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Network pharmacology-based study on the lipid-lowering effect of Bidens bipinnata L. extract with experimental validation in a hyperlipidemia rat model

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Abstract

Bidens bipinnata L. (BBL), a traditional Chinese medicinal herb commonly known as “Popozhen”, has demonstrated significant efficacy in treating hyperlipidemia. However, its active components and the mechanisms underlying its therapeutic effects have yet to be fully clarified. This study was undertaken to identify the bioactive components of BBL and to elucidate the mechanisms underlying its therapeutic action against hyperlipidemia, through a combination of a hyperlipidemic rat model and network pharmacology. Firstly, a rat model of hyperlipidemia established through high-fat diet (HFD) feeding was used to assess the lipid-lowering potential of BBL extract. Findings from the animal experiments indicated that BBL produced a marked improvement in the blood lipid profile of hyperlipidemic rats, characterized by substantial decreases in serum triglycerides (TG), total cholesterol (TC), and low-density lipoprotein cholesterol (LDL-C), accompanied by an increase in high-density lipoprotein cholesterol (HDL-C). Subsequently, network pharmacology analysis identified 7 active components (rutin, quercetin, hyperoside, luteolin, protocatechuic acid, gallic acid, and bipinnatapolyacetyloside) and 18 key targets. Additionally, KEGG pathway analysis revealed 17 significantly enriched pathways, including PPAR signaling pathway, adipocytokine signaling pathway, metabolism of xenobiotics by cytochrome P450, AMPK signaling pathway, and steroid hormone biosynthesis. Finally, data obtained from quantitative real-time polymerase chain reaction (qRT-PCR) validation revealed that BBL markedly elevated the mRNA expression of CYP1A2 and APOA2, while suppressing the expression of CYP3A4, HMGCR, RXRA, and RXRB. In conclusion, BBL alleviates hyperlipidemia by regulating fatty acid oxidation, cholesterol synthesis and metabolism, and apolipoprotein transport through multicomponent, multitarget, and multipathway interactions.

Introduction

Hyperlipidemia is defined as an increase in circulating lipid levels; it is a systemic metabolic disorder characterized by abnormal lipid metabolism or transport mechanisms that result in plasma lipid concentrations exceeding the normal physiological range (Alloubani et al. 2021). Currently, the incidence of hyperlipidemia has been increasing annually, primarily driven by modern lifestyle factors such as excessive caloric intake and sedentary behaviors. This disorder represents a principal risk factor for a spectrum of cardiovascular and metabolic conditions, encompassing obesity, non-alcoholic fatty liver disease, atherosclerosis, coronary artery disease, diabetes mellitus, and metabolic syndrome. (Yao et al. 2020). Contemporary clinical management of hyperlipidemia typically involves pharmacological interventions such as statins and fibrates. However, these therapeutic agents are frequently associated with chronic adverse effects, including myalgia, rhabdomyolysis, and sleep disturbances (Singh et al. 2022). Given the high prevalence of hyperlipidemia coupled with the undesirable side effects of conventional lipid-lowering medications, developing highly effective hypolipidemic agents with improved safety profiles has become a critical focus in modern medical research.

Traditional Chinese Medicine (TCM) serves as a crucial therapeutic approach for preventing and managing hyperlipidemia. Characterized by multicomponent formulations acting through multiple

biological targets and metabolic pathways, TCM demonstrates a favorable safety profile while effectively modulating lipid homeostasis with minimal adverse effects. Within TCM diagnostic frameworks, hyperlipidemia is often categorized as a manifestation of “Blood Stasis Syndrome”. Clinicians specializing in TCM recognize that hyperlipidemic patients frequently present with pathophysiological manifestations consistent with phlegm accumulation, dampness retention, and blood stasis patterns, guiding therapeutic strategies rooted in syndrome differentiation(Xiang-Jun et al. 2019). TCM has been practiced in China since antiquity and has gained recognition as a therapeutic intervention for hyperlipidemia management. *Bidens bipinnata* L. (BBL), a member of the Asteraceae family and an annual herb within the *Bidens* genus, is commonly referred to as “Popozhen” in China. This plant is widely distributed across China and was first documented in the classical Chinese pharmacopeia text “Bencao Shiyi”, where it is described as having a bitter-sweet flavor, slightly cold nature, and non-toxic. Its primary pharmacological actions include heat-clearing, detoxification, antidiarrheal effects, blood stasis resolution, and reducing swelling (Zhu et al. 2013; Zhuang et al. 2020). In China, BBL has been employed in traditional folk medicine for centuries to manage hyperlipidemia. Furthermore, pharmacological studies have demonstrated its therapeutic potential in anti-hyperlipidemic (Zhang et al. 2012), anti-hypertensive (Bilanda et al. 2017), anti-microbial (Singh et al. 2017), anti-inflammatory (Fotso et al. 2014), and anti-tumor (Yang et al. 2013) applications, particularly through mechanisms involving lipid metabolism modulation. The plant contains phytochemical constituents such as flavonoids, chalcones, polyacetylenes, and terpenoids (Lima Silva et al. 2011; Wang et al. 2014; Yang et al. 2020). Nevertheless, the specific bioactive components responsible for its therapeutic effects in hyperlipidemia remain unidentified, and their pharmacological mechanisms of action have not been systematically elucidated.

Network pharmacology, grounded in systems biology, bioinformatics, and polypharmacology, is an emerging field that applies network analysis to biological systems to identify key molecular nodes for multitarget drug design (Nogales et al. 2022; Shu et al. 2024). This methodology constructs biological networks and conducts network topology analysis using database mining, computational modeling, and high-throughput omics data integration (Li et al. 2023). By analyzing interconnections between network nodes, it evaluates drug-disease interactions, predicts therapeutic targets holistically, and elucidates multimechanistic drug actions.Contemporary research aligned with TCM's holistic paradigm acknowledges that herbal formulations, which comprise diverse bioactive compounds, achieve therapeutic effects through synergistic pathway modulation. The rise of network pharmacology has facilitated systematic exploration of TCM-disease interrelationships, particularly in polygenic and multifactorial disorders (Zhao et al. 2023). This approach not only enables molecular-level assessment of TCM's pharmacological effects but also synthesizes network biology and polypharmacology principles, revealing mechanistic complexities of herbal therapeutics. Applied to TCM research, network pharmacology has advanced the study of multi-target compounds within biological networks, decoding their polyvalent mechanisms of action.

In this study, we constructed component-target-pathway and disease-target-component networks using network pharmacology to elucidate the active components, key targets, and molecular mechanisms of BBL against hyperlipidemia. Additionally, we conducted experimental validation by establishing high-fat

diet (HFD)-induced hyperlipidemic rat models and performing quantitative real-time polymerase chain reaction (qRT-PCR) to clarify the efficacy and mechanism underlying BBL's therapeutic effects on hyperlipidemia. By combining network pharmacology with experimental verification, this study revealed the bioactive components, key targets, and molecular mechanisms of BBL against hyperlipidemia. This research approach, which integrates virtual prediction and experimental validation, provided new insights for studying the mechanisms of action of complex components in TCM. Additionally, this study represents the first systematic exploration of the bioactive constituents and therapeutic mechanisms of BBL against hyperlipidemia, thereby offering a theoretical foundation and experimental support for the development of highly effective, low-toxicity natural lipid-lowering drugs.

