



Original Article

Asian Pacific Journal of Tropical Biomedicine



apjtb.org

doi: 10.4103/apjtb.apjtb_197_26

Hypolipidemic and antioxidant potential of *Trigonella foenum-graecum* leaf extract via MAPK/JNK signaling pathway in high-fat diet-induced obese ratsSahar Javed¹, Faiza Hassan¹✉, Bilal Aslam¹, Misbah Ijaz²¹Institute of Physiology and Pharmacology, University of Agriculture, Faisalabad, Pakistan²Department of Clinical Medicine & Surgery, University of Agriculture, Faisalabad, Pakistan

ABSTRACT

Objective: To determine the qualitative and quantitative phytochemical components in the aqueous leaf extract of *Trigonella foenum-graecum* L. (AqE-TFG) and evaluate its therapeutic potential against high-fat diet-associated dyslipidemia in high-fat diet-induced obese rats.

Methods: A total of 24 healthy adult male albino rats were randomly divided into 4 groups ($n=6$): Group 1 (normal control), Group 2 (untreated dyslipidemic control), Group 3 and 4 (low and high dose treatments of AqE-TFG 250 mg/kg and 500 mg/kg). Blood samples were collected for the estimation of lipid profile, oxidative markers, biochemical parameters, and histopathology of adipose tissue. Gene expression analysis was performed using qRT PCR.

Results: AqE-TFG showed antioxidant activity ($IC_{50} = 4.82 \mu\text{g/mL}$). HPLC analysis showed the presence of sinapic acid, *p*-coumaric acid, gallic acid, salicylic acid, kaempferol, ferulic acid, chlorogenic acid, quercetin, and HB acid. AqE-TFG remarkably inhibited body weight gain and restored the lipid profile. Furthermore, AqE-TFG elevated superoxide dismutase and catalase levels. Besides, AqE-TFG significantly downregulated mRNA expression levels of dual oxidase-related genes (*Duox*, *Duoxa-1*, *Duox2*) and downstream MAPK/JNK signaling cascade genes (*MAPK8*, *Traf4*, *Traf6*). Adipose tissue histology showed well-organized, intact adipocytes in treatment groups.

Conclusions: AqE-TFG has both preventive and therapeutic effects against high-fat diet-induced dyslipidemia. AqE-TFG could enhance antioxidant defense and restore histopathological lesions via MAPK/JNK signaling pathway.

KEYWORDS: Dyslipidemia; *Trigonella foenum-graecum* L.; Phytochemical; Adipose cells; Antioxidant; Lipid

1. Introduction

Dyslipidemia is a metabolic disorder manifested by abnormally low or high levels of all plasma lipids, including triglycerides (TG), total cholesterol (TC), phospholipids, and lipoproteins[1]. Hypercholesterolemia is a prevalent subtype of dyslipidemia, characterized by increased low-density lipoprotein cholesterol (LDL-C), elevated TG, and decreased high-density lipoprotein

Summary

Question: Could the aqueous leaf extract of *Trigonella foenum-graecum* (AqE-TFG) alleviate high-fat diet-triggered dyslipidemia by mitigating oxidative stress and modulating the DUOX and MAPK/JNK signaling cascades?

Findings: AqE-TFG decreased excessive body weight gain and ameliorated abnormal serum lipid profiles. Additionally, it enhanced the activity of superoxide dismutase and catalase, and restored normal adipose tissue morphology. Furthermore, AqE-TFG regulated the DUOX family gene expression and modulated the downstream MAPK/JNK pathway.

Meaning: AqE-TFG possesses remarkable antihyperlipidemic properties and may serve as a promising natural therapeutic agent for the management of obesity-associated dyslipidemia by regulating oxidative stress-mediated DUOX and MAPK/JNK signaling pathways.

✉To whom correspondence may be addressed. E-mail: faiza.hassan@uaf.edu.pk

This is an open access article distributed under the terms of the Creative Commons Attribution-Non-Commercial-No Derivatives License 4.0 (CCBY-NC-ND), where it is permissible to download and share the work provided it is properly cited. The work cannot be changed in any way or used commercially without permission from the journal.

For reprints contact: reprints@medknow.com

©2026 Asian Pacific Journal of Tropical Biomedicine Produced by Wolters Kluwer-Medknow.

How to cite this article: Javed S, Hassan F, Aslam B, Ijaz M. Hypolipidemic and antioxidant potential of *Trigonella foenum-graecum* leaf extract via MAPK/JNK signaling pathway in high-fat diet-induced obese rats. Asian Pac J Trop Biomed 2026; 16(9): 402-410.

Article history: Received 8 April 2026; Revision 6 May 2026; Accepted 23 July 2026; Available online 6 August 2026

cholesterol (HDL-C). Elevated LDL-C levels in dyslipidemia raise the risk of cardiovascular disease, and thus LDL-C lowering is considered an effective therapeutic strategy to hinder the disease progression[2].

Metabolic disorders cover a spectrum of pathological states such as obesity/overweight, hyperlipidemia, diabetes mellitus, hypertension, and hyperuricemia. Over the past two decades, global shifts in dietary habits and lifestyle have contributed to a rise in the prevalence of these metabolic diseases. Patients with metabolic disorders or diabetes exhibit greater susceptibility to atherogenic dyslipidemia and are more likely to have cardiovascular diseases[3]. With the increasing global prevalence of dyslipidemia, lipid-lowering medications may reduce the risk of cardiovascular disease in vulnerable patients. Epidemiological studies report a higher obesity rate in women relative to men, and obese females are prone to reproductive issues, metabolic disorders, and cardiac diseases in later life[4]. There is no permanent cure for dyslipidemia, and current treatment strategies focus on the prevention of disease relapse. Statins, classified as HMG-CoA reductase inhibitors, serve as a rational frontline drug against dyslipidemia with low-density lipoproteins (LDL) as the primary target. Statins lower the LDL-C levels by blocking 3-hydroxy-3-methyl glutaryl coenzyme A reductase and upregulating LDL receptors. Overall, statins are considered safe and effective agents for the treatment of dyslipidemia, but long-term administration may have adverse effects in certain patients[5].

