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**CLINICAL RESEARCH**

# Study on Hyperlipidemia Mechanism of Lotus Leaves by Network Pharmacology and Molecular Docking

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## ABSTRACT

### OBJECTIVE

Based on network pharmacology and molecular docking, analyzing the mechanism of lotus leaves regulating hyperlipidemia.

### METHODS

The main active chemical components and targets of lotus leaves were obtained though the TCMSP database and screened by ADME; The main targets of hyperlipidemia were obtained by Genecards, OMIM, TTD and DRUGBANK databases; Protein interactions were analyzed through the STRING database; Finding potential protein functions by constructing a PPI network; Metascape software was used to analyze “drug-component-target” and the biological processes and pathways involved. Build a “lotus leaves component-hyperlipemia target” network though Cytoscape 3.7.1 software. Molecular docking verification was performed by Autodock tools software.

### RESULTS

The main active ingredients of lotus leaves regulation of hyperlipidemia are Quercetin, Kaempferol, Isorhamnetin, etc.; The main targets are TNF, IL1B, IFNG, IL-6, CCL2, ICAM1, MAPK14, CXCL8, SELE, VCAM1, AKT1, FOS, IL-10, etc.; The biological pathway of lotus leaves regulating hyperlipidemia mainly acts on HIF-1 signaling pathway, Apelin signaling pathway, PPAR signaling pathway, etc. The functions are mainly to regulate hormone response, cell response to lipid, inflammatory response, smooth muscle cell proliferation response, etc. The binding energy of molecular docking is <-5 KJ/mol (-1.2 KJ/mol), indicating that the target and the composition are more binding.

**CONCLUSION**

The preliminary study shows that lotus leaves regulation of hyperlipidemia have a multi-component, multi-target and multi-pathway mechanism, which provides data basis for traditional Chinese medicine to regulate hyperlipidemia.

## KEYWORD

Lotus leaves; Pharmacology network; Hyperlipidemia; Target prediction; Molecular docking

## INTRODUCTION

Hyperlipidemia, also known as "dyslipidemia", usually refers to elevated levels of plasma triglycerides, total cholesterol, and LDL cholesterol, and reduced levels of HDL cholesterol. The Guidelines for the Prevention and Treatment of Dyslipidemia in Chinese Adults (2016 Revision) [1] states that the overall prevalence of dyslipidemia in Chinese adults is as high as 40.40%, which significantly increases the incidence of atherosclerosis, stroke, coronary heart disease, hypertension, diabetes and other diseases. Currently approved and marketed drugs can reduce lipid levels in hyperlipidemic populations, but there are still problems of drug intolerance and low compliance, leading to poor prognosis. The regulation of hyperlipidemia by drugs and food has potential advantages, and its regulatory mechanism is characterized by multi-target, stable efficacy and high safety.

Cyberpharmacology can systematically reflect the intervention mechanism of drugs on diseases, and is a basic research method of medicine, biology and other multidisciplinary disciplines, which helps to elucidate the mechanism of action and drug targets of drugs and food, and guides the development of drugs and food and the development of theoretical research of Chinese medicine. Molecular docking is a theoretical simulation method that mainly studies molecule-molecule interactions and predicts their binding modes and affinity, and is a key technology in the field of computer-aided drug research. In this study, we used network pharmacology and molecular docking validation to analyze the mechanism of lotus leaf in regulating hyperlipidemia based on the material basis of lotus leaf, and provide the theoretical basis for the subsequent research.

## MATERIALS AND METHOD

### *Target Screening of Active Ingredients of Lotus Leaves*

The chemical composition of lotus herbal medicine was obtained through the pharmacology platform of TCM system, and the active ingredients and their protein action targets were obtained by preliminary screening based on two ADME properties (oral utilization ≥30% and drug-like properties ≥0.18), and the unavailable active ingredients and their protein action targets were supplemented by reference to literature reports. To ensure the standardization of the protein action targets, the Uniprot database was used to standardize the protein action target information after the screening.

### *Hyperlipidemia-Related Target Screening*

Using "hyperlipidemia" and "dyslipidemia" as keywords, hyperlipidemia target information was obtained from Gencards, OMIM, TTD and DRUGBANK databases. The targets from the four databases were combined and duplicate items were removed to obtain the hyperlipidemia target collection.