Materials and Methods

Materials and instruments

Plant samples of *Bidens bipinnata* L. (Asteraceae) were harvested in October 2023 from Nanyang City, Henan Province, China (32°59'23" N, 112°31'42" E). The taxonomic identity was confirmed by Professor Yu-Qing Wang. Voucher specimens (No. NYMC-20231015) were lodged in the herbarium housed at the Center of Scientific Research, Nanyang Medical College (Nanyang, China). A standard diet and a HFD were obtained from Hunan SJA Laboratory Animal Co., Ltd. The HFD comprised 0.2% propylthiouracil, 0.3% bile salts, 1% cholesterol, 5% soybean meal, 7% pork fat, 10% egg yolk powder, and 76.5% basal diet. Lovastatin tablets (sinopharm: 23051041; specification: 20 mg) were obtained from Beijing Wanwei Pharmaceutical Co., Ltd. The kits for measuring total cholesterol (TC), triglycerides (TG), low-density lipoprotein cholesterol (LDL-C), and high-density lipoprotein cholesterol (HDL-C) were obtained from Nanjing Jian Cheng Biological Engineering Research Institute Co. Ltd. The Ultrapure RNA kit was obtained from CimWin Biotech Co., Ltd. SYBR® Premix Ex Taq™ II and PrimeScript™ 1st-Strand cDNA Synthesis Kit were obtained from Takara Bio Inc. A full-wavelength microplate reader was obtained from BioTek® Instruments, Inc. 7500 Real-Time PCR instrument was obtained from Applied Biosystems™. The NanoDrop™ Lite spectrophotometer was obtained from Thermo Fisher Scientific Inc.

Preparation of BBL extract

The preparation method of BBL extract was based on the our previous research results(Li et al. 2022). 3 kg of dried BBL herb pieces were extracted twice with boiling water (1:10, w/v) for 2 h each, and the resulting extracts were then filtered sequentially through 200-mesh gauze (75 μm). The pooled filtrates were concentrated by rotary evaporation under reduced pressure at 50°C, yielding BBL extract with a mass yield of 1.5:1 (crude herb to extract).

Animal experiments

Induction of hyperlipidemia rat model

48 male Sprague-Dawley rats, each weighing 200 ± 20 g, were procured from Hunan SJA Laboratory Animal Co., Ltd. (Changsha, China; certificate No. SCXK (Xiang) 2019-0004). These animals were housed in a controlled environment (23 ± 2°C, 50 ± 10% relative humidity) and exposed to a 12-h light/12-h dark cycle. The rats were allowed free access to standard laboratory chow and purified water. After a 1-week acclimatization period, the rats were randomly assigned to six groups (n = 8 per group). With the exception of the control (C) group, all remaining groups received an HFD. To verify the successful induction of the hyperlipidemia model, blood lipid levels were assessed after 4 weeks of dietary intervention. Blood specimens were drawn from the orbital venous plexus, maintained at 4°C for 2 h, and subsequently subjected to centrifugation at 13,000 × g for 10 min. The concentrations of TG, TC, HDL-C, and LDL-C in serum were then determined. Relative to the C group, rats fed an HFD exhibited markedly elevated serum levels of TG, TC, and LDL-C. Rats whose serum TC was ≥ 6.2 mmol/L were regarded as hyperlipidemic.

Assessment of *in vivo* efficacy

The following groups were established for the hyperlipidemic rat model: the model group (M), the lovastatin group (LFTD) (2.0 mg/kg/day), the BBL high-dose group (BBL-H) (10.0 g/kg/day), the BBL medium-dose group (BBL-M) (5.0 g/kg/day), and the BBL low-dose group (BBL-L) (2.5 g/kg/day). The BBL extract was administered by gavage, whereas the C and M groups were given an equivalent volume of saline. The treatment schedule consisted of one administration per day for 8 weeks. Doses were calculated through body surface area normalization using the following equation: human clinical dose × 0.018 / 200 × 1000 × clinical equivalence multiple. At 0.5 h after the final administration, anesthesia was induced in all rats using isoflurane. Carotid arterial blood was then collected, followed by euthanasia via cervical dislocation. Liver samples were collected immediately and preserved at − 80°C.

Blood specimens collected from rats in each group were maintained at 4°C for 2 h and then subjected to centrifugation at 13,000 × g for 10 min to harvest serum. The harvested serum was subsequently assayed for TG, TC, HDL-C, and LDL-C concentrations using commercially available kits and a microplate reader, according to the manufacturers' instructions.

The total anti-hyperlipidemic effect for each group was calculated using a multi-index comprehensive evaluation method based on normalized values of TC, TG, HDL-C, and LDL-C. First, the data for each indicator were normalized. For indicators where the value in the M group was higher than in the C group, $V_{normalization} = (V_M - V_{indicator})/V_M$. Conversely, for indicators where the M group value was lower than the C group value, $V_{normalization} = (V_{indicator} - V_M)/V_M$. The weight of each indicator was determined based on its clinical relevance. A review of 5,000 hyperlipidemia-related articles published in the past five years (reporting frequencies: 1,879 for TC, 1,863 for TG, 2,004 for LDL-C, and 1,630 for HDL-C) informed a scoring system to evaluate the relative importance of each indicator in anti-hyperlipidemic efficacy assessment. Accordingly, weight coefficients of 2, 2, 5, and 1 were assigned to TC, TG, LDL-C, and HDL-C, respectively. The total anti-hyperlipidemic effect value was then computed as the weighted sum of each normalized indicator multiplied by its corresponding weight coefficient.

Network pharmacology

Components and targets

Based on our team's previous research, eleven biologically active components of BBL were identified by high-performance liquid chromatography–quadrupole–time-of-flight/mass spectrometry (HPLC-Q-TOF/MS) and served as the components for this study (Wang et al. 2020). To predict the targets of these components, they were first entered separately into three databases: PharmMapper (https://www.lilab-ecust.cn/pharmmapper/), Bioinformatics Analysis Tool for Molecular mechANism of Traditional Chinese Medicine (BATMAN-TCM, http://bionet.ncpsb.org.cn/batman-tcm/) and Traditional Chinese Medicine Systems Pharmacology Database and Analysis Platform (TCMSP, https://www.tcmsp-e.com/). The results from these three databases were then integrated to comprehensively predict component-associated targets. Second, to identify disease-associated targets, the keywords "hyperlipidemia," "hypercholesterolemia," and "hypertriglyceridemia" were searched in the DrugBank (https://go.drugbank.com) and Therapeutic Target Database (TTD, http://bidd.nus.edu.sg/group/cjttd). The retrieved targets were subsequently filtered to remove putative false positives. Afterward, duplicate entries and non-standardized names were eliminated from the targets obtained from the above databases, and only targets associated with *Homo sapiens* were retained for further analysis. Finally, with the UniProtKB search function on the UniProt database (http://www.uniprot.org), all retained targets (both component-associated and disease-associated) were uniformly converted to official gene symbols. The common targets between the component-associated set and the disease-associated set were designated as BBL-antihyperlipidemic-associated targets. These targets were subsequently input into the Traditional Chinese Medicines Integrated Database (TCMID, http://www.megabionet.org/tcmid/) to retrieve their corresponding target-associated components.

KEGG pathway enrichment

The BBL-anti-hyperlipidemic-associated targets were input into the MAS 3.0 database (http://bioinfo.capitalbio.com/mas3) to identify hyperlipidemia-associated pathways. A *P* value of less than 0.01 was set as the threshold for statistical significance. Enrichment analysis of the identified pathways was performed using the Kyoto Encyclopedia of Genes and Genomes (KEGG) database (http://www.genome.jp/kegg).

