

Article

Exploring the Potential Lipid-Lowering and Weight-Reducing Mechanisms of FH06 Fermented Beverages Based on Non-Targeted Metabolomics and Network Pharmacology

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Abstract: Investigating the intricate pathways through which FH06 fermentation broth exerts lipid-lowering and weight-loss effects is pivotal for advancing our comprehension of metabolic regulation and therapeutic interventions. Ultrahigh-performance liquid chromatography quadrupole electrostatic field orbit trap mass spectrometry (UHPLC-QE-MS) detection and the ChEMBL database were used to determine the effective compounds in the FH06 fermentation broth and predict their targets. The TTD database and DisGeNET database were used to query obesity-related targets. The STRING database was used to construct protein interaction information. The Gene Ontology (GO) database and the Kyoto Encyclopedia of Genes and Genomes (KEGG) database were used to perform biological function annotation (GO) and KEGG pathway enrichment analyses of the targets. Results: A total of 85 effective compounds were screened from the fermentation broth of FH06; these compounds may act on TP53, PPARG, TNF, and other targets through 10 signaling pathways, such as the chemical carcinogenesis-receptor activation and lipid and atherosclerosis pathways, and exert pharmacological effects, such as hypoglycemic effects and weight loss. They also have anti-inflammatory, antioxidant, antitumor, and immunoregulatory effects. These findings reveal the active ingredients of FH06 fermentation broth and its multi-target and multi-channel characteristics in lipid lowering and weight loss. This study has positive implications for the clinical treatment of obesity using FH06, providing a theoretical and scientific basis for further developing of FH06-assisted lipid-lowering products.

Keywords: fermented beverages; anti-obesity; network pharmacology; metabolomics; the homology of medicine and food



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1. Introduction

Obesity occurs when the body's long-term intake of energy is greater than the energy consumed, resulting in a systemic fat increase or excessive fat accumulation. Obesity is a major risk factor for metabolic diseases. In particular, the distribution of visceral adipose tissue is closely related to diseases such as diabetes, cardiovascular disease, nonalcoholic fatty liver disease, inflammatory bowel disease, and cancer [1]. Statins, fibrates, and other drugs have good lipid-lowering and weight-loss effects, but they may cause adverse reactions, such as digestive system disorders, liver dysfunction, peripheral nerve paresthesia,

and rhabdomyolysis, and even increase the risk of internal bleeding [2]. Both medicinal and edible homologous substances have therapeutic effects on drugs and the nutritional and nourishing properties of food. Compared with simple Western medicine, drugs and homologous food substances have fewer side effects and are safer [3]. They have good application prospects for the prevention and treatment of lipid-lowering disorders and weight loss.

Shenheling fermentation broth (FH06) is a functional herbal beverage prepared by the fermentation of *Lactobacillus fermentum* [4]. FH06, a plant-based compound with anti-obesity effects, contains six kinds of plant raw materials, such as ginseng, poria cocos, lotus leaf, tangerine peel, red bean, and cinnamon, which have been used as herbs and food for thousands of years in Asian countries such as China, Japan, and South Korea [5]. The fermentation broth of FH06 contains various types of compounds, including terpenes, alkaloids, organic acids and their derivatives, flavonoids, phenylpropanoids, phenols, aromatic compounds, and amino acid derivatives. Previous studies have shown that FH06 has a variety of effects, such as inhibiting lipase activity, α -glucosidase activity, and antioxidant activity in vitro, and has potential weight-loss and hypoglycemic effects [6]. However, the mechanism by which FH06 inhibits obesity has not been reported.

Network pharmacology is an interactive network based on “disease–gene target medicine” using computer analysis technology combined with biology and pharmacology to explain the occurrence and development of diseases from the perspective of systems biology and biological network balance [7]. From the perspective of the system and molecular level, this study reveals the complex mechanism of action of traditional Chinese medicine on the body and guides the discovery of new drugs. From the original “one target, one drug” model to the new network target, multi-component model, a new herbal network pharmacology method is established, which opens up a new research model for predicting the targeting map and pharmacological effects of herbal compounds and reveals the correlation of drug–gene–disease synergy modules. Network pharmacology has become an effective strategy for transforming traditional Chinese medicine from empirical medicine to evidence-based medicine.

Therefore, based on UHPLC-QE-MS detection and network pharmacology methods, this study explored the possible lipid-lowering and weight-loss-related active ingredients, targets, and related pathways of FH06, a fermented beverage compound, providing a basis and guidance for further verification experiments on the efficacy of weight loss in animals and the exploration of the possible underlying mechanisms.

2. Materials and Methods

2.1. Preparation of FH06

The preparation and fermentation of the FH06 extract were performed according to our previous research [1]. Briefly, the Shenheling powder and purified water were mixed at a ratio of 1:10 (g/mL), soaked for 30 min, and then extracted for 30 min at 100 °C in a water bath. After chilling, the activated *Lactobacillus fermentum* grx08 strain was inoculated and cultured at 37 °C for 49.5 h. The supernatant was centrifuged at 5000 × g for 5 min to obtain the FH06 fermentation broth. The samples were prepared in three batches, one per week, and then frozen at −80 °C. Finally, the fermented and non-fermented samples from all three batches were tested.

2.2. UHPLC-QE-MS Conditions

LC–MS/MS analysis was performed on a UHPLC system (Vanquish, Thermo Fisher Scientific, Waltham, MA, USA) with a Waters UPLC BEH C18 column (1.7 μ m 2.1 × 100 mm). The flow rate was set at 0.5 mL/min, and the sample injection volume was set at 5 μ L. The mobile phase consisted of 0.1% formic acid in water (A) and 0.1% formic acid in acetonitrile (B). The multistep linear elution gradient program was as follows: 0–11 min, 85–25% A; 11–12 min, 25–2% A; 12–14 min, 2–2% A; 14–14.1 min, 2–85% A; 14.1–16 min, 85–85% A. An Orbitrap Exploris 120 mass spectrometer (Thermo Fisher Scientific, Waltham, MA, USA)

coupled with Xcalibur software 4.1 (Thermo Fisher Scientific, Waltham, MA, USA) was employed to obtain the MS and MS/MS data based on the IDA acquisition mode. During each acquisition cycle, the mass range was from 100 to 1500, the top four of every cycle were screened, and the corresponding MS/MS data were further acquired. Sheath gas flow rate: 35 Arb, aux gas flow rate: 15 Arb, ion transfer tube temp: 350 °C, vaporizer temp: 350 °C, full ms resolution: 60,000, MS/MS resolution: 15,000, collision energy: 16/32/48 in NCE mode, spray voltage: 5.5 kV (positive) or −4 kV (negative). Detection and analysis were provided by Shanghai Biotree Biomedical Technology Co., Ltd., Shanghai, China.

