Exploring the Mechanism of Danggui Shaoyao San in the treatment of Non-alcoholic Fatty Liver Disease: A Study based on Network Pharmacology and Molecular Docking

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Research

Keywords: Danggui Shaoyao San (DSS), Non-alcoholic Fatty Liver Disease (NAFLD), network pharmacology, molecular docking

Posted Date: January 11th, 2022

DOI: https://doi.org/10.21203/rs.3.rs-1216657/v1

Abstract

Background

Non-alcoholic fatty liver disease (NAFLD), the most prevalent chronic liver disease in the world, has yet to identify a particular medicine for treatment. Danggui Shaoyao San (DSS), a traditional Chinese medicine formula, has steadily been employed to treat NAFLD in recent years.

Methods

The active ingredients of the DSS were screened from the Traditional Chinese Medicine Systems Pharmacology (TCMSP), and the candidate targets of the ingredients were collected through the PharmMapper platform. NAFLD-related targets were acquired from NCBI, DisGeNet, Genecards databases. Venn diagram was used to identify possible DSS drug strategies in the treatment of NAFLD. Active ingredients - potential therapeutic targets network constructed in Cytoscape. The STRING database provides PPI networks. Metascape was used to evaluate targets using Gene Ontology (GO) and the Kyoto Encyclopedia of Genes and Genomes (KEGG). Finally, molecular docking simulations were performed using Pymol 2.4.0, AutoDuck Tools 1.5.6 and LigPlot 2.2.4 software to assess the affinity of key ingredients and targets.

Results

51 compounds were screened in the DSS, including paeoniflorin, poric acid A and poric acid B. There are a total of 38 cross-targets between herbs and NAFLD. PPI network analysis identified AKT1, ALB, PPARG, and CASP3 as priority targets. GO analysis focused on vesicle lumen, nutrition levels, and nitrous-oxide synthase regulator activity. DSS may have therapeutic benefits via the pathways in cancer,foxo signaling pathway,IL-17 signaling pathway, Th17 cell differentiation, PI3K-Akt signaling pathway according to KEGG analysis. Sitosterol and β- sitosterol were proven to be true promising compounds with excellent affinity in the final molecular docking.

Conclusions

DSS entails a number of components, targets, and routes, and it provides novel therapy and preventative alternatives for NAFLD.

1 Introduction

Non-alcoholic fatty liver disease (NAFLD)is a clinical disease marked by diffuse hepatocyte steatosis in the absence of heavy alcohol use and other known liver-damaging factors [1]. Non-alcoholic fatty liver (NAFL) and non-alcoholic steatohepatitis (NASH) are two types of the illness, and NASH can lead to liver cirrhosis and hepatocellular carcinoma [2]. NAFLD affects one-quarter of the adult population in Europe and the United States [3]. In China, the total prevalence of NAFLD has risen to 29.6% in the last 20 years [4]. NAFLD is a major cause of end-stage liver disease (cirrhosis and hepatocellular carcinoma) that has a significant economic and health impact across the world. However, due to the disease's complicated pathophysiology and wide individual variances [4], there is currently no particular medicine for therapy. The quest for effective NAFLD treatment and preventive medications

has become a pressing issue. Traditional Chinese medicine (TCM) natural medications have showed considerable potential in both research and clinical application.

Danggui Shaoyao San (DSS) is a formula from *the Golden Chamber Synopisis*. The ancient book is one of the four classics of TCM, written by Zhongjing Zhang during the Eastern Han dynasty. DSS consists of six herbs: DangGui [DG, Angelicae Sinensis Radix], FuLing [FL, Poria Cocos(Schw.) Wolf], ChuanXiong [CX, Chuanxiong Rhizoma], BaiZu [BZ,Atractylodes Macrocephala Koidz], ZeXie [ZX, Alisma Orientale (Sam.) Juz], BaiShao [BS, Paeoniae Radix Alba]. DSS is skilled in treating gynecological discomfort in *the Golden Chamber Synopisis*. DSS has been also widely established in the application of clinical disorders in recent years, based on the principle of "Treating the different diseases with the same therapies"in TCM. Alzheimer's disease, nephrotic syndrome, liver cirrhosis ascites, and other disorders have all been proven to benefit from the formula [5–7]. Meanwhile, some studies have shown that treating patients with NAFLD by DSS combined with acupuncture can significantly improve clinical symptoms and serum indexes [8]. Furthermore, it has been demonstrated that DSS may substantially enhance the NAS score of the liver tissue in a NAFLD mice model [9]. DSS has a protective impact on the mechanical barrier of the intestinal mucosa in a rat model of NAFLD [10]. Although some studies support the use of DSS in the treatment of NAFLD, its precise components and mechanism remain unknown.

Network pharmacology is an integrated systemic method that finds numerous active chemicals in a crude drug and anticipates several pharmacological targets, and its holistic examination of the human body and disease, as well as the medication, aligns with the "holistic view" in Chinese medicine's underlying concept [11]. When combined with molecular docking based on network pharmacology, affinity testing of potential compounds and targets can identify key pharmacodynamic components and reveal the pharmacodynamic material basis of complex chemical systems, improving the efficiency and relevance of the TCM screening process [12]. He et al. used network pharmacology and molecular docking to discover the potential pathways through which Liu Wei Di Huang Wan may be used in the treatment of type 2 diabetes and its complications [13]. Gan et al. used network pharmacology-molecular docking technique to reveal the potential mechanism of Rhizoma drynariae against osteoporosis [14]. Through network pharmacology and molecular docking simulations, this study identified key bioactive components of DSS for the treatment of NAFLD as well as putative molecular processes and biological pathways. The workflow is depicted in Figure 1.

2 Methods

2.1 Screening for active ingredients and candidate targets of Danggui Shanyao San

TCMSP(https://old.tcmsp-e.com/tcmsp.php) [15] included all active compounds for the six Chinese herbs in DSS, which contains all 499 herbs, 29,384 ingredients registered in the Chinese pharmacopoeia. The following two metrics were employed as inclusion criteria for candidate ingredients in order to obtain compounds with better oral absorption, utilisation, and biological properties for future analysis: (1) oral bioavailability (OB) ≥ 30%; (2) drug-likeness (DL) ≥ 0.18. The active ingredients that satisfied the criteria were acquired after screening and deleting duplicates, and the mol2 files were downloaded.

The pharmmapper server [16] (http://www.lilab-ecust.cn/pharmmapper/) is used to find drug candidate targets. The targets were screened with an inclusion criterion of a parameter norm fit ≥ 0.9. The screened targets were submitted

into the Uniprot website [17] (https://www.uniprot.org/), with "Homo sapiens" and "Reviewed" as criteria. The drug candidate targets are therefore changed from protein names to gene names in this manner.

2.2 Prediction of candidate therapeutic targets in NAFLD

The keyword "non-alcoholic fatty liver disease" from the Medical Subject Headings was used to identify possible therapeutic targets for disease. The candidate therapeutic targets retrieved from the following three websites: (i) NCBI (https://www.ncbi.nlm.nih.gov/) [18] : the GENE resource on the NCBI integrates data from a variety of species, provides a searchable genetic database focusing on completely sequenced genomes, and includes an active research community that contributes gene-specific data. Includes all of the targets gathered by the website; (ii) DisGeNet (https://www.disgenet.org/home/) [19]: the DisGeNet database is one of the most comprehensive collections of genes and variations associated to human disease that is publicly available. The current version consists of 1,134,942 gene-disease associations (GDAs) and 369,554 variant-disease associations (VDAs). After considering the database's median DSI (Disease Specificity Index for the gene) as the parameter, the targets retrieved from the website were incorporated.; (iii) Genecards (https://www.genecards.org/) [20]: Genecards is a searchable, comprehensive database that contains detailed information on all human genes that have been annotated or predicted. The objective from the website is calculated by using the database's reference scores as the parameter and dividing by the median. To screen the candidate therapeutic targets for this study, the three sets of data gathered above were pooled and duplicate values were deleted.