Construction of network

The component-target-pathway and disease-target-component networks were constructed by Cytoscape 3.10.1 to evaluate the multicomponent, multitarget, and multipathway features of hyperlipidemia. In these networks, the nodes represent components, targets, pathways, or diseases, and the edges represent component-target interactions, target-pathway interactions, or target-disease interactions.

Validation of the key targets by qRT-PCR

The liver samples from the group with the highest total effect value among BBL-H, BBL-M, and BBL-L were selected for experimental validation. Total RNA was isolated from these liver specimens using an Ultrapure RNA kit following the manufacturer's protocol. RNA concentration was determined using a NanoDrop Lite spectrophotometer, after which the RNA was reverse-transcribed into cDNA using the PrimeScript™ 1st-Strand cDNA synthesis kit. Finally, qRT-PCR was conducted using SYBR® Premix Ex Taq™ II reagent on a 7500 Real-Time PCR system to determine the mRNA expression levels of the key target genes, with GAPDH serving as the reference gene. Primer sequences are provided in Table S2.

Statistical analysis

Statistical computations were carried out with GraphPad Prism 9.0. Comparisons among groups were made using one-way analysis of variance (ANOVA). Values are reported as the mean ± standard deviation (SD). A threshold of $P < 0.05$ was considered to indicate statistical significance.

Results

BBL exerts therapeutic effects in rats with hyperlipidemia

As shown in Fig. 1, marked differences were observed in TG, TC, HDL-C, and LDL-C levels between the M and C groups. Specifically, compared with the C group, the M group exhibited significantly elevated TG, TC, and LDL-C levels, whereas HDL-C levels were significantly reduced. These findings confirm the successful establishment of the hyperlipidemic rat model. Furthermore, compared with the M group, the lovastatin group (LFTD), BBL high-dose group (BBL-H), BBL medium-dose group (BBL-M), and BBL low-dose group (BBL-L) showed significant reductions in TG, TC, and LDL-C levels. In contrast, HDL-C levels were significantly increased in the LFTD and BBL-M groups, whereas no statistically significant changes were detected in the BBL-H or BBL-L groups. As shown in Table 1, the overall anti-hyperlipidemic efficacy ranking was LFTD > BBL-M > BBL-H > BBL-L. These results indicate that both lovastatin tablets and BBL exert therapeutic effects against hyperlipidemia, with BBL-M demonstrating optimal efficacy. Therefore, the BBL-M dose was selected for subsequent validation.

Table 1
Total effect value of BBL anti-hyperlipidemia in each group

Group	Normalized value				Total effect value
	TG	TC	LDL	HDL	
C	0.65	0.79	0.72	1.66	8.13
M	0	0	0	0	0
LFTD	0.58	0.60	0.61	1.06	6.48
BBL-L	0.50	0.41	0.39	0.24	4.02
BBL-M	0.60	0.60	0.60	1.00	6.38
BBL-H	0.41	0.50	0.42	0.58	4.48

Network pharmacology analysis

Eleven active components were obtained after HPLC-Q-TOF/MS analysis (Supplementary Fig. S1 and Table S1). A total of 652 component-associated targets were retrieved from the PharmMapper, BATMAN-TCM, and TCMSP databases. Ninety disease-associated targets were obtained from the DrugBank and TTD databases. Eighteen BBL-antihyperlipidemic-associated targets were identified as key targets mediating the therapeutic action of BBL against hyperlipidemia (Table 2). Additionally, a total of 1,527 components were retrieved from TCMID database, seven of which overlapped with those identified in BBL.

Table 2
Information on BBL-antihyperlipidemic-associated targets

No.	UniProtKB	Target name	Gene name
1	P37231	Peroxisome proliferator-activated receptor gamma	PPARG
2	P04035	3-hydroxy-3-methylglutaryl-coenzyme A reductase	HMGCR
3	P14324	Farnesyl pyrophosphate synthase	FDPS
4	P06213	Insulin receptor	INSR
5	P19793	Retinoic acid receptor RXR-alpha	RXRA
6	Q03181	Peroxisome proliferator-activated receptor delta	PPARD
7	Q07869	Peroxisome proliferator-activated receptor alpha	PPARA
8	P04798	Cytochrome P450 1A1	CYP1A1
9	P05177	Cytochrome P450 1A2	CYP1A2
10	P08684	Cytochrome P450 3A4	CYP3A4
11	P10632	Cytochrome P450 2C8	CYP2C8
12	P11712	Cytochrome P450 2C9	CYP2C9
13	P28702	Retinoic acid receptor RXR-beta	RXRB
14	P02652	Apolipoprotein A-II	APOA2
15	P23141	Liver carboxylesterase 1	CES1
16	P28845	Corticosteroid 11-beta-dehydrogenase isozyme 1	HSD11B1
17	P04150	Glucocorticoid receptor	NR3C1
18	P10828	Thyroid hormone receptor beta	THRB

KEGG enrichment analysis (Fig. 2) identified 17 significantly enriched pathways. The results demonstrated that key signaling pathways included the peroxisome proliferator-activated receptor (PPAR), retinol metabolism, metabolism of xenobiotics by cytochrome P450, linoleic acid metabolism, metabolic pathways, steroid hormone biosynthesis, drug metabolism–cytochrome P450, adipocytokine signaling, bile secretion, AMP-activated protein kinase (AMPK) signaling, non-alcoholic fatty liver disease (NAFLD), terpenoid backbone biosynthesis, tryptophan metabolism, drug metabolism–other enzymes, ovarian steroidogenesis, arachidonic acid metabolism, and neuroactive ligand-receptor interaction.

The component-target-pathway network diagram (Fig. 3) displayed 42 nodes and 94 edges, including 7 components, 18 targets, and 17 pathways. Seven targets were regulated by four or more pathways, and the PPAR signaling pathway was regulated by six targets (PPARA, PPARD, PPARG, RXRA, RXRB, and APOA2). These results indicate that BBL exhibits multicomponent, multitarget, and multipathway

pharmacological characteristics. Analysis of the disease-target-component network (Fig. 4) revealed one disease, 18 targets, and 7 components, comprising 26 nodes and 51 edges. Rutin, hyperoside, quercetin, and bipinnata polyacetyloside were all associated with HMGCR. These seven components (quercetin, rutin, hyperoside, luteolin, protocatechuic acid, gallic acid, and bipinnatapolyacetyloside), which are linked to hyperlipidemia, were identified as potential active components of BBL (Fig. 5).

qRT-PCR analysis

The mRNA levels of CYP1A2, CYP3A4, HMGCR, RXRA, RXRB, and APOA2 were quantified using qRT-PCR. The expression level of each gene in the M group was normalized to 1 (Fig. 6). Compared with the M group, significant differences in the mRNA expression of CYP3A4, RXRA, and APOA2 were observed in the C and BBL groups. These results indicate that hyperlipidemia exerts dual effects on hepatic mRNA expression, significantly upregulating CYP3A4 and RXRA while downregulating APOA2. However, BBL group suppressed CYP3A4 and RXRA mRNA expression, and enhanced APOA2 mRNA expression. The mRNA levels of CYP1A2, RXRB, and HMGCR in the M group showed no statistical difference compared to the C group, but were significantly altered in the BBL group. This demonstrates that hyperlipidemia did not significantly affect CYP1A2, RXRB, and HMGCR expression, whereas BBL significantly increased CYP1A2 mRNA and decreased RXRB and HMGCR mRNA levels. Collectively, BBL regulates hyperlipidemia by suppressing CYP3A4, HMGCR, RXRA, and RXRB mRNA expression, and promoting CYP1A2 and APOA2 mRNA expression.