***Construction of PPI Network of Active Ingredient-Hyperlipidemic Targets of Lotus Leaves***

To clarify the interactions between the active ingredient targets of lotus leaf and hyperlipidemic targets, the common targets of the active ingredient targets of lotus leaf and hyperlipidemic targets were screened in R language, and Venn diagrams were drawn, and the common targets were submitted to the String database to construct the PPI network model, setting the species as "homo sapiens", and further analyzed with Cytoscape 3.1 PPI network. 7.1 to obtain protein functions and characterize the protein functions by analyzing the biological processes involved in the protein function.

***Lotus Leaf Active Ingredient-Hyperlipidemia Target and Pathway Enrichment Analysis***

The targets of lotus leaf to modulate hyperlipidemia were submitted to Metascape software, P range was set, enrichment analysis was performed for major biological processes and metabolic pathways, and the data results were saved for visualization and analysis.

***Construction of Active Ingredient-Hyperlipidemia Target Map of Lotus Leaves***

Cytoscape software was applied to construct the active ingredient-hyperlipidemia target map of lotus leaf, analyze the network topology parameters (connectivity, mediocrity, tightness, etc.) of active ingredients and targets, and identify the core targets and the main active ingredients that exert drug effects according to the network topology parameters.

***Validation of Molecular Docking***

The targets in the lotus leaf component-hyperlipidemia network were ranked from highest to lowest Degree value, and the top 5 targets were potential targets of lotus leaf for regulating hyperlipidemia. The 3D structures of the potential targets were obtained from the RCSB PDB database, and the mol2 files of the main active ingredients of lotus leaf were obtained from the TCMSP database. The targets and active ingredients were pre-processed with AutoDock tools software, molecular docking was performed, binding energy was calculated, binding intensity was analyzed, and then the results were visualized.

## RESULTS

***Obtaining the Targets of Active Ingredients of Lotus Leaf***

Initially, 93 chemical components of lotus leaf were obtained, and 14 chemical components of lotus leaf were obtained after ADME screening, including quercetin, kaempferol, glutathione, etc., (Table 1). 168 action targets of lotus leaf chemical components were obtained.

**Table 1:** Active components of lotus leaves in TCMSP for hyperlipidemia.

| No. | Molecule ID | Active Ingredients | MW | OB ≥30% | DL ≥0.18 |
|---|---|---|---|---|---|
| HY1 | MOL007207 | Machiline | 285.37 | 79.64 | 0.24 |
| HY2 | MOL007206 | Armepavine | 313.43 | 69.31 | 0.29 |
| HY3 | MOL000096 | (-)-catechin | 290.29 | 49.68 | 0.24 |
| HY4 | MOL000354 | Isorhamnetin | 316.28 | 49.60 | 0.31 |
| HY5 | MOL000073 | Ent-Epicatechin | 290.29 | 48.96 | 0.24 |
| HY6 | MOL000098 | Quercetin | 302.25 | 46.43 | 0.28 |
| HY7 | MOL000422 | Kaempferol | 286.25 | 41.88 | 0.24 |

| | | | | | |
|---|---|---|---|---|---|
| HY8 | MOL007218 | Remerin | 279.36 | 40.75 | 0.52 |
| HY9 | MOL003578 | Cycloartenol | 426.8 | 38.69 | 0.78 |
| HY10 | MOL007214 | (+)-Leucocyanidin | 306.29 | 37.61 | 0.27 |
| HY11 | MOL000359 | Sitosterol | 414.79 | 36.91 | 0.75 |
| HY12 | MOL007213 | Nuciferin | 295.41 | 34.43 | 0.40 |
| HY13 | MOL007210 | o-Nornuciferine | 281.38 | 33.52 | 0.36 |
| HY14 | MOL007217 | Leucodelphinidin | 322.29 | 30.02 | 0.31 |

***Hyperlipidemia-Related Targets Obtained***

1309 dyslipidemic targets, 1632 hypercholesterolemic targets, 1535 hypertriglyceridemic targets and 8 hyperlipidemic targets were obtained from Genecards database, and targets with scores greater than the median were set as hyperlipidemia-related targets. Combining OMIM, TTD and DRUGBANK databases to supplement target information, merging target information and removing duplicate values, 782 hyperlipidemia-related targets were obtained.

