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 **Study on effective components and mechanism of lowering uric acid in Safflower**

First (Bachelor's) level of higher education

Specialty 162 "Biotechnology and Bioengineering"

Educational and professional program "Biotechnology"

Completed: student of group BEBT-20
Zhang ZHIHENG

Scientific supervisor
Olga IUNGIN, Ph.D., Assoc. Prof.

Reviewer
Ihor HRETSKYI, Ph.D., Assoc. Prof.

Kyiv 2024

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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
Zhang Zhiheng

1. Thesis topic **Study on effective components and mechanism of lowering uric acid in Safflower**
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 _____ Zhang ZHIHENG

Scientific supervisor _____ Olga IUNGIN

SUMMARY

Zhiheng Zhang。Study on the active component and mechanism of uric acid lowering in safflower – Manuscript.

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

Hyperuricemia (Hyperuricemia, HUA) is a relatively common metabolic disease, mainly manifested by increased blood uric acid concentration. As a traditional herbal medicine, safflower (Crocus sativus) is widely used in traditional Chinese medicine to treat many diseases including hyperuricemia, but the interaction between its multi-components, multi-targets and multi-pathways is not clear. In this study, network pharmacology and molecular docking techniques were used to explore the interaction mechanism between safflower active components and target proteins, in order to provide theoretical basis and guidance for the design and development of safflower drugs.

In this study, using the network pharmacology method, we collected the active ingredients and targets of safflower through TCMSP database and used Uniprot to screen the active components and targets of lowering uric acid. When hyperuricemia targets were collected in GeneCards and OMIM databases, 22 active ingredients and 941 potential targets of hyperuricemia were obtained. GO and KEGG enrichment analysis were performed in this study, and the results showed that safflower may treat hyperuricemia through several signaling pathways, such as Lipid and atherosclerosis signaling pathway, AGE-RAGE signaling pathway, TNF signaling pathway, IL-17 signaling pathway, and tumor necrosis factor signaling pathway.

In this thesis, using Discovery Studio and AutoDockVina molecular docking software to verify the interaction of safflower active components and key targets, after the molecular docking validation, found that kaempferol, quercetin and luteolin showed good binding ability, its mechanism of action involves uric acid transporter

and related signaling pathway, including TP53, CASP 3, IL 6, TNF may be the key target of safflower for uric acid, speculated that safflower can treat hyperuricemia.

In conclusion, safflower is treated by multi-component, multi-target and multi-pathway cooperation. This study provides a basis for further research on the active components and mechanism of action of safflower, and provides a new idea for related research.

Key words: safflower; hyperuricemia; network pharmacology; molecular docking

TABLE OF CONTENTS

INTRODUCTION

The relevance of the topic is : Safflower is a traditional Chinese medicine that contains a variety of active ingredients, has a variety of biological activities such as antioxidant, anti-inflammatory and anticoagulant, and is used to treat a variety of diseases including hyperuricemia

The purpose of the study is: the Network pharmacology and molecular docking techniques were used to explore the mechanism of safflower in the treatment of hyperuricemia, explore the synergistic and antagonistic effects of Safflower with other components of Chinese medicine, and search for effective active components of Chinese medicine in the treatment of hyperuricemia.

The objectives of the study : Based on the results of network pharmacology and molecular docking, the key targets and signaling pathway regulation of safflower in the treatment of hyperuricemia were analyzed, and the significance of the effect and mechanism of safflower in the treatment of hyperuricemia was evaluated and analyzed.

The object of the study S: afflower, hyperuricemia

The subject of the study : Study on effective components and mechanism of lowering uric acid in Safflower

Research methods : Network pharmacology, molecular docking

The scientific novelty: There is a lack of research on the mechanism of safflower in the treatment of hyperuricemia at home and abroad, and the specific mechanism of safflower in the treatment of hyperuricemia is not clear. In this study, the innovative use of network pharmacology and molecular docking technology to clarify the mechanism of safflower treatment of hyperuricemia, providing a new idea for related research.

The practical significance of the results obtained is: When uric acid is produced too much or excreted too little, it can lead to elevated levels of uric acid in the blood and form hyperuricemia. Safflower has the effect of promoting blood circulation, dissipating blood stasis and relieving pain. It is often used to treat injuries such as

bruises and joint pain. At present, there is no clear classical medical record of using safflower to treat hyperuricemia. Therefore, through the method of network pharmacology and molecular docking, this study explored the therapeutic mechanism of safflower on hyperuricemia, and provided scientific basis and reference for the prevention and treatment of this disease.

CHAPTER 1 LITERATURE REVIEW

1.1 Research background and significance

Uric acid is an end product of purine metabolism, and its level in the human body is closely related to diseases such as gout and uric acid nephropathy. Hyperuricemia is a metabolic disease due to an imbalance in the synthesis and excretion of uric acid[1], Its clinical manifestations mainly include gout, uric acid arthritis, etc., which seriously affect people's quality of life, and bring a huge burden to the society and families.

Safflower is a traditional Chinese medicine that has been widely used since ancient times to treat many diseases, including hyperuricemia[2]. Modern pharmacological studies show that safflower contains a variety of active ingredients, with antioxidant, anti-inflammatory, anticoagulant and other biological activities, and has a good effect on the treatment of hyperuricemia, but its specific mechanism of action is not yet clear.

Network pharmacology and molecular docking technology are widely used technologies in the field of drug research and development, which can conduct multi-dimensional and comprehensive analysis of traditional Chinese medicine compound, so as to deeply study its pharmacological action mechanism. And the interaction between TCM compounds and multiple targets can be predicted by network pharmacology analysis[3]To find the potential mechanism of TCM compound treatment for diseases; through molecular docking technology, we can explore the mechanism between active ingredient in TCM compound and target protein to provide support for the research and development of TCM compound.

Therefore, based on the network pharmacology and molecular docking technology of the safflower treatment of hyperuricemia mechanism research, to insight into the treatment of safflower hyperuricemia mechanism, mining the safflower potential treatment mechanism, explore the interaction mechanism between the safflower active ingredient and target protein mechanism, has important theoretical and practical significance.

1.2 Current research status of hyperuricemia and safflower

1.2.1 Etiology and pathogenesis of hyperuricemia

The etiology and pathogenesis of hyperuricemia are multifaceted: genetic factors, dietary factors, metabolic disorders, and circulatory mechanisms. The circulatory mechanism of hyperuricemia refers to the process related to the circulating metabolism of uric acid in the body. After uric acid enters the blood, most of it is excreted outside the body through the kidney, and a small part is excreted through the intestine. When uric acid excretion is blocked, the concentration of uric acid in the blood will rise, forming hyperuricemia. Hyperuricemia can lead to uric acid crystals[4]Deposition in the joints, kidneys and other sites, causing the corresponding clinical symptoms.