Discussion

Hyperlipidemia represents a principal risk factor driving the onset and exacerbation of cardiovascular disorders. In this study, BBL demonstrated a significant lipid-lowering effect. The results demonstrated that the BBL extract significantly reduced blood lipids in hyperlipidemic rats, significantly lowering serum concentrations of TG, TC, and LDL-C while increasing HDL-C levels. However, the intriguing finding was that the hypolipidemic efficacy of BBL-M surpassed both BBL-L and BBL-H. The inferior efficacy of BBL-L compared to BBL-M may be attributed to subtherapeutic dosing, resulting in insufficient active component concentrations to effectively modulate lipid metabolism pathways. As for BBL-H, its reduced efficacy could stem from BBL-induced inhibition of key cytochrome P450 enzymes (e.g., CYP3A4, CYP1A2, CYP2C8 and CYP2C9), which are critical hepatic drug-metabolizing enzymes (Kirchner et al. 2022). This inhibition may lead to potential accumulation of toxic metabolites or drug-drug interactions, thereby disrupting lipid metabolic pathways. Currently, research on the material basis and mechanisms of BBL in treating hyperlipidemia remains limited. In the past few years, with the advancement of systems biology, network pharmacology has emerged as a crucial strategy for clarifying the multicomponent, multitarget, and multipathway actions of TCM. This study addressed these gaps by employing a comprehensive methodology that integrates network pharmacology with experimental validation.

In this study, the component-target-pathway network analysis revealed key targets including PPARA, PPARD, PPARG, RXRA, RXRB, APOA2, HMGCR, CYP1A1, CYP1A2, CYP3A4, CYP2C8, CYP2C9 etc. Among them, PPARA, PPARD, and PPARG were three subtypes (α, δ, and γ) of PPAR, which function as ligand-activated transcriptional regulators and are pivotal in glucose and lipid metabolism (Mirza et al. 2019). PPARA is abundantly expressed in tissues characterized by active fatty acid metabolism, most notably the liver, where it governs the expression of genes associated with β-oxidation in peroxisomes and mitochondria, fatty acid uptake, and the breakdown of triglycerides (Ren et al. 2019). Stimulation of PPARD might produce anti-atherogenic benefits through an increase in plasma HDL, possibly facilitating reverse cholesterol transport from peripheral tissues to the liver, followed by its eventual excretion via the biliary route (van der Veen et al. 2005). PPARG is a nuclear hormone receptor that regulates adipocyte differentiation, lipid metabolism, and glucose homeostasis (Han et al. 2019). RXRA and RXRB are retinoic acid receptors (RXR-α and RXR-β). Retinoic acid receptors bind to ligands such as all-trans-retinoic acid or 9-cis-retinoic acid, forming heterodimers that regulate gene expression by targeting response elements in diverse biological processes. The RXRA/PPARA heterodimer is essential for PPARA's transcriptional activity in fatty acid oxidation-related genes (Tahri-Joutey et al. 2021). APOA2 stabilizes HDL structure through lipid association and modulates HDL metabolism (Pownall 2018). The PPAR signaling pathway plays a critical role in cholesterol homeostasis and efflux, mitigating hepatic lipid accumulation and oxidative stress (Chen et al. 2020; Zhang et al. 2024). This study focuses on the regulation of the PPAR signaling pathway through PPARA, PPARD, PPARG, RXRA, RXRB, and APOA2 targets, which act as ligand-activated transcriptional regulators and are fundamental in the metabolic processes of lipoproteins. HMGCR is the rate-limiting enzyme in cholesterol biosynthesis. Inhibiting HMGCR in the liver and stimulating the LDL receptors lead to enhanced clearance of LDL from the bloodstream, resulting in a reduction in blood cholesterol levels. It was reported that the amount of free cholesterol in the body affects the expression and functionality of HMGCR-reducing enzymes in the liver, and this effect occurs at the transcriptional and translational levels (Sakai et al. 2018; Wang et al. 2018). CYP1A1, CYP1A2, CYP3A4, CYP2C8, and CYP2C9 are a group of heme-thiolate monooxygenases, commonly known as cytochrome P450 enzymes, which are the most important drug-metabolizing enzymes in the liver. These enzymes participate in the oxidation and metabolism of a range of endogenous compounds, including fatty acids, arachidonic acid, and steroid hormones, as well as in the anabolism and catabolism of cholesterol, bile acids, and steroids (Jarrar et al. 2019). Additionally, this study demonstrated that these enzymes regulate the retinol metabolism signaling pathway, the cytochrome P450-mediated xenobiotic metabolism signaling pathway, and the linoleic acid metabolism signaling pathway. Furthermore, our previous research indicated that the mRNA expression levels of PPARA, PPARD, and PPARG in hyperlipidemic rats differed from those in normal rats; these alterations were partially reversed, with the levels approaching normal after BBL intervention (Jarrar et al. 2019). In this study, the mRNA levels of CYP3A4, HMGCR, RXRA, and RXRB were downregulated, while the mRNA levels of CYP1A2 and APOA2 were upregulated following BBL treatment. Thus, our results demonstrated that BBL could improve abnormal lipid metabolism in hyperlipidemia by regulating key targets and critical signaling pathways associated with fatty acid oxidation, cholesterol biosynthesis and metabolism, and apolipoprotein transport.

In the disease-target-component network, we identified potential bioactive components of BBL against hyperlipidemia through network pharmacology, including quercetin, rutin, hyperoside, luteolin, protocatechuic acid, gallic acid, and bipinnatapolyacetyloside. These components primarily belong to flavonoids, polyacetylenes, and phenolic acids. Quercetin was found to inhibit hepatic cholesterol synthesis by reducing HMGCR mRNA expression, protein levels, and enzyme activity, while promoting hepatic LDL-C uptake through upregulation of LDL receptor expression and its upstream regulatory factor, sterol regulatory element-binding protein 2 (SREBF2), at both mRNA and protein levels (Danesi et al. 2016). Rutin inhibited adipogenic differentiation in preadipocytes and affected hepatocyte function by reducing the expression of pivotal adipogenic transcription factors, including PPARG (Choi et al. 2006). Hyperoside reduced cholesterol levels, regulated blood lipid profiles, enhanced serum superoxide dismutase activity, suppressed endogenous cholesterol synthesis, and inhibited HMGCR (Zhang et al. 2017). Luteolin was shown to reduce cholesterol accumulation in peripheral tissues and prevent extensive damage to vascular endothelial cells, while also inhibiting atherosclerosis formation by promoting cholesterol transport to the liver in hyperlipidemic rats (Nekohashi et al. 2014). Elevated serum lipid concentrations promote the accumulation of cholesterol and triglycerides in cardiac tissues, thereby contributing to pathological effects on the heart. However, administration of protocatechuic acid significantly restored serum lipid levels to normal and ameliorated both hyperlipidemia and diabetic cardiomyopathy (Nekohashi et al. 2014). Gallic acid alleviated hypertriglyceridemia and reduced fat deposition by enhancing key glycolysis and lipolysis pathways in the perirenal adipose tissue of HFD-fed rats (Huang et al. 2018). Bipinnatapolyacetyloside mitigates CCl_4-induced acute liver injury in mice by lowering serum alanine aminotransferase (ALT) / aspartate aminotransferase (AST) levels, reducing hepatic malondialdehyde (MDA) and nitric oxide (NO) content, enhancing hepatic superoxide dismutase (SOD) and glutathione peroxidase (GSH-Px) activity, and suppressing rabbit anti-human NF-κB (p65) polyclonal antibody expression (Jian-Jun et al. 2012). Therefore, these active components may collectively constitute the pharmacodynamic material basis for the hypolipidemic effect of BBL.