***Construction of PPI Network of Active Ingredient-Hyperlipidemia Targets of Lotus Leaves***

The intersection of active ingredient targets of lotus leaf and hyperlipidemia-related targets was obtained, and 63 common targets of lotus leaf active ingredient-hyperlipidemia were obtained by Venn diagram in R language (Figure 1). 63 common targets of lotus leaf active ingredient-hyperlipidemia were submitted to String database, and PPI network of lotus leaf targets was constructed (Figure 2). In order to clarify the mechanism of action of lotus leaf in treating hyperlipidemia, the PPI network obtained needs to further identify the intrinsic Using Cytoscape, the interactions were analyzed by molecular complex detection algorithm to obtain the module (Figure 3). based on the P value, the PPI network was retained with the three highly scored biological processes in the module and their functions were described (Table 2).

**Table 2:** Functional description of PPI network diagram of active ingredient-hyperlipidemic targets of lotus leaves.

| GO | Description | Log10(P) |
|---|---|---|
| GO: 0009725 | Response to Hormone | -27.8 |
| GO: 0071396 | Cellular Response to Lipid | -24.5 |
| GO: 0032496 | Response to Lipopolysaccharide | -23.9 |

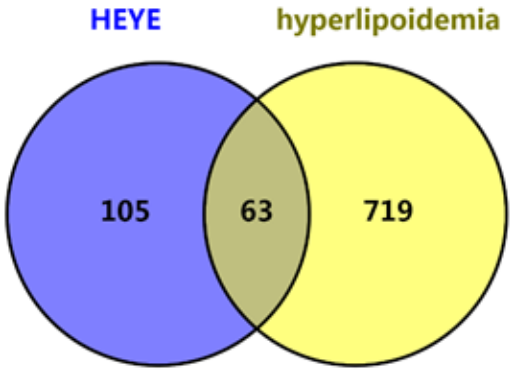


**Figure 1:** Venn diagram of active ingredient-hyperlipidemic target of lotus leaf.

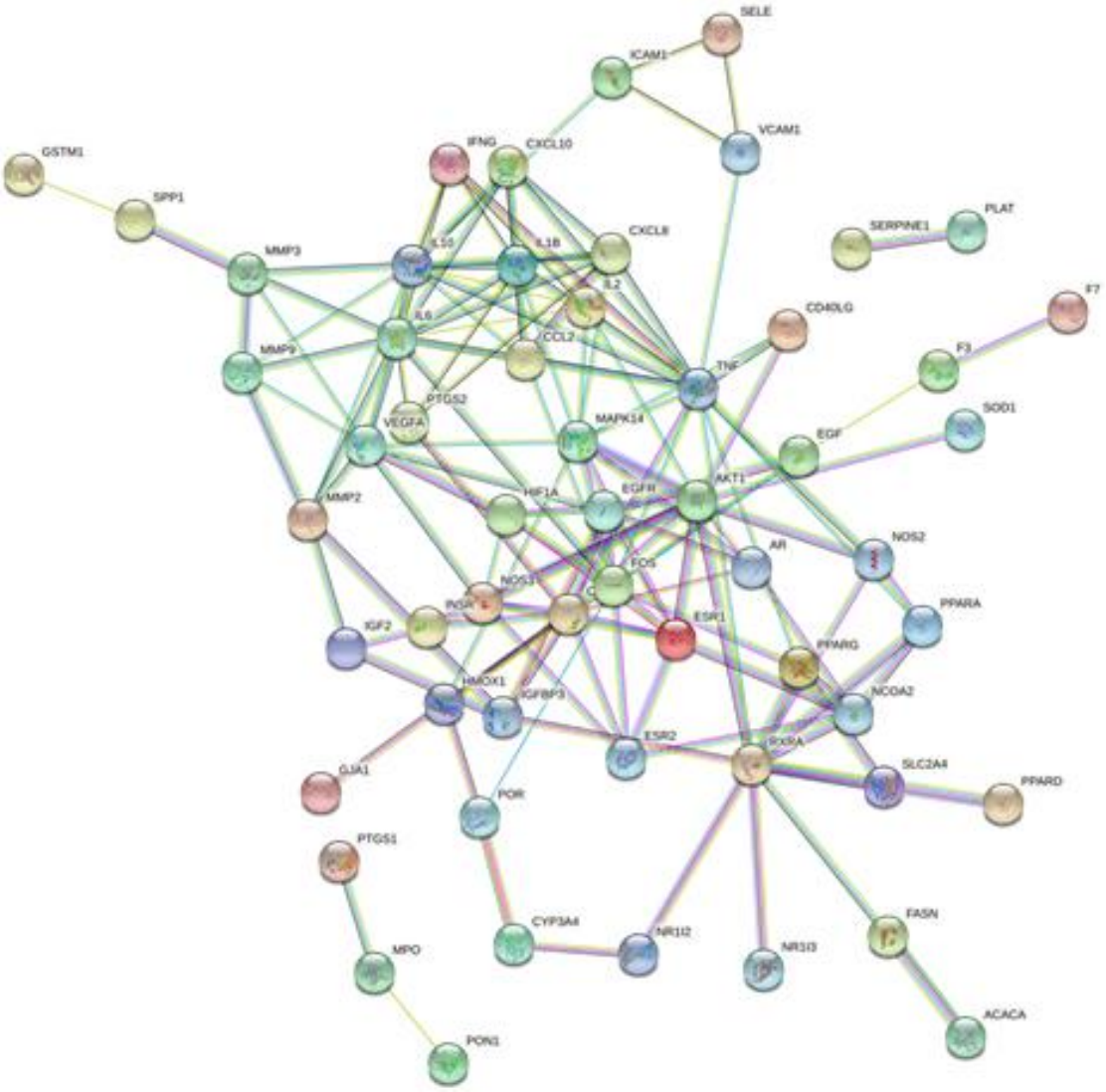


**Figure 2:** PPI network diagram of active ingredient-hyperlipidemia target of lotus leaf.

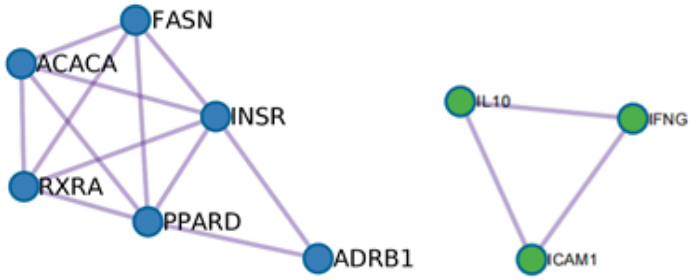


**Figure 3:** Active ingredient of lotus leaf - hyperlipidemia target PPI network diagram in module.

***Enrichment Analysis of Hyperlipidemic Targets and Pathways***

The signaling pathways of hyperlipidemia-related targets of lotus leaf were analyzed by Metascape software, and the results were visualized and analyzed. The data showed that multi-target functions are closely related to the development of hyperlipidemia, and lotus leaves are mainly involved in biological processes such as hormonal responses, cellular responses to lipids, positive regulatory processes of small molecule metabolism, regulation of apoptotic signaling pathways, and cell adhesion regulatory effects; cellular composition such as endoplasmic

reticulum lumen early endosomes; molecular functional binding such as phosphatase-binding proteases, serine-type endopeptidase-activated steroids; involvement in HIF-1, cancer, insulin resistance, PPAR and other signaling pathways (Figure 4).