1.2.2 Study on the chemical composition and pharmacological effects of safflower

Safflower, also known as gold and saffron, is the style and dye of saffron, with blood tonic, blood circulation, meridian regulation, pain relief, anti-inflammatory, lipid lowering and blood pressure5And many other pharmacological effects. Honghua contains many active ingredients, such as flavonoids, carboxylic acid, coumarins, flavonoids, sterols, flavonoid glycosides, etc. Among them, flavonoids are the main active ingredients of safflower, such as phenolic acid and ruin. The combined effect of these active ingredients in safflower makes it have many pharmacological effects, especially for the treatment of hyperuricemia.

Danphenolic acid and flavonoid components in safflower[6]It has obvious anti-inflammatory, anti-oxidation and apoptosis. Honghua extract has obvious analgesic and anti-inflammatory effects, and can inhibit the release of inflammatory mediators and reduce the inflammatory response in the experimental arthritis model, thus reducing the symptoms of arthritis. In addition, safflower also has lipid-lowering, anti-platelet condensation, antibacterial, antiviral, blood pressure, blood sugar and other effects.

1.2.3 Current research status of safflower in the treatment of hyperuricemia

As a traditional Chinese medicine, safflower has the effects of clearing away heat and detoxification, promoting blood circulation and removing blood stasis, and relieving pain. It is mostly used for the treatment of rheumatic bone pain, dysmenorrhea, falling injury and other diseases. In recent years, studies have found that safflower also treats hyperuricemia.

(1) The effect of safflower on reducing blood uric acid

safflower was found to reduce serum uric acid levels. An experiment based on a rat model showed that total flavonoids in safflower significantly reduced hyperuricemia in rats caused by a high-fat diet[7], It can also reduce the liver damage that causes hyperuricemia.

(2) The effect of safflower on improving renal function

Hyperuricemia is one of the important causes of kidney disease, and safflower is also helpful to improve kidney function[8]. Studies have found that safflower reduces the amount of uric acid in the kidneys, reduces the burden on the kidneys, and also improves sodium excretion in the renal tubules.

Figure 1.1 – for safflower

1

1.3 Study purpose, method, and content

1.3.1 Study Purpose

(1) This study used network pharmacology to analyze the main chemical components of safflower and its possible targets for the treatment of hyperuricemia. Network pharmacology (Network Pharmacology) is an emerging research method for predicting the targets of drug effects and their mechanisms of action in biological processes. The analysis of network pharmacology can provide a theoretical basis for the subsequent molecular docking.

(2) The interaction between the chemical composition of safflower and the key targets was also investigated by molecular docking method. Molecular docking technology is a computational chemical method that can be used to predict intermolecular interactions and then reveal the mechanism of action between drug molecules and target sites. Through molecular docking methods, the interaction between safflower chemical components associated with treating hyperuricemia and key targets was investigated, and thus the mechanism by which hyperuricemia may play a role in safflower treatment was revealed.

1.3.2 Research methods and content

This paper focuses on the mechanism of safflower for hyperuricemia, using network pharmacology and molecular docking. The specific research methods and contents include the following aspects:

(1) Screof active compounds using TCMSP database

(2) Human gene database (GeneCards) screening targets

(3) Construction of the key target PPI network

(4) Molecular docking

In conclusion, this study will use TCMSP database, GeneCards database, Omim, Cytoscape 3.6.0 and other software to study the mechanism of network pharmacology and molecular docking methods of safflower, aiming to reveal the molecular mechanism of hyperuricemia and provide theoretical support for the medicinal value of safflower.

Conclusions to chapter 1

1. Research background and significance: The importance of research is emphasized.
2. Current research status of hyperuricemia and safflower: including the etiology and pathogenesis of hyperuricemia, the chemical composition and pharmacological effects of safflower, and the current research status of safflower in the treatment of hyperuricemia.
3. Research purpose, method and content: The research purpose is clarified, and the research method and content are introduced.

CHAPTER 2 OBJECT, PURPOSE AND METHODS OF THE STUDY

2.1 Study methods and content

2.1.1 Screof active compounds using the TCMSP database

TCM system pharmacology database: TCMSP[9](http://tcmspw.com /tcms. The php) is a database covering TCM chemical composition, targets, mechanism of action, providing comprehensive molecular information and systematic pharmacology information of TCM. This study was based on the ADME principles[10]12, Oral bioavailability (oral bioavailability, OB) of 30% and drug-like (drug-likeness, DL) of 0.18 were selected, and the core safflower active ingredients and targets were obtained. And imported into Uniprot to derive the gene name.

2.1.2 Use Uniprot to obtain the gene name

In the process of converting target information into gene name, target information into Uniprot database[13]14（https://www.uniprot. To org /) to achieve. First, this study needs to visit the official website of the Uniprot database, and then enter the safflower target information such as protein name and sequence into the search box. Uniprot The database returns the protein data related to the input information.

Protein entries associated with the target information can all be found in the search results. In this entry, the sequence, structure, functional description and gene information associated with it, which can find the gene name corresponding to the safflower target information. Gene names are often presented in a Uniprot database in a specific series of formats. In this study, the safflower target information was added to the specific gene name, so as to provide an important reference basis for the subsequent research and analysis.

2.2 Results and analysis

Potential active ingredients of safflower in TCMSP

Table 2.2 – **potential active components in safflower**

numerator ID	Molecular name	OB /%	DL
MOL001771	poriferast-5-en-3beta-ol	36.91	0.75
MOL002680	Flavoxanthin	60.41	0.56
MOL002694	4-[(E)-4-(3,5-dimethoxy-4-oxo-1-cyclohexa-2,5-dienylidene)but-2-enylidene]-2,6-dimethoxycyclohexa-2,5-dien-1-one	48.47	0.36
MOL002695	lignan	43.32	0.65
MOL002698	lupeol-palmitate	33.98	0.32
MOL002706	Phytoene	39.56	0.5
MOL002707	phytofluene	43.18	0.5
MOL002710	Pyrethrin II	48.36	0.35
MOL002712	6-Hydroxykaempferol	62.13	0.27
MOL002714	baicalein	33.52	0.21
MOL002717	qt_carthamone	51.03	0.2
MOL002719	6-Hydroxynaringenin	33.23	0.24
MOL002721	quercetagetin	45.01	0.31
MOL002757	7,8-dimethyl-1H-pyrimido[5,6-g]quinoxaline-2,4-dione	45.75	0.19
MOL002773	beta-carotene	37.18	0.58

MOL002776	Baicalin	40.12	0.75
MOL000358	beta-sitosterol	36.91	0.75
MOL000422	kaempferol	41.88	0.24
MOL000449	Stigmasterol	43.83	0.76
MOL000006	luteolin	36.16	0.25
MOL000953	CLR	37.87	0.68
MOL000098	quercetin	46.43	0.28

2.3 Hyperuricemia and Safflower common target gene acquisition and construction of PPI network

2.3.1 Gene collection

The human gene database, GeneCards[15]16 （https://genecards.weizmann.ac. And il /v3 /), the human online Mendelian genetic platform OMIM[17] （https://omim. Both org /) for the same kind of database, included all human certified, not certified genes, similarly also included have identified human can suffer from all disease genes, in two sites respectively input hyperuricemia (Hyperuricemia), in two databases search related target genes, remove the results after repeat the same genes, and then match the safflower targets from Uniprot. Through the Draw Venn Diagram web site (http: / /bioinformatics.psb.ugent. Be / webtools / Venn /) analyzed the Wayn diagram of the common target genes of safflower and hyperuricemia, and the middle overlap was the common target genes of hyperuricemia-safflower, which are theoretically the target genes of effective components of safflower that have therapeutic effect on hyperuricemia.