2.3. FH06 Compound Screening and Target Prediction

The above experimental test results were used as keywords to search the TCMSP (<https://www.tcmsp-e.com/>, accessed on 12 March 2024) database. (Traditional Chinese Medicine Systems Pharmacology Database and Analysis Platform) ADME filtering was performed on the condition that the oral bioavailability (OB) was greater than or equal to 30% and the drug likeness (DL) was greater than or equal to 0.18, and finally, the effective compounds of FH06 were obtained. ADME refers to the metabolic dynamics of drugs, mainly studying the dynamic changes in the body's disposal of drugs. This includes the absorption, distribution, biochemical conversion (or metabolism), and excretion processes of drugs in the body, especially the temporal variation in blood drug concentration. The metabolism of drugs is related to human age, gender, individual differences, and genetic factors. Then, the protein targets of the effective compounds were predicted from the ChEMBL (<https://www.ebi.ac.uk/chembl/>, accessed on 12 March 2024) database, and the corresponding gene target names were retrieved and collected from the UniProt database.

2.4. Disease Target Prediction

In the TTD (<https://db.idrblab.net/ttd/>, accessed on 12 March 2024) and DisGeNET (<https://www.disgenet.org>, accessed on 12 March 2024) databases, “obesity” was used as the keyword to search for obesity-related gene targets. The genes screened in the two disease databases were integrated, duplicate genes were eliminated, and irrelevant genes were deleted. Finally, the disease genes closely related to obesity were identified, and they were intersected with the targets of the above compounds to obtain effective targets.

2.5. PPI Network Construction Analysis of Effective Target Proteins

Proteins maintain temporal and spatial coherence by forming protein–protein interaction (PPI) networks related to biological functions, constructing interaction networks of target proteins, and further searching for key nodes in target proteins. The above effective targets were imported into the STRING database (<https://cn.string-db.org>, V11, accessed on 12 March 2024), the species was set to *Homo sapiens*, the minimum interaction requirement score was set to greater than 0.700, the nodes with network interruption were hidden to construct the protein interaction relationships, and Cytoscape v.3.9.1 software was used for visualization.

2.6. Effective Target-GO Enrichment and KEGG Pathway Analysis

The targets of the above-screened compounds and the targets of the disease were intersected, and R language (clusterProfiler package, 3.12.0) was used to map the results against the GO database (<http://www.geneontology.org>, accessed on 12 March 2024) and Kyoto Encyclopedia of Genes and Genomes (KEGG) database for target pathway and enrichment analysis. The species was set as *Homo sapiens*, and the GO enrichment analysis included BP, CC, and MF. The higher the ranking, the more likely FH06 is to act on the body through these biological pathways, exerting its pharmacological and health benefits. Therefore, the top 10 genes were selected and visualized using R language (ggplot). KEGG enrichment analysis was performed, and the first 10 results were visualized using R language (networkD3, ggplot).

2.7. Metabolite–Target–Pathway Network Construction Analysis

The results of 2.3 and 2.5 were integrated, and network interaction analysis was performed using the FH06 effective compound constructed in the R language (ggalluvial) as well as the corresponding target and the pathway mapped by the target in the KEGG database.

3. Results and Analysis

3.1. FH06 Compound Screening and Target Prediction

A total of 445 compounds were obtained from FH06 by UHPLC-QE-MS (Table S1). After ADME screening, 85 effective compounds were obtained (Table 1), and the top 20 compounds with degree values are shown in Table 2.

Table 1. Effective compounds of FH06.

No.	Id	Compound	OB	DL
1	M375.249T650.399	Glyceryl linolenate	100.00	0.81
2	M280.133T192.065	Remerine	100.00	0.37
3	M373.128T336.748	Sinensetin	100.00	0.75
4	M373.128T305.926	Isosinensetin	100.00	0.67
5	M441.371T206.852	Sebiferic acid	100.00	0.92
6	M343.117T343.045	6-Demethoxytangeretin	100.00	0.58
7	M301.034T93.965	Demethylwedelolactone	54.37	0.30
8	M282.149T143.561	Floribundine	96.37	0.33
9	M303.050T64.081	Herbacetin	54.18	0.27
10	M295.226T315.850	Isopalmitic acid	87.27	0.69
11	M301.072T152.445	Hematoxylin	61.51	0.32
12	M287.055T126.555	Kaempferol	64.44	0.24
13	M389.124T299.923	Artemetin	100.00	0.77
14	M409.163T399.221	9-Hydroxycalabaxanthone	100.00	0.97
15	M283.152T143.561	Artemisinin	96.33	0.28
16	M421.163T63.588	Mulberrin	89.55	0.88
17	M489.214T300.675	Glimepiride	76.66	0.72
18	M423.180T68.924	Flavanone base + 4O, 2Prenyl	85.94	0.89
19	M403.138T328.764	Nobiletin	100.00	0.80
20	M395.109T306.267	Tangeritin	100.00	0.70
21	M299.056T261.149	Diosmetin	74.04	0.33
22	M301.071T260.804	Kaempferide	78.85	0.32
23	M314.138T191.030	N-cis-Feruloyltyramine	87.65	0.64
24	M279.159T605.154	Di-n-butyl phthalate	100.00	0.36
25	M273.076T128.611	Rubrofusarin	91.08	0.23
26	M287.055T202.797	Luteolin	61.50	0.25
27	M255.102T37.307	5-Methoxyflavanone	100.00	0.22
28	M287.091T224.607	Flavanone base +2O, 1MeO	88.64	0.28
29	M391.285T805.080	Di(2-ethylhexyl)phthalate (DEHP)	100.00	0.82
30	M347.219T557.971	Reichstein's substance S	80.36	0.66
31	M469.181T109.966	Epiyangambin	100.00	0.99
32	M315.255T507.075	Methyl hexadecanoate	100.00	0.72
33	M389.123T459.238	5-O-Demethylnobiletin	100.00	0.73
34	M357.098T301.842	Corymbosin	100.00	0.64
35	M293.179T626.901	2,5-dihydroxy-3-undecylcyclohexa-2,5-diene-1,4-dione	81.20	0.66
36	M287.128T267.648	Loureirin A	100.00	0.36
37	M419.134T465.131	5-hydroxy-6,7,8-trimethoxy-2-(3,4,5-trimethoxyphenyl)chromen-4-one	100.00	0.88
38	M373.093T374.031	Casticin	93.05	0.67
39	M308.220T494.010	Dihydrocapsaicin	100.00	0.75
40	M305.068T43.711	Flavanol base + 5O	35.45	0.31
41	M315.232T750.032	Progesterone	100.00	0.52
42	M251.125T252.620	Dipropyl phthalate	100.00	0.25

