Journal of Polyphenols
2025, 7 (3): 127–136
journal homepage: http://jpolyph.magtechjournal.com

Network pharmacology and molecular docking were used to study the mechanism of flavonoid in the treatment of alcoholic liver

Yufei Wang, Ning Wang, Kexin Huang, Xiaoshu Zhang *

Faculty of Functional Food and Wine, Shenyang Pharmaceutical University, Shenyang 110016, China

Abstract The aim of this project is to explore the mechanism of the treatment of alcoholic liver by means of network pharmacology and molecular docking. In this study, the chemical components of the extract of *Callistephus chinensis* (L.) Nees. were characterized by LC-MS/MS, identifying 6 compounds by positive and negative total ion flow maps. The target of chrysanthemum was derived from SwissTargetPrediction database, and the target related to alcoholic liver was derived from GeneCards and OMIM database. Add the target to the String database to build the protein interaction platform Microbiology software was used for GO bioprocess enrichment analysis and KEGG pathway enrichment analysis, and the target pathway network was constructed. In Discovery Studio 2016 Client software verified molecular docking, China aster flavonoids compounds and the adhesion strength of the key targets. Five potential active components were screened from the flavonoid monomer compounds of the chrysanthemum Cupressus. 546 Bioprocess (BP), 75 cell composition (CC) and 185·molecular function (MF) were obtained by GO enrichment analysis. KEGG enrichment analysis for each cross target involved a pathway. Network analysis showed that it was the main active component of flavonoids in the treatment of alcoholic liver, and other related signals were related to the treatment of alcoholic liver. This study showed that the flavonoids of *Callistephus chinensis* (L.) Nees were involved in the treatment of alcoholic liver by regulating multi-target and multi-pathway.

Keywords: *Callistephus chinensis* (L.) Nees; alcoholic liver disease; flavonoid compounds; network pharmacology; molecular docking

1 Introduction

Alcoholic liver disease (ALD) is a liver disease caused by continued drinking, every year there are up to 50% of the global cirrhosis death is due to alcohol intake. Alcoholic liver disease can be divided into the relatively mild and reversible fattyliver and alcoholichepatitis, and the more serious fibrosis and cirrhosis [1]. Human liver is the main place for alcohol metabolism. Alcohol can be converted into acetic acid under the catalysis of two corresponding dehydrogenase enzymes in the liver, and acetic acid further reacts to produce carbon dioxide and water. Long-term heavy drinking will lead to the intake of alcohol exceeding the metabolic capacity of the body, thus causing alcohol and its metabolite acetaldehyde to accumulate in the body [2]. Studies have shown that ethanol is metabolized by the liver to

* Corresponding author: Xiaoshu Zhang (xiaoshu2397@163.com).
These authors have no conflict of interest to declare.

produce acetaldehyde, then oxidized by acetaldehyde dehydrogenase to produce acetic acid, and finally oxidized to water and carbon dioxide. However, the conversion rate of acetaldehyde into acetic acid is very slow, resulting in acetaldehyde accumulation in the body, thus destroying the Redox balance and causing oxidative stress injury of the liver. Current studies believe that the pathogenesis of alcoholic liver disease is relatively complex, and its occurrence and development are related to many factors, including ethanol and lipid metabolism, inflammatory response, oxidative stress, etc [3]. These metabolic and immune abnormalities can lead to necrosis and apoptosis of liver cells, leading to liver damage, and ultimately lead to liver disease. It is precisely because of its very complex pathological mechanism, there is still no specific drug and treatment plan.

Callistephus chinensis (L.) Nees(CA) is a plant in the Asteraceae family, and chrysanthemum is a common flower used in both medicine and food, with the effects of clearing heat, brightening eyes, dissipating wind and detoxifying [4]. It is widely distributed in Jilin, Liaoning, Hebei, Shanxi, Shandong, Yunnan and Sichuan. Chrysanthemum flowers can be used as medicine, taste slightly bitter, flat [5]. According to classical records, China has cultivated chrysanthemums for more than 3,000 years. The Han Dynasty "Shennong Materia Medica" recorded: "chrysanthemums for a long time can be lightly worn to prolong life". Modern pharmacological studies have shown that chrysanthemum has antibacterial [6], antiviral antioxidant [7], anti-inflammatory [8] and liver protection effects [9], so it is often taken as a health drink. It was found that chrysanthemum extract can inhibit oxidative stress and apoptosis of liver cells caused by alcohol, and inhibit liver inflammation. In addition, chrysanthemum can also improve steatosis, which significantly improves liver histopathological changes, thus playing a role in protecting the liver. Wang Baowei [10] et al. found that wild chrysanthemum flower flavonoids can reduce TNF-α in the course of alcoholic fatty liver disease and play an anti-inflammatory role.

