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Lycium barbarum leaves ameliorate diabetic retinopathy by regulating MAPK/NF- κ B/VEGF signaling axis and gut microbiota-inflammation crosstalk

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ABSTRACT: *Lycium barbarum* leaves (LBL), a medicinal and edible plant traditionally used in China, exert proven anti-diabetic effects, however, their potential for ameliorating diabetic retinopathy (DR) remains unclear. We aimed to screen LBL bioactive fractions (LBLBFs) and clarify their underlying mechanisms. LBL fractions were evaluated in high-glucose-injured zebrafish and retinal cells to identify LBLBFs; Ultra-high performance liquid chromatography with quadrupole time-of-flight mass spectrometry analysis identified flavonoids and phenolic acids as the core bioactive components, which guide network pharmacology-based mechanism prediction. In the screening models, LBLBFs significantly reduced oxidative stress and ROS levels. In mice with DR induced by a high-fat/high-sugar diet and streptozotocin, multi-dimensional validation showed that LBLBFs alleviated DR-related inflammation, retinal damage, and aberrant angiogenesis by downregulating the MAPK/NF- κ B/VEGF axis. They also regulated gut microbial homeostasis to reduce circulating lipopolysaccharide levels and synergistically inhibit this pathway. In conclusion, LBLBFs improve DR via dual mechanisms, highlighting their potential as multicomponent, multitargeted functional food ingredients suitable for long-term dietary intervention.

Keywords: Diabetic retinopathy; *Lycium barbarum* leaves; Gut microbiota

1. Introduction

In recent years, the ageing global population and lifestyle changes have contributed to a steady annual increase in the incidence and disability rates of diabetes and its complications. Diabetes has become the third most significant threat to global health, following cancer and cardiovascular diseases [1]. The Global Diabetes Report released by the IDF Diabetes Atlas indicated that as of 2021, an estimated 537 million adults worldwide were living with diabetes. This number is projected to rise to 783 million by 2045. Diabetic retinopathy (DR),

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one of the most common microvascular complications of diabetes, is also classified as a top three sight-threatening eye disease by the World Health Organization. In 2022, approximately 103.12 million adults were affected by DR globally. This number is expected to increase to 160.5 million by 2045 [2]. As the leading cause of visual impairment among the working-age population, DR poses substantial challenges for prevention and clinical management [3, 4]. Therefore, enhancing research on its prevention and control to delay its onset and progression is of critical societal importance.

The pathogenesis of DR is complex. Currently, the widely recognised pathogenic mechanisms include microvascular lesions induced by hyperglycaemia, neuronal lesions, and moderate to low-grade inflammatory responses [5]. Currently, DR clinical treatment mainly includes control of blood glucose and lipid levels, laser photocoagulation, and intravitreal injection of antibody-based drugs. However, each of these therapeutic approaches has inherent limitations, glycemic and lipid control only delays disease progression and cannot reverse established retinal injury; laser photocoagulation destroys retinal tissue and may cause visual field defects; and intravitreal injections carry risks of infection, elevated intraocular pressure, the burden of repeated treatments. Therefore, further exploration of the pathogenesis of DR and the development of safe and effective treatment strategies are particularly important. Although traditional Chinese medicine (TCM) does not have a specific disease name for DR, based on its clinical manifestations, signs, and disease characteristics, it can be classified into the TCM categories of ‘dim vision’, ‘cloudy eyes’, ‘sudden blindness’, and ‘blood filling the pupil’. According to TCM theory, DR is often attributed to long-term diabetes, which depletes ‘yin’ and ‘qi’, damages the meridians over time, and causes blood stasis and obstruction, resulting in meridian malnutrition. In clinical practice, herbal medicines with functions such as nourishing ‘yin’, clearing ‘heat’, and improving vision are often used as first-line treatments for DR. In recent years, TCM has demonstrated notable advantages in delaying DR progression, reducing its incidence, and improving the quality of life of patients because of its mild and long-lasting effects, multicomponent composition, and multitarget mechanisms [6-9]. Therefore, under the guidance of TCM theory, in-depth exploration of its material basis and mechanisms of action in regulating DR onset and progression has become a major research focus in recent years and an important approach for developing new drugs for DR prevention and treatment.

Lycium barbarum L. from Ningxia is a traditional Chinese plant with medicinal and edible value. Its fruits, known as *Lycium barbarum* fruits (LBF), are valued for their effects in nourishing essence and blood, improving eyesight, and alleviating deficiency-related symptoms [10]. Modern pharmacological research has further confirmed that LBF possess therapeutic potential in DR treatment [11-13]. *Lycium barbarum* leaves (LBL), which share the same botanical origin as LBF, also have a long history of application. As the dried tender leaves of *Lycium barbarum* L. from Ningxia, LBL have been used in China for centuries and are traditionally consumed as daily edible ingredients via boiling, stir-frying, tea-making, or being added to soups. They are believed to offer effects such as tonifying deficiency, nourishing essence, clearing ‘heat’, quenching ‘thirst’, dispelling ‘wind’, and improving vision. Contemporary research indicates that LBL are rich in various chemical constituents, including flavonoids, phenolic acids, alkaloids, and polysaccharides [14]. Furthermore,

the content of certain bioactive components, such as flavonoids and phenolic acids, is substantially higher in LBL than in LBF [15]. These components are relatively stable under common cooking conditions, rendering LBL a promising candidate for long-term dietary intervention in diabetes management. Specifically, LBL and its extracts exhibit marked inhibitory activity against α -glucosidase and α -amylase, thereby delaying digestion and absorption of dietary carbohydrates in the small intestine and subsequently suppressing postprandial hyperglycemia [16]. Animal studies indicate that LBL extracts significantly reduce fasting blood glucose and lipid levels in streptozotocin-nicotinamide-induced diabetic rats. They also lower alkaline phosphatase, aspartate transaminase, alanine transaminase activities, reduce oxidative stress and inflammatory factor levels (TNF- α , IL-6, and IL-1 β), and delay the onset and progression of complications such as diabetic nephropathy and cardiomyopathy [17-19]. Research also indicates that flavonoid components in LBL may mitigate obesity by regulating glucose and lipid levels, oxidative stress, and gut microbiota composition in mice fed a high-fat diet [20]. Notably, as a natural edible material, LBL avoids the side effects of synthetic drugs, making it suitable for development as a functional food or dietary supplement for diabetic patients [21]. However, the underlying mechanisms by which LBL improves DR remain to be elucidated.

We aimed to isolate LBL fractions and evaluate their efficacy using high-glucose-induced injury zebrafish and cell models to identify the optimal fraction. The therapeutic effects were further assessed in a DR mouse model induced by a high-sugar/high-fat diet (HHFD) and streptozotocin (STZ). The chemical profile was characterized using ultra-high performance liquid chromatography with quadrupole time-of-flight mass spectrometry (UPLC-Q-TOF/MS); potential targets were predicted using network pharmacology (NP) with validation by western blot analysis. Collectively, 16S rRNA sequencing was employed to explore the role of gut microbiota regulation in ameliorating DR. Together, these findings highlight the nutritional and functional potential of LBL and provide a scientific basis for its development as a functional food, dietary supplement, or nutraceutical to support eye health in individuals with diabetes.

2. Materials and methods

2.1 Materials

L. barbarum leaves were purchased from Mingde Chinese Herbal Medicine Decoction Pieces Co., Ltd (Ningxia, China) (batch No. 20240925). Glucose (GLU), glutathione (GSH), malondialdehyde (MDA) and superoxide dismutase (SOD) assay kits were purchased from Jiancheng Biotech (Nanjing, China). Betaine (CAS# 107-43-7) was purchased from Yuanye Biotech Co., Ltd. (Shanghai, China). HHFD for mice was purchased from Shuyu Biotech Co., Ltd. (Shanghai, China). All other chemicals and solvents were purchased from local reagent suppliers and were of analytical or chromatographic grade.

2.2 Preparation of LBL fractions

A specified weight of dried LBL was divided into two portions. Each portion was extracted twice by reflux heating for 1 h using 10 times its mass of distilled water and 80% ethanol, respectively. The extracts were concentrated under reduced pressure and freeze-dried to obtain two types of total LBL extracts: the water

extract (WE) fraction and ethanol extract (EE) fraction. Another EE was prepared following the same procedure, concentrated to an appropriate volume, and fully adsorbed onto D101 microporous resin for 24 h. The resin was eluted with 2–3 column volumes each of distilled water, 10% ethanol, and 30% ethanol; the eluates were discarded. Subsequently, 70% ethanol was used as the eluent. After collecting 2–3 column volumes of the 70% ethanol eluate, the solution was concentrated under reduced pressure and freeze-dried to obtain the LBL flavonoid-phenolic acid fraction (FPF). The EE residue was air-dried and divided into two parts. One part was added with 10 times its mass of distilled water, and refluxed twice for extraction (1 h for each extraction). After concentration under reduced pressure, anhydrous ethanol was added until ethanol concentration reached 80%. The mixture was left at 4 °C overnight, after which the supernatant was discarded, and the residue was dried to obtain crude polysaccharides. The protein was removed using the Sevag (chloroform: n-butanol = 4:1) method to obtain the refined polysaccharide fraction (PF). Remaining portion of the EE residue was combined with 10 times its mass of 1% NaOH, stirred thoroughly, and centrifuged. The supernatant was discarded; the precipitate was treated with 10 volumes of ethanol solution (pH = 3.0). Extraction was performed at 60 °C under ultrasonic assistance. The product was vacuum-concentrated and freeze-dried to obtain the LBL alkaloids fraction (AF). Two extract fractions (WE and EE) and three refined component fractions (FPF, PF, and AF) of LBL were obtained.

2.3 Animal experiments

Wild-type (AB strain) and *Tg (fli1: EGFP)* transgenic (CZ16 strain) zebrafish were purchased from Yao Shunyu Biotechnology Co., Ltd. and reared in a circulating water system. The zebrafish were maintained under a 14-h:10-h light–dark cycle at water temperature of 28 ± 0.5 °C. All adult zebrafish were fed brine shrimp twice daily. Healthy embryos were collected by natural mating of adult zebrafish (male: female = 2:1), and cultured in embryo water (1.3 mmol/L CaCl_2 , 0.137 mol/L NaCl, 5.4 mmol/L KCl, 0.25 mmol/L Na_2HPO_4 , 4.2 mmol/L NaHCO_3 , 0.44 mmol/L K_2HPO_4 , 1.0 mmol/L MgSO_4). The embryo water was replaced every 24 h. Healthy embryos 3 days post fertilization (dpf) were selected under a stereomicroscope for subsequent experiments.

Eight-week-old male SPF C57BL/6J mice, weighing (20–30) g, were purchased from Shanghai Slack Experimental Animal Co., Ltd. [SCXK (shanghai) 2022-0004] were used in this research. Before formal experiment, the mice underwent a 7-d acclimatisation period under the following experimental conditions: 20–26 °C temperature, 30–70% relative humidity, and 12 h light/dark cycle. All procedures were approved by the Institutional Animal Care and Use Committee of Nanjing University of Chinese Medicine. (approval number: 202409A068).

2.4 Cell culture

The ARPE-19 cell line is a human retinal pigment epithelial cell line purchased from American Type Culture Collection (USA). The cells were cultured in DMEM/F12 medium (Gibco, Thermo Fisher Scientific, USA) containing 10% fetal bovine serum (Gibco, Thermo Fisher Scientific, USA) and 1%

penicillin/streptomycin (Gibco, Thermo Fisher Scientific, USA) at 37 °C in a humidified atmosphere containing 5% CO₂. Cells were inoculated into culture plates at a specific density and divided into 12 groups: the control group, model group, and high- and low-dose groups of WE, EE, FPF, PF and AF. After 24 h, medium of the control group cells was replaced with a fresh medium, whereas the model group cells were cultured in a medium containing 300 mM glucose for 48 h. The remaining high-dose treatment groups were exposed to a 300 mM glucose medium supplemented with 10 µg/mL of the corresponding lyophilized powder of LBL fractions, the low-dose group received 1 µg/mL of the corresponding fractions powder. The cultures were maintained for an additional 48 h before subsequent experiments.

2.5 High-glucose-induced injury zebrafish models

Zebrafish (3 dpf) were cultured in embryo water and randomly divided into 13 groups: the control group, model group, metformin (MET) group, and high and low dose groups of WE, EE, FPF, PF and AF, with 30 fish in each group. The control group was fed normally, the model group was given 180 mM glucose, and the MET group was given 180 mM glucose and 400 µg/mL MET. High-dose groups of the remaining treatment groups were given 180 mM glucose and 40 µg/mL of the corresponding fractions, whereas the low-dose groups received 20 µg/mL of the same fractions. All doses of the LBL fractions described above were equivalent to crude drug doses. All embryos were treated for 3 days, zebrafish (6 dpf) were collected for subsequent experiments.

2.6 Biochemical analysis of zebrafish

Wild-type (AB) zebrafish embryos were collected after 3 d of intervention according to the above-mentioned method. Tissue homogenates were prepared at low temperature. GLU content, GSH and MDA levels, and SOD activity in zebrafish embryos were detected according to the biochemical detection kit's instructions.

2.7 Cell viability assay

ARPE-19 cells were seeded in 96-well plates at a density of 5×10^3 cells per well and cultured as described above. At the end of the culture period, the medium was discarded, fresh medium containing 10% Cell Counting Kit-8 (Abbkine, China) was added. The cells were incubated at 37 °C for 1 h. Subsequently, absorbance at 450 nm was measured using a microplate reader (Spectrum MAX190, Molecular Devices, USA).

2.8 Intracellular reactive oxygen species (ROS) assay

ARPE-19 cells were seeded in 6-well plates at a density of 1.5×10^4 cells per well and cultured as previously described. Following removal of the culture medium, the cells were washed twice with PBS and subsequently incubated with a serum-free medium containing 10 mM 2',7'-dichlorofluorescein-diacetate (Glpbio, USA) fluorescent probe at 37 °C for 30 min in the dark. After incubation, the cells were washed twice with PBS to remove residual probe. Fluorescence imaging was performed using an AxioScope A1

fluorescence microscope (Zeiss, Germany) with excitation at 540 nm and emission at 590 nm. Quantitative analysis of fluorescence intensity was conducted using ImageJ.

