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 **Bioinformatic modelling of the action of active components of *Morus alba***

First (Bachelor's) level of higher education

Specialty 162 "Biotechnology and Bioengineering"

Educational and professional program "Biotechnology"

Completed: student of group BEBT-20
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Educational and professional program Biotechnology

APPROVE
Head of Department of Biotechnology,
Leather and Fur, Professor,
Doctor of Technical Science
Olena MOKROUSOVA

_____
«___»_____2024

ASSIGNMENTS
FOR THE QUALIFICATION THESIS
Cui Zitong

1. Thesis topic **Bioinformatic modelling of the action of active components of *Morus alba***
Scientific supervisor Olga Iungin, Ph.D., Assoc. Prof.

approved by the order of KNUTD "____"_____2024, №____

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_____

EXECUTION SCHEDULE

№	The name of the stages of the qualification thesis	Terms of performance of stage	Note on performance
1	Introduction	From 01 April 2024 to 11 April 2024	
2	Chapter 1. Literature review	From 06 April 2024 to 20 April 2024	
3	Chapter 2. Object, purpose, and methods of the study	From 21 April 2024 to 30 April 2024	
4	Chapter 3. Experimental part	From 01 May 2024 to 10 May 2024	
5	Conclusions	From 07 May 2024 to 12 May 2024	
6	Draw up a bachelor's thesis (final version)	From 20 May 2024 to 25 May 2024	
7	Submission of qualification work to the supervisor for feedback	From 25 May 2024 to 27 May 2024	
8	Submission of bachelor's thesis to the department for review (14 days before the defense)	27 May 2024	
9	Checking the bachelor's thesis for signs of plagiarism (10 days before the defense)	31 May 2024	
10	Submission of bachelor's thesis for approval by the head of the department (from 7 days before the defense)	03 June 2024	

I am familiar with the task:

Student _____Cui ZITONG

Scientific supervisor _____Olga IUNGIN

SUMMARY

Cui Zitong. Bioinformatic modelling of the action of active components of *Morus alba*– Manuscript.

Qualification thesis on the specialty 162 «Biotechnology and Bioengineering». – Kyiv National University of Technologies and Design, Kyiv, 2024.

Objective: To explore the mechanism of mulberry leaf in the treatment of hyperuricemia based on network pharmacology, and to verify the experimental results by molecular docking technology. How: The common target genes of mulberry leaf active components and hyperuricemia were found using TCMSP, Uniprot and Draw Venn Diagram database, and the PPI network diagram of key target was drawn. GO and KEGG concentration were carried out using David database, and molecular docking was carried out by Cytoscape software. Predict the binding degree of major compounds to key targets. Result: In this study, a total of 29 active components and 657 potential targets in mulberry leaves were screened, 908 disease targets related to hyperuricemia were identified, and 78 common targets of mulberry leaves and hyperuricemia were identified, and several key targets GAPDH, AlB, JUN, TNF, PPARG and ESR1 were screened for molecular docking. The results of docking showed that JUN, TNF, PPARG and ESR1 had good binding activity with anisodamine, arachidonic acid, quercetin and sanocin. It is predicted that JUN, TNF, PPARG and ESR1 may be the key targets of mulberry leaf in the treatment of hyperuricemia. The results of KEGG pathway enrichment analysis showed that mulberry leaves may treat hyperuricemia through several signaling pathways, such as TNF signaling pathway, cancer signaling pathway, PI3K-Akt signaling pathway, and AGE-RAGE signaling pathway in diabetic complications. Conclusion: Multiple active components in mulberry leaves have a network mechanism of synergistic action on hyperuricemia through multi-component, multi-target and multi-pathway, possibly by acting on core target genes such as JUN, TNF, PPARG and ESR1. Then, it regulates the cancer signaling pathway, PI3K-Akt signaling pathway, TNF signaling pathway,

NF-κB signaling pathway and other pathways to play a therapeutic role in hyperuricemia.

Keywords: Mulberry leaf; Hyperuricemia; Network pharmacology; Molecular docking; Mechanis

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INTRODUCTION

In this study, traditional methods such as high performance liquid chromatography were improved to extract active ingredients from mulberry leaves, a traditional Chinese medicine with the same origin as medicine and food. Bioinformatics methods were used to quickly and efficiently screen out each active ingredient, and the target protein gene of the active ingredient could be deeply mined to explore the potential molecular mechanism of interaction with the target protein of disease. It provides a strong basis for the treatment of hyperuricemia.

The relevance of the topic is screening of active components of mulberry leaf and treatment of hyperuricemia.

The purpose of the study is to explore the therapeutic mechanism of active ingredients in mulberry leaves on hyperuricemia, and to verify the experimental results by molecular docking technology. To be more specific, in this paper, I aiming to find the potential molecular basis of anti-inflammatory and anti-bone damage effects in the treatment of diseases, so as to provide some help for the follow-up treatment of traditional Chinese medicine and the research and development of new drugs, with a view to expanding the research on the mechanism of action of more diseases.

The objectives of the study rapid screening of the active ingredients in mulberry leaves provides evidence for the treatment of hyperuricemia.

The object of the study is mulberry leaf, a traditional Chinese medicine with the same origin as medicine and food, plays an irreplaceable role in the treatment of inflammation, anti-aging and diabetes. Its flavonoid components have been proved to regulate abnormal glucose and lipid metabolism, reduce inflammation and inhibit xanthine oxidase in uric acid metabolism, which is widely used.

The subject of the study is in order to explore the mechanism of action of mulberry leaves in the treatment of hyperuricemia, a series of bioinformatics methods were used to screen the active ingredients and their targets and genes, and the related target genes of the disease were obtained. Then the common genes are obtained and the genes are enriched to find more targeted treatments. Finally, molecular docking technology was used to verify whether the active ingredients could successfully dock with the disease targets, and the effective ingredients in mulberry leaves were obtained.

Research methods are network pharmacology and molecular docking.

The scientific novelty the traditional methods such as high performance liquid chromatography (HPLC) to extract the active components of traditional Chinese medicine have the disadvantages of cumbersome operation, long time and insufficient extraction of trace active components. In this paper, each active ingredient can be quickly and efficiently screened through bioinformatics methods, and the active ingredient of the drug can be deeply mined and matched with the target protein and related genes of the disease, so as to explore the potential molecular mechanism of the therapeutic effect of the drug, and provide a strong basis for finding efficient treatment methods and new drug synthesis.

The practical significance of the results obtained is based on the concept of drug and food homology, it is expected to expand the study of the mechanism of action of more diseases by using bioinformatics analysis methods such as network pharmacology and molecular docking technology.

CHAPTER 1
LITERATURE REVIEW

1.1 Hyperuricemia

Hyperuricemia (HUA) can be classified as a class of metabolic diseases, the main cause is purine metabolism disorders, affecting factors mainly include heredity and environment. The evaluation is based on two fasting serum uric acid tests on different days under the conventional purine diet, which is defined as hyperuricemia when the serum uric acid content is higher than 0.7mg/L(420μmol/L) in male and 0.6mg/L(357μmol/L) in female, respectively. It has been found in clinical studies to be closely related to a variety of different types of chronic diseases such as gout, hypertension, diabetes, etc[1]. The White Paper on Trends of Hyperuricemia and Gout in China (2021) indicates that the number of hyperuricemia patients in China is increasing year by year, and the age of patients is trending younger. At present, the number of patients with hyperuricemia in China is about 177 million, accounting for about 13.3% of the total population, among which the corresponding proportions of patients aged 18-25, 26-35 and 36-45 are 22%, 38% and 6%, respectively[2], posing a serious threat to social and public health. Based on this background, how to prevent and treat hyperuricemia has become the focus of global attention today.