At present, the mechanism of action of flavonoid in CA in treating alcoholic liver injury is not clear. In this paper, six kinds of flavonoids were obtained by LC-MS analysis: Rotenone, Eriodictyol, Kaempferol, Quercetin, Apigenin, Catechin, etc. Based on the characteristics of multiple components and multiple targets, network pharmacology analyzed and elaborated the mechanism of action of the CA which is conducive to promoting the study of the mechanism of action of the CA. In order to further explore the mechanism of flavonoids in CA in the treatment of ALD, the target and pathway of flavonoids in CA in the treatment of ALD were predicted by network pharmacology, and the mechanism of action of CA in the treatment of ALD was further explored by molecular docking technology.

2 Experimental materials and methods

2.1 Extraction and LC-MS analysis of Callistephus chinensis (L.) Nees

2.1.1 Experimental instruments

Rotary evaporator (Zhengzhou Great Wall Science, Industry and Trade Co); Circulating Water Vacuum Pumps (Zhengzhou Great Wall Science, Industry and Trade Co); Electronic Analytical Balance (Sartorius, Germany); Electrothermal constant temperature water bath (Beijing Changfeng Instrumentation Company); CNC Ultrasonic Cleaner (Kunshan Ultrasonic Instrument Co); Electrothermal blast thermostatic drying oven (Shaoxing Supe Instrument Co); Triple UV Analyser (Shanghai LiChen Bang Xi Instrument Technology Co); High Performance Liquid Chromatograph (Beijing Innovation Tongheng Technology Co).

2.1.2 Experimental materials

Drugs and reagents: Ethanol, petroleum ether, dichloromethane, *n*-butanol, ethyl acetate, methanol, acetone, formic acid, sulphuric acid are all analytically pure. Tianjin Lianlong Bohua Pharmaceutical & Chemical Co; Deuterium reagent: DMSO-D6,

CD3OD. Cambridge Isotope Laboratories, Inc.USA.

Sulfuric acid ethanol chromogenic agent: acid: ethanol = 1:9 preparation.

Thin-layer plate: mix thin-layer chromatography silica gel GF254 with 0.7% CMC-Na solution (1:4), grind it evenly, spread it uniformly on a clean glass plate, dry it naturally for 24 h, and collect it in a desiccator for spare.

2.1.3 LC-MS/MS analysis

Extraction of total flavonoids from *Callistephus chinensis* (L.) Nees The material was processed into 100 mesh particle size, extracted with 70% ethanol, solid-liquid ratio of 1:25, ultrasonic extraction 25 min, extraction temperature 25 ℃.

The total extracts of SDL were analyzed by full scanning under positive and negative ion modes. Parameters for liquid chromatography: Agilent 1260 HPLC system equipped with Agilent ZORBAX bsC_{18} column, column temperature set at 30 ℃. The temperature of the automatic sampler was set at 4 ℃, the sample volume was set at 1 μL, and the flow rate was set at 1 mL/min. The mobile phase consisted of 0.1% phosphoric water and methanol. Gradient conditions: 0−5 min, 2% methanol; 5−10 min, 2%−5% methanol; 10−40 min, 5%−45% methanol; 40−60 min, 45% methanol.

Parameters used for mass spectrum: Agilent 6 530 QTOF/MS mass spectrometer, positive and negative ion modes using electrospray ionization (ESI). The MS scanning range is set to 100−500 Da, and the MS/MS scanning range is set to 50−500 Da. The capillary voltage was set at 3.5 kV, the dry gas flow was set at 9 mL/min, and the temperature was set at 350 ℃. The atomizer gas pressure is set at 45 psi. Sheath gas flow and temperature were maintained at 12 mL/min and 400 ℃, respectively. Nitrogen is used as an auxiliary gas as well as an atomizing gas. The fragmentation voltage and collision energy (CE) were set at 150 V and 40 V, respectively. Agilent MassHunter workstation was used for data acquisition (version B.05.01). Data were processed using Agilent MassHunter qualitative analysis (version B.06.00).

2.2 Network pharmacology research

2.2.1 Screening of the active ingredients and their related targets of Callistephus chinensis (L.) Nees

The LC-MS/MS analyzed components of the extracts of CA were searched in the PubChem (https://pubchem.ncbi.nlm.nih.gov/) database, and the corresponding Isomeric SMILES numbers were queried according to the names of the components, which were imported into Swiss ADME (http://www.swissadme.ch), and one “High” and two “Yes” were set as the screening conditions to select the active components; Then the active ingredients were imported into the Swiss Target Prediction (http://swisstargetprediction.ch/) database, and the species was limited to “Homo sapiens”, and the official gene name of the target was obtained, and the preliminary screening condition was set as “Probability > 0”; the compound-target network diagram was constructed by using Cytoscape 3.9.0 software.

2.2.2 Screening of targets for alcoholic liver

In the GeneCards database (https://www.genecards.org/), the OMIM database (https://omim.org/) and TTD database (http://bidd.nus.edu.sg/group/cjttd), “alcoholic liver” is set as the keyword to search for related target. After the name of the target is obtained, Excel spreadsheets are established to eliminate repetition, and it is used to build a library of disease targets.