2.9 Biochemical analysis of ARPE-19

Following treatment in 6-well plates as previously described, the cells were trypsinised, harvested, and counted using a hemocytometer. Cell suspensions were centrifuged at 1,000 rpm for 4 min at 4 °C, after which the supernatant was carefully aspirated. The cell pellets were washed three times with pre-chilled PBS. Subsequently, the cells were lysed by ultrasonication on ice, followed by centrifugation at 12,000 rpm for 10 min at 4 °C to obtain the homogenate supernatant. Oxidative stress parameters including GSH and MDA levels, and SOD activity, were measured according to the biochemical detection kit's instructions.

2.10 Multivariate analysis

The original data from 10 pharmacodynamic indicators, including GLU content, GSH and MDA levels, SOD activity in zebrafish, as well as cell viability, ROS levels, and GSH and MDA levels, SOD activity in ARPE-19 cells—were normalized. Principal component analysis (PCA) was performed using SIMCA (v14.1) to evaluate the model, followed by 200 permutation tests to assess potential overfitting. In the PCA model, the first principal component (t_1) captures the largest variance, whereas the second component (t_2) accounts for the second largest variance. The Euclidean distance between each sample's coordinates (t_{1s} , t_{2s}) and centroid of the control group (t_{1c} , t_{2c}) was calculated to quantify group separation. The formula is as follows:

$$d = \sqrt{(t_{1s} - t_{1c})^2 + (t_{2s} - t_{2c})^2}$$

The optimal LBL fraction was determined by PCA and used for subsequent experiments.

2.11 UPLC-Q-TOF/MS analysis

The WE freeze-dried powder was dissolved in pure water, whereas the EE and FPF freeze-dried powders were dissolved in 80% ethanol; they both yielded solutions containing 20 mg/ml of dried LBL fractions. The resulting solution was centrifuged at 12,000 rpm for 10 min and filtered through a 0.22 µm microporous membrane to obtain the analytical sample.

Chromatographic separation was performed on an ACQUITY UPLC BEH C₁₈ column (100 mm × 2.1 mm, 1.7 µm, Waters) using 0.1% formic acid water (A) and acetonitrile (B) as the eluents. Gradient elution conditions were as follows: 0–2 min, 5%–6% B; 2–6 min, 6%–10% B; 6–7 min, 10%–12% B; 7–15 min, 12%–16% B; 15–17 min, 16%–30% B; 17–19 min, 30%–52% B; 19–22 min, 52%–70% B; 22–23 min, 70%–5% B; 23–24 min, 5%–95% B; and 24–25 min, 95%–95% B. The injection volume was 2 µL.

Mass spectrometry data were acquired using the UPLC-Q-TOF-MS (SCIEX Corporation, USA) equipped with an electrospray ionization (ESI) source operating in positive and negative ion scanning modes. ESI source settings were set as follows: curtain gas pressure: 35 psi; gas source pressure: 55 psi; spray voltage: 5,500 V/ -4,500 V; declustering potential: 80 V; ion source temperature: 550 °C; mass scan range: 80 ~ 1,200 m/z .

2.12 NP analysis

Chemical constituents derived from the three active fractions of LBL were identified using UPLC-Q-TOF/MS analysis. These compounds were pooled and had their duplicates removed; a total of 104 chemical components were obtained. To initially filter candidate active compounds, the criteria of oral bioavailability $\geq 30\%$ and drug-likeness ≥ 0.18 were applied, which served as the basis for subsequent network pharmacology analysis. Potential targets of the chemical components in LBL were predicted using the Swiss Target Prediction database (<http://www.swisstargetprediction.ch/>), with a confidence threshold of possibility > 0 to retain highly credible compound-target interactions. Therapeutic targets for DR were retrieved from the GeneCards (<https://www.genecards.org/>), OMIM (<https://omim.org/>), DisGeNET (<https://www.disgenet.org/>), and DrugBank (<https://go.drugbank.com/>) databases using "Diabetic retinopathy" as the keyword. Common targets between LBL compounds and DR were identified using Venn diagrams and were considered as key targets via which LBL may alleviate DR. A "component-target-disease" network and a protein-protein interaction (PPI) network was constructed. Furthermore, Gene Ontology (GO) and Kyoto Encyclopedia of Genes and Genomes (KEGG) pathway analyses were performed using the online platform (<https://www.bioinformatics.com.cn/>) to elucidate the biological processes and key signaling pathways regulated by LBL in DR treatment. To avoid introducing irrelevant targets, only those significantly enriched in DR-related pathways ($P < 0.05$) were retained for subsequent analysis.

2.13 DR mice modeling and experimentation

Mice were randomly divided into 9 groups, the control group, model group, MET group, and high-dose and low-dose groups of WE, EE, and FPF, with 6 mice in each group. Following an acclimatization period, the control group was maintained on a standard maintenance diet, whereas the model and treatment groups were fed a HHFD. Body weight was recorded weekly. After eight weeks of dietary intervention, all animals were fasted for 12 h. The model and treatment groups received intraperitoneal injections of 1% STZ solution (60 mg/kg, dissolved in 0.1 mol/L sodium citrate buffer) for 3 d consecutively, whereas the control group received equivalent volumes of the citrate buffer alone. After 24 h, fasting blood glucose levels were measured. Animals with blood glucose levels ≥ 16.7 mM were considered to have developed diabetes successfully. Following model establishment, the model group continued to receive a HHFD without treatment. The MET group received metformin (400 mg/kg/d) via oral gavage while remaining on HHFD. High-dose treatment groups received their respective LBL fractions at 4.5 g/kg/d, whereas low-dose groups received 2.25 g/kg/d, with all treatments administered via oral gavage under HHFD conditions. The control group received daily oral gavage of an equivalent volume of normal saline and remained on the standard maintenance diet. All fractions' dosages were expressed in terms of crude herb equivalent. The experimental protocol was maintained for an additional 8 weeks.

2.14 Optical coherence tomography (OCT) and optical coherence tomography angiography (OCTA) scans

Mice were anesthetized via intraperitoneal injection of 1% sodium pentobarbital (dose: 50 mg/kg, 0.5 mL/100 g) 24 h after the final drug administration. Spectralis OCT and OCTA systems (Heidelberg, Germany) were used to measure retinal thickness and vascular density. Retinal thickness was measured at three randomly selected points near the optic disc using ImageJ; the average value was calculated for each sample. Retinal vascular density was quantified by calculating the percentage area of blood vessels in binarized images using ImageJ. After completion of the imaging session, the mice were euthanized; tissue samples were collected.

2.15 Retinal hematoxylin and eosin (H&E) staining

Mouse eyeballs were enucleated and washed with physiological saline. The eyeballs were fixed in 4% paraformaldehyde solution, embedded in paraffin, and sectioned. Subsequently, sections underwent dewaxing, H&E staining, alcohol dehydration, xylene clearing, and neutral resin mounting. Histological observations were performed under a microscope at 400× magnification.

2.16 Retinal TdT-mediated dUTP Nick-End Labeling (TUNEL) staining

Paraffin-embedded mouse ocular tissue sections were baked at 65 °C for 2 h. After removal from the oven, the sections were dewaxed and rehydrated in ethanol. A Proteinase K working solution was added; the sections were incubated at 37 °C for 30 min. The sections were washed three times with PBS for 5 min each. TUNEL reagent was added and incubated in the dark at 42 °C for 1 h. Subsequently, excess reagent was removed; DAPI was added for nuclear staining, followed by incubation in the dark for 3 min. Fluorescence images were observed and captured using a fluorescence microscope.

2.17 Western blot analysis

Mouse ocular tissues were homogenized in RIPA lysis buffer containing protease and phosphatase inhibitors. Total protein was extracted by centrifugation; concentration was determined using a microspectrophotometer (DeNovix, USA). Subsequently, the protein expression levels of p38 (8690T, Cell Signaling Technology), p-p38 (4511T, Cell Signaling Technology), JNK (67096T, Cell Signaling Technology), p-JNK (4668T, Cell Signaling Technology), ERK (4695T, Cell Signaling Technology), p-ERK (4370T, Cell Signaling Technology), VEGFA (19003-1-AP, Proteintech), VEGFR2 (8696T, Cell Signaling Technology), p-VEGFR2 (2478T, Cell Signaling Technology), TLR4 (19811-1-AP, Proteintech), p65 (8242T, Cell Signaling Technology) and p-p65 (310013, Zenbio) in mouse ocular tissue were detected. Protein signals were visualized with ECL reagent (Abbkine, China) under a chemiluminescence blot scanner (Tanon 1600, Shanghai, China) and quantified using Image J.

2.18 Measurement of retinal vascular diameter in zebrafish

Tg (fli1: EGFP) zebrafish embryos were cultured as described above. Zebrafish embryos (6 dpf) were fixed on slides using 3% CMC-Na solution; the diameters of retinal blood vessels were observed using a stereofluorescence microscope (Leica DFC7000T, Leica, Germany). Retinal vessels were visualised in the

green fluorescence channel. Retinal blood vessel diameters were measured at three randomly selected points using ImageJ; the values were averaged.

2.19 Retinal periodic Acid-Schiff (PAS) staining

Retinal tissues were microdissected from paraffin-embedded mouse eyeball sections in PBS under a microscope. The isolated retina was washed in distilled water (800 μ L) with the solution changed every 30 min for at least four cycles, followed by overnight immersion. After aspiration of the water, the tissues were digested with 3% trypsin at 37 °C with gentle agitation until initial dissociation was observed. Following trypsin removal, retinal blood vessels were separated, transferred to a slide, and air-dried. Slides were oxidized with periodic acid (10 min) and rinsed under running water for 5 min. Staining was performed with Schiff reagent (15 min), followed by a final 5-min water rinse. Finally, the samples were routinely dehydrated, cleared in xylene, and mounted with neutral gum for microscopic examination.

2.20 Biochemical analysis in mice

After collecting mouse serum, IL-1, IL-2, IL-6, IL-10, TNF- α , VEGF, and lipopolysaccharide (LPS) levels were measured using enzyme-linked immunosorbent assay (ELISA). Simultaneously, by following operational procedures specified in the biochemical assay kit manual, serum GSH and MDA concentrations and SOD activity were determined.

2.21 16S rRNA sequencing of gut bacteria

Colonic content samples from each group (n = 6) were collected; total genomic DNA of the microbial community was extracted using a Fast Pure Stool DNA Isolation Kit (MJYH, Shanghai, China). The V3-V4 region of 16S rRNA was amplified using the F338/R806 primer pair. PCR conditions included an initial denaturation at 95 °C for 3 min, 27 cycles of 95 °C denaturation for 30 s, annealing at 55 °C for 30 s, and an extension at 72 °C for 30 s, followed by a final extension at 72 °C for 10 min. Finally, it was stored at 4 °C (PCR instrument: T100 Thermal Cycler). A Next Flex Rapid DNA-SeqKit (BioScientific, USA) was used to build a library of purified PCR products; the NextSeq2000PE300 (Illumina, USA) was used for sequencing. Statistical differences in gut microbial community composition and diversity indices among different groups were further analyzed using the Majorbio Cloud Platform (www.majorbio.com) and GraphPad Prism 9.5 (GraphPad Software, Inc., CA, USA).

2.22 Statistical analysis

All experimental results were derived from at least three independent experiments. The data are expressed as mean $\pm$ standard deviation. GraphPad Prism 9.5 (GraphPad Software, Inc., CA, USA) was used for statistical analysis. One-way analysis of variance (95% confidence interval) was used to compare differences between groups. For the lifespan assay, a log-rank (Mantel–Cox) test based on the Kaplan–Meier method was used to assess its statistical significance.

3. Results

3.1 Evaluation of the efficacy of LBL fractions and screening of the optimal bioactive fraction

Oxidative stress is a critical upstream trigger of chronic low-grade inflammation, which is a core pathogenic factor in DR [5, 22]. It serves as a key target for evaluating the protective effects of LBL fractions against DR-related injury. In high-glucose-induced ARPE-19 cells, 300 mM glucose significantly reduced cell viability and induced oxidative stress imbalance, which were characterized by decreased GSH levels, diminished SOD activity, and elevated MDA and ROS levels. All LBL fractions except for PF significantly reversed these alterations (Fig. 1A–D). Consistent with the cellular findings, results from the zebrafish model—which exhibits high similarity to humans in ocular structure and gene expression—showed that high-glucose induction increased glucose and MDA levels while decreasing GSH levels and SOD activity. Similarly, LBL fractions significantly ameliorated these pathological changes in all groups except PF (Fig. 1E). These results collectively indicated that LBL fractions effectively mitigated high-glucose-induced oxidative injury in ocular-related models, whereas PF lacked such bioactivity and was therefore excluded from subsequent studies.

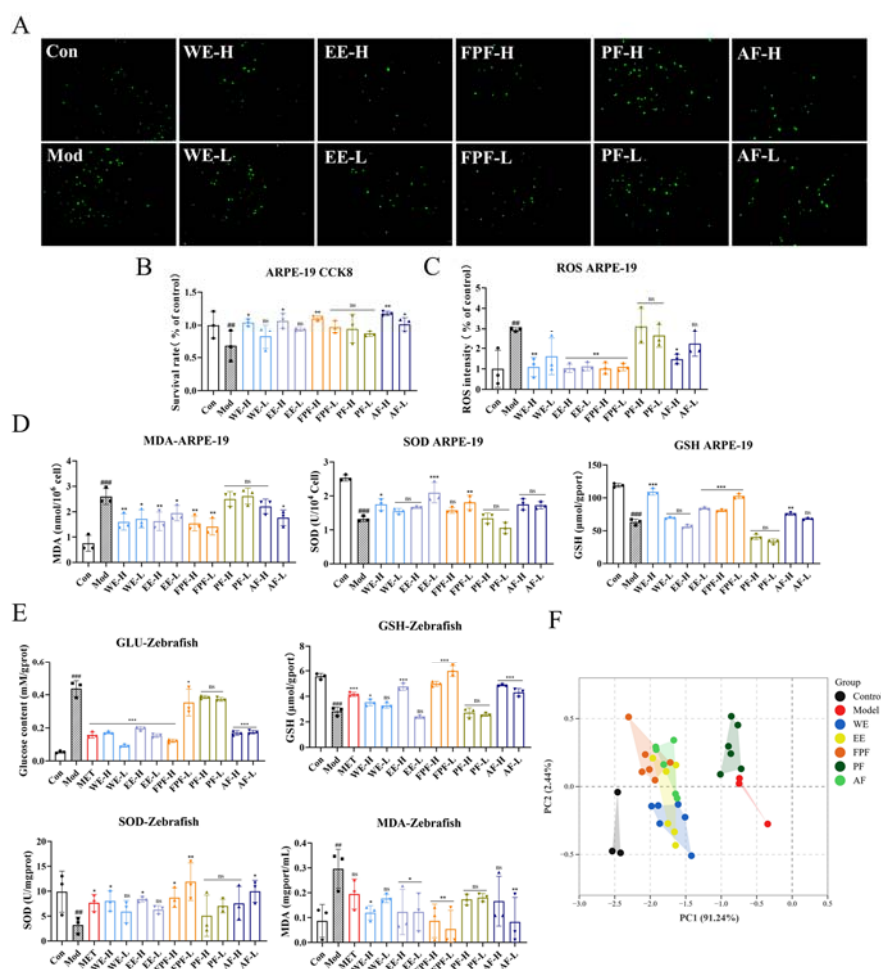


Fig. 1 Evaluation of the efficacy of LBL fractions and screening of the optimal bioactive fraction. (A) Representative fluorescence images of ROS in ARPE-19 cells (n = 3). (B) Cell viability of ARPE-19 cells (n=3). (C) Statistical analysis of ROS fluorescence intensity (n=3). (D) GSH level, SOD activity and MDA activity in ARPE-19 cells (n = 3). (E) Statistical analysis of ROS fluorescence intensity (n=3). (F) PCA analysis. Data were expressed as mean $\pm$ SD. $^{\#}P < 0.05$, $^{\#\#}P < 0.01$, $^{\#\#\#}P < 0.001$ vs control group; $^{*}P < 0.05$, $^{**}P < 0.01$, $^{***}P < 0.001$ vs model group.