Recently, several folk medicinal herbs have attracted the attention of researchers for their cholesterol-reducing efficacy. Their antihyperlipidemic effect arises from promoting fecal bile acid secretion and excretion, as well as inhibiting cholesterol synthesis and esterification. A variety of phytochemicals widely identified in medicinal plants, including phenolics, saponins, fibers, polysaccharides, alkaloids, and terpenoids, exert antioxidant and hypolipidemic effects. They constitute vital components of the antioxidant defense system and inhibit lipid peroxidation. Among these phytocompounds, phenolic compounds are distinguished for their ability to mitigate oxidative stress, thereby maintaining the integrity of cell membranes and tissues[6].

Many plants exhibit antihyperlipidemic activity. Fenugreek (*Trigonella foenum-graecum* L., *T. foenum-graecum*), an annual herb belonging to the Fabaceae family, possesses abundant nutritional and bioactive ingredients in both its leaves and seeds. Various phytochemicals within its seeds and leaves contribute to its medicinal benefits and spicy flavor[7]. Current research on the hypolipidemic actions of *T. foenum-graecum* L. is still limited, which underscores the importance of exploring its anti-dyslipidemia activity.

2. Methods

2.1. Main reagents

CHOD-POD method kit (QCA[®], Spain) and ELISA kits (Bioassay Technology Laboratory, Shanghai, China) were used. HPLC-grade solvents such as acetic acid, methanol, acetonitrile, and dimethylsulfoxide were purchased from Sigma (Sigma-Aldrich, Germany). Reference compounds like gallic acid, sinapic acid, quercetin, *p*-coumaric acid, salicylic acid, and kaempferol were also purchased from Sigma-Aldrich (Sigma-Aldrich, Germany).

2.2. Ethical approval

All *in vivo* experiment protocols were approved by the Institutional Biosafety and Bioethical Committee, with protocol number D. No. 3937/ORIC, dated 10-07-2024. All animal care and experimental procedures strictly complied with the Guidelines of National Biosafety Rules 2005 (Amended 2024), the Punjab Animal Health Act 2019, and institutional bioethics protocols.

2.3. Plant collection and preparation of extract

T. foenum-graecum L. leaves were morphologically authenticated by a taxonomist from the Department of Botany, and the voucher specimen was deposited with herbarium accession number 47-2-24. The leaves were washed and air-dried in the shade at room temperature for two weeks. After complete drying, the sample was ground into a fine powder for extraction. Then 10 g of the leaf powder was immersed in 100 mL of distilled water, heated and stirred at 75 °C and 120 rpm for 30 min using a magnetic stirrer. The mixture was filtered using a muslin cloth, followed by a Whatman filter paper. Then, the filtrate was concentrated using a rotary evaporator, and the extract was preserved for further formulation[8].

2.4. Quantification by high-performance liquid chromatography (HPLC)

Phytochemicals of *T. foenum-graecum* leaf extract were quantified and identified *via* HPLC by comparing their retention times with reference standards[9]. Chromatographic separation was performed using a C18 column (25 cm × 4.6 mm, 5 μm; Shimadzu, Japan) as the stationary phase. Pure acetonitrile was used as mobile phase B with a gradient elution program set as follows: 15% B from 0 to 15 min, 45% B from 15 to 30 min, and 100% B from 30 to 45 min. The injection volume of the sample was 20 μL, with a constant flow rate of 1 mL/min. The detection wavelength was set in the range of 280–360 nm.

2.5. DPPH assay

The DPPH scavenging activity of leaf extracts was assayed according to Neupane and Lamichhane's method with minor modifications. A fresh DPPH working solution was prepared by dissolving 0.004 g DPPH reagent in 100 mL of methanol. Subsequently, 20 μ L serially diluted leaf extract or standard solution was mixed with 180 μ L freshly prepared DPPH solution in a 96-well plate. The plate was incubated in the dark for 15 min, and absorbance was detected at 517 nm using a spectrophotometer (Thermo Fisher Scientific, Type 1510). Methanol blank and methanolic ascorbic acid standard were assayed simultaneously. Serial concentrations of leaf extracts were tested to calculate the half-maximal inhibitory concentration (IC_{50}). Each sample was analyzed in triplicate, and the radical scavenging rate was calculated using the following equation[10].

$$\% \text{Inhibition} = \frac{(\text{Blank absorbance} - \text{Sample absorbance}) \times 100}{\text{Blank absorbance}}$$

2.6. Experimental animals

A total of 24 healthy adult male albino rats, weighing 150–200 g, were included in this study. They were acclimatized for one week before the initiation of the experiments. All animals were maintained under standardized laboratory conditions: $(23 \pm 2)^\circ\text{C}$, a 12 h/12 h light-dark cycle, and 40%–60% humidity, with free access to diet and tap water. The study lasted 48 days, comprising two consecutive phases: a 20-day modeling phase (Phase I) for dyslipidemia induction, followed by a 28-day treatment phase (Phase II) for pharmacological intervention.

Dyslipidemia was induced in rats *via* administration of a 30% high-fat diet (HFD) (20% proteins, 50% carbohydrates, 30% fats). Rats were randomly divided into 4 groups ($n=6$): Group 1 (normal control), consisting of healthy rats with no intervention; Group 2 (untreated dyslipidemic control), comprising HFD-induced dyslipidemic rats without drug treatment; Group 3 (low-dose treatment), where dyslipidemic rats were orally administered *T. foenum-graecum* aqueous leaf extract at 250 mg/kg body weight; and Group 4 (high-dose treatment), where dyslipidemic rats received oral *T. foenum-graecum* aqueous extract at 500 mg/kg body weight. All dosage were determined by a pilot study.

Rat body weights were measured at the beginning and end of the experiment to verify successful dyslipidemia induction.

2.7. Determination of serum biomarkers

At the end of the 48-day experimental period, all rats were decapitated, and whole blood samples were immediately harvested. Serum was separated by centrifugation at 4 000 rpm and preserved at -20°C for subsequent biochemical analysis.

Serum levels of TC, TG, HDL-C, LDL-C, aspartate aminotransferase (AST), alanine aminotransferase (ALT), and alkaline phosphatase (ALP) were determined using a commercial assay kit (QCA[®], Spain).

