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 the signaling pathways regulated by the biological activity of Xiaoyao San**
First (Bachelor's) level of higher education
Specialty 162 "Biotechnology and Bioengineering"
Educational and professional program "Biotechnology"

Completed: student of group
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APPROVE
Head of Biotechnology, Leather and Fur
Department, Professor,
Dr. Sc., Prof.
___________Olena MOKROUSOVA
«___»_______________2025

ASSIGNMENTS
FOR THE QUALIFICATION THESIS
Zhao Haojun

1. Thesis topic **Study on the signaling pathways regulated by the biological activity of Xiaoyao San**
Scientific supervisor Ph.D., Assoc. Prof. Olena Okhmat
approved by the order of KNUTD “05” March 2025, № 50-уч

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

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

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WORK CALENDAR

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

I am familiar with the task:

Student ____________ Zhao Haojun

Scientific supervisor ____________ Olena OKHMAT

Abstract

Zhao Haojun. Study on the signaling pathways regulated by the biological activity of Xiaoyao San. – Manuscript.

Qualification thesis on the specialty 162 «Biotechnology and Bioengineering». – Kyiv National University of Technologies and Design, Kyiv, 2025.

As a classic prescription for soothing the liver and strengthening the spleen, Xiaoyao San takes "harmonizing the liver and spleen" as the core, taking into account the dual regulation of emotion and physiology, and is a model of "holistic view" and "syndrome differentiation and treatment" in traditional Chinese medicine. Its modern research has revealed the scientific connotation of traditional prescriptions through multidisciplinary technology, providing an important reference for the treatment of complex chronic diseases. The network pharmacology system can analyze its "multi-component-multi-target-multi-pathway" action characteristics: pharmacological system analysis, and pharmacophysical system analysis can obtain: pharmacological system analysis. In this study, 8 herbs such as *Bupleurum chinensis, Angelica sinensis, Licorice glabra* and *Paeonia alba* were used as the object, and highly bioavailable active ingredients such as saikosaponin and peony glycosides were screened through TCMSP and other databases, which can be used to predict their targets and intersect with disease genes such as depression and irritable bowel syndrome, and obtain core regulatory targets such as SIRT1 and MAOA. Websites such as bioinformatics platforms can also be used to analyze their pathways. Network analysis showed that Xiaoyao San played a synergistic role in the pathology of liver depression and spleen deficiency by regulating neurotransmitters (5-HT, dopamine), inflammation (NF-B) and hormone metabolism (HIF-1, estrogen). The binding activity of key components to targets has been verified by molecular docking. This study reveals the molecular mechanism of "holistic regulation" of Xiaoyao San to provide a theoretical basis for the treatment of incurable diseases in traditional Chinese medicine, and also provides an example for the modern application of network pharmacology in traditional Chinese medicine

Key words: Xiaoyao San, Network Pharmacology, TCMSP

TABLE OF CONTENTS

INTRODUCTION

Xiaoyao San is a commonly used therapeutic medicine. Xiaoyao San first appeared in the Taiping Huimin and Pharmaceutical Bureau Prescription , and the components include Bupleurum chinensis, Angelica sinensis, Paeonia alba, Poria cocos, Atractylodes macrocephala, and licorice, all of which are used together to relieve liver depression, promote blood circulation and eliminate blood stasis, and mainly treat patients with liver depression, spleen deficiency, qi stagnation and blood stasis [1.] Xiaoyao San is mainly used to treat liver stagnation and blood deficiency and spleen weakness, but the focus is on liver qi stagnation, so the treatment method is mainly to relieve liver depression and cooperate with the nourishing method of nourishing blood and strengthening the spleen. Peppermint can evacuate the stagnation of the liver meridian; Simmering ginger can reduce adversity and medium, and has the characteristics of pungent and scattered; Licorice plays a role in harmonizing the properties of various medicines. The overall formula can not only dredge, but also contain health effects, taking into account qi and blood, and regulating the liver and spleen at the same time. This prescription is a representative prescription for regulating the liver and nourishing blood, and it is also a common prescription for gynecological menstruation, "Gynecological Menstrual Regulation" has a detailed introduction in "Gynecological Menstrual Regulation", with the pain of the two hypochondria as the key points of syndrome differentiation, fatigue and lack of food, irregular menstruation, and pulse deficiency are the focus of syndrome differentiation in clinical application. Angelica sinensis and white peony are used in Xiaoyao San, plus Bupleurum, Poria cocos, Shucao, ginger and mint; It is suitable for liver stagnation, blood deficiency and lack of spleen, and has a significant effect on reconciling the liver and spleen. Among them, Bupleurum is the king's medicine, which can clear liver qi, alleviate depressed feelings, and has a good regulating effect on the liver; Pharmacological studies have shown that Bupleurum has antipyretic, anti-inflammatory, immune, hepatoprotective, anti-radiation damage and other effects, and the active components are: chai saponins, volatile oils, fatty acids, etc. Studies have shown that white peony has the effects of regulating immunity, sedation, liver protection, antiplatelet coagulation, anti-inflammatory and antibacterial; Angelica sinensis has the effect of nourishing blood and blood, mediating menstrual blood, analgesic and analgesic, returning to the heart, liver and spleen, anti-thrombosis, anti-inflammatory, analgesic, hypoglycemic, and improving circulation. Atractylodes macrocephalus and Poria cocos can strengthen the spleen and dehumidify, so that the function of transporting the spleen can be exerted. Atractylodes atractylodes has certain benefits in lowering blood sugar, antitumor, antibacterial, anticoagulant platelet coagulation, and promoting hematopoietic function; Poria cocos has a diuretic effect, can resist tumors, and can also increase myocardial contractility; Licorice has the functions of nourishing the heart and replenishing qi, which can play a role in sedation, anti-inflammatory, immune regulation, and anti-virus. All three of these drugs are adjuvant.

CHAPTER 1

LITERATURE REVIEW

1.1 LOOSE COMPOSITION

It belongs to the Apiaceae family, Bupleurum is a perennial herbaceous plant, which was first published in the Shennong Materia Medica, and is listed as the top grade [2]. At present, there is a shortage of wild Bupleurum chinensis, and the market is in short supply, and the artificial planting of Bupleurum chinensis makes up for the shortage of wild resources. Saikosaponin is the main pharmacodynamic component of Bupleurum chinensis, and the content of saikosaponin is affected by a variety of factors, such as growth conditions, harvest time, fertilization period, and cultivation method [3]. Fertilization plays a very important role in yield, quality and economic benefits, and the previous research on formula application of Bupleurum chinensis was mainly based on fertilization [4-5], but the fertilization strategy is still extensive and the degree of refinement is insufficient, resulting in low fertilizer efficiency utilization rate, relatively insufficient yield and quality improvement, and unnecessary economic expenditure, so it is of great significance to carry out the refined fertilization strategy of Bupleurum chinensis to improve the fertilizer efficiency utilization rate and yield and quality of Bupleurum chinensis. The main effects and uses are antipyretic, liver soothing and depressing, lifting yang qi, and the active ingredients are saikosaponin (anti-inflammatory, antiviral), volatile oil (antipyretic), polysaccharide (immunomodulatory). The classic formulas are: Xiao Chai Hu Tang (Bupleurum Skullcap Banxia Ginseng), Buzhong Yiqi Tang (Bupleurum Astragalus Cimicifuga Cimicifuga), Xiaoyao San (Bupleurum White Paeonia Angelica).