2.2.3 Acquisition of drug-disease common targets and construction of PPI networks

In the Venny 2.1 online platform(https://www.liuxiaoyuyuan.cn) the CA compound targets and disease targets are entered for Veen mapping and the intersection of the resulting active ingredient targets and disease targets are the key targets. The common targets obtained from the analysis were imported into

the String database (https://string-db.org/) to construct a target network map with "human" as the species, and the constructed PPI was imported into the software Cytoscape 3.9.0 software to screen the *Callistephus chinensis* (L.) *Nees* core targets.

2.2.4 GO biofunctional and analysis and KEGG pathway enrichment analysis

The common targets were analyzed using the David database (http://www.david.ncifcrf.gov) andvisualized using the online mapping platform Microbiology (http://www.bioinformatics.com.cn/).

2.2.5 Key active ingredient prediction

Cytoscape 3.9.0 software was used to predict the key active ingredients.

2.3 Molecular docking

Search for protein structure from PDB data (https://www.rcsb.org/), download 3D structure *PDB Format of PPARG, AKT1, HIF1, BCL-2, ESR1 modify the structure of protein and compound with Discovery Studio 2016 Client software.

3 Results

3.1 Structural identification

After screening and target prediction, 6 activeingredients were obtained. According to molecular weight, retention time (t_R), fragment ions, chemical class and name of each compound (Table 1), their identities were finally determined. The LC-MS/MS positive and negative ion chromatogram of CA was shown in Figs. 1-2.

Table 1 The potential active ingredient of the *Callistephus chinensis* (L.) Nees

No.	t_R/min	Precursor Ion $[M+H]^+$ (m/z)	Theoretical mass (m/z)	Elemental composition	Name of the compound
1	7.229	395.0946	394.4230	$C_{23}H_{22}O_6$	Rotenone
2	9.585	289.0652	288.0634	$C_{15}H_{12}O_6$	Eriodictyol
3	9.745	287.1243	286.0477	$C_{15}H_{10}O_6$	Kaempferol
4	10.174	303.0498	302.0427	$C_{15}H_{10}O_7$	Quercetin
5	13.590	293.2117	270.2370	$C_{15}H_{10}O_5$	Apigenin
6	14.296	313.2740	290.2700	$C_{15}H_{14}O_6$	Catechin