2.3.2 Build the key target PPI network

Introduction of the hyperuricemia-safflower common target gene into the STRING[18]19Website (https://string-db. In org/), the selected species is "Hom o sapiens"[20], To obtain the interaction relationship between the target proteins. In this study, the STRING database will be used to build key target PPI networks, which can predict protein function and interaction by analyzing the interrelationships among various nodes. Through the network analysis tool Cytoscape 3.9.1[21]PPI network[22]23Visual processing and analysis were performed to further select the core targets closely related to the development and development of hyperuricemia. In the PPI network, the targets are displayed as nodes and connected by edges. The more dense the edges of the key targets, the more role they play in the PPI network[20]。

Conclusions to Chapter 2

The research scheme, database and methods were described

CHAPTER 3 EXPERIMENTAL PART

3.1 Acquisition of hyperuricemia genes

After entering hyperuricemia (Hyperuricemia) in the human gene database (GeneCards and OMIM), 941 hyperuricemia genes were combined by Excel form. First, this study further analyzed these 941 genes on functional annotation and bioinformatics. The analysis of the functional categories, pathway enrichment, and interaction networks of these genes through bioinformatics tools such as STRING can help to reveal the mode of action of these genes in the pathogenesis of hyperuricemia. Secondly, these genes were analyzed jointly with the data of clinical phenotypes and related genetic variants. The potential pathogenic genes and variant loci can be obtained by comparing the differential genes between patients and normal people, and this method is helpful for the development of individualized diagnosis and treatment and prevention strategies.

3.2 Wayn diagram of target genes shared by safflower and hyperuricemia

The resulting hyperuricemia effective genes were matched to the 386 safflower target genes obtained from Uniprot. Import Draw Venn Diagram website to draw the Wayn diagram, yielding 51 intersection genes. This result can be shown visually by drawing the Venn Diagram (Wayn diagram). Venn Diagram Is a commonly used statistical chart that can clearly show the overlap and differences between different data sets, and can help to understand the data intuitively.

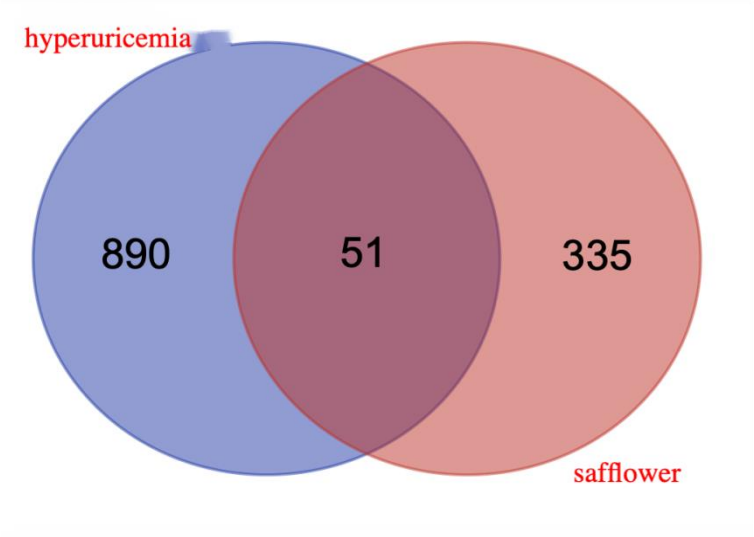


Figure 3.1 – Venn diagram of hyperuricemia – safflower target genes

3.3 Construction of the key target PPI network

After importing the above 51 common genes into the STRING database, the ke y target PPI network map is obtained (see Figure 2.4.1). After the PPI network is save d in the TSV format, it is opened in the Cytoscape software[20],Can be visualized and a nalyzed. The top 10 of the core genes: TP53, CASP3,IL6, TNF, IL1B, PTGS2, ESR1, PPARG, MYC, and BCL2 degree values were 43,43,42,42,41,41,41,41,40,40,40, res pectively (see Figure 2.4.2). First, TP53 gene is an important tumor suppressor gene, i ts mutation is closely related to the occurrence and development of a variety of tumor s, and has important regulatory effects on cell apoptosis, cycle regulation and so on. S econdly, the cysteine protease encoded by the CASP3gene is an important factor in th e regulation of cell fate and plays a key role in apoptosis[24]. Genetically encoded cytok ines, such as IL6, TNF, and IL1B, have important roles in inflammatory response and immune regulation, and are closely related to the occurrence and development of vari ous diseases. Furthermore, for the PTGS2 gene[25]The encoded cyclooxygenase-2 plays an important role in the inflammatory response and is one of the targets of nonsteroid al anti-inflammatory drugs; related studies indicate the ESR1 gene[26]The encoded estr ogen receptor is a target of endocrine therapy, and it plays a crucial role in the treatme nt of hormone-related neoplastic diseases; the PPARG gene[27]Involved in the regulatio

n of lipid metabolism, the disordered expression of this gene leads to the production o f metabolic diseases; MYC and BCL2 genes[28]Involved in the regulation of cell prolife ration and apoptosis, the overexpression of MYC and BCL2 genes will lead to uncont rolled cell proliferation and excessive survival of cells, thus leading to tumorigenesis. The above genes play important roles in regulating cell survival, proliferation, and ap optosis. Further exploring the interaction mechanisms between genes can help to prov ide new research ideas and directions for the diagnosis and treatment of clinical diseas es.