Table 1. Cont.

No.	Id	Compound	OB	DL
43	M317.066T102.739	2-(3,4-dihydroxyphenyl)-5,7-dihydroxy-3-methoxychromen-4-one	65.16	0.32
44	M315.052T321.661	Eupafolin	66.17	0.37
45	M337.105T475.812	Psoralidin	91.75	0.63
46	M315.052T267.813	Isorhamnetin	65.76	0.34
47	M268.104T53.746	Adenosine	42.37	0.20
48	M301.036T247.331	Quercetin	52.23	0.27
49	M313.035T211.574	Wedelolactone	68.16	0.38
50	M279.232T477.337	Alpha-linolenic acid	87.30	0.46
51	M343.083T359.527	Eupatilin	93.25	0.54
52	M272.128T93.913	Higenamine	66.38	0.23
53	M275.162T261.545	2-Naphthaleneacetic acid, decahydro-8-hydroxy-4a,8-dimethyl-alpha-methylene-	84.87	0.18
54	M277.179T577.922	Ginsenoynes C	94.40	0.61
55	M330.133T154.081	N-trans-Feruloyloctopamine	80.75	0.59
56	M461.324T753.044	Polyporusterone E	91.69	0.84
57	M326.139T125.820	Nornantenine	100.00	0.57
58	M379.158T751.393	8-Desoxygartanin	100.00	0.75
59	M423.180T118.178	Kushenol F	90.75	0.93
60	M431.211T588.064	Grandisin	100.00	0.92
61	M328.154T117.982	Boldine	83.23	0.51
62	M313.274T752.302	2,3-dihydroxypropyl hexadecanoate	100.00	0.58
63	M333.061T100.848	Flavonol base + 4O, 1MeO	54.06	0.38
64	M327.159T169.368	Dehydrodiisoeugenol	100.00	0.62
65	M375.107T236.493	Skullcapflavone II	93.75	0.56
66	M259.167T418.190	Ginsenoynes E	100.00	0.58
67	M343.082T336.916	Lysionotin	96.66	0.54
68	M255.233T20.106	Palmitic acid	87.40	0.72
69	M294.149T131.469	Dehydronuciferine	100.00	0.38
70	M262.144T577.956	Suberosin	100.00	0.20
71	M269.046T918.356	Genistein	76.33	0.22
72	M301.036T313.918	Morin	53.80	0.27
73	M340.154T255.885	Crebanine	100.00	0.63
74	M283.025T337.240	Rheic acid	47.04	0.20
75	M283.265T26.412	Stearic acid	91.98	0.68
76	M275.092T162.583	Phloretin	72.81	0.25
77	M359.078T271.495	Irigenin	83.91	0.60
78	M271.060T246.155	Galangin	77.70	0.20
79	M322.107T398.936	Cepharadione B	93.08	0.42
80	M305.247T310.008	Arachidonic acid (not validated)	90.26	0.48
81	M359.149T221.852	Dihydrocubebin	100.00	0.61
82	M353.143T742.445	Xanthohumol	100.00	0.69
83	M363.214T474.995	11,17,21-Trihydroxypregn-4-ene-3,20-dione	73.69	0.68
84	M453.337T279.747	Zizyberanolic acid	84.02	0.85
85	M285.208T343.820	Hexadecanedioic acid	65.23	0.74

Among these effective compounds, genistein is an isoflavone compound that can regulate insulin levels, promote lipid metabolism, prevent lipid oxidation, and prevent cardiovascular and cerebrovascular diseases. Genistein has become a potential drug for the prevention and treatment of various chronic diseases. Studies have shown that genistein can significantly alleviate liver lesions caused by a high-fat diet, and its mechanism may involve increasing the expression level of adiponectin and inhibiting inflammatory factors in the liver. In mice, genistein blocked Δ^9 -THC-induced endothelial dysfunction in wire myography, reduced atherosclerotic plaque, and had minimal penetration of the central nervous system [8].

Table 2. Effective compounds (top 20 in degree value).