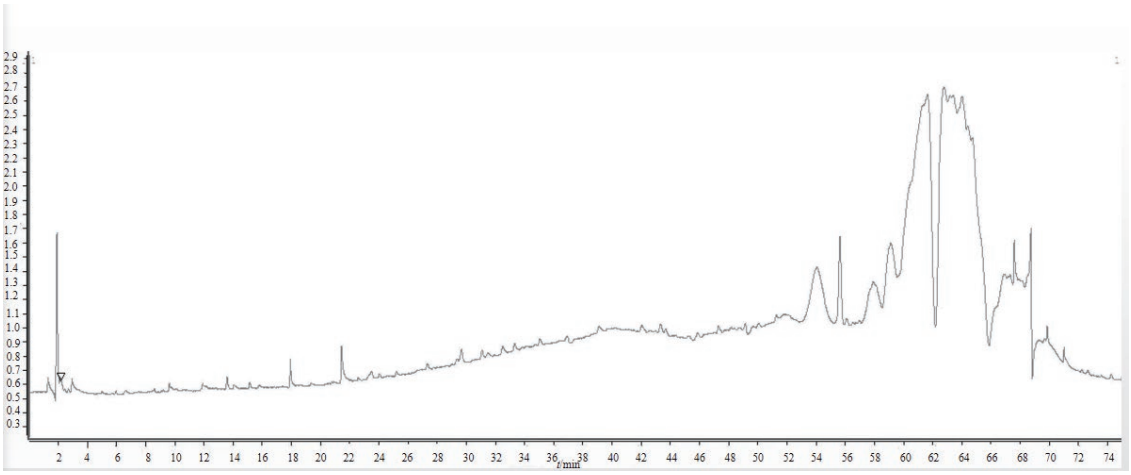

Fig. 1 Total ion chromatogram of positive ion LC-MS/MS of CA extract

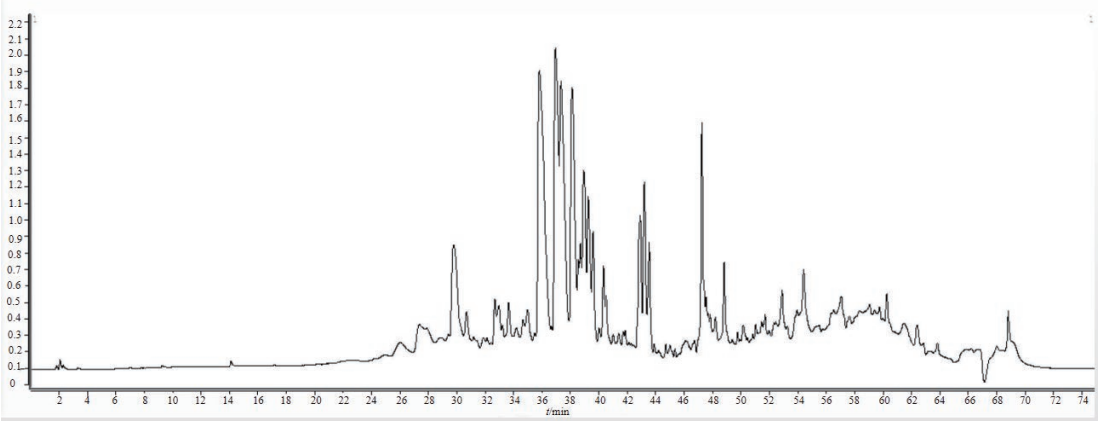

Fig. 2 Total ion chromatogram of negative ion LC-MS/MS of CA extract

3.2 Prediction of target, network and pathway of alcoholic liver in Callistephus chinensis (L.) Nees

3.2.1 Sreening of drug and disease targets of Callistephus chinensis (L.) Nees

The six compounds isolated from *Callistephus chinensis* (L.) Nees involucrata should be screened in the Swiss Target Prediction database. In order to better demonstrate the relationship between active components and targets, Cytoscape 3.9.0 software was used to construct the compound-target network diagram of *Callistephus chinensis* (L.) Nees involucrate. (Fig. 3).

The target targets were screened by GeneCards and OMIM databases, and a total of 2416 alcoholic liver disease targets were obtained after deleting duplicates. By importing the Venn diagram of Venny 2.1.0 online tool together with 267 active ingredient targets for comparison, 114 core gene targets were obtained. (Fig. 4).

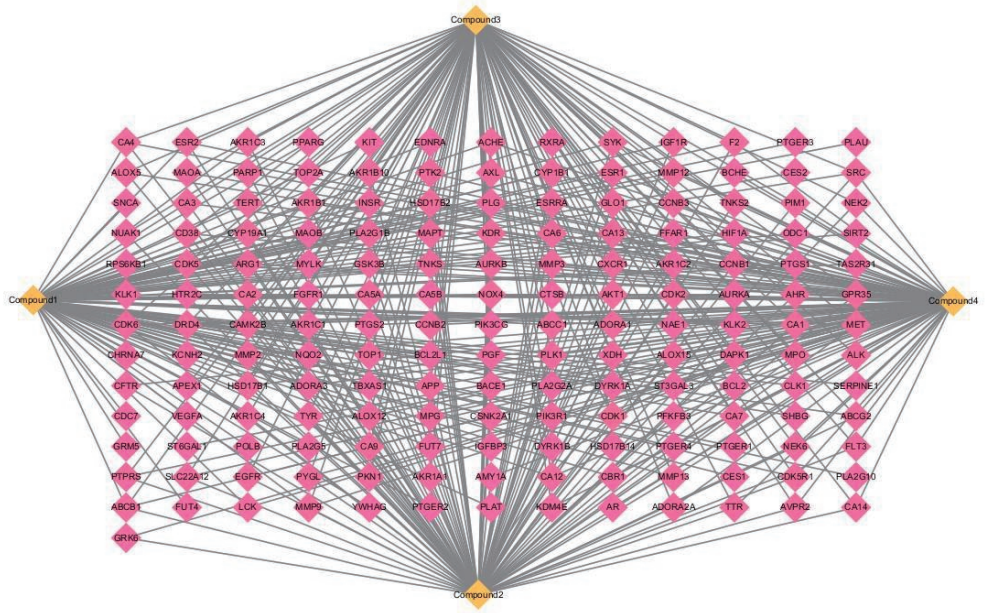

Fig. 3 "Compounds-targets" network

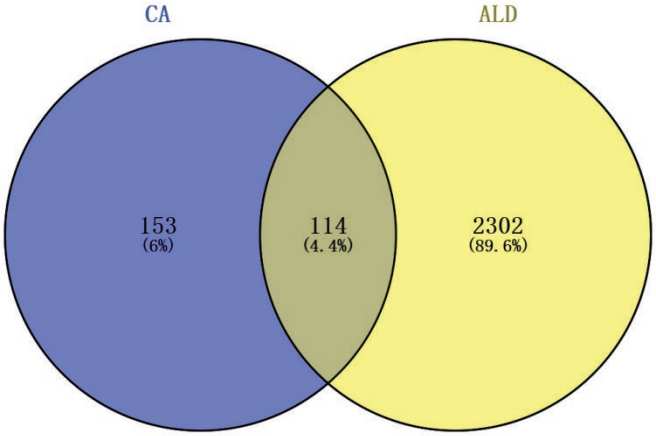

Fig. 4 "Disease-compound" target Venn diagram

3.2.2 PPI network construction

The information of compounds and corresponding targets were imported into the String database to map the interaction network. The core targets were saved and imported into Cytoscape 3.9.0, and the protein-protein interaction network of the core proteins was drawn, as shown in Fig. 5. The greater the number of targets connected to each target, the larger the node area and the darker the color, the more important the target is in the protein interaction network.