2.8. Determination of hepatic oxidative stress biomarkers

Liver tissues were harvested. A 10% (*w/v*) liver homogenate was prepared by homogenizing in pre-cooled 10% potassium chloride, followed by centrifugation at 3 000 rpm for 15 min. The activities of superoxide dismutase (SOD) and catalase (CAT) were determined by using the commercial assay kits.

2.9. Quantitative gene expression by RT-PCR analysis

TRIzol reagent was utilized to extract total RNA from liver tissues, which was quantified by a NanoDrop spectrophotometer. mRNA was reverse-transcribed to cDNA, and qRT-PCR was conducted on a Bio-Rad iQ5 system with Maixma SYBR Green/ROX qPCR Master Mix (2 $\times$). The amplification program included 15 s denaturation at 95°C , 30 s annealing at 58°C , and 20 s extension at 72°C [11]. Relative mRNA expression was quantified by the $2^{-\Delta\Delta Ct}$ method. Primers for the *Duox*, *Duoxa-1*, *Duox2*, *MAPK8*, *Traf4*, *Traf6*, and β -*actin* (housekeeping gene) were used following the manufacturer's instructions (Supplementary Table 1).

2.10. Histopathological analysis

The liver and adipose tissues were collected after the rats were euthanized. The tissues were washed with 0.9% NaCl solution, fixed in 10% neutral buffered formalin, and embedded in paraffin. After sectioning into 5 μ m slices, all specimens were stained with hematoxylin and eosin (H&E) and observed under a light microscope[12].

2.11. Statistical analysis

The data were analyzed by one-way analysis of variance (ANOVA) using the GraphPad Prism software (version 8.1.0). Tukey's test was used to compare the means of the different treatment groups. A *P* value less than 0.05 was regarded as statistically significant. The results were expressed as mean $\pm$ SD.

3. Results

3.1. Quantitative analysis

Quantitative analysis of the leaves of *T. foenum-graecum* using HPLC identified multiple phytochemicals, including sinapic acid,

p-coumaric acid, gallic acid, salicylic acid, kaempferol, ferulic acid, chlorogenic acid, quercetin, and HB acid, as illustrated in Figure 1A. The retention times and concentrations of phytochemicals are detailed in Supplementary Table 2.

3.2. DPPH activity

The antioxidant potential of *T. foenum-graecum* L. aqueous leaf extract (AqE-TFG) was evaluated by DPPH assay (Table 1). The result showed that the plant extract had dose-dependent free radical scavenging effects. The IC₅₀ of AqE-TFG was 4.82 µg/mL, and ascorbic acid was 2.43 µg/mL.

Table 1. Inhibition percentage of AqE-TFG (%).

Concentration (µg/mL)	AqE-TFG	AA
20	20.57 ± 0.01	21.30 ± 0.01
40	25.26 ± 0.01	46.10 ± 0.01
60	34.40 ± 0.01	62.87 ± 0.01
80	41.78 ± 0.01	79.71 ± 0.01
100	53.32 ± 0.01	85.87 ± 0.02

Results are expressed as mean ± SD. AA: ascorbic acid; AqE-TFG: *Trigonella foenum-graecum* aqueous leaf extract.

3.3. Effect of AqE-TFG on body weight

The average body weight of the rats in all groups was similar at the start of the experiment. All groups except the normal control received HFD throughout the experiment. HFD induced body weight gain in rats, with the model group showing a significant ($P<0.05$) increase in body weight at week 7. Oral administration of AqE-TFG significantly ($P<0.05$) reduced body weight (Figure 1B).

3.4. Effect of AqE-TFG on biochemical and antioxidant markers

The model group showed significant increases in serum AST, ALT, and ALP levels and a significant decrease in hepatic CAT and SOD activities ($P<0.05$). AqE-TFG significantly reduced AST, ALT, and ALP levels and significantly elevated hepatic CAT and SOD activities ($P<0.05$) (Figure 2).

3.5. Effect of AqE-TFG on lipid profile

A significant increase in TC, TG and LDL-C level, along with a significant ($P<0.05$) decrease in HDL-C level, was noticed in the

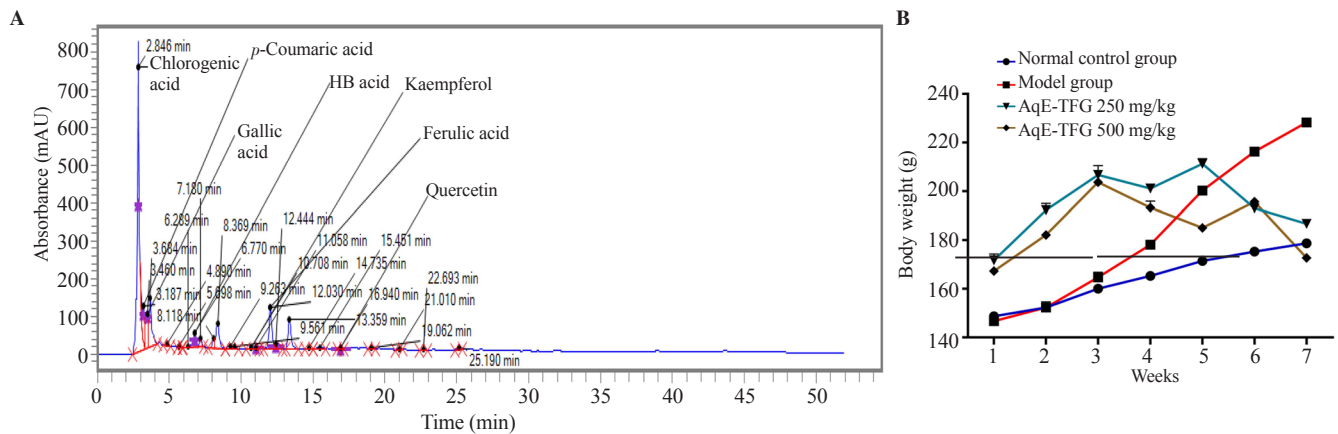


Figure 1. HPLC result (A) and changes in body weight (B). AqE-TFG: aqueous leaf extract of *Trigonella foenum-graecum* L.

