Identify Key Active Ingredients and Molecular Mechanisms of Jiangzhi Decoction on Non-alcoholic Fatty Liver Disease by Network Pharmacology Analysis

Lei Wang  
Shanghai Seventh People's Hospital

Yin Zhi  
Shanghai Seventh People's Hospital

Ying Ye  
Shanghai Seventh People's Hospital

Miao Zhang  
Shanghai Seventh People's Hospital

Xing Ma  
Shanghai Seventh People's Hospital

Hongyun Tie  
Shanghai Seventh People's Hospital

Wei Xia  
Shanghai Seventh People's Hospital

Yanan Song (synabc.123@163.com)  
Shanghai Seventh People's Hospital

Research

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Abstract

Background: Jiangzhi Decoction (JZD), a traditional herb mixture, has shown significant clinical efficacy against non-alcoholic fatty liver disease (NAFLD). However, its multicomponent and multitarget characteristics bring difficulty in deciphering its pharmacological mechanisms. Our study aimed to identify the key active ingredients and core molecular mechanisms of JZD against NAFLD.

Methods: The active ingredients were searched from Traditional Chinese Medicine Systems Pharmacology (TCMSP) database and Traditional Chinese Medicine Integrated Database (TCMID). The targets of those ingredients were identified using ChemMapper database based on 3D-structure similarity. NAFLD-related genes were searched from DisGeNET database and Gene Expression Omnibus (GEO) database. Then we obtained candidate targets of JZD against NAFLD by overlapping the active ingredient targets and NAFLD-related genes. We performed protein-protein interaction (PPI) analysis, functional enrichment analysis and constructed pathway networks of “herbs-active ingredients-candidate targets”, and identified the key active ingredients and core molecular mechanisms in the network.

Results: We found 147 active ingredients in JZD, 1285 targets of active ingredients, 401 NAFLD-related genes, and 59 overlapped candidate targets of JZD against NAFLD. 22 core targets were obtained by PPI analysis. Finally, nuclear receptor transcription and lipid metabolism regulation were found as the core molecular mechanisms of JZD against NAFLD by functional enrichment analysis, and emodin, emodin anthrone, hyperin, questin, rhein, etc. were speculated as the key active ingredients of JZD.

Conclusion: Our study will provide the scientific evidences of the clinical efficacy of JZD against NAFLD.

1. Introduction

Non-alcoholic fatty liver disease (NAFLD) encompasses a spectrum of liver pathology that is characterized by the excessive accumulation of fat in the liver, including simple steatosis non-alcoholic fatty liver (NAFL), non-alcoholic steatohepatitis (NASH), steatofibrosis, cirrhosis, and hepatocellular carcinoma [1]. In the face of a global obesity epidemic, NAFLD has emerged as the most common form of chronic liver disease, affecting an estimated 25% of the general population worldwide [2, 3]. Epidemiological researches have reported that NAFLD is one of the three main causes of cirrhosis [4], and NASH is rapidly becoming the leading cause of end-stage liver disease [5, 6]. However, except for lifestyle interventions, such as exercise and dietary, there is still no approved pharmacotherapy for NAFLD. Therefore, it is important to explore and develop valid pharmacotherapies for NAFLD [7].

In recent years, traditional Chinese medicine (TCM) has been proved effective in the case of alone or integrated with western medicine, and has attracted more and more people’s attention [8, 9]. Jiangzhi Decoction (JZD), a clinically used herbal formula developed in accordance with TCM pathogenesis, is composed of the following five medicinal herbs: *Trichosanthes Kirilowii* (TK), *Alisma Orientale* (AO), *Angelica Sinensis* (AS), *Crataegus Pinnatifida* (CP) and *Polygonum Multiflorum* (PM). Previous evidence
has proved the efficacy of JZD on regulating lipid metabolism [10]. However, the key active ingredients and molecular mechanisms of JZD are still unclear and need further exploration.

