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Research Article

Identification and mechanism elucidation of medicative diet for food therapy XQCSY in NAFLD prevention: an integrative *in silico* study

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Abstract: XingQiChuShiYin (XQCSY), a conventional Chinese medicine dietary therapy, is effective in preventing non-alcoholic fatty liver disease (NAFLD), but its effective constituents and functional mechanisms are yet vague for the reason conventional modes are not suitable. Therefore, this study is centered on exploring the NAFLD prevention mechanisms of XQCSY via an *in-silico* method. The potential targets of XQCSY and NAFLD are obtained from public databases, and there are 81 intersected targets. Then six key targets including RAC-alpha serine/threonine-protein kinase, tumor necrosis factor, interleukin-6, interleukin-1 beta, cellular tumor antigen p53, and epidermal growth factor receptor are screened from the "herb-compound-common target" network. Subsequently, Gene Ontology and Kyoto Encyclopedia of Genes and Genomes enrichment analysis reveals that 513 of biological processes, 51 of cellular components, and 90 of molecular functions in XQCSY are related to the treatment of NAFLD. Results from network pharmacology show that XQCSY plays a pivotal role in preventing NAFLD with a "multicomponent-multitarget-multichannel" mechanism. Molecular docking also confirms active ingredients from XQCSY are well affinitive to the key genes and thus greatly affect NAFLD prevention. In conclusion, this research may provide a vital theoretical basis for determining the prevention mechanism of XQCSY on NAFLD.

Keywords: non-alcoholic fatty liver disease; XingQiChuShiYin; network pharmacology; *in silico*; molecular docking

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1 Introduction

Owing to the epidemic of obesity and related metabolic syndromes, non-alcoholic fatty liver disease (NAFLD) featured by excessive fat accumulation in the liver[1] has become the most common chronic liver disease[2]. Even worse, NAFLD may progress to severe diseases, including steatohepatitis, hepatic cirrhosis, and hepatocellular carcinoma[3,4]. Therefore, the prevalence of NAFLD has become a significant public health issue worldwide. However, the treatments for NAFLD are limited. Although the disease can be prevented and treated by changing lifestyle (e.g., doing more physical exercises and trying to lose weight), few people can adhere to the way for long time[5]. Therefore, the treatment of most NAFLD patients still relies on adjuvant medicine. Nowadays, there are several treatments for medical therapy of NAFLD, such as ursodeoxycholic acid[6] and vitamin E[7], but the treatments do not always work and even can lead to substantial adverse effects[8,9]. Therefore, searching for safe and effective treatments is of great urgency.

Conventional Chinese medicine dietary therapy, which shows remarkable efficacy but few side effects, has a long history in treating various diseases. Zha Qu Ping Wei San (ZQPWS), a dietary therapy for fatty liver disease, is originally from "*Chinese Medicine Disease Mechanisms and Treatments*". It is composed of 13 herbs: *Atractylodes lancea*, *Citri Reticulatae Pericarpium*, *Raphani Semen*, *Cassiae Semen*, *Magnolia officinalis* Rehd et Wils., licorice, *Crataegi Fructus*, *Massa Fermentata*, *Galli Gigerii Endothelium Corneum*, *Hordei Fructus Germinatus*, *Aurantii Fructus Immaturus*, *Alisma orientale* (Sam.) Juz., and *Curcumae Radix*. XingQiChuShiYin (XQCSY) is a dietary therapy based on ZQPWS. Although both ZQPWS and XQCSY have been proved to own the potential to regulate NAFLD, they play a therapeutic role in indirect and direct ways respectively. The core idea of ZQPWS is to promote the harmonious operation of spleen and stomach, thereby indirectly regulating nonalcoholic fatty liver[10,11]. Relatively speaking, XQCSY can directly act on the liver, thus dispeling the moisture in the body and promote the smooth flow of blood. Comparatively speaking, XQCSY can reduce the burden on the liver and promote the recovery of liver function in a more

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direct way and shorter time, which is undoubtedly an extremely effective therapy for NAFLD patients. XQCSY comprises of four herbs: *A. lancea*, *Citri Reticulatae Pericarpium*, *Raphani Semen*, and *Cassiae Semen*, which possess potential for multiple activities, such as antioxidant and anti-inflammatory activities[12-15]. For example, *A. lancea* alleviates NAFLD by regulating Nrf2-mediated ferroptosis[16]. Reportedly, *Citri Reticulatae Pericarpium* may function as a dietary supplement for ameliorating fatty liver diseases[17]. *Raphani Semen* has the potential for improving NAFLD by inhibiting de novo lipogenesis[18]. *Cassiae Semen* has been used for relieving constipation and treating various liver diseases[19]. Nevertheless, the therapeutic mechanisms of XQCSY on NAFLD remain yet vague, and the effective constituents and key targets remain to be identified.

To maximize drug efficacy, we adopt network pharmacology to analyze the biological network of herb candidates. With the "herb-compound-common target" network multidisciplinary fusion theory developed as a benchmark[20], network pharmacology emphasizes the concept of "multicomponent-multitarget-multichannel". Generally, the tech clarifies the connection between XQCSY and NAFLD from a holistic perspective. For example, Observation based on network pharmacology from Xu *et al.*[21] shows saffron has a therapeutic effect on NAFLD through various mechanisms, including inhibition of oxidative stress and attenuation of inflammatory responses. In addition, Hong *et al.*[22] clearly identified vascular endothelial growth factor-C (VEGF-C) as a key target of pure total flavonoids from Citrus (PTFC) against NAFLD. The integrity and systematicness of the network pharmacology research strategy are consistent with the overall concept and dialectical therapeutic theory of conventional Chinese medicine, which provides a novel approach for modern therapeutic interpretation of conventional Chinese medicine[23].