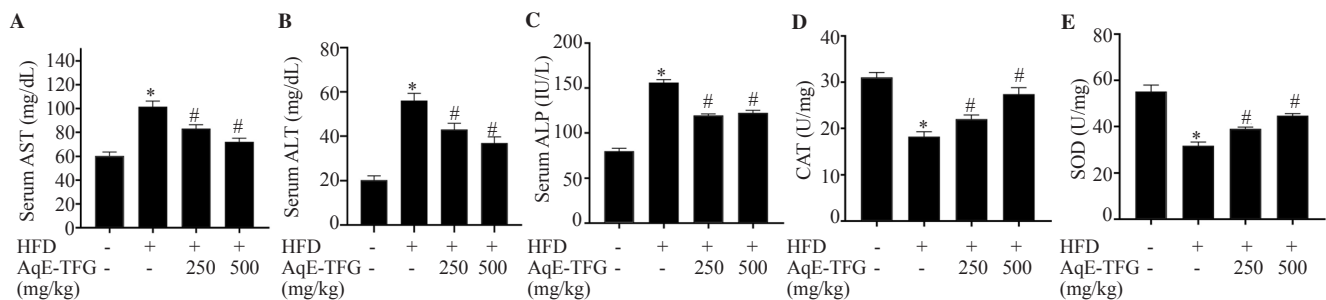


Figure 2. Effect of AqE-TFG treatment on aspartate aminotransferase (AST) (A), alanine aminotransferase (ALT) (B), alkaline phosphatase (ALP) (C), catalase (CAT) (D), and superoxide dismutase (SOD) (E). # $P<0.05$ compared with the model group. * $P<0.05$ compared with the control group. HFD: high fat diet.

model group as compared to the normal control (Figure 3). In all treatment groups, TC, TG and LDL-C levels were significantly ($P<0.05$) decreased and HDL-C level was markedly ($P<0.05$) increased as compared to the model group.

3.6. Quantitative gene expression

The mRNA expression levels of dual oxidase-related genes (*Duox*, *Duoxa-1*, *Duox2*) and downstream MAPK/JNK signaling cascade genes (*MAPK8*, *Traf4*, *Traf6*) were significantly upregulated in the model group ($P<0.05$). By contrast, all treatment groups exhibited marked downregulation of these genes ($P<0.05$) (Figure 4).

3.7. Histological examination of liver and adipose tissues

As shown in Figure 5, the normal group displayed an intact hepatic structure with normal hepatocytes without necrosis. The model group exhibited hepatic lipid accumulation and inflammatory cell infiltration within hepatic lobules. All treatment groups showed reduced lipid deposition and restored hepatocyte nuclei.

H&E staining of adipose tissue showed normal adipocytes with neat arrangement in the normal control group. The model group showed hypertrophic, irregular adipocytes and partial cytoplasmic structural damage. Treatment groups exhibited markedly decreased adipocyte size and volume compared to the model group (Figure 5).

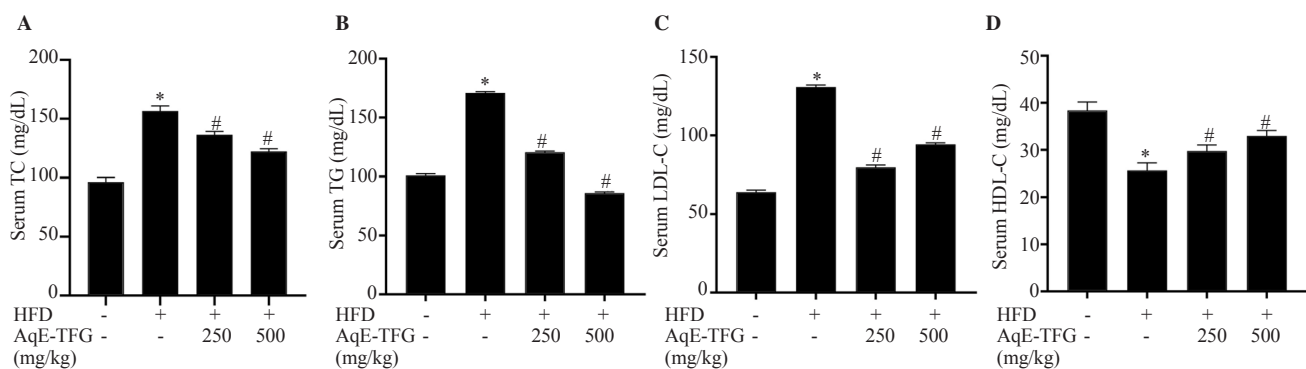


Figure 3. Effect of AqE-TFG treatment on lipid profile; total cholesterol (TC) (A), triglycerides (TG) (B), low-density lipoprotein cholesterol (LDL-C) (C) and high-density lipoprotein cholesterol (HDL-C) (D). # $P<0.05$ compared with the model group. * $P<0.05$ compared with the control group.