Hyperuricemia is a kind of disease closely related to diet structure. In the treatment of hyperuricemia, diet can play an important auxiliary role to improve the condition of patients and help optimize the quality of life of patients. With the steady development of society and the increasing income level, people are more and more concerned about their health, and the research on screening the active ingredients of drugs for the treatment of hyperuricemia is more and more extensive. TCM with the same origin of medicine and food not only meets the actual nutritional needs of people in daily life, but also helps to treat and prevent related diseases[3]. Through reviewing the Chinese medicinal materials included in Shennong Materia Medica and Compendium of Materia Medica, it was found that most of the Chinese medicinal materials that are helpful for body nourishing can be eaten normally, effectively

verifying the concept of TCM with the same origin of medicine and food. The use of TCM with the same origin of medicine and food in the treatment of diseases has obvious advantages, including conditioning the whole body, less toxic and side effects, and remarkable therapeutic effect[4]. In addition, due to the large population in China, the aging degree continues to deepen, medicine and food homologous health products in all sectors of society have been widely concerned, more and more people hope to use daily health to increase the autoimmune ability, optimize body function, with the help of health diet mode, improve the level of health, it can be said that the concept of medicine and food homology is gradually deeply rooted in people's hearts. And spread in social life.

1.2 The idea of the same origin of medicine and food

"Medicine and food have the same origin" means "medicine and food have the same origin, medicine and food have the same root" [5]. Tonic food is a very important content in traditional Chinese medicine. In ancient China, medicinal materials with the same origin as food and medicine have appeared in the field of health care. For example, "cerealose" belongs to the same origin of medicine and food and was recorded in the Notes on the Herbal Classics, which has the effects of alleviating emergency, supplementing deficiency and relieving pain[6]. With the development of social modernization, the per capita income level is increasing day by day, and health problems have attracted much attention. Medicinal and edible herbs are widely used in dishes to achieve health care effects. For example, Tangerine peel is widely used in stewed dishes, the former can play the role of "supplementing lung qi, tonifying middle and eliminating cold and heat" [7], while the latter can play the role of stopping vomiting and lowering qi[8]. Star anise is often used in stewed dishes, which can not only enhance the flavor of dishes, but also achieve the effect of regulating the middle and dispelling wind and regulating qi with the stomach[9]. The main characteristics of the homologous medicinal materials are non-toxic, mild efficacy, acceptable taste, and can be used in large quantities without side effects.

1.3 Mulberry leaf

As one of the medicinal materials, mulberry leaves contain rich functional components, and have preventive and therapeutic effects on a variety of diseases, which is an ideal health food and medicine. According to the records of Shennong Materia Medica, mulberry leaves are known as "fairy grass", which has the effect of dispersing wind and heat, lowering blood pressure and diuresis, promoting liver ventilation, nourishing Yin and tonifying blood. According to the Compendium of Materia Medica, mulberry leaves are the medicine of Yangming, hands and feet, and decocting tea with juice can relieve summer heat and thirst, improve eyes and long hair[10], with excellent efficacy. Mulberry leaves are the dry leaves of Mulberry, a plant in the Mulberry family. Flavonoids contained in mulberry leaves have been proved to regulate abnormal glucose and lipid metabolism, reduce inflammation, and inhibit xanthine oxidase in uric acid metabolism [11]-[13]. As one of the very important components of traditional Chinese medicine with the same origin as medicine and food, mulberry leaves have obvious activities in lowering blood sugar, lowering blood pressure, anti-aging, anti-virus, etc., and are widely used. In the treatment of hyperuricemia, mulberry leaf is an important traditional Chinese medicine with the same origin as medicine and food, which has many obvious advantages, such as low price, wide source, convenient and simple access, and outstanding safety, etc. It has a long history of use in the field of traditional Chinese medicine[14][15]. Therefore, it is undoubtedly the best choice to use mulberry leaves to treat gouty arthritis caused by hyperuricemia. However, there are many targets of Chinese medicine with the same origin of medicine and food, and the data volume is complicated. And the research on its active components and mechanism of action is still in a blank state, which limits its further basic research and clinical application to a certain extent. Therefore, this paper will take mulberry leaf as the research object, explore and analyze different active ingredients in it with the help of network pharmacology methods, study the mechanism of action in the treatment of hyperuricemia, and further verify relevant results with molecular docking technology. It is found that mulberry leaves have the effect of lowering uric acid because of its active ingredients such as scopolamine, sangocin,

quercetin, arachidonic acid, etc., which has certain implications for the follow-up research and development of new drugs and traditional Chinese medicine treatment.

Conclusions to chapter 1

This paper focuses on the mechanism of action of mulberry leaves in the treatment of hyperuricemia, and finds that quercetin, scopolamine and cyranine have stronger matching degree to the target proteins of related pathways involved in the disease. It is expected to expand the research on the mechanism of action of more diseases by using bioinformatics analysis methods such as network pharmacology and molecular docking technology according to the concept of homology of medicine and food.

CHAPTER 2
OBJECT, PURPOSE, AND METHODS OF THE STUDY

2.1 The object and purpose of study

The object of the study is mulberry leaf, a traditional Chinese medicine with the same origin as medicine and food, plays an irreplaceable role in the treatment of inflammation, anti-aging and diabetes. Its flavonoid components have been proved to regulate abnormal glucose and lipid metabolism, reduce inflammation and inhibit xanthine oxidase in uric acid metabolism, which is widely used.

The purpose of the study is to explore the therapeutic mechanism of active ingredients in mulberry leaves on hyperuricemia, and to verify the experimental results by molecular docking technology. To be more specific, in this paper, I aiming to find the potential molecular basis of anti-inflammatory and anti-bone damage effects in the treatment of diseases, so as to provide some help for the follow-up treatment of traditional Chinese medicine and the research and development of new drugs, with a view to expanding the research on the mechanism of action of more diseases.

2.2 Experimental method

2.2.1 Network pharmacology

At present, the scientific field urgently needs to conduct rapid and accurate screening of effective ingredients of hyperuricemia treatment drugs. Network pharmacology breaks through the limitations of traditional disciplines by means of bioinformatics. It mainly involves grid construction, network analysis and network verification. It is an innovative discipline. Among them, the ultimate purpose of network construction is to promote the interaction between active chemicals and target proteins and between different target proteins, and to complete the search and verification of key target proteins through network analysis and network verification, which mainly includes the screening of active substances, the discovery of target proteins, the evaluation of drug toxicity, and the study of mechanism of action[16]. The outstanding advantages of network pharmacology mainly lie in its extensive biological

theoretical system and comprehensiveness of biological system network analysis, which can also realize multi-target design of drug molecules[17]. Network pharmacology can explore different signaling pathways of drugs through a variety of different pathways, which not only can understand the main efficacy of drugs, but also can effectively exclude the toxic side effects of some drugs, thus reducing the capital cost of new drug research and development and greatly increasing the probability of successful research and development[18][19]. Traditional Chinese medicine network pharmacology is a bioinformatics method that uses the data analysis of the network database, uses the TCMSP database to screen the active ingredients of drugs, finds the corresponding target proteins in the Uniprot database, obtains the target genes for diseases through the GeneCards and OMIM databases, and then carries out the data analysis. This paper mainly analyzes the complex system of traditional Chinese medicine from the perspective of biological network, and uses the software Cytoscape system as a whole to reveal the mystery of the association between disease, target protein and drug, observe the intervention and influence of drugs on disease from the level of network, reveal the mystery of the synergistic action of complex drugs on human body, so as to find multi-target new drugs with high efficiency and low toxicity. It is widely used and has obvious significance, such as improving the therapeutic effect of drugs, reducing toxic side effects, and providing support for theoretical analysis before the experiment and reducing the cost of artificial materials.

2.2.2 Molecular docking

Molecular docking is the main link of drug development, which can reveal the role of drug molecules and biological macromolecules, provide a reasonable active conformation for the study of structure-activity relationship and pharmacophore model, and can also be used for the discovery and optimization of lead compounds. He mainly used the bioinformatics software Discovery Studio to verify whether the target of the active ingredient of the drug could closely dock with the target of the disease, so as to explore the potential mechanism of therapeutic action, and presented the experimental results in 2D and 3D visual pictures. Its purpose is to find the best binding

position of drug molecules and biological macromolecules, and finally find suitable small molecules from the drug molecule database as the ligand of macromolecules, that is, candidate drugs for new drug research and development, and molecular docking research provides a strong theoretical support for guiding the synthesis of new drugs and practical experiments.