2.3 Construction of active Ingredients - potential therapeutic targets network.

Bioinformatics (http://www.bioinformatics.com.cn/) generated Wayne maps of DSS and NAFLD potential targets. The possible targets of DSS in the therapy of NAFLD are the targets in the Wayne diagram intersection. Then, using Cytoscape 3.7.0 [21] software, the active ingredients - potential therapeutic targets network was built and displayed, and the primary active ingredients and potential therapeutic targets of DSS was confirmed.

2.4 Construction of Protein-Protein interaction network

STRING (https://string-db.org/) [22] is used to build the Protein-Protein interaction (PPI)network. Potential therapeutic targets were added to the STRING site, the organism was defined as "Homo sapiens," irrelevant or free proteins were removed from the network, and other settings were set to default. The core sub-modules of the PPI network were then mined using MCODE tool of Cytoscape, with the parameter score set to 5 and the rest set to default settings.

2.5 GO and KEGG pathway enrichment analysis

The association between putative biological pathways and therapeutic targets may be further explored using gene ontology (GO) and Kyoto Encyclopedia of Genes and Genomes (KEGG) analyses. After screening, Metascape (https://metascape.org) is utilized for enrichment analysis of possible therapeutic targets, followed by bioinformatics for visual presentation.

2.6 Molecular docking of the main target with the core active ingredients

Molecular docking can verify the binding affinity between a potential therapeutic targets and active ingredients of DSS. To begin, the finest protein of the targes was sought in the PDB [23] (https://www.rcsb.org/) and downloaded in PDF format. The targets' PDF files and the components' mol2 files are then imported into pymol2.4.0 for ligand

removal and water molecule removal pretreatment. After that, AutoDuck tools 1.5.6 is used to dock the protein targets and active components one by one. Finally, ligplot 2.2.4 is employed to determine the primary link between small compounds and large proteins.

3 Results

3.1 Active ingredients- potential therapeutic targets network

A total of 51 ingredients from six herbs in Dss were screened, including 2 of DG,13 of SY,7 of CX,10 of ZX,15 of FL.7of BZ. Among these, sitosterol is the common ingredient of BS, CX and ZX, beta-sitosterol is the common ingredient of DG and BS (Table 1). From 51 active components, the PharmMapper website yielded 133 possible pharmacological targets. The sites NCBI, DisGeNet, and Genecards were used to collect 1048 possible disease targets. DSS has 38 possible therapeutic targets in the treatment of NAFLD, according to a Venn diagram (Figure 2). In Cytoscape 3.7.0, the ingredients-potential therapeutic targets network has 95 nodes and 584 (Figure 3).

Table1 The active ingredients of DSS

Num	MolID	Molecule Name	OB	DL	Drug
CX01	MOL000433	FA	68.96	0.71	CX
CX02	MOL002140	Perlolyrine	65.95	0.27	CX
CX03	MOL002151	senkyunone	47.66	0.24	CX
CX04	MOL002157	wallichilide	42.31	0.71	CX
CX05	MOL001494	Mandenol	42	0.19	CX
CX06	MOL002135	Myricanone	40.6	0.51	CX
DG01	MOL000449	Stigmasterol	43.83	0.76	DG
FL01	MOL000273	(2R)-2-[(3S,5R,10S,13R,14R,16R,17R)-3,16-dihydroxy-4,4,10,13,14-pentamethyl-2,3,5,6,12,15,16,17-octahydro-1H-cyclopenta[a]phenanthren-17-yl]-6-methylhept-5-enoic acid	30.93	0.81	FL
FL02	MOL000275	trametenolic acid	38.71	0.8	FL
FL03	MOL000276	7,9(11)-dehydropachymic acid	35.11	0.81	FL
FL04	MOL000279	Cerevisterol	37.96	0.77	FL
FL05	MOL000280	(2R)-2-[(3S,5R,10S,13R,14R,16R,17R)-3,16-dihydroxy-4,4,10,13,14-pentamethyl-2,3,5,6,12,15,16,17-octahydro-1H-cyclopenta[a]phenanthren-17-yl]-5-isopropyl-hex-5-enoic acid	31.07	0.82	FL
FL06	MOL000282	ergosta-7,22E-dien-3beta-ol	43.51	0.72	FL
FL07	MOL000283	Ergosterol peroxide	40.36	0.81	FL
FL08	MOL000285	(2R)-2-[(5R,10S,13R,14R,16R,17R)-16-hydroxy-3-keto-4,4,10,13,14-pentamethyl-1,2,5,6,12,15,16,17-octahydrocyclopenta[a]phenanthren-17-yl]-5-isopropyl-hex-5-enoic acid	38.26	0.82	FL
FL09	MOL000287	3beta-Hydroxy-24-methylene-8-lanostene-21-oic acid	38.7	0.81	FL
FL10	MOL000289	pachymic acid	33.63	0.81	FL
FL11	MOL000290	Poricoic acid A	30.61	0.76	FL
FL12	MOL000291	Poricoic acid B	30.52	0.75	FL
FL13	MOL000292	poricoic acid C	38.15	0.75	FL
FL14	MOL000296	hederagenin	36.91	0.75	FL
FL15	MOL000300	dehydroeburicoic acid	44.17	0.83	FL
BS01	MOL001910	11alpha,12alpha-epoxy-3beta-23-dihydroxy-30-norolean-20-en-28,12beta-olide	64.77	0.38	BS
BS02	MOL001918	paeoniflorgenone	87.59	0.37	BS
BS03	MOL001919	(3S,5R,8R,9R,10S,14S)-3,17-dihydroxy-4,4,8,10,14-pentamethyl-2,3,5,6,7,9-hexahydro-1H-cyclopenta[a]phenanthrene-15,16-dione	43.56	0.53	BS

BS04	MOL001921	Lactiflorin	49.12	0.8	BS
BS05	MOL001924	paeoniflorin	53.87	0.79	BS
BS06	MOL001925	paeoniflorin_qt	68.18	0.4	BS
BS07	MOL001928	albiflorin_qt	66.64	0.33	BS
BS08	MOL001930	benzoyl paeoniflorin	31.27	0.75	BS
BS09	MOL000211	Mairin	55.38	0.78	BS
BS10	MOL000422	kaempferol	41.88	0.24	BS
BS11	MOL000492	(+)-catechin	54.83	0.24	BS
ZX01	MOL000830	Alisol B	34.47	0.82	ZX
ZX02	MOL000831	Alisol B monoacetate	35.58	0.81	ZX
ZX03	MOL000832	alisol,b,23-acetate	32.52	0.82	ZX
ZX04	MOL000849	16β-methoxyalisol B monoacetate	32.43	0.77	ZX
ZX05	MOL000853	alisol B	36.76	0.82	ZX
ZX06	MOL000854	alisol C	32.7	0.82	ZX
ZX07	MOL000856	alisol C monoacetate	33.06	0.83	ZX
ZX08	MOL002464	1-Monolinolein	37.18	0.3	ZX
ZX09	MOL000862	[(1S,3R)-1-[(2R)-3,3-dimethyloxiran-2-yl]-3-[(5R,8S,9S,10S,11S,14R)-11-hydroxy-4,4,8,10,14-pentamethyl-3-oxo-1,2,5,6,7,9,11,12,15,16-decahydrocyclopenta[a]phenanthren-17-yl]butyl] acetate	35.58	0.81	ZX
BZ01	MOL000020	12-senecioyl-2E,8E,10E-atractylentriol	62.4	0.22	ZX
BZ02	MOL000021	14-acetyl-12-senecioyl-2E,8E,10E-atractylentriol	60.31	0.31	BZ
BZ03	MOL000022	14-acetyl-12-senecioyl-2E,8Z,10E-atractylentriol	63.37	0.3	BZ
BZ04	MOL000028	α-Amyrin	39.51	0.76	BZ
BZ05	MOL000033	(3S,8S,9S,10R,13R,14S,17R)-10,13-dimethyl-17-[(2R,5S)-5-propan-2-yloctan-2-yl]-2,3,4,7,8,9,11,12,14,15,16,17-dodecahydro-1H-cyclopenta[a]phenanthren-3-ol	36.23	0.78	BZ
BZ06	MOL000049	3β-acetoxyatractylone	54.07	0.22	BZ
BZ07	MOL000072	8β-ethoxy atractylenolide Ⅱ	35.95	0.21	BZ
B	MOL000359	sitosterol	36.91	0.75	CX, BS, ZX
A	MOL000358	beta-sitosterol	36.91	0.75	DG, BS