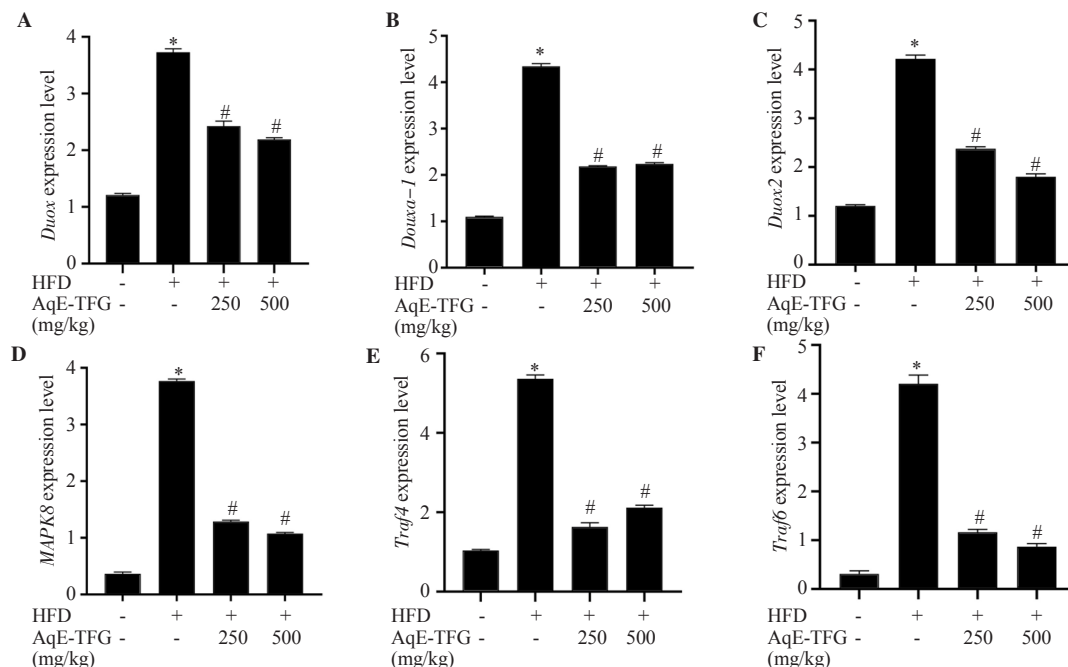


Figure 4. Effect of AqE-TFG treatment on mRNA expression of dual oxidase pathway and downstream MAPK/JNK pathway. A: *Duox*, B: *Duoxa-1*, C: *Duox2*, D: *MAPK8*, E: *Traf4*, F: *Traf6*. # $P<0.05$ compared with the model group. * $P<0.05$ compared with the control group.

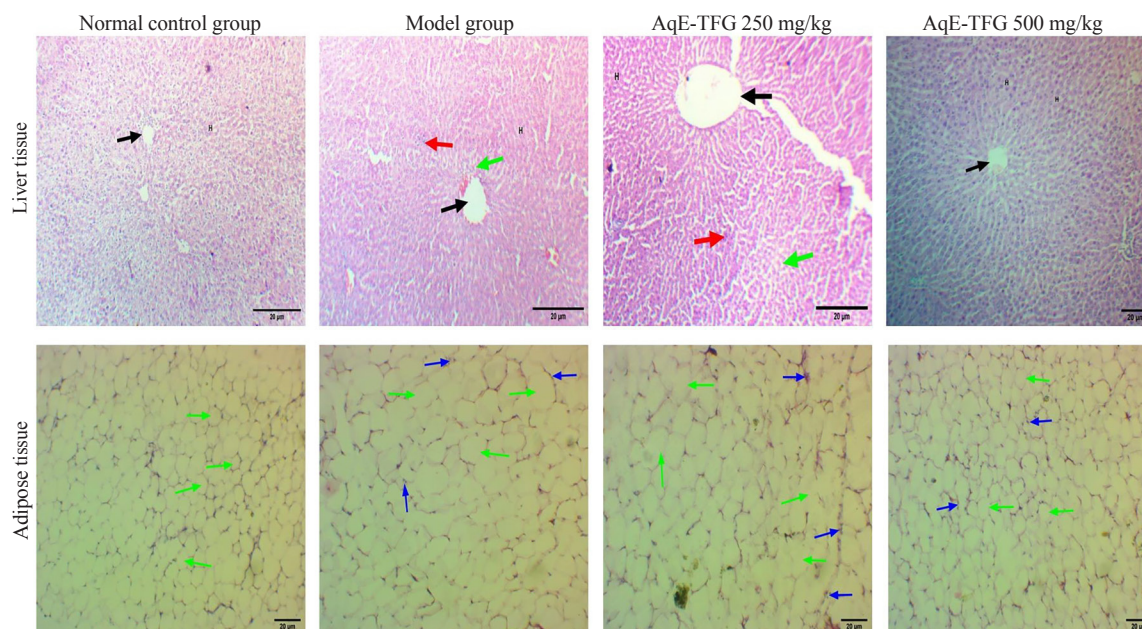


Figure 5. Effect of AqE-TFG treatment on histopathology of liver and adipose tissue. In liver tissues, black arrow shows central vein, red arrow shows infiltration of inflammatory cells, green arrow shows fatty vacuoles and H shows hepatocytes. In adipose tissue, green arrow shows adipocytes and blue arrow shows infiltration of inflammatory cells (20 μ m and 100 $\times$).

4. Discussion

Obesity is a chronic metabolic disorder characterized by disrupted lipid and carbohydrate homeostasis, which arises from excessive fat deposition in adipose tissues and other internal organs. Obesity could lead to many other disorders, such as insulin resistance, dyslipidemia, type 2 diabetes, osteoarthritis, and coronary heart disease. Women are more susceptible to obesity than men. Alterations in lifestyle and dietary patterns are major contributors to the global prevalence of obesity and related metabolic disorders[13]. Medicinal plants have long served as an important part of traditional therapeutic systems[14] and are abundant natural sources of bioactive antioxidants, *e.g.*, phenolics, anthocyanins, flavonoids, and stilbenes, which are widely used in the food industries[15]. Therefore, growing research attention has focused on dietary supplements and herbal medicines for the prevention, alleviation, and reversal of obesity and dyslipidemia. *T. foenum-graecum* is a classic medicinal herb with a long history as folk medicine against various diseases.

Plants and herbs are thought to be copious sources of antioxidants. Their phytoconstituents, especially phenolic compounds, are mainly accountable for their antioxidant property[16]. HPLC analysis was performed to qualitatively and quantitatively analyze the flavonoids and phenolic profiles of *T. foenum-graecum* leaf extract. Standards (caffeic acid and gallic acid) were selected based on their well-documented medicinal properties, such as anticancer,

antioxidant, and anti-inflammatory activities[17,18]. HPLC results demonstrated that AqE-TFG contains many bioactive compounds like gallic acid, chlorogenic acid, salicylic acid, chlorogenic acid, kaempferol, sinapic acid, *p*-cumaric acid, ferulic acid, and syringic acid, which are consistent with the findings reported by Neda *et al*[19]. Vanillic acid possesses potent anti-inflammatory and anticarcinogenic properties[20]. In addition, coumaric acid, caffeic acid, and chlorogenic acid exert antipyretic, hepatoprotective, anti-carcinogenic, neuroprotective, anti-inflammatory, and analgesic activities because of their antioxidant potential[21,22].