Because Chinese herbal medicines have the characteristics of multi-component and multi-targeted effects, conventional strategies can be hardly used to explore their pharmacological mechanisms. Network pharmacology, a novel approach based on systems biology, has been proved suitable for analyzing the complex relationships of various ingredients and effects in Chinese herbal medicines [11]. In this study, network pharmacology was carried out to investigate the key active ingredients and molecular mechanisms according to screening a great deal of candidate ingredients, predicting multiple drug targets, analyzing possible signaling pathways and conducting herbs-ingredients-targets networks. A flowchart of the network pharmacology approach is presented in Fig. 1.

2. Methods

2.1 Searching for active ingredients of JZD

Active ingredients of JZD were collected from two databases. One is Traditional Chinese Medicine Systems Pharmacology (TCMSP) database [12] (http://lsp.nwu.edu.cn/tcmsp.php), which contains large number of herbal entries, drug-disease networks and drug-target networks. A great deal of herbal information can be obtained from TCMSP database, including ingredients, molecule name, molecular weight (MW), drug-likeness (DL), human oral bioavailability (OB), half-life (HL), water partition coefficient (AlogP), number of hydrogen bond donors and receptors (Hdon/Hacc), Caco-2 permeability (Caco-2), blood-brain barrier (BBB) and so on. The active ingredients of JZD were screened out according to the ADME parameter, and the ingredients with DL ≥ 0.18 were regarded as active ingredients [13].

If the herbal information could not be found in TCMSP database, the other database would be used. It's Traditional Chinese Medicine Integrated Database (TCMID) (http://www.megabionet.org/tcmid/), which contains 46929 prescriptions, 8159 herbs, 43413 total ingredients, 8182 drugs, 4633 diseases, 1045 prescription ingredients, 778 herbal mass spectra, 3895 mass spectrometry of ingredients [14]. By combining the information from two databases above, the active ingredients of JZD were identified.

2.2 Identification of targets of active ingredients

ChemMapper database (http://lilab.ecust.edu.cn/chemmapper/) is a versatile web server for exploring pharmacology and chemical structure association based on molecular 3D similarity method [15]. We searched the predicted targets of each active ingredient in JZD from ChemMapper database and screened according to the criteria of 3D structure similarity above 1.0 and prediction score above 0 [16]. The full names of targets were converted to gene symbol based on the UniProt ID in UniProt database (http://www.uniprot.org/) for further analysis.

2.3 Searching for NAFLD-related genes
DisGeNET database (http://www.disgenet.org/web/DisGeNET/menu/home) is a knowledge management platform integrating and standardizing data about disease associated genes and variants from multiple sources, including the scientific literature [17]. Known genes of NAFLD were searched from DisGeNET database using “non-alcoholic fatty liver disease” as the keyword, and the top 30% of genes were regarded as important genes for further analysis.

In addition, Gene Expression Omnibus (GEO) database (http://www.ncbi.nlm.nih.gov/geo/) is an international public repository for high-throughput microarray and next-generation sequence functional genomic data sets submitted by the research community [18]. The main differentially expressed genes (DEGs) between mild NAFLD and advanced NAFLD were extracted from microarray data GSE31803 and GSE49541 in GEO database, with a cutoff value of \( P<0.05 \) and fold change \(|FC|\geq3\).

### 2.4 Protein-protein interaction (PPI) analysis

The overlapped genes between the target genes of active ingredients and NAFLD-related genes were imported into STRING database to construct PPI network. STRING database integrates the quality-controlled protein-protein association networks of a large number of organisms. We selected the core PPI targets according to the degree score above the average value and the confidence score above 0.9.

### 2.5 Functional enrichment analysis

ReactomeFIViz and ClueGO, two kinds of plug-ins for Cytoscape, were used to perform functional enrichment analysis. ReactomeFIViz is a highly reliable protein functional interaction network covering around 60% of total human genes based on Reactome database, the most popular and comprehensive open-source biological pathway knowledgebase [19]. ClueGO integrates Gene Ontology (GO) terms and KEGG/BioCarta pathways, and creates a functionally organized GO/pathway term network [20]. \( P<0.01 \) was regarded as the significant cutoff in this study.