Conventional Chinese medicine dietary therapy, with remarkable efficacy and small side effects, is an ideal treatment for various diseases. While different people have different physical conditions, so XQCSY shows different anti-NAFLD effects, and even that it may only be effective for some people[24]. Besides, XQCSY, a dietary intervention with few side effects, needs to be drinked for a long time to show an anti-NAFLD effect compared with drug treatment[25]. However, it is obvious that long-term diet control is a huge challenge for people, so it is difficult for NAFLD patients to prepare and drink a daily diet of XQCSY[26]. The limitations above-mentioned affect the effects of dietary intervention. But it is undeniable that XQCSY does shows an anti-NAFLD effect. Since XQCSY comprises of complex mixtures and has various multi-target chemicals, it is impractical to systematically explore the mechanisms of XQCSY using conventional modes. Therefore, the possible active ingredients and potential mechanisms of XQCSY in preventing NAFLD are comprehensively investigated using network pharmacology and molecule docking. The relevant flowchart is exhibited in Fig. 1.

2 Materials and methods

2.1 Retrieval of main components of XQCSY

XQCSY decoction comprises of Cangzhu (*A. lancea*), Chenpi (*Citri Reticulatae Pericarpium*), Laifuzi (*Raphani Semen*), and Juemingzi (*Cassiae Semen*). The active constituents and related targets in XQCSY were obtained from the conventional Chinese Medicine Systems Pharmacology Database (https://old.tcmsp-e.com/tcmsp.php), Analysis Platform (http://tcmspw.com/tcmsp.php), and references[27-42] with Cangzhu, Chenpi, Laifuzi, and Juemingzi as the keywords. Then, two screening parameters viz. oral bioavailability (OB) ≥ 30% and drug-likeness (DL) ≥ 0.18 were adopted to select the constituents for subsequent analysis[43]. The target protein names obtained were converted into a unified format with "*Homo sapiens*"[44] via Unified Protein Database (https://www.uniprot.org/).

2.2 Identification of NAFLD-related targets of XQCSY

"Non-alcoholic fatty liver disease" was used as the keyword to collect potential targets from GeneCards (http://www.genecards.org/), DisGeNET (http://www.Disgenet.org/search), OMIM (https://omim.org/), MalaCards (https://www.malacards.org/),

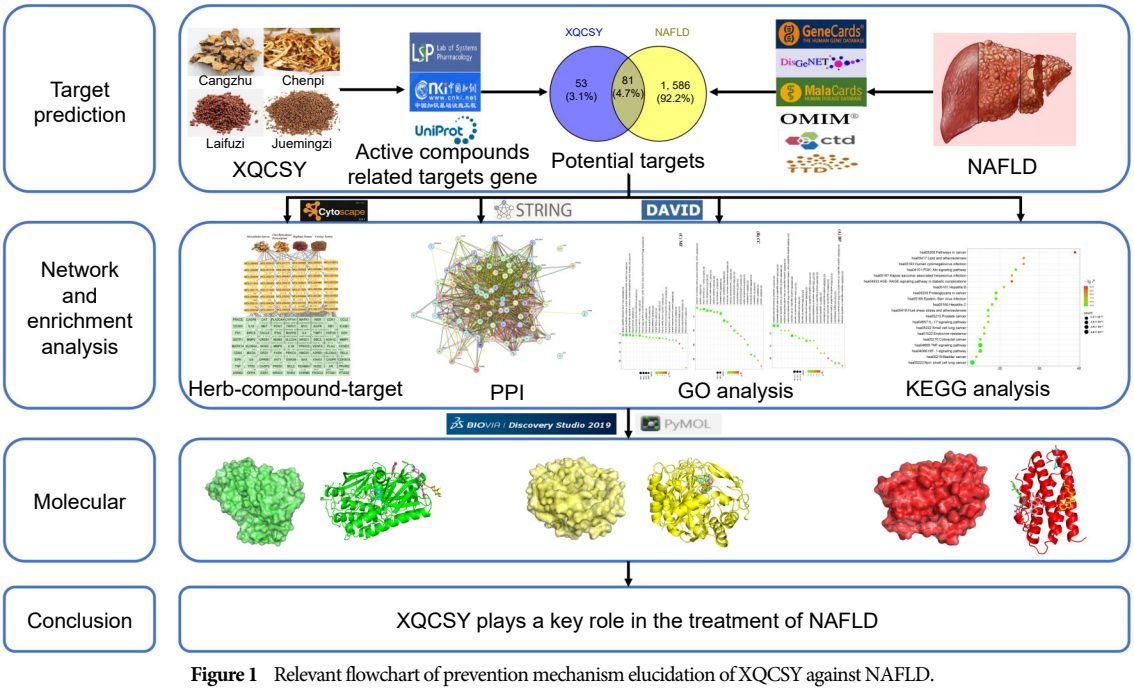

Figure 1 Relevant flowchart of prevention mechanism elucidation of XQCSY against NAFLD.