In summary, the method of network pharmacology and molecular docking breaks through the traditional concept of "one drug, one target, one disease" and represents a change in the philosophy and research model of modern biomedical research. The maturity of this technology and method provides a new idea for further research on the interaction between potential active ingredients and targets of TCM compounds.

2.3 Experimental Subjects and procedures

2.3.1 Search Of Active Ingredients And Targets Of Drugs

Mulberry leaves were input into the Traditional Chinese Medicine System Pharmacological Analysis Platform database (TCMSP, https://tcms-e.com)[20], which was used to search and screen the names of active components and targets of target drugs, and to obtain the absorption, distribution, metabolism and excretion data of various active components of mulberry leaves. According to the ADME principle, The screening criteria included oral bioavailability (OB) and drug-likeness (DL), and the screening criteria were set at: Drug similarity (DL) ≥0.18, oral bioavailability (OB) ≥30%, to obtain the core drug active ingredients, and understand their matching action targets. The search of each active ingredient was completed through TCMSP database, and its associated target proteins were sorted out. Targeted search was carried out by protein database UniProt (https://www.uniprot.org/) to obtain each target protein name (proteinID). "HomoSapiens" was selected as the analysis species, and the target gene name (geneID) was matched with each target protein for subsequent analysis. On this basis, the name of the target gene is merged, the duplicate gene is deleted, and the only item is retained. This result can be regarded as the target of active ingredient prediction.

2.3.2 Disease-Drug Common Target Gene Acquisition

The purpose of screening disease-drug common target genes is to find the relevant target proteins that the active ingredients of traditional Chinese medicine can treat diseases, that is, the target proteins that the drugs can play a role in. Therefore, we must first obtain the common target genes of disease-drug and intuitively reveal the common genes through the Venn diagram. With keyword "Hyperuricemia" in the human genome database (GeneCards)[21][22](https://genecards.weizmann.ac.il/v3/), human online Mendelian inheritance platform (OMIM)[23] (https://omim.o rg/) were searched, and the genes associated with hyperuricemia were finally obtained. The results were de-duplicated and combined to effectively integrate the disease targets, and then matched with the active components of mulberry leaf. In DrawVennDiagram website (http://bioinformatics.psb.ugent.be/webtools/Venn/) import UniProt database to get the target genes and drug target genes of disease, complete Wayne figure drawing, Finally, the acquisition of disease-drug common target genes can be realized. Subsequently, a protein-protein interaction (PPI) network of key targets was constructed. The above disease-drug common target genes were input into STRING website[24][25] (https://string-db.org/), and the species "Homosapiens" was selected and identified. The interaction between the target proteins was further obtained. Key targets were screened according to the degree values, protein interaction network files were exported, and graphic optimization was carried out by Cytoscape software[26].

2.3.3 Gene Enrichment Analysis

In order to further obtain the number and number of target genes for drug therapy in related disease pathways, it is necessary to conduct enrichment analysis of target genes and screen out the pathways with a large number of genes, so as to further dig the genes of the obtained pathways, so as to play the role of active ingredients in drugs more efficiently and find disease treatment methods more targeted.

Disease-drug common genes were imported into the David database, species screening was carried out, and the specific Homosapiens was identified. GO enrichment analysis[27] and KEGG enrichment analysis[28][29] were carried out, and

the action pathway map of signaling pathway closely related to hyperuricemia was obtained. In "micro letter" website (http://www.bioinformatics.com.cn/) to import more than as a result, around the implementation of relevant results visualization data operation, drawing histogram GO enrichment and KEGG bubble chart, or intuitive that gene is path which has the most number of genes, Thus, gene enrichment was achieved, and key disease pathways were obtained, which laid the foundation for subsequent research.

Currently, genes corresponding to each target protein of active components and genes corresponding to disease-related pathways have been obtained. In order to more clearly show the relationship between drug - target gene - disease pathway, Cytoscape_v3.9.1 is used to map the network diagram of mulberry leaf active component-target-signal pathway, and the number of edges in the diagram represents the connections with other nodes. The function of nodes in the network diagram is closely related to the number of nodes, and the two are in direct proportion.

2.3.4 Molecular Docking Verification

Molecular docking is an important part of drug development, which can reveal the interaction between drug molecules and biological macromolecules, and can also be used for the discovery and optimization of lead compounds. Its purpose is to discover the optimal binding position of drug molecules and disease-related target protein macromolecules, and finally find suitable small molecules from the drug molecular database as the ligand of macromolecules. It mainly uses the bioinformatics software DiscoveryStudio to verify whether the target of the active ingredient of the drug can be closely connected with the target of the disease. To explore the potential mechanism of therapeutic action, and the experimental results are presented in the form of 2D and 3D visual images. It provides a strong theoretical support for exploring the treatment of diseases and guiding the synthesis of new drugs.

First 2D structure of access to medicines effective component, using the PubChem database (https://pubchem.ncbi.nlm.nih.gov/) to download 3D structure of the 29 kinds of effective components in mulberry leaves, save as "SDF" format, as a small

molecules ligand molecules of molecular docking. Then the receptor molecule is prepared, the first six core protein targets are screened according to the degree value, and the six core protein targets are searched in Uniprot to obtain the corresponding gene names. For the target proteins containing more conformation, a target gene of a peptide chain is selected as far as possible to reduce the error in the docking process. Finally, the receptor molecules are selected on RCSBPDB (https://www.rcsb.org) Download the 3D structure of the protein and save it in "pdb format" as a macromolecular receptor molecule.

After the ligands and receptors are ready, DiscoveryStudio is used for molecular docking verification, small molecular ligands are pretreated, water molecules are removed from the protein receptor, original ligands are separated, hydrogen atoms are added, etc., and molecular docking verification is then carried out. The matching degree and binding degree are observed through the 2D and 3D maps obtained. The degree of action of active ingredients was obtained.

Conclusions to chapter 2

By means of network pharmacology and molecular docking technology, the active ingredients in mulberry leaves were screened out, action targets were found, and the interaction relationship between disease targets was revealed, so as to explore the potential mechanism of action of mulberry leaves in the treatment of hyperuricemia, providing a strong support for bioinformatics research on this disease. Through the study, we determined that multiple active components in mulberry leaves have the network mechanism of synergistic action on hyperuricemia through multi-component, multi-target and multi-pathway.It is expected to be extended to the study of other disease mechanisms and provide new ideas and methods for future drug development and innovation.

CHAPTER 3
EXPERIMENTAL PART

3.1 Active components and target of mulberry leaves

A total of 269 active ingredients of mulberry leaves were obtained through TCMSP database search, and 29 active ingredients were found in the filter conditions

of OB≥30% and DL≥0.18. Including moracin (moracin), scopolin (scopolin), quercetin (quercetin), beta-carotene (beta-carotene), arachidonicacid (arachidonicacid) and so on. (Tab.3.1) Then, the target corresponding to each active ingredient was input into the UniProt database to obtain the target gene name. In this experiment, the UniProtKB knowledge base was mainly used to predict the target genes of the compound obtained by the above approach and combine to de-weight, and the "mulberry leaf active ingredient-target database" was established. A total of 657 targets and 1438 genes related to active components were obtained.

Table 3.1 – Effective ingredients of mulberry leaf

Name of Chinese medicine	ID	Molecular name	OB	DL
Sang Ye	MOL002218	scopolin	0.39	56.45
	MOL002773	beta-carotene	0.58	37.18
	MOL001771	poriferast-5-en-3beta-ol	0.75	36.91
	MOL003975	icosa-11,14,17-trienoic acid methyl ester	0.23	44.81
	MOL003842	Albanol	0.24	83.16
	MOL003847	Inophyllum E	0.85	38.81
	MOL003850	26-Hydroxy-dammara-20,24-dien-3-one	0.79	44.41
	MOL003851	Isoramanone	0.51	39.97
	MOL000098	quercetin	0.28	46.43
	MOL003856	Moracin B	0.23	55.85
	MOL003857	Moracin C	0.29	82.13
	MOL000422	kaempferol	0.24	41.88
	MOL003858	Moracin D	0.38	60.93
	MOL003859	Moracin E	0.38	56.08

MOL001439	arachidonic acid	0.2	45.57
MOL003860	Moracin F	0.23	53.81
MOL003759	Iristectorigenin A	0.34	63.36
MOL003861	Moracin G	0.42	75.78
MOL003862	Moracin H	0.51	74.35
MOL003879	4-Prenylresveratrol	0.21	40.54
MOL000433	FA	0.71	68.96
MOL000729	Oxysanguinarine	0.87	46.97
MOL007879	Tetramethoxyluteolin	0.37	43.68
MOL000358	beta-sitosterol	0.75	36.91
MOL000449	Stigmasterol	0.76	43.83
MOL007179	Linolenic acid ethyl ester	0.2	46.1
MOL001506	Supraene	0.42	33.55
MOL006630	Norartocarpetin	0.24	54.93
MOL013083	Skimmin (8CI)	0.32	38.35

3.2 Disease-Drug Common Target Gene Acquisition

(1) Acquisition of disease genes: Disease genes were obtained from GeneCards and OMIM, and 908 effective genes were obtained after the results were collated and duplicated.