OB, oral bioavailability; DL, drug-like; DG, DangGui; FL, FuLing; CX,ChuanXiong; BZ,BaiZu; ZX, ZeXie; BS, BaiShao

3.2 Result of core targets and pathways analyses

The PPI network of treatment targets include 38 nodes and 177 edges (Figure. 4A). The targets including albumin(ALB)、AKT Serine/Threonine Kinase 1(AKT1)、peroxisome proliferator-activated receptor gamma (PPARG)、caspase 3(CASP3)、heat shock protein 90 alpha family class A member 1(HSP90AA1)、epidermal growth factor receptor (EGFR)、and estrogen receptor 1 (ESR1) have a higher degree of node(Figure 4). There are three core clusters modules of PPI network screened by MCODE plug-in. Cluster 1 gets ALB、AKT1、HSP90AA1、EGER、ESR1、and mitogen-activated protein kinase 8 (MAPK8) with high degree value. Cluster 2 includes GC vitamin D binding protein (GC)、apolipoprotein A2 (APOA2)、transthyretin (TTR)、coagulation factor II, thrombin (F2)、and retinoid X receptor alpha (RXRA). Cluster 3 consists of glutathione-disulfide reductase (GSR), glutathione S-transferase pi 1(GSTP1), NAD(P)H quinone dehydrogenase 1(NQO1) (Figure 5).

Simultaneously, 38 potential disease targets were imported into Metascape, species were limited to Homo sapiens, then GO enrichment analysis was conducted 647 items were obtained. Among them, there were 586 items related to biological processes (BP) category, including nutrient levels, response to extracellular stimulus, cellular response to hormone stimulus, response to reactive oxygen species, and etc. The category cellular components (CC) contained 16 items, involving vesicle lumen, secretory granule lumen, cytoplasmic vesicle lumen, and etc. In the molecular functions (MF) category, there are 45 entries for nitric-oxide synthase regulator activity, monocarboxylic acid binding, fatty acid binding, nuclear receptor activity, and etc. According to the P value(P0.01), each category displays the top 20 entries at most, as shown in Figure 6. KEGG pathway enrichment analysis resulted in 161 pathways, including pathways in cancer, foxo signaling pathway, IL-17 signaling pathway, Th17 cell differentiation, PI3K-Akt signaling pathway, Non-alcoholic fatty liver disease (NAFLD). The top 20 pathways for degree values are shown in Figure 7.

3.3 Molecular docking

The molecular docking targets consisted of twelve targets with high repetition rates: from active ingredients-potential therapeutic targets network, PPI network and KEGG signaling pathway. The docking compounds included the top ten active compounds from the active ingredients- potential therapeutic targets network, as well as two repeat compounds, for a total of 12 compounds (Table 2). The lower the binding energy in the autoduck tools 1.5.6 output findings, the higher the affinity between the active component and the target protein. The results of molecular docking reveal that all binding energies between the target and the compound are less than 0, indicating that each core compound has a significant binding affinity for the target (Figure 8). Additionally, small molecules and big proteins interact in a variety of ways, including hydrogen bonding, van der Waals force interactions, and hydrophobic interactions, among others. Small molecules bind to big proteins in a range of methods (Figure 9).

Table 2
The molecule docking results of molecules and proteins (kj/mol)

Protein	A	B	BS02	BS05	BZ02	BZ03	FL03	FL09	FL11	FL12	ZX06	ZX09
AKT1	-5.17	-4.06	-3.96	-1.36	-1.64	-1.49	-3.38	-3.96	-2.05	-1.86	-4.52	-3.18
ALB	-3.56	-3.84	-4.04	-1.54	-0.84	-1.62	-4.13	-3.24	-1.91	-1.04	-2.64	-2.75
CASP3	-4.32	-4.72	-3.83	-2.02	-3.03	-2.21	-3.99	-4.35	-2.05	-1.03	-3.13	-3.27
ESR1	-4.08	-3.66	-3.86	-1.8	-2.23	-1.59	-3.75	-3.71	-2.03	-0.97	-3.07	-3.48
F2	-5.51	-5.09	-4.67	-2.9	-2.27	-3.37	-4.4	-4.54	-3.12	-1.84	-3.89	-3.18
GC	-3.84	-4.49	-3.99	-1.63	-2.09	-1.51	-3.58	-3.39	-2.18	-1.05	-4.03	-3.01
GSTP1	-4.74	-4.64	-5.31	-2.83	-1.91	-1.42	-4.59	-4.01	-1.4	-1.08	-4.65	-3.36
MAPK14	-4.31	-4.23	-4.54	-1.36	-1.74	-2.13	-3.59	-4.24	-2	-1.07	-3.61	-2.57
MAPK8	-5.09	-4.08	-4.52	-2.27	-2.51	-1.94	-3.86	-4.39	-2.03	-0.44	-3.5	-2.86
PPARG	-4.39	-4.5	-3.93	-1.63	-1.6	-1.8	-3.74	-3.44	-1.41	-1.68	-3.43	-2.87
TTR	-5.46	-4.26	-4.64	-1.99	-2.91	-3.39	-4.34	-4.43	-2.9	-0.87	-4.25	-2.95
RXRA	-4.32	-4.32	-3.81	-2.06	-2.21	-1.65	-3.68	-3.73	-2.15	-0.6	-3.11	-2.43
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4 Discussion

The Pathogenesis of NAFLD has not been unified across time. The "two hits" theory offered by Day, C P et al in the 1990s suggested that NAFLD is produced by a double whammy of steatosis and oxidative stress generated by lipid peroxidation [24]. With further research, it's becoming that NAFLD is caused by a combination of factors, including lipid accumulation in the liver, insulin resistance, inflammation, oxidative stress, ER stress, apoptosis, and autophagy[25]. As a result, not all patients have the same causative factors, and disease mechanisms are exceedingly varied. However, the diagnostic criteria for NAFLD are generally accepted. For the diagnosis of NAFLD, liver biopsy has become the gold standard. NAFLD is diagnosed when at least 5% of liver biopsy specimens show steatosis, with lobular inflammation, ballooning, and fibrosis as the disease progresses. In the absence of a significant history of alcohol use or other known liver disease, NAFLD is diagnosed when at least 5% of liver biopsy specimens show steatosis [26, 27]. Therefore, the primary therapeutic strategy for NAFLD is to reduce hepatic steatosis and mitigate hepatocellular damage.

In this study, the top three active components in the network are BS05, FL11, and FL12. BS05 is Paeoniflorin. According to prior study, paeoniflorin can activate the LKB1/AMPK pathway to reduce hepatic steatosis and has beneficial advantages in the treatment of NAFLD [28]. Paeoniflorin has also been shown to cure NAFLD by modulating the insulin signaling pathway IRS/AKT/GSK3 and antioxidation [29]. Poricoic Acid A and Poricoic Acid B are FL11 and FL12. Poria cocos is made up primarily of them. Interleukin 1 (IL-1), tumor necrosis factor alpha (TNF-α) and other proinflammatory mediators and cytokines could be inhibited by poria cocos extract [30]. Through the AMPK (AMP-activated protein kinase) pathway, poria cocos could also control lipid metabolism, reduce ER stress, and protect hepatic steatosis in obese mice fed a high-fat diet (HFD) and hepatocellular carcinoma (HepG2) cells treated with free fatty acid (FFA)-palmitic [31]. Intriguingly, there are varied numbers of common targets among

herbs in a network of active ingredients-potential therapeutic targets. More than 70% of paeonia lactiflora's targets overlap with those of other herbs, implying that paeonia lactiflora has a close interaction with the other herbs in the formula. This is in line with the seven relations of medicinal compatibility, a way of constructing traditional Chinese medical prescriptions that aims to improve medicine efficacy.