CTD (http://ctdbase.org/), and TTD (http://db.idrblab.net/ttd/). Then, the duplicates were deleted after the targets were merged from the databases above. Besides, the targets of NAFLD and XQCSY and their common targets were acquired via Venny 2.1.0.

2.3 Construction of "herb-compound-common target" network

To construct the "herb-compound-common target" network, the herbs, active compounds, and common targets of XQCSY were imported into Cytoscape 3.9.1[45]. Then, to analyze the network systematically, various plug-ins were applied, such as CytoHubba app[46], Centiscape2.2[47], and NetworkAnalyzer[48].

2.4 Topological analysis of protein-protein interaction (PPI) network

The overlapped targets between NAFLD and XQCSY were introduced into the STRING network platform (https://stringdb.org/) to construct a PPI network. The parameter was set to be "*Homo sapiens*", and the protein type was set as the rating criterion (confidence level > 0.4). Afterwards, the PPI information was imported into Cytoscape 3.9.1 before the significant topology parameters of the network were calculated using the CytoHubba app. Subsequently, the betweenness, closeness, and the degree between nodes were calculated on Centiscape 2.2.

2.5 Gene Ontology (GO) enrichment analyses and Kyoto Encyclopedia of Genes and Genomes (KEGG) signal pathway

To further explore the role of the above screening targets with biological signaling pathways, the core targets of XQCSY in NAFLD treatment were subjected to GO[49] and KEGG[50] signal pathway enrichment analyses via David 6.8 (https://david.ncifcrf.gov/). In this way, the specific mechanism was uncovered through biological processes (BP), cellular components (CC), molecular functions (MF), and key signaling pathways. The result set was sorted as per −lg *P*, and the top 20 GO and KEGG pathways were screened out for subsequent research. The *P* value is of statistic importance for target enrichment, and $P < 0.05$ indicates the pathway is significant. Consequently, the main signaling pathways involved within the efficacy of XQCSY were obtained by screening the target genes with $P < 0.05$. A lower *P* value implies more crucial enrichment.

2.6 Molecular docking

Molecular docking was adopted via AutoDock Vina to further clarify the link between active components and key genes and to access the intensity and mode of interaction[51]. On basis of the degrees from section 2.3, six targets and active ingredients with the highest degrees were selected for molecular docking. The crystal structures of RAC-alpha serine/threonine-protein kinase (AKT1), tumor necrosis factor (TNF), interleukin-6 (IL6), interleukin-1 beta (IL1B), cellular tumor antigen p53 (TP53), and epidermal growth factor receptor (EGFR) were obtained from RCSB Protein Data Bank. The proteins were discarded off water and added with polar hydrogens and demanded charges via AutoDock Tools. Then the minimum binding energy was searched via Autodock Vina. Generally, a negative value of binding energy represents a stable level of ligand binding to the receptor[52]. The binding energy ≤ −5 kcal/mol represents the ligand and receptor can bind spontaneously. The binding effect is better when the score is higher. The receptor protein was dehydrated on PyMOL 2.4.0 and Discovery Studio 2018.

3 Results and discussion

3.1 Screening active compounds and related genes

Totally 48 effective active compounds of XQCSY were obtained on basis of OB ≥ 30% and DL ≥ 0.18 in the TCMSP database. Results show 20 species from *A. lancea*, 7 species from *Citri Reticulatae Pericarpium*, 6 species from *Raphani Semen*, and 18 species from *Cassiae Semen*. Among them, three compounds (*β*-sitosterol, sitosterol, stigmasterol) are from more than one herbs in XQCSY and may play a major anti-NAFLD role. Then the protein targets corresponding to the active components were imported into the Uniport data platform, and 134 active compound targets were left after removing the duplications.

In addition, the GeneCards, DisGeNET, OMIM, MalaCards, CTD, and TTD databases were adopted to identify the reviewed therapeutic targets in NAFLD. Finally, 1,667 targets were acquired, including 992 genes in GeneCards, 447 in DisGeNET, 529 in OMIM, 60 in MalaCards, 87 in CTD, and 14 in TTD by using "non-alcoholic fatty liver disease" as the keyword (Fig. 2B). Among 134 XQCSY-related targets and 1,667 NAFLD-related targets, there are 81 common targets (Fig. 2A), which are considered as the hub targets of the subsequent research.

3.2 Construction and analysis of "herb-compound-common target" network

To comprehensively reveal the molecular mechanisms underlying the anti-NAFLD effects of XQCSY, a "herb-compound-