(2) Acquisition of disease-drug common target genes: 908 effective targets of disease and 657 targets of effective active ingredients of drugs are respectively introduced in the DrawVennDiagram to complete the drawing of the Venndiagram. (Fig. 3.1) A total of 78 intersection genes are found.

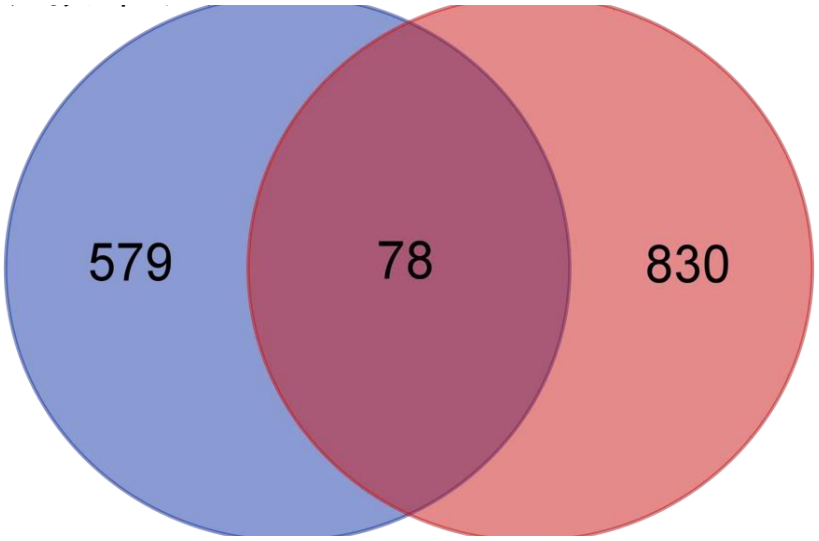

Figure 3.1 – Venny map of disease-drug intersection genes

3.3 Construction Of PPI Networks

Protein interaction network (PPI network) is used to clarify the potential interaction mechanism between proteins, which aims to describe the relationship between genes or proteins, so as to obtain the proteins that have the greatest influence in the whole network, screen and obtain key targets, and provide a strong basis for further gene in-depth mining.

Construction of key target PPI network: Input the above common genes into the STRING database(Fig. 3.2), in order to form the PPI network diagram of key target. There is only one isolated node in the network, and the connectivity between nodes is good, indicating that the effective components of mulberry leaves can regulate multi-target synergistic treatment of hyperuricemia, and store PPI network according to TSV format. Cytoscape software is used for data processing, core genes are sequenced according to degree value, and the top 10 genes are as follows: The degree values of GAPDH, ALB, TNF, PPARG, ESR1, JUN, BCL2, MYC, PTGS2 and CASP3 correspond to 59,50,50,47,45,45,43,43,42,41 in sequence. The targets are displayed as nodes in the PPI network and connected by edges. The key targets have relatively dense edges, which means that they play a more important role in the PPI network, so it can be determined which target is more effective.

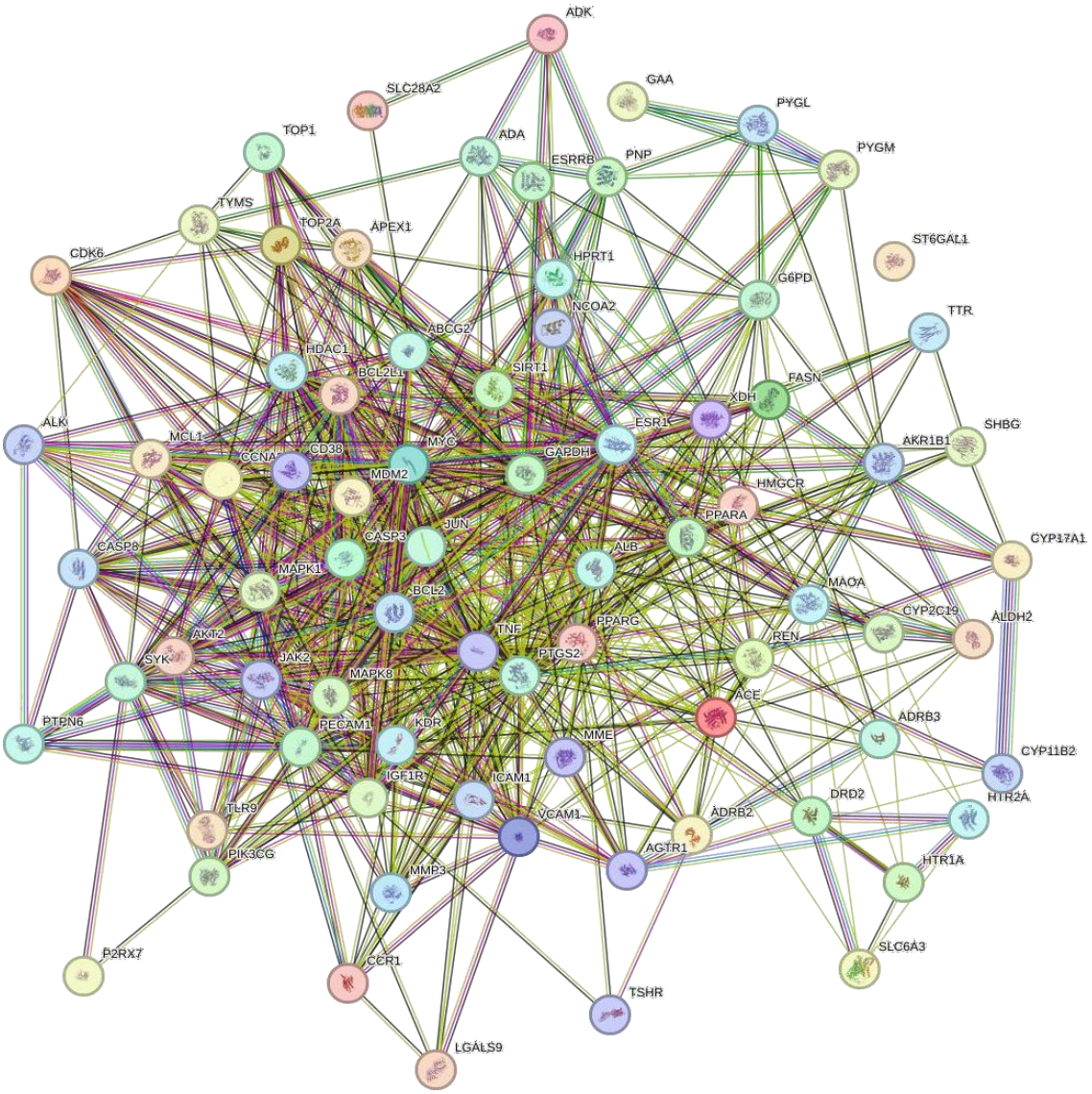

Figure 3.2 – Disease-drug intersection gene PPI network

3.4 Gene enrichment result and component-target-path network diagram

(1) GO enrichment: A total of 78 disease-drug common target genes obtained in 3.2 were imported into the David database for GO enrichment analysis. The "Weishengxin" website is used to produce bar charts for three ontologies: biologicalprocess (BP), cellularcomponent (CC), and molecularfunction (MF). (Fig. 3.3) The longer the length of each rectangle in the bar chart indicates that the more genes in the process and the more significant the effect after enrichment. BP is generally used to describe the biological process to be completed by a certain protein.