It's worth noting that carbonic anhydrase II (CA2) is the network's objective with the highest degree. Although it does not appear to have been tested in an experiment on NAFLD, research on NAFLD-related disorders has progressed. Carbonic anhydrase inhibitors paired with a hypocaloric diet were linked with better weight loss and fewer side effects in obese individuals than placebo and a hypocaloric calorie diet alone in a randomized controlled clinical trial [32]. In rats with type 2 diabetes, carbonic anhydrase inhibitor has also been demonstrated to minimize oxidative stress and protect the blood-brain barrier [33]. As we all know, NAFLD is intimately linked to type 2 diabetes and obesity. As a result, CA2 could be a good candidate for NAFLD therapy and prevention strategies in the future.

In the PPI network, ALB has the highest node value of 30 and the highest edges of 29, indicating that ALB and the screened potential therapeutic targets have a broad and intimate interaction. Albumin encoded by the ALB gene is the most abundant circulating protein and performs a variety of biological functions, including binding and transporting endogenous (e.g., fatty acids) and exogenous molecules, as well as antioxidant and anti-inflammatory functions, all of which are important in the prevention and treatment of NAFLD [34, 35]. Lower albumin concentrations have been linked to weight and fat increase; the mechanism underlying this link is unknown, although weight and fat gain are linked to NAFLD[36]. Furthermore, because albumin is mostly produced by the liver, impaired hepatic function will influence albumin synthesis, resulting in a decrease in serum albumin concentration[37]. Therefore, albumin is being considered as a potential biomarker for the diagnosis of NAFLD, which is receiving relevant discussion and research.

AKT1, a subtype of protein kinase B(PLB/AKT), exhibits a very high degree of enrichment in both the PPI network and the GO enrichment and KEGG pathways. Cancer, cardiovascular disease, insulin resistance, type 2 diabetes, and inflammation are all linked to Akt[38]. Akt is the fundamental protein of the PI3K-Akt pathway, which is the central regulator in glucose and insulin signal transduction homeostasis [39, 40]. It has been shown that AKT activation causes sterol regulatory element binding transcription factor 1 (SREBP1) mRNA accumulation in primary hepatocytes and that this may be the major signaling event for insulin-induced SREBP1 gene expression in the liver [41]. The SREBP family of genes regulates lipid absorption, de novo biosynthesis, and oxidative catabolism[42]. Hence, DSS regulates lipogenesis via modulating the PI3K-AKT pathway, which is a therapeutic approach to NAFLD. In addition, glycogen synthase kinase 3 (GSK3) is a serine/threonine protein kinase. As the substrate of Akt, it is a key enzyme involved in glycogen metabolism. GSK3 is also involved in the inflammatory response, ER stress, mitochondrial dysfunction, and other processes[43]. The results demonstrated that inhibiting GSK3 might reduce liver damage, decrease TNF-α, interleukin 6 (IL-6), and IL-1 mRNA expression, decrease caspase-3 expression, and reduce hepatocyte apoptosis [44]. For this reason, DSS may have anti-NAFLD effect through regulating GSK3.

Further investigation of the KEGG pathway indicated that DSS can be used to prevent and treat NAFLD in a variety of pathways. The Foxo signaling pathway is involved in the regulation of cell cycle, apoptosis, and metabolism, as well as glucose and lipid homeostasis [45]. The FOXO signal pathway induced autophagy by regulating autophagy related 14 (ATG14), enhancing the breakdown of lipid droplets in the liver, in addition to limiting fat synthesis via inhibiting SREBP-1c and glucokinase[46–48]. When Th17 cells are developed from naive T cells, they produce pro-inflammatory cytokines and chemokines[49]. Th17 cells release IL-17, which plays a key role in bacterial and fungal infection barrier immunity and promotes a variety of inflammatory diseases in the body [49]. In the livers of Nash

mice caused by lipopolysaccharide (LPS) and high fat, the frequency of Th17 cells was dramatically raised, however, when an IL-17 inhibitor was applied, inflammatory cell infiltration and serum alanine aminotransferase (ALT) levels were reduced[50, 51]. The transition from benign uncomplicated steatosis to Nash requires persistent liver inflammation. The preceding findings suggest that Dss can prevent future deterioration by altering fundamental targets such as the Foxo signaling pathway, Th17 cell differentiation, and IL-17 signaling pathway.

Molecular docking simulation is a very useful tool in drug discovery and design, which can be used to predict the position of ligands in their receptor binding sites. The chemical components with strong affinity screened are sitosterol and β- sitosterol, they all belong to phytosterols. Phytosterols have been shown to contribute to prevent diseases such as fatty liver, inflammation and obesity [52]. As the most representative of phytosterols, β-sitosterol has anti-inflammatory, lipid-lowering, antioxidant and other effects [53]. It has been shown that β-sitosterol added into the diet can reduce hepatic steatosis in rats without affecting liver quality or impairing liver function [54]. The protein targets with strong affinity for β-sitosterol are CASP3, GC, PPARG, RXRA. CASP3 encodes caspase-3, which is a frequently activated death protease that plays a key role in apoptosis [55]. Thapaliya et al found that inhibition of caspase-3 reduced liver cell injury and proinflammatory signal transduction in Nash mouse models, and prevented hepatic stellate cell activation [56]. PPARG encodes PPAR γ. PPAR γ is a peroxisome receptor that can regulate inflammation, lipid and glucose metabolism, and overall energy homeostasis [57]. It has been found that β-sitosterol can decrease the level of caspase-3, increase the level of PPAR-γ and protect cardiomyocytes through PPAR γ signal transduction. These finding are consistent with the results of molecular docking [58]. Clinically, PPAR γ natural agonists have shown good efficacy in patients with NAFLD, but no specific conclusions have been drawn[59]. DSS, a natural herbal formula widely used in Chinese medicine since the Eastern Han dynasty, could provide more choices and references for the development of drugs to treat patients with NAFLD.

5 Conclusions

In conclusion, this study used network pharmacology and molecular docking to examine the active components and possible therapeutic targets of DSS in the treatment of NAFLD, revealing the multi-component, multi-target, and multi-pathway characteristics. It established a solid foundation for optimizing the trial design and further elucidating DSS's mode of action in the prevention and treatment of NAFLD. There may be differences in the chemical constituents and targets. As a result, a literature study and associated studies should be conducted to validate the mechanism of DSS in the treatment of NAFLD.

Abbreviations

NAFLD, Non-alcoholic Fatty Liver Disease; DSS, Danggui Shaoyao San; TCMSP, Traditional Chinese Medicine Systems Pharmacology; GO, Gene Ontology; KEGG, Kyoto Encyclopedia of Genes and Genomes; TCM, Traditional Chinese medicine; ER, endoplasmic reticulum; CA2, carbonic anhydrase II; PLB/AKT□protein kinase B; DG, DangGui, Angelicae Sinensis Radix; FL, FuLing, Poria Cocos(Schw.) Wolf; CX, ChuanXiong, Chuanxiong Rhizoma; BZ, BaiZu, Atractylodes Macrocephala Koid; ZX, ZeXie, Alisma Orientale (Sam.) Juz; BS, BaiShao, Paeoniae Radix Alba; OB, oral bioavailability; DL, drug-likeness; PPI, Protein-Protein interaction; ALB, albumin; AKT1, AKT Serine/Threonine Kinase 1; PPARG, peroxisome proliferator-activated receptor gamma; CASP3, caspase 3; HSP90AA1, heat shock protein 90 alpha family class A member 1; EGER, epidermal growth factor receptor; ESR1, estrogen receptor 1; MAPK8, mitogen-activated protein kinase 8; GC, GC vitamin D binding protein; APOA2, apolipoprotein A2; TTR, transthyretin; F2, coagulation factor II, thrombin; RXRA, retinoid X receptor alpha; GSR, glutathione-disulfide reductase; GSTP1, glutathione S-transferase pi 1; NQO1, NAD(P)H quinone dehydrogenase 1; BP, biological processes; CC, cellular

components; MF, molecular functions; CA2, carbonic anhydrase II; SREBP1, sterol regulatory element binding transcription factor 1; GSK3, glycogen synthase kinase 3; MCODE, Molecular Complex Detection

Declarations

7.1 Ethics approval and consent to participate

Not applicable

7.2 Consent for publication

Not applicable

7.3 Availability of data and materials

All data are available in the manuscript and they are showed in figures and tables

7.4 Competing interests

The authors declare that they have no competing interests.