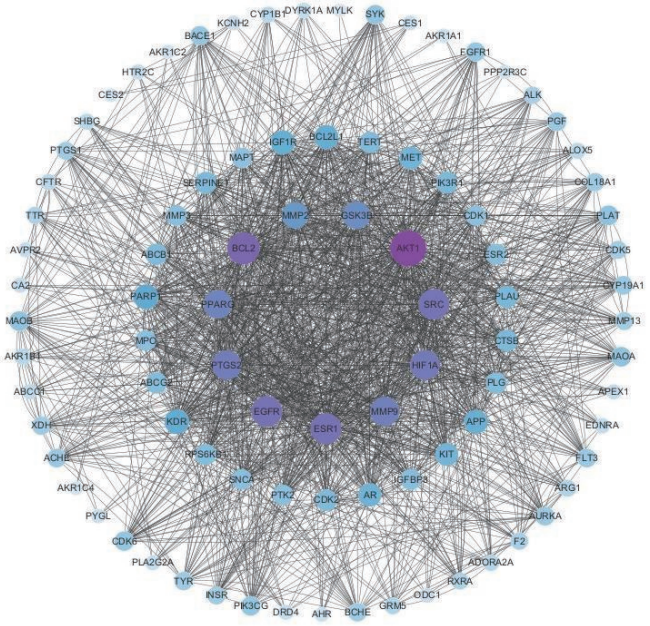

Fig. 5 PPI network

3.2.3 GO biofunctional and analysis and KEGG pathway enrichment analysis

GO functional analysis was performed on 114 key targets through the DAVID database, and a total of 986 GO entries were obtained, of which, 546 entries were covered for biological processes, including cellular response to mitrogen compound, positive regulation of cell migration, positive regulation of cell motility, positive regulation of locomotion, cellular response to organonitrogen compound, etc.; 75 entries for cellular composition, including receptor complex, cytoplasmic vesicle lumen, dendrite, dendritic tree; In terms of molecular function, there are 185 entries,

including protein kinase activity, growth factor binding, kinase activity, phosphotransferase activity, alcohol group as acceptor, protein serine kinase activity. The results are shown in Fig. 6.

KEGG enrichment analysis yielded a total of 145 pathways involving PI3K-Akt signaling pathway, Endocrine resistance, EGFR tyrosine kinase inhibitor resistance, Prostate cancer, Proteoglycans in cancer etc. We selected the top 10 GOs and the top 20 pathways with small *P*-values for visualization and analysis, and the results are shown in Fig. 7.