CC is used to describe the position where the protein plays a role, that is, where it is expressed; MF is mainly concerned with the function of the protein, such as catalytic capacity, transport capacity and so on. Through GO enrichment analysis, a large number of target protein genes were grouped into different ontology categories according to functional similarity, so as to more efficiently screen the required active ingredient genes.

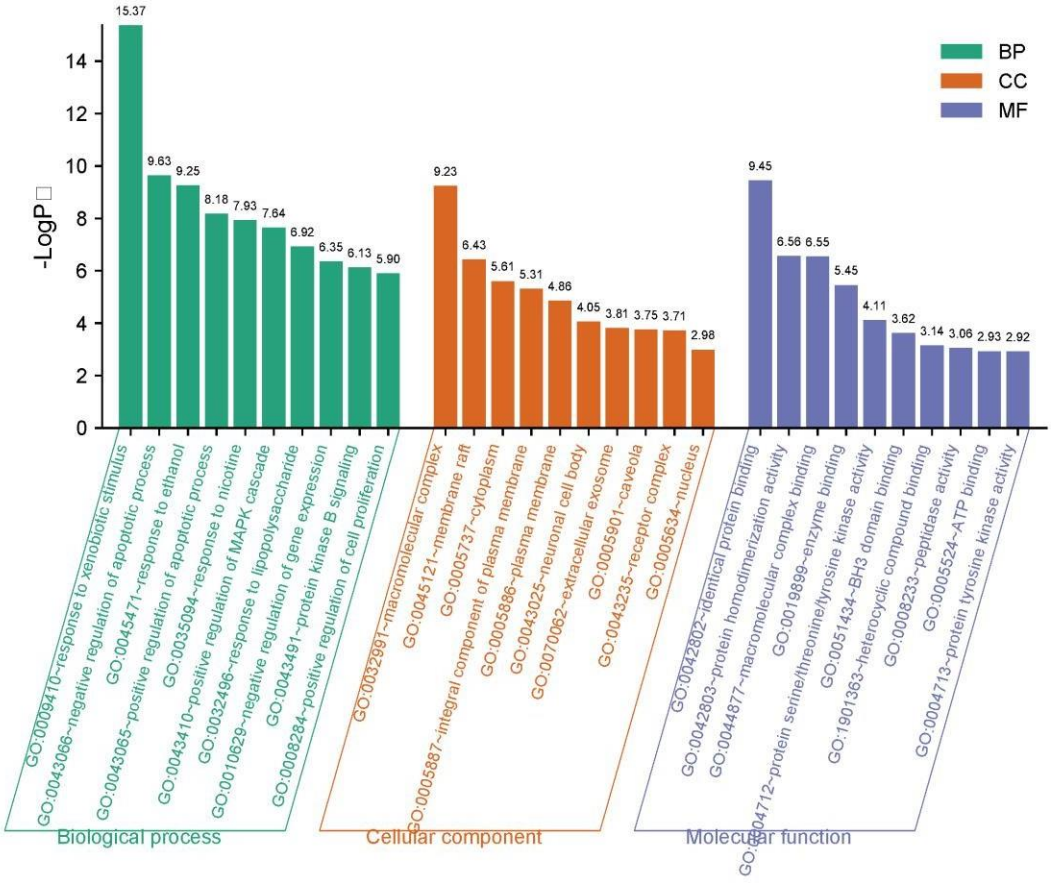

Figure 3.3 – GO enrichment analysis results

(2) KEGG enrichment: KEGG enrichment analysis with high uric acid hematic disease pathways involved mainly includes TNF signaling pathway, AGE-RAGE signaling pathway in diabetic complications, Toxoplasmosis, NF-kappaB signaling pathway, Pathways in cancer, NOD like receptor signaling pathway, PI3K-Akt signaling pathway etc.(Fig. 3.4) . By analyzing the KEGG enrichment map, we can see that the larger the number of circles, the more genes are enriched in this pathway, so

the most genes are enriched in cancer-related pathways. The smaller the p value, the smaller the chance, that is, the more likely the gene is enriched in the relevant pathway, which provides a powerful basis for guiding us how to treat diseases. In the David database, the access of the lookup and high uric acid hematic disease is closely related to the AGE-RAGE signaling pathway in diabetic complications and Pathwaysincancer(Fig. 3.5 and Fig. 3.6)