7.5 Funding

This work was supported by the Key Research and Development Program of Science and Technology Department of Sichuan Province (grant number 2020JDZH0018),the Sichuan Provincial Famous TCM Doctor Studio Construction Project (Sichuan TCM 003113005003), the fourth National TCM Doctor (Clinical And Basic) Excellent Talents Research And Study Program (grant number CMM No. 24 [2017]), the Special Research Project of Sichuan Provincial Administration of Traditional Chinese Medicine (grant number 2021MS099),Technological innovation research and development projects of Chengdu Science and Technology Bureau(grant number 2021-YF05-02379-SN),the Sichuan Provincial Department of Education project (grant number 17ZB0146),and the “Double First-class” construction project of Chengdu University of Traditional Chinese Medicine (030041043).

7.6 Authors' contributions

Conception and design: XLY, ZTE; administrative support: XLY; provision of study materials or patients: TYQ, WYQ, HWY; Collection and assembly of data: HLL, ZCJ, FZY, YQZ; Data analysis and interpretation: XLY, LZ, ZHY; manuscript writing: XLY, LZ; final approval of manuscript: TEZ, YZZ All the author(s) read and approved the final manuscript.

7.7 Acknowledgements

The authors would like to express their gratitude to the editor, anonymous reviewers, and all reference writers.