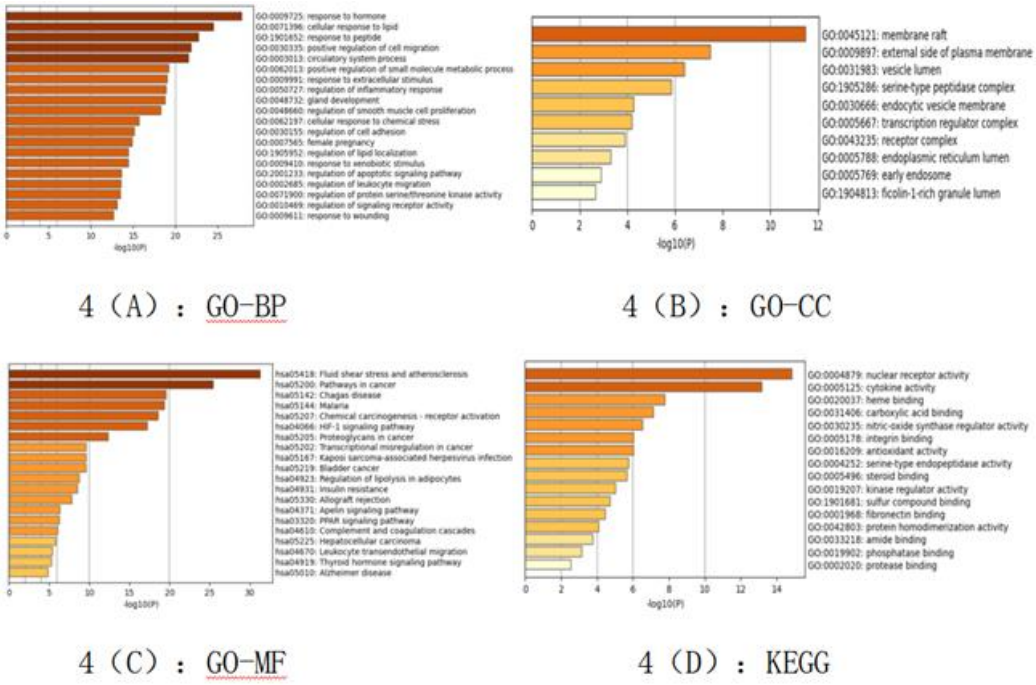


**Figure 4:** Target enrichment analysis of active ingredients of lotus leaves.

***Construction of Network Diagram of Active Ingredients-Hyperlipidemia Targets of Lotus Leaf***

The active ingredient-hyperlipidemia target network map of lotus leaf was constructed using Cytoscape software (Figure 5). Network Analyzer was used to analyze the topological parameters of lotus leaf network for regulating hyperlipidemia, and core components and core targets of action were obtained (Figure 6). Betweenness centrality value of 0.05306884 and CC (Closeness centrality) value of 0.53725166; Hordein Degree value of 823, BC value of 0.02666347 and CC value of 0.5252934; Catechin degree value of 764 and BC value of 0.02646775 and CC value of 0.52449742; kaempferol Degree value of 453, BC value of 0.00491657 and CC value of 0.48243821; isorhamnetin Degree value of 217, BC value of 0.00651376 and CC value of 0.46319921 (Table 3).

TNF had a connectivity value of 113 in the component-target network, a mediator value of 0.03, and a tight connectivity value of 0.41, thus predicting TNF to be an important target for the regulation of hyperlipidemia in Hordeum vulgare. In addition, IL1B, IFNG, IL-6, CCL2, ICAM1, MAPK14, CXCL8, SELE, VCAM1, AKT1, FOS, and IL-10 were also important targets (Table 4).

**Table 3:** Characteristic parameters of main active components of lotus leaf.

| MOL ID | Active components | GENE SYMBOL | Degree | BC | CC |
|---|---|---|---|---|---|
| MOL000098 | Quercetin | EGFR | 934 | 0.05306884 | 0.53725166 |
| MOL007213 | Nuciferin | TP53 | 823 | 0.02666347 | 0.5252934 |
| MOL000096 | (-)-Catechin | ESR1 | 764 | 0.02646775 | 0.52449742 |
| MOL000422 | Kaempferol | VCAM1 | 453 | 0.00491657 | 0.48243821 |
| MOL000354 | Isorhamnetin | FOS | 217 | 0.00651376 | 0.46319921 |