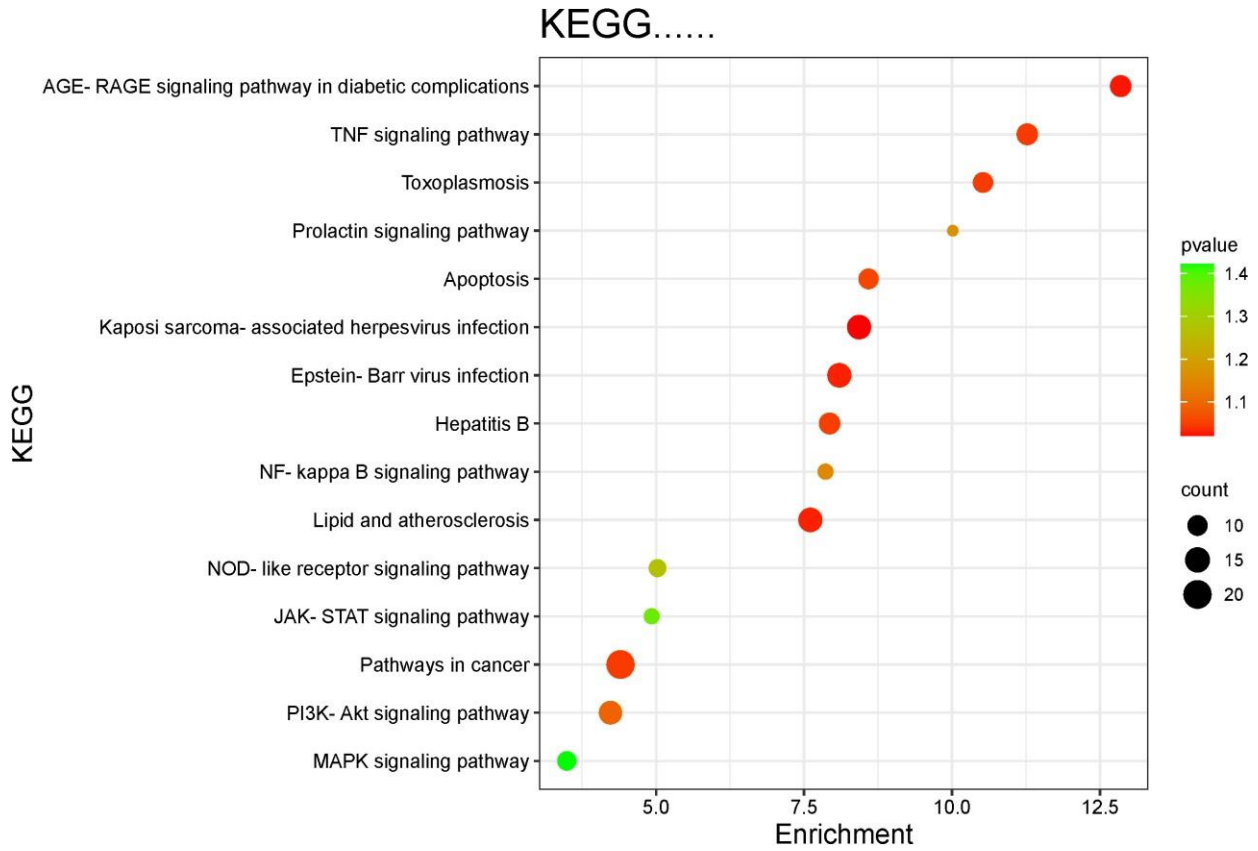

Figure 3.4 – KEGG enrichment

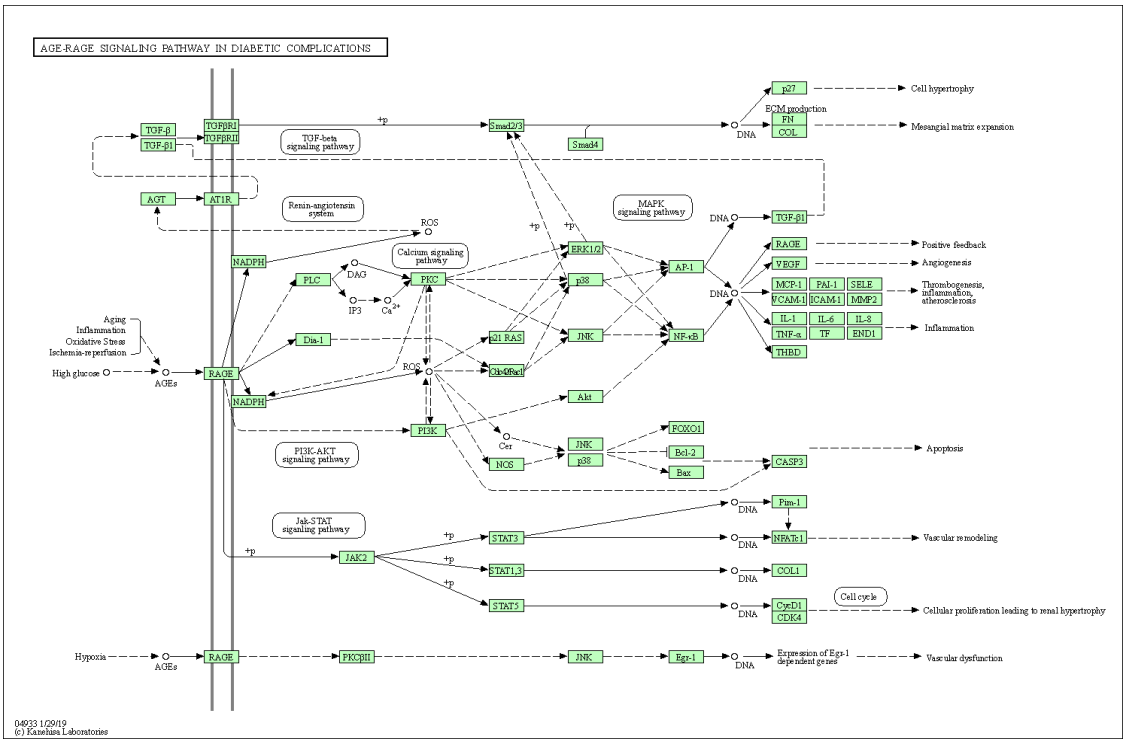

Figure 3.5 – AGE-RAGE signaling pathway in diabetic complications signal path action diagram

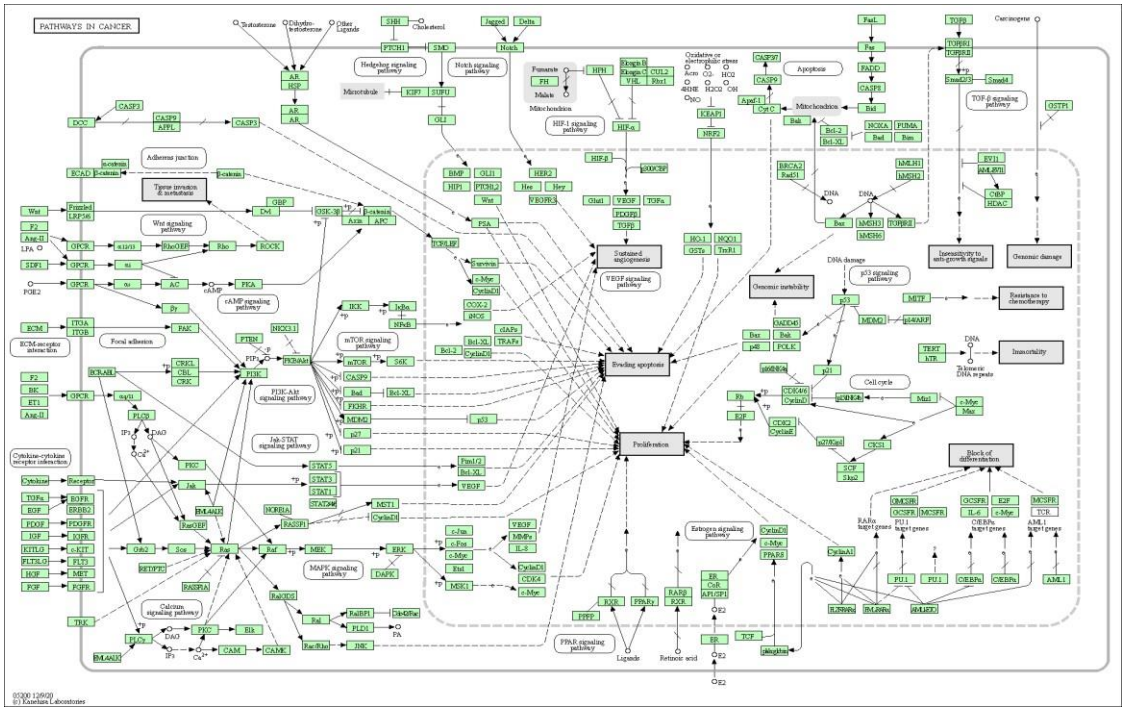

Figure 3.6 – Pathways in cancer signal path action diagram

(3) After gene enrichment analysis is completed and disease-related pathways are obtained, bioinformatics software is required to take a series of visual operations to

obtain the composition-target-pathway network diagram, so as to reveal the relationship between the three more directly and provide a strong basis for disease treatment.

Analysis of mulberry leaf active ingredient-Target-signal pathway network: Related information and data obtained from mulberry leaf, including active ingredients and corresponding protein targets, are imported into Cytoscape software to complete the construction of the "drug active ingredient-target-disease pathway" network,(Fig. 3.7). Combined with the information presented in the network diagram, it can be intuitively concluded that the effect of mulberry leaves on hyperuricemia depends on the coordination of multi-components, multi-targets and multi-pathways. The blue square node represents the active component of the drug, the purple square node indicates that the disease involves pathway signals, the yellow square node represents the target gene, and the edge represents the connection between the nodes. The more the number of edges indicates that they are closely connected with other nodes, and the greater the role they play in the entire network.

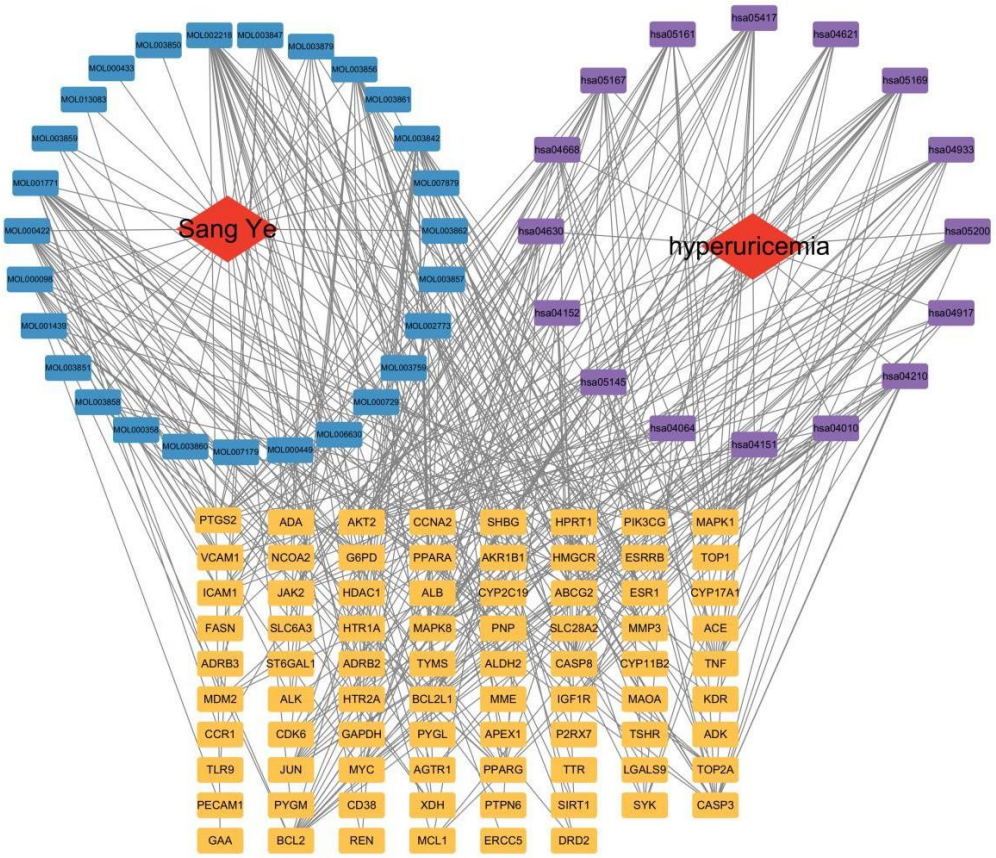

Figure 3.7 – Active ingredient-target-signal pathway network diagram of mulberry leaf