Node	Degree
Genistein	130
Quercetin	124
Luteolin	68
Kaempferol	59
Rheic acid	35
Morin	33
Galangin	33
Phloretin	29
Wedelolactone	27
Alpha-linolenic acid	27
2,5-dihydroxy-3-undecylcyclohexa-2,5-diene-1,4-dione	27
Hematoxylin	21
Arachidonic acid (not validated)	21
Diosmetin	17
Stearic acid	16
Palmitic acid	16
Nobiletin	16
2-(3,4-dihydroxyphenyl)-5,7-dihydroxy-3-methoxychromen-4-one	16
Isorhamnetin	15
Eupafolin	15

Quercetin is an important flavonoid compound that has antihypertensive, antihyperlipidemic, antihyperglycemic, antioxidant, antiviral, antitumor, anti-inflammatory, antimicrobial, neuroprotective, and cardioprotective effects. Quercetin can reduce health risks such as high blood pressure, diabetes, and cardiac disease, which are closely related to obesity. Previous studies have shown that quercetin exerts anti-lipid peroxidation effects by inhibiting the adipocyte-specific transcription factors PPARG and C/EBP α and activating the AMPK signaling pathway in 3T3-L1 cells. Quercetin supplementation significantly reduced weight gain and increased epididymal adipose tissue and liver weight in mice fed a high-fat diet. Quercetin reduced hepatic lipid accumulation by decreasing the expression levels of Aldh1b1, Apoa4, Abcg5, Gpam, Acaca, Fdft1, and Fasn in hepatocytes [9].

Sinensetin is a polymethoxyflavone that is widely found in the fruits and peels of citrus plants, tangerine peels, and other medicinal materials [10]. Sinensetin has pharmacological activities such as lipid metabolism regulation, anti-inflammatory effects, and anticancer effects. Sinensetin exerts its anti-lipidation effect by downregulating the expression of sterol regulatory element-binding protein 1c (SREBP1c) and enhancing lipolysis by activating the cAMP-dependent PKA pathway in mature 3T3-L1 adipocytes [11].

Sinensetin can inhibit the proliferation of human MDA-MB-468 breast cancer cells by interacting with the cytochrome P450 enzyme CYP1 to produce metabolites [12].

Kaempferol is a plant flavonoid compound with antibacterial and anti-inflammatory effects. It can be used to treat obesity, diabetes, and cardiovascular diseases and to inhibit tumor growth. Kaempferol promotes anti-obesity effects by modulating adipogenesis and lipolytic pathways. Kaempferol is an efficient compound for modulating the differentiation capacity of adipocytes by selectively reducing Cebpa mRNA levels without affecting PPARG expression [13].

Isosinensetin is a methoxyflavonoid compound found in green husk and Bupleurum falcatum L. (BF) that has anti-inflammatory and other effects [14]. The anti-apoptotic protein Bcl-2 and the G1 phase regulatory proteins CK4 and CDK6 were significantly reduced, and the expression of the apoptotic proteins Bax and cleaved caspase8 was significantly increased, which could significantly inhibit proliferation and induce apoptosis and cell cycle arrest in AGS gastric cancer cells [15]. Isoflavone activated hTAS2R50 and increased the secretion of GLP-1 in NCI-H716 cells through a G $\alpha\beta$ -mediated pathway.

Demethylwedelolactone (DWEL) is a naturally occurring coumestan derived from Wedelia calendulacea and Eclipta alba that can inhibit insulinase activity and has anti-

cancer effects. DWL exerted an anti-inflammatory effect by inhibiting the degranulation of mast cells induced by compound 48/80 (C48/80) and inhibiting the production of NO, proinflammatory cytokines such as TNF- α , IL-1 β , IL-6, and the expression of costimulatory molecules such as CD40, CD80, and CD86 in LPS-stimulated macrophages [16].

Therefore, the effective compounds identified above are the basis for the anti-obesity effects of FH06. FH06 exerts antioxidative, hypoglycemic, lipid-lowering, anti-inflammatory, anti-atherosclerotic, and other pharmacological effects through these effective compounds.

3.2. Construction of the PPI Network of the FH06 Target Protein

The STRING database was used to determine the interaction relationships of the effective FH06 targets, and Cytoscape v.3.9.1 software was used to analyze the topology of the FH06 effective target protein–protein interaction (PPI) network. With the degree value as the condition, the effective targets were sorted, the dots were the effective targets, and each edge was the target interaction relationship. The number of nodes in the PPI network diagram (Figure 1) was 193, the number of edges was 1360, and the clustering coefficient was 0.411. The larger the degree value, the larger the node, and the darker the color, the more biological functions the node plays and the stronger its biological importance [17]. The diagram shows that TP53, SRC, AKT1, STAT3, ESR1, and other target networks play important roles in this process and may be key targets of FH06 in the regulation of fat metabolism.