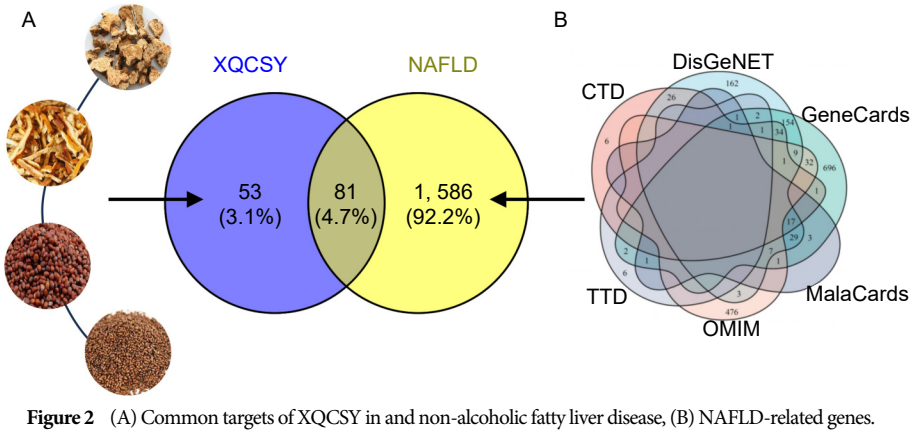

Figure 2 (A) Common targets of XQCSY in and non-alcoholic fatty liver disease, (B) NAFLD-related genes.

common target" network was constructed with 4 herbs, 48 active compounds, and 81 targets of XQCSY (Fig. 3). The orange octagons and green rectangles in the network symbolize active compounds and targets, respectively. The network includes 133 nodes and 306 edges that represent between-node interactions. The network diagram is aimed to clarify the "multicomponent-multitarget-multichannel" mechanism. The basic characteristics of all 48 active constituents are presented in Table S1. NetworkAnalyzer, a Cytoscape plug-in to analyze the network, was adopted to rank the ingredients in the descending order of degrees. The following components have higher degrees: Luteolin (MOL000006, n = 37), wogonin (MOL000173, n = 32), stigmasterol (MOL000449, n = 26), nobiletin (MOL005828, n = 22), aloe-emodin (MOL000471, n = 18), β-sitosterol (MOL000358, n = 17). For instance, stigmasterol and β-sitosterol were proved by Feng *et al.*[53] to potentially alleviate high-fat Western-style diet-induced NAFLD. Chen *et al.*[54] concluded that wogonin had therapeutic effects on NAFLD via the alleviation of inflammatory response and oxidative stress. References show all the top 6 components (luteolin, wogonin, stigmasterol, nobiletin, aloe-emodin, β-sitosterol) can be detected in blood[55-60]. In addition, many other components of XQCSY can also be detected in blood, but the concentration and existing forms may vary with individual differences[61,62].

3.3 Topological analysis of PPI network and selection of key targets

The 81 common targets between XQCSY and NAFLD were imported into STRING 12.0 to obtain PPI (Fig. 4A). In total, the PPI network involved 80 nodes and 1,226 interactive associations, with average node degree of 30.6, and average local clustering coefficient of 0.723, and 490 interactive proteins with the PPI enrichment $P < 1.0 \times 10^{-16}$. Then to acquire the network diagram of target interaction, the PPI results were introduced into Cytoscape 3.9.1. After being screened, the top 30 targets were

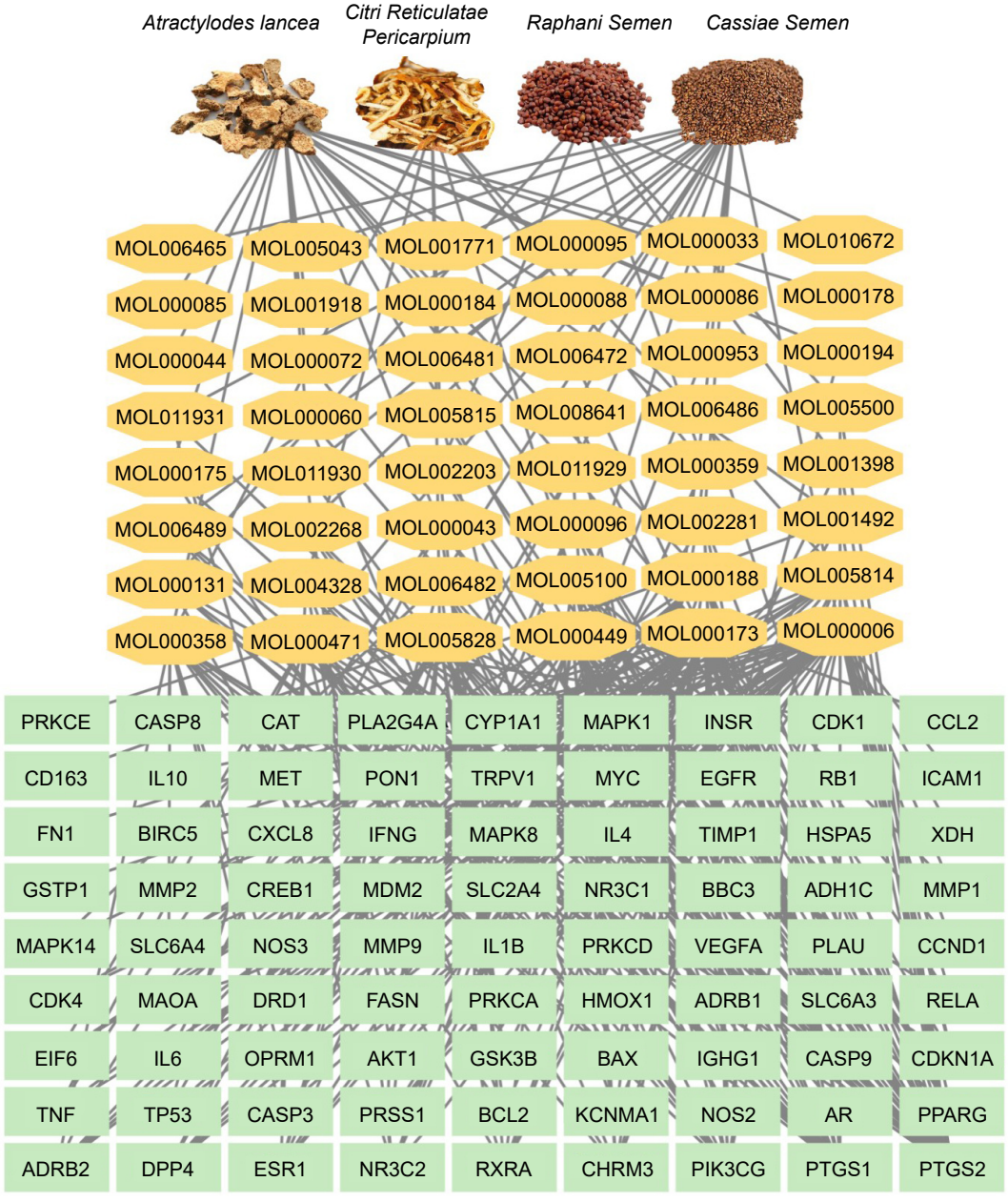

Figure 3 "Herb-compound-common target" network of XQCSY.

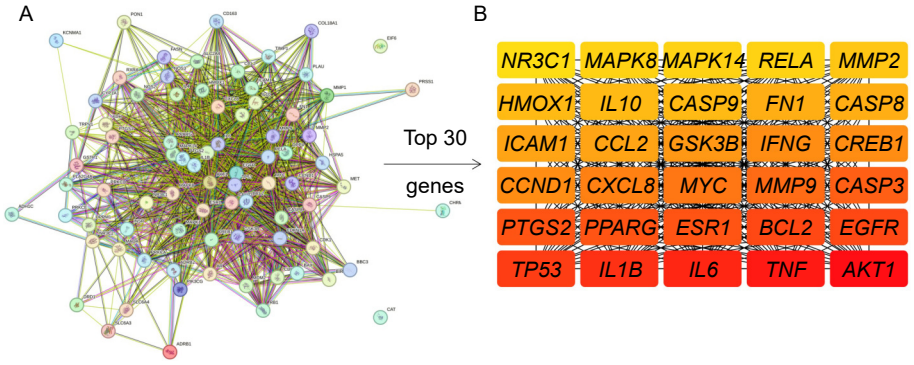

Figure 4 (A) Protein-protein interaction networks of XQCSY for the treatment of NAFLD, (B) Top 30 genes in terms of degree.

shown in Fig. 4B. AKT1 is the most relevant target, followed by *TNF*, *IL6*, *IL1B*, etc. The importance of targets decreases as the degree of red weakens. The findings of our study were in line with those reported in the literature that Akt1 signaling pathway shows the potential to ameliorate NAFLD[63]. Besides, the anti-NAFLD role of TNF, IL6 and IL1B has also been confirmed in the literatures[64-66]. Based on the betweenness, closeness, and degree, AKT1, TNF, IL6, represented the crucial targets of XQCSY, which play a vital role in the anti-NAFLD process. For example, Fang *et al.*[67] found the anti-NAFLD effects were achieved via crosstalk between muscle IL6 production and liver STAT3 activation. Furthermore, Liu *et al.*[68] concluded that AKT1 plays a key role in the activation of pro-inflammatory macrophages and the progress of NAFLD.

3.4 GO enrichment analysis

The characteristics of XQCSY-related targets were investigated through GO enrichment analysis to systematically elucidate the potential mechanism of XQCSY against NAFLD. The GO functions consist of BP, CC, and MF. In the present study, a total of 654 significant GO terms were acquired, including 513 BPs, 51 CCs, and 90 MFs. Subsequently, the top 20 significantly enriched items of BP, CC and MF were selected based on *P* values that represent the degree of enrichment, and a redder dot represents lower *P* value and greater enrichment (Fig. 5).

Based on the above principle, Fig. 5 shows the dots which represents greatest enrichment among BP, CC and MF are response to xenobiotic stimulus (GO: 0009410), macromolecular complex (GO: 0032991) and enzyme binding (GO: 0019899), respectively. In detail, in terms of BP (Fig. 5A), the therapeutic effects of XQCSY on NAFLD are mainly due to response to xenobiotic stimulus (GO: 0009410), negative regulation of apoptotic process (GO: 0043066), positive regulation of gene expression (GO: 0010628), positive regulation of apoptotic process (GO: 0043065), positive regulation of nitric oxide biosynthetic process (GO: 0045429). NasiriAnsari *et al.*[69] conducted experiments and found that the hepatocyte apoptotic process can be inhibited by empagliflozin treatment so as to treat NAFLD. Besides, evidence showed response to xenobiotic stimulus destroys metabolic homeostasis, and some of them are risk factor candidates for NAFLD[70]. In terms of CC (Fig. 5B), XQCSY may influence macromolecular complex (GO: 0032991), mitochondrion (GO: 0005739), cytosol (GO: 0005829), extracellular region (GO: 0005576), extracellular space (GO: 0005615), and so on. Studies have confirmed macromolecular complex[71], mitochondrion[72], cytosol[73] are related to the regulation of NAFLD, thereby demonstrating the anti-NAFLD potential of XQCSY. For MF (Fig. 5C), the targets were mostly participated in enzyme binding (GO: 0019899), identical protein binding (GO: 0042802), protein kinase activity (GO: 0004672), protease binding (GO: 0002020), and protein binding (GO: 0005515). Ferreira *et al.*[74] conducted experiments and found that the enzyme binding plays an important role in regulating NAFLD. Additionally, Ahmed *et al.*[75] discovered that sterol regulatory element binding proteins show the potential to regulate hepatic lipid metabolism, which is the key to the anti-NAFLD process. In conclusion, the results above indicate the multiple synergies of XQCSY on BP.