3.5 Molecular docking result

Discovery Studio was used to verify molecular docking, and it was found that some of the active components could not bind to the active site of the receptor protein, so the docking conformation could not be obtained. The results were screened and ranked, and it was found that santisinine, scopolamine and quercetin had higher binding to estrogen receptor and tumor necrosis factor, which provided strong evidence for proving the mechanism of action of effective active ingredients. The two-dimensional plan of the ligand-receptor interaction is shown in (Figure 3.8), and the three-dimensional diagram is shown in (Figure 3.9).

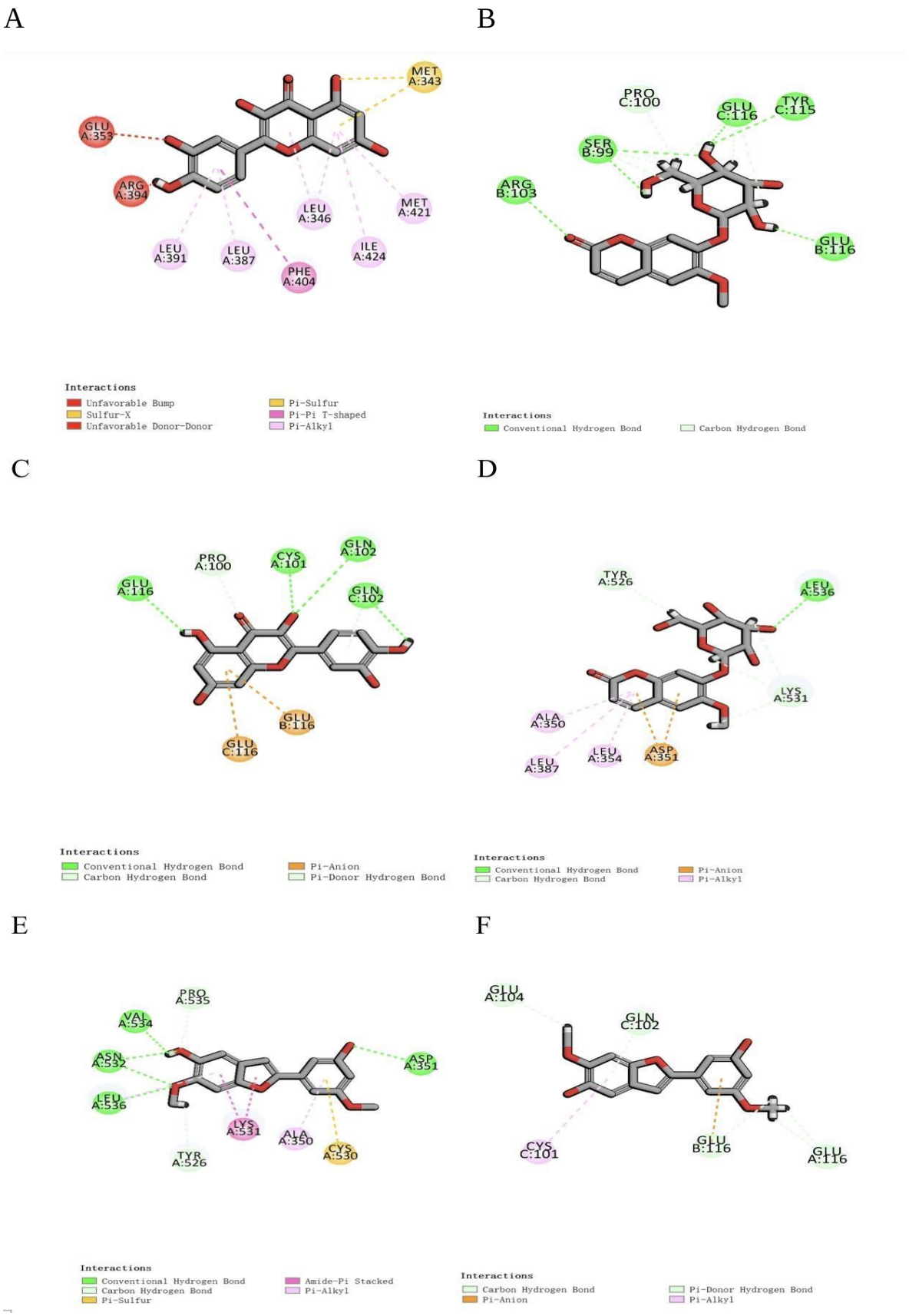

Figure 3.8 – Two-dimensional diagram of ligand-protein interactions

Note: A. Result of the docking between Estrogen receptor and quercetin; B. Result of the docking between TNF-α and scopolamine; C. Result of the docking between TNF-α and quercetin; D. Result of the docking between Estrogen receptor and scopolamine; E. Result of the docking between Estrogen receptor and Santhine B; F. Result of the docking between TNF-α with santhine B