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Figures

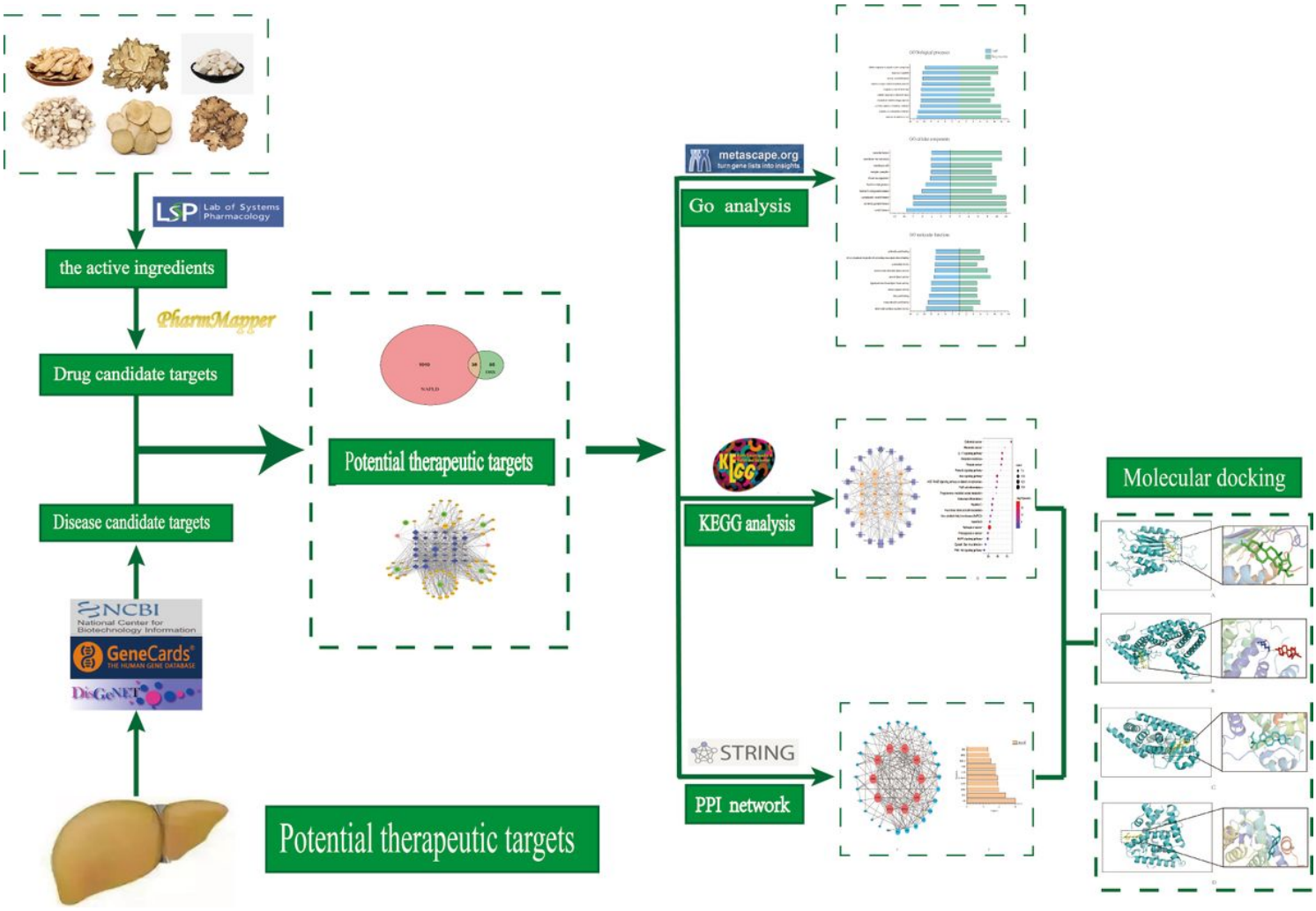

Figure 1

Flowchart shows the study's design in detail

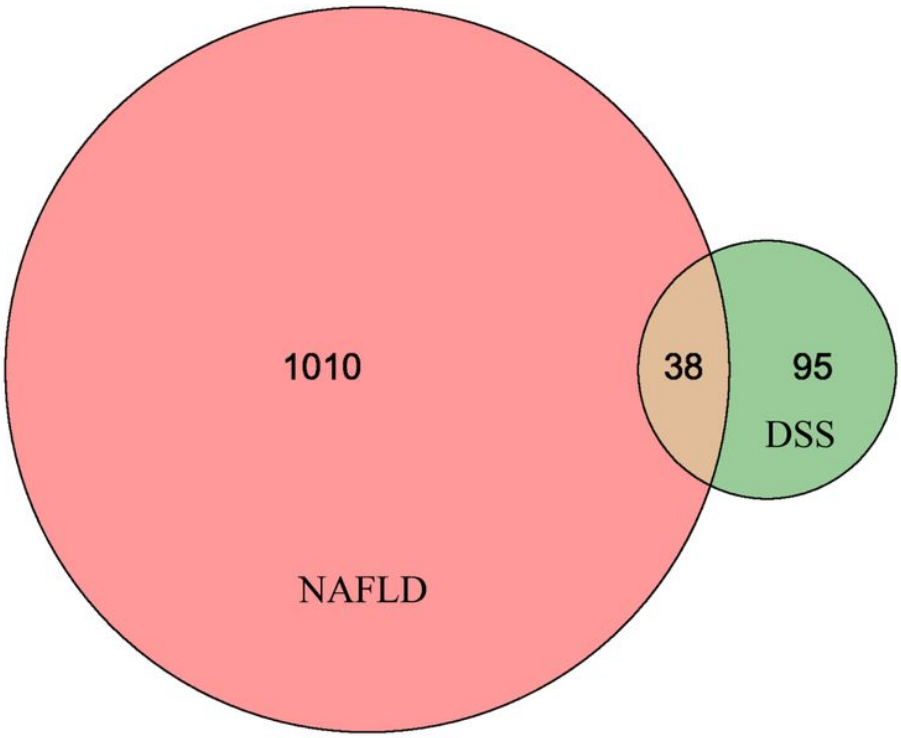

Figure 2

Venn diagram of intersection targets of FLD and NAFLD

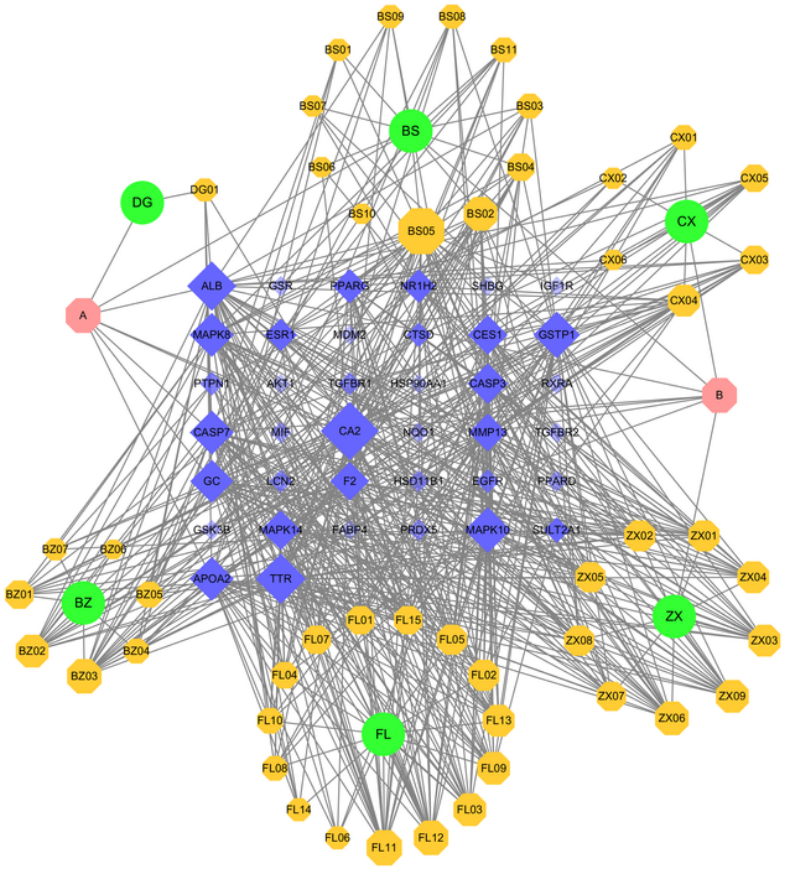

Figure 3

Active ingredients- potential therapeutic targets network; potential therapeutic targets in purple; active compounds contained in each herb in orange; active ingredients shared by the herbs in pink; See Table 1 for details of cingredient number

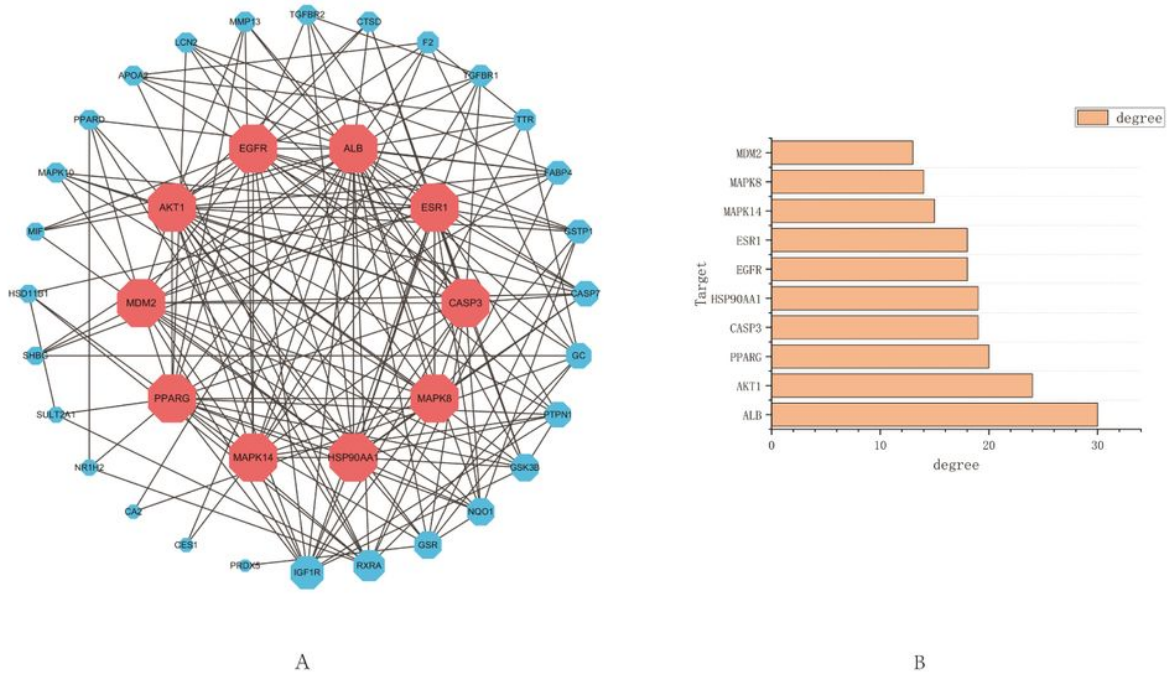

Figure 4

A：PPI network of potential therapeutic targets；B: top 10 protein targets in the PPI network