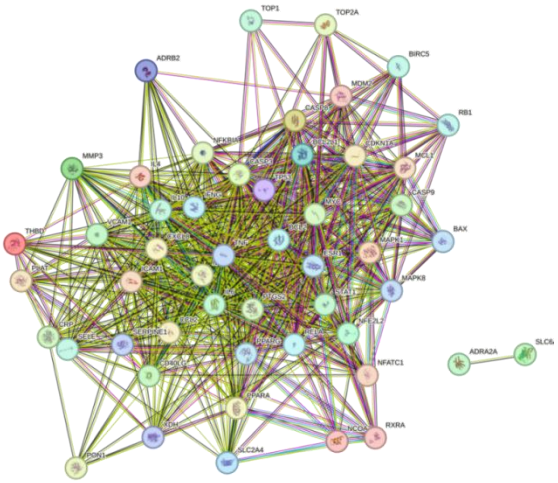


Figure 2.4.1 – The PPI network plots of the key targets obtained in the STRING database

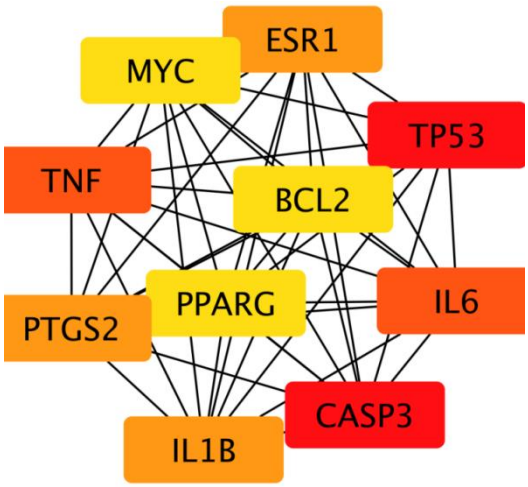


Figure 2.4.2 – A PPI network diagram of the top ten core genes in the Cytoscape software

Table 2.4 – **Top ten core gene annotation in the Cytoscape software**

sort	name	The degree of value
1	TP53	43
1	CASP3	43
3	IL6	42
3	TNF	42
5	PTGS2	41
5	ESR1	41
5	IL1B	41
8	PPARG	40
8	MYC	40
8	BCL2	40

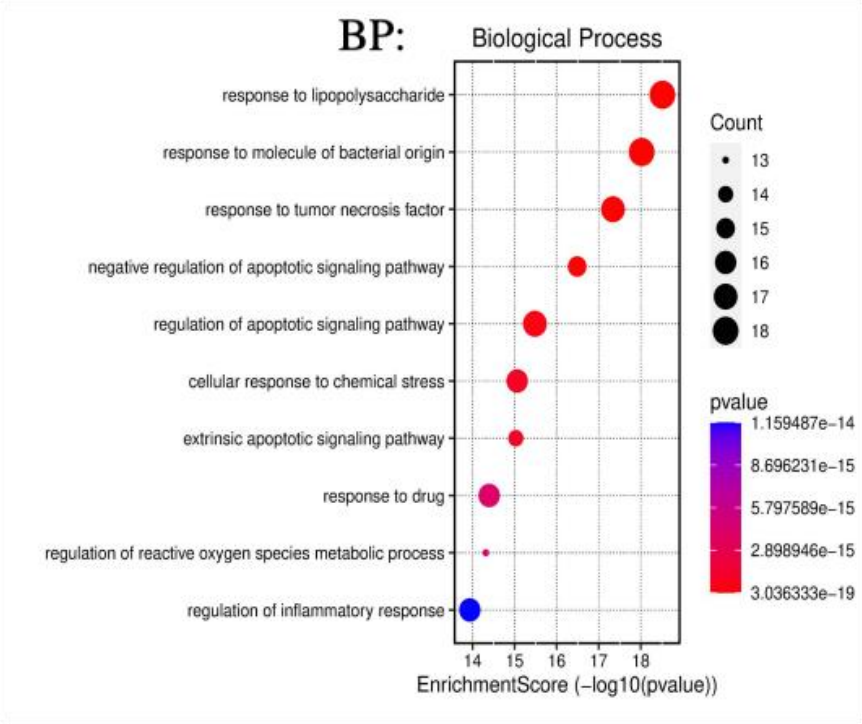
3.4 Methods and content of GO enrichment analysis

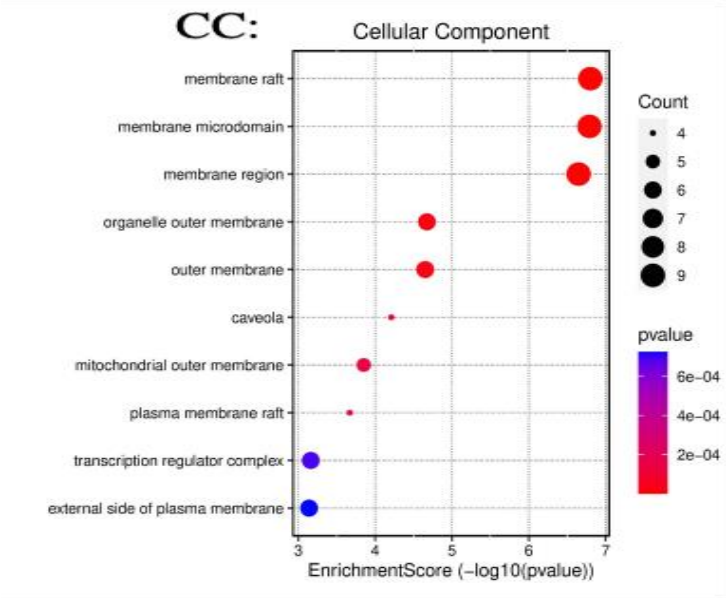
3.4.1 GO (Gene Ontology) enrichment analysis

GO（Gene Ontology）[29]It is a database established by the Gene Ontology Federation (Gene Ontology Consortium) to establish a semantic vocabulary standard suitable for various species, qualifiers and protein functions, and can be updated with the deepening of research. A comprehensive description of the properties of gene products in an organism can be achieved by establishing a set of control characters (controlled vocabulary) in a dynamic form that describe the roles of genes and proteins within the cell.

3.4.2 Research methods and content

The above 224 disease-drug co-target genes were introduced into the Metascape database (https://metascape.org/gp/index.html/main/step1) GO enrichment analysis includes three aspects, namely biological process (biological process, BP), cell component (cellular component, CC) and molecular function (molecular function, MF). The data from the above GO enrichment analysis were collated and imported into the "Wechat Letter" website (http://www.bioinformatics.com. Cn) visualize the results and data, draw the GO enrichment bubble map, and intuitively get the genes or which gene pathways are the most, so as to achieve the purpose of gene enrichment and obtain the research of key pathways of diseases.