HFD induces excessive body weight gain and obesity, largely due to its high energy and fat content that promotes adiposity[23]. Excess TG accumulate with white adipose tissue and other organs like the kidneys, heart, liver, and muscle, leading to fat deposition[24]. In the present study, rats given a 48-day HFD in the model group exhibited significantly higher body weight as compared to the normal control group receiving a standard chow, which is similar to the finding reported by Kumar *et al*[13]. Notably, all AqE-TFG treatment groups displayed markedly reduced body weight. Some studies have attributed the weight-lowering effect of the anorexigenic potential of *T. foenum-graecum* to fiber content and delayed gastric emptying[25].

Long-term high-fat feeding is closely associated with the development of dyslipidemia and metabolic syndrome. Previous studies have confirmed that HFD-induced dyslipidemia serves as a major trigger for systemic oxidative stress. Results of

the present study have shown that the model group exhibited markedly elevated serum TC, TG and LDL-C levels alongside reduced HDL-C concentrations, which are hallmark biomarkers of dyslipidemia, which is similar to the findings reported by Sajid *et al*[8]. Additionally, dysregulated lipid profiles, especially hypercholesterolemia, are strongly correlated with the progression of cardiovascular complications. The administration of AqE-TFG extract effectively ameliorated these metabolic disorders. The lipid-regulating efficacy of the extract can be attributed to its abundant phytochemicals, including saponins, flavonoids, and phenolic acids. These bioactive compounds modulate lipid metabolism *via* multiple pathways, notably suppressing HMG-CoA reductase, the vital enzyme involved in cholesterol biosynthesis[26].

Previous research has demonstrated that abnormal elevation of serum AST, ALT, and ALP results from the leakage of intracellular enzymes into the bloodstream, which are typically considered an indicator of liver injury[24,27]. HFD-fed rats displayed significantly elevated serum AST and ALT, which indicate liver damage. AqE-TFG exhibited hepatoprotective effects by markedly decreasing the levels of these enzymes. The favorable liver-protective effects of *T. foenum-graecum* leaves can be largely attributed to their rich phytochemical components, including flavonoids, phenols, saponins, alkaloids, *etc.* Previous studies revealed that these bioactive constituents effectively attenuate the elevation of liver injury biomarkers and alleviate hepatocellular damage. It is reported that pretreatment with flavonoids suppresses elevated AST and ALT and protects hepatocytes against oxidative damage *in vitro*[28]. The hepatoprotective effect of flavonoids is likely mediated by their antioxidant properties against reactive oxygen species (ROS). Variable intracellular ROS levels trigger a variety of pathological responses, such as apoptosis, autophagy, and necrosis[29]. Pretreatment with flavonoids enhances the antioxidant capacity of the liver compared with nontreated groups[30].

Metabolic syndrome is linked to oxidative stress and visceral fat accumulation. Research indicated that HFD-induced obese rats displayed severe hepatic oxidative stress, which arises from excessive ROS and impaired antioxidant defense system[31]. Abnormal alterations in hepatic CAT and SOD activities are well-recognized biomarkers of aggravated lipid peroxidation and oxidative damage under obese conditions[32]. In the present study, hepatic SOD and CAT were decreased, indicating an impaired antioxidant defense system due to HFD. The antioxidant defense system relies primarily on functional antioxidant enzymes: SOD catalyzes the dismutation of superoxide anion (O_2^-) into hydrogen peroxide, while CAT decomposes hydrogen peroxide into water[33]. Compared with the normal control group, HFD increased ROS generation. Results of our study indicated that AqE-TFG significantly enhanced SOD and CAT activities, which could effectively restore antioxidant capacity

and protect the liver from HFD-induced oxidative toxic injury[34]. The enhanced SOD and CAT activity indicates that AqE-TFG alleviated HFD-induced oxidative stress. This antioxidant activity can be attributed to the presence of numerous phenolic compounds such as quercetin and chlorogenic acid[12].

In our study, mRNA levels of genes involved in the downstream MAPK signaling pathway were significantly upregulated in the model group. AqE-TFG downregulated these genes, showing the efficacy of *T. foenum-graecum* in alleviating oxidative stress and modulating the downstream MAPK/JNK signaling pathway. Previous research also found that the stress-activated MAPK/JNK pathway is crucial for the onset and progression of inflammatory disorders. Inhibition of this pathway can effectively block inflammation-driven disease development and progression[35,36].

Dual oxidases (DUOX) are members of the NADPH oxidases family. DUOXs are involved in many biological processes and play a critical role in epithelial ROS generation[37]. To further validate the antioxidant potential of AqE-TFG, expressions of *Duox*, *Duox2*, and *Duoxa-1* genes were determined in this study. Compared to the model group, AqE-TFG significantly downregulated these DUOX-related genes.

Biochemical findings were further supported by a histopathological examination of the liver and adipose tissues. The model group showed hepatic lipid accumulation and enlarged adipocytes. In the treatment groups, AqE-TFG reversed these morphological abnormalities, confirming the potential of the extracts against obesity and dyslipidemia. Such beneficial effects may be attributed to increased mitochondrial β -oxidation and decreased lipid accumulation, which are well-characterized metabolic regulatory properties of polyphenol-rich plant extracts.

T. foenum-graecum exhibits superior safety profiles compared with conventional lipid-lowering agents, such as statins or fibrates, which are frequently associated with adverse effects, including hepatotoxicity, myopathy, and gastrointestinal disturbance. Nevertheless, the present study has several limitations, including the lack of dose-response validation and long-term intervention evaluation. Further in-depth mechanistic investigations, clinical trials, and pharmaceutical formulation optimization are warranted to translate the current preclinical findings into viable clinical applications.

In conclusion, our findings showed the value of AqE-TFG in the management of dyslipidemia, as evidenced by its favorable lipid-regulating, antioxidant activities, and hepatoprotective effects. Such herbal extracts can be integrated into functional foods or complementary therapy to reduce reliance on synthetic lipid-lowering chemical pharmaceuticals and promote holistic health.

Conflict of interest statement

The authors declare that there is no conflict of interest.

Funding

The article received no extramural funding.

Data availability statement

The data supporting the findings of this study are available from the corresponding author upon request.