3.5 KEGG pathway enrichment analysis

The KEGG pathways of XQCSY were investigated via Metascape to further understand the NAFLD treatment mechanism of the compound. There were 172 significant NAFLD-XQCSY-related pathways, and the top 20 KEGG pathways retrieved based on *P* values were displayed in Fig. 6. Results show XQCSY plays a key role in preventing NAFLD through multiple targets and pathways. The KEGG analysis illustrates the main pathways related to NAFLD are Pathways in cancer, AGE-RAGE signaling pathway in diabetic complications, Lipid and atherosclerosis, Human cytomegalovirus infection, and Kaposi sarcoma-associated herpesvirus infection. Specifically, the AGE-RAGE signaling pathway increases the fat accumulation in the liver, which leads to inflammation, fibrosis, insulin resistance and other complications of NAFLD[76]. Moreover, Lipid and atherosclerosis increase the risk of NAFLD[77]. In a word, XQCSY may exert its anti-NAFLD effects by influencing the above-mentioned pathways.

3.6 Molecular docking

To figure out the potential anti-NAFLD action of XQCSY, molecular docking was adopted to study the binding behavior between active compounds and hub targets[78]. On basis of degrees, the top three targets (AKT1, TNF, IL6) and six components (luteolin, wogonin, stigmasterol, nobiletin, aloe-emodin, β-sitosterol) were chosen as receptors and ligands for molecular docking via AutoDock Vina, respectively. The 3D structures of AKT1 (PDB ID: 1unq), TNF (PDB ID: 1vyr), and IL6 (PDB ID: 1alu) were acquired from RCSB Protein Data Bank. Figure 7 displays the theoretical binding sites and conformations of the hub targets of XQCSY. The interaction was conducted mainly through eight forms: hydrogen bond, unfavorable donor–donor, C–H, van der Waals, pi–sigma, pi–cation, pi–alkyl, and alkyl. The docking binding energy (kcal/mol) was demonstrated in Fig. 8. The ranges of binding energy for each target protein were: AKT1 (−7.3–−5.7 kcal/mol), TNF (−9.8–−8.3 kcal/mol), and IL6 (−7.8–−6 kcal/mol). The structure is more stable when the binding

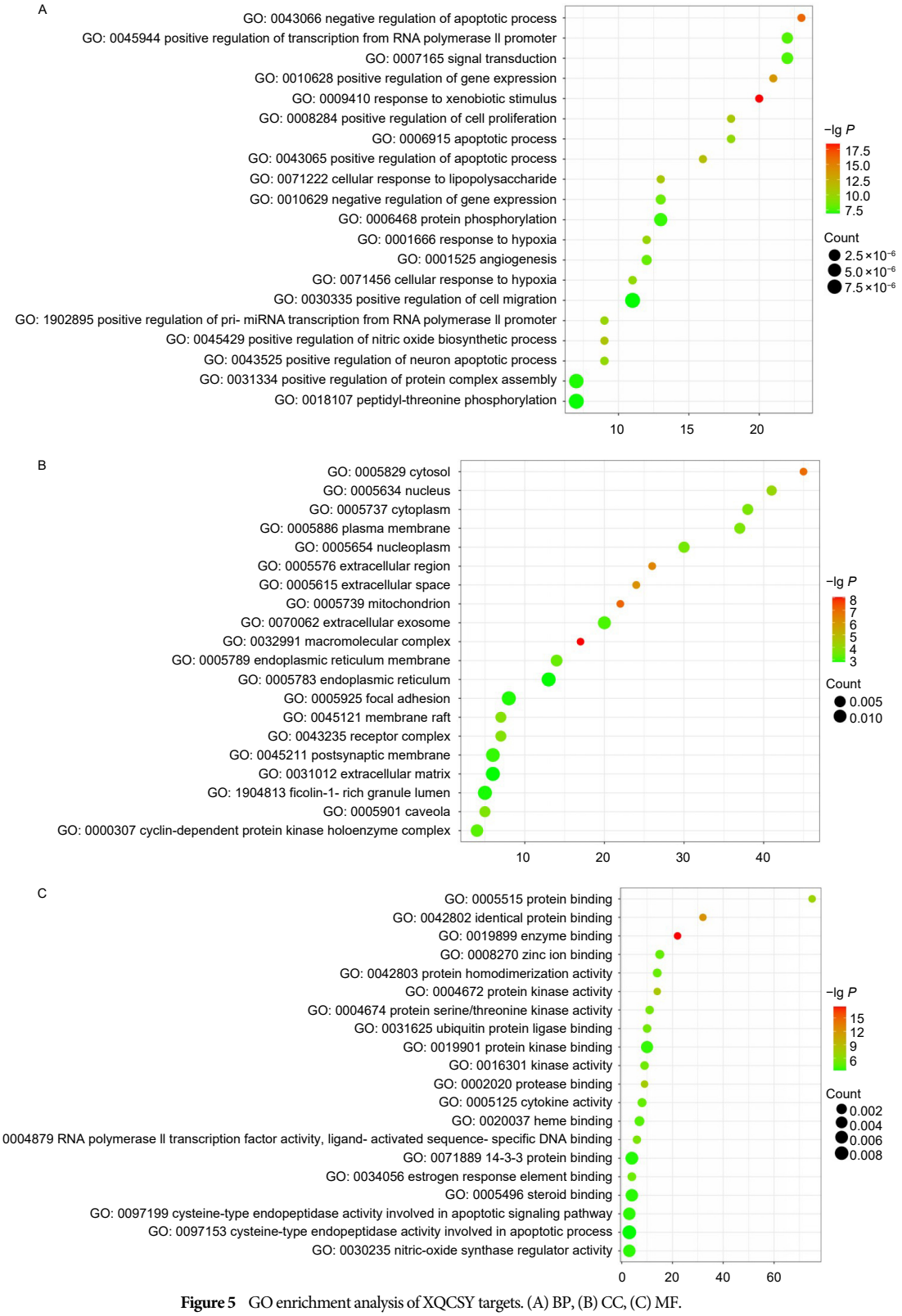

Figure 5 GO enrichment analysis of XQCSY targets. (A) BP, (B) CC, (C) MF.

energy is lower. In general, the observed free energy changes of binding between the key targets and the corresponding components are all below −6 kcal/mol. It is suggested the hub targets including AKT1, TNF, and IL6 can interact well with luteolin, stigmasterol, wogonin, β-sitosterol, nobiletin, tangeretin, and aloe-emodin, respectively. Besides, TNF is more affinitive to the six core bioactive components, indicating TNF may be critical for the potential molecular mechanisms of NAFLD treatment. Research shows that phytochemicals may affect the activities of targets in various ways. For example, aloe-emodin induces mitochondrial dysfunction and pyroptosis by activating caspase-3 in cells[79]. In addition, the components may also bind to cytokine receptors, thus affecting the targets. Stigmasterol and tangeretin have been proved to be effective in inhibiting the activity of IL6

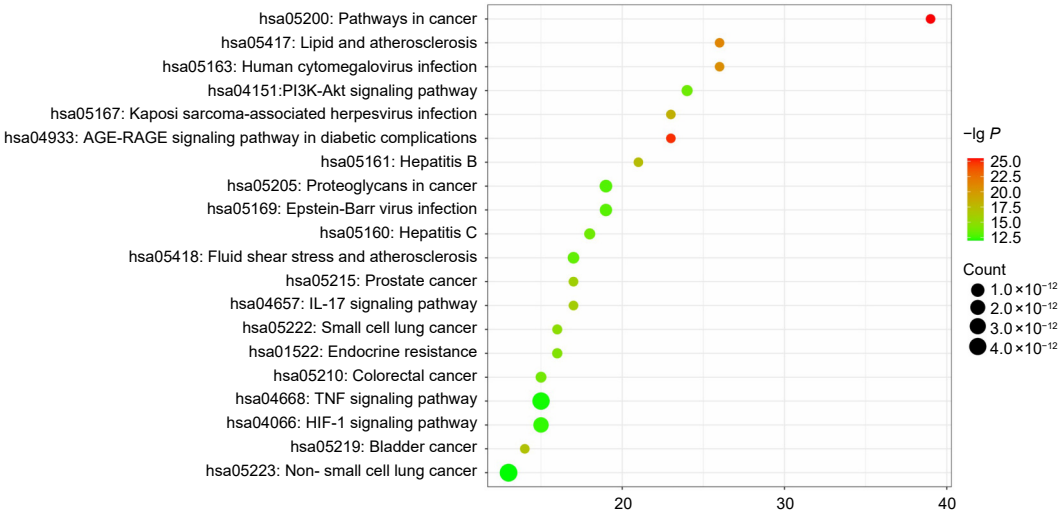

Figure 6 Pathways enrichment analysis of XQCSY targets.