To identify the optimal LBL fractions, PCA was performed on various indicators from high-glucose-induced injury cell and zebrafish models; Euclidean distance was calculated to quantify similarity of each fraction group to the control group (Table S1). The results showed that among the three refined fractions (FPF, PF, AF), FPF exhibited the smallest Euclidean distance to the control group, indicating the most potent bioactivity (Fig. 1F). Based on the above efficacy screening results, two total extract LBL fractions (WE and EE) and the optimal bioactive fraction (FPF) were selected for further. They are collectively referred to as the LBL bioactive fractions (LBLBFs) hereafter.

Table 1 Chemical characterisation and analysis of components in WE fraction of LBL

T (min)	Theoretical value	Measured value	Name	Formula	Adduct	Fragment Ions (m/z)	ppm	Category
0.71	179.0561	179.0559	D-Glucose	C ₆ H ₁₂ O ₆	[M-H] ⁻	89.0298	-1	Carbohydrates
0.71	191.0561	191.0560	Quinic acid	C ₇ H ₁₂ O ₆	[M-H] ⁻	191.0590;127.0407;93.0353;85.0305	-0.9	Organic Acids
0.75	133.0143	133.0143	Malic acid	C ₄ H ₆ O ₅	[M-H] ⁻	133.0145;115.0075;89.0245	0.5	Organic Acids
1.16	191.0197	191.0198	Citric acid	C ₆ H ₈ O ₇	[M-H] ⁻	129.0196;111.0176;87.0164	0.4	Organic Acids
1.2	117.0193	117.0193	Succinic acid	C ₄ H ₆ O ₄	[M-H] ⁻	116.9281;99.9255	0	Organic Acids
3.29	315.0722	315.0721	2-Hydroxybenzoic acid 5-O-glucoside	C ₁₃ H ₁₆ O ₉	[M-H] ⁻	315.0743;152.0120;108.0226	-0.3	Phenolic Derivatives
3.43	329.0878	329.0873	Vanillic acid 4-O-glucoside	C ₁₄ H ₁₈ O ₉	[M-H] ⁻	167.0364;123.0456	-1.4	Phenolic Derivatives
3.43	167.0350	167.0342	4-Hydroxy-3-methoxybenzoic acid	C ₈ H ₈ O ₄	[M-H] ⁻	123.0449;108.0209	-5	Phenolic Derivatives
3.44	183.0299	183.0300	Methyl gallate	C ₈ H ₈ O ₅	[M-H] ⁻	124.0196	0.3	Phenolic Derivatives
3.55	109.0295	109.0295	1,2-Benzenediol	C ₆ H ₆ O ₂	[M-H] ⁻	108.0219;109.0294;91.0190	0	Phenolic Derivatives
3.55	153.0193	153.0190	Protocatechuic acid	C ₇ H ₆ O ₄	[M-H] ⁻	109.0298;108.0220;81.0374	-2.4	Phenolic Derivatives
4.18	197.0456	197.0456	4-Hydroxy-3,5-dimethoxybenzoic acid	C ₉ H ₁₀ O ₅	[M-H] ⁻	182.0222;166.9980;123.0083;95.0145	0.2	Phenolic Derivatives
4.46	353.0878	353.0875	1-O-Caffeoylquinic acid	C ₁₆ H ₁₈ O ₉	[M-H] ⁻	191.061	-0.9	Phenolic Derivatives
4.46	353.0878	353.0875	Coumaroylquinic acid	C ₁₆ H ₁₈ O ₉	[M-H] ⁻	191.061	-0.9	Phenolic Derivatives
4.84	137.0244	137.0243	Protocatechualdehyde	C ₇ H ₆ O ₃	[M-H] ⁻	137.0248;136.0166;108.0216;91.9189	-0.9	Phenolic Derivatives
4.89	137.0244	137.0244	4-Hydroxybenzoic acid	C ₇ H ₆ O ₃	[M-H] ⁻	93.0352	-0.1	Phenolic Derivatives

5.12	299.1136	299.1134	p-Hydroxyphenethyl β -D-glucoside	C ₁₄ H ₂₀ O ₇	[M-H] ⁻	119.0492;89.0250	-0.8	Phenolic Derivatives
6.16	163.0390	163.0390	4-Hydroxycoumarin	C ₉ H ₆ O ₃	[M+H] ⁺	135.0443	0.4	Coumarins
6.16	163.0390	163.0390	3-Hydroxycoumarin	C ₉ H ₆ O ₃	[M+H] ⁺	135.0443	0.4	Coumarins
6.16	163.0390	163.0390	6-Hydroxycoumarin	C ₉ H ₆ O ₃	[M+H] ⁺	163.0393;135.0443;145.0283;117.0334	0.4	Coumarins
6.21	353.0878	353.0875	5-O-Caffeoylquinic acid	C ₁₆ H ₁₈ O ₉	[M-H] ⁻	353.0903;191.0610;179.0377;135.0478	-1	Phenolic Derivatives
6.21	353.0878	353.0875	3-O-Caffeoylquinic acid	C ₁₆ H ₁₈ O ₉	[M-H] ⁻	353.0903;191.0610;179.0377;135.0478	-1	Phenolic Derivatives
6.21	353.0878	353.0875	4-O-Caffeoylquinic acid	C ₁₆ H ₁₈ O ₉	[M-H] ⁻	353.0903;191.0610;179.0377;135.0478	-1	Phenolic Derivatives
6.43	525.1614	525.1590	Isatis oxoneolignan glycoside A	C ₂₄ H ₃₀ O ₁₃	[M-H] ⁻	363.1100;195.0663;167.0352	-4.5	Lignans
6.5	177.0193	177.0193	Esculetin	C ₉ H ₆ O ₄	[M-H] ⁻	133.0296;105.0345;177.0194	-0.2	Coumarins
6.62	121.0295	121.0293	Benzoic acid	C ₇ H ₆ O ₂	[M-H] ⁻	92.027	-1.5	Organic Acids
6.67	325.0929	325.0919	p-Coumaric acid glycosides	C ₁₅ H ₁₈ O ₈	[M-H] ⁻	163.0402;113.0504	-3	Phenolic Derivatives
6.67	325.0929	325.0919	p-Coumaric acid 4-O-glucoside	C ₁₅ H ₁₈ O ₈	[M-H] ⁻	163.0402;119.0504	-3	Phenolic Derivatives
6.77	179.0350	179.0346	Caffeic acid	C ₉ H ₈ O ₄	[M-H] ⁻	135.0452	-2.2	Phenolic Derivatives
8.13	337.0929	337.0926	3-O-p-Coumaroylquinic acid	C ₁₆ H ₁₈ O ₈	[M-H] ⁻	191.0566;119.0503	-0.9	Phenolic Derivatives
9.09	165.0546	165.0545	p-Coumaric Acid	C ₉ H ₈ O ₃	[M+H] ⁺	119.0496	-0.7	Phenolic Derivatives
9.09	163.0401	163.0399	p-Coumaric acid	C ₉ H ₈ O ₃	[M-H] ⁻	119.0504	-1	Phenolic Derivatives
9.13	163.0401	163.0399	3-Hydroxycinnamic acid	C ₉ H ₈ O ₃	[M-H] ⁻	119.0504	-1	Phenolic Derivatives
9.15	163.0401	163.0397	p-Hydroxycinnamic acid	C ₉ H ₈ O ₃	[M-H] ⁻	119.0504	-2.2	Phenolic Derivatives
9.16	163.0401	163.0399	m-Coumaric acid	C ₉ H ₈ O ₃	[M-H] ⁻	119.0504	-1	Phenolic Derivatives
9.28	367.1035	367.1027	5-O-Feruloylquinic acid	C ₁₇ H ₂₀ O ₉	[M-H] ⁻	193.0508;191.0566;173.0456	-1.9	Phenolic Derivatives
9.28	367.1035	367.1027	Feruloylquinic acid	C ₁₇ H ₂₀ O ₉	[M-H] ⁻	-	-1.9	Phenolic Derivatives
13.57	609.1461	609.1454	Quercetin 3-O-rhamnoglucoside	C ₂₇ H ₃₀ O ₁₆	[M-H] ⁻	609.1596;151.0034;301.0411	-1.1	Flavonoids

13.62	611.1607	611.1611	Rutin	$C_{27}H_{30}O_1$ 6	[M+H] ⁺	465.1045;303.0529	0.7	Flavonoids
13.62	609.1461	609.1451	Rutin	$C_{27}H_{30}O_1$ 6	[M-H] ⁻	301.0363;300.0311;2 71.0253;151.0038	-1.7	Flavonoids
13.62	609.1461	609.1451	Quercetin 3-O-neohesperidoside	$C_{27}H_{30}O_1$ 6	[M-H] ⁻	301.0363;300.0311;2 71.0253	-1.7	Flavonoids
13.62	609.1461	609.1451	Quercetin 3-O-glucoside-7-O-rhamnoside	$C_{27}H_{30}O_1$ 6	[M-H] ⁻	301.0363;300.0311;2 71.0253;151.0038	-1.7	Flavonoids
14.11	465.1028	465.1031	Isoquercitrin	$C_{21}H_{20}O_1$ 2	[M+H] ⁺	303.0505	0.8	Flavonoids
14.11	463.0882	463.0873	Isoquercitrin	$C_{21}H_{20}O_1$ 2	[M-H] ⁻	301.0367;300.0283;2 71.0243;178.9980;15 1.0033	-2	Flavonoids
14.11	463.0882	463.0873	Quercetin 3-O-glucoside	$C_{21}H_{20}O_1$ 2	[M-H] ⁻	301.0367;300.0283;2 71.0243;178.9980;15 1.0033	-2	Flavonoids
16.43	287.0550	287.0550	Luteolin	$C_{15}H_{10}O_6$	[M+H] ⁺	287.0556;153.0192	-0.2	Flavonoids
16.43	287.0550	287.0550	Kaempferol-3-O-rutinoside	$C_{15}H_{10}O_6$	[M+H] ⁺	287.0563	-0.2	Flavonoids
16.5	593.1512	593.1502	Luteolin 7-O-rutinoside	$C_{27}H_{30}O_1$ 5	[M-H] ⁻	285.0413;284.0330	-1.8	Flavonoids
16.5	593.1512	593.1502	Kaempferol 3-O-rutinoside	$C_{27}H_{30}O_1$ 5	[M-H] ⁻	285.0413;284.0331	-1.8	Flavonoids
16.74	447.0933	447.0930	Luteolin 5-O-glucoside	$C_{21}H_{20}O_1$ 1	[M-H] ⁻	47.0943;285.0328;28 4.0328	-0.7	Flavonoids
16.74	447.0933	447.0930	Luteolin 6-O-glucoside	$C_{21}H_{20}O_1$ 1	[M-H] ⁻	47.0943;285.0328;28 4.0328	-0.7	Flavonoids
16.74	447.0933	447.0930	Kaempferol 3-O-glucoside	$C_{21}H_{20}O_1$ 1	[M-H] ⁻	285.0422;284.0323;2 55.0309;227.0399	-0.7	Flavonoids
16.8	317.0656	317.0657	Eupatilin	$C_{16}H_{12}O_7$	[M+H] ⁺	302.0427;153.0181	0.3	Flavonoids
17.47	515.1195	515.1179	1,5-Dicaffeoylquinic acid	$C_{25}H_{24}O_1$ 2	[M-H] ⁻	515.1209;353.0876;1 91.0511;179.0359;17 3.0463;135.0464	-3.1	Phenolic Derivatives
17.47	515.1195	515.1179	3,5-Dicaffeoylquinic acid	$C_{25}H_{24}O_1$ 2	[M-H] ⁻	515.1209;353.0876;1 91.0511;179.0359;17 3.0463;135.0464	-3.1	Phenolic Derivatives
17.47	515.1195	515.1179	3,4-Dicaffeoylquinic acid	$C_{25}H_{24}O_1$ 2	[M-H] ⁻	515.1209;353.0876;1 91.0511;179.0359;17 3.0463;135.0464	-3.1	Phenolic Derivatives
17.47	515.1195	515.1179	4,5-Dicaffeoylquinic acid	$C_{25}H_{24}O_1$ 2	[M-H] ⁻	515.1209;353.0876;1 91.0511;179.0359;17 3.0463;135.0464	-3.1	Phenolic Derivatives
18.98	327.2177	327.2171	Trihydroxyoctadecadienoic acid isomer	$C_{18}H_{32}O_5$	[M-H] ⁻	229.1448;211.1342	-1.9	Fatty Acids and Derivatives
18.98	327.2177	327.2171	Trihydroxyoctadecadienoic acid	$C_{18}H_{32}O_5$	[M-H] ⁻	229.1448;211.1342	-1.9	Fatty Acids and Derivatives
19.02	287.0550	287.0550	Kaempferol	$C_{21}H_{20}O_1$ 2	[M+H] ⁺	153.0192;121.0282	-0.2	Flavonoids
19.25	329.2334	329.2328	Trihydroxyoctadecenoic acid	$C_{18}H_{34}O_5$	[M-H] ⁻	229.1453;221.1342	-1.6	Fatty Acids and Derivatives