There are two main types of Angelica sinensis medicinal materials recorded in ancient documents, namely Horsetail Angelica sinensis and Grass Angelica (also known as silkworm head Angelica). The "New Revision of Materia Medica" mentions: "There is a plant similar to Big-leaved Xiong, and another is similar to Richmond fine-leaved, but their stems and leaves are lower than Xiongxiong.[6] "Notes on the Collection of Materia Medica" also recorded: "Today, Longxi Nayang, Heishui Angelica, the name of Horsetail Angelica, a little rare. . .[7] "Longxi Nayang" is the area of Shouyang, Gansu Province, which produces horsetail angelica with high quality; "Liyang" is now Hexian County, Anhui Province, and the grass Angelica [8]. "Shennong's Materia Medica" for the first time described the effects of Angelica sinensis: warming and relieving pain, removing blood congestion, stroke, spasm, sweating, dampness, evil, politeness and coldness, replenishing the five organs, and generating muscles and bones. The qi and blood are drowsy, and the clothes are fixed. It has the power of qi and blood every time, hence the name Angelica. [9] The Tang Dynasty's "Theory of Medicinal Properties: Classification of Medicinal Properties" recorded: "It can relieve fatigue, regulate fatigue and hunger, remove blood stasis and promote tissue repair, regulate abnormal uterine bleeding, and improve intestinal and diarrhea." [10] The Yuan Dynasty's "Materia Medica" supplemented Angelica sinensis with the effect of "breaking the evil blood, moistening the dryness, and rushing up the main postpartum bad blood" [11]. The "Compendium of Materia Medica" of the Ming Dynasty recorded: "Angelica sinensis cures the bad blood of pregnant women after childbirth, and it is effective in a hurry." Those who are in a comatose state of qi and blood will be determined if they take it. It can make the qi and blood have their own home, and I am afraid that the name of Angelica will come out because of this. The 2020 edition of the Pharmacopoeia of the People's Republic of China (Part I) records that Angelica sinensis can replenish blood and invigorate blood, regulate menstruation and relieve pain, moisten the intestines and laxative, and is used to treat insufficient blood, yellowish complexion, frequent heartbeat, irregular menstruation, and constipation [13]. Modern pharmacology has found that Paeonia alba has anti-inflammatory, immunomodulatory, antioxidant, intestinal function and liver protection [14]. The total glycosides extracted from the dried roots of

Paeonia alba have anti-inflammatory, hepatoprotective, analgesic, and blood-nourishing effects, and are often compatible with licorice [15]. The anti-inflammatory effect of Paeonia alba is mainly achieved by inhibiting the production of inflammatory mediators (TNF-α, IL-1β, IL-6, etc.), thereby regulating the human immune system, resisting oxidative stress, and regulating the activation and migration of inflammatory cells.

Poria cocos: widely used in food, health products, cosmetics and other fields, with high edible and economic value, its chemical composition includes polysaccharides, triterpenoids, sterols, metal elements, etc., with a variety of medicinal value. Poria cocos has its unique role in the treatment of limb stiffness, musculoskeletal pain, scrofula, and scabies caused by syphilis and mercury poisoning [17-22], and is often used together with honeysuckle, comfrey, chebula, etc. The inhibition of cellular immune response by Poria cocos is mainly due to various pathways such as affecting the function of T lymphocytes, regulating cytokine balance, upregulating the number of Treg cells, and inhibiting the maturation and antigen presentation ability of dendritic cells. The anti-inflammatory effect is due to the presence of flavonoids, alkaloids, and polysaccharides, which reduce the inflammatory response by inhibiting the production and release of inflammatory mediators, and alkaloids and polysaccharides that enhance the body's anti-inflammatory ability and inhibit the activation and aggregation of inflammatory cells [16]. Poria cocos has been regarded as a medicine and food homology since ancient times, and it is recorded in the "Shennong Materia Medica" that Poria cocos has the effect of "serving the soul for a long time to nourish the spirit, not starving and prolonging life". With the implementation of the "Healthy China" strategy, the demand for medicinal and edible homologous functional foods has risen, and it is of great significance to develop health care products and medicinal and edible homologous foods with exact health care functions, and it will also help to extend the Poria cocos industry chain.

Atractylodes: return to the spleen and stomach meridians. It has the effects of strengthening the spleen and invigorating qi, drying dampness and improving water, stopping sweating, and stabilizing the fetus, and is suitable for the treatment of spleen deficiency, less food, abdominal distension and diarrhea, phlegm dizziness, edema, self-sweating, fetal restlessness and other diseases. With the depletion of wild resources of Atractylodes macrocephalus, most of the Atractylodes macrocephalus used in traditional Chinese medicine are conventional cultivation products. The main applications are spleen and qi, dampness and water, antiperspiration, fetal stability, etc., and in modern pharmacological research, there are also regulating gastrointestinal function, anti-diarrhea, enhancing immunity, antioxidant and anti-tumor and other effects.

Licorice: The main components of licorice include triterpene saponins, flavonoids, polysaccharides, coumarins, and volatile oils. Cai Shuhui et al. analyzed multiple batches of fried licorice from different origins by high performance liquid chromatography (HPLC) fingerprinting and multi-component quantification, and pointed out that glycyrrhizic acid, glycyrrhizin, glycyrrhizin, isoglycyrrhizin, isoglycyrrhizin and celebris isoglycyrrhizin were the main components of licorice, and the glycyrrhizic acid content was the highest. The results showed that compared with raw licorice, the content of flavonoids in glycyrrhiza was increased, while the content of triterpenoid saponins was decreased.

1.2 CYBER PHARMACOLOGY

Cyberphysics is an encompassing multidisciplinary field of study, which includes computational and experimental methods with large amounts of information. Network pharmacology is based on the theory of systems biology and its role is to analyze biological systems networks. is a promising method for revealing the pharmacological mechanisms of traditional Chinese medicine. Network pharmacology focuses on channel regulation signals, enhances the precision therapeutic effect and efficacy of drugs, and reduces toxic side effects, which can improve the success rate of clinical trials of new drugs, explain the principle of action of drugs, and reduce the cost of drug development [23-25], and network pharmacology can reveal the effective targets of new drugs, provide clues for the mechanism of action of drugs and subsequent improvements, thereby promoting the development of medical precision and side effect reduction [26-29]. Network pharmacology research integrates a large number of authoritative databases in the medical field, through which the relationship between drugs and diseases or symptoms can be established, among which the databases dedicated to TCM research include TCMSP (Traditional Chinese Medicine System Pharmacology, https://old.tcmsp-e.com/tcmsp.php)[30] , TCMID (Traditional Chinese Medicines Integrated Database, http://47.100.169.139/tcmid/)[31]， HIT (Herbal Ingredients' Targets Database, http://lifecenter.biosino.org/hit/)[32] 、HERB (a high-throughput experiment-and reference-guided database of TCM, http://herb.ac.cn/)[33] ， TCMGeneDIT (http://tcm.lifescience.ntu.edu.tw/), BATMAN-TCM (Bioinformatics Analysis Tool for Molecular mechANism of Traditional Chinese Medicine, http://bionet.ncpsb.org/batman tcm/), etc. Drug databases include ChEMBL (https://www.ebi.ac.uk/chembl/) [34], Drugbank (https://go.drugbank.com/), and STITCH (search tool for interaction of chemical datasets, http://stitch.embl.de) [35]. Disease databases include OMIM (Online Mendelian Inheritance in Man, http://www.ncbi.nlm.nih.gov/omim) [36], GAD (Genetic Association Database, http://geneticassociationdb.nih.gov/) [37], HPO (The Human Phenotype Ontology, https://hpo.jax.org/app/) etc. Protein-related. Protein-related databases include STRING (search tool for searching interacting genomes/proteins, https://cn.string-db.org/) [38], HPRD (Human Protein Reference Database, http://www.hprd.org/) [39], and others.databases include STRING (search tool for searching interacting genomes/proteins, https.)