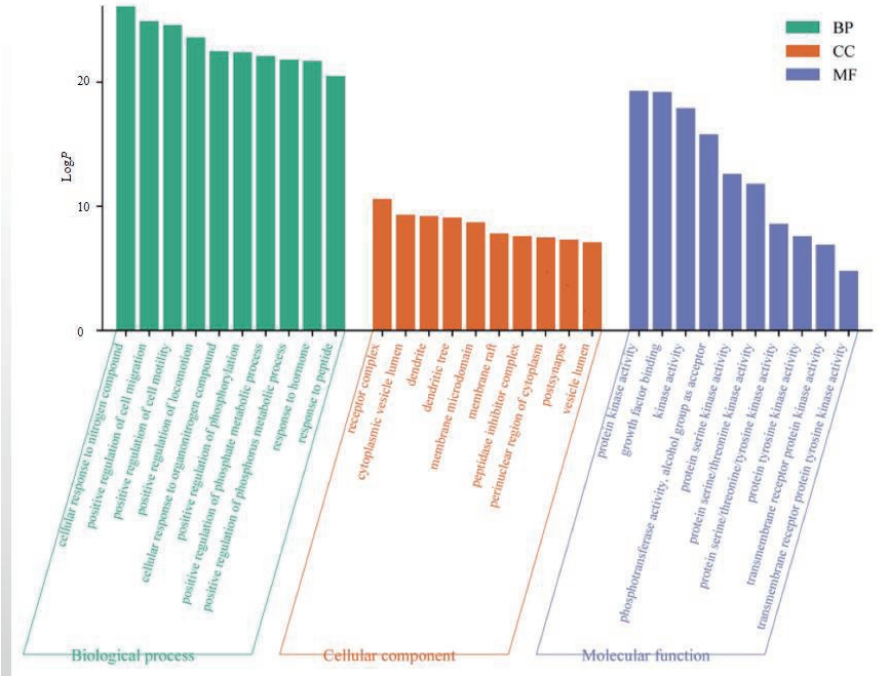

Fig. 6 GO functional enrichment analysis diagram

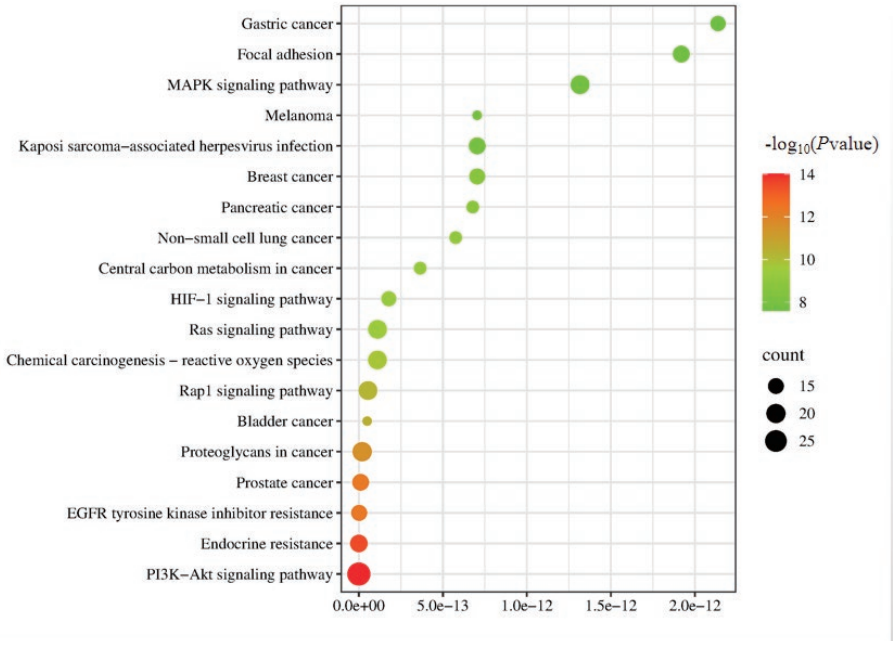

Fig. 7 KEGG pathway enrichment analysis bubble chart

3.3 Molecular docking results

In order to further clarify the binding effect between the key targets and active ingredients of *Callistephus chinensis* (L.) Nees in the treatment of diabetes, the top 4 components of the "disease pathway-targeted drug" regulatory network medium were selected in the PPI network analysis, and molecular docking was performed with the core targets of the top 4 degrees. It is generally believed that the higher the LibDock score, the richer the form of force, the stronger the binding ability. In this study, 4 target proteins were docked with 4 active component molecules, and the results showed that compounds had relatively good interaction with BCL2 and Quercetin with AKT1. The binding mode of the target proteins to component docking is shown in Figs. 8-9.

Table 2 Interaction between the active substance and the target protein

Entry	Protein	PDB ID	LibDock score	Interaction
Apigenin	PPARG	9CK0	107.35	Carbon Hydrogen Bond; Unfavorable Donor-Donor; Unforvable Acceptor-Acceptor; Pi-Signa; Pi-Pi Stacked; Pi-Alkyl
	AKT1	7FCV	123.42	Attractive Charge; Conventional Bydrogen Bond; Carbon Hydrogen Bond; Unfavorable Negative-Negative; Pi-Alkyl
	HIF1	4NQ0	125.32	Attractive Charge; Conventional Bydrogen Bend; Carbon Hydrogen Bond; Unforvable Donor-Donor; Pi-Donor Hydrogen Bond; Pi-Slgna; Pi-Pi Stacked; Pi-Alkyl
	BCL-2	4HW3	107.12	Unfavorable Bump; Attractive Charge; Conventional Hydrogen Bond; Pi-Cation; Pi-Anion; Pi-Signa; Pi-Pi T-shaped; Pi-Alkyl
Eriodictyol	PPARG	9CK0	105.34	Conventional Hydrogen Bond; Carbon Hydrogen Bond; Pi-Cation; Pi-Alkyl
	AKT1	7FCV	114.65	Conventional Hydrogen Bond; Pi-Pi Stacked; Pi-Alkyl
	ESR1	6PSJ	120.35	Conventional Hydrogen Bond; Pi-Sulfur; Pi-Pi T-shaped; Pi-Alkyl
	BCL-2	4HW3	104.74	Unforvable Bump; Conventional Hydrogen Bond; Carbon Hydrogen Bond; Pi-Pi T-shaped; Pi-Alkyl