### 2.6 Pathway network construction

Pathway networks of “herbs-active ingredients-candidate targets” were constructed using the Cytoscape 3.3.0 software. The Network Analyzer plug-in was used to identify key active ingredients and critical candidate targets based on the criterion below: nodes with degree values exceeding the average value of all nodes in the network. The degree value is the number of edges a node has in a network, which indicates how many herbs/ingredients/targets one node is related with. If the degree value of a node is larger, the node is believed to play a more important role in the network.

### 3. Results

#### 3.1 Active ingredients of JZD

Among the five main herbs of JZD, TK, AO and AS were searched from TCMSP database, while the other herbs, CP and PM, couldn't be found in TCMSP database and were searched from TCMID database. Based on the criteria of \( DL \geq 0.18 \), a total of 147 active ingredients were finally screened out in this study.
(Fig. 2 and Supplementary Table 1). The numbers of active ingredients in TK, AO, AS, CP and PM were 17, 15, 7, 66 and 46, respectively. There were four ingredients overlapped in two herbs, including emodin in AO and PM, epicatechin in CP and PM, tricin in TK and PM, and β-sitosterol in AS and PM.

A note about those results was that ADME parameters were not provided in TCMID database. Thus, the ingredients in CP and PM didn’t be filtered according to the ADME parameters. Maybe it was the reason why the numbers of ingredients in CP and PM were much larger than those in TK, AO and AS.

### 3.2 Targets of active ingredients of JZD

The direct target of each chemical ingredient in JZD were identified from ChemMapper database. According to the criteria of 3D structure similarity above 1.0 and prediction score above 0, a total of 1285 targets of active ingredients of JZD were obtained (Supplementary Table 2). Our analysis showed that gallic acid, citric acid and succinic acid were the top three active ingredients targeting 525, 481 and 440 targets, respectively.

### 3.3 Genes related to NAFLD

A total of 333 known genes were found from DisGeNET database, and the top 30%, that were 100 genes, were chosen for further analysis. Based on the cutoff value of $P < 0.05$ and fold change $|\text{FC}| \geq 3$, a total of 315 DEGs were extracted from microarray data GSE31803 and GSE49541. After duplicates of genes from DisGeNET database and GEO data were eliminated, a total of 401 genes were identified as NAFLD-related genes (Supplementary Table 3).

### 3.4 Core targets in protein-protein interaction (PPI)

According to the search and analysis above, we obtained a total of 59 overlapped genes between the target genes of active ingredients and NAFLD-related genes (Table 1). The 59 candidate targets were imported into STRING database to construct the PPI network. According to the screening criteria of the degree score above the average value and the confidence score above 0.9, 22 core targets were obtained, including TNF, IGF1, IL1B, GPT, CCL2, HGF, TLR4, PPARG, GOT2, LDLR, MMP1, F2, DCN, ACE, GLUL, PPARA, THBS1, JAK2, CFTR, PLAT, OAT and GPX8 (Fig. 3). The 22 core targets may be the potential targets in the treatment of JZD against NAFLD.
Table 1
The overlapped genes between the target genes of active ingredients and NAFLD-related genes