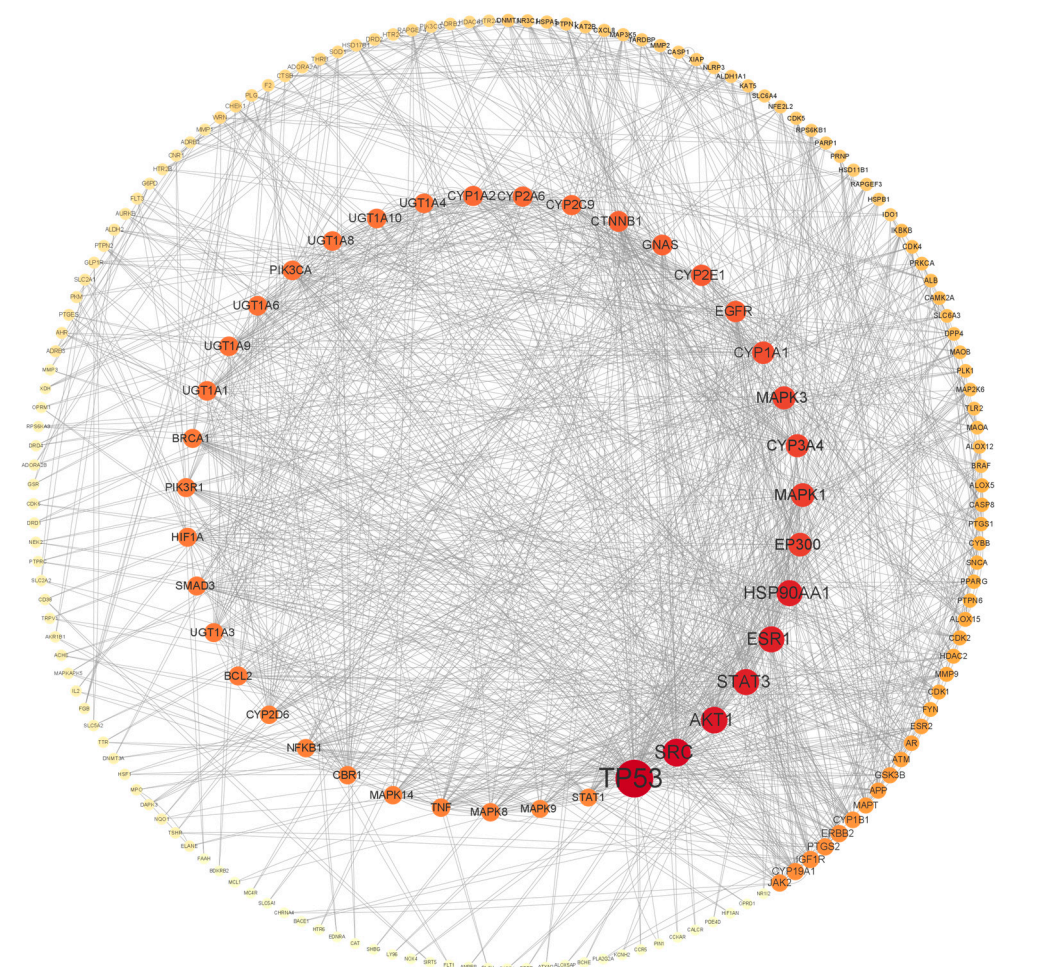


Figure 1. Target protein interaction network diagram.

TP53, tumor protein 53, is also known as p53. In addition to its classic tumor suppressor effect, TP53 is involved in the regulation of cellular metabolic pathways. TP53 has

been confirmed to be involved in the metabolism of carbohydrates, amino acids, and lipids. TP53 binds to the promoters of PLTP, Abca12, and Cel and activates their transcription to regulate the metabolism of fat and lipoprotein in liver cells, thereby affecting systemic lipid homeostasis and the development of atherosclerosis [18]. Therefore, effective compounds such as genistein in FH06 may participate in the regulation of metabolic pathways such as lipids and atherosclerosis through TP53.

SRC is a member of the membrane-associated nonreceptor protein tyrosine kinase superfamily and can act as a key factor in a variety of signaling pathways to regulate tumor cell proliferation, metastasis, angiogenesis, glycolysis, and lipid metabolism. In the HIF-1 α signaling pathway, SRC can participate in the regulation of glycolysis in tumor cells by regulating the coupling of related genes [19]. In addition, studies have shown that SRC mediates fat metabolism by inhibiting the transcriptional activity of PPAR γ , a key regulator of lipid metabolism, promoting fat accumulation, and inhibiting the conversion of white fat to brown fat [20,21].

Evidence shows that SRC is involved in the occurrence and development of DN. Taniguchi et al. reported that the activation of Sre is involved in the pathogenesis of type 1 diabetic nephropathy. This study revealed that the phosphorylation of Sre in mesangial cells stimulated by high glucose caused the activation of the epidermal growth factor receptor, resulting in mitogen-activated protein kinase activation and collagen IV synthesis [22].

AKT1, also known as protein kinase B (PKB), is a key molecule in the cell signal transduction pathway and is involved in the regulation of cell growth, survival, metabolism, and differentiation. AKT1 is widely expressed in mammals and has a variety of biological effects that regulate cell proliferation, survival, and metabolism [23]. Its dysregulation can lead to cancer, diabetes, metabolic syndrome, and cardiovascular and neurological diseases. Studies have shown that AKT1 is closely related to glucose metabolism and atherosclerosis. The excessive activation of AKT1 can lead to an imbalance in glucose homeostasis, which is characterized by insulin resistance with a compensatory increase in insulin secretion and a delayed decrease in blood glucose, which is an early feature of diabetes [24]. After hemorrhage, vascular smooth muscle cell (VSMC) apoptosis promotes vascular remodeling and atherosclerosis. AKT1 plays an important role in inhibiting VSMC apoptosis and may be a potential therapeutic target for diabetic vascular complications [25].

STAT3 belongs to the family of STAT transcription factors that participate in the regulation of a variety of cellular processes, including proliferation, differentiation, inflammation, and stemness. It can regulate lipid metabolism and carbohydrate metabolism. STAT3 was found to inhibit ferroptosis via suppression of the expression of acyl-CoA synthetase long-chain family member 4 (ACSL4) [26]. Studies have shown that under hypoxic conditions, STAT3 activates LDHA by downregulating the expression of LINC00671, regulating glycolysis, growth, and metastasis in thyroid cancer. In hepatocellular carcinoma, targeted inhibition of STAT3 can prevent the expression of key enzymes involved in glycolysis and induce immunogenic cell death to reconstruct the tumor immune microenvironment [27,28]. In addition, under hypoxic conditions, STAT3 activates LDHA by downregulating the expression of LINC00671, regulating glycolysis, growth, and metastasis in thyroid cancer. In hepatocellular carcinoma, targeted inhibition of STAT3 can prevent the expression of key enzymes involved in glycolysis and induce immunogenic cell death to reconstruct the tumor immune microenvironment [27,28].

ESR1 mediates the corresponding biological effects of estrogen, regulates the expression of different genes in different tissues, and plays an important role in maintaining blood glucose homeostasis, immune robustness, bone and cardiovascular health, reproductive capacity, and neurological function.

In summary, it can be seen that the TP53, SRC, AKT1, STAT3, and ESR1 targets that play a bridging role in the PPI network are closely related to the anti-inflammatory and antioxidant effects of FH06, and antitumor and immune regulation may be the core of FH06's series of effects. The above results can provide a theoretical basis for further research on FH06 and its lipid-lowering effects.