20.68	309.2071	309.2065	Hydroperoxyoctadecatrienoic acid isomer	C ₁₈ H ₃₀ O ₄	[M-H] ⁻	209.1546	-2.1	Fatty Acids and Derivatives
22.17	297.2424	297.2426	9(10)-Epoxy-12Z-octadecenoic Acid	C ₁₈ H ₃₂ O ₃	[M+H] ⁺	279.2319;123.0801;123.1154;123.1040;109.1009;95.0858	0.7	Fatty Acids and Derivatives
23.12	279.1591	279.1592	Diisobutyl Phthalate	C ₁₆ H ₂₂ O ₄	[M+H] ⁺	149.0245;121.0288	0.5	Esters

Table 2 Chemical characterisation and analysis of components in EE fraction of LBL

T (min)	Theoretical value	Measured value	Name	Formula	Adduct	Fragment Ions (m/z)	ppm	Category
0.7	341.1089	341.1083	D-(+)-Cellobiose	C ₁₂ H ₂₂ O ₁₁	[M-H] ⁻	341.1105;179.0559;179.8741;119.0354;89.0244	-1.8	Carbohydrates
0.71	191.0561	191.0560	Quinic acid	C ₇ H ₁₂ O ₆	[M-H] ⁻	191.0590;127.0407;93.0353;85.0305	-0.9	Organic Acids
0.76	115.0037	115.0034	Maleic acid	C ₄ H ₄ O ₄	[M-H] ⁻	97.9312	-2.4	Organic Acids
3.25	315.0722	315.0711	3,4-Dihydroxybenzoic acid O-glucoside	C ₁₃ H ₁₆ O ₉	[M-H] ⁻	153.0182;109.0295	-3.5	Phenolic Derivatives
3.43	167.0350	167.0342	4-Hydroxy-3-methoxybenzoic acid	C ₈ H ₈ O ₄	[M-H] ⁻	123.0449;108.0209	-5	Phenolic Derivatives
4.84	137.0244	137.0243	Protocatechualdehyde	C ₇ H ₆ O ₃	[M-H] ⁻	137.0248;136.0166;108.0216;91.9189	-0.9	Phenolic Derivatives
5.97	341.0878	341.0872	Caffeic acid 3-O-glucoside	C ₁₅ H ₁₈ O ₉	[M-H] ⁻	179.5540;179.0354;179.2352;179.5422;135.0455	-1.8	Phenolic Derivatives
5.99	375.1297	375.1292	Loganic acid	C ₁₆ H ₂₄ O ₁₀	[M-H] ⁻	101.0234;89.0644;89.0241	-1.2	Terpenoids
6.16	163.0390	163.0390	7-Hydroxycoumarin	C ₉ H ₆ O ₃	[M+H] ⁺	135.0443	0.4	Coumarins
6.16	163.0390	163.0390	4-Hydroxycoumarin	C ₉ H ₆ O ₃	[M+H] ⁺	135.0443	0.4	Coumarins
6.16	163.0390	163.0390	3-Hydroxycoumarin	C ₉ H ₆ O ₃	[M+H] ⁺	135.0443	0.4	Coumarins
6.16	163.0390	163.0390	6-Hydroxycoumarin	C ₉ H ₆ O ₃	[M+H] ⁺	163.0393;135.0443;145.0283;117.0334	0.4	Coumarins
6.16	355.1024	355.1026	5-O-Caffeoylquinic acid	C ₁₆ H ₁₈ O ₉	[M+H] ⁺	135.0442	0.6	Phenolic Derivatives
6.16	355.1024	355.1026	3-O-Caffeoylquinic acid	C ₁₆ H ₁₈ O ₉	[M+H] ⁺	163.0414;135.0442;145.0289;117.0337	0.6	Phenolic Derivatives
6.16	355.1024	355.1026	Cyclohexanecarboxylic Acid	C ₁₆ H ₁₈ O ₉	[M+H] ⁺	163.0414;135.0442	0.6	Organic Acids

6.21	353.0878	353.0875	5-O-Caffeoylquinic acid	C ₁₆ H ₁₈ O ₉	[M-H] ⁻	353.0903;191.0610;179.0377;135.0478	-1	Phenolic Derivatives
6.21	353.0878	353.0875	3-O-Caffeoylquinic acid	C ₁₆ H ₁₈ O ₉	[M-H] ⁻	353.0903;191.0610;179.0377;135.0478	-1	Phenolic Derivatives
6.21	353.0878	353.0875	4-O-Caffeoylquinic acid	C ₁₆ H ₁₈ O ₉	[M-H] ⁻	353.0903;191.0610;179.0377;135.0478	-1	Phenolic Derivatives
6.49	193.0495	193.0496	Scopoletin	C ₁₀ H ₈ O ₄	[M+H] ⁺	178.0266;133.0289	0.3	Coumarins
6.49	191.0350	191.0349	Scopoletin	C ₁₀ H ₈ O ₄	[M-H] ⁻	133.0959	-0.2	Coumarins
6.57	325.0929	325.0926	p-Coumaric acid glycoside isomer	C ₁₅ H ₁₈ O ₈	[M-H] ⁻	119.3297	-1	Phenolic Derivatives
6.57	325.0929	325.0926	Coumaroyl hexoside	C ₁₅ H ₁₈ O ₈	[M-H] ⁻	119.0510	-1	Phenolic Derivatives
6.62	121.0295	121.0293	Benzoic acid	C ₇ H ₆ O ₂	[M-H] ⁻	92.027	-1.5	Organic Acids
6.63	223.0612	223.0609	4-Hydroxy-3,5-dimethoxycinnamic acid	C ₁₁ H ₁₂ O ₅	[M-H] ⁻	97.2226;163.0238	-1.3	Phenolic Derivatives
6.77	179.0350	179.0346	Caffeic acid	C ₉ H ₈ O ₄	[M-H] ⁻	135.0452	-2.2	Phenolic Derivatives
7.61	355.1035	355.1032	Eucalyptol 1,7-oxide	C ₁₆ H ₂₀ O ₉	[M-H] ⁻	193.0517	-0.6	Terpenoids
7.95	355.1035	355.1032	1-O-trans-Feruloyl-β-D-glucopyranoside	C ₁₆ H ₂₀ O ₉	[M-H] ⁻	175.0408;160.0169	-0.6	Phenolic Derivatives
8.13	337.0929	337.0926	3-O-p-Coumaroylquinic acid	C ₁₆ H ₁₈ O ₈	[M-H] ⁻	191.0566;119.0503	-0.9	Phenolic Derivatives
8.44	387.1661	387.1649	Hydroxyjasmonic acid hexoside	C ₁₈ H ₂₈ O ₉	[M-H] ⁻	207.1015;163.1116;101.0232	-2.9	Jasmonic Acid Derivatives
9.09	165.0546	165.0545	p-Coumaric Acid	C ₉ H ₈ O ₃	[M+H] ⁺	119.0496	-0.7	Phenolic Derivatives
9.09	163.0401	163.0399	p-Coumaric acid	C ₉ H ₈ O ₃	[M-H] ⁻	119.0504	-1	Phenolic Derivatives
9.13	163.0401	163.0399	3-Hydroxycinnamic acid	C ₉ H ₈ O ₃	[M-H] ⁻	119.0504	-1	Phenolic Derivatives
9.16	163.0401	163.0399	m-Coumaric acid	C ₉ H ₈ O ₃	[M-H] ⁻	119.0504	-1	Phenolic Derivatives
9.24	165.0557	165.0555	3-Ethoxy-4-hydroxybenzaldehyde	C ₉ H ₁₀ O ₃	[M-H] ⁻	121.0526	-1.4	Phenolic Derivatives
9.33	371.0984	371.0972	4-(2-Carboxyethyl)-2-methoxyphenyl β-D-glucuronide	C ₁₆ H ₂₀ O ₁₀	[M-H] ⁻	371.0975;121.0299	-3	Phenolic Derivatives

10.43	163.0401	163.0399	2-Hydroxycinnamic acid	C ₉ H ₈ O ₃	[M-H] ⁻	119.0504;117.0342; 93.0346	-1	Phenolic Derivatives
10.8	193.0506	193.0497	Ferulic acid	C ₁₀ H ₁₀ O ₄	[M-H] ⁻	178.0259;149.0620; 134.0375	-5	Phenolic Derivatives
12.38	595.1305	595.1304	Quercetin 3-O-glucoside-3"-O-arabinoside	C ₂₆ H ₂₈ O ₁₆	[M-H] ⁻	300.0278;301.0349; 595.1318;595.2076. 595.2464;151.0055	0	Flavonoids
13.48	449.1078	449.1080	Quercitrin	C ₂₁ H ₂₀ O ₁₁	[M+H] ⁺	303.0499	0.3	Flavonoids
13.57	609.1461	609.1454	Quercetin 3-O-rhamnogluco-side	C ₂₇ H ₃₀ O ₁₆	[M-H] ⁻	609.1596;151.0034; 301.0411	-1.1	Flavonoids
13.57	609.1461	609.1454	Luteolin 7-O-diglucoside	C ₂₇ H ₃₀ O ₁₆	[M-H] ⁻	609.1596;151.0034	-1.1	Flavonoids
13.62	611.1607	611.1611	Rutin	C ₂₇ H ₃₀ O ₁₆	[M+H] ⁺	465.1045;303.0529	0.7	Flavonoids
13.62	609.1461	609.1451	Rutin	C ₂₇ H ₃₀ O ₁₆	[M-H] ⁻	301.0363;300.0311; 271.0253;151.0038	-1.7	Flavonoids
13.62	609.1461	609.1451	Quercetin 3-O-neohesperidoside	C ₂₇ H ₃₀ O ₁₆	[M-H] ⁻	301.0363;300.0311; 271.0253	-1.7	Flavonoids
13.99	465.1028	465.1031	Hyperoside	C ₂₁ H ₂₀ O ₁₂	[M+H] ⁺	303.0505	0.8	Flavonoids
13.99	463.0882	463.0873	Hyperoside	C ₂₁ H ₂₀ O ₁₂	[M-H] ⁻	463.0893;301.0352; 300.0274;151.0026; 255.0280	-1.9	Flavonoids
14.11	465.1028	465.1031	Isoquercitrin	C ₂₁ H ₂₀ O ₁₂	[M+H] ⁺	303.0505	0.8	Flavonoids
14.11	463.0882	463.0873	Isoquercitrin	C ₂₁ H ₂₀ O ₁₂	[M-H] ⁻	301.0367;300.0283; 271.0243;178.9980; 151.0033	-2	Flavonoids
15.9	577.1563	577.1556	Apigenin 6-C-rhamnoside-8-C-glucoside	C ₂₇ H ₃₀ O ₁₄	[M-H] ⁻	577.1567	-1.3	Flavonoids
16.43	287.0550	287.0550	Luteolin	C ₁₅ H ₁₀ O ₆	[M+H] ⁺	287.0556;153.0192	-0.2	Flavonoids
16.43	287.0550	287.0550	Kaempferol-3-O-rutinoside	C ₁₅ H ₁₀ O ₆	[M+H] ⁺	287.0563	-0.2	Flavonoids
16.57	549.1978	549.1969	Tracheloside	C ₂₇ H ₃₄ O ₁₂	[M-H] ⁻	387.145	-1.6	Lignans
16.59	187.0976	187.0974	Azelaic acid	C ₉ H ₁₆ O ₄	[M-H] ⁻	123.0815;125.0974; 187.0978;169.0870	-0.9	Fatty Acids and Derivatives
16.73	447.0933	447.0918	Quercetin 3-O-rhamnoside	C ₂₁ H ₂₀ O ₁₁	[M-H] ⁻	153.7095	-3.4	Flavonoids
16.74	447.0933	447.0918	Luteolin 7-O-glucoside	C ₂₁ H ₂₀ O ₁₁	[M-H] ⁻	447.0932;285.0405	-3.4	Flavonoids
16.8	317.0656	317.0657	Eupatilin	C ₁₆ H ₁₂ O ₇	[M+H] ⁺	302.0427;153.0181	0.3	Flavonoids
16.8	317.0656	317.0657	Capillarisin	C ₁₆ H ₁₂ O ₇	[M+H] ⁺	302.0427	0.3	Flavonoids
17.04	477.1039	477.1023	Isorhamnetin 3-O-glucoside	C ₂₂ H ₂₂ O ₁₂	[M-H] ⁻	314.043	-3.2	Flavonoids
17.29	631.2971	631.2953	2α,3β,19α-Trihydroxyursolic acid	C ₃₀ H ₄₈ O ₁₄	[M-H] ⁻	469.2425	-3	Terpenoids
17.46	361.1657	361.1643	Epinodosin	C ₂₀ H ₂₆ O ₆	[M-H] ⁻	315.121	-3.7	Terpenoids