BP:
Biological Process
response to lipopolysaccharide
response to molecule of bacterial origin
response to tumor necrosis factor
negative regulation of apoptotic signaling pathway
regulation of apoptotic signaling pathway
cellular response to chemical stress
extrinsic apoptotic signaling pathway
response to drug
regulation of reactive oxygen species metabolic process
regulation of inflammatory response
14
15
16
17
18
EnrichmentScore (−log10(pvalue))
Count
13
14
15
16
17
18
pvalue
1.159487e−14
8.696231e−15
5.797589e−15
2.898946e−15
3.036333e−19


CC:
Cellular Component
membrane raft
membrane microdomain
membrane region
organelle outer membrane
outer membrane
caveola
mitochondrial outer membrane
plasma membrane raft
transcription regulator complex
external side of plasma membrane
3
4
5
6
7
EnrichmentScore (−log10(pvalue))
Count
4
5
6
7
8
9
pvalue
6e−04
4e−04
2e−04

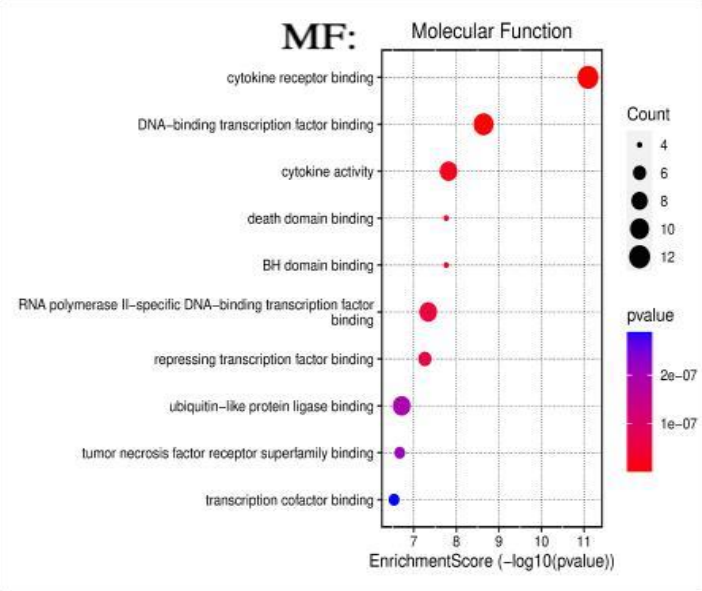


Figure 2.5 – GO enrichment bubble diagram

3.5 Methods and content of KEGG enrichment analysis

3.5.1 KEGG, and the enrichment analysis

KEGG, Enrichment analysis[30]31Is a method used for the analysis of gene or protein function. It is based on the Kyoto Encyclopedia of Genes and Genomes (Kyoto Encyclopedia of Genes and Genomes, KEGG) database[32], The database contains a large amount of information on biochemical pathways, molecular interactions, and gene function.

The purpose of KEGG enrichment analysis is to determine which biological pathways or functional categories are significantly enriched in a set of genes or proteins. By comparing gene or protein expression differences between experimental and control groups, this study can discover pathways related to specific biological processes or diseases. KEGG enrichment analysis can help this study to gain insight into gene function and regulatory mechanisms in biological systems, reveal the biological processes underlying the occurrence and progression of diseases, and provide clues for drug development and treatment. However, when conducting the analysis, attention is needed to choose the appropriate threshold and correction method to avoid false positive or false negative results.

3.5.2 Research methods and content

KEGG enrichment analysis mainly involved Lipid and atherosclerosis signal transduction pathway, AGE-RAGE signaling pathway (AGE-RAGE signaling pathway), IL-17 signaling pathway (IL-17 signaling pathway) tumor necrosis factor signaling pathway (TNF signaling pathway), etc. (see Figure 4-2).

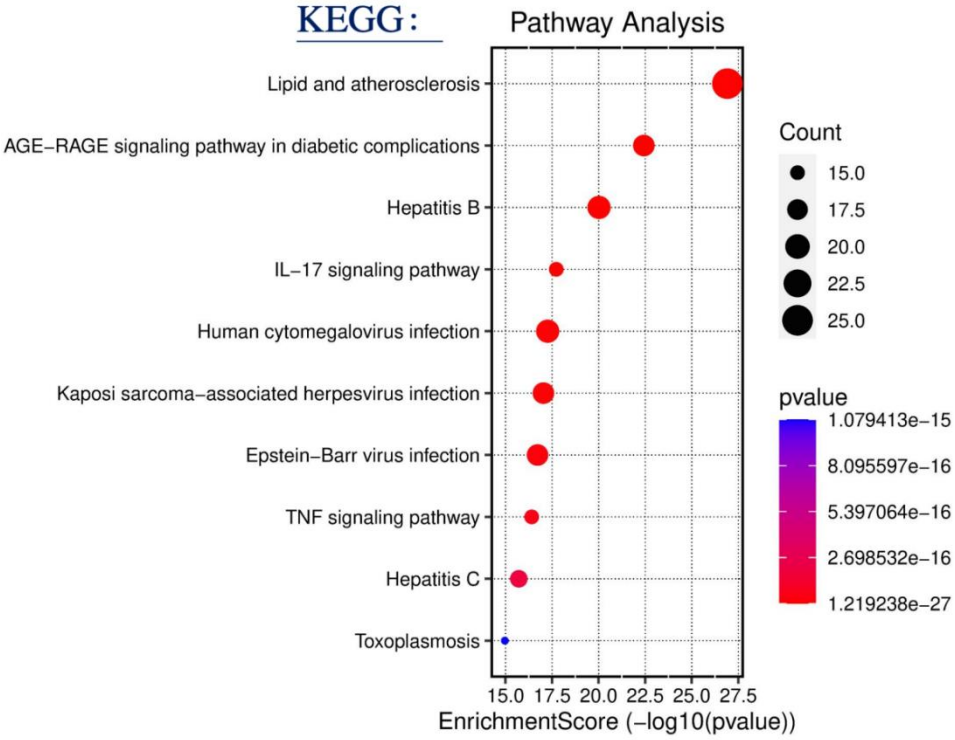


Figure 2.6 – KEGG enrichment bubble plot

3.6 Honghua-component-target-hyperuricemia network construction

Honghua-component-target-hyperuricemia The network network diagram of "safflower-component-target-hyperuricemia" interaction was drawn by Cystoscap 3.9.1 software ."honghua" represents "safflower", blue represents 14 active ingredients in the coptis, and "Hyperuricemia" represents "hyperuricemia".

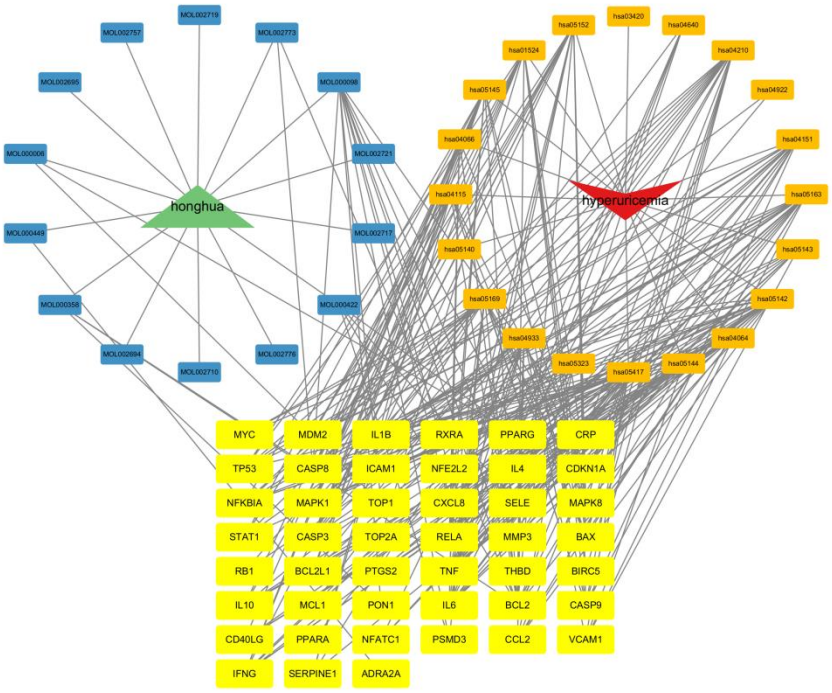


Figure 2.7 – The safflower-component-target-hyperuricemia network construction diagram Figure