Conclusions

This study integrated network pharmacology with animal experiments to elucidate the mechanism by which BBL ameliorates hyperlipidemia. Screening identified active components of BBL — including quercetin, rutin, hyperoside, luteolin, protocatechuic acid, gallic acid, and bipinnatopolyacetyloside — as well as key targets (e.g., PPARA, PPARD, PPARG, RXRA, RXRB, APOA2, HMGCR, CYP1A1, CYP1A2, CYP3A4, CYP2C8, and CYP2C9) as critical mediators of its anti-hyperlipidemic effects. KEGG pathway analysis revealed 17 significantly enriched pathways, including the PPAR signaling pathway, retinol metabolism, xenobiotic metabolism by cytochrome P450, and linoleic acid metabolism, which may be pivotal for alleviating hyperlipidemia. In vivo experiments demonstrated that BBL significantly modulated blood lipid profiles in hyperlipidemic rats, as evidenced by reduced serum levels of TG, TC, and LDL-C, alongside elevated HDL-C levels. Furthermore, BBL's lipid-lowering mechanisms involved downregulation of CYP3A4, HMGCR, RXRA, and RXRB mRNA expression and upregulation of CYP1A2 and APOA2 mRNA expression. Collectively, these findings indicate that BBL alleviates hyperlipidemia by regulating key

targets and critical signaling pathways. The lipid-lowering efficacy of BBL is attributed to its flavonoids, phenolic acids, and polyacetylenes, providing a scientific foundation for further investigation into its bioactive components and mechanistic actions.

Abbreviations

ALT, alanine aminotransferase

AMPK, AMP-activated protein kinase

ANOVA, one-way analysis of variance

AST, aspartate aminotransferase

BATMAN-TCM, a Bioinformatics Analysis Tool for Molecular mechANism of Traditional Chinese Medicine

BBL, *Bidens bipinnata* L.

BBL-H, BBL high-dose group

BBL-L, BBL low-dose group

BBL-M, BBL medium-dose group

C, control group

GSH-Px, glutathione peroxidase

HDL-C, high-density lipoprotein cholesterol

HFD, high-fat diet

HPLC-Q-TOF/MS, high-performance liquid chromatography - quadrupole - time of flight / mass spectrometry

KEGG, the Kyoto Encyclopedia of Genes and Genomes

LDL-C, low-density lipoprotein cholesterol

LFTD, lovastatin group

M, model group

MDA, malondialdehyde

NAFLD, non-alcoholic fatty liver disease

NO, nitric oxide

PPAR, peroxisome proliferator-activated receptor

qRT-PCR, quantitative real-time polymerase chain reaction

SD, standard deviation

SOD, superoxide dismutase

SREBF2, sterol regulatory element-binding protein 2

TC, total cholesterol

TCM, traditional Chinese medicine

TCMID, Traditional Chinese Medicines Integrated Database

TCMSP, Traditional Chinese Medicine Systems Pharmacology Database and Analysis Platform

TG, triglycerides

TTD, Therapeutic Target Database

Declarations

Declarations

Conflicting interests

The authors declare no conflicts of interest.

Ethics Approval

The authors declare that all animal procedures performed in this study were in accordance with the institutional guidelines for the care and use of laboratory animals. The animal experiments were approved by the Animal Ethics Committee of Nanyang Medical College (Approval No. ACU-25-2024-1561).

Declarations

Conflicting interests

The authors declare no conflicts of interest.

Ethics Approval

The authors declare that all animal procedures performed in this study were in accordance with the institutional guidelines for the care and use of laboratory animals. The animal experiments were approved by the Animal Ethics Committee of Nanyang Medical College (Approval No. ACU-25-2024-1561).

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Data Availability The authors confirm that the data supporting the findings of this study are available within the article and its supplementary materials.

Authors contribution

Xu Jiang: Writing–original draft, Methodology, Investigation, Data curation, Funding acquisition. Yu-Qing Wang: Project administration, Supervision, Writing – review and editing, Conceptualization, Resources, Funding acquisition. Shu-Jiao Li: Data curation, Formal analysis, Funding acquisition. Yong-Hong Man: Validation. Guo Zhuang: Software, Visualization. Xiao-Yu Wang: Visualization. All authors contributed to the study and approved the final manuscript. Xu Jiang and Yu-Qing Wang have contributed equally to this work and must be considered as co-first authors.