17.47	515.1195	515.1179	1,5-Dicaffeoylquinic acid	C ₂₅ H ₂₄ O ₁₂	[M-H] ⁻	515.1209;353.0876;191.0511;179.0359;173.0463;135.0464	-3.1	Phenolic Derivatives
17.47	515.1195	515.1179	3,5-Dicaffeoylquinic acid	C ₂₅ H ₂₄ O ₁₂	[M-H] ⁻	515.1209;353.0876;191.0511;179.0359;173.0463;135.0464	-3.1	Phenolic Derivatives
17.47	515.1195	515.1179	3,4-Dicaffeoylquinic acid	C ₂₅ H ₂₄ O ₁₂	[M-H] ⁻	515.1209;353.0876;191.0511;179.0359;173.0463;135.0464	-3.1	Phenolic Derivatives
17.47	515.1195	515.11792	4,5-Dicaffeoylquinic acid	C ₂₅ H ₂₄ O ₁₂	[M-H] ⁻	515.1209;353.0876;191.0511;179.0359;173.0463;135.0464	-3.1	Phenolic Derivatives
17.56	461.10894	461.10715	4',5-Dihydroxy-6-methoxyisoflavone 7-O-glucoside	C ₂₂ H ₂₂ O ₁₁	[M-H] ⁻	461.1089	-3.9	Flavonoids
18.39	303.04993	303.04993	Quercetin	C ₁₅ H ₁₀ O ₇	[M+H] ⁺	153.0183	0	Flavonoids
18.39	301.03538	301.0346	Quercetin	C ₁₅ H ₁₀ O ₇	[M-H] ⁻	273.0394;151.0035	-2.6	Flavonoids
18.66	315.05103	315.05038	Isorhamnetin	C ₁₆ H ₁₂ O ₇	[M-H] ⁻	300.0283;271.0253	-2.1	Flavonoids
18.9	271.0612	271.06131	Naringenin	C ₁₅ H ₁₂ O ₅	[M-H] ⁻	119.0499;271.0615	0.4	Flavonoids
19.02	287.05501	287.05496	Kaempferol	C ₂₁ H ₂₀ O ₁₂	[M+H] ⁺	153.0192;121.0282	-0.2	Flavonoids
19.02	285.04046	285.03867	Kaempferol	C ₁₅ H ₁₀ O ₆	[M-H] ⁻	239.0407	-6.3	Flavonoids
19.15	501.32216	501.32136	Medicagenic acid	C ₃₀ H ₄₆ O ₆	[M-H] ⁻	501.3200;501.2895;501.2375	-1.6	Terpenoids
19.27	329.23335	329.23244	9,10,13-Trihydroxy-11-octadecenoic acid	C ₁₈ H ₃₄ O ₅	[M-H] ⁻	329.2365;127.1128;157.1232;171.1029;229.1450;99.0815	-2.8	Fatty Acids and Derivatives
19.28	401.08781	401.08672	Apigenin 8-C-arabinoside	C ₂₀ H ₁₈ O ₉	[M-H] ⁻	401.0876;121.0296;225.0563;181.0659	-2.7	Flavonoids
20.46	227.12888	227.12831	trans-Traumatic acid	C ₁₂ H ₂₀ O ₄	[M-H] ⁻	227.1284;183.1394;165.1287	-2.5	Fatty Acids and Derivatives
21.08	313.23843	313.23823	9,10-Dihydroxy-12Z-octadecenoic acid	C ₁₈ H ₃₄ O ₄	[M-H] ⁻	295.2275;277.2167;201.1133;171.1020	-0.7	Fatty Acids and Derivatives
21.7	293.21222	293.21144	13-Hydroxyoctadecatatrienoic acid isomer	C ₁₈ H ₃₀ O ₃	[M-H] ⁻	171.1026	-2.7	Fatty Acids and Derivatives
22.17	279.23186	279.23164	α-Linolenic acid	C ₁₈ H ₃₀ O ₂	[M+H] ⁺	149.0235;121.0283	-0.8	Fatty Acids and Derivatives
22.17	277.2173	277.21619	α-Linolenic acid	C ₁₈ H ₃₀ O ₂	[M-H] ⁻	233.1512;	-4	Fatty Acids and Derivatives
22.17	297.24242	297.24264	9(10)-Epoxy-12Z-octadecenoic Acid	C ₁₈ H ₃₂ O ₃	[M+H] ⁺	279.2319;123.0801;123.1154;123.1040;109.1009; 95.0858	0.7	Fatty Acids and Derivatives

22.44	295.22787	295.22707	Hydroxyoctadecadienoic acid	C ₁₈ H ₃₂ O ₃	[M-H] ⁻	277.2166	-2.7	Fatty Acids and Derivatives
23.12	279.15909	279.15922	Diisobutyl Phthalate	C ₁₆ H ₂₂ O ₄	[M+H] ⁺	149.0245; 121.0288	0.5	Esters
23.91	223.20564	223.20547	α-Bisabolol	C ₁₅ H ₂₆ O	[M+H] ⁺	149.0231; 109.0997; 107.0855; 95.0695	-0.8	Terpenoids

Table 3 Chemical characterisation and analysis of components in FPF of LBL

T (min)	Theoretical value	Measured value	Name	Formula	Adduct	Fragment Ions (m/z)	ppm	Category
0.7	341.1089	341.1083	D-(+)-Cellobiose	C ₁₂ H ₂₂ O ₁₁	[M-H] ⁻	341.1105;179.0559;179.8741;119.0354;89.0244	-1.8	Carbohydrates
0.71	191.0561	191.0560	Quinic acid	C ₇ H ₁₂ O ₆	[M-H] ⁻	191.0590;127.0407;93.0353;85.0305	-0.9	Organic Acids
0.76	115.0037	115.0034	Maleic acid	C ₄ H ₄ O ₄	[M-H] ⁻	97.9312	-2.4	Organic Acids
3.25	315.0722	315.0711	3,4-Dihydroxybenzoic acid O-glucoside	C ₁₃ H ₁₆ O ₉	[M-H] ⁻	153.0182;109.0295	-3.5	Phenolic Derivatives
6.16	163.0390	163.0390	Hydroxycoumarin 4-	C ₉ H ₆ O ₃	[M+H] ⁺	135.0443	0.4	Coumarins
6.16	163.0390	163.0390	Hydroxycoumarin 3-	C ₉ H ₆ O ₃	[M+H] ⁺	135.0443	0.4	Coumarins
6.16	163.0390	163.0390	Hydroxycoumarin 6-	C ₉ H ₆ O ₃	[M+H] ⁺	135.0443	0.4	Coumarins
6.16	163.0390	163.0390	Hydroxycoumarin 5-O-	C ₉ H ₆ O ₃	[M+H] ⁺	163.0393;135.0443;145.0283;117.0334	0.4	Coumarins
6.16	355.1024	355.1026	Caffeoylquinic acid 3-O-	C ₁₆ H ₁₈ O ₉	[M+H] ⁺	135.0442	0.6	Phenolic Derivatives
6.16	355.1024	355.1026	Caffeoylquinic acid Cyclohexane carboxylic Acid	C ₁₆ H ₁₈ O ₉	[M+H] ⁺	163.0414;135.0442;145.0289;117.0337	0.6	Phenolic Derivatives
6.16	355.1024	355.1026	5-O-Caffeoylquinic acid	C ₁₆ H ₁₈ O ₉	[M+H] ⁺	163.0414;135.0442	0.6	Organic Acids
6.21	353.0878	353.0875	3-O-Caffeoylquinic acid	C ₁₆ H ₁₈ O ₉	[M-H] ⁻	353.0903;191.0610;179.0377;135.0478	-1	Phenolic Derivatives
6.21	353.0878	353.0875	4-O-Caffeoylquinic acid	C ₁₆ H ₁₈ O ₉	[M-H] ⁻	353.0903;191.0610;179.0377;135.0478	-1	Phenolic Derivatives
6.21	353.0878	353.0875	5-O-Caffeoylquinic acid	C ₁₆ H ₁₈ O ₉	[M-H] ⁻	353.0903;191.0610;179.0377;135.0478	-1	Phenolic Derivatives
6.49	193.0495	193.0496	Scopoletin	C ₁₀ H ₈ O ₄	[M+H] ⁺	178.0266;133.0289	0.3	Coumarins
6.49	191.0350	191.0349	Scopoletin	C ₁₀ H ₈ O ₄	[M-H] ⁻	133.0959	-0.2	Coumarins

6.63	223.0612	223.0609	4-Hydroxy-3,5-dimethoxycinnamic acid	C ₁₁ H ₁₂ O ₅	[M-H] ⁻	97.2226;163.0238	-1.3	Phenolic Derivatives
9.09	165.0546	165.0545	p-Coumaric Acid	C ₉ H ₈ O ₃	[M+H] ⁺	119.0496	-0.7	Phenolic Derivatives
9.09	163.0401	163.0399	p-Coumaric acid	C ₉ H ₈ O ₃	[M-H] ⁻	119.0504	-1	Phenolic Derivatives
12.38	595.1305	595.1304	Quercetin 3-O-glucoside-3"-O-arabinoside	C ₂₆ H ₂₈ O ₁₆	[M-H] ⁻	300.0278;301.0349;595.1318;595.2076;595.2464;151.0055	0	Flavonoids
13.48	449.1078	449.1080	Quercitrin	C ₂₁ H ₂₀ O ₁₁	[M+H] ⁺	303.0499	0.3	Flavonoids
13.57	609.1461	609.1454	Quercetin 3-O-rhamnoglucoside	C ₂₇ H ₃₀ O ₁₆	[M-H] ⁻	609.1596;151.0034;301.0411	-1.1	Flavonoids
13.57	609.1461	609.1454	Luteolin 7-O-diglucoside	C ₂₇ H ₃₀ O ₁₆	[M-H] ⁻	609.1596;151.0034	-1.1	Flavonoids
13.62	611.1607	611.1611	Rutin	C ₂₇ H ₃₀ O ₁₆	[M+H] ⁺	465.1045;303.0529	0.7	Flavonoids
13.62	609.1461	609.1451	Rutin	C ₂₇ H ₃₀ O ₁₆	[M-H] ⁻	301.0363;300.0311;271.0253;151.0038	-1.7	Flavonoids
13.62	609.1461	609.1451	Quercetin 3-O-neohesperid oside	C ₂₇ H ₃₀ O ₁₆	[M-H] ⁻	301.0363;300.0311;271.0253	-1.7	Flavonoids
13.99	465.1028	465.1031	Hyperoside	C ₂₁ H ₂₀ O ₁₂	[M+H] ⁺	303.0505	0.8	Flavonoids
13.99	463.0882	463.0873	Hyperoside	C ₂₁ H ₂₀ O ₁₂	[M-H] ⁻	463.0893;301.0352;300.0274;151.0026;255.0280	-1.9	Flavonoids
14.11	465.1028	465.1031	Isoquercitrin	C ₂₁ H ₂₀ O ₁₂	[M+H] ⁺	303.0505	0.8	Flavonoids
14.11	463.0882	463.0873	Isoquercitrin	C ₂₁ H ₂₀ O ₁₂	[M-H] ⁻	301.0367;300.0283;271.0243;178.9980;151.0033	-2	Flavonoids
15.9	577.1563	577.1556	Apigenin 6-C-rhamnoside-8-C-glucoside	C ₂₇ H ₃₀ O ₁₄	[M-H] ⁻	577.1567	-1.3	Flavonoids
16.43	287.0550	287.0550	Luteolin	C ₁₅ H ₁₀ O ₆	[M+H] ⁺	287.0556;153.0192	-0.2	Flavonoids
16.43	287.0550	287.0550	Kaempferol-3-O-rutinoside	C ₁₅ H ₁₀ O ₆	[M+H] ⁺	287.0563	-0.2	Flavonoids
16.8	317.0656	317.0657	Eupatilin	C ₁₆ H ₁₂ O ₇	[M+H] ⁺	302.0427;153.0181	0.3	Flavonoids
16.8	317.0656	317.0657	Capillarisin	C ₁₆ H ₁₂ O ₇	[M+H] ⁺	302.0427	0.3	Flavonoids
18.9	271.0612	271.0613	Naringenin	C ₁₅ H ₁₂ O ₅	[M-H] ⁻	119.0499;271.0615	0.4	Flavonoids
19.02	287.0550	287.0550	Kaempferol	C ₂₁ H ₂₀ O ₁₂	[M+H] ⁺	153.0192;121.0282	-0.2	Flavonoids
19.15	501.3222	501.3214	Medicagenic acid	C ₃₀ H ₄₆ O ₆	[M-H] ⁻	501.3200;501.2895;501.2375	-1.6	Terpenoids

19.27	329.2334	329.2324	9,10,13-Trihydroxy-11-octadecenoic acid	C ₁₈ H ₃₄ O ₅	[M-H] ⁻	329.2365;127.1128;157.1232;171.1029;229.1450;99.0815	-2.8	Fatty Acids and Derivatives
21.08	313.2384	313.2382	9,10-Dihydroxy-12Z-octadecenoic acid	C ₁₈ H ₃₄ O ₄	[M-H] ⁻	295.2275;277.2167;201.1133;171.1020	-0.7	Fatty Acids and Derivatives
22.17	279.2319	279.2316	α -Linolenic acid	C ₁₈ H ₃₀ O ₂	[M+H] ⁺	149.0235;121.0283	-0.8	Fatty Acids and Derivatives
22.17	277.2173	277.2162	α -Linolenic acid	C ₁₈ H ₃₀ O ₂	[M-H] ⁻	233.1512	-4	Fatty Acids and Derivatives
22.17	297.2424	297.2426	9(10)-Epoxy-12Z-octadecenoic Acid	C ₁₈ H ₃₂ O ₃	[M+H] ⁺	279.2319;123.0801;123.1154;123.1040;109.1009;95.0858	0.7	Fatty Acids and Derivatives
23.12	279.1591	279.1592	Diisobutyl Phthalate	C ₁₆ H ₂₂ O ₄	[M+H] ⁺	149.0245;121.0288	0.5	Esters
23.91	223.2056	223.2055	α -Bisabolol	C ₁₅ H ₂₆ O	[M+H] ⁺	149.0231;109.0997;107.0855;95.0695	-0.8	Terpenoids

3.2 Analysis of the chemical constituents of LBLBFs

To characterize chemical constituents of the LBLBFs, UPLC-Q-TOF/MS analysis was performed. Total ion flow chromatograms in positive and negative ion modes were obtained (Fig. 2A); combined with PeakView and literature databases, 64, 83 and 45 chemical constituents were identified in WE, EE, and FPF, respectively. These constituents primarily comprised phenolic acids, flavonoids, phenolic derivatives, fatty acids, and other components. The specific components are listed in Table 1-3. These results indicated that phenolic acids and flavonoids were the main chemical constituents of LBLBFs. They may represent key active components responsible for the ameliorative effect of LBL's on DR.

3.3 NP analysis

Based on identification of the core chemical constituents of LBLBFs, NP was employed to predict the potential molecular targets and signaling pathways of LBLBFs involved in ameliorating DR, thereby providing a basis for subsequent mechanistic validation experiments. A total of 257 potential targets corresponding to 104 identified components in LBLBFs were obtained from the Swiss Target Prediction database, and these targets intersected with 829 DR-related targets, resulting in 69 drug-disease common targets (Fig. 2B). A "component-target-disease" network diagram was constructed, consisting of 139 nodes (1 drug node, 68 compound nodes, 1 disease node, and 69 target nodes) and 371 edges (Fig. 2C). The PPI network analysis showed 259 edges and 59 nodes (including 20 core nodes), with MAPK1, MAPK14, TLR4, VEGFA, etc. as the core targets (Fig. 2D), suggesting that these targets may play a key role in the anti-DR effect of LBLBFs. GO enrichment analysis revealed that LBLBFs were involved in the regulation of apoptotic signaling pathway, ROS metabolic processes and positive regulation of the MAPK cascade (Fig. 2E), which are closely related to the core pathological processes of DR, such as oxidative stress and inflammation. KEGG

enrichment analysis further indicated that the potential targets of LBLBFs were significantly enriched in the VEGF, MAPK and NF- κ B signaling pathways (Fig. 2F), which are the classical signaling pathways mediating DR-related inflammation, oxidative stress and pathological angiogenesis [23–28]. These NP prediction results identified the core signaling pathways (MAPK/NF- κ B/VEGF) for LBLBFs to ameliorate DR; these pathways were selected as the key targets for subsequent in vivo molecular mechanism verification in DR mice.