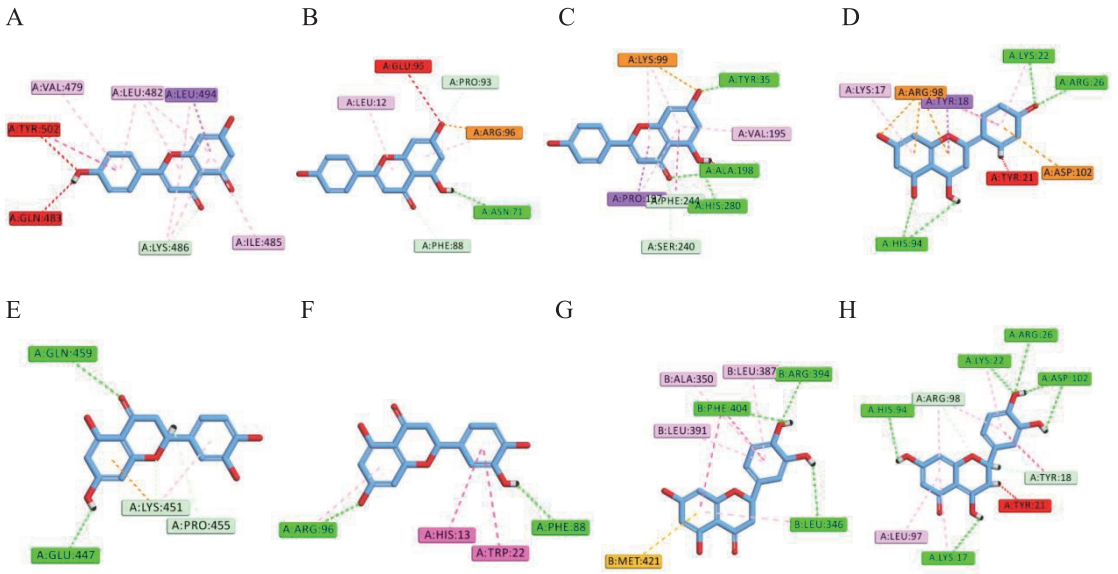

Fig. 8 2D model diagram of the docking between CA components and target proteins

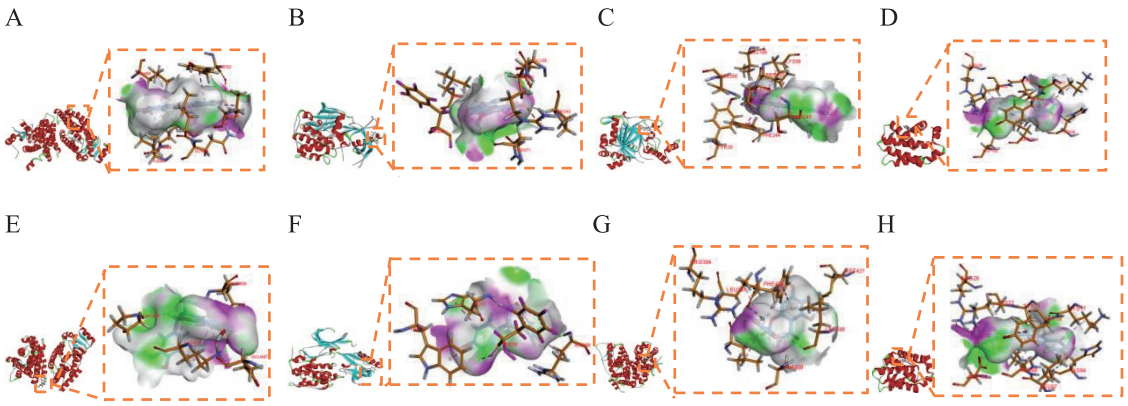

Fig. 9 CA of molecular docking between key compounds and core target proteins

4 Discussion

Studies have shown that lipid metabolism, oxidative stress and inflammatory response are important pathogenesis of ALD [11].

6 compounds were identified by LC-MS/MS and analyzed the mechanism of the effects of CA extract on ALD by network pharmacology and molecular docking techniques. The results of the analysis using Cytoscape showed that the active compounds with the highest number of flavonoid compound targets in CA were Apigenin and Eriodictyol. Wang et al. [12] found that apigenin can regulate the expression of lipid metabolism-related proteins PPARα, SREBP-1c and Fasn, reduce excessive lipid accumulation in mouse liver, and regulate liver lipid metabolism. Wang [13] et al found that Eriodictyol can inhibit MAPK signaling pathway, and then activate Nrf2/HO-1 signaling pathway to play the role of antioxidant stress, so as to alleviate non-alcoholic fatty liver disease. The above results indicate that the screening of this study has a certain theoretical basis, and provides a research direction for the treatment of alcoholic liver disease mellitus with CA. The results of molecular intercalation showed that the active components of flavonoids of CA were well bound to the core target proteins, among which the active compound Apigenin was the best bound to PPARG, AKT1, HIF1A, BCL-2; Eriodictyol was the best bound to PPARG, AKT1, ESR1, BCL-2. In summary, active ingredients can achieve the effect of treating ADL by modulating key targets.

GO analysis indicated that ALD related biological processes were mainly cellular response to nitrogen compound, positive regulation of cell migration, positive regulation of cell motility and positive regulation of locomotion. KEGG pathways were further confirmed in present research, mainly including Proteoglycans in cancer, Transcripitional misregulation in cancer, Prostate in cancer, Phospolipase D signaling way, Calcium signaling way, NF-κB signaling pathway, P_{53} signaling pathway, IL-17 signaling pathway. The Phospolipase D induces the release of the inflammasome-dependent cytokines IL1b and IL-18, and the induction of cell death independently of the pore-forming proteins gasdermin D, MLKL and the cell death effector protein ninjurin-1 or NINJ1. The NF-κB is an important transcription factor that regulates inflammatory response. It inhibits the phosphorylation and degradation of protein IκB under oxidative stress, which leads to the release of NF-κB into the nucleus, thereby regulating the synthesis of TNF-α and IL-6 and other inflammatory factors, causing inflammation and injury in the body [14]. The IL-23 can promote the proliferation and differentiation of helper T cell 17 and induce its production of IL-17, which can promote the production of various inflammatory factors of neutrophils and aggravate the inflammatory response of the body. Meanwhile, IL-17 can also promote the production of IL-6 and tumor necrosis factor-α in hepatocytes, promote the production of reactive oxygen species and aggravate abnormal oxidation of lipids. It also reduces insulin sensitivity [15]. The results

indicate that CA can achieve therapeutic effect on ALD through multi-component mult-target and multi-pathway.