1.3 APPLICATION OF NETWORK PHARMACOLOGY IN MEASAN ANALYSIS

Network pharmacology is characterized by its ability to systematically elucidate the complex network relationship between drug active ingredients and biological targets. Through the integration of bioinformatics analysis and experimental verification, this technology can not only identify potential targets (such as the 155 key targets screened by the TCMSP database), but more importantly, reveal the unique systematic mode of action of "multi-component synergy-multi-target dynamic regulation-multi-pathway cross-interaction" unique to TCM compounds. Taking the treatment of deep vein thrombosis as an example, it was found that baicalin in the compound can regulate TLR4/MyD88 signal transduction, while paeoniflorin affects the PI3K/Akt/mTOR pathway, and the two synergistically act on multiple pathological links such as inflammatory response, coagulation function, and vascular remodeling, reflecting the characteristics of the overall regulation of traditional Chinese medicine [40], and this mechanism of action indicates that the therapeutic characteristics of traditional Chinese medicine compound lie in its "active component group-biological target network-signaling pathway cluster" multi-dimensional integration. Different from single-target drugs, the compound exerts synergistic effects through a variety of pathways, including the metabolic transformation of components (such as the hydrolysis of glycyrrhizic acid into more active glycyrrhetinic acid), the regulation of key enzyme activity (such as the selective inhibition of COX-2 by paeoniflorin), and epigenetic regulation (such as the regulation of specific miRNA expression by Angelica sinensis polysaccharides). These findings suggest that it is necessary to combine metabolomics analysis, computer-aided molecular simulation and other technical means to deeply explore the dynamic change law and synergistic effect principle of compound active ingredients.

Conclusions to chapter 1

1. The effective biological components and mechanism of action in TCM formulas can be understood through network pharmacological analysis, which can be of good help to the composition and dosage of Chinese medicines, improve the therapeutic effect, and reduce toxic side effects.

2. It has a promoting effect on the treatment of new drugs, and at the same time can improve the treatment effect of new drugs and save research and development costs. At present, Xiaoyao San treatment has been widely used in life, but some patients still do not have a good treatment effect; Moreover, there are few network pharmacological analysis studies on Xiaoyao San at home and abroad, because the specific mechanism of action of Xiaoyao San treatment is not understood, which leads to the indiscriminate use of drugs.

3. In this paper, the interaction between the targets of each component of Xiaoyao San was discussed by using network pharmacology, and by clarifying the effective components and targets of Xiaoyao San in the treatment of diseases, the combination and potential mechanism of action of Xiaoyao San in the treatment of diseases were clarified, so as to provide experimental reference and basis for the selection and use of drugs in the clinical treatment of diseases and provide a new way for the clinical research of traditional medicine in China.

CHAPTER 2

OBJECT, PURPOSE, AND METHODS OF THE STUDY

Network pharmacology is an encompassing multidisciplinary field of study based on the theory of systems biology for the analysis of biological systems networks. Ability to reveal effective targets for new drugs.

2.1 PREFACE

In order to ensure the safety and effectiveness of traditional Chinese medicine preparations, the methods and means of quality control are particularly important. At present, the domestic quality control standards for Chinese herbal medicines are mainly based on the reference mode of foreign chemical quality control. The Chinese Pharmacopoeia 2020 Edition includes 2711 kinds of traditional Chinese medicines, and there are two ways to select the quality control ingredients of a certain traditional Chinese medicine, one is to select pharmacologically active ingredients, and the other is to select representative ingredients, both of which are relatively high; The latter may be the active ingredient. High performance liquid chromatography (HPLC) is widely used in the quality control of active ingredients and index components in traditional Chinese medicine, and its advantages are high separation efficiency, fast analysis speed, good reproducibility, wide flow phase selectivity, and reusable chromatographic columns. The key targets in the network were screened out according to the degree weights of the PPI-protein interaction map. Some studies have shown that Xiaoyao San contains a variety of complex components, and network pharmacology analysis can explore the possible therapeutic mechanisms of TCM from the aspects of gene molecular function, protein-protein interaction relationship, biological processes involved, and signaling pathways, combined with the chemical composition of TCM and disease-related genes. The Xiaoyao San component enrichment analysis was carried out, and the key targets in the network were screened out according to the degree weights of the PPI protein interaction map.

2.2 EXPERIMENTAL MATERIALS AND METHODS

TCMSP database (https://old.tcmsp-e.com/tcmsp.php)
Uniport database (https://www.uniprot.org/)
STRING database (https://string-db.org)
WeBio online platform (https://www.bioinformatics.com.cn)
Cytoscape 3.10.1

Collection of TCMSP ingredients and corresponding targets of Xiaoyao San
The TCMSP database was used to screen out the components of traditional Chinese medicine, and the Uniport database was used to convert the target name into a unified gene name, and the species was limited to humans. Search for the formula of Xiaoyao San from the network, record the composition of Xiaoyao San and its corresponding traditional Chinese medicine, establish excel, query the name of traditional Chinese medicine in TCMSP and screen, the screening conditions are OB (oral bioavailability) greater than 30%, DL value (drug-like) greater than 0.18 to obtain the required compound components, and import the table.

Find out the corresponding target by looking up the compound name and establish a table, use the VLOOKUP function formula in the table to obtain the target name and former name, screen and remove the duplicate value and import the target processing platform into the processing sequence respectively, obtain the network diagram and the file in tsv format and download it and import it into Cytoscape 3.10.1 software, by adjusting the size, color and shape of the node to reflect different information, the size of the node should be based on the degree value (that is, the number of connections of the node) The color shade and shape are set according to the type of node (e.g., TCM ingredients, potential targets, etc.). Degree calculations can be done using the CytoNCA module in the software, and the "Without weight" option should be selected when using the CytoNCA plugin.

Finally, according to the role and affinity of each target gene, the plain film was sorted, each part of the drug was analyzed, and finally the final data was integrated into the 'WeBioxin' platform to obtain the drug target and channel action map to understand its biological process, cellular components and molecular functions.