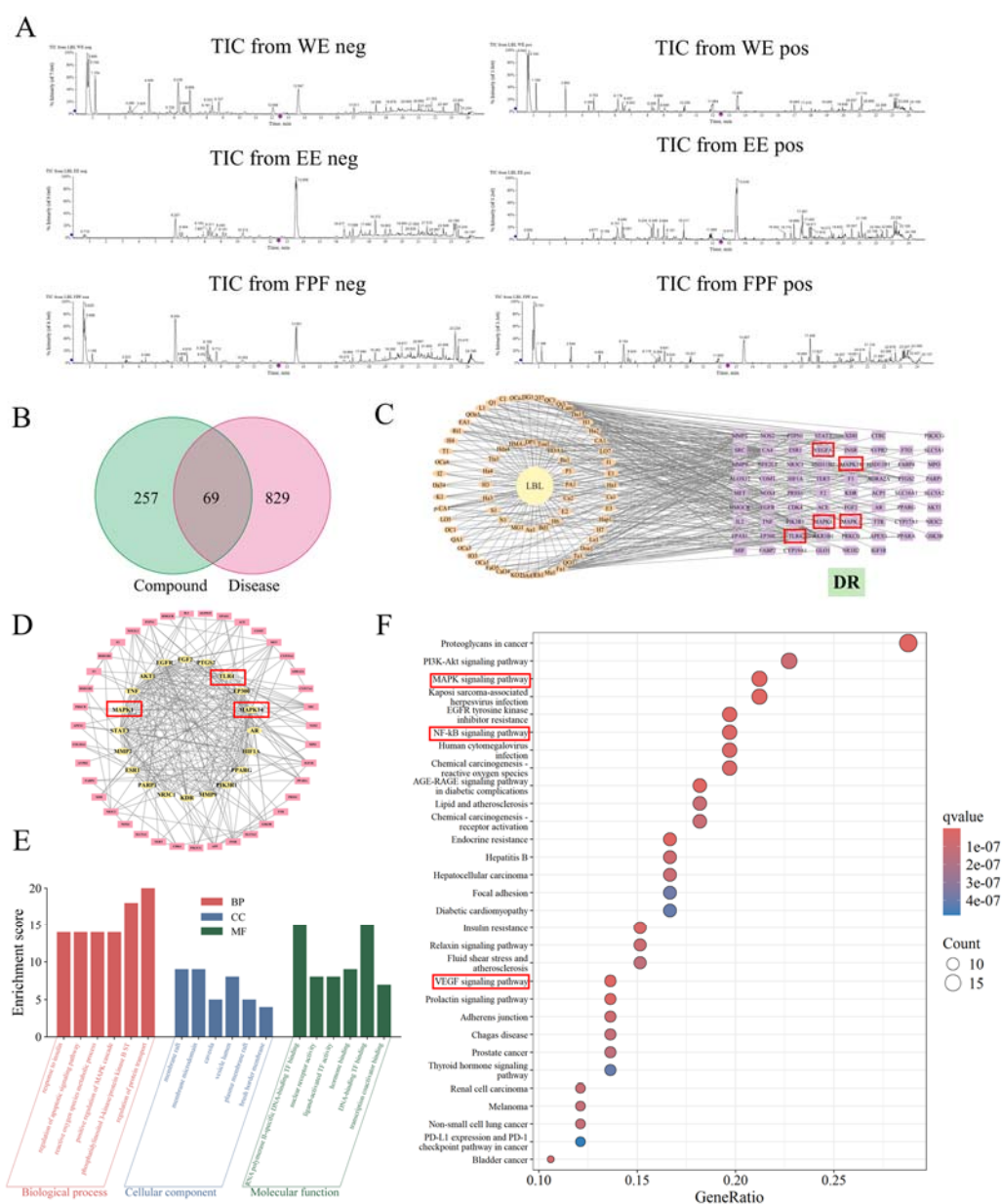


Fig. 2 Analysis of chemical constituents and network pharmacology analysis of LBLBFs. (A) Total Ion Chromatograms (TIC) of LBLBFs. (B) Venn diagram of DR and LBLBFs targets. (C) "Component-target-disease" network. (D) PPI network of the 69 common genes. (E) GO enrichment analysis of the 53 common genes. (F) KEGG enrichment analysis of the 53 common genes.

3.4 Effects of LBLBFs on basic phenotypes and retinal pathology in DR mice

Following the prediction of core anti-DR pathways of LBLBFs using NP, a DR mouse model induced by a combination of HHFD and STZ (a classic model for type 2 diabetes-related DR) was used to validate the in vivo therapeutic effects of LBLBFs (Fig. 3A). Compared with non-DR mice in the control group, DR mice

in the model group exhibited elevated blood glucose levels and an early trend of weight gain under HHFD feeding followed by significant weight loss after STZ injection (an effect attributable to the toxic side effects of STZ and diabetes-induced metabolic disorders). Oral administration of LBLBFs significantly reduced blood glucose levels in DR mice, with no significant effect on body weight (Fig. 3B, C). This finding indicates that LBLBFs can improve the core metabolic abnormality of hyperglycemia in DR mice.

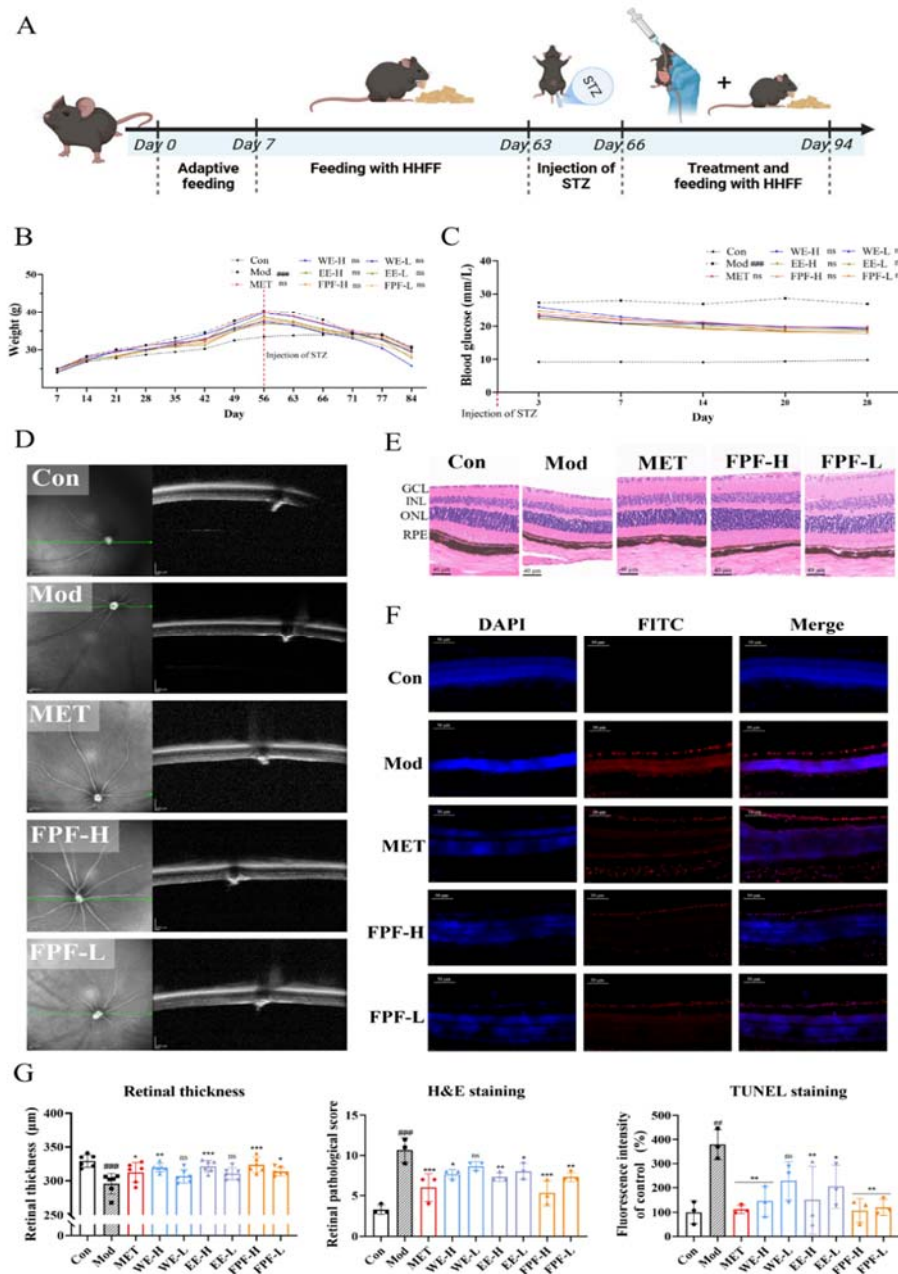


Fig. 3 Effects of LBLBFs on basic phenotypes and retinal pathology in DR mice. (A) Experimental procedure of the DR mouse model. (B) Body weight of mice (n = 6). (C) Blood glucose of DR mice (n = 6). (D) Representative pictures of OCT scan. (E) Representative pictures of H&E staining. (F) Representative pictures of TUNEL staining. (G) Statistical analysis of retinal thickness (n = 6), pathological score of retinal (n = 3) and positive rate of TUNEL in retinal (n = 3). Data were expressed as mean $\pm$ SD. #P < 0.05, ##P < 0.01, ###P < 0.001 vs control group; *P < 0.05, **P < 0.01, ***P < 0.001 vs model group.

Retinal injury constitutes the definitive pathological target of DR; therefore, subsequent analyses prioritized the assessment of retinal pathological alterations. OCT scans revealed that, retinal thickness of DR

mice in the model group was significantly reduced compared with that of mice in the control group. After oral administration of LBLBFs, the retinal thickness of DR mice increased significantly (Fig. 3D, G). H&E staining revealed extensive cavitation in the retinal pigment epithelium (RPE) layer of DR mice in the model group, blurred cell boundaries and marked thinning in the outer nuclear layer (ONL) and inner nuclear layer (INL), along with disorganized cell arrangement and substantial cell loss in the ganglion cell layer (GCL). Oral administration of LBLBFs markedly improved cellular depletion and structural disorganization during DR progression (Fig. 3E, G). TUNEL staining revealed a markedly elevated TUNEL-positive rate in DR mice of the model group, which was significantly reduced following oral administration of LBLBFs (Fig. 3F, G). Representative images from selected groups are depicted in Fig. 3, with the complete set for all groups provided in Figure S1-3. These results demonstrated that LBLBFs could effectively alleviate retinal structural damage and cellular apoptosis in DR mice, thereby exerting a significant protective effect on the retina.

3.5 LBLBFs inhibit inflammatory response by regulating the MAPK and NF- κ B pathways

Oxidative stress and subsequent inflammatory responses are the core upstream pathological processes of DR; NP prediction identified MAPK and NF- κ B as the core pathways by which LBLBFs regulate inflammation and oxidative stress. Based on confirmation of the retinal protective effect of LBLBFs in DR mice, this section further verified the regulatory effects of LBLBFs on oxidative stress and the MAPK/NF- κ B inflammatory signaling pathway. Detection of oxidative stress indicators in mouse serum showed that LBLBFs significantly reversed the decrease in SOD activity and GSH levels, and the increase in MDA levels in DR mice, thereby effectively improving systemic oxidative stress imbalance (Fig. 4A).

Western blotting analysis revealed significant activation of the MAPK and NF- κ B signaling pathways in DR mouse ocular tissues. Compared with that in the control group, the phosphorylation ratios of key MAPK proteins (p-JNK/JNK, p-ERK/ERK, and p-p38/p38) were significantly elevated in the DR model group; LBLBFs treatment markedly reduced these phosphorylation levels (Fig. 4B, C). Similarly, in the NF- κ B pathway, TLR4 protein expression and the p65 phosphorylation ratio (p-p65/p65) were significantly increased in the DR model group, and LBLBFs significantly decreased TLR4 expression and p65 phosphorylation (Fig. 4D). These results confirmed that LBLBFs could inhibit abnormal overactivation of the MAPK and NF- κ B signaling pathways in DR mouse ocular tissues.

Inflammatory cytokines are key downstream effectors of the MAPK/NF- κ B pathways; their abnormal secretion mediates DR-related retinal inflammation and damage. Detection of serum inflammatory factors showed that LBLBFs significantly reduced the levels of pro-inflammatory cytokines IL-1, IL-2, IL-6, and TNF- α and increased that of the anti-inflammatory cytokine IL-10 in DR mice, thereby reversing the abnormal expression of inflammatory factors (Fig. 4E).

Collectively, these findings indicated that LBLBFs ameliorated DR-related inflammatory responses by inhibiting overactivation of the MAPK and NF- κ B signaling pathways, which was consistent with the NP prediction results.