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Figures

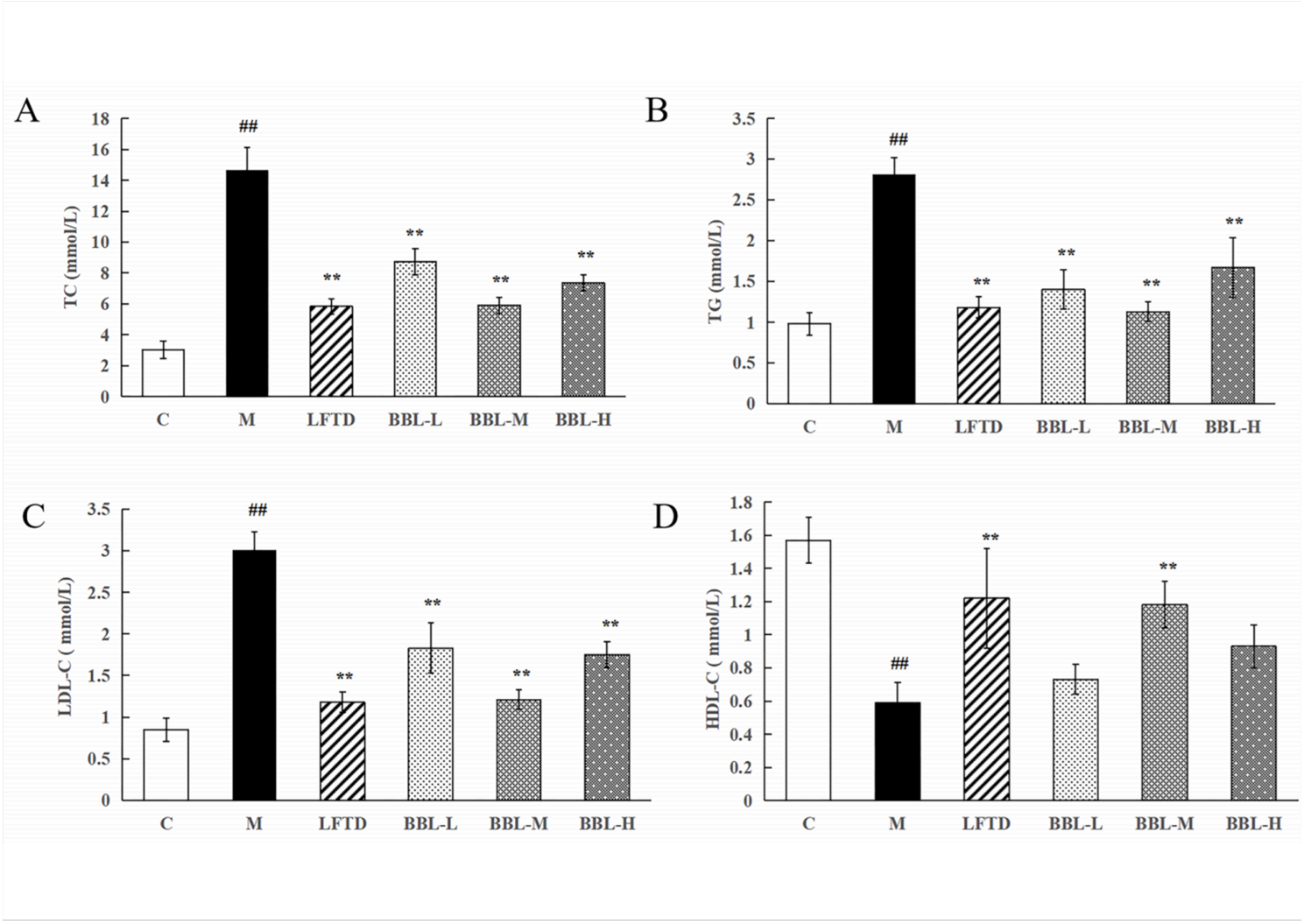


Figure 1

Effect of BBL on serum lipid levels in hyperlipidemic rats. (**A**) TC. (**B**) TG. (**C**) LDL-C. (**D**) HDL-C. Data are presented as mean ± SD (n = 8). Compared with M group, **P* < 0.05; ***P* < 0.01, compared with C group, $^{\#}P < 0.05$, $^{\#\#}P < 0.01$.

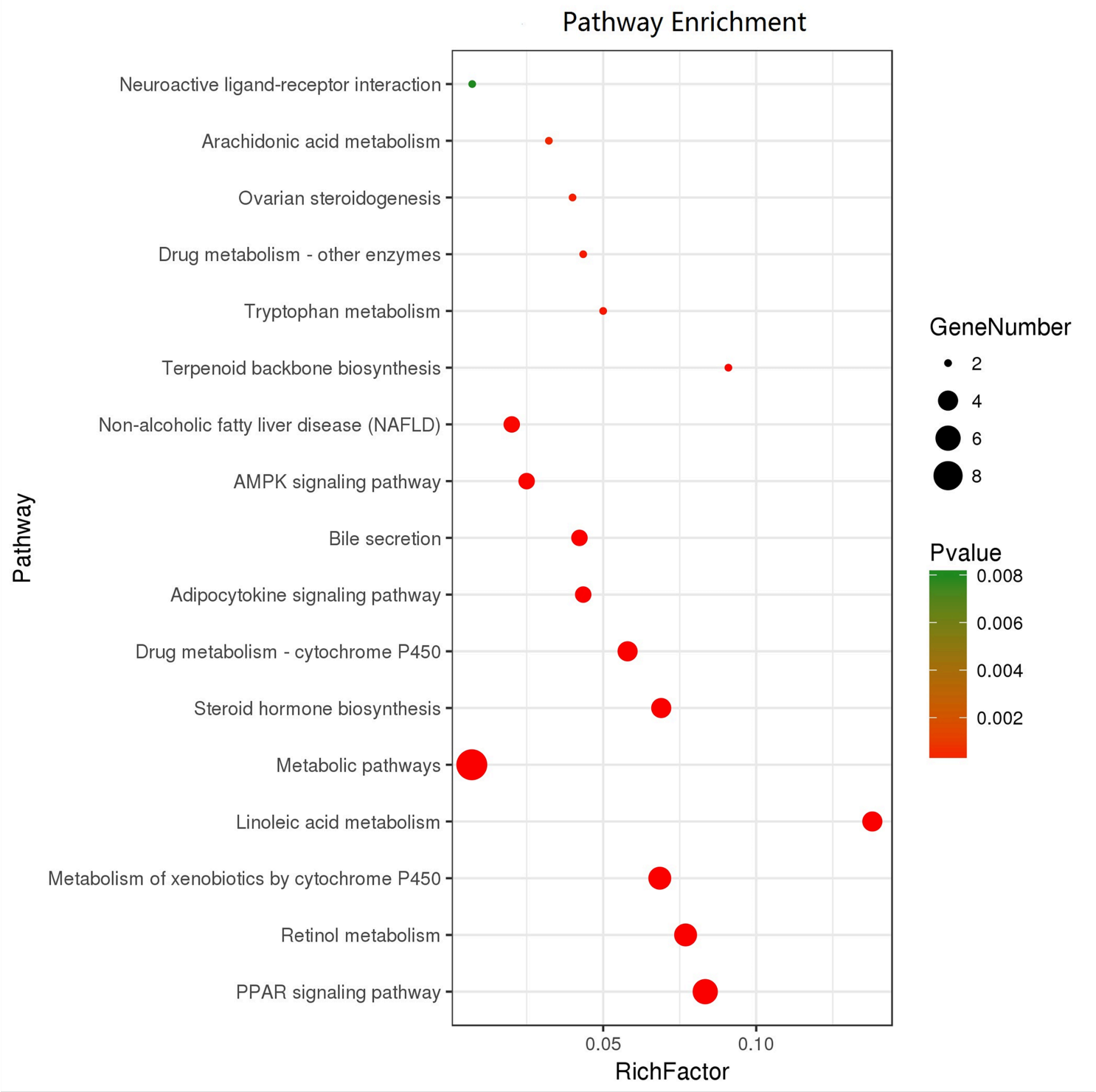


Figure 2

KEGG pathway enrichment analysis of BBL in hyperlipidemia treatment. Rich Factor = (Number of target genes mapped to a pathway) / (Total annotated genes in the pathway). Higher Rich Factor indicates a higher level of enrichment.

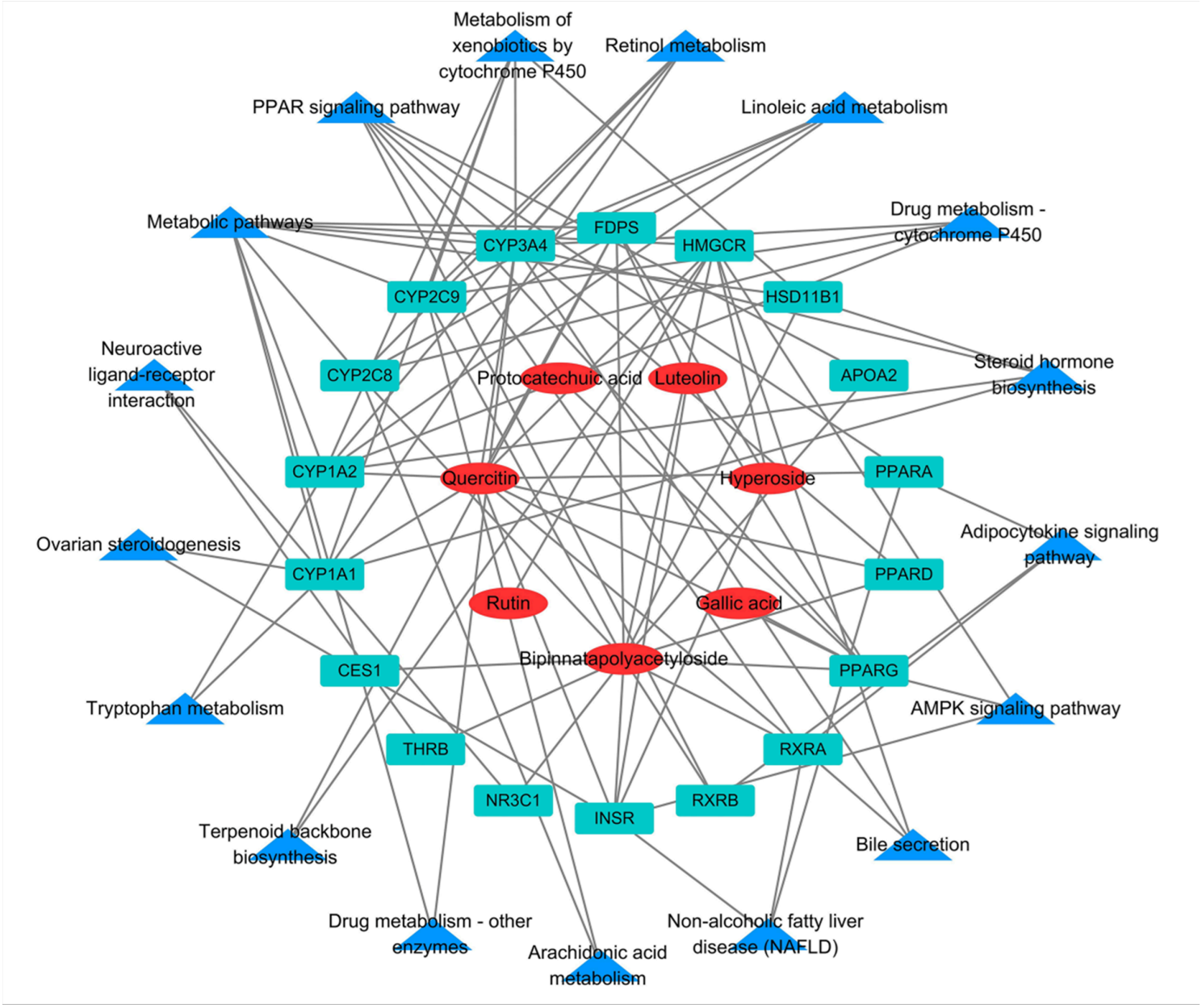


Figure 3

Network diagram of component-target-pathway. Red oval nodes representcomponents; green rectangle nodes represent targets; blue triangular nodes represent pathways.

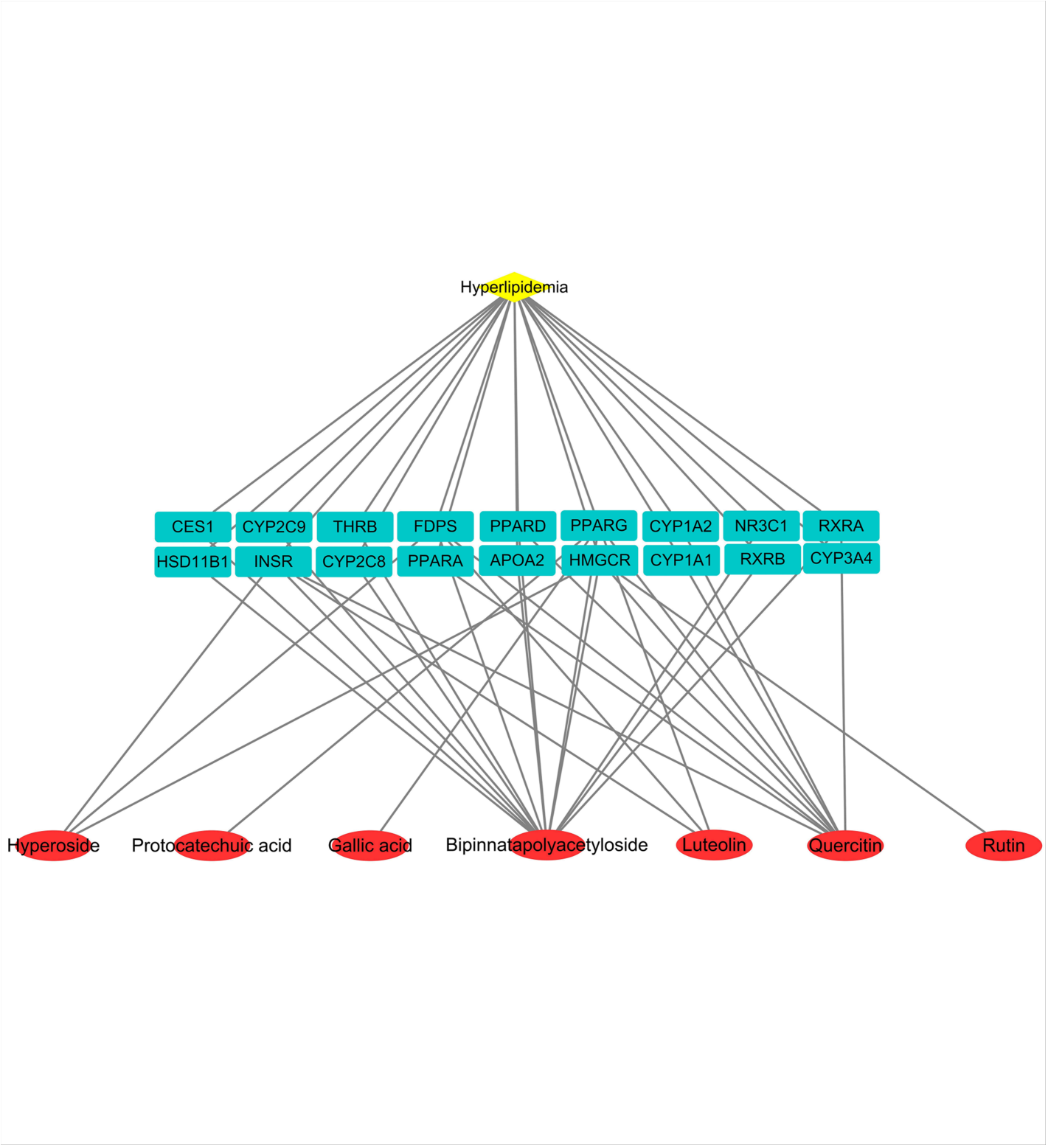


Figure 4

Network diagram of disease-target-component. Yellow diamond nodes represent diseases; green rectangular nodes represent targets; red oval nodes represent components.