**Table 4:** Enrichment results of the target pathway for hyperlipidemia modulation by lotus leaves.

| GO | Description | LogP | Enrichment | Hits |
|---|---|---|---|---|
| hsa05418 | Fluid shear stress and atherosclerosis | -31.30410345 | 68.99851547 | AKT1\|CCL2\|MAPK14\|FOS\|GSTM1\|GSTP1\|HMOX1\|ICAM1\|IFNG\|IL1B\|MMP2\|MMP9\|NOS3\|PLAT\|CAV1\|SELE\|THBD\|TNF\|VCAM1\|VEGFA |
| hsa04933 | AGE-RAGE signaling pathway in diabetic complications | -25.72088799 | 76.72634921 | AKT1\|IL1B\|IL6\|MAPK14\|F3\|ICAM1\|CXCL8\|MMP2\|NOS3\|SERPINE1\|CCL2\|SELE\|THBD\|TNF\|VCAM1\|VEGFA |
| hsa05200 | Pathways in cancer | -25.42726401 | 21.67410995 | IFNG\|AR\|EGF\|EGFR\|ESR1\|ESR2\|FOS\|GSTM1\|GSTP1\|HIF1A\|HMOX1\|AKT1\|IGF2\|IL2\|IL6\|CXCL8\|MMP2\|MMP9\|NOS2\|PPARD\|PPARG\|PTGS2\|RXRA\|VEGFA |
| hsa05417 | Lipid and atherosclerosis | -23.68281036 | 40.14750831 | AKT1\|CD40LG\|MAPK14\|FOS\|ICAM1\|IL1B\|IL6\|CXCL8\|MMP3\|MMP9\|NOS3\|OLR1\|PPARG\|RXRA\|CCL2\|SELE\|TNF\|VCAM1 |
| hsa04668 | TNF signaling pathway | -20.89177238 | 59.94246032 | AKT1\|MAPK14\|FOS\|ICAM1\|IL1B\|IL6\|CXCL10\|MMP3\|MMP9\|PTGS2\|CCL2\|SELE\|TNF\|VCAM1 |
| hsa04920 | Adipocytokine | -19.51544382 | 61.11780268 | AKT1\|MAPK14\|FOS\|IFNG\|IL1B\|IL2\|IL6\|CXCL8\|IL10\|NOS2\|SERPINE1\|CCL2\|TNF |
| hsa05144 | Malaria | -19.34486735 | 105.4987302 | CD40LG\|ICAM1\|IFNG\|IL1B\|IL6\|CXCL8\|IL10\|CCL2\|SELE\|TNF\|VCAM1 |
| hsa05207 | Chemical carcinogenesis - receptor activation | -18.56719065 | 33.92969452 | ADRB1\|ADRB2\|AKT1\|AR\|CYP3A4\|EGF\|EGFR\|ESR1\|ESR2\|FOS\|GSTM1\|PPARA\|RXRA\|VEGFA\|NR1I3 |
| hsa04657 | IL-17 signaling pathway | -18.03103515 | 61.21783181 | MAPK14\|FOS\|IFNG\|IL1B\|IL6\|CXCL8\|CXCL10\|MMP3\|MMP9\|PTGS2\|CCL2\|TNF |
| hsa04066 | HIF-1 signaling pathway | -17.22418433 | 52.79335955 | AKT1\|EGF\|EGFR\|HIF1A\|HMOX1\|IFNG\|IL6\|INSR\|NOS2\|NOS3\|SERPINE1\|VEGFA |