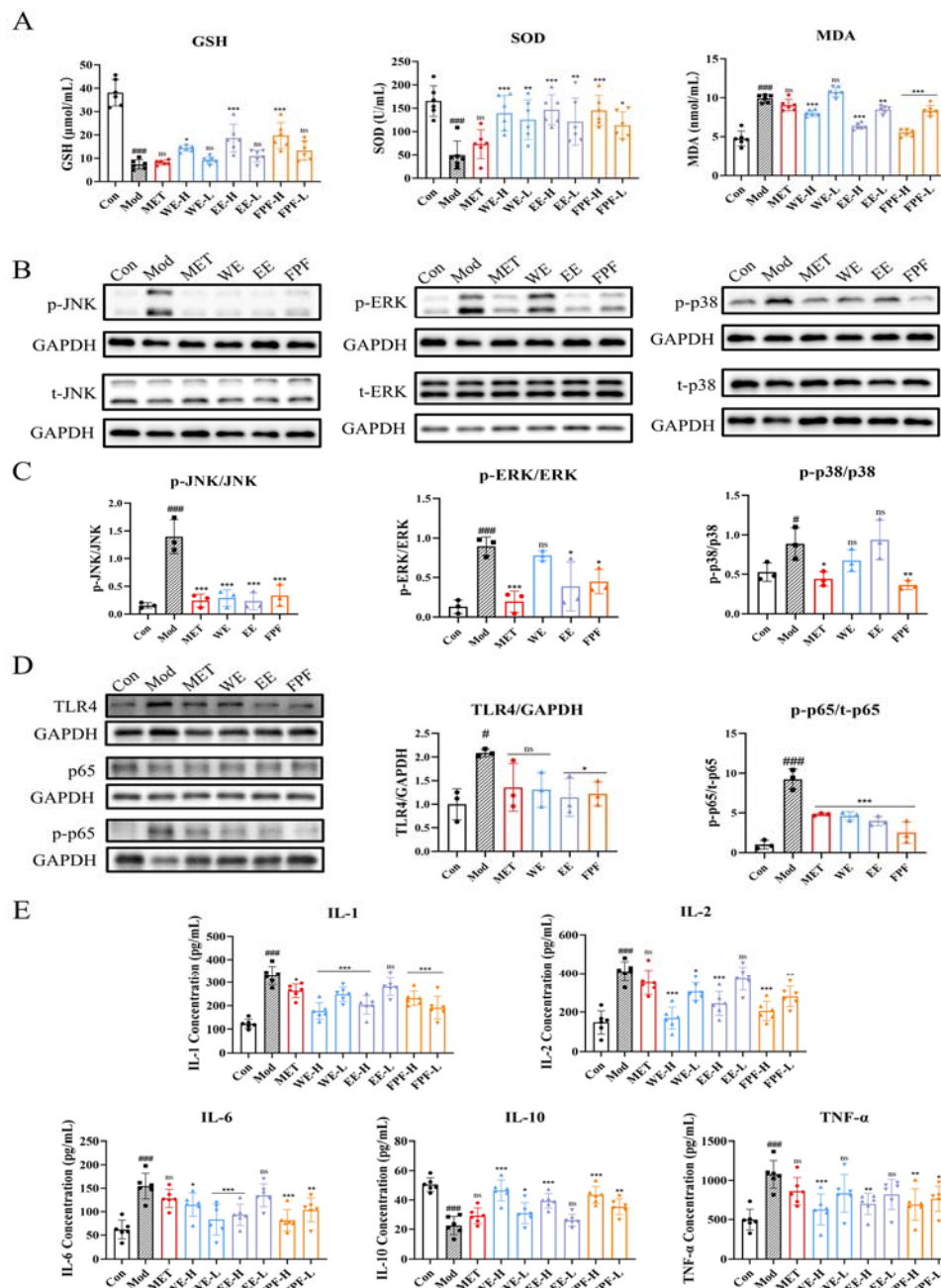


Fig. 4 LBLBFs inhibit inflammatory response by regulating MAPK and NF- κ B pathways. (A) Oxidative stress level of mice ($n = 6$). (B, C) Western blotting analysis for levels of p-JNK, JNK, p-ERK, ERK, p-p38 and p38 ($n = 3$). (D) Western blotting analysis for levels of TLR4, p-p65 and p65 ($n = 3$). (E) Expression levels of inflammatory factors of mice ($n = 6$). Data were expressed as mean $\pm$ SD. $^{\#}P < 0.05$, $^{\#\#}P < 0.01$, $^{\#\#\#}P < 0.001$ vs control group; $^*P < 0.05$, $^{**}P < 0.01$, $^{***}P < 0.001$ vs model group.

3.6 LBLBFs inhibit angiogenesis by regulating the VEGF signaling pathway

Pathological angiogenesis is another core hallmark of DR [28]; the VEGF signaling pathway is a classical regulatory pathway of retinal neovascularization, which was identified as a key target of LBLBFs in NP prediction. Based on verification of the anti-inflammatory effect of LBLBFs, this section further validated their inhibitory effect on DR-related pathological angiogenesis and its regulatory effect on the VEGF signaling pathway. In the high-glucose-induced *Tg (fli1: EGFP)* zebrafish model, high glucose caused significant dilation of retinal blood vessels; intervention with different LBLBFs concentrations effectively alleviated this

vascular dilation, normalizing the retinal vascular morphology (Fig. 5A, B). Retinal flat-mount PAS staining showed that density of retinal angiogenesis in the DR mouse model group was significantly higher than that in the control group, LBLBFs treatment significantly reduced the density of retinal neovascularization (Fig. 5C, D). OCTA scanning further confirmed that deep retinal vascular density in the model group was significantly elevated; LBLBFs markedly downregulated this parameter (Fig. 5E, F). These results from zebrafish and mouse models collectively demonstrated that LBLBFs had a significant inhibitory effect on DR-related pathological retinal angiogenesis.

To further elucidate the potential molecular mechanism of the anti-angiogenic effect of LBLBFs, expression of key proteins in the VEGF signaling pathway was detected. ELISA results showed that the VEGF expression was significantly higher in model group than in the control group; LBLBFs treatment significantly decreased VEGF levels (Fig. 5G). Consistently, western blotting showed that the relative expression level of VEGFA protein and ratio of p-VEGFR2/VEGFR2 in mouse ocular tissues in the model group were significantly upregulated compared with those in the control group; LBLBFs significantly downregulated VEGFA expression and VEGFR2 phosphorylation (Fig. 5H). These findings confirmed that LBLBFs exerted an inhibitory effect on DR-related pathological angiogenesis by targeting and inhibiting VEGF signaling pathway activation.

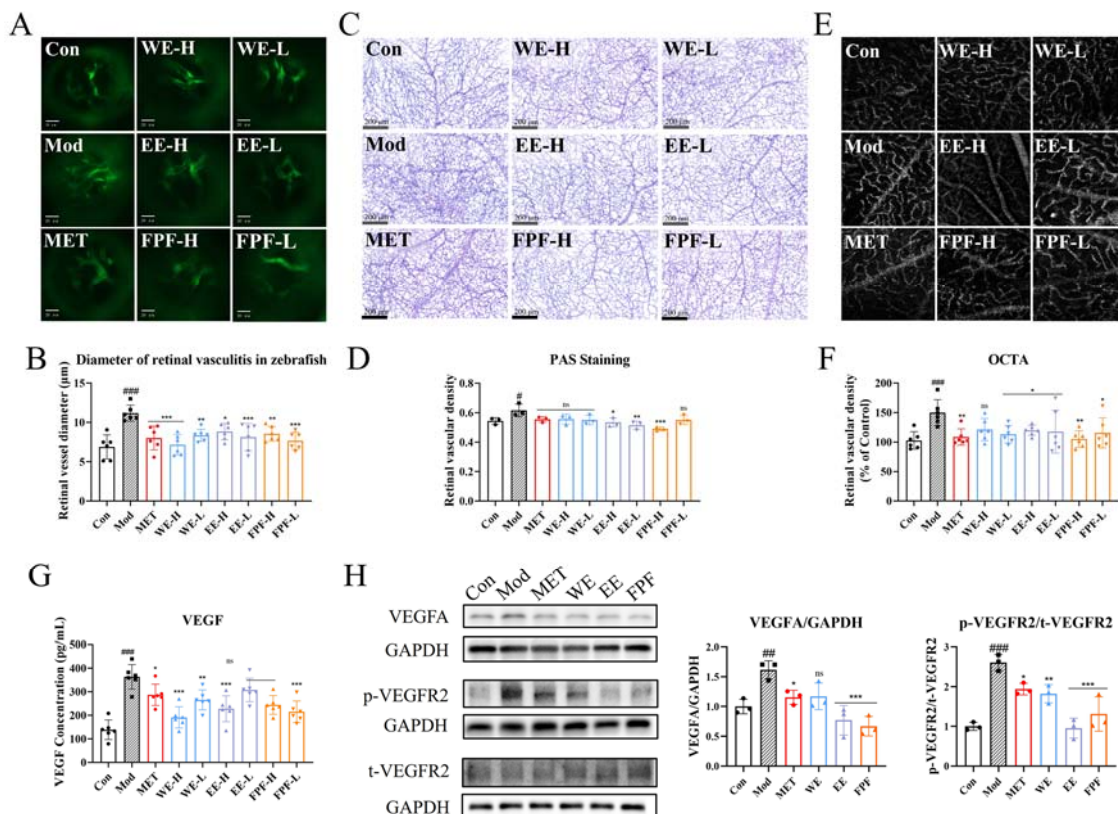


Fig. 5 LBLBFs inhibits angiogenesis by regulating the VEGF signaling pathway. (A, B) Analysis of retinal blood vessel diameter in Tg (fli1: EGFP) zebrafish (n = 6). (C, D) PAS staining analysis of mice retinal flat mounts (n = 3). (E, F) OCTA scanning of deep retinal vessels in mice (n = 6). (G) Serum VEGF expression levels mice (n = 6). (H) Expression levels of key proteins in the VEGF signaling pathway in ocular tissues of mice (n = 3). Data were expressed as mean $\pm$ SD. #P < 0.05, ##P < 0.01, ###P < 0.001 vs control group; *P < 0.05, **P < 0.01, ***P < 0.001 vs model group.

3.7 LBLBFs improve DR by regulating gut microbial homeostasis

The above studies have clarified the local ocular mechanism of LBLBFs in ameliorating DR by inhibiting the MAPK/NF- κ B/VEGF signaling axis; however, the systemic regulatory mechanism of LBLBFs on DR-related inflammation remains to be explored. The "gut-eye axis" is a newly discovered regulatory network linking gut microbiota and ocular diseases; hyperglycemia-induced gut microbial dysbiosis is a key driver of systemic inflammation in diabetic complications [29–34]. Therefore, this section further investigated the systemic regulatory mechanism of LBLBFs in ameliorating DR by regulating gut microbial homeostasis, and explored the crosstalk between gut microbiota regulation and the local ocular MAPK/NF- κ B pathway.

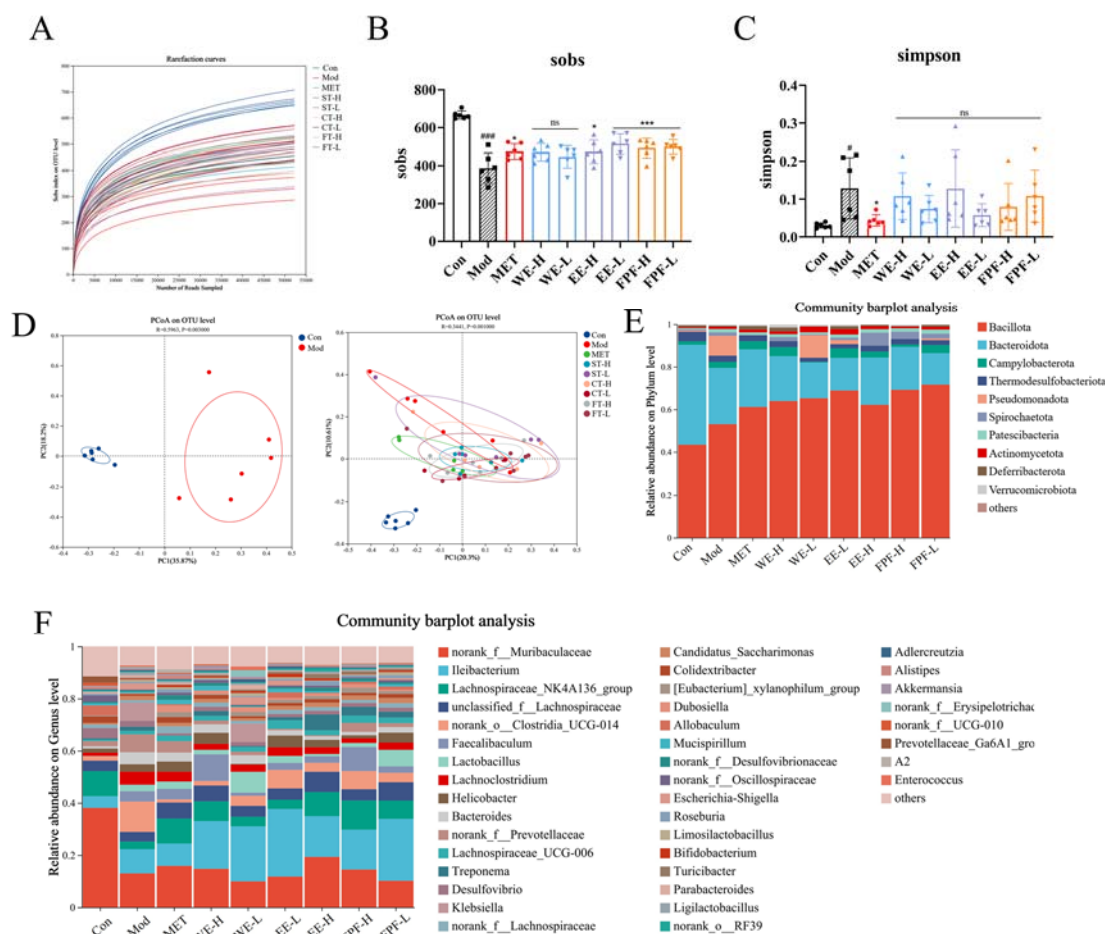


Fig. 6 Effects of LBLBFs on the diversity and community composition of gut microbiota in DR mice (A) Rarefaction curves (n = 6). (B) sobs index (n = 6). (C) simpson index (n = 6). (D) PCoA. (E) Relative abundance at phylum level. (F) Relative abundance at genus level. Data were expressed as mean $\pm$ SD. #P < 0.05, ##P < 0.01, ###P < 0.001 vs control group; *P < 0.05, **P < 0.01, ***P < 0.001 vs model group.

16S rRNA gene sequencing of mouse colonic contents showed that rarefaction curves based on the Sobs index tended to plateau (Fig. 6A), indicating sufficient sequencing depth. α -diversity (as reflected by the Sobs index and Simpson index) of gut microbiota in the model group was significantly lower than in the control group, and LBLBFs treatment restored the α -diversity to varying degrees (Fig. 6B, C). Principal coordinate analysis (PCoA) of β -diversity showed a significant separation in gut microbiota structure between the model and control group; LBLBFs treatment shifted the gut microbial structure of DR mice to be similar to that of the control group (Fig. 6D). This finding indicates that LBLBFs can reverse hyperglycemia-induced gut

microbial dysbiosis in DR mice. Subsequently, gut microbial community composition was analyzed at the phylum (Fig. 6E) and genus levels (Fig. 6F). At the phylum level, LBLBFs significantly increased the abundance of Bacteroides but decreased that of Proteobacteria in DR mice. At the genus level, LBLBFs treatment elevated the abundance of *g_norank_f_Lachnospiraceae_NK4A136_group* while reducing the proportions of *o_norank_c_Clostridia_UCG-014* and *Lachnoclostridium*. Heatmap analysis and the Wilcoxon rank-sum test further identified differential microbial taxa between the control and model groups; LBLBFs significantly reversed the abnormal abundance of these differential genera (Fig. 7A-C). Results indicated that Bacteroidetes exhibited the strongest association with DR pathogenesis, with 10 differentially abundant genera identified at the genus level (Fig. 7D), including *g_norank_f_Muribaculaceae*, *g_norank_f_Lachnospiraceae_NK4A136_group*, *Faecalibaculum*, and *g_Colidextribacter*. Furthermore, LBLBFs enhanced the relative abundance of beneficial genera *g_norank_f_UCG-010* [35] and *g_norank_f_Lachnospiraceae_NK4A136_group* [36], and that of the potentially beneficial genus *g_Colidextribacter* [37].