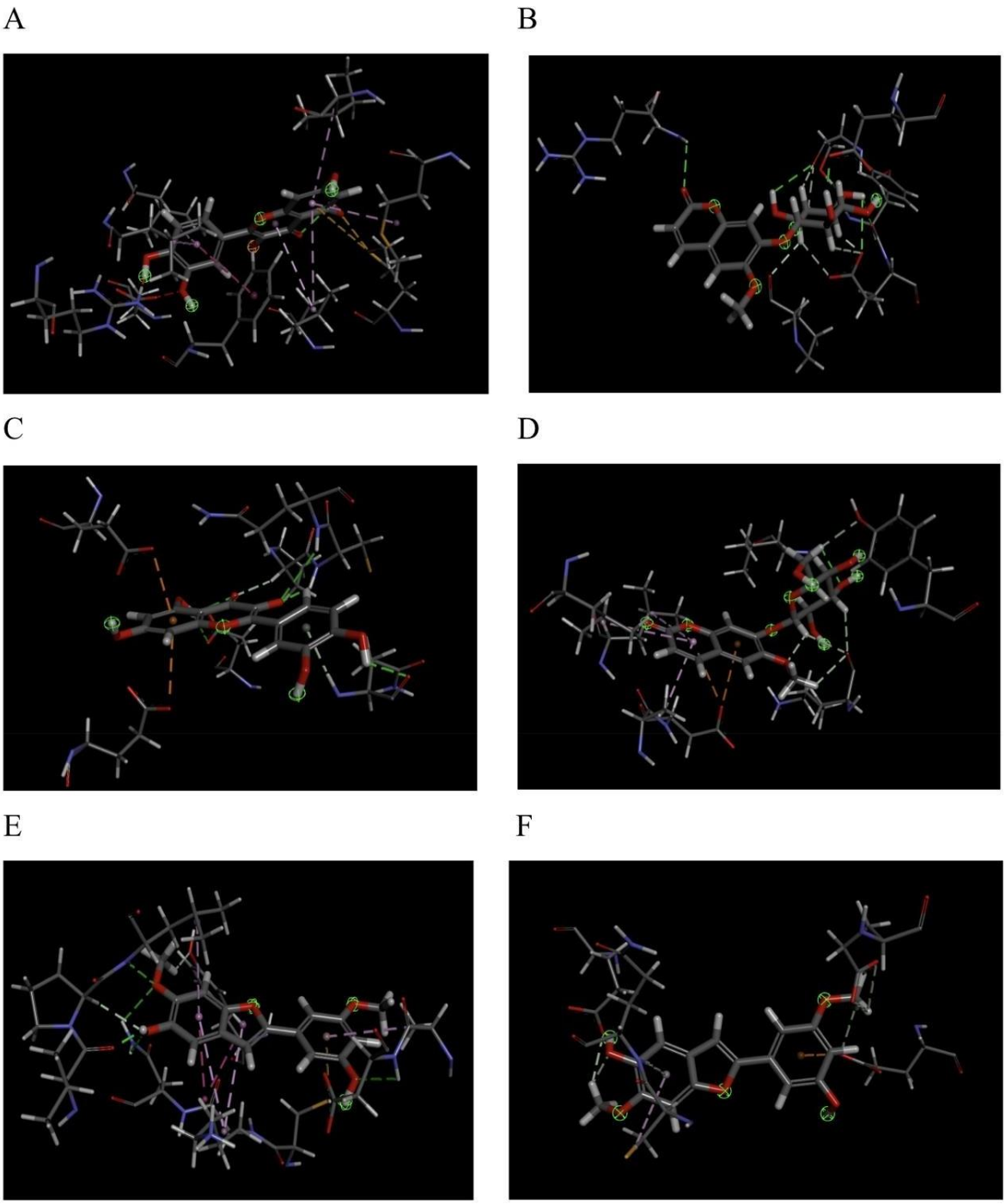

Figure 3.9 – Three-dimensional plan of ligand-protein interactions

Note: A. Result of the docking between Estrogen receptor and quercetin; B. Result of the docking between TNF-α and scopolamine; C.Result of the docking between TNF-α and quercetin; D. Result of the docking between

Estrogenreceptor and scopolamine; E.Result of the docking between Estrogen receptor and Santhine B; F.Result of the docking between TNF-α with santhine B

Conclusions to chapter 3

Quercetin, scopolamine and moracin were identified as active components through molecular docking. Quercetin, a flavonoid widely found in plants, can inhibit the activity of xanthine oxidase. Appropriate intake of quercetin can enhance the body's ability to clear oxygen free radicals and reduce lipid peroxidation to prevent oxidative stress. Reduce the activity of xanthine oxidase in liver to reduce serum uric acid level and alleviate kidney damage. Through gene enrichment, the disease-related pathways involved include TNF signaling pathway, cancer signaling pathway, PI3K-Akt signaling pathway, AGE-RAGE signaling pathway in diabetic complications and other metabolic pathways, so the drugs designed are more targeted.

CONCLUSIONS

The main manifestation of hyperuricemia is gouty arthritis, an immunoinflammatory disease mediated by uric acid crystal deposition in synovial tissue, which is a key pathological factor in the onset of gout. The occurrence of inflammation is closely related to tumor necrosis factor α and other inflammatory factors. Therefore, patients with gouty arthritis can optimize and improve their clinical symptoms through the regulation of immune-inflammatory response[30]. According to clinical research results, mulberry leaves can help patients with gouty arthritis reduce the response of inflammatory factors in the body, but the molecular mechanism has not yet been clarified. The active ingredients involved mainly include quercetin, sangxin and scopolamine, which can inhibit the production and expression of inflammatory cytokines such as tumor necrosis factor α, thereby improving the human immune response. To achieve analgesic and anti-inflammatory effects[31][32]. KEGG analysis results showed that the mechanism of mulberry leaves in the treatment of gout arthritis involved the regulation of TNF signaling pathway, AGE-RAGE signaling pathway in diabetic complications, NF-kappaB signaling pathway, cancer signaling pathway, PI3K-Akt signaling pathway, and inflammatory response, etc. These reactions are also closely related to human immune inflammatory response. Results showed that tumor necrosis factor signaling pathway mediated eosinophils and lymphocytes to regulate inflammatory factors such as interleukin 1 and interleukin 6, thus affecting the degree of inflammation in the body[33]. AGE-RAGE pathway is closely related to the occurrence of inflammatory response. It can up-regulate the phosphorylation level of MAPK and promote the expression of inflammatory factors, including interleukin 1, tumor necrosis factor α, interleukin 6, etc.[34]. The NOD-like receptor pathway and nuclear transcription factor κB pathway are closely related to inflammatory diseases, and activation of these pathways can promote the expression of inflammatory

cytokines such as interleukin-1 and tumor necrosis factor α[35]. Therefore, mulberry leaves mainly play a role in inhibiting inflammation and immune regulation, and regulate inflammatory factors in the blood, adjust human immune function, and inhibit human inflammatory response by acting on multi-component, multi-target-multi-pathway, so as to achieve the treatment effect of gouty arthritis.

According to the results of modern pharmacological studies, mulberry leaves have good effects in anti-bone injury and regulating apoptosis, so as to protect bone and joint and alleviate bone and joint injury. Chondrocytes play a crucial role in the maintenance of articular cartilage function and stroma secretion. Studies have shown that articular cartilage is often destroyed during the development of gouty arthritis, and the apoptosis of chondrocytes is closely related to the reduction of articular cartilage stroma secretion[36]. Studies have shown that quercetin, the active ingredient in drugs, can play a protective role in joints, mainly through reducing the apoptosis of knee cartilage cells and eliminating cartilage degeneration[37]. Sangxin has anti-inflammatory effects, such as inhibiting the infiltration of inflammatory cells by inhibiting the activity of PDE4[38]. As for the receptor protein, abnormal expression of estrogen receptor gene will activate the TP53 target gene and further accelerate the apoptosis rate[39]. Studies have shown that the occurrence of gouty arthritis will cause chondrocyte apoptosis, resulting in reduced secretion of articular cartilage matrix and joint degeneration[40]. Therefore, estrogen receptor may delay joint degeneration and alleviate joint pain in gouty arthritis by regulating chondrocyte apoptosis. In terms of regulatory pathways, mulberry leaf mainly regulates NOD-like receptor pathway, nuclear transcription factor κB pathway, TNF signaling pathway, pathway in cancer and AGE-RAGE signaling pathways in diabetic complications to regulate chondrocyte proliferation, differentiation, and apoptosis. The NOD-like receptor signaling pathway can affect the catabolism of articular chondrocytes, and this regulatory pathway may be a useful target to prevent the development and progression of arthritis[41]. Meanwhile, the nuclear transcription factor κB pathway can regulate the apoptosis of

chondrocytes and thus affect the development of osteoarthritis[42]. Therefore, mulberry leaves can alleviate the destruction of articular cartilage in patients with gouty arthritis by regulating the proliferation, differentiation and apoptosis of chondrocytes, further relieve the inflammatory response of gouty arthritis, and significantly improve the clinical symptoms of patients.

In summary, this paper breaks through the research mode of single target through the method of network pharmacology, which provides great convenience for the research of traditional Chinese medicine. The network mechanism of synergistic action of multiple active ingredients in mulberry leaves on hyperuricemia through multi-component, multi-target and multi-pathway is discussed, and the correlation is verified through molecular docking technology. It was found that Sangxin, scopolamine and quercetin in mulberry leaves were the potential molecular basis for their anti-inflammatory effects and alleviating bone and joint injury, which provided some help for the subsequent treatment of diseases with other medicinal and food homologous Chinese medicines. This study also has shortcomings. For example, in the process of analyzing the interaction between active drug ingredients and disease targets, although various databases, network pharmacology, molecular docking and other bioinformatics methods have been used to reveal the relationship between the two, it is still necessary to further verify the potential molecular mechanism of action between the two by in vitro or in vivo experiments. In order to ensure that the experimental research results are real and effective, and provide experimental basis for the clinical application of drugs. Moreover, this experiment only carried out in-depth exploration and research on the target proteins and genes of the active ingredients, while the study on the effects of other active ingredients in mulberry leaves is not thorough enough. A more comprehensive understanding of mulberry leaves as a traditional Chinese medicine is needed, with a view to expanding it to the treatment of more diseases.

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