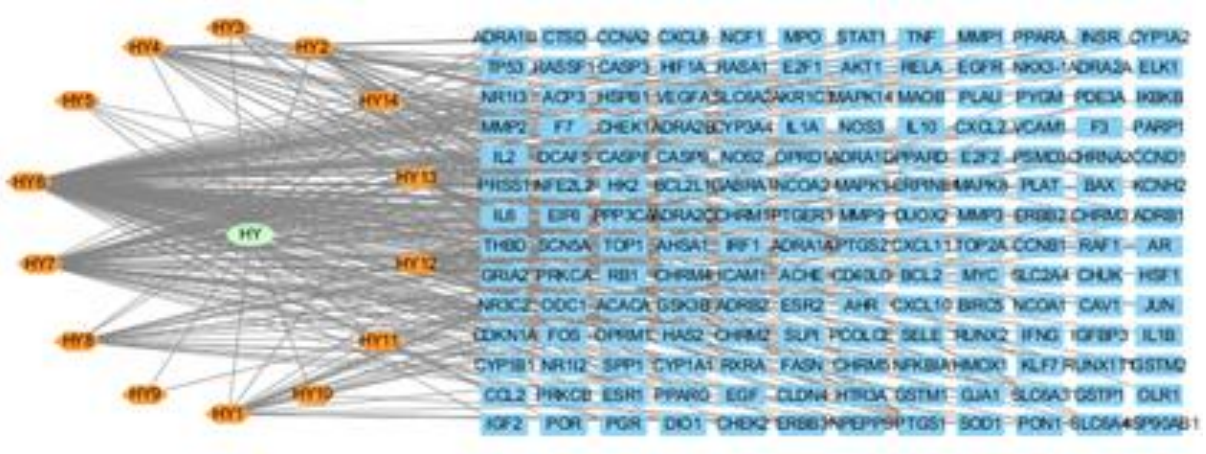


**Figure 5:** Network map of lotus leaf active ingredient-hyperlipidemia target.

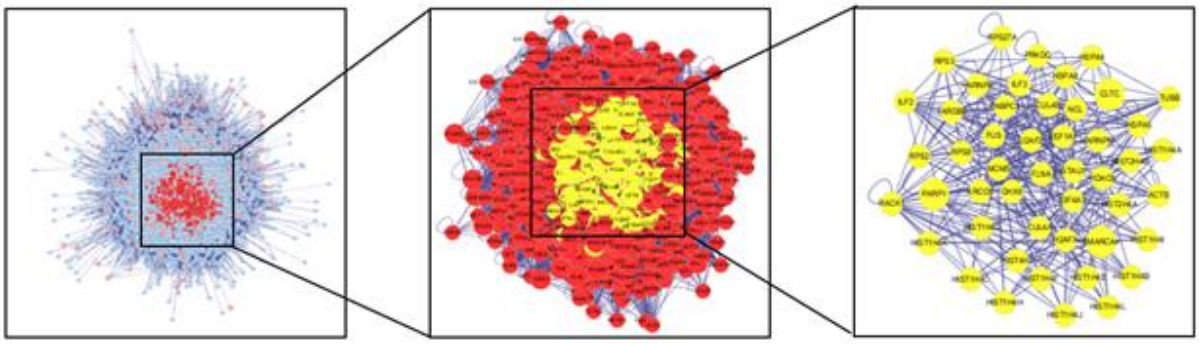

**Figure 6:** Network topological analysis diagram of lotus leaf regulated hyperlipidemia.

***Molecular Docking Validation***

The top five targets with higher Degree values, namely quercetin, nuciferin, catechin, Kaempferol, and isorhamnetin, were selected as potential targets for the regulation of hyperlipidemia in lotus leaves, and those closely related to the development of hyperlipidemic disease (TNF, IL1B, IFNG, IL-6, CCL2, IL-10). The molecular docking of TNF, IL1B, IFNG, IL-6, CCL2 and IL-10 was verified. The molecular docking binding energy was <-7 in 20%, binding energy >-7 and <-6 in 33%, binding energy >-6 and <-5 in 23%, and binding energy >-5 in 23%. Docking binding energy <0 means that docking is possible in the natural state, and docking binding energy <-5 KJ/mol (-1.2 KJ/mol) means good docking results [2], and the molecular docking results are shown in Figure 7.