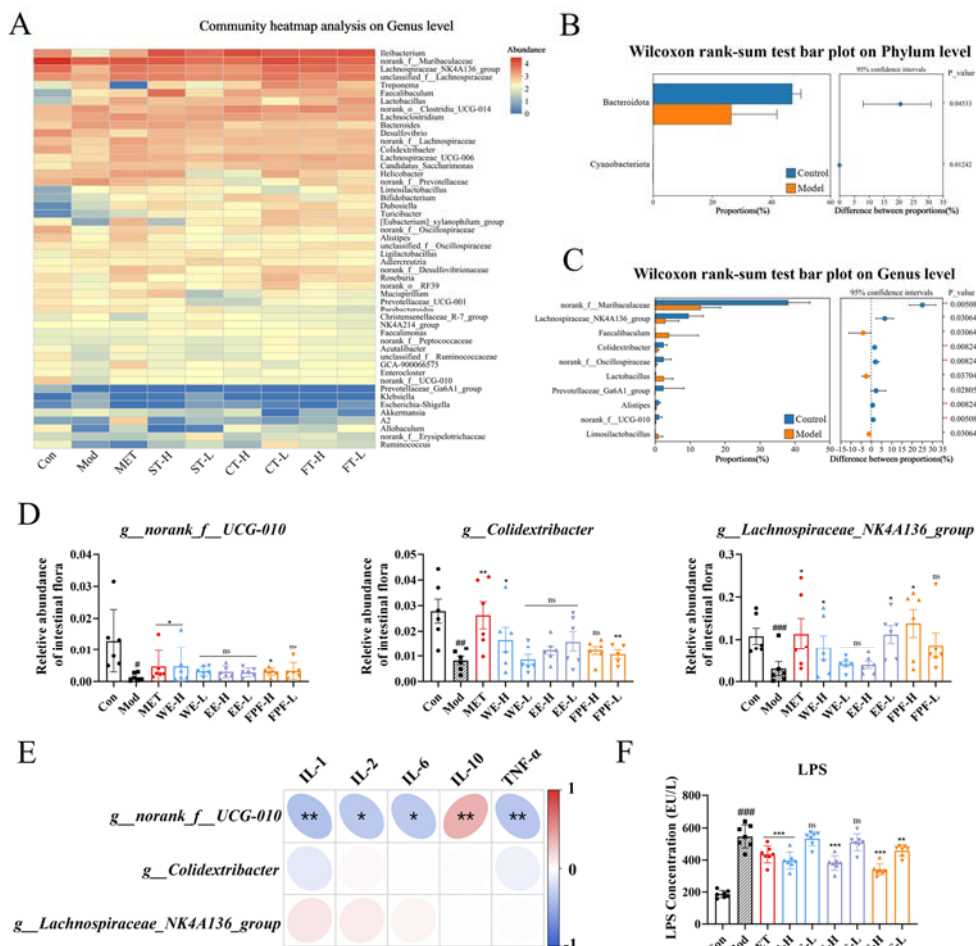


Fig. 7 Analysis of the difference of gut microbiota and the regulation and correlation of LBLBFs in DR mice. (A) Heat map analysis. (B) Analysis of the difference of phylum level between the control group and model group based on Wilcoxon rank sum test. (C) Analysis of the difference of genus level between the control group and model group based on Wilcoxon rank sum test. (D) The regulatory effect of LBLBFs on three key bacteria (n = 6). (E) Spearman correlation analysis. (A) Serum LPS concentration of mice (n = 6). Data were expressed as mean ± SD. #P < 0.05, ##P < 0.01, ###P < 0.001 vs control group; *P < 0.05, **P < 0.01, ***P < 0.001 vs model group.

Correlation analysis based on the Pearson correlation coefficient revealed that the relative abundance of the beneficial genus *g__norank__f__UCG-010* was significantly negatively correlated with the serum levels of key pro-inflammatory cytokines (IL-1, IL-2, IL-6, TNF- α) and positively correlated with the anti-inflammatory cytokine IL-10 (Fig. 7E). This correlation validates the functional relevance of *g__norank__f__UCG-010* in regulating of systemic inflammatory responses and establishes a direct mechanistic link between LBLBFs-mediated modulation of gut microbial composition and the attenuation of DR-associated systemic inflammation. Additionally, we determined the concentration of LPS, the major harmful metabolite of gut microbes, and found that serum LPS level was significantly elevated in the model group DR mice; LBLBFs treatment markedly reduced this level (Fig. 7F). This reduction in LPS levels may be associated with the improvement of intestinal barrier integrity mediated by LBLBFs via regulating gut microbial homeostasis; decreased LPS translocation into the bloodstream further inhibits overactivation of the MAPK/NF- κ B pathways in ocular tissues [32, 33, 38–40]. This systemic regulatory mechanism of LBLBFs is intrinsically linked to direct inhibition of the MAPK/NF- κ B pathways in ocular tissues; the two mechanisms synergistically ameliorate DR-related inflammation and retinal damage.

4. Discussion

DR, a leading cause of vision loss in patients with diabetics, presents substantial clinical challenges owing to its complex pathogenesis. LBL, the leaves of a plant with a long history of medicinal and dietary use, have shown potential in ameliorating type 2 diabetes and its complications [20, 41]. We systematically investigated the therapeutic effects and underlying mechanisms of LBL in DR. The results indicate that phenolic acids and flavonoid are the primary bioactive constituents of LBL. These compounds exert protective effects through a dual-pathway mechanism: inhibiting the VEGF signaling pathway to suppress pathological retinal angiogenesis and regulating gut microbial homeostasis to attenuate systemic inflammation.

High glucose-injured cell and zebrafish models were used to establish the bioactivity of different LBL fractions against oxidative stress, which is a key upstream event that drives disease progression in DR pathogenesis [22]. Among these fractions, PF exhibited no significant protective effect against high-glucose-induced oxidative damage, which may be attributed to its high molecular weight limiting cellular uptake and bioavailability [42] or absence of specific bioactive polysaccharide structures under the current experimental conditions. Combined with PCA, FPF was identified as the optimal bioactive fraction. This pharmacodynamically-guided screening ensured that the subsequent mechanistic investigation focused on the most therapeutically relevant fraction.

Following confirmation of the efficacy of LBLBFs, their chemical basis and mechanistic network were delineated. First, UPLC-QTOF-MS analysis revealed that flavonoids and phenolic acids were the core components of LBLBFs (WE, EE, and FPF). Subsequently, NP screening based on these constituents identified a series of potential molecular targets. Chronic low-grade inflammation and pathological retinal angiogenesis are the core pathological mechanisms of DR. Therefore, subsequent validation focused on the VEGF, MAPK and NF- κ B signaling pathways.

Chronic low-grade inflammation is a key pathological feature in DR progression [5]; oxidative stress serves as a critical upstream inducer driving this inflammatory response. Based on this premise, the effect of LBLBFs on oxidative stress in DR mice was first examined. The results showed that LBLBFs significantly reversed abnormal oxidative stress in DR mice. Western blot analysis was conducted to link this phenotypic improvement to molecular events. Its results demonstrated that LBLBFs markedly suppressed abnormal phosphorylation of JNK, ERK, and p38 in the MAPK pathway and downregulated TLR4 expression and p65 phosphorylation in the NF- κ B pathway. These findings indicate that LBLBFs mitigate inflammatory responses in DR by inhibiting the abnormal activation of both pathways, thereby contributing to the overall improvement of DR pathology.

Angiogenesis is another key pathological feature in DR progression; it represents a major therapeutic target [26, 43]. Prompted by NP predictions implicating the VEGF pathway, the effect of LBL on vascular parameters was evaluated. In *Tg (fli 1: EGFP)* zebrafish, LBL reduced high-glucose-induced vascular dilation. In the DR mouse model, OCTA and PAS staining showed that LBLBFs significantly ameliorated retinal angiogenesis. Mechanistically, western blot analysis revealed that LBLBFs downregulated abnormal VEGFA expression and inhibited VEGFR2 phosphorylation. Hence, LBLBFs exert a direct anti-angiogenic effect by suppressing the VEGF pathway.

Notably, these three pathways form an interconnected network. Hyperglycemia-induced oxidative stress leads to excessive production of ROS, which directly activates the MAPK signaling pathway [27, 44]. Activated MAPK can subsequently trigger the NF- κ B pathway, leading to the transcription of inflammatory factors; these factors, in turn, can feedback to reactivate MAPK signaling, forming a positive feedback loop [44–46]. Inflammation-driven vascular pathology is a core pathological feature of DR, wherein the MAPK and NF- κ B pathways link inflammation to pathological angiogenesis [47–49]. Phosphorylated ERK/MAPK and p65/NF- κ B can directly promote VEGFA expression [49, 50]. Moreover, inflammatory factors can induce VEGF secretion, thereby initiating angiogenesis. The resulting pathological vessels are prone to leakage, triggering additional inflammation and reactivating NF- κ B/MAPK pathways, thereby forming a vicious cycle [25, 26]. The results demonstrate that LBLBFs break this cycle by synergistically inhibiting the VEGF, MAPK, and NF- κ B signaling pathways, thereby reducing the release of inflammatory factors.

Although the local ocular mechanisms were elucidated, systemic triggers of chronic inflammation required further exploration. Hyperglycemia-induced gut microbial dysbiosis is a key driver of systemic inflammation in diabetic complications [32, 33]; microbial metabolites translocated into the bloodstream can amplify distant tissue damage. LBLBFs significantly reduced serum LPS levels in DR mice. LPS is a major harmful metabolite of gut microbes and a classical agonist of TLR4/MAPK/NF- κ B pathways [38, 39]. This reduction was closely associated with the modulation of gut microbial communities by LBLBFs, as evidenced by 16S rRNA sequencing data: LBLBFs restored gut microbial α -diversity, which is critical for maintaining intestinal barrier integrity and the competitive exclusion of pathogenic bacteria [30]; simultaneously, it shifted the β -diversity of gut microbiota in DR mice towards that of normal mice, indicating reversal of diabetes-

induced microbial community dysbiosis. At the compositional level, LBLBFs increased the abundance of beneficial *Bacteroides* (a producer of anti-inflammatory short-chain fatty acids ^[51]) and decreased pro-inflammatory *Proteobacteria*, while upregulating genus-level beneficial bacteria (*g__norank__f__Lachnospiraceae_NK4A136_group*, *g__Colidextribacter*) and downregulating pro-inflammatory genera (*o__norank__c__Clostridia__UCG-014*, *Lachnoclostridium*). Importantly, the beneficial genus *g__norank__f__UCG-010*, whose abundance was enhanced by LBLBFs, exhibited a significant negative correlation with pro-inflammatory cytokines (IL-1, IL-2, IL-6, TNF- α) and positive correlation with the anti-inflammatory IL-10, thereby directly linking gut microbial modulation to systemic inflammation regulation ^[52]. Collectively, these findings suggest that LBLBFs modulate gut microbial homeostasis to reduce circulating LPS levels, which may be attributed to improvements in intestinal barrier integrity ^[34, 53], thereby indirectly suppressing the overactivation of MAPK/NF- κ B pathways. However, this hypothesis requires further experimental verification. This mechanism forms an intrinsic logical correlation with direct inhibition of these pathways by the bioactive components of LBLBFs, thereby synergistically ameliorating DR-related inflammation and retinal damage.

However, this study also has certain limitations. Although the FPF of LBL has been identified as the principal bioactive fraction mediating its anti-DR effects, the specific contributions of individual monomeric compounds and their potential synergistic interactions remain to be elucidated. As a typical medicinal and edible natural product, LBL exerts its biological effects through the multicomponent synergy characteristic of such botanicals. UPLC-Q-TOF/MS analysis identified several constituents, including rutin, isoquercitrin, and chlorogenic acid, which exhibit antioxidant, anti-inflammatory, and anti-angiogenic activities ^[54-56]. Regardless, the current experimental design, which employed mixed fractions (WE, EE, and FPF), precludes definitive attribution of the observed anti-DR effects to specific constituents. For instance, flavonoids may preferentially target the VEGF pathway to suppress pathological angiogenesis ^[57, 58], whereas phenolic acids may primarily regulate the MAPK/NF- κ B axis to mitigate inflammation ^[24, 59]. However, such functional specialization, requires direct validation using bioactivity-guided isolation and systematic evaluation of purified monomers in future studies. Therefore, subsequent investigations should focus on isolating key monomeric components from LBLBFs and evaluating their individual and combined anti-DR activities. This approach will clarify structure-activity relationships of the bioactive constituents of LBL and lay a foundation for the development of superiorly targeted and potent functional products. Another limitation lies in the lack of direct evidence confirming a causal relationship between gut microbiota modulation and DR amelioration. Although the gut microbiota sequencing data and serum LPS levels strongly suggest involvement of the gut-eye axis, the specific role of gut microbial changes in driving retinal pathology improvement remains to be fully elucidated. The results showed that LBLBFs regulated the abundance of specific gut microbial genera and reduced LPS translocation, which was correlated with inflammation inhibition. However, LBLBFs may directly act on intestinal epithelial cells to enhance barrier function, thereby reducing LPS leakage, or regulate immune cells in the retina independently of gut microbiota. Faecal microbiota transplantation experiments

will help determine whether gut microbiota alone is sufficient to mediate the protective effect of LBL on DR. Additionally, further studies can investigate the role of specific microbial metabolites in DR improvement, as these metabolites may serve as key messengers in the gut-eye axis. Despite the lack of direct causal evidence, our findings still provide important insights into the systemic regulatory mechanism, particularly the proposed gut microbiota-LPS-inflammatory pathway axis, which offers a promising direction for future mechanistic exploration.

In conclusion, despite these limitations, the present study systