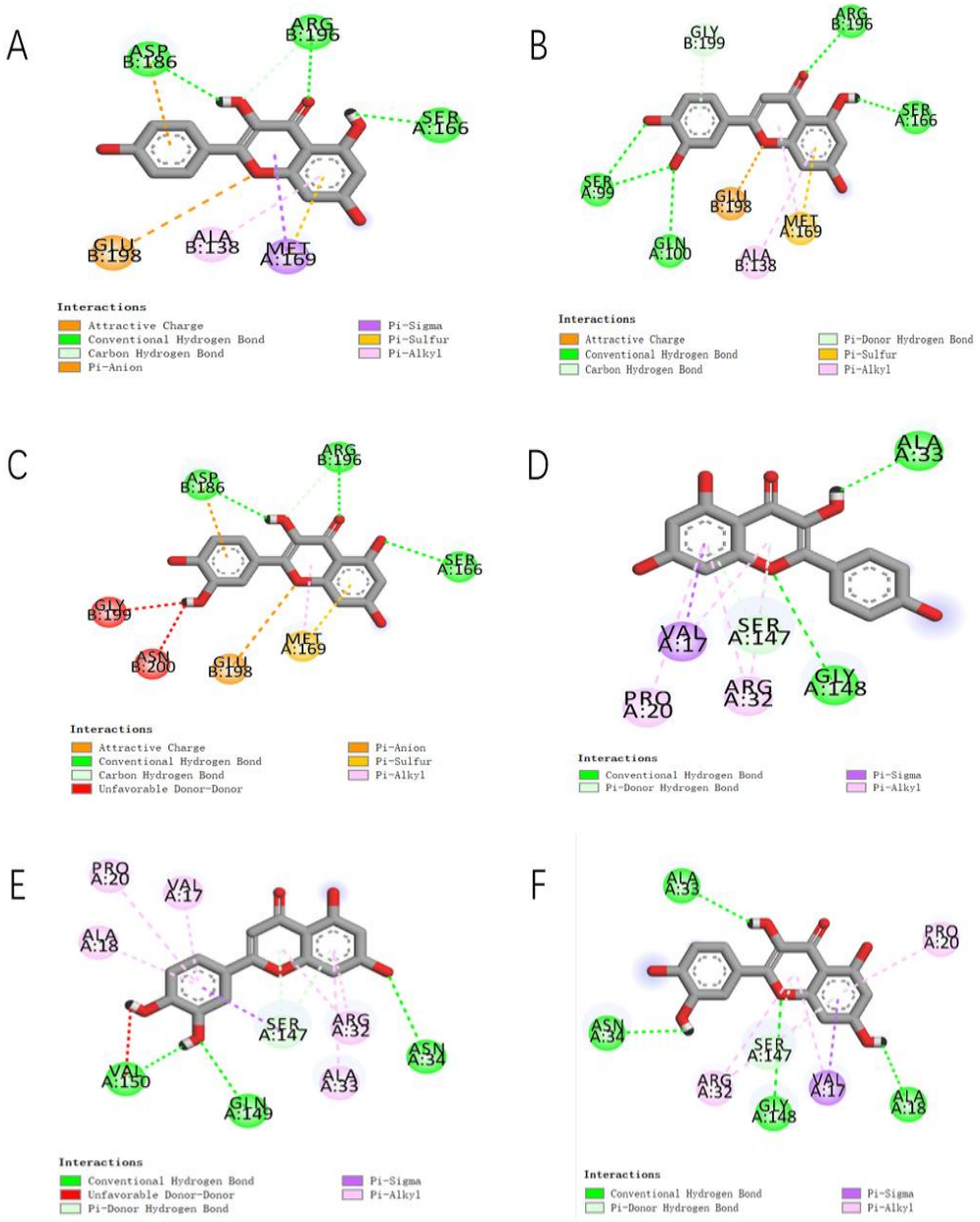

Figure 3.8 Two-dimensional planar diagram of ligand-protein interactions

Note: A. Molecular docking result of TP53 with kaempferol; B. Molecular docking result of TP53 with luteolin; C. Molecular docking result of TP53 with quercetin; D. Molecular docking result of TNF-α with kaempferol; E. Molecular docking result of TNF-α with luteolin; F. Molecular docking result of TNF-α with quercetin.

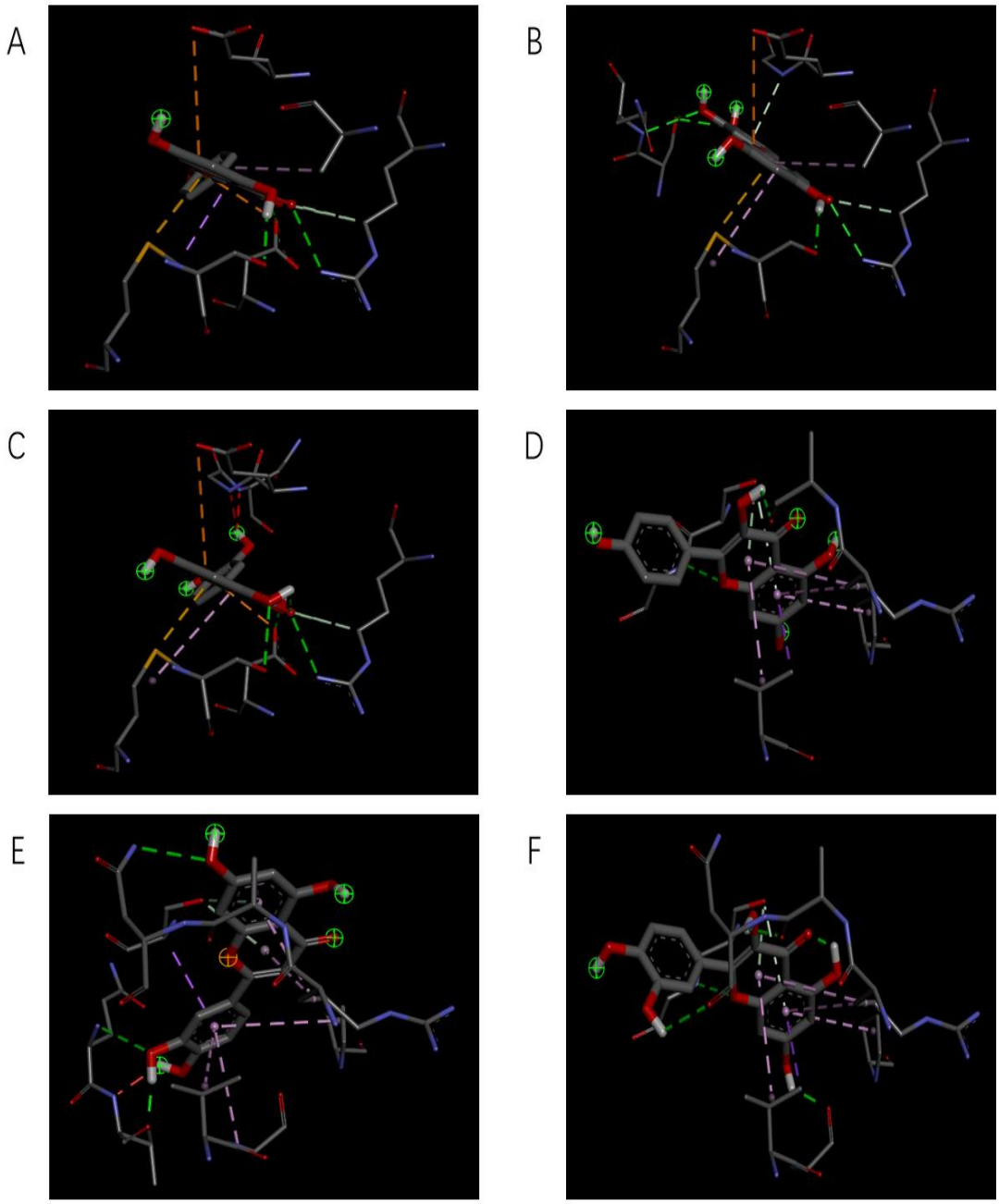

Figure 3.9 Three-dimensional planar diagram of ligand-protein interactions

Note: A. Molecular docking result of TP53 with kaempferol; B. Molecular docking result of TP53 with luteolin; C. Molecular docking result of TP53 with quercetin; D. Molecular docking result of TNF-α with kaempferol; E. Molecular docking result of TNF-α with luteolin; F. Molecular docking result of TNF-α with quercetin.

3.7 Analysis and discussion

In this study, the mechanism of action of honeysuckle in the treatment of hyperuricemia was analyzed and discussed through network pharmacology combined with molecular docking. A total of 23 active components of honeysuckle were obtained, corresponding to 502 targets, 1,375 hyperuricemia-related targets, and 125 intersection targets between drug and disease. By constructing and analyzing the regulatory network of" active ingredients of "honeysuckle-activeingredient-target-signaling pathway", it is concluded that the most core active components of honeysuckle in the treatment of hyperuricemia are quercetin, luteolin and kaempferol.

Quercetin, as a natural flavonoid compound, has a rather wide distribution range and can be found in the stem epidermis, seed inner core and fruit tissue of many plants in nature. In recent years, pharmacological studies have revealed that quercetin possesses various pharmacological functions such as anti-inflammation, antioxidation, tumor growth intervention, hypoglycemia and lipid-lowering[34]. Studies have shown that quercetin can prevent and treat hyperuricemia by inhibiting the activity of key enzymes like xanthine oxidase (XOD), thereby reducing uric acid production. It can also regulate renal transporter protein levels to promote urate excretion. Moreover, quercetin can reduce the inflammatory response by inhibiting the secretion of inflammatory factors and the activation of the NLRP3 inflammatory vesicles, among other pharmacological effects to prevent and control hyperuricemia[35].

Luteolin, a plant-derived flavonoid active component, is commonly found in various traditional medicinal plants and has multiple effects including anti-inflammation, anti-tumor, anti-fibrosis, anti-allergy and so on[36]. Yu[37] et al. further studied through a mouse model of hyperuricemia and found that luteolin could significantly reduce the levels of serum uric acid, creatinine and urea nitrogen, inhibit the activity of xanthine oxidase, and thereby reduce the synthesis of uric acid. It can

also up-regulate the expression levels of uric acid transportation-related proteins such as ABCG2, OAT1, and OAT3, and promote the excretion process of uric acid in the body.

Kaempferol is a major flavonoid compound in the daily diet, accounting for 22-29% of the total flavonoid intake[38]. It is widely present in food, vegetables and medicinal plants, and shows significant antioxidant, antibacterial, anti-inflammatory and anti-tumor effects[39]. Studies have found that kaempferol as one of the main polyphenols in sophora japonica, can be transformed into a more active form through deglycosylation during thermal processing, which can significantly enhance the XOD inhibitory activity of sophora japonica polyphenols. In the mouse model of hyperuricemia, kaempferol exerts a uric acid-lowering effect through multiple mechanisms such as inhibiting XOD activity, regulating uric acid transporters, improving renal function, and regulating the intestinal flora[40].

By analyzing the protein-protein interaction PPI network and conducting network topology analysis based on the degree, multiple core targets of honeysuckle in the treatment of hyperuricemia were screened out, including IL6, TNF, TP53, PPARG, ESR1, etc.

As one of the key pro-inflammatory cytokines, interleukin-6 (IL-6) belongs to the chemokine family and can play a core regulatory part in various biological processes. For example, IL-6 can induce hepatocytes to synthesize acute-phase proteins to mediate the inflammatory response process, and can also stimulate T cell proliferation and the activation of cytotoxic T lymphocytes (CTL). In addition, IL-6 participates in promoting the development of blood cells, and acts an indispensable role in maintaining the normal physiological functions of the body and responding to pathological conditions. Some studies have suggested that when hyperuricemia is complicated with disorders of glycolipid metabolism, serum IL-6 levels of patients with hyperuricemia significantly elevated. This indicates that hyperuricemia may cause an inflammatory response, leading to an increase in IL-6 levels and thereby taking part in the occurrence of metabolic disorders[41].

Tumor necrosis factor (TNF), which includes TNF-α and TNP-β two subtypes, has a significant impact on maintaining immune homeostasis and antiviral defense, and is also crucial in the initiation and maintenance of inflammatory responses as well as the regulation of programmed cell death. Studies have shown that elevated uric acid levels can promote inflammatory responses. Inflammatory factors such as TNF-α take a pivotal part in the pathogenesis of hyperuricemia and gout, and may aggravate tissue damage by mediating cellular inflammatory responses. Changes in their expression levels may be related to kidney function and inflammatory regulation[42].

KEGG pathway enrichment analysis revealed that the mechanism of honeysuckle in treating hyperuricemia mainly involves multiple signaling pathways such as the AGE-RAGE signaling pathway in diabetic complications, lipid and atherosclerosis, Kaposi sarcoma-associated herpesvirus infection, hepatitis B. Among them, the AGE-RAGE signaling pathway in diabetic complications can have a vital part in various pathological conditions. The activation of this pathway can induce the release of various inflammatory factors, such as IL-6, IL-1β, etc. By inhibiting the AGE-RAGE signaling pathway, the inflammatory response of hyperuricemia combined with gouty arthritis can be significantly alleviated, and the clinical symptoms of patients can be improved[43]. Lipid and atherosclerosis are closely related to hyperuricemia, which can accelerate the process of atherosclerosis through direct damage to the vascular endothelium by soluble uric acid, inflammatory responses triggered by urate crystals, and through synergistic effects with hyperlipidemia[44].

In the molecular docking, three active components of honeysuckle, including kaempferol, luteolin and quercetin, were used with three core targets, IL6, TNF and TP53. The docking results indicated that the binding activities of all receptor-ligand docking were good, which indicates that the active components of honeysuckle may exert potential effects by interacting with these proteins, thereby contributing to the regulation of purine metabolism and the inhibition of inflammation. These findings

provide a deeper scientific basis for the pharmacological research of honeysuckle in the treatment of hyperuricemia.