At present, the clinical research of flavonoids in the prevention and treatment of ALD is still in its infancy, and the specific mechanism of related compounds affecting ALD, and the safety and long-term effectiveness of its clinical application need to be further studied.

5 Conclusion

This study was conducted to identify the components of CA by LC-MS/MS analysis to determine their key active compositions and a network pharmacology approach was used to initially explore the active components, core targets and signaling pathways of CA for the treatment of diabetes, and further validate the active components and core targets by molecular docking. The results provide the basis for further experiments on the treatment of alcohol liver disease with the active components such as the flavonoids of CA. In addition, ideas and methods for the research on the pharmacological substances of other components are provided.

References

[1] Li SQ, Wang YR, Xie ZL, et al. NLRP3 activation maintains intestinal epithelial barrier and reduces liver injury in alcoholic liver disease mice [J]. Clin Transl Med, 2024, 14 (12): 70099.

[2] Gao B, Xu MJ, Bertola A, et al. Animal Models of Alcoholic Liver Disease: Pathogenesis and Clinical Relevance [J]. Gene Expr, 2017, 17 (3): 173-186.

[3] Mandrekar P, Mandal A. Pathogenesis of Alcohol-Associated Liver Disease [J]. Clin Liver Dis, 2024, 28 (4): 647-661.

[4] Shi GH, Yang SL, Zhang XS, et al. Simultaneously determined five flavonoids in different parts of Chrysanthemum morifolium using HPLC method [J]. Chin Herb Med, 2015, 46 (3): 428-431.

[5] Liu X. Study on the Preventive and Therapeutic Effects and Mechanisms of Cuiju Polyphenols on Liver Fibrosis [D]. Liaoning Univ.

[6] Yang WS, Kim D, Yi YS, et al. AKT-targeted anti-inflammatory activity of the methanol extract of Chrysanthemum indicum var [J]. J Ethnopharmacol, 2017, 201 (0): 82-90.

[7] Yang L, Cheng P, Wang JH, et al. Analysis of Floral Volatile Components and Antioxidant Activity of Different Varieties of Chrysanthemum morifolium [J]. Molecules, 2017, 22 (10): 1790.

[8] Xue GM, Li XQ, Chen C, et al. Highly Oxidized Guaianolide Sesquiterpenoids with Potential Anti-inflammatory Activity from Chrysanthemum indicum [J]. J Nat Prod, 2018, 81 (2): 378-386.

[9] Kung C, Lu D, Shen G, et al. Chemical composition and antimicrobial activities of volatile oil extracted from Chrysanthemum morifolium Ramat. Sichuan [J]. Int J Food SCI Tech, 2018, 55 (7): 2786-2794.

[10] Wang BW, Li J, Cheng WM, et al. The preventive and therapeutic effects of total flavonoids from wild chrysanthemum on alcoholic fatty liver in rats [J]. Anhui Med J, 2011, 46 (10): 1022-1025.

[11] Xia T, Zhang J, Yao JH, et al. Research progress in mechanism of oxidative stress in alcoholic liver disease [J]. Chin Pharmacol Bull, 2017, 33 (10): 1353-1356.

[12] Wang F, Liu JC, Zhou RJ, et al. Apigenin protects against alcohol-induced liver injury in mice by regulating hepatic CYP2E1-mediated oxidative stress and PPARα-mediated lipogenic gene expression [J]. Chem-bio Interact, 2017, 275 (0): 171-177.

[13] Shen BY, Feng HH, Cheng JQ, et al. Geniposide alleviates non-alcohol fatty liver disease via regulating Nrf2/AMPK/mTOR signalling pathways [J]. J Cell Mol Med, 2020, 24 (9): 5097-5108.

[14] Liu YD, Liu JZ, Ji GX, et al. Exploring the protective effect of Zhizichi decoction on alcoholic liver injury based on the NF - κ B/NLRP3 signaling pathway [J]. J FSQT, 2022, 13 (22): 7446-7453.

[15] Jiang Y, Yan Y, Zhuang L, et al. Serum macrophage migration inhibitory factor transforming growth factor B interleukin 17 and interleukin 23 concentrations are associated with the severity of liver disease in patients with nonalcoholic fatty liver disease [J]. Hepatol Int, 2015, 9 (1): S366-S367.