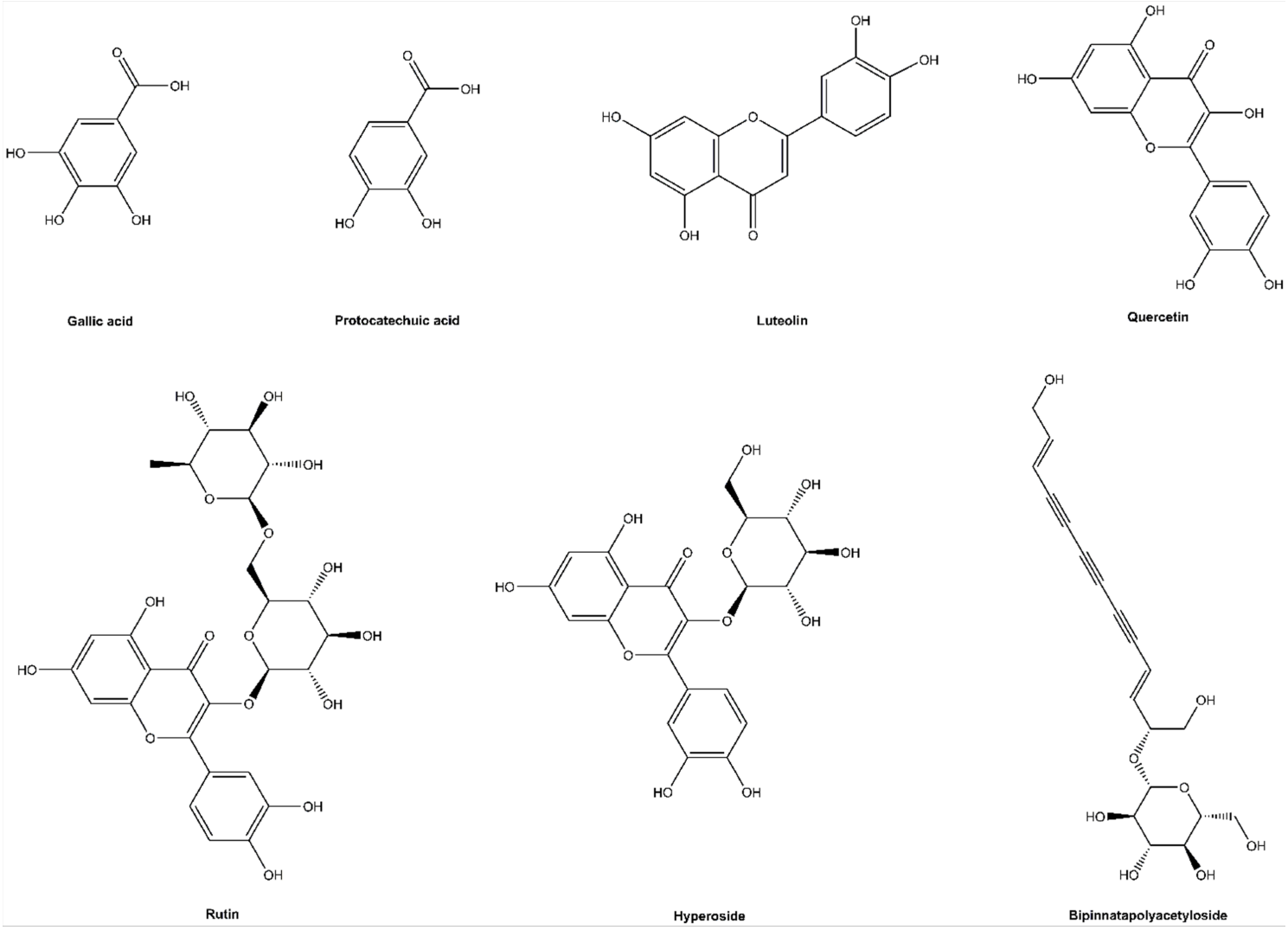


Figure 5

The active components of BBL

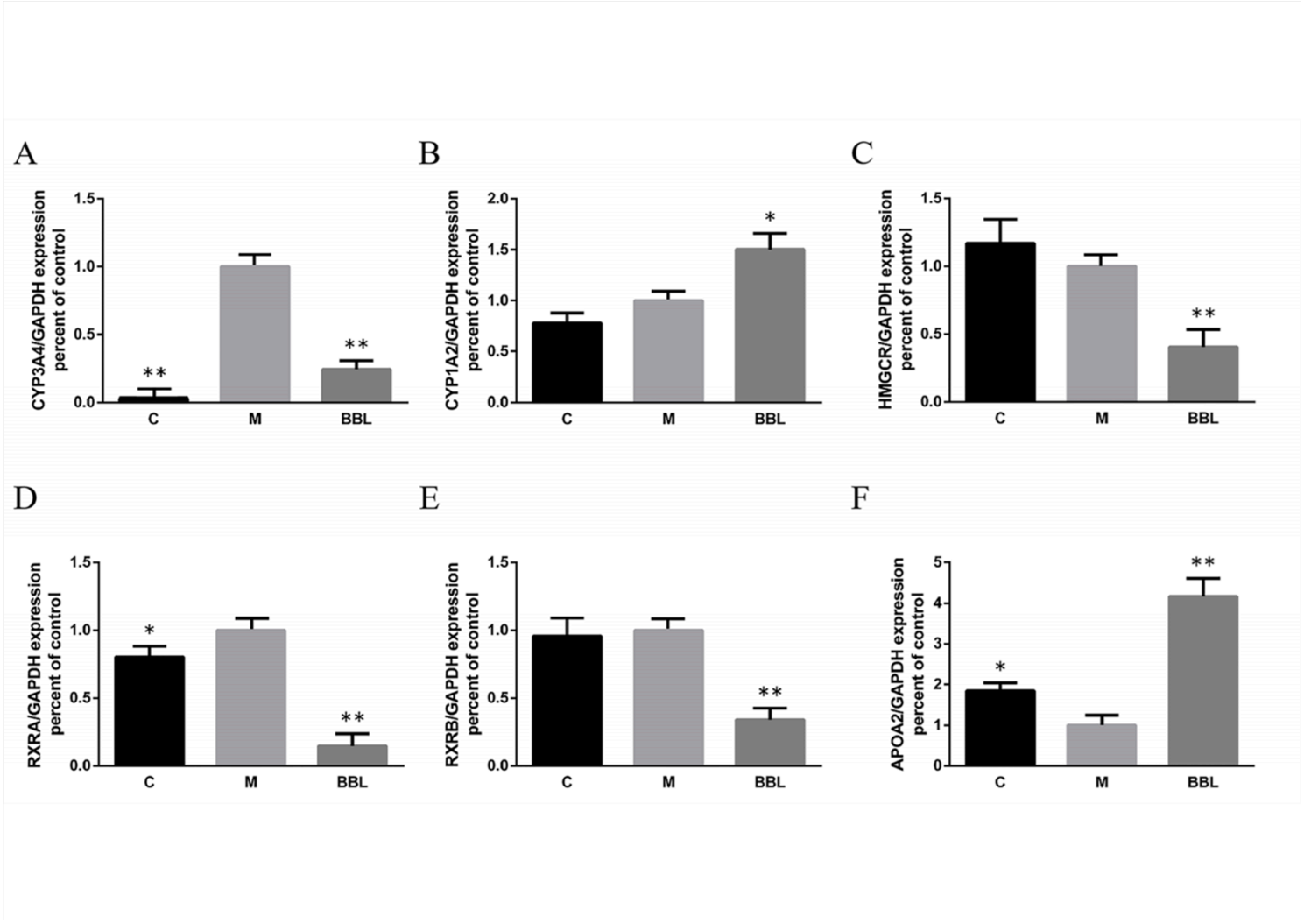


Figure 6

Effect of BBL on key target genes' mRNA expression in the liver of hyperlipidemic rats. (**A**) CYP3A4. (**B**) CYP1A2. (**C**) HMGCR. (**D**) RXRA. (**E**) RXRB. (**F**) APOA2. Data were normalized to the M group (set as 1) and are presented as mean ± SD (n = 8). Compared with the M group, *$P < 0.05$, **$P < 0.01$.

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