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Exploring the molecular mechanism of *Carthami Flos* against liver cirrhosis using network pharmacology and molecular docking

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Author contributions

Xie JM conceived the study. Chen HR, Song L, Bai HZ and Zhu Y investigated and analyzed the data. Xie JM supervised the study. Xie JM wrote and revised the manuscript. All authors read and agreed to publish the paper.

Competing interests

The authors declare no conflicts of interest.

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Peer review information

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Abbreviations

TCM-ID, Traditional Chinese Medicine Information Database; TCMSP, Traditional Chinese Medicine Systems Pharmacology Database and Analysis Platform; GeneCards, Human Genes | Gene Database | Gene Search; MalaCards, The Human Disease Database; DisGeNET, Disease Gene Network; DAVID, Database for Annotation, Visualization and Integrated Discovery.

Citation

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Abstract

Background: The molecular mechanism of *Carthami Flos* in treating cirrhosis was investigated using network pharmacology and molecular docking. **Methods:** We employed the TCM-ID (Traditional Chinese Medicine Information Database) and TCMSP (Traditional Chinese Medicine Systems Pharmacology Database and Analysis Platform) databases, the SwissADME (http://www.swissadme.ch/) platform to screen active components in *Carthami Flos*. Subsequently, on the SwissTargetPrediction (http://swisstargetprediction.ch/) analysis platform, we inputted the 'Isomeric SMILES' of the active ingredients from *Carthami Flos* to collect related targets, with a selective criteria of probability ≥ 0.1, specifically targeting '*Homo sapiens*'. Liver cirrhosis-related targets were sourced from GeneCards (Human Genes | Gene Database | Gene Search), MalaCards (The Human Disease Database), TTD (Therapeutic Target Database), and DisGeNET (Disease Gene Network) disease databases. The intersection target of *Carthami Flos*'s active components and liver cirrhosis was identified using the Jvenn (an interactive Venn diagram viewer) online platform, and the '*Carthami Flos* - Active ingredients - Targets - Liver Cirrhosis' network diagram was constructed with Cytoscape 3.9.1 software. The target protein interaction network was analyzed using the STRING (Search Tool for the Retrieval of Interacting Genes) database, through which core targets were identified, screened, and visualized. GO functions and KEGG pathway enrichments were analyzed using the DAVID (The Database for Annotation, Visualization and Integrated Discovery) database. Molecular docking between the core target and the active ingredient was performed using PyMOL 2.6.0 and AutoDock Vina 1.5.7. **Results:** Fourteen active components of *Carthami Flos*, including Luteolin, Kaempferol, and Quercetin, were identified from the Traditional Chinese Medicine database, with seven potential targets for cirrhosis treatment. GO-BP analysis primarily involves processes related to the cell cycle, protein phosphorylation, and cellular responses to reactive oxygen species. KEGG pathway enrichment is mainly associated with viral hepatitis B, VEGF signaling, and the HIF-1 signaling pathway, among others. The active components of *Carthami Flos* exhibited effective molecular docking with core target molecules, demonstrating strong affinity. **Conclusion:** *Carthami Flos* contributes to the treatment of cirrhosis through a multi-component, multi-target, and multi-pathway approach. SRC (Proto-oncogene tyrosine-protein kinase Src) and HSP90AA1 (Heat shock protein HSP 90-alpha) are key therapeutic targets, impacting significant signaling pathways such as Wnt/β-catenin and MAPK/Erk.

Keywords: *Carthami flos*; liver cirrhosis; network pharmacology; molecular docking

Introduction

The 2017 Global Burden of Disease (GBD) study on cirrhosis and chronic liver diseases revealed a higher incidence among men, with an estimated 112 million people worldwide having compensated cirrhosis, and cirrhosis accounting for 2.4% of global deaths [1]. Liver cirrhosis, a chronic disease, primarily results from extensive liver cell necrosis and represents an advanced stage of damage in the liver parenchyma. Currently, cirrhosis can stem from various causes, with the most common being viral hepatitis (types B, C, D, and occasionally E, with hepatitis B being predominant in China), chronic alcoholic liver disease (the leading cause of cirrhosis in Europe and the United States, accounting for 50% to 90% of cases), non-alcoholic fatty liver disease, prolonged cholestasis, disorders of liver blood circulation, genetic and metabolic diseases, parasitic infections, immune disorders, and exposure to drugs or toxins [2, 3].

Cirrhosis is a progressive condition classified into different clinical stages, including compensated and decompensated cirrhosis, based on symptomatology and liver function changes. In the compensated stage (clinical stages 1 and 2), patients may be asymptomatic or exhibit non-specific symptoms such as fatigue, loss of appetite, and diarrhea, often linked to the underlying liver disease. Decompensated cirrhosis (clinical stages 3, 4, and 5) involves severe liver function impairment, presenting as jaundice, fever, hepatic pleural effusion, esophageal varices, enlargement of the liver and spleen, ascites, bleeding tendencies, and potentially leading to hepatic encephalopathy, sepsis, hepatorenal syndrome, and liver cancer [4].

The clinical diagnosis of cirrhosis typically involves a comprehensive assessment that includes a review of the patient's history, a thorough examination of symptoms and signs, imaging studies, laboratory tests, and histological analysis, with liver biopsy remaining the 'gold standard' for diagnosing and assessing cirrhosis [3, 5]. Currently, treatment for cirrhosis primarily consists of pharmacological and surgical approaches. Pharmacological treatment often employs antiviral drugs such as Entecavir and Tenofovir [6], although long-term use can lead to significant side effects [7, 8]. Surgical intervention mainly involves liver transplantation [9], which carries substantial risks and potential postoperative complications, including male hypogonadism and arterial complications [10, 11].

Increasingly, traditional Chinese medicine (TCM) is garnering attention for the prevention and treatment of cirrhosis due to its efficacy and fewer adverse reactions. Research into TCM for synergistic cirrhosis treatment is ongoing. *Carthami Flos*, from the plant *Carthamus tinctorius L.* in the Asteraceae family, consists of dried flowers rich in flavonoids, quinolizidines, alkaloids, polyacetylenes, fatty acids, proteins, lignans, steroids, and polysaccharides [12]. Modern pharmacological studies have highlighted *Carthami Flos*'s diverse biological effects, including neuroprotection [13], hepatoprotection [14], anti-inflammatory properties [15], antifibrotic effects on the liver [16], anticancer activities [17, 18], anticoagulant [19], antioxidative [20], myocardial ischemia relief [21], and antithrombotic effects [22].

Carthami Flos has demonstrated therapeutic effects on a range of conditions including cardiovascular and cerebrovascular diseases [23], neurodegenerative disorders [24], psychological illnesses [25], and osteoporosis [26, 27]. Currently, *Carthami Flos* is also widely utilized in the clinical management of diverse ailments such as space sickness, diabetes, edema, psoriasis, arthritis, depression, anxiety, cardiovascular disease, and amenorrhea [28]. While numerous studies have explored the pharmacological functions of *Carthami Flos* in treating liver fibrosis, research into its effectiveness against liver cirrhosis remains limited, and the specific targets and mechanisms involved are not yet well understood.

Using network pharmacology and molecular docking techniques, this study elucidates the disease progression from the dual perspectives of systemic biology and biological network equilibrium. It constructs the "Ingredient-target-pathway" interactive network for *Carthami Flos* and cirrhosis, investigating the potential molecular mechanisms by which *Carthami Flos* may treat cirrhosis. This research aims to provide a theoretical foundation for the development of new pharmaceuticals for the clinical treatment of cirrhosis (Figure 1).

Materials and methods

Active ingredients screening of *Carthami Flos* and target prediction

In the TCM-ID (https://www.bidd.group/TCMID/) [29] and TCMSP (https://old.tcmsp-e.com/tcmsp.php) [30] databases, '*Carthami Flos*' was used as a keyword to retrieve the ingredients. Active ingredients were identified using Venny 2.0.2 (https://bioinfogp.cnb.csic.es/tools/venny/index2.0.2.html), with criteria of oral bioavailability (OB) ≥ 30%, drug-likeness (DL) ≥ 0.18. For ingredients with insufficient data in the TCMSP, their Isomeric SMILES were copied from the PubChem database (https://pubchem.ncbi.nlm.nih.gov/) [31] and imported into the SwissADME platform (http://www.swissadme.ch/) [32], where gastrointestinal absorption values were categorized as 'High'. The criteria for screening included MWT ≤ 500, logP ≤ 5, hydrogen bond donors ≤ 5, and hydrogen bond acceptors ≤ 10, with at least three criteria needing to be met. Target prediction was performed on the SwissTargetPrediction platform (http://www.swisstargetprediction.ch/) [33] with the species set to 'Homo sapiens' and a minimum probability of 0.1. Targets related to *Carthami Flos*'s effective components were identified.

Target collection for cirrhosis

GeneCards (https://www.genecards.org) [34], MalaCards (https://www.malacards.org) [35], and other databases such as the Therapeutic Target Database (HTTPS: //db.idrblab.org/ttd/) [36] and DisGeNET (https://www.disgenet.org) [37] provided data on gene and disease associations. Using liver cirrhosis as a keyword, all genes associated with cirrhosis were identified. Cirrhosis targets were subsequently confirmed using EVenn (www.ehbio.com/test/venn/#/) [38].

Carthami Flos-ingredients-targets-liver cirrhosis network construction

Using the Jvenn online platform (https://jvenn.toulouse.inrae.fr/app/example.html) [39], we analyzed the intersection of targets related to *Carthami Flos*'s active ingredients and cirrhosis of the liver. This analysis identified targets relevant for treating liver cirrhosis. Data files and network tables were established and imported into Cytoscape 3.9.1 software to create a network diagram illustrating the relationship between *Carthami Flos*, active ingredients, targets, and cirrhosis.

Protein-protein interaction network construction and core targets screening

The intersected targets of *Carthami Flos* and cirrhosis were then imported into the STRING database (https://cn.string-db.org/) [40] for protein-protein interaction (PPI) analysis, conducted with a confidence level of ≥ 0.9. The resulting target protein interaction map was visualized using Cytoscape 3.9.1. The software's 'analyze network' plugin was utilized to analyze topological properties and export the data to a CSV file. In the PPI network, nodes with a higher degree appeared larger and more prominent. Core targets were selected based on the criteria of a degree ≥ twice the median value, betweenness ≥ twice the median value, and closeness ≥ the median value.

GO function and KEGG pathway enrichment analysis

Intersected targets was further analyzed in the DAVID database (https://david.ncifcrf.gov/) [41], selecting '*Homo sapiens*' for gene ontology (GO) and Kyoto Encyclopedia of Genes and Genomes (KEGG) pathway enrichment analyses. Results were organized in ascending order of p-value, selecting the top 10 for GO functional analysis and the top 20 for KEGG pathway enrichment analysis. The SangerBox website (http://sangerbox.com/) [42] was used to visualize these

Pharmacology Discovery 2024;4(4):20. https://doi.org/10.53388/PD202404020

analyses with a GO functional enrichment analysis bubble chart and a KEGG pathway enrichment analysis string diagram. The top 20 signaling pathways and their corresponding targets were imported into Cytoscape 3.9.1 software to construct the 'Target-pathway' network diagram.

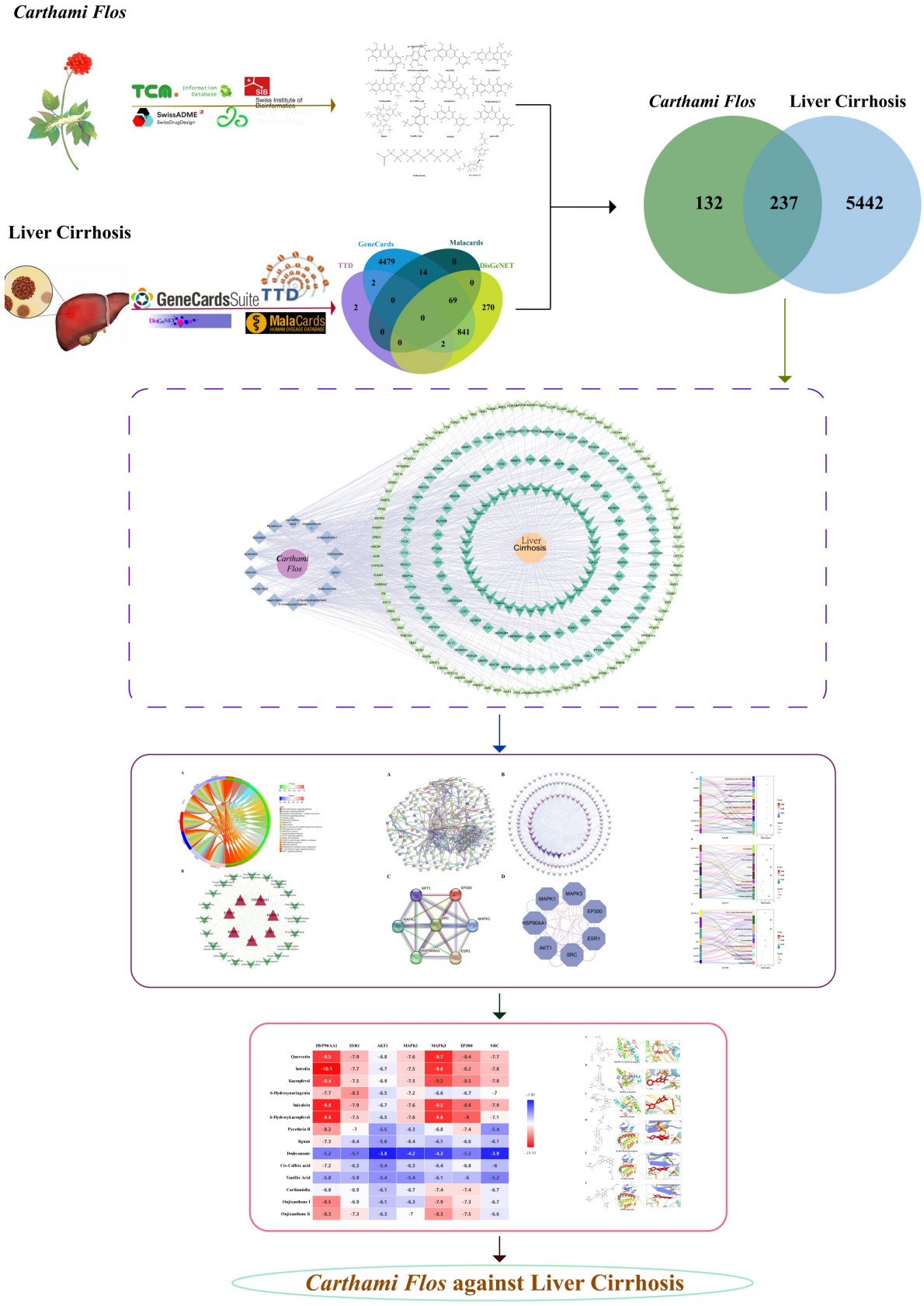

Figure 1 Flowchart of *Carthami Flos* against liver cirrhosis

Molecular docking validation

In the Uniprot (Universal Protein) database (https://www.uniprot.org/) [43], core target protein were searched by selecting, Status 'Reviewed', and their corresponding entry numbers were copied. These entries were then imported into the RCSB PDB (RCSB Protein Data Bank) database (https://www.rcsb.org/) [44] for further investigation. Species '*Homo sapiens*' was specified, with protein chosen as the Polymer Entity Type.

Using PyMOL 2.6.0 [45], water and residues were removed from the core target. The 2D structure of active ingredients from *Carthami Flos* was downloaded in SDF format from the PubChem database, and their structures were optimized using Chem 3D software. AutoDock Vina 1.5.7 software was employed to hydrogenate the protein and small drug molecules, calculate charge numbers, define rigid structures, and configure parameters in the Grid and Docking panels for molecular docking predictions. The resulting docking energies were recorded and analyzed.

The 6 macromolecule-ingredient molecule combinations with the lowest binding energy were selected for visual analysis to verify hydrogen bonding. Molecular docking results and binding energy data were imported into the CNSknowall website (https://cnsknowall.com/index.html#/HomePage) for heat map generation.

Results

Main active ingredients of *Carthami Flos* and correlated targets

Seventy-four *Carthami Flos* components were retrieved from the TCM-ID database, 189 from the TCMSP database, and a total of 261 primary ingredients were identified using Venny (Consejo Superior de Investigaciones Científicas) 2.0.2. The main ingredients of *Carthami Flos* were further analyzed using the SwissTargetPrediction online platform, adhering to the screening criteria of the TCMSP database and the SwissADME website. This analysis identified 14 effective *Carthami Flos* ingredients (Table 1) and 369 associated targets. The 2D molecular structure diagrams of these active components were obtained from the PubChem database (Figure 2).

Table 1 Detailed information of the active ingredients in *Carthami Flos*

NO.	Name	Formula	CAS-ID	MW (g/mol)
1	Onjixanthone Ii	$C_{15}H_{12}O_7$	136083-93-7	304.25
2	Onjixanthone I	$C_{16}H_{14}O_6$	136083-92-6	302.28
3	Carthamidin	$C_{15}H_{12}O_6$	479-54-9	288.25
4	Vanillic Acid	$C_8H_8O_4$	121-34-6	168.15
5	Cis-Caffeic acid	$C_9H_8O_4$	331-39-5	180.16
6	Dodecanoate	$C_{12}H_{23}O_2$	115-05-9	199.31
7	Lignan	$C_{25}H_{30}O_8$	6549-68-4	458.5
8	Pyrethrin II	$C_{22}H_{28}O_5$	121-29-9	372.5
9	6-Hydroxykaempferol	$C_{15}H_{10}O_7$	4324-55-4	302.23
10	Baicalein	$C_{15}H_{10}O_5$	491-67-8	270.24
11	6-Hydroxynaringenin	$C_{15}H_{12}O_6$	479-54-9	288.25
12	Kaempferol	$C_{15}H_{10}O_6$	520-18-3	286.24
13	Luteolin	$C_{15}H_{10}O_6$	491-70-3	286.24
14	Quercetin	$C_{15}H_{10}O_7$	117-39-5	302.23

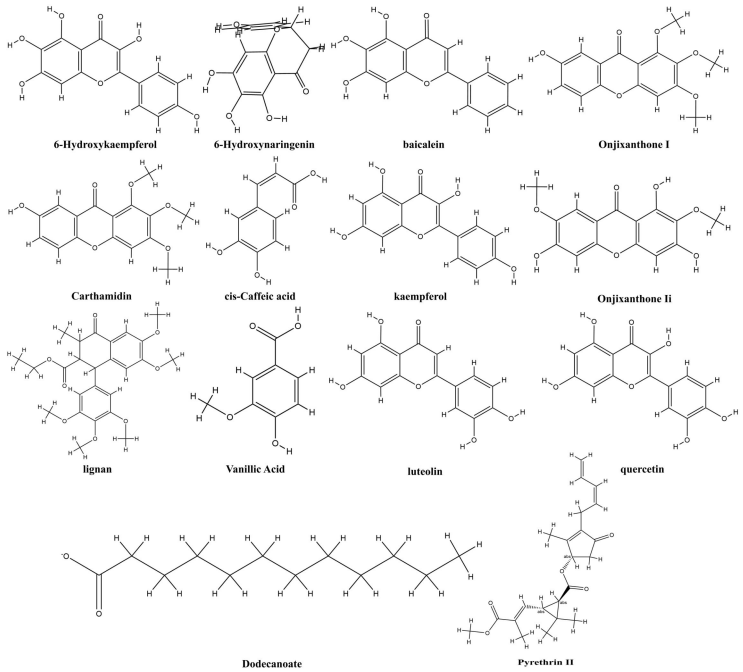

Figure 2 Molecular structure of the 14 active ingredients in *Carthami Flos*

Pharmacology Discovery 2024;4(4):20. https://doi.org/10.53388/PD202404020

Ingredients-liver cirrhosis intersection target acquisition

Cirrhosis-related targets were retrieved from several databases: GeneCards, MalaCards, DisGeNET, and the Therapeutic Target Database (TTD). After consolidating and removing duplicates, a total of 5680 cirrhosis-related targets were identified (Figure 3A). Using Venny 2.0.2, an intersection of these 5680 targets with the 369 *Carthami Flos* component targets yielded 237 disease-drug molecular intersection targets (Figure 3B).

'*Carthami Flos*-ingredients-targets-liver cirrhosis' network construction

Cytoscape 3.9.1 software was used to create a network diagram representing the relationship among *Carthami Flos*, active ingredients, targets, and cirrhosis. The 'analyze network' function revealed that luteolin, kaempferol, quercetin, and lignan are associated with a significant number of cirrhosis targets, indicating their pivotal role in treating the disease. Specifically, there were 77 targets linked to luteolin, 76 to kaempferol, 76 to quercetin, and 76 to lignan (Figure 3C), highlighting these four molecules as the most critical effective ingredients of *Carthami Flos* in cirrhosis treatment.

PPI network construction

The 237 targets identified between *Carthami Flos* and cirrhosis were uploaded to the STRING website to generate a PPI diagram (Figure 4A). The resulting TSV file was then imported into Cytoscape 3.9.1 for visualization and topological attribute analysis (Figure 4B). This analysis identified seven core targets: HSP90AA1(Heat shock protein HSP 90-alpha), SRC(Proto-oncogene tyrosine-protein kinase Src), AKT1 (RAC-alpha serine/threonine-protein kinase), MAPK1 (Mitogen-activated protein kinase 1), ESR1(Estrogen receptor), MAPK3 (MAP kinase-activated protein kinase 3), and EP300(Histone acetyltransferase p300) (Figures 4C–4D).

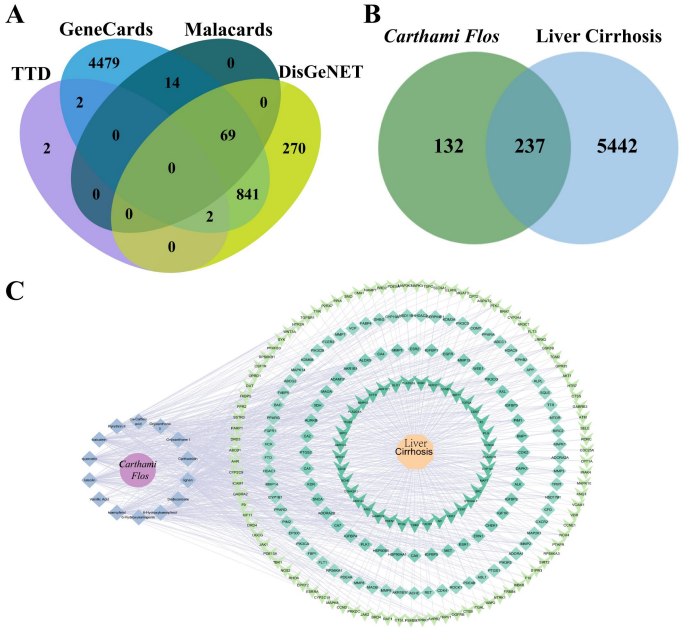

Figure 3 Targets of *Carthami Flos* against liver cirrhosis. (A) Liver cirrhosis targets were collected from TTD, GeneCards, Malacards and DisGeNET databases. (B) Intersection targets of active ingredients of *Carthami Flos* and liver cirrhosis. (C) Network of *Carthami Flos*-ingredients-targets-liver cirrhosis.

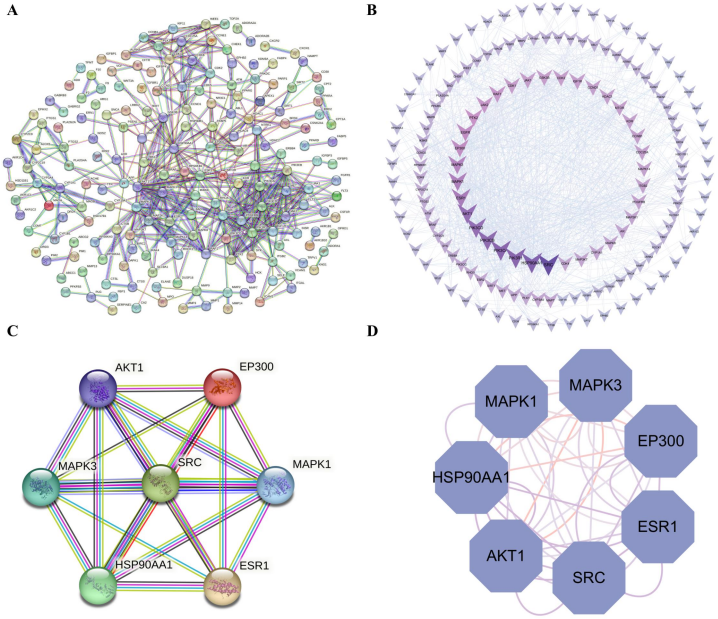

Figure 4 PPI network of *Carthami Flos*-liver cirrhosis intersection targets and core targets. (A, B) PPI network and visualization of *Carthami Flos* against liver cirrhosis. (C, D) PPI network and visualization of the core targets.

Pharmacology Discovery 2024;4(4):20. https://doi.org/10.53388/PD202404020

GO function and KEGG pathway enrichment analysis

These 7 core targets were then analyzed using the DAVID database for GO function and KEGG pathway analyses, with results sorted by ascending p-value. The analysis yielded 77 GO-BP items, 18 GO-CC items, and 20 GO-MF items. The top 10 items with the highest significance from each category were visualized as Sankey charts (Figures 5A–5C). The GO-BP analysis indicated that *Carthami Flos*'s treatment of cirrhosis is notably linked to processes such as the regulation of early to late endosome transport, insulin-like growth factor receptor signaling pathway, positive regulation of telomerase activity, lipopolysaccharide-mediated signaling pathway, positive regulation of nitric oxide biosynthesis, cellular response to reactive oxygen species, and positive regulation of TORC1 signal transduction. The GO-MF results primarily involved functions like nitric oxide synthase regulator activity, ATP binding, protein binding, MAP kinase activity, protein serine/threonine kinase activity, 14-3-3 protein binding, transcription cofactor binding, and tau protein binding. The GO-CC findings highlighted that carthamus treatment of cirrhosis primarily affects the nucleus, cell membrane invaginations, mitochondria, and cytoplasm. Finally, KEGG pathway enrichment identified a total of 115 pathways, with the top 20 pathways of greatest significance further analyzed in a loop graph (Figure 6A). The core target KEGG pathway for *Carthami Flos* treatment of cirrhosis predominantly involves the EGFR signaling pathway, HIF-1 signaling pathway, and viral hepatitis B, among others. The target network diagram of the KEGG pathway, constructed using Cytoscape 3.9.1, features 27 nodes and 93 edges (Figure 6B).