Research methods:

1. TCMSP database screens out the ingredients of traditional Chinese medicine
2. The Uniport database converts the target name into a unified gene name
3. The names of traditional Chinese medicines were queried and screened in TCMSP, and the screening conditions were OB (oral bioavailability) greater than 30% and DL value (drug-like) greater than 0.18 to obtain the required compound composition.
4. Find the name of the compound to find out the corresponding target and establish a table, and use the VLOOKUP function formula in the table to get the target name and former name. Filter to remove duplicate values.
5. Import the target processing platform to process the sequence, obtain the network diagram and the file in tsv format, download and import it into Cytoscape 3.10.1 software.
6. Adjust the size, color and shape of the node to reflect different information, and the size of the node should be adjusted according to the degree value (that is, the number of connections of the node).The color shade and shape are set according to the type of the node (e.g., TCM ingredients, potential targets, etc.).
7. Integrate the final data into the 'WeBioxin' platform to obtain drug targets, channel action maps, and understand its biological processes, cellular components and molecular functions.

Conclusions to chapter 2

The key targets in the network were screened out according to the degree weights of the PPI protein interaction map.

CHAPTER 3

EXPERIMENTAL PART

3.1 RESULTS OF THE PROTEIN NETWORK

Through the screening of the TCMSP database (Fig. 1-14), 919 chemical components were found, and the number of 6 drug components was 85 Paeonia alba, 55 Atractylodes macrocephalus, 349 Bupleurum chinensis, 125 Angelica sinensis, 34 Poria cocos, and 280 licorice. A total of 14 Paeonia alba Radix, 8 Atractylodes macrocephalus, 18 Bupleurum chinensis, 2 Angelica sinensis, 16 Poria cocos and 93 licorice were obtained by screening and deduplication with OB≥30% and DL≥0.18. Then, the above chemical components were put into the TCMSP database, and the corresponding components were searched for the targets, and the duplicate values were merged and deleted, and the effective targets were obtained.