| No. | Uniprot ID | Gene ID | Gene Symbol | Gene Full Name |
|-----|------------|---------|-------------|----------------|
| 1   | P54710     | 486     | FXYD2       | FXYD Domain Containing Ion Transport Regulator 2 |
| 2   | P06396     | 2934    | GSN         | Gelsolin       |
| 3   | P28472     | 2562    | GABRB3      | Gamma-Aminobutyric Acid Type A Receptor Beta3 Subunit |
| 4   | P07996     | 7057    | THBS1       | Thrombospondin 1 |
| 5   | P54289     | 781     | CACNA2D1    | Calcium Voltage-Gated Channel Auxiliary Subunit Alpha2delta 1 |
| 6   | P04818     | 7298    | TYMS        | Thymidylate Synthetase |
| 7   | P17302     | 2697    | GJA1        | Gap Junction Protein Alpha 1 |
| 8   | P55011     | 6558    | SLC12A2     | Solute Carrier Family 12 Member 2 |
| 9   | P07585     | 1634    | DCN         | Decorin |
| 10  | P08729     | 3855    | KRT7        | Keratin 7 |
| 11  | P14210     | 3082    | HGF         | Hepatocyte Growth Factor |
| 12  | P13569     | 1080    | CFTR        | CF Transmembrane Conductance Regulator |
| 13  | Q96SL4     | 2882    | GPX7        | Glutathione Peroxidase 7 |
| 14  | P13500     | 6347    | CCL2        | C-C Motif Chemokine Ligand 2 |
| 15  | O94925     | 2744    | GLS         | Glutaminase |
| No. | Uniprot ID | Gene ID | Gene Symbol | Gene Full Name |
|-----|------------|---------|-------------|----------------|
| 16  | P21802     | 2263    | FGFR2       | Fibroblast Growth Factor Receptor 2 |
| 17  | Q99584     | 6284    | S100A13     | S100 Calcium Binding Protein A13 |
| 18  | Q13133     | 10062   | NR1H3       | Nuclear Receptor Subfamily 1 Group H Member 3 |
| 19  | P13716     | 210     | ALAD        | Aminolevulinate Dehydratase |
| 20  | P82251     | 11136   | SLC7A9      | Solute Carrier Family 7 Member 9 |
| 21  | Q8N159     | 162417  | NAGS        | N-Acetylglutamate Synthase |
| 22  | P21549     | 189     | AGXT        | Alanine–Glyoxylate And Serine–Pyruvate Aminotransferase |
| 23  | P34896     | 6470    | SHMT1       | Serine Hydroxymethyltransferase 1 |
| 24  | Q08828     | 107     | ADCY1       | Adenylate Cyclase 1 |
| 25  | O43708     | 2954    | GSTZ1       | Glutathione S-Transferase Zeta 1 |
| 26  | P51570     | 2584    | GALK1       | Galactokinase 1 |
| 27  | P15104     | 2752    | GLUL        | Glutamate-Ammonia Ligase |
| 28  | Q14749     | 27232   | GNMT        | Glycine N-Methyltransferase |
| 29  | P36222     | 1116    | CHI3L1      | Chitinase 3 Like 1 |
| 30  | Q8TED1     | 493869  | GPX8        | Glutathione Peroxidase 8 (Putative) |
| No. | Uniprot ID | Gene ID | Gene Symbol | Gene Full Name |
|-----|------------|---------|-------------|----------------|
| 31  | P11137     | 4133    | MAP2        | Microtubule Associated Protein 2 |
| 32  | P00750     | 5327    | PLAT        | Plasminogen Activator, Tissue Type |
| 33  | Q9H2S1     | 3781    | KCNN2       | Potassium Calcium-Activated Channel Subfamily N Member 2 |
| 34  | P04181     | 4942    | OAT         | Ornithine Aminotransferase |
| 35  | Q07869     | 5465    | PPARA       | Peroxisome Proliferator Activated Receptor Alpha |
| 36  | P01130     | 3949    | LDLR        | Low Density Lipoprotein Receptor |
| 37  | P09488     | 2944    | GSTM1       | Glutathione S-Transferase Mu 1 |
| 38  | P09211     | 2950    | GSTP1       | Glutathione S-Transferase Pi 1 |
| 39  | Q03181     | 5467    | PPARD       | Peroxisome Proliferator Activated Receptor Delta |
| 40  | P30711     | 2952    | GSTT1       | Glutathione S-Transferase Theta 1 |
| 41  | P08263     | 2938    | GSTA1       | Glutathione S-Transferase Alpha 1 |
| 42  | P03956     | 4312    | MMP1        | Matrix Metallopeptidase 1 |
| 43  | Q9P2W7     | 27087   | B3GAT1      | Beta-1,3-Glucuronyltransferase 1 |
| 44  | O60674     | 3717    | JAK2        | Janus Kinase 2 |
| No. | Uniprot ID | Gene ID | Gene Symbol | Gene Full Name                              |
|-----|------------|---------|-------------|---------------------------------------------|
| 45  | P08700     | 3562    | IL3         | Interleukin 3                               |
| 46  | P12821     | 1636    | ACE         | Angiotensin I Converting Enzyme             |
| 47  | P00734     | 2147    | F2          | Coagulation Factor II, Thrombin             |
| 48  | Q8WTVO     | 949     | SCARB1      | Scavenger Receptor Class B Member 1         |
| 49  | P06576     | 506     | ATP5F1B     | ATP Synthase F1 Subunit Beta                |
| 50  | O14594     | 1463    | NCAN        | Neurocan                                    |
| 51  | P24298     | 2875    | GPT         | Glutamic–Pyruvic Transaminase               |
| 52  | P01375     | 7124    | TNF         | Tumor Necrosis Factor                       |
| 53  | P37231     | 5468    | PPARG       | Peroxisome Proliferator Activated Receptor Gamma |
| 54  | O00206     | 7099    | TLR4        | Toll Like Receptor 4                        |
| 55  | P06213     | 3643    | INSR        | Insulin Receptor                            |
| 56  | P00505     | 2806    | GOT2        | Glutamic-Oxaloacetic Transaminase 2         |
| 57  | P01584     | 3553    | IL1B        | Interleukin 1 Beta                          |
| 58  | P05019     | 3479    | IGF1        | Insulin Like Growth Factor 1                |
| 59  | P41235     | 3172    | HNF4A       | Hepatocyte Nuclear Factor 4 Alpha           |