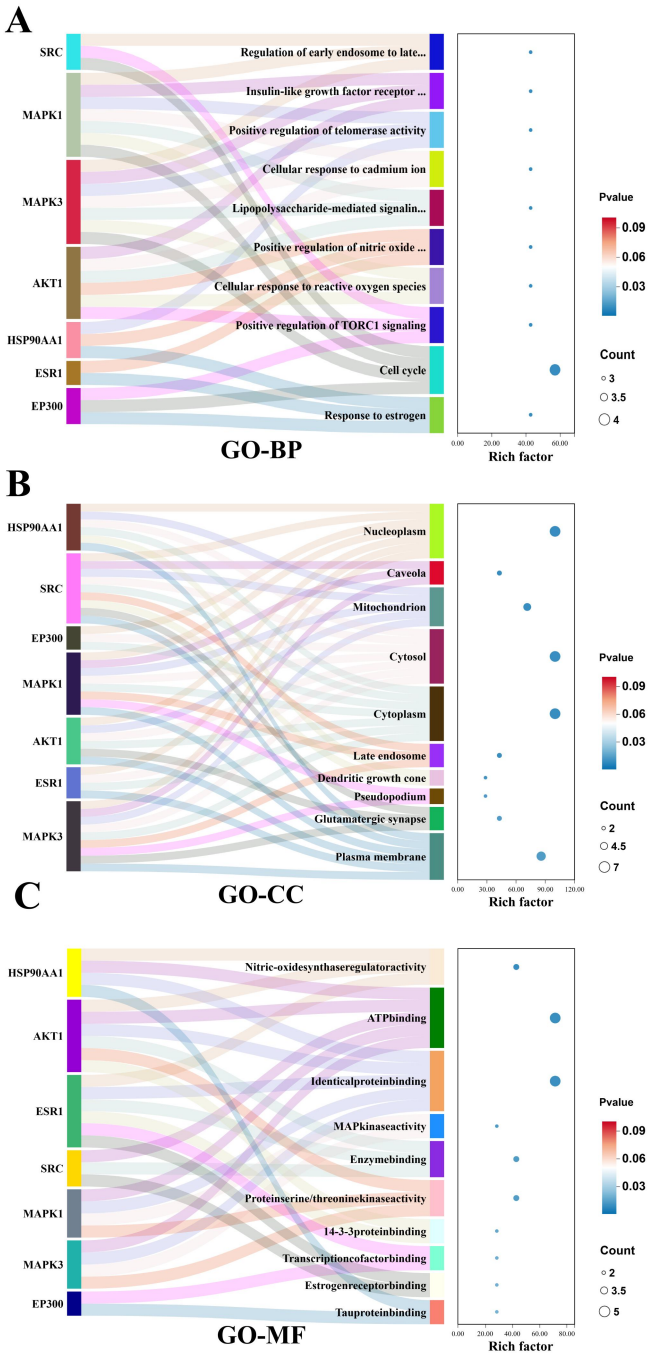

Figure 5 GO function enrichment analysis of targets in *Carthami Flos* against liver cirrhosis. (A) Biological process (BP) enrichment analysis. (B) Cellular component (CC) enrichment analysis. (C) Molecular function (MF) enrichment analysis. Bubble size represents the number of enrichment targets, and the depth of bubble color represents the *P*-value size.

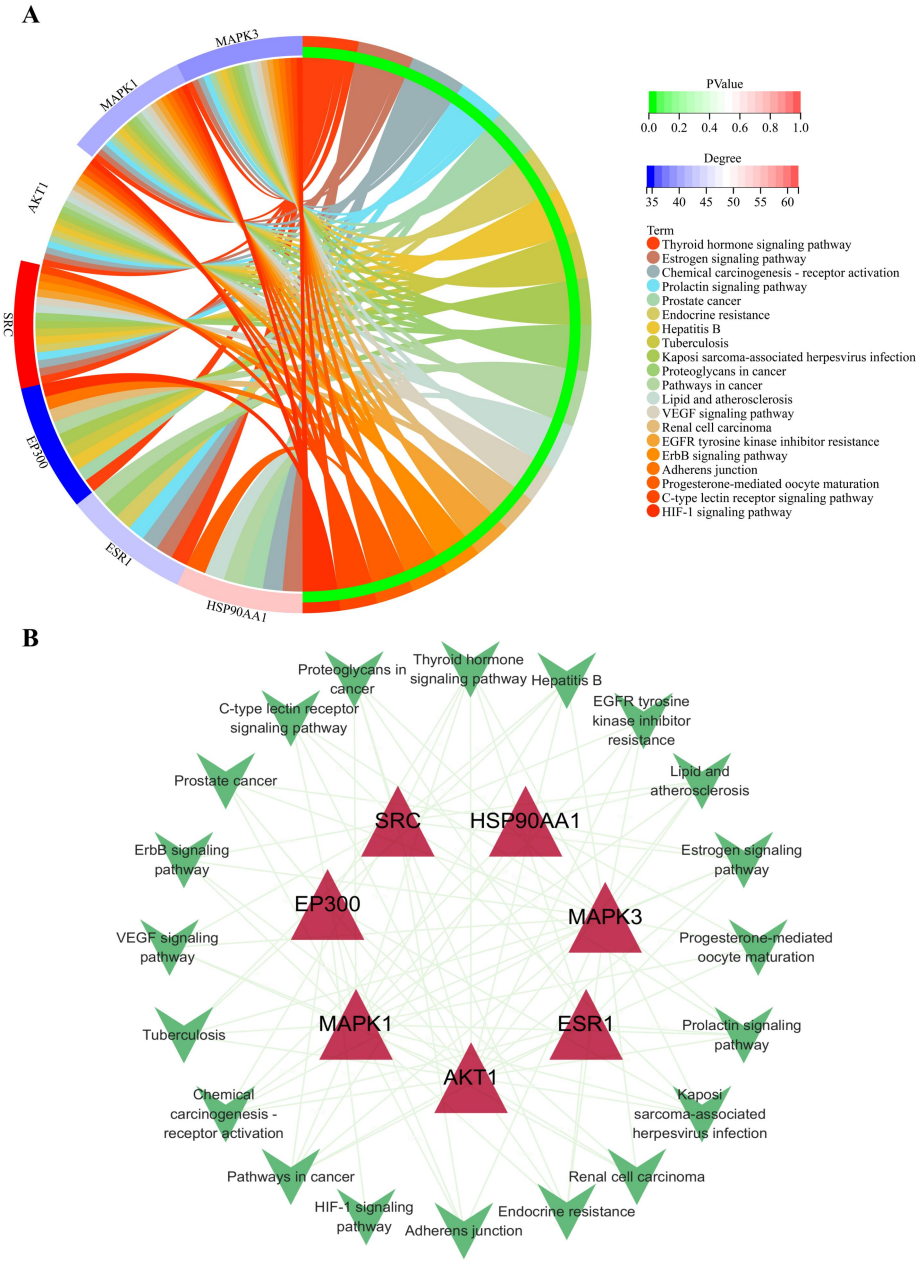

Figure 6 KEGG enrichment analysis and 'target-pathway' diagram. (A) Visualization of KEGG top 20 enriched pathways. (B) Visualization of "target-pathway" diagram of *Carthami Flos* treatment for liver cirrhosis. Green represents pathways, red represents core targets.