Summary of chapter III

1.Acquisition of active ingredients and corresponding targets of honeysuckle: 23 active components were obtained (e.g., quercetin, luteolin) from 236 ingredients via OB≥30% and DL≥0.18 criteria and gained 502 drug-related targets through database integration and annotation.

2.Hyperuricemia targets identification and intersection targets: 1,375 hyperuricemia-related targets were collected from the GeneCards and OMIM databases, with 125 intersection targets identified as potential therapeutic targets

3.PPI Network and core genes: Constructed a protein-protein interaction (PPI) network which has 125 nodes, 1,913 edges and obtained core genes such as IL6, TNF, TP53, PPARG, ESR1 via Cytoscape_v3.9.1 software.

4.GO function and KEGG pathway enrichment analysis:a total of 677 GO function terms were obtained, which showed targets were mainly participated in biological processes such as anti-inflammatory, anti-apoptotic, and gene expression regulation, involving molecular functions like enzyme binding and protein kinase activity. And 135 KEGG pathways linked to inflammation and metabolism were acquired including the AGE-RAGE signaling pathway in diabetic complications, lipid and atherosclerosis and so on.

5."Active ingredient-target-signaling pathway" network: The network constructed and visualized using Cytoscape_v3.9.1 software demonstrated the potential of honeysuckle to treat hyperuricaemia through multi-ingredient, multi-target and multi-pathway.

6.Molecular docking verification: It confirmed strong binding affinities between core ingredients (kaempferol, luteolin, quercetin) and core targets (IL6, TNF, TP53), supporting the mechanism of action of honeysuckle in reducing hyperuricemia.

6.Multi-component synergistic effects: Flavonoids represented by quercetin, luteolin, and kaempferol reduce uric acid production by inhibiting xanthine oxidase, regulate renal transport proteins to promote excretion , and lower the levels of serum uric acid and inflammation-related substances.

7.Multi-target regulation: Core targets (IL6, TNF, etc.) improve tissue damage and complications of hyperuricemia by regulating inflammatory responses and metabolic disorders.

8.Multi-pathway integration: The AGE-RAGE pathway plays a key role by mediating inflammation (e.g., IL-6, IL-1β), and its inhibition may alleviate gouty arthritis symptoms. Additionally, uric acid-induced vascular damage, inflammation, and synergy with hyperlipidemia link hyperuricemia to atherosclerosis. Molecular docking confirmed that the active ingredient has a high affinity for targets such as TP53 and TNF, providing a structural basis for the mechanism.

9.Honeysuckle intervenes in hyperuricemia through the synergistic effects of multiple components (mainly flavonoids), multiple targets (inflammation/metabolism-related), and multiple pathways (AGE-RAGE pathway, etc.) from some aspects like inhibiting uric acid production, promoting excretion, and reducing inflammation, providing a scientific basis for subsequent basic research and clinical applications.

CONCLUSION

Based on network pharmacology and molecular docking techniques, this study analyzed the complex relationship network between honeysuckle and hyperuricemia, deeply explored the potential mechanisms of honeysuckle in the treatment of hyperuricemia. A total of 23 active compounds from honeysuckle and 1,375 hyperuricemia-related targets were screened, identifying thepotential therapeutic targets and associated signaling pathways. The results indicate that key targets include IL6, TNF, TP53, PPARG, ESR1 and so on, which may be through components such as luteolin, quercetin, and kaempferol, participating in the regulation of multiple signaling pathways like the AGE-RAGE signaling pathway in diabetic complications, lipid and atherosclerosis, and p53 signaling pathway. These findings demonstrate that the multi-component, multi-target, and multi-pathway characteristics of honeysuckle in the prevention and treatment of hyperuricemia.

Although this study combined the novel methodologies of network pharmacology and molecular docking to elucidate the pharmacological mechanisms of honeysuckle in treating hyperuricemia from the systemic to the molecular level, and achieved certain results, providing novel perspectives and theoretical basis for traditional Chinese medicine treatment. However, there are still some deficiencies in this study. The exploration of the specific pharmacological mechanisms of honeysuckle in the treatment of hyperuricemia is still in the preliminary stage, requiring further in-depth research and analysis of its related mechanisms. For instance, this study adopted the method of integrating data from several databases, which might be some deviations in the acquisition of the active ingredients of the drug. Additionally, due to the continuous updates of database platforms, there may be some differences and influences in the data results.And the validation of findings solely utilized molecular docking technology, lacking relevant in vitro experimental evidence. Therefore, the reliability of the results

still needs to be strengthened. In the future, these aspects need to be combined to further verify the biological functions of the core targets and pathways, thereby providing a scientific basis for the development of traditional Chinese medicine.

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