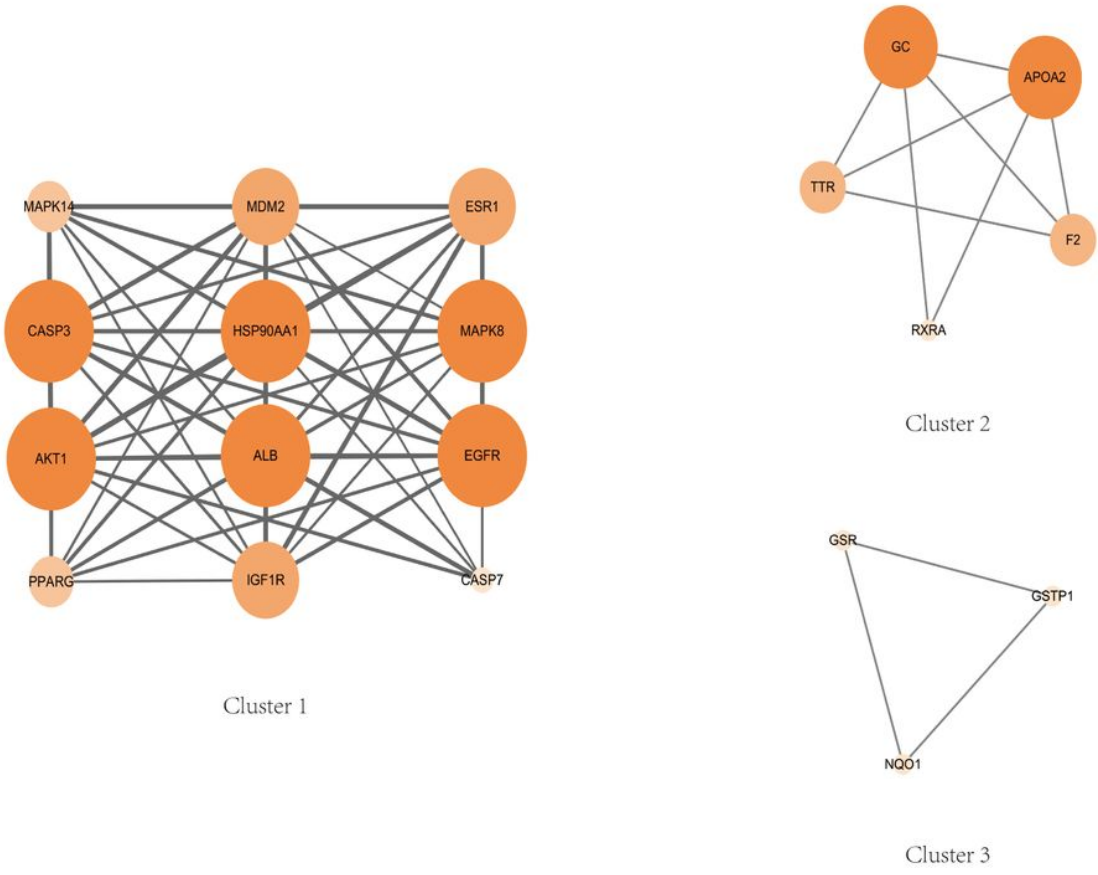

Figure 5

The core sub-modules of the PPI network

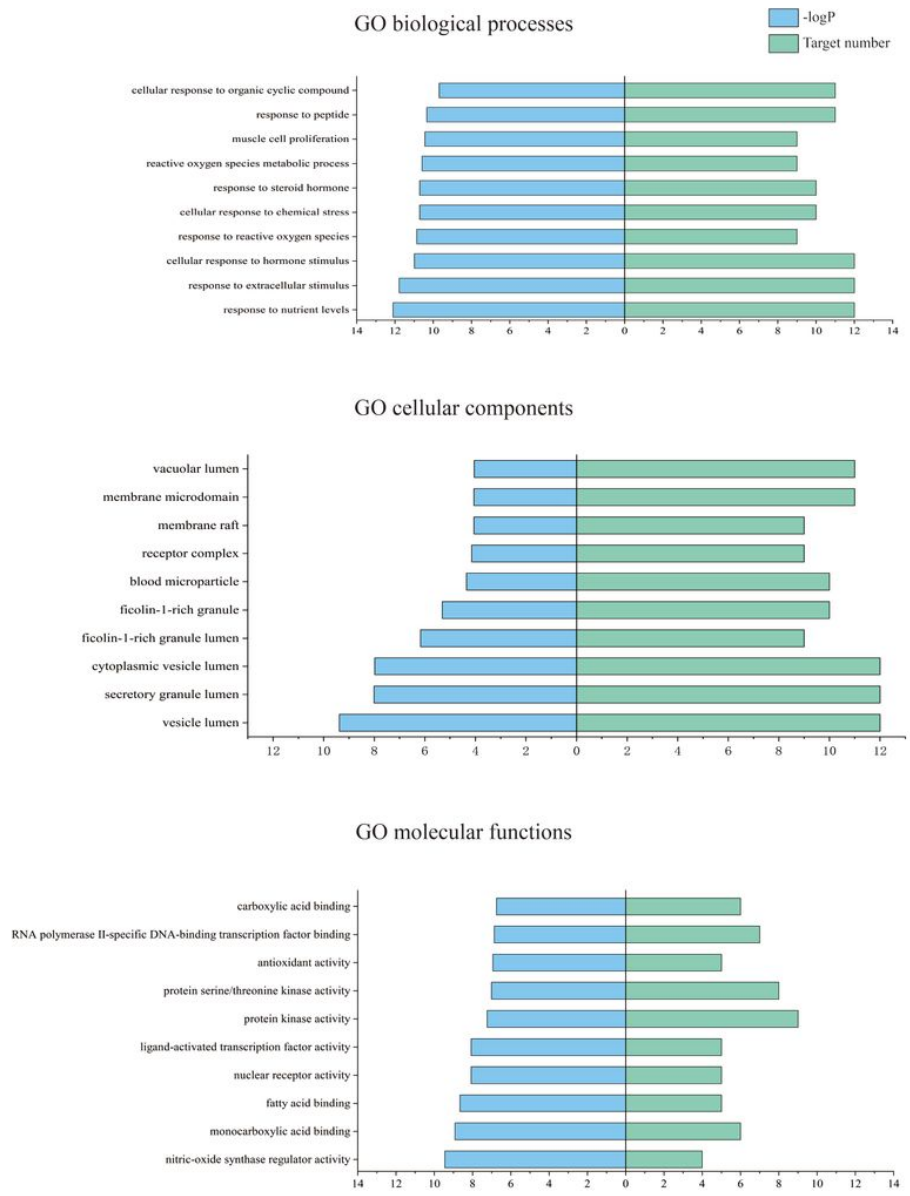

Figure 6

Histogram of GO enrichment analysis for DSS treatment of NAFLD targets, with -logP values in blue and the number of target in green

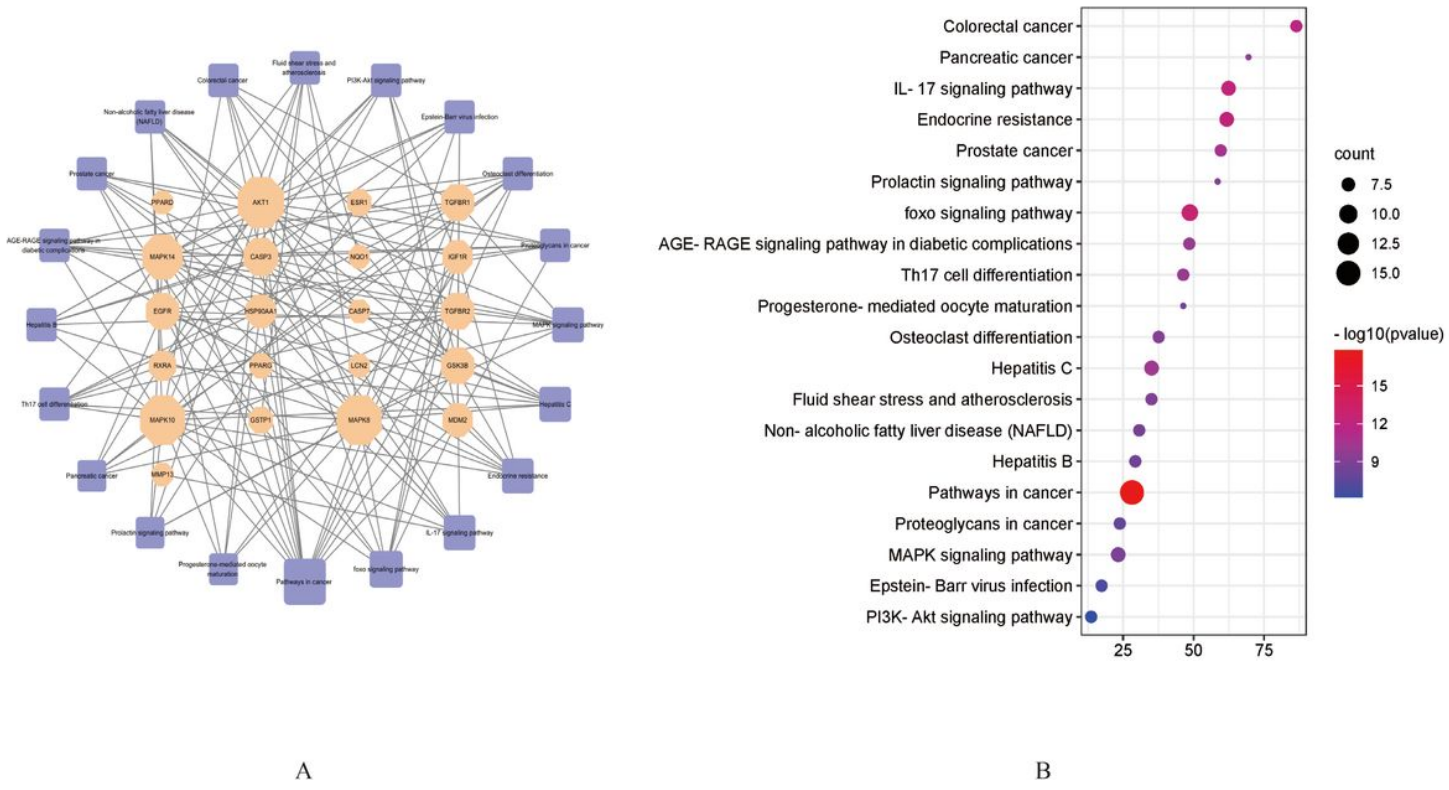

Figure 7

A: the network of the KEGG pathway - the network of targets included in the pathway; B: the bubble diagram of KEGG analysis of potential therapeutic targets