Molecular docking and visualization

Molecular docking of 7 core target proteins with 14 ingredient molecules was performed using AutoDock Vina 1.5.7, selecting 6 pairs with binding energies lower than −9.5 Kcal/mol. These pairs are: HSP90AA1-Kaempferol, HSP90AA1-Luteolin, HSP90AA1-Quercetin, SRC-Kaempferol, SRC-Luteolin, and SRC-Quercetin. Corresponding binding energy data was presented as a heat map in (Figure 7), and Visualization of the molecular docking results is shown in (Figure 8).

Discussion

Liver cirrhosis is a chronic condition typically resulting from prolonged liver damage due to causes such as viral hepatitis, alcoholic liver disease, or fatty liver disease [46]. Characterized by structural changes and functional decline, current treatments for cirrhosis primarily aim to manage the underlying cause, delay disease progression, and alleviate symptoms [47].

Network pharmacology is an interdisciplinary field that merges systems biology, information networking, and computer science to use simulations for screening drug molecules and disease targets. It employs high-throughput screening, target interaction analysis, visualization, and network analysis to uncover the intricate connections among drugs, targets, and diseases, thus predicting drug mechanisms of action [48].

Traditional Chinese medicine is characterized by its multi-component, multi-target, and multi-pathway approaches to treat diseases [49]. *Carthami Flos* is extensively utilized in traditional Chinese medicine to enhance blood circulation and alleviate stasis [50]. In recent years, increasing research has focused on *Carthami Flos*'s potential role in treating cirrhosis.

This study marks the first analysis of *Carthami Flos*'s active ingredients in treating liver cirrhosis through network pharmacology. Fourteen effective drug molecules, including quercetin, lignan, kaempferol, and luteolin, were identified. Quercetin, in particular, exhibits notable anti-inflammatory and immunosuppressive properties [51].

The results indicated 237 potential therapeutic targets for these 14 active ingredients in cirrhosis treatment. Core targets included SRC,

Pharmacology Discovery 2024;4(4):20. https://doi.org/10.53388/PD202404020

HSP90AA1, AKT1, ESR1, MAPK1, MAPK3, and EP300. KEGG enrichment analysis revealed that the overlapping targets between quercetin and cirrhosis primarily involved the HIF-1, PI3K-AKT, and MAPK signaling pathways. Integrating PPI network and KEGG pathway analyses showed that SRC and HSP90AA1 were central in multiple pathways, including those associated with viral hepatitis B, HIF-1, and PI3K-AKT, all pivotal to the progression of cirrhosis. Molecular docking demonstrated quercetin's strong binding affinity to SRC and HSP90AA1, underscoring their crucial roles in *Carthami Flos*-based cirrhosis treatment.

SRC is key to the activation and transformation of hepatic stellate cells (HSCs) into myofibroblasts [52]. By modulating SRC activity, one can effectively inhibit HSC activation and reduce collagen synthesis, thereby alleviating liver fibrosis [53]. Additionally, SRC regulates signal transduction in inflammatory cells and, by controlling the release of pro-inflammatory factors, may help reduce liver inflammation, a crucial factor in cirrhosis treatment [54]. SRC also influences liver regeneration by regulating the proliferation and migration of liver stem cells [55].

Similarly, HSP90AA1 (heat shock protein 90α1), a critical molecular chaperone, plays a vital role in protein folding, stabilization, and degradation within cells [56]. It is instrumental in the development and progression of cirrhosis, maintaining liver cell function by aiding the proper folding and stabilization of essential signaling proteins, such as cyclins and transcription factors. Research indicates that protein misfolding, which increases in cirrhosis, can lead to cell damage or death. HSP90AA1 assists in correct protein structuring, thereby minimizing the formation of insoluble fibers or aggregates that result from misfolding [57].