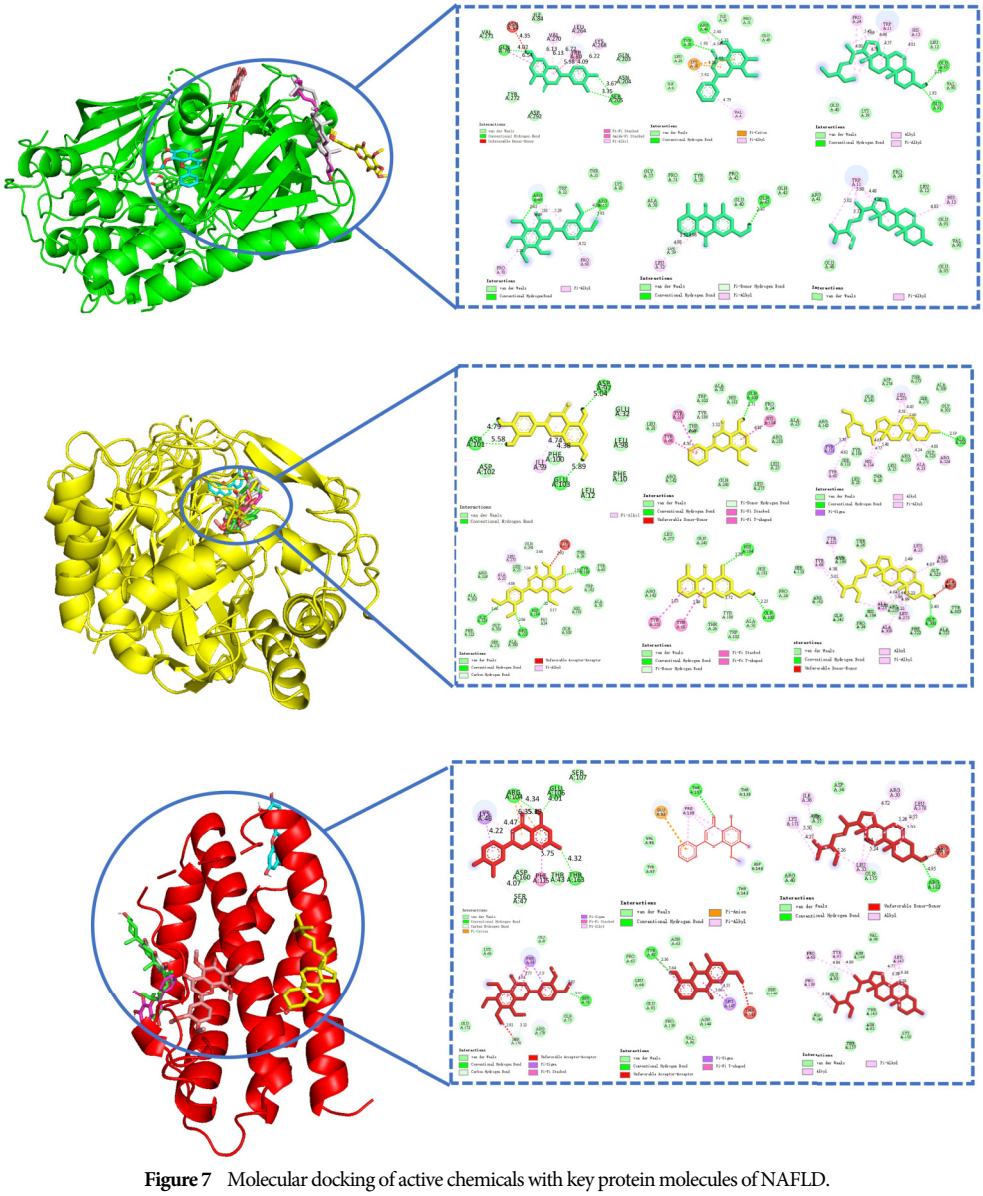

Figure 7 Molecular docking of active chemicals with key protein molecules of NAFLD.

and TNF receptors, respectively[80, 81]. Although the above phytochemicals show different mechanisms on the targets, they all show the potential in anti-NAFLD and lay a foundation for exploring the biological activities and potential applications.

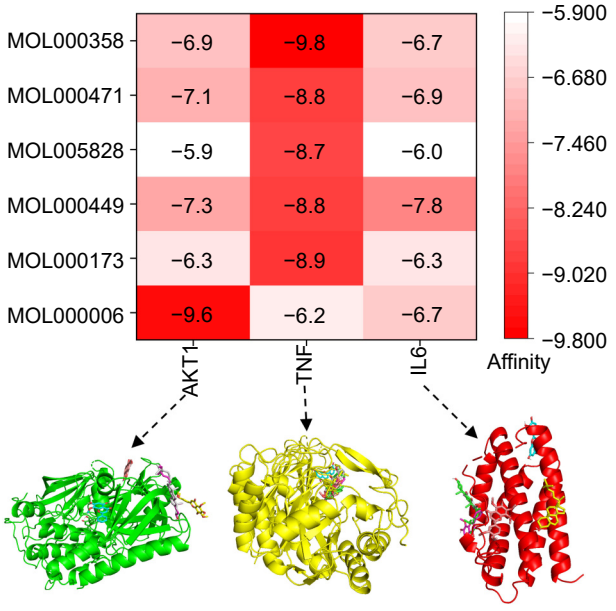

Figure 8 Heatmap of top six targets in the protein-protein interaction network and the top six components of XQCSY. The colors range from white to red represents the docking binding correlation range from negative to positive.

4 Conclusion

This study provides a reference to probe into the mechanism of XQCSY in preventing NAFLD. The results clearly prove the potential therapeutic effects of XQCSY on NAFLD, with a multi-compound, multi-target and multi-pathway mode of action. Besides, active ingredients from XQCSY are well affinitive to the key genes, among which AKT1, TNF, and IL6 plays the most important part and thus greatly affect NAFLD prevention. In conclusion, the results provide theoretical support for XQCSY to treat NAFLD. In the long run, it may also yield practical therapeutic results of great value.

Conflicts of interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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Electronic Supplementary Material (ESM)

The online version contains supplementary material available at https://doi.org/10.26599/FMH.2024.9420015.

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