3.7 Basic principle and application method of molecular docking technology

Discovery Studio (DS, V2016) is a new generation of molecular simulation software, application method mainly includes preparing protein structure and small molecule structure, parameter setting, docking calculation, results analysis steps using the kinetic method of CDOCKER module random search small molecule conformation, then adopt the method of simulated annealing optimized each conformation in the receptor active site region[33]34. When studying the mechanism of hyperuricemia in safflower, it is necessary to obtain the possible active component structure in safflower, and then make protein-small molecule docking calculation using Discovery Studio to predict the binding mode and affinity of the two.

In conclusion, the application of molecular docking technology in the treatment of hyperuricemia is of great significance, contribute to the deep understanding of the interaction mechanism between safflower active components and target proteins, and

provide theoretical basis and guidance for the design and development of safflower drugs.

3.8 Molecular docking results and analysis

Molecular docking analysis and docking simulation technology is a convenient and efficient means to explore the interaction between small molecules and target targets. With the help of AutoDockVina software, the top three Degree active components, K aempferol (kaempferol), Quercetin (quercetin), L uteolin (luteolin), and core targets TP53, CASP 3, IL 6, and TNF, and the results are presented in Table 5-1. The binding affinity is negative, indicating that there is a possibility of binding, and usually the values less than-6.0kcal / mol are considered to have a large binding possibility. As shown in Table 5-2, the binding energy of 12 groups of complexes were obtained, and all the binding energy score values were below-6.0kcal / mol, indicating the better binding status of these combinations[35]. Among them, Kaempferol-CASP3, Quercetin-CASP3, Quercetin-IL6and Luteolin-CASP3 are the four complex complexes for optimal binding, with binding energy below-9.0kcal / mol, indicating that these four combinations are extremely important (see Figure 5-1)[35]. Dark blue dashed lines represent hydrogen bonds, yellow dashed lines represent ionic interactions, dark gray dashed lines represent hydrophobic interactions, and green dashed lines represent π-π conjugation[35]。

(1) The interaction mode between small molecule Kaempferol and CASP3 protein is that small molecules mainly act through hydrophobic interaction, hydrogen bond and protein. For example, hydrophobic with F207, F183 and H202 on small molecules and hydrogen bond with D172, Q138 and S139 (see Figure 2-8A)[35]。

(2) The small molecule Quercetin forms hydrophobic interactions with N167, G166, F183, V182 and F181 on the CASP3protein, and hydrogen bonding with F183,

R136, H137, V182 and F181. In addition, π-π conjugation with F207 on the protein (Figure 2-8B)[35]。

(3) The small molecule Quercetin forms a hydrophobic interaction with Y320, P107, E172, A192, Q281 on the IL6protein, and forms a hydrogen bond interaction with R179, S176, E172, Q175, Q281, Y230 (Figure 2-8C).

(4) The small molecule L uteolin forms a hydrophobic interaction with F183, F184, G185 on the CASP3protein, and forms hydrogen bond with H137, R136, Q138, S139, D172, D230, H231, F232, F233, and π - π conjugate with F 207, F, F183, F184, and G185 on the protein (Figure 2-8D)[35]。

In conclusion, after molecular docking validation, the active ingredients, kaempferol, quercetin, and luteolin, showed better binding ability with TP53, CASP3, IL6, and TNF core targets.

Table 2.8 – **Docking of the core components to the core target molecules**

ingredient	Target and binding energy / (kcal / mol)			
	TP53	CASP3	IL6	TNF
Kaempferol	-8.1	-9.2	-8.8	-7.5
Quercetin	-8.1	-9.4	-9.7	-7.5
Luteolin	-8.3	-9.9	-8.8	-7.8