### 3.5 Functional enrichment analysis of the overlapped genes

ReactomeFIViz and ClueGO pathway analyses were further performed for the overlapped genes. The top 10 significantly related pathways were shown in Fig. 4A by ReactomeFIViz analysis, including nuclear receptor transcription pathway, glutathione conjugation, interleukin-4 and 13 signaling, regulation of
insulin-like growth factor (IGF) transport and uptake by insulin-like growth factor binding proteins (IGFBPs), phase II conjugation and so on. At the same time, according to ClueGo analysis, signaling pathways were divided into 13 enriched categories based the kappa coefficient, including positive regulation of lipid metabolic process, regulation of smooth muscle cell proliferation, fatty acid transport, positive regulation of reactive oxygen species metabolic process, regulation of phosphatidylinositol 3-kinase signaling, alpha-amino acid metabolic process, regeneration, response to mechanical stimulus, muscle cell proliferation, cellular detoxification, exocrine system development, regulation of glucose transport, and cell-cell signaling involved in cardiac conduction (Fig. 4B and 4C). The functional enrichment analysis indicated that nuclear receptor transcription and lipid metabolism regulation might play an important role in the treatment of JZD against NAFLD.

3.6 Pathway network construction of herb-ingredient-target

To identify key active ingredients and molecular mechanisms of JZD against NAFLD, we further constructed herb-ingredient-target networks based on the top significantly related pathways from functional enrichment analysis (Fig. 5). We found that 12 ingredients appeared in both nuclear receptor transcription pathway and lipid metabolism regulation pathway, including emodin, emodin anthrone, hyperin, questin, rhein, tricin, aloe emodin, 2-acetylemodin, epicatechin, chrysazin, chrysophanol, 4,4′-dihydroxydiphenyl methane. The results implied that those key active ingredients all participated in nuclear receptor transcription and lipid metabolism regulation, which might be the key molecular mechanisms of JZD against NAFLD.

4. Discussion

NAFLD is characterized by abnormal lipid metabolism and excessive lipid accumulation in hepatocytes. Most of the herbs in JZD have been reported to take part in the regulation of lipid metabolism in NAFLD or other related diseases. Some researches showed that *Alisma Orientale* (AO) could prevent hepatic triglyceride accumulation through suppressing de novo lipogenesis and increasing lipid export, and control oxidative stress markers, lipoapoptosis, liver injury panels and inflammatory and fibrotic mediators, eventually influencing steatohepatitis and liver fibrosis [21, 22]. The components of *Angelica Sinensis* (AS) have been proved to regulate lipid and glucose metabolism [23–25]. Liu et al. found that a diet formula of *Crataegus Pinnatida* (CP) and three other herbs could alleviate hepatic steatosis and insulin resistance *in vivo* and *in vitro* [26]. Yu et al. performed a serious of experiments to confirm that the active components of *Polygonum Multiflorum* (PM) could promote the lipolysis of cholesterol and triglyceride, increase the content of HTGL, and reduce LDL and VLDL [27–29]. However, the synergetic mechanisms of all the herbs in JZD were still unclear.