3.3. GO Enrichment Analysis of the Effect of FH06 on Therapeutic Targets for Obesity

The GO database contains functional information about genes involved in biological processes (biological process, BP), cellular components (cellular component, CC), and molecular functions (molecular function, MF). In this chapter, we mapped genes to each node of the Gene Ontology database and performed functional enrichment analysis using GO (<http://www.geneontology.org/>, accessed on 12 March 2024), obtaining 2906 BP, 112 CC, and 369 MF terms. The GO analysis results of the target proteins are displayed in the form of a bubble chart (taking the top 10 entries of each GO category for display, Figure 2).

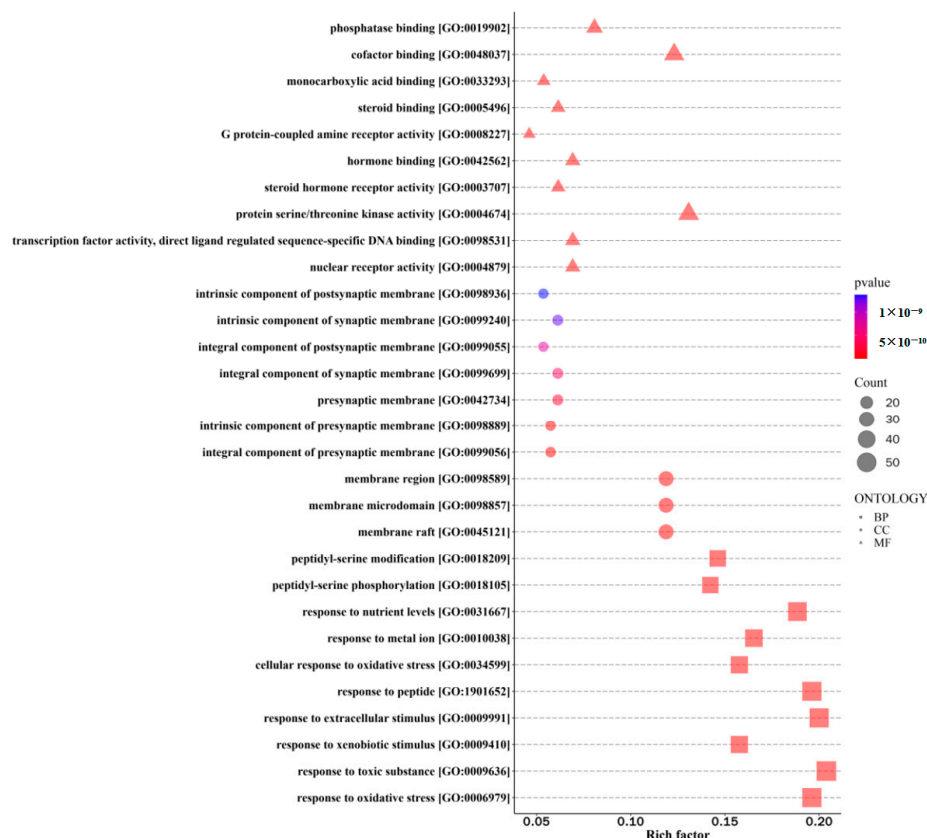


Figure 2. Target protein GO enrichment analysis classification bubble chart.

Biological processes involve responses to oxidative stress, responses to lipopolysaccharides, responses to steroid hormones, fatty acid metabolism processes, regulation of lipid metabolism processes, and aging; cellular locations involve membrane regions, presynaptic membranes, and neuronal cell bodies; and molecular functions involve nuclear receptor activity, steroid hormone receptor activity, adrenergic receptor activity, and fatty acid binding.

Figure 2 shows a bubble map of the target protein GO enrichment analysis classification. There are three categories of GO enrichment analysis for target proteins, with each category containing 10 entries. In the figure, the abscissa is the RichFactor value of the enrichment degree, and the ordinate is the GO term. The sizes of the squares, circles, and triangles represent the number of differentially expressed proteins, and the larger the number is, the greater the number is. The color of the graph represents the corrected *p*-value, and the redder the color is, the smaller the *p*-value.

3.4. KEGG Analysis of the Effective Compounds of FH06 That Act on Therapeutic Targets for Obesity

The KEGG pathway database (www.kegg.jp/kegg/pathway.html, accessed on 12 March 2024) contains functional information on genes and genomes, including information

on cellular biochemical processes such as metabolism, membrane translocation, signaling, and the cell cycle, as well as information on conserved subpathways of homologous lines. By analyzing the metabolic pathways that are significantly enriched by target proteins, we can determine which pathways are affected by the active ingredients used to treat diseases. The KEGG enrichment analysis in Figure 3 shows that 172 pathways are significantly enriched ($p < 0.05$), and the pathway enrichment analysis results of the target protein are displayed in the form of a histogram. The 40 pathways affected by FH06 in the treatment of obesity were mainly related to chemical carcinogenesis, receptor activation, lipid and atherosclerosis, neuroactive ligand-receptor interaction, prostate cancer, endocrine resistance, the AGE-RAGE signaling pathway in diabetic complications, the HIF-1 signaling pathway, inflammatory mediator regulation of TRP channels, regulation of adipocyte lipolysis, the FoxO signaling pathway, and the sphingolipid signaling pathway.

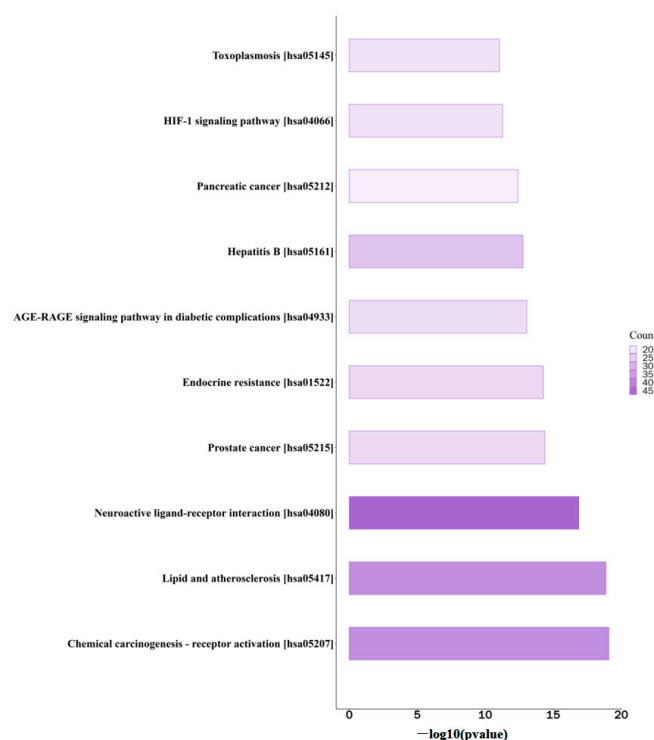


Figure 3. Histogram of target protein pathway enrichment analysis.