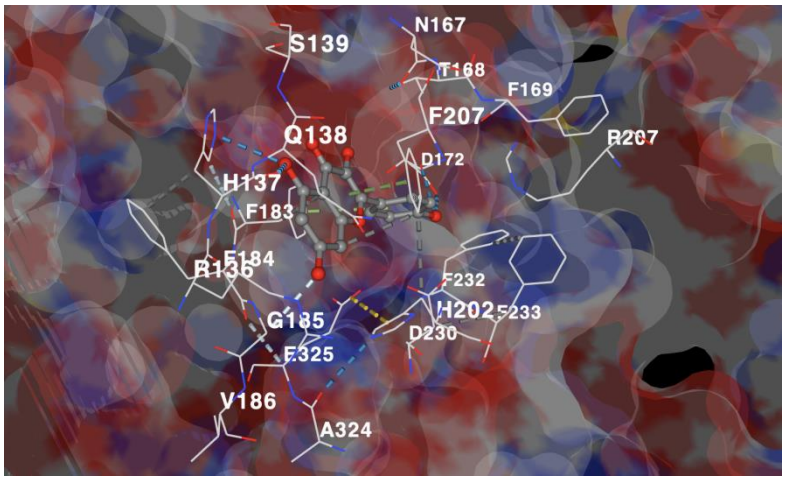


Figure 2.8A – The A K aempferol-CASP3complex

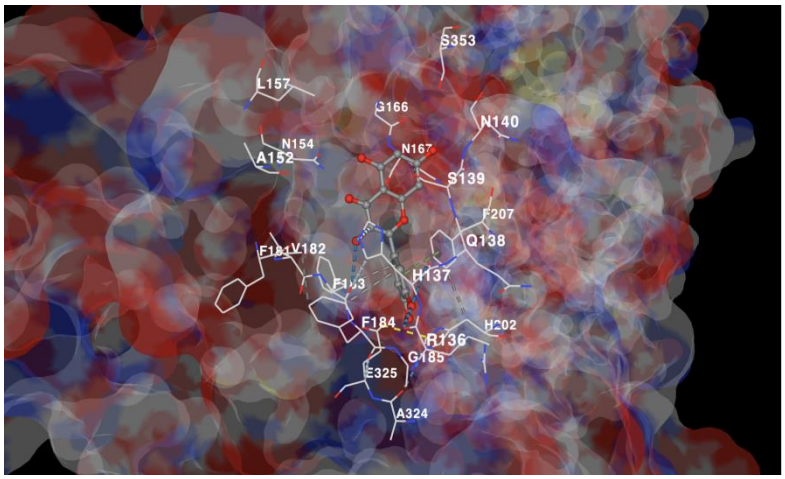


Figure 2.8B – The B Quercetin-CASP3complex

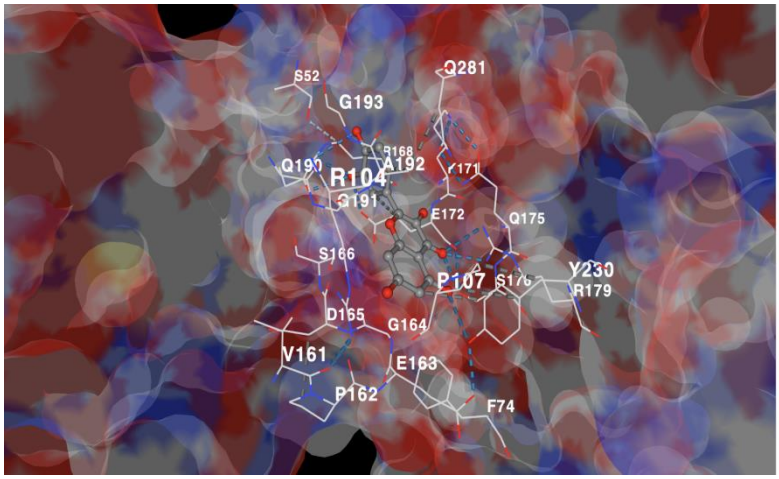


Figure 2.8C – The C Quercetin – IL6complex

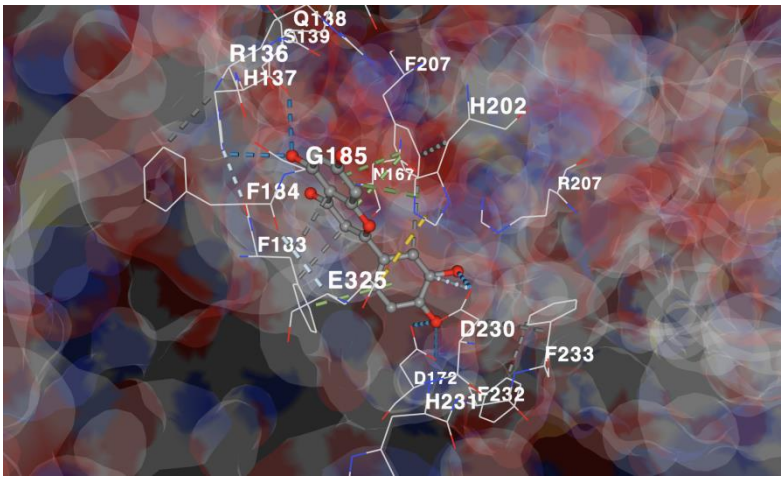


Figure 2.8D – The D L uteolin-CASP3complex

In this study, the mechanism of safflower action in the treatment of hyperuricemia was explored through network pharmacology and molecular docking. Through the TCMSP database, 22 safflower active ingredients were selected: phenol, octohydrolycopene, pyrethroid II, baicalin, baicalin, β -carotene, baicalin, kaempferol, stigmasterol, fotein, quercetin, etc. The results showed that K aempferol (kaempferol), Quercetin (quercetin), L uteolin (luteolin) and other active ingredients in safflower are more important in the pharmacological effect of reducing uric acid. Through the method of network pharmacology, the targets of various components of mulberry leaf-chrysanthemum medicine were obtained and the Wayn diagram was analyzed with disease targets, and the intersection genes were obtained. Then, the PPI network map was used to obtain the top 10 core targets of safflower in the treatment of hyperuricemia:

TP53: TP53 is an important tumor suppressor gene that plays a key role in cell growth, division, and apoptosis.

Mutations or functional abnormalities of TP53 have been implicated in the initiation and progression of many cancers.

CASP3: CASP3 is a member of the cysteine protease family, involved in the process of apoptosis (programmed cell death). It is activated when the cell is stimulated by specific signals, triggering a cascade of responses to apoptosis.

IL6: IL6 is an interleukin and is a cytokine. It plays an important role in the immune system and participates in the inflammatory response and the immune response.

TNF: TNF usually refers to tumor necrosis factor, an inflammatory cytokine that participates in immune responses and inflammatory processes, and plays a role in host defense and immune regulation.

PTGS2: PTGS2 is also known as cyclooxygenase-2 (COX-2). It is an enzyme involved in the arachidonic acid metabolic pathway associated with inflammatory and pain responses.

ESR1: ESR1 is the gene for estrogen receptor- α, associated with estrogen signaling and estrogen receptor function. It plays roles in many biological processes, including the development and function of the reproductive system.

IL1B: IL1B is the interleukin-1β, which is a pro-inflammatory cytokine involved in the inflammatory response and immune regulation.

PPARG: PPARG is a gene for the peroxisome proliferator-activated receptor-γ. It is involved in regulating processes such as adipose metabolism, insulin sensitivity and inflammatory response.

MYC: MYC is a transcription factor that has important effects on cell proliferation, apoptosis, and regulation of gene expression. It is associated with tumorigenesis and progression.

BCL2: BCL2 is an anti-apoptotic protein, which prevents the development of apoptosis. Overexpression of BCL2 was observed in some tumors and has been associated with tumor drug resistance and survival.

One of the most critical targets is TP53, CASP3, IL6, and TNF.

Zhang class[36]In animal experiments, potassium oxide and hypoxanthine induced the mouse model of hyperuricemia, and it was found that the flavonoids quercetin effectively reduced the serum uric acid levels, by the mechanism of inhibiting XOD activity and expression, and reducing uric acid production. Compared with allopurinol, quercetin has the advantage of no renal toxicity. Zhu class[37]In animal

experiments, we studied the effects of flavonoids on the kidneys of the mice with hyperuricemia, and found that luteolin had an inhibitory effect on XOD, treated hyperuricemia by reducing the production of uric acid, and improved the kidney injury in the mice. Secondly, luteolin also intervened in uric acid transporter and related signaling pathways. It has been suggested that kaempferol may be a potential xanthine oxidase inhibitor, which was shown to block substrate entry by inserting into the hydrophobic active site of xanthine oxidase and inhibiting xanthine oxidase activity by competing sites, thus reducing blood uric acid levels in hyperuricemia mice[38-39]。