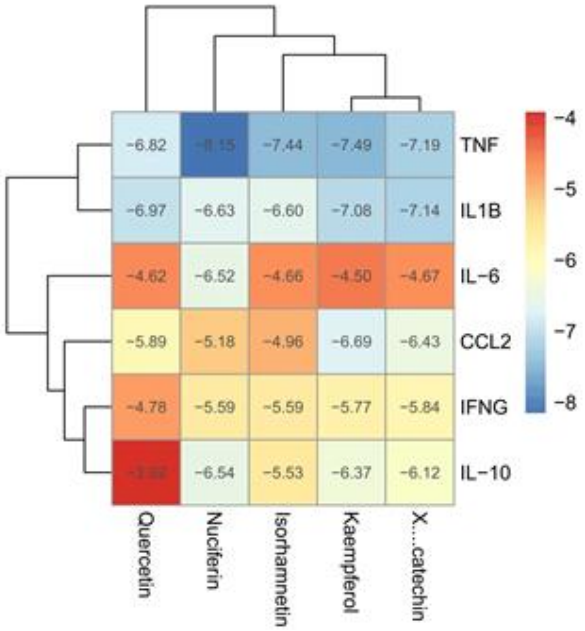


**Figure 7:** Molecular docking results.

## DISCUSSION

In this study, the main potential active components of lotus leaf for the regulation of hyperlipidemia were obtained by applying network pharmacological screening, including quercetin, lotarine, catechin, kaempferol, and isorhamnetin. Studies on the mechanism of hyperlipidemia have also shown that quercetin decreased the gene and protein expression levels of endoplasmic reticulum stress-related molecules IRE1α, s XBP1, and C/EBPα and

C/EBPβ in a concentration-dependent manner, thereby reducing plasma amylase levels and down-regulating the expression of inflammatory factors in hypertriglyceridemic rats [3]. The results of real-time fluorescence quantitative PCR showed that huperzine could significantly down-regulate the expression of genes of HMGCR and SREBP-2, which affect cholesterol anabolism, and up-regulate the expression of LXRα and CYP7A1, which affect cholesterol catabolism, to reduce the serum TC level in mice. Catechins can significantly down-regulate the expression of the genes of ACC and SREBP-1c, proteins that affect fatty acid anabolism, and up-regulate the expression of CPT-1, a protein that affects fatty acid catabolism, to achieve the effect of reducing serum TG levels in mice [4]. Catechins may have a hypolipidemic effect by downregulating the expression of genes related to cholesterol metabolism, NPC1L1 and SREBP-2, and thus reducing cholesterol uptake and accumulation in Caco-2 cells [5]. Continuous administration of kaempferol alone can elevate SOD activity by altering blood rheological parameters and serum MDA concentration, inhibit macrophage foam formation, and reduce lipids in experimental hyperlipidemic animals [6]. Isorhamnetin can alleviate FFA-induced lipid deposition in L-02 cells by reducing oxidative stress, and significantly increase total ROS and MDA levels in cells; inhibit SOD enzyme activity and reduce SOD1, SOD2, and GPx1 expression; inhibiting the Nrf2 pathway, decreasing its downstream HO-1, GCLC, and GCLM expression, promoting Nrf2 activation into the nucleus and increasing its downstream protein expression level [7]. The results of molecular docking validation indicated that quercetin, loticin, catechin, kaempferol, and isorhamnetin bind extremely well to the main targets of hyperlipidemic diseases (TNF, IL1B, IFNG, IL-6, CCL2, IL-10). Therefore, lotus leaves may be regulating hyperlipidemic diseases through quercetin, loticin, catechin, kaempferol, and isorhamnetin.

Studies have shown that the main pathways of lotus leaf that regulate hyperlipidemia include Fluid shear stress and atherosclerosis (hsa05418), diabetic complications AGE-RAGE signaling pathway (hsa04933), cancer signaling pathway (hsa05200), and lipids and atherosclerosis (hsa05417). High-fat and high-sugar diets lead to elevated levels of total cholesterol, total triglycerides, and low-density lipoprotein cholesterol (LDL-C) in the body. Leptin secreted by the white adipose tissue of the body contributes to the activation of CPT-1 signaling molecule by activating JAK2, and the activated JAK2 phosphorylation activates the leptin signaling factor LEPRb. CPT-1 is a rate-limiting enzyme that promotes fatty acid oxidation, reduces fat accumulation and TG synthesis, and decreases the development of hyperlipidemia [8]. Therefore, lotus leaves may act on the Adipocytokine signaling pathway to regulate hyperlipidemic diseases.

The results of the present study suggest that lotus compounds can modulate multiple targets and thus intervene in different biological processes and signaling pathways, demonstrating the synergistic multi-target and multi-pathway action of lotus leaves in regulating hyperlipidemia. Based on the results of this study, animal models of hyperlipidemia will be established to further clarify the targets and signaling pathways of lotus leaf in regulating hyperlipidemia.

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