Authors' contributions

SJ and FH contributed to conceptualization, methodology, and data collection. BA and MI were involved in data analysis. All authors have read and agreed to the published version of the manuscript.

References

- [1] Dybiec J, Baran W, Dąbek B, Fularski P, Młynarska E, Radzioch E, et al. Advances in treatment of dyslipidemia. *Int J Mol Sci* 2023; **24**(17): 13288.
- [2] Ceja-Galicia ZA, Aranda-Rivera AK, Amador-Martínez I, Aparicio-Trejo OE, Tapia E, Trujillo J, et al. The development of dyslipidemia in chronic kidney disease and associated cardiovascular damage, and the protective effects of curcuminoids. *Foods* 2023; **12**(5): 921.
- [3] Li L, Li T, Liang X, Zhu L, Fang Y, Dong L, et al. A decrease in *Flavonifractor plautii* and its product, phytosphingosine, predisposes individuals with phlegm-dampness constitution to metabolic disorders. *Cell Discov* 2025; **11**(1): 25.
- [4] Vekic J, Stefanovic A, Zeljkovic A. Obesity and dyslipidemia: A review of current evidence. *Curr Obes Rep* 2023; **12**(3): 207-222.
- [5] Wang WZ, Liu C, Luo JQ, Lei LJ, Chen MH, Zhang YY, et al. A novel small-molecule PCSK9 inhibitor E28362 ameliorates hyperlipidemia and atherosclerosis. *Acta Pharmacol Sin* 2024; **45**(10): 2119-2133.
- [6] Belguith-Hadriche O, Ammar S, Contreras MD, Turki M, Segura-Carretero A, El Feki A, et al. Antihyperlipidemic and antioxidant activities of edible *Tunisian ficus carica* L. fruits in high fat diet-induced hyperlipidemic rats. *Plant Foods Hum Nutr* 2016; **71**(2): 183-189.
- [7] Olaiya CO, Soetan KO. A review of the health benefits of fenugreek (*Trigonella foenum-graecum* L.): Nutritional, biochemical and pharmaceutical perspectives. *Int J Adv Soc Sci Humanit* 2014; **4**: 3-12.
- [8] Sajid MR, Aslam B, Hassan F. Determination of phytochemical constituents and anti-dyslipidemic potential of *Cassia absus* seed extract to combat high-fat diet-induced dyslipidemia in rats model. *Pak J Agri Sci* 2025; **62**(2): 261-267.
- [9] AlMasoud N, Rabail R, Alomar TS, Munir S, Hassan SA, Aadil RM. Therapeutic impact of bitter gourd seed-fortified crackers on alloxan-induced diabetic rats. *Pak Vet J* 2024; **44**(3): 629-636.
- [10] Rueangsri K, Lasunon P, Kwantrairat S, Taweejun N. Effect of ultrasound-assisted aqueous two-phase extraction on phenolic compounds from *Nymphaea pubescens* Willd. and its antioxidant and antimicrobial properties. *Int J Agric Biosci* 2025; **14**(1): 1-10.
- [11] Abbas I, Majeed W, Aslam B, Faisal MN. *Salsola imbricata* attenuates type 2 diabetes mellitus in rats by regulating oxidative stress and insulin signaling. *Asian Pac J Trop Biomed* 2025; **15**(5): 210-218.
- [12] Choe DW, Foo HL, Loh TC, Hair-Bejo M, Awis QS. Inhibitory property of metabolite combinations produced from *Lactobacillus plantarum* strains. *Pertanika J Trop Agric Sci* 2013; **36**(1): 79.
- [13] Kumar DS. *Advances in food fortification with phytonutrients*. USA: CRC Press; 2017, p. 161-244.
- [14] Aslam B, Sajid MR, Hussain A, Abbas I. Pharmacological profiling of medicinal plants: A contemporary overview. *Nat Prod Res* 2024; **40**(10): 2963-2979.
- [15] Djeridane A, Yousfi M, Nadjemi B, Boutassouna D, Stocker P, Vidal N. Antioxidant activity of some Algerian medicinal plants extracts containing phenolic compounds. *Food Chem* 2006; **97**(4): 654-660.
- [16] Shahid Chatha S, Hussain A, Asad R, Majeed M, Aslam N. Bioactive components and antioxidant properties of *Terminalia arjuna* L. extracts. *J Food Process Technol* 2014; **5**(298): 2.
- [17] Shabbir M, Syed DN, Lall RK, Khan MR, Mukhtar H. Potent anti-proliferative, pro-apoptotic activity of the *Maytenus royleanus* extract against prostate cancer cells: Evidence in *in-vitro* and *in-vivo* models. *PLoS One* 2015; **10**(3): 119859.
- [18] Yang J, Guo J, Yuan J. *In vitro* antioxidant properties of rutin. *LWT Food Sci Technol* 2008; **41**(6): 1060-1066.
- [19] Neda B, Feyissa T, Dagne K, Assefa E. The study of morphological characteristics and statistics of the phenotypes and correlation in faba bean (*Vicia faba* L.) germplasm. *Plant Breed Biotechnol* 2021; **9**(2): 139-163.
- [20] Yrbas MA, Morucci F, Alonso R, Gorzalczy S. Pharmacological mechanism underlying the antinociceptive activity of vanillic acid. *Pharmacol Biochem Behav* 2015; **132**: 88-95.
- [21] Naveed M, Hejazi V, Abbas M, Kamboh AA, Khan GJ, Shumzaid M, et al. Chlorogenic acid (CGA): A pharmacological review and call for further research. *Biomed Pharmacother* 2018; **97**: 67-74.
- [22] Espindola KMM, Ferreria RG, Narvaez LEM. Chemical and pharmacological aspects of caffeic acid and its activity in hepatocarcinoma. *Front Oncol* 2019; **9**: 541.
- [23] Ilić I, Oršolić N, Rodak E, Odeh D, Lovrić M, Mujkić R, et al. The effect of high-fat diet and 13-*cis* retinoic acid application on lipid profile, glycemic response and oxidative stress in female Lewis rats. *PLoS One* 2020; **15**(9): 0238600.