In the KEGG enrichment pathway analysis of hyperuricemia, the Lipid and atherosclerosis signal transduction pathway plays a key role in the development of cardiovascular diseases such as atherosclerosis. It involves many aspects, including lipid metabolism, cholesterol transport, and inflammatory response, which are closely related to the pathogenesis of hyperuricemia. The AGE-RAGE signaling pathway is a pathway with high AGE-receptor expression before glycosylation end-product-receptor activation. AGEs play an important role in the pathophysiological process of hyperuricemia, further inducing inflammation, oxidative stress, fibrosis and other responses through RAGE signaling, aggravating tissue damage. Related studies have shown that IL-17 is an inflammatory mediator[40], Involved in the regulation of the inflammatory response and the immune response, and its hyperactivation may lead to the development of other inflammatory diseases, including hyperuricemia; IL6is also an inflammatory factor[41]In IL-17 signaling pathway, IL6 may aggravate the inflammatory response by inducing inflammatory cell activation, leading to the deterioration of inflammatory diseases; tumor necrosis factor signaling pathway (TNF)[42], Related literature indicates that it participates in the regulation of biological processes such as inflammatory response and cell apoptosis by inducing inflammatory cell infiltration and activating inflammatory signaling pathways. In the context of hyperuricemia, abnormal activation of the TNF signaling pathway leads to an increased inflammatory response; TP53 is also known as oncoprotein p53[41]. In tumor necrosis factor signaling pathway, TP53 can induce abnormal expression of genes involved in

apoptosis and inflammation; CASP3 belongs to cysteine protease[42], Play a critical role in the apoptosis processes. CASP3 may lead to cardiovascular diseases, neurological diseases, such as: Alzheimer's disease, Parkinson's disease.

Kaempferol (quercetin) is a flavonoid compound with antioxidant, anti-inflammatory, and antitumor activities. Kaempferol is able to affect TP53 stability, activity, or shadow its phosphorylation status and function. Thus exerting a regulatory role on the TP53 signaling pathway. Quercetin (quercetin) is a flavonoid compound that also has various antioxidant and anti-inflammatory biological activities. In molecular docking, quercetin may form specific interactions with CASP3 protein to inhibit CASP3 activity and then intervene with the apoptotic pathway to exert an anti-tumor effect. Finally, luteolin (luteolin) is a flavonoid compound with anti-inflammatory, anti-oxidation and anti-tumor biological activities[43]. The molecular docking technology shows that luteolin may exert its anti-inflammatory and anti-tumor effects by binding to inflammatory factors such as IL6and TNF to inhibit the conduction of its signaling pathways.

3.2 Research outlook

In this paper, we studied the mechanism of safflower in the treatment of hyperuricemia through network pharmacology and molecular docking technology, which laid a foundation for the further study of the active components and action mechanism of safflower. However, the interaction between complex mechanisms such as multi-component and multi-target TCM is still unclear, so further in vivo experiments are needed to verify the therapeutic effect of safflower and determine the safety of safflower. At the same time, it is necessary to pay attention to the synergistic and antagonistic effects of safflower and other TCM components, and to find the effective active ingredients in TCM to treat hyperuricemia.

At present, safflower has widely used clinical applications for uric acid lowering, but few pharmacological studies have been reported. In this study, the therapeutic mechanism of safflower on hyperuricemia is proved through the method of network

pharmacology and molecular docking, which provides a new idea for further research on the mechanism of safflower on uric acid lowering. This paper studied three main active components in safflower: K aempferol (kaempferol), Quercetin (quercetin) and L uteolin (luteolin). However, the mechanism of action of other active active ingredients is still unclear and has not been thoroughly studied. Related studies have shown that caffeic acid, lutin and luteolin in safflower may treat hyperuricemia by inhibiting the activity of XOD enzyme. At the same time, pigment and safflower polysaccharide may have immunomodulatory effects. Therefore, the active components and mechanism of action of safflower need to be deeply explored.

In conclusion, hyperuricemia has multiple targets, and multiple pathways interact to jointly affect the occurrence of hyperuricemia. Although the interconnection between active drug components and disease-related targets is revealed by using computer databases and other electronic information technologies such as molecular docking technology, the interaction between the two factors requires further experimental verification by in vivo and in vivo experiments. There are still many problems to be solved in the study on the mechanism of safflower in the treatment of hyperuricemia. In the future, we can explore the mechanism in the treatment of other diseases, so as to provide a more powerful scientific basis for the clinical application of safflower.

Conclusions to Chapter 3

1. Safflower is entered into the TCMSP database, and according to the ADME principle, set oral bioavailability (OB) ≥30% and drug-likeness (DL) ≥0.18 for screening, and 22 effective components and targets of safflower are obtained. When the target was obtained, the structural formula of the active component was retrieved from TCMSP and saved in MOL2 format. The above targets were imported into the Uniprot website for gene mapping to obtain the gene names of the corresponding targets, and it was concluded that there were 386 targets.

2. The common target genes of Safflower and hyperuricemia were obtained by drawing Venn Diagram, with 51 common target genes of hyperuricemia and Safflower overlapping in the middle. These genes are the target genes of the effective components of Safflower that theoretically have therapeutic effects on hyperuricemia.
3. Visualization processing and analysis were carried out in Cytoscape software to reveal the interaction between the target of effective components of safflower and the target of hyperuricemia, and topological calculations were carried out to find the key target. The top 10 core genes were ranked according to the degree value: TP53, CASP3, IL6, TNF, IL1B, PTGS2, ESR1, PPARG, MYC and BCL2. The above targets were found to play an important role in the treatment of hyperuricemia by safflower, and the correlation with other targets was particularly significant. Therefore, the key targets of the top four topological scores (TP53, CASP3, IL6, TNF) were selected as receptors for molecular docking experiments.
4. We observed that these targets are mainly involved in biological processes (GO-BP), including the response to lipopolysaccharides, the response to bacterial molecules, and the negative regulation of apoptosis signaling pathways. The analysis of cell composition (GO-CC) showed that these targets mainly involved membrane rafts, membrane microregions, plasma membrane, etc. The results of molecular function (GO-MF) analysis showed that these targets mainly involved DNA-binding transcription factor binding, cytokine receptor binding, cytokine activity, etc.The results of KEGG pathway enrichment analysis mainly include: lipid and atherosclerosis, age-rage signaling pathway in diabetes complications, hepatitis B, IL-17 signaling pathway, etc.
5. Through the image of drug-active ingredient-target-pathway-disease network, we can clearly see that the compounds MOL000098(quercetin), MOL000422 (kaempferol) and MOL000006 (luteolin) of the key active

ingredients of safflower, as the most important active ingredients, are associated with hyperuricemia through shared target genes. These include the hsa04933 Age-rage signaling pathway, which is most directly related to hyperuricemia, thus providing a strong theoretical support for our understanding of its pharmacodynamic mechanism.

6. Basic principle and application method of molecular docking technology: The principle of molecular docking technology is elaborated, that is, the binding mode and affinity of compound and target are predicted by simulating the interaction between molecules. At the same time, the specific application method of the technology is introduced, including the selection of appropriate docking software and parameter setting.
7. Molecular docking screening of the active components of Safflower: This paper describes how to use molecular docking technology to screen the active components of Safflower, so as to determine which components may have better binding ability with the related targets of hyperuricemia, and provide a basis for further research on its therapeutic effect.

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