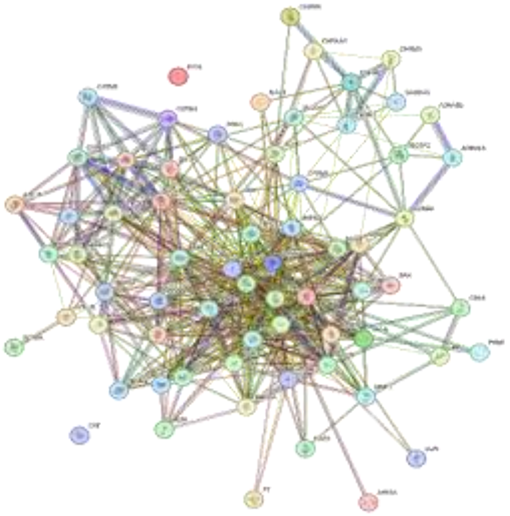

Figure 3.1 – Target network diagram of Paeonia alba

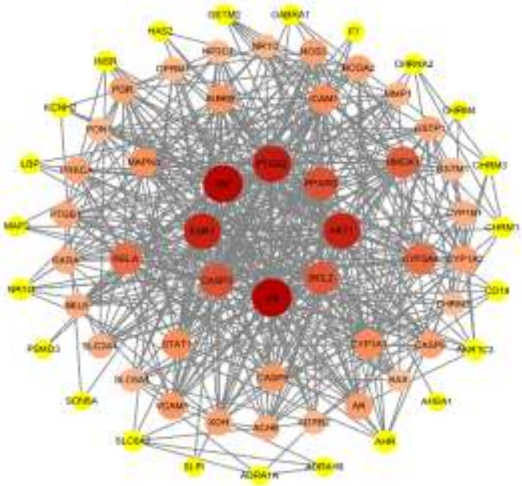

Figure 3.2 – Arrange according to degree values

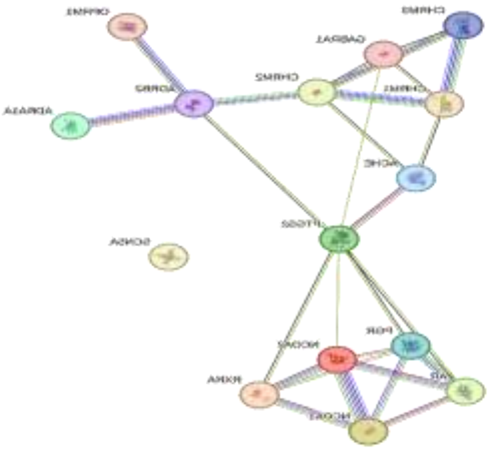

Figure 3.3 –Atractylodes target network diagram

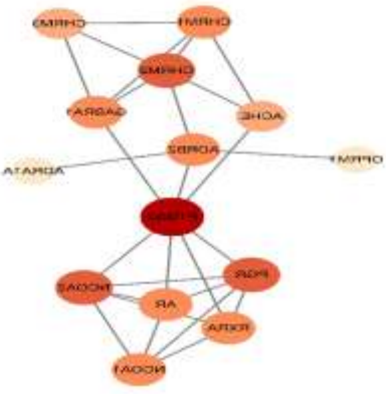

Figure 3.4 – Arrange according to degree values

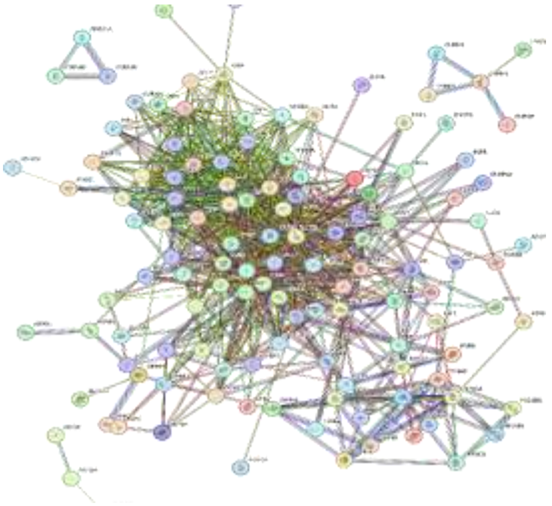

Figure 3.5– Licorice target network diagram

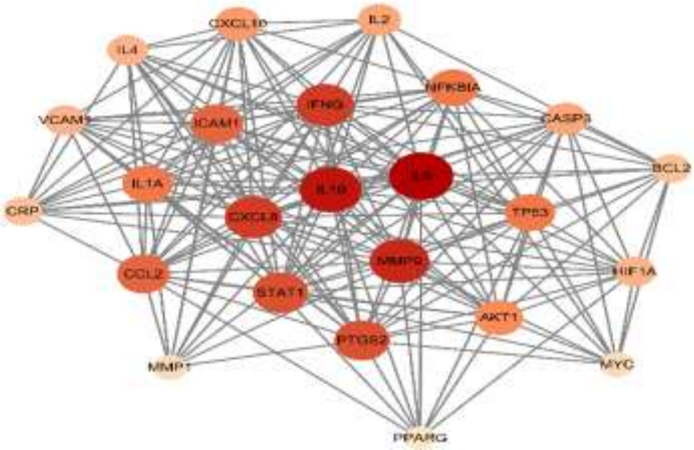

Figure 3.6 – Arrange according to degree values

Figure 3.7 – Angelica target network diagram

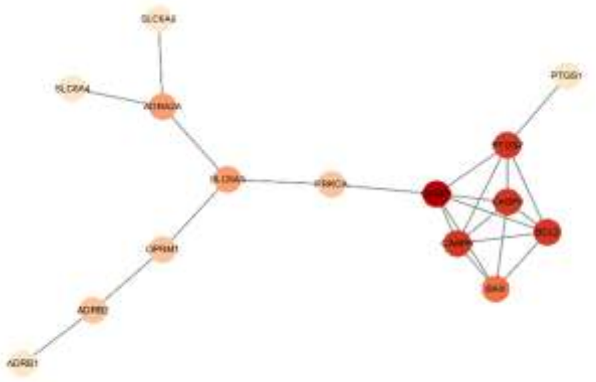

Figure 3.8 –Arrange according to degree values

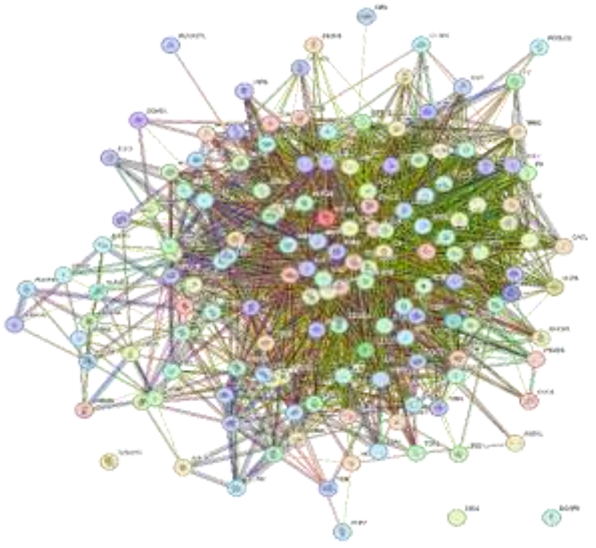

Figure 3.9 – Bupleurum target network diagram

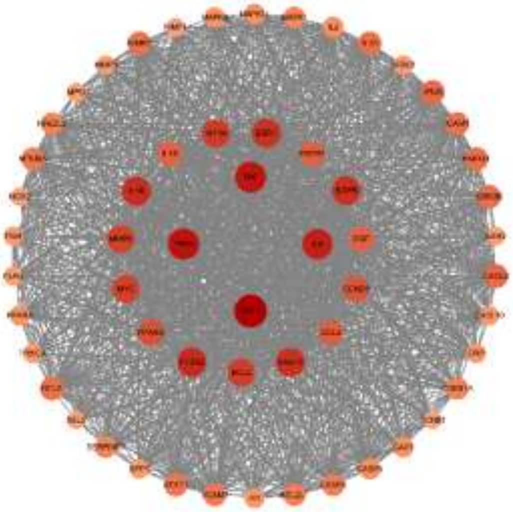

Figure 3.10 – Arrange according to degree values

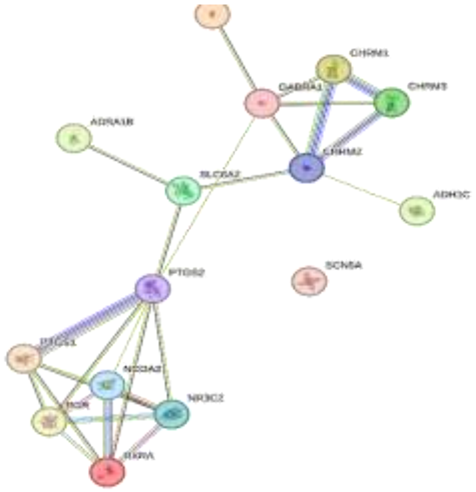

Figure 3.11– Poria cocos target network diagram nodes represent genes

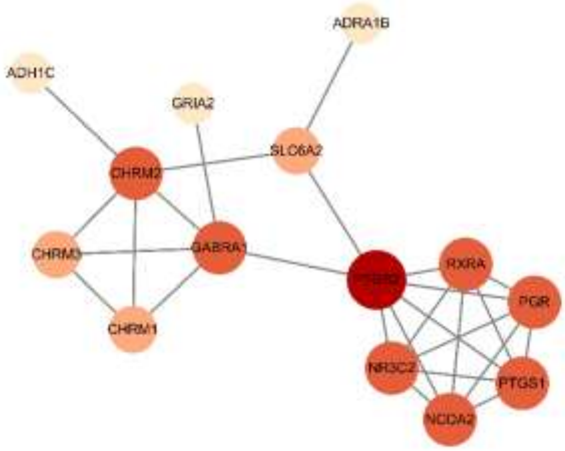

Figure 3.12 – Arrange according to degree values

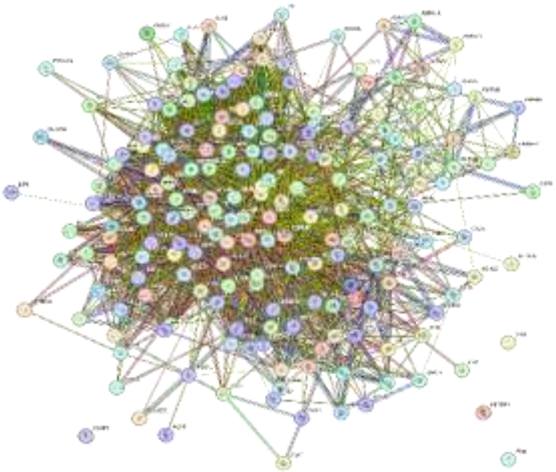

Figure 3.13 – Network diagram of loose targets

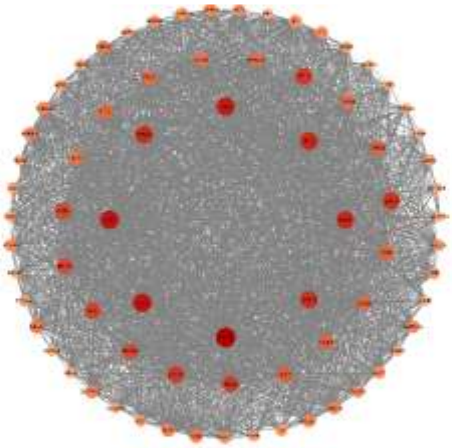

Figure 3.14 – Arrange according to degree values（Less than 40 is not counted）

3.2 GO AND KEGG ENRICHMENT ANALYSIS

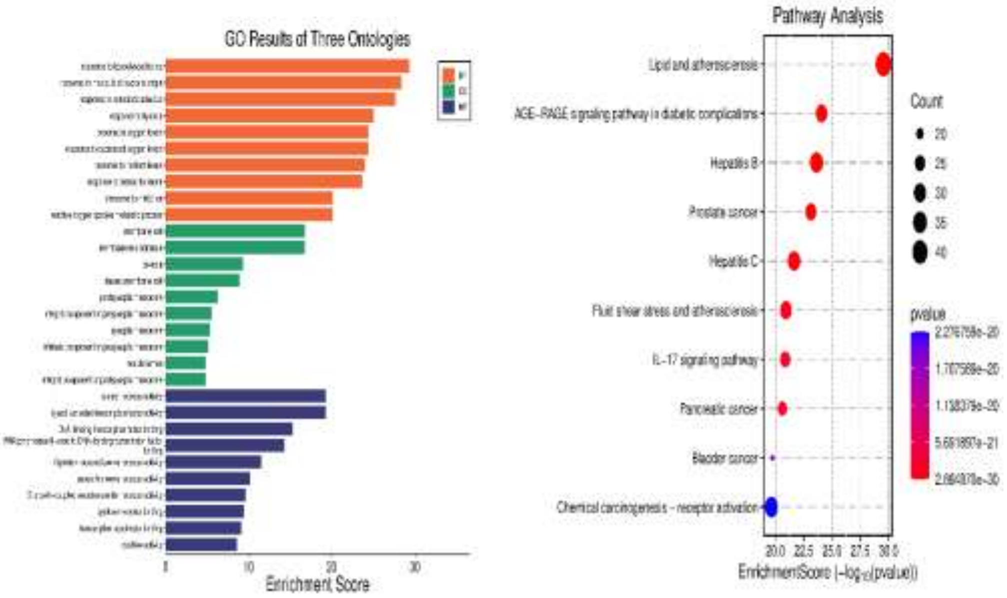

Figure 3.15: (1) Histogram of GO enrichment;