In our study, nuclear receptor transcription and lipid metabolism regulation were found as the core pathways which JZD mainly participated in when alleviating NAFLD. As we know, there are 48 nuclear receptors categorized into 7 subfamilies designated as NR0-NR6 [30]. Of particular importance in NAFLD are specific members of NR1 subfamily [31]. Most potential targets of JZD in Fig. 2 belong to NR1
subfamily, including PPARs (peroxisome proliferator-activated receptors, PPARA/PPAR\(\alpha\), PPARD/PPAR\(\beta/\delta\), PPARG/PPAR\(\gamma\); NR1C1-3) and LXR\(\alpha\) (liver X receptor \(\alpha\); NR1H3). PPAR\(\alpha\) activation induces the increase in fatty acid oxidation, ketogenesis and gluconeogenesis \[32\]. PPAR\(\beta/\delta\) activation exerts regulatory effects on fatty acid catabolism, reverse cholesterol transport and energy metabolism, and even reduces insulin resistance and plasma glucose \[33\]. PPAR\(\gamma\) shifts lipids from non-adipose organs such as the liver and skeletal muscles to white adipose tissue, leading to the attenuation of lipotoxicity \[34\]. In general, PPAR activation is thought to be beneficial in NAFLD, and clinical trials of single/dual receptor agonists are underway \[31\]. Another important nuclear receptor LXR\(\alpha\) acts as the negative regulator of cholesterol metabolism through the induction of hepatocyte cholesterol catabolism, excretion, and the reverse cholesterol transport pathway \[35\]. Furthermore, HNF4A/HNF4\(\alpha\) (hepatocyte nuclear factor 4\(\alpha\); NR2A1) also belongs to the subfamily of nuclear receptors. Previous study reported that HNF4\(\alpha\) could prevent liver steatosis by controlling hepatic carboxylesterase 2 expression and modulating lipolysis, lipogenesis and endoplasmic reticulum in NAFLD \[36\]. Therefore, they are all potential therapeutic targets for the treatment of NAFLD.

Our research found that some ingredients in JZD might be the key ones for NAFLD treatment, such as emodin, hyperin and rhein. Many previous studies have reported that those ingredients could take part in the regulation of nuclear receptor. Emodin has been proved to increase the mRNA level of PPAR\(\gamma\) and play a protective role in alcohol-mediated liver steatosis \[37\]. According to the activation of PPAR\(\gamma\) signaling pathway, emodin could also alleviate atherosclerosis followed by promoting cholesterol efflux \[38\], or play other roles though regulating inflammatory response \[39, 40\] and nitric oxide production \[41\]. Furthermore, emodin has also been reported to regulate the expression of LXR\(\alpha\) in atherosclerosis \[38\] and melanogenesis \[42\]. Hyperin is one of the chief flavonoid components of Ericaceae, Guttifera, Leguminosae and Celastraceae, and could remarkably induce the expression of PPAR\(\gamma\) and attenuate inflammation of acute liver injury \[43\]. In addition, some studies also reported that rhein could target PPAR\(\gamma\) signaling pathway and play anti-inflammatory activity \[44, 45\]. Moreover, rhein has been confirmed to ameliorate NAFLD and obesity and recover metabolic disorders through directly binding to LXR\(\alpha\) \[46, 47\]. Thus, those key ingredients in JZD might improve NAFLD via regulating the nuclear receptors.