- [24]Othman AI, Amer MA, Basos AS, El-Missiry MA. *Moringa oleifera* leaf extract ameliorated high-fat diet-induced obesity, oxidative stress and disrupted metabolic hormones. *Clin Phytosci* 2019; **5**(1): 48.
- [25]Rahman Z, Shaikh N, Abdurrahman OA, Akhtar J, Ahmad NZ. Natural agents used for the management of obesity in the traditional unani system of medicine: A comprehensive review. *Acta Sci Med Sci* 2025; **9**(2):114-126.
- [26]Kang H, Yan G, Lin X, Tian Y, Yin J, Liu J, et al. The substructure of the endothelial glycocalyx in rat aorta and its interaction with the low-density lipoproteins (LDL). *Am J Pathol* 2025; **195**(10): 1936-1958.
- [27]Karabekir SC, Gultekin B, Ayan IC, Savas HB, Cuce G, Kalkan S. Protective effect of astaxanthin on histopathologic changes induced by bisphenol A in the liver of rats. *Pak Vet J* 2024; **44**(2): 244-251.
- [28]Chi L, Niu H, Niu Y, Yao R, Shi D, Lu B, et al. *Trigonella foenum-graecum* L. ameliorates metabolism-associated fatty liver disease in type 2 diabetic mice: A multi-omics mechanism analysis. *J Ethnopharmacol* 2025; **348**: 119862.
- [29]Riaz R, Asif M, Afzal G, Ur-Til-Wusqa, Talib M, Afzal M, et al. Investigation of oxidative stress and antioxidative enzymes in erythrocytes and bone marrow of albino rats treated with different concentrations of copper ferrite nanoparticles. *Int J Agric Biosci* 2025; **14**(5): 1035-1041.
- [30]Sozen ME, Savas HB, Cuce G. Protective effects of selenium against acrylamide-induced hepatotoxicity in rats. *Pak Vet J* 2024; **44**(2): 274-279.
- [31]Fardet A, Llorach R, Martin JF, Besson C, Lyan B, Pujos-Guillot E, et al. A liquid chromatography-quadrupole time-of-flight (LC-QTOF)-based metabolomic approach reveals new metabolic effects of catechin in rats fed high-fat diets. *J Proteome Res* 2008; **7**(6): 2388-2398.
- [32]Firdous H. Ameliorative effects of foliar thiourea on growth, yield, and antioxidant defense in wheat (*Triticum aestivum* L.) under differential irrigation regimes. *Plant Crop Lett* 2025; **4**: 47-53.
- [33]Rocha KKR, Souza GA, Ebaid GX, Seiva FRF, Cataneo AC, Novelli ELB, et al. Resveratrol toxicity: Effects on risk factors for atherosclerosis and hepatic oxidative stress in standard and high-fat diets. *Food Chem Toxicol* 2009; **47**(6): 1362-1367.
- [34]Ijaz MU, Nadeem A, Hayat MF, Ehsan N, Al-Ghanim KA, Atique U. Evaluation of possible ameliorative role of robinetin to counteract polystyrene microplastics instigated renal toxicity in rats. *Pak Vet J* 2024; **44**(2): 400-404.
- [35]Jia JH, Zhai SY, Cai TQ, Guo PP, Yang GA, Chang ZK, et al. Rutin alleviates high-fat diet-induced MAFLD: Insights from 16S rRNA sequencing, metabolome, network pharmacology and transcriptome analyses. *Food Sci Hum Wellness* 2026; **15**(1): 9250908.
- [36]Kragholm K, Newby LK, Melloni C. Emerging treatment options to improve cardiovascular outcomes in patients with acute coronary syndrome: Focus on losmapimod. *Drug Des Dev Ther* 2015; **9**(2015): 4279.
- [37]Wu M, Yang S, Wang S, Cao Y, Zhao R, Li X, et al. Effect of berberine on atherosclerosis and gut microbiota modulation and their correlation in high-fat diet-fed ApoE^{-/-} mice. *Front Pharmacol* 2020; **11**: 223.

Publisher's note

The Publisher of the *Journal* remains neutral with regard to jurisdictional claims in published maps and institutional affiliations.

Edited by Tan BJ, Liang Q

Supplementary Table 1. List of primer sequence.

Primers	Sequence
Housekeeping gene	
<i>Beta actin-F</i>	CGAGTACAACCTTCTTGCAGC
<i>Beta actin-R</i>	TATCGTCATCCATGGCGAACTG
MAPK downstream JNK pathway	
<i>MAPK8-F</i>	CTCAGCATCCGGTCTCTTCG
<i>MAPK8-R</i>	CTGCTGTCTGTATCCGAGGC
<i>Traf-4-F</i>	CGACTACAAGTTCCTGGAGAAGC
<i>Traf-4-R</i>	AGGTGTCGCAGAAGCGGTG
<i>Traf-6-F</i>	GGCGCCTAGTAAGACAGGAC
<i>Traf-6-R</i>	GGCGCCTAGTAAGACAGGAC
Dual oxidase pathway	
<i>Duox-F</i>	TCATGTGGATGGCACCAGAC
<i>Duox-R</i>	CGAAACCACAACAAGCCCAG
<i>Duoxa-1-F</i>	GAAAGGAAGCATCAACACCCC
<i>Duoxa-1-R</i>	GTGGTGTCCATTGGGAAGGT
<i>Duox2-F</i>	CTCTCCAGCTCTCAGGGTCT
<i>Duox2-R</i>	GCGAGAGTATCTGTCTGGGC

Supplementary Table 2. Phytoconstituents retention time and concentrations in *Trigonella foenum graceum* (fenugreek).

Extract	Phytochemicals	Peak #	Retention time (min)	Area	Height

AqE-TFG	Chlorogenic acid	1	2.846	7,933,887.3	708,298.7
	<i>P</i> -coumaric acid	2	3.187	1,420,363.7	89,960.4
	Gallic acid	3	3.460	1,133,368.5	95,867.0
	HB acid	8	6.770	594,540.3	37,400.5
	Kaempferol	14	10.708	72,185.9	4,824.3
	Ferulic acid	16	12.030	1,587,796.6	109,727.6
	Quercetin	21	16.940	32,308.1	2268.8