(2) KE enrichment diagram of GO of GO

IL-6 is a pleiotropic pro-inflammatory and immunomodulatory cytokine, mainly secreted by skin cells, alveolar macrophages, adipocytes and muscle cells on the airways, and is a multifunctional cytokine.

IL-1β is involved in a variety of cellular activities, including cell proliferation, differentiation, and apoptosis, and acts as an important mediator of inflammation. IL-1β is characterized by high expression in tongue cancer, and it is speculated that it may play a role as a pro-tumor gene.

ESR1, as a type of estrogen receptor, plays a key role in intracellular signaling, especially in the occurrence and development of precocious puberty, an endocrine disorder.

PTGS2 (prostaglandin intraperoxide synthase 2, also known as COX-2) is a key enzyme in inflammation, pain, and disease development, and its role spans both physiological and pathological processes.

TNF is a class of pro-inflammatory cytokines that are mainly produced by macrophages that play a key role in immune regulation, inflammatory response, and apoptosis.

TP53 protein is known as the "genome guardian" in the process of cell cycle regulation, DNA repair, apoptosis and aging, and is one of the most important tumor suppressor genes in the human body. More than 50% of human cancers have TP53 mutations, and their loss of function is closely related to tumorigenesis, progression and drug resistance.

AKT1 is a core member of the AGC kinase family and a key node in the PI3K/AKT/mTOR signaling pathway, which plays a central role in cell survival, proliferation, metabolism, and angiogenesis. Its abnormal activation has a lot to do with cancer, metabolic diseases, and neurodegenerative diseases.

Caspase-3 is a core member of the caspase family, a protease that plays a key role in apoptosis, programmed necrosis, and neurodegenerative diseases. It is a core member of the caspase family. It has a lot to do with cancer, neurodegenerative diseases, ischemic injury, etc., such as abnormal activation or inhibition.

HIF1A is hypoxia-inducible factor 1α and plays a central role in the cell's response to a hypoxic environment. It regulates the expression of many genes and helps cells adapt to hypoxia, such as promoting angiogenesis, glycolysis, etc.

Analysis of the pathway map shows that "Lipid and atherosclerosis" is at the top and has the highest enrichment fraction, indicating that Xiaoyao San may have a significant effect on lipid metabolism and atherosclerosis。The "Chemical carcinogenesis–receptor activation" pathway has the lowest p-value of all pathways. The results showed that the receptor activation pathways associated with chemocarcinogenesis were the most significant。A variety of cancer pathways were significantly enriched, including prostate cancer, liver cancer, pancreatic cancer, bladder cancer, etc. Viral infection-related pathways such as hepatitis B and hepatitis C are also significantly correlated. The pathway related to diabetic complications (AGE-RAGE signaling pathway) also showed a strong association, so it can be concluded that the mechanism of action of Xiaoyao San presents the characteristics of multiple targets and multiple pathways, which not only affects the pathways related to liver depression and spleen deficiency in traditional Chinese medicine theory, but also may play a pharmacological role by regulating lipid metabolism, affecting the pathways of the occurrence and development of various cancers, and intervening in the pathways related to viral infection. These findings provide important clues for modern pharmacological research in Xiaoyao San.

Biological process (BP) dominance was found by observing the enrichment map. The enrichment score was significantly higher than that of the other two groups (the highest 35.8 vs CC/MF <15). The top 5 were all stress response correlated (lipopolysaccharide, bacterial molecule, hypoxia, etc.). Suggesting that the study system may be in a state of pathogen infection or environmental stress. Cellular composition (CC) and molecular function (MF) are weak. The highest score of CC (membrane raft related) was only 7.3 points, and the highest MF score (transcription factor binding) was only 5.9 points, suggesting that the experimental design may not adequately cover the subcellular localization or molecular interaction level

CC Special Findings:

- Enrichment of membrane raft structures and their subtypes (synaptic membrane, presynaptic membrane).
- Suggesting that signal transduction may take place in lipid raft microstructures

MF Potential Value:

- Co-occurrence of nuclear receptor activity (6.8 points) with transcription factor binding (5.9 points).
- May involve nuclear receptor-mediated transcriptional regulation pathways

1. Significance verification

The top 10 items of BP all > 20 points, and the Z-score may be >2 (to be confirmed by raw data) CC/MF lacks a high-confidence signal, so it is recommended to combine the FDR value to judge

2. Biological consistency

Stress response and metabolic regulation form a logical closed loop: pathogen recognition→ hypoxic response→ ROS management→ nutrient mobilization. The association of membrane structural features with hormonal response may suggest a lipid raft-mediated steroid signaling axis

3.3 SUBSTANTIATION OF EXPERIMENTAL DATA

The potential of IL-10 family members in licorice in the treatment of anti-liver fibrosis agents. High-concentration hydrogen drinking water can enhance HO-1 expression and ameliorate NASH-related liver injury by upregulating the levels of IL-10 and silent information regulator 1 (SIRT1). In a rat model of liver fibrosis induced by bile duct ligation, lactoferrin attenuates liver inflammation and fibrosis by increasing IL-10 levels and inhibiting the TGF-β1/Smad2/α-SMA pathway. By increasing the level of IL-10 and other levels, it can improve the abnormal morphology and structure of the liver, showing its potential for the treatment of liver fibrosis.

CASP3 in Paeonia alba is a member of the CASP family, which plays an important role in the occurrence and development of apoptosis, and its activation is stimulated by the apoptosis signal generated by the internal and external interactions of various factors, which is closely related to the normal growth and development process of organisms. Studies have shown that CASP3 is usually activated by many apoptosis signals to cleave a variety of important cellular proteins, which is an essential factor for DNA fragmentation and apoptosis in cells, and Casp3 is activated by many apoptosis signals. [47-49]

The anti-inflammatory effect of Xiaoyao San has the characteristics of multiple targets and multiple pathways, mainly by inhibiting pro-inflammatory pathways such as NF-κB and MAPK to activate the Nrf2 antioxidant pathway. NF-κB is a core transcription factor of the inflammatory response, which activates the expression of pro-inflammatory cytokines (e.g., TNF-α, IL-1β, IL-6) and chemokines. The release of inflammatory factors downstream is inhibited and reduced by the nuclear translocation of NF-φB. The MAPK family (p38, ERK, JNK) is involved in the signal amplification of inflammatory responses and regulates cell proliferation, differentiation and apoptosis. Inhibits phosphorylation of p38, MAPK and JNK, and reduces the expression of inflammatory mediators (e.g., COX-2, iNOS). Regulates the ERK pathway and affects the transcription of inflammation-related genes. Down-regulation of upstream signaling molecules such as TLR4/MyD88 to block the initiation of inflammatory signaling.