In the figure, the x-coordinate is $-\log_{10}(p\text{-value})$, and the y-coordinate is the path name. In the figure, the length of the column represents the size of the p -value, and the longer the column is, the smaller the p -value. The darker the color is, the more target proteins are mapped to the pathway.

The rapid division of tumor cells leads to tumor hypoxia. At this time, hypoxia-inducible factor (HIF)-1 α promotes angiogenesis, thereby supporting the further growth and metastasis of tumors. Obesity is also associated with tissue hypoxia, which is due to the expansion of adipose tissue beyond its vascular supply, which also promotes the formation of new blood vessels. In addition, hypoxia-induced VEGF (vascular endothelial growth factor) expression can promote the expansion and inflammation of adipose tissue, which can produce a microenvironment that promotes tumor growth [29].

3.5. Construction of the FH06 Effective Compound–Target–Pathway Network

For construction of the metabolite–target–pathway network, a network construction analysis of the same target protein was performed. This analysis revealed the relationships between the metabolites and target protein nodes and between the target proteins and pathway nodes. KEGG pathway enrichment analysis revealed that the effective components

of FH06 were mainly involved in 10 pathways, such as lipid metabolism and atherosclerosis, the AGE-RAGE signaling pathway in diabetic complications, insulin resistance, and the HIF-1 signaling pathway.

Among these pathways, the PI3K-Akt signaling pathway is the key signaling pathway regulating cell proliferation, differentiation, autophagy, and apoptosis and is the main signaling pathway regulating glucose metabolism [30,31]. Activation of the PI3K-Akt signaling pathway inhibits glycogenolysis and gluconeogenesis by phosphorylating forkhead transcription factor (FOXO), resulting in reduced glucose production [32,33]. Therefore, when the PI3K-Akt signaling pathway is dysregulated, FOXO1 expression is increased, resulting in elevated blood glucose. Elevated blood glucose increases the oxidative breakdown of glucose to produce acetyl coenzyme A (CoA), which is the raw material for fatty acid synthesis. Elevated levels increase fatty acid synthesis, which in turn increases the formation of TG from the esterification of fatty acids with glycerol, leading to dyslipidemia [34,35]. Therefore, the PI3K-Akt signaling pathway indirectly regulates lipid metabolism.

In the PPAR signaling pathway, the peroxisome proliferator PPAR forms a heterodimer with RXRA, and under the action of the ligand, the heterodimer conformationally changes and then binds to the PPREs in the sequence of the promoter region of the target genes, thus regulating the expression of the target genes of fat metabolism and subsequently exerting the physiological effect of regulating adipocyte proliferation and differentiation, as well as lipid deposition, at the transcriptional level [36]. FH06 plays a role in regulating lipid metabolism through the PPAR signaling pathway.

The metabolite–target–GO interaction network diagram (Figure 4) and metabolite–target–KEGG pathway interaction network diagram (Figure 5) show that each metabolite corresponded to multiple targets and that each target corresponded to multiple pathways, which reflected the characteristics of the compound fermentation broth FH06, including multiple components, multiple targets, and multiple pathways.

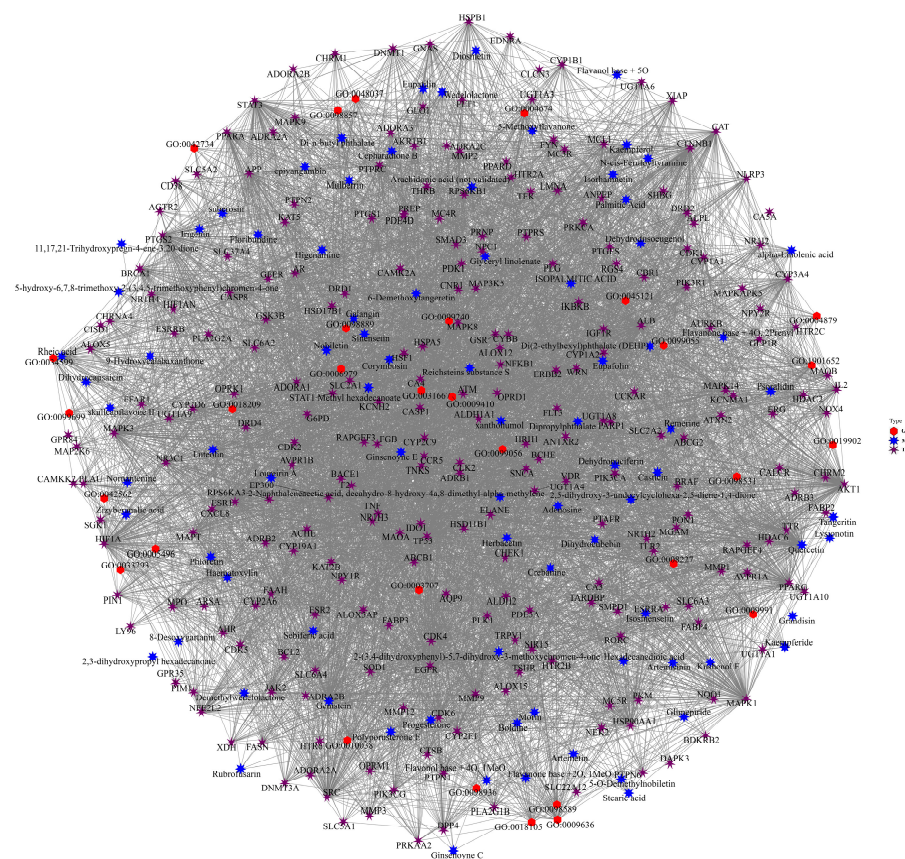


Figure 4. Metabolite–target–GO interaction network.