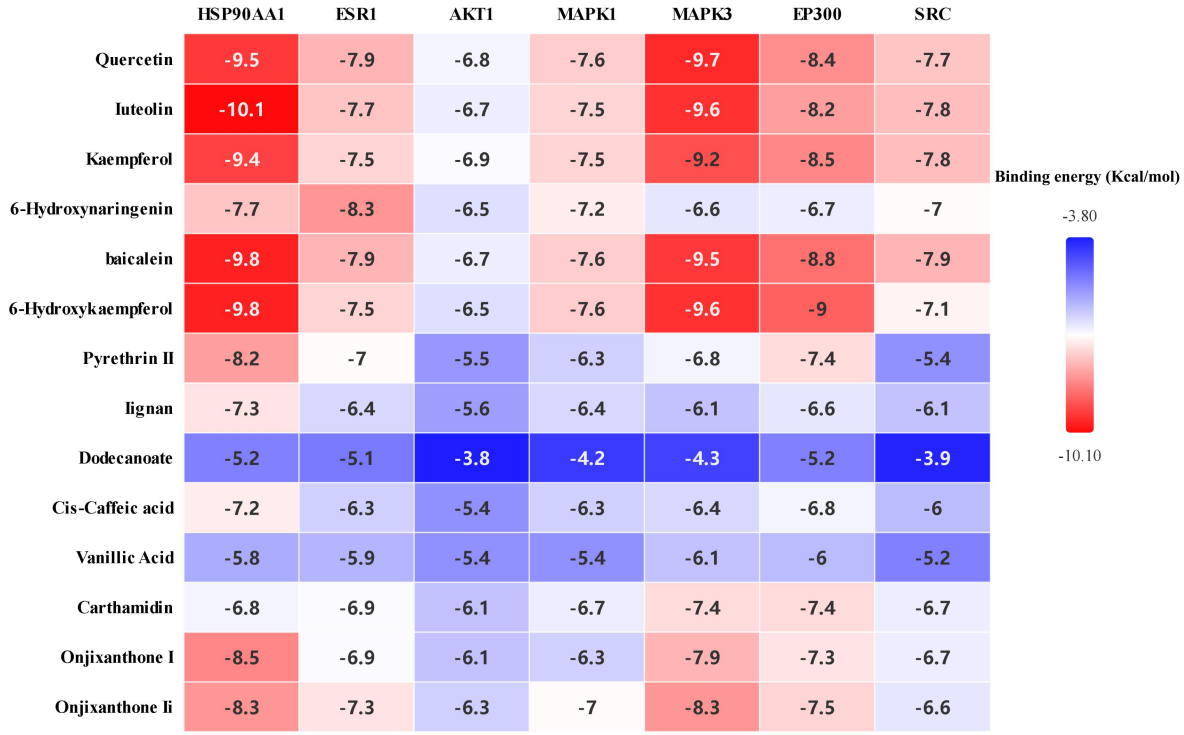

Figure 7 Heat map of binding energy between active ingredients and cirrhosis core targets. The heat map of binding energy between 14 active ingredients of *Carthami Flos* and 7 core targets showed that the smaller the value, the more stable the binding, and the darker the color, the stronger the affinity.

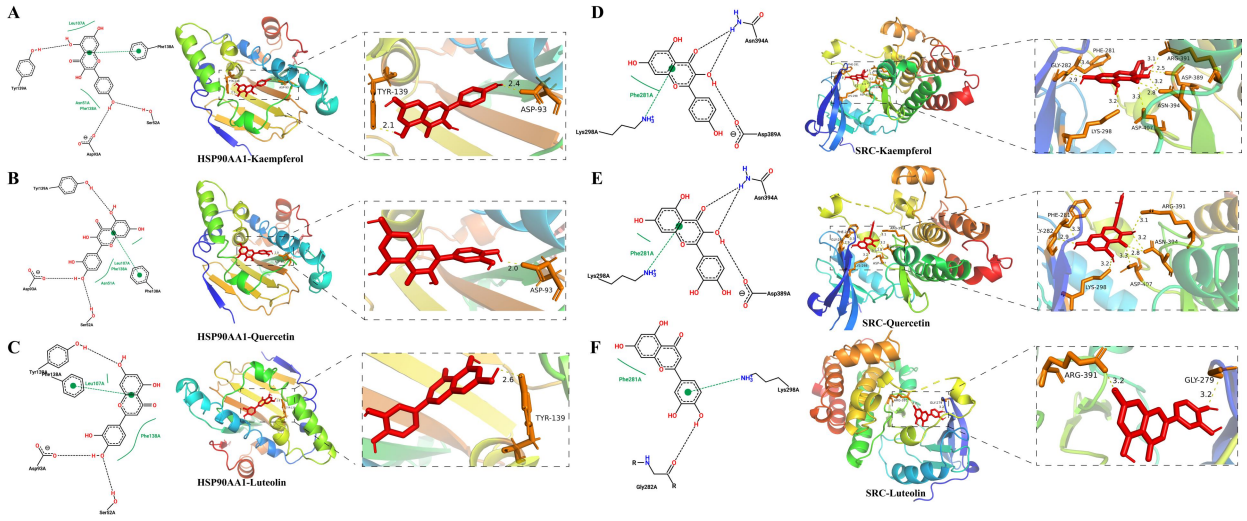

Figure 8 Molecular docking and visualization of *Carthami Flos* active ingredients-core targets. (A–F) HSP90AA1-Kaempferol, HSP90AA1-Quercetin, HSP90AA1-Luteolin, SRC-Kaempferol, SRC-Quercetin and SRC-Luteolin.

During the initial stage of liver injury, inflammation and fibrosis can induce hepatocyte stress. SRC, by binding to PYK2 (a non-receptor tyrosine protein kinase), promotes cellular responses to stress and injury through tyrosine phosphorylation of GRB2 (growth factor receptor-binding protein 2) [58]. This action mitigates apoptosis, enhances hepatocyte proliferation and regeneration, and helps alleviate cirrhosis. SRC also interacts with various cell signaling pathways, including the Wnt/β-catenin and MAPK pathways [59, 60]. Normally, β-catenin is degraded in a complex that includes proteins like GSK-3β [61]. Activation of SRC can inhibit this degradation by reducing GSK-3β activity, allowing β-catenin to accumulate in cells. This accumulation regulates genes associated with the cell cycle and proliferation, such as c-Myc and Cyclin D1, and promotes cell proliferation [62]. This ability to integrate signals provides a complex response mechanism in cirrhosis and enhances liver repair.

Despite its insights, this study has limitations, such as a lack of substantial experimental evidence. Nevertheless, it identifies potential components, targets, and signaling pathways of *Carthami Flos* in treating cirrhosis through network pharmacology and molecular docking, offering a theoretical basis for further experimental validation.

Conclusion

This study, utilizing network pharmacology and molecular docking technology, is the first to demonstrate that quercetin, an active component of *Carthami Flos*, exhibits multi-target and multi-pathway characteristics in the treatment of cirrhosis. It investigates the therapeutic effects of safflower on liver cirrhosis at the molecular level, systematically elucidating the potential mechanisms and therapeutic targets. This research provides a novel approach for exploring the pharmacological mechanisms of *Carthami Flos* in cirrhosis treatment and establishes a theoretical foundation for future drug development against this disease.

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