HIF-1α (hypoxia-inducible factor-1α) is the core regulator of cells in response to the hypoxic environment, and participates in angiogenesis, metabolic adaptation, cell survival and other processes by regulating downstream gene expression. The intervention (activation or inhibition) of the HIF-1α pathway has shown potential in the treatment of a variety of diseases, and the mechanism of action in the treatment of liver cancer is that the tumor microenvironment hypoxia activates HIF-1α and promotes the expression of angiogenesis (VEGF, etc.), glycolysis metabolism and metastasis-related genes. In treatment, HIF-1α can be blocked by inhibition of its pro-tumor effects by small molecule inhibitors (e.g., PX-478, YC-1) or HIF-1α-degrading drugs (e.g., EZN-2968). [45]

TP53 (tumor protein 53) is one of the most important tumor suppressor genes in humans, and its encoded p53 protein is responsible for cellular stress (e.g., DNA damage, hypoxia, carcinogenesis). Abnormal TP53 pathway (e.g., mutation, inactivation, or overexpression) is associated with more than 50% of human cancers, as well as certain genetic diseases, aging-related diseases. The intervention strategy for the TP53 pathway is a hot topic in the treatment of solid tumors, such as gastric cancer, and the mechanism of action is that TP53 mutations lead to loss of p53 function, tumor cells escape apoptosis, accelerate proliferation and develop drug resistance. Its function can be activated by delivery of the wild-type TP53 gene (e.g., Gendicine, Advexin) via adenoviral vectors. [43]The core of TP53 pathway therapy is to restore its tumor suppression function or bypass its missing effects, and multiple drugs have entered clinical trials (such as the phase III trial of APR-246 in MDS/AML).

According to the etiological theory of traditional Chinese medicine, the etiology of emotion first affects the qi in the body, and its disease is located in the liver, and liver disease is the most likely to spread to the spleen, so liver depression and spleen deficiency are the most common clinical symptoms caused by emotion. Xiaoyao San is a representative prescription for liver depression and spleen deficiency, in the formula, Bupleurum soothes the liver and relieves depression, Angelica sinensis and white peony nourish blood and soften the liver; Atractylodes macrocephalus and Poria cocos have the function of strengthening the spleen and dispelling dampness, and have the source of qi and blood; Licorice has the ability to benefit qi and relieve liver urgency; Ginger warms the stomach and medium, add a little mint to help Bupleurum soothe the liver and clear depression, clear depression and relieve depression; The liver and spleen of the whole formula are harmonized, so that the liver and body can be smooth, the blood deficiency can be nourished, and the spleen deficiency can be replenished. Modern studies have found that Xiaoyao San can reduce the serum CORT content and improve the behavioral and learning and memory functions of CUMS rats. Xiaoyao San can exert a neuroprotective effect by modulating TGF-β and regulate abnormal behavioral changes in animal models of depression. Saikosaponin d21 in Bupleurum chinensis and paeoniflorin in Paeonia alba can inhibit lipopolysaccharide (LPS)-induced microglia activation and the release of pro-inflammatory cytokines such as IL-6 in mice, thereby improving the behavioral indexes of mice. Saikosaponin A can reduce Th17 cells, increase Treg cells, and decrease Th17/Treg cell ratio in serum, thereby reducing Th17 cells in serum of CUMS rats. In this study, it was found that high doses of Xiaoyao San could reduce the levels of pro-inflammatory cytokines and anti-inflammatory cytokines in the serum of mice after stress while reducing serum CORT levels. The therapeutic effect of Xiaoyao San is still to be discovered in the later stage.

Salvia miltiorrhiza is one of the most commonly used traditional Chinese medicines in clinical practice to promote blood circulation and dispel blood stasis, which has the effect of dredging blood vessels and dissipating blood congestion. cinnabar Ginseng 20 is the dry root and rhizome of the herbaceous plant Lamiaceae, a perennial plant native to China and Japan. Salvia it plays an increasingly important role in the treatment of cardiovascular disease (CVD). Cause it is valued for its unique pharmacological effects and low side effects, especially in improving cardiovascular and cerebrovascular function and preventing blood embolism and myocardial ischemia have been shown to be significant. Modern pharmacological studies have shown that salvia has dilated coronary arteries and peripheral blood vessels, improving hemorheological properties and antiplatelet aggregation 21. To date, it has been separated and more than 200 compounds from Salvia miltiorrhizae were identified. They can be divided into two main groups: water-soluble (hydrophilic) phenolic compounds and non-polar (fat-soluble) diterpenoids, with salvianolic acids and the diterpene tanshinone being two representative in this plant Hydrophilic and lipophilic components, which are the main pharmacological activities of Salvia miltiorrhiza . Basic chemistry of water-soluble phenolic acids in salvia the structure consists of C6C3 units. The total phenolic acids in this plant are usually produced by water, methanol, ethanol, or aqueous acetone separation extraction. Most salvianolic acids are colorless or tan amorphous powders. Due to the presence of α-3,4-dihydroxybenzene in the structure phenolic acids are very sensitive to light and heat and are easily oxidized in air. Phenolic acids in salvia such as salvianolic acid A,Salvianolic acid B and danshensu can regulate the dysfunction of vascular endothelial cells associated with atherosclerosis and protect blood vessels Injuries . Among the phenolic acids, salvianolic acid B has antioxidant effects and has a protective effect on cardiovascular endothelial cells. SAA It has been shown to have a variety of pharmacological effects, such as anti-inflammatory, antioxidant , anti-apoptotic , and neuroprotective activities .

Unlike aspirin, SAA has a mild inhibitory effect on platelet aggregation and comes by reducing the risk of bleeding To prevent thrombosis-induced ischemic stroke, SAA inhibits the upregulation of the Src signaling pathway in vivo and in vitro, and:Reverses the increase in matrix metalloproteinases (MMPs) expression after ischemic stroke. Tanshinone in salvia Mostly diterpenes, fat-soluble, the basic parent nucleus of the diterpenoid tanshinone contains furan or dihydrofuran ring of 1,2-o-naphthalene Quinones, such as tanshinone I, dihydrotanshinone I, tanshinone A. The general structural characteristics of tricyclic diterpene tanshinone are the same as isopropylene Base linkages, e.g., salshennovane, dehydrodanshenxinone. Most compounds of the tanshinone class are red in color and stable in solid state.