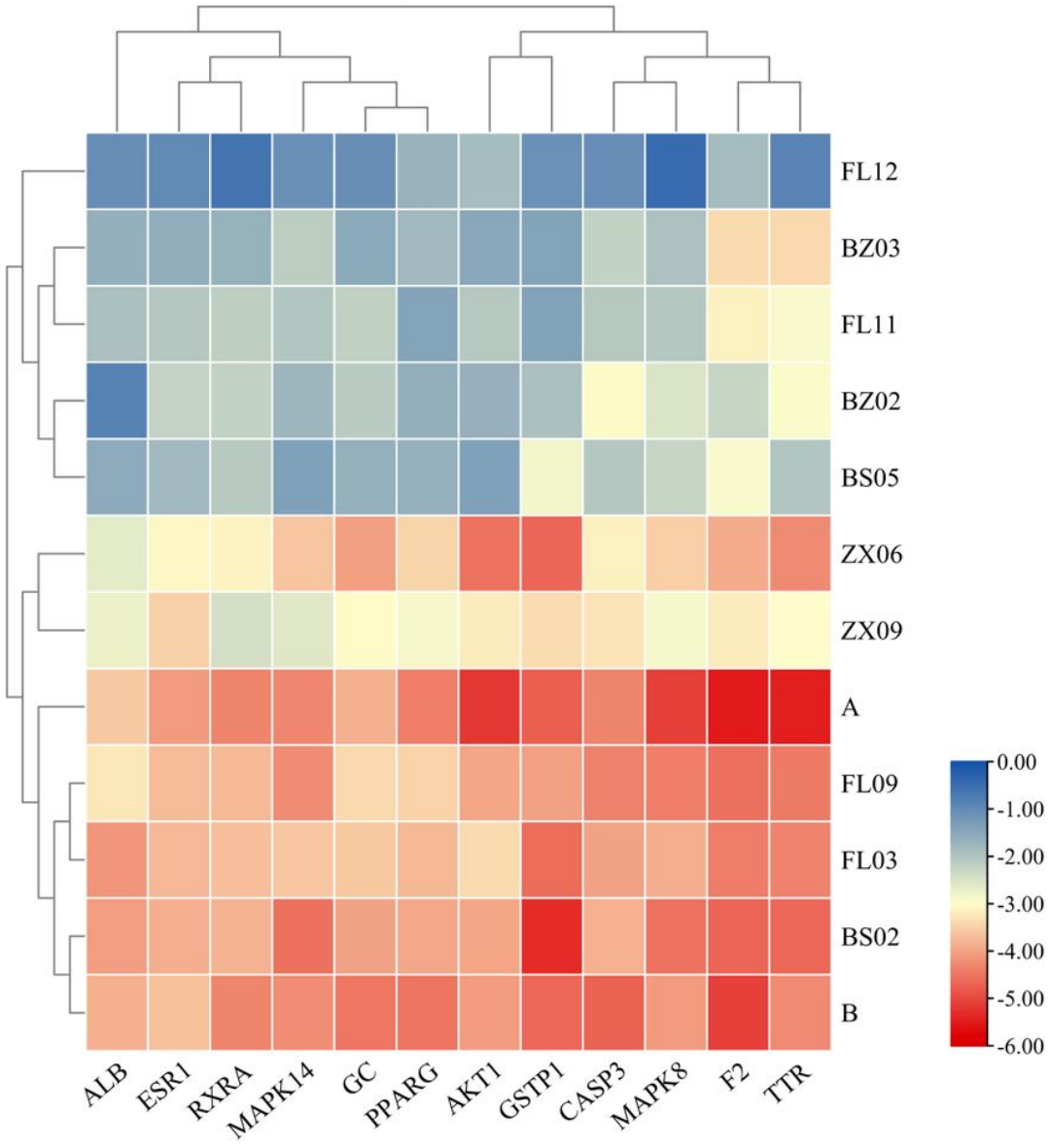

Figure 8

The heat map of active ingredients and potential therapeutic targets

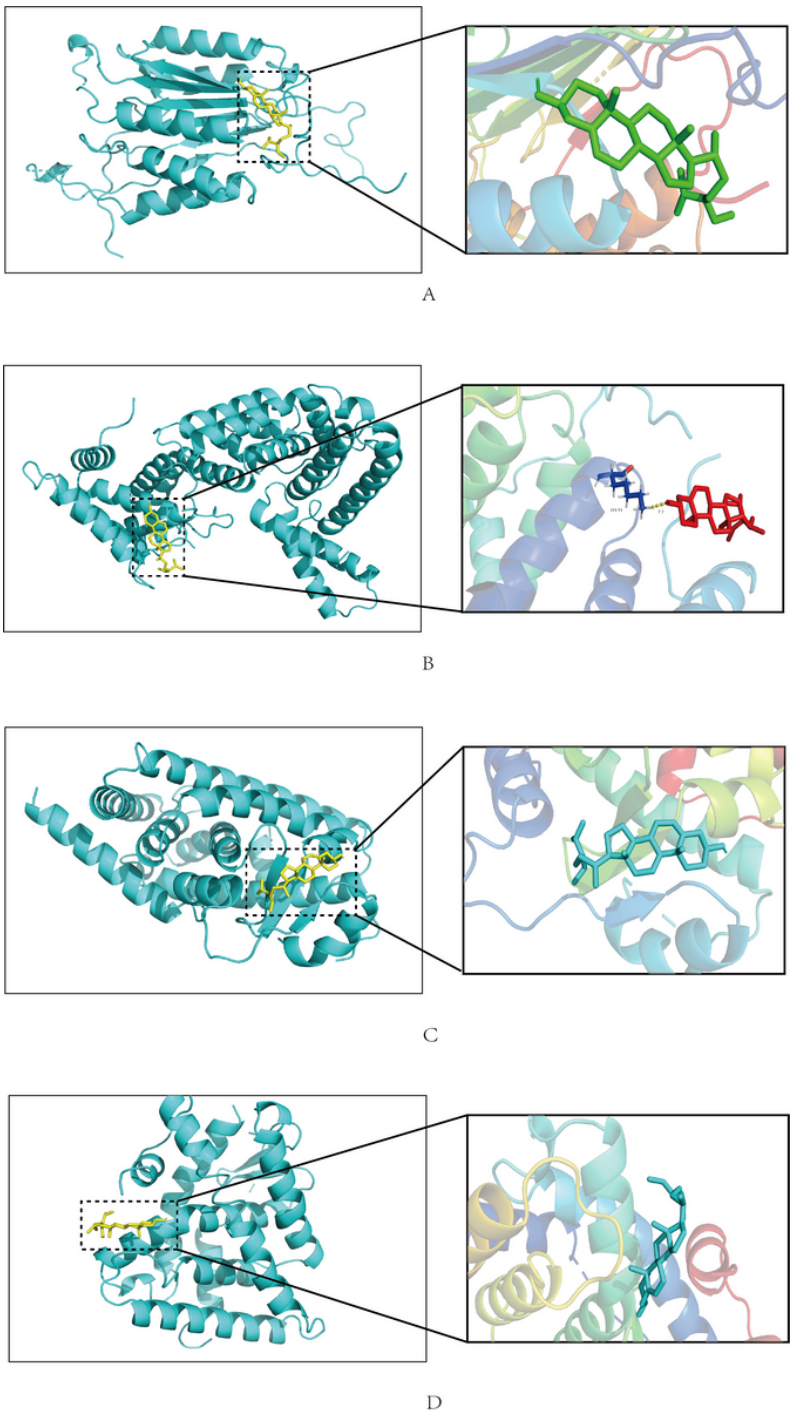

Figure 9

Molecular docking diagrams of β-sitosterol and four high-affinity targets for it. A: molecular docking of β-sitosterol with CASP3; B represents molecular docking of β-sitosterol with GC; C: molecular docking of β-sitosterol with PPARG; D: represents molecular docking of β-sitosterol with RXRA.