In conclusion, the multicomponent and multitarget characteristics of the therapeutic effects of JZD against NAFLD were effectively elucidated through network pharmacology approach. Emodin, emodin anthrone, hyperin, questin, rhein were speculated as the key active ingredients of JZD, and nuclear receptor transcription and lipid metabolism regulation were found as the core molecular mechanisms by which JZD alleviated NAFLD (Fig. 6). Therefore, our study will provide the scientific evidences of the clinical efficacy of JZD against NAFLD.

**Declarations**

**Ethics approval and consent to participate**
Consent for publication

Not applicable.

Availability of data and materials

The data of our research can be acquired from the Supplementary Materials uploaded with this article.

Competing interests

The authors declare that they have no competing interests.

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Authors’ contributions

Song YN and Xia W conceived the study. Wang L, Zhi Y, Zhang M and Ma X analyzed the data. Zhi Y and Tie HY made the charts. Wang L wrote the paper. Song YN and Xia W revised the paper. Ye Y provided technical support.

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Figures
Jiangzhi Decoction (JZD)

TCMSP Database
TCMID Database

147 Active Ingredients

ChemMapper Database

1285 Targets of Active Ingredients

DisGeNET Database
GEO Database

401 NAFLD-Related Genes

59 Overlapped Candidate Targets of JZD against NAFLD

PPI Analysis
Enrichment Analysis
Network Analysis

Herbs-Active ingredients-Candidate targets

Key Active Ingredients and Core Molecular Mechanisms
Figure 1

The flowchart of the network pharmacology analysis for JZD against NAFLD.

Figure 2

147 active ingredients found in JZD. The red square represents herbs in JZD, and green triangle represents ingredients of those herbs.
Figure 3

PPI network of 22 core targets of JZD against NAFLD. In the PPI diagram, each solid circle represents a target, and the middle of the circle shows the structure of the protein.
**Figure 4**

Functional enrichment analysis from ReactomeFIViz and ClueGO. (A) The column bar graph from ReactomeFIViz. It shows the top 10 significantly related pathways, including nuclear receptor transcription pathway, glutathione conjugation, interleukin-4 and 13 signaling, regulation of insulin-like growth factor (IGF) transport and uptake by insulin-like growth factor binding proteins (IGFBPs), phase II conjugation and so on. (B) The pie chart from ClueGO. It shows the enriched signaling pathway categories based on the kappa coefficient, including positive regulation of lipid metabolic process, regulation of smooth muscle cell proliferation, fatty acid transport, positive regulation of reactive oxygen species metabolic process, regulation of phosphatidylinositol 3-kinase signaling, alpha-amino acid metabolic process and so on. (C) The functional enrichment network from ClueGO. The node represents the signaling pathway, and the size of each node represents the enrichment significance of each signaling pathway. The larger the node is, the more significant the pathway is. The line represents the correlation between functions, and the thickness of each line represents the kappa coefficient between functions. The thicker the line is, the greater the kappa coefficient is.

**Figure 5**

The top significantly related pathways of “herb-ingredient-target” networks in JZD against NAFLD. (A) Nuclear Receptor transcription pathway; (B) Regulation of lipid metabolic process. The red square represents herbs in JZD, green triangle represents ingredients of those herbs, and orange circle represents candidate targets of JZD against NAFLD.
Trichosanthes Kirilowii (TK), Alisma Orientale (AO), Angelica Sinensis (AS), Crataegus Pinnatifida (CP) and Polygonum Multiflorum (PM)

Key Active Ingredients
Emodin, Emodin anthrone, Hyperin, Questin, Rhein ……

Core Molecular Mechanisms
Nuclear Receptor Transcription and Lipid Metabolism Regulation
The key active ingredients and the core molecular mechanisms of JZD against NAFLD. Emodin, emodin anthrone, hyperin, questin, rhein were speculated as the key active ingredients of JZD, and nuclear receptor transcription and lipid metabolism regulation were identified as the core molecular mechanisms of JZD against NAFLD.

Supplementary Files

This is a list of supplementary files associated with this preprint. Click to download.

- SupplementaryTable3.xlsx
- SupplementaryTable2.xlsx
- SupplementaryTable1.xlsx