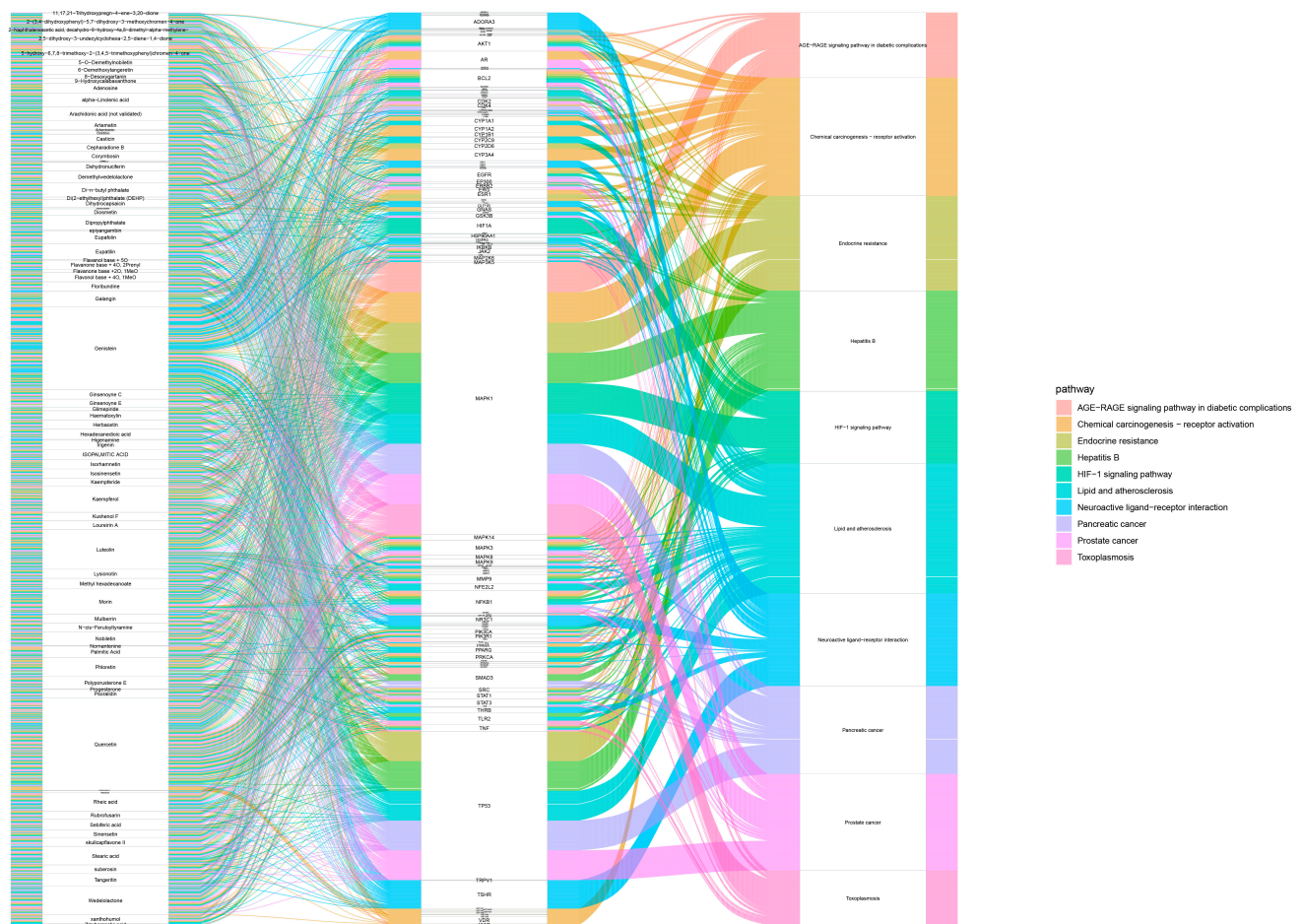


Figure 5. Interaction network diagram of the metabolite–target–KEGG pathway.

4. Conclusions

In this study, a network pharmacology method was used to explore the mechanism by which FH06 reduces lipid levels and decreases weight loss. The results showed that glyceryl linolenate, 9-Hydroxycalabaxanthone, sinensetin, isosinensetin, sebiferic acid, 6-demethoxytangeretin, demethylwedelolactone, sodium glycocholate, herbacetin, isopalmitic acid, and 85 other components are effective compounds through which FH06 can exert anti-inflammatory, antioxidative, antitumor, immunoregulatory, and other pharmacological effects and lipid-lowering and other health effects.

The effective compounds of FH06 can act on TP53, SRC, AKT1, STAT3, ESR1, PPARG, TNF, and other targets through 10 pathways, such as lipid and atherosclerosis, the AGE-RAGE signaling pathway in diabetic complications, insulin resistance, the HIF-1 signaling pathway, etc. Other pharmacological benefits include anti-inflammatory, antioxidant, anticancer, immunological modulation, lipid-lowering, and so on. This finding reflects the multitarget and multichannel characteristics of FH06 in lipid reduction and weight loss. Our research opens a promising avenue for obesity through FH06-based interventions. By providing a robust theoretical foundation, we advocate for the development of FH06-assisted lipid-lowering products, potentially revolutionizing current approaches to weight management. The elucidation of FH06's pharmacological profile leads to diverse applications, spanning medicinal therapeutics to functional foods. Future research may explore novel formulations and delivery systems, maximizing the therapeutic potential of FH06 and harnessing its medicinal and edible value to its fullest extent.

Supplementary Materials: The following materials are available online at <https://www.mdpi.com/article/10.3390/fermentation10060294/s1>. Table S1: Total metabolite annotation of FH06.

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