Compared to the model group, XYS treatment improved histopathological changes, liver index, serum ALT, AST, TBIL, HA, LN, PC-III, IV-C, liver tissue Hyp, protein expression of αSMA and Collagen I ($P < 0.05$); compared to the model group, LLAAF and XYS could improve the steatosis and collagen fiber deposition, reduce the expression of αSMA and Collagen I protein and Hyp content ($P < 0.05$). (4) Compared with the XYS and LLAAF network, the shared pathway including Jak-STAT and FoxO signaling pathways and c-Myc target protein were obtained; The core subnetwork a of the LLAAF is mainly related to the PI3K-Akt signaling pathway and the FoxO signaling pathway, the core subnetwork b affects both the Notch and Wnt signaling pathway, and the core subnetworks c and d affect the Jak-STAT signaling pathway; In cells and rat liver tissues, compared to the model group, LLAAF treatment reduced p FoxO3a/FoxO3a, p-STAT3/STAT3, p-Akt/Akt, p-Smad3/Smad3 ratio, and c-Myc protein expression ($P < 0.05$). Conclusion: The LLAAF from XYS can anti-liver fibrosis; its efficacy is related to synergistic effect of four compounds; the LLAAF has better efficacy; the anti-fibrosis effect of LLAAF may be related to the down-regulation of Jak-STAT, PI3K-Akt-FoxO signaling pathway and c-Myc protein expression, which in inhibits HSC activation. It may be able to play a certain role in the clinical use and drug development of XYS. Keywords: liver fibrosis; Xiaoyao San Decoction; network pharmacology; LLAAF; synergistic effect; Jak-STAT signaling pathway; PI3K-Akt-FoxO signaling pathway.

According to the literature, Xiao The pharmacological mechanism of remote dispersion corresponds to the pathogenesis of liver depression and spleen deficiency syndrome. Huang NN et al. , Zhou et aland Zhao Bo et al. evaluated immune function and a variety of indicators, including behavior, and found that there was a strong presence in the liver and spleen in the model rats, the material basis of liver depression and spleen deficiency syndrome was related to immune function and other factors. Liang et al. found the mechanism of Xiaoyao San in improving liver depression and spleen deficiency syndrome is related to AMPA receptors. Jin Zhongye et al. reported on the loose ginseng and regulate Ghrelin to improve liver depression and spleen deficiency syndrome. Kuang Xiangnan et al. found that Xiaoyao San reduced the pattern of liver depression and spleen deficiency. Expression of ob-R and α-MSH in rats. Li Xiaohong et al. reported that Xiaoyao San could affect rats with liver depression and spleen deficiency Genetic changes in gastric tissue. Jiao Haiyan et al. detected the expression of 5-HT in brain tissue of rats with liver depression and spleen deficiency syndrome level, it was found that Xiaoyao San could regulate the expression of TPH2 and IDO1. Shi Xueying found that Xiaoyao San could be changed Immune function in patients with liver depression and spleen deficiency syndrome. At the same time, liver depression and spleen deficiency syndrome are common in the course of liver fibrosis In the process, it is also more common to report Xiaoyao San in the treatment of liver fibrosis and animal liver fibrosis models. Although Xiaoyao San is a commonly used prescription for liver depression and spleen deficiency syndrome, Xiaoyao San is a pharmacodynamic for the treatment of liver fibrosis with liver depression and spleen deficiency the qualitative basis and its regulatory mechanism are still not well understood.

As a new field of modern pharmacology research in TCM, network pharmacology can be used by many existing databases, enrichment analyses, and network analyses are used to explore the mechanism of action of TCM in the treatment of diseases.

Network pharmacology focuses on the interaction of compounds and targets. The drug target network is an important part of network pharmacology components, which play an important role in explaining the mechanism of action of complex compounds. Based on a number of documents Dao, network pharmacology has been shown to be a suitable approach to elucidate the mechanism of action of TCM and its effects Synergistic effects in disease treatment. Shi SH et al. used a 5-principle approach to screen high-scoring sections in the network point, so as to find the target Chinese medicine compound ingredients and key targets. Liu et al. and Du Wei et al. adopted it network robustness and other parameters are discussed, and the impact of key targets on network stability is discussed, which in turn provides information in the network Methods for finding key targets. In the research of TCM compounds, network pharmacology technology also has its limitations. The late start of the research on TCM compounds has led to the lack of data on some TCMs, which also gives a negative impact on the pharmacology of TCM compounds The study has brought some difficulties, such as the use of network topological parameters for compound screening.The absence of envoys. Considering that the current database information related to TCM compounds and TCM syndrome types is still imperfect, it is in a certain process. The results of network prediction are limited, which requires us to combine the results of multiple studies, including in vitro and in vitro drugs.

Results of physical experiments, gene chip technology, and whole transcriptome sequencing (RNA-Seq) technology and other relevant experiments were carried out to verify it. Gene chips can be detected and fractionated by whole gene expression.

The molecular mechanism of TCM compounds was evaluated. Compared with gene chip technology, it has become rapidly popular in recent years. RNA-Seq technology is less costly, yielding intact RNA sequences and the ability to detect single base differences.

It is highly sensitive and can detect low-abundance expressed genes and quantify the expression of RNA. These technologies can be used to conduct a more comprehensive study on the material basis of TCM syndromes and the efficacy of TCM compounds. Because microarray technology can reflect the mRNA, long non-coding RNA (Long non-coding RNA,lncRNA), circRNA, and alternative splicing, thereby revealing the effects of disease on transcriptional regulation Drug intervention in transcriptional regulation. In recent years, many studies have reported the role of transcriptional regulation in the occurrence and development of liver fibrosis played an important role in the process. Wu et al. reported that TGF-1 was included in the liver tissue of rats with liver fibrosis. The mRNA expression of a variety of growth factors in the company increased. Gong et al. reported 11 lncRNA references. The process of liver fibrosis in rats with CCl4.

Snider NT found that the selective cleavage of NT5E can be involved in the development of liver fibrosis and liver cancer. Meantime Studies on the influence of TCM compounds on the disease process by interfering with transcriptional regulation have also been frequently reported. Shuai Feng et al. Reported Daoxiao Chai Hu Tang improved liver fibrosis by regulating the mRNA expression of MMPs and TIMPs. Yang Y et al.

Panax notoginseng can promote the expression of iNOS and the concentration of NO by regulating lncRNA AK094567 and then improve high blood pressure. Chen CY et al. reported that the TCM psoralen component can upregulate tumor inhibition Gene lncRNA-specific transcript product 5 (GAS5) inhibits hepatocellular carcinoma. Zhou et al. Found.artesunate extract, an extract of Artemisia annua, can regulate lncRNA UCA1, thereby affecting miR184 and then improving it prostate cancer symptoms. Shang Jiawei et al. and Shi Miaojuan et al. reported that traditional Chinese medicine can regulate the liver through a variety of lncRNAs Cancer. The above report is the use of RNA-Seq technology to obtain transcriptional regulation data for the intervention of liver fiber by traditional Chinese medicine compound.The molecular mechanism of chemistry is studied.

Conclusions to chapter 3

Analysis of the pathway map showed that "Lipid and atherosclerosis" was at the top with the highest enrichment fraction, suggesting that Xiaoyao San may have a significant effect on lipid metabolism and atherosclerosis.

CONCLUSIONS

1. Through the analysis of the pathway map, it can be found that Lipid and atherosclerosis are located at the top, and the enrichment fraction is the highest, indicating that Xiaoyaosan may have a significant effect on lipid metabolism and atherosclerosis."

2. The "chemical carcinogenesis–receptor activation" pathway value was the lowest among all pathways, indicating that the receptor activation pathway related to chemical carcinogenesis was the most significantly correlated, and a variety of cancer pathways were significantly enriched, including prostate cancer, liver cancer, pancreatic cancer, bladder cancer, etc.

3. Viral infection-related pathways such as hepatitis B and hepatitis C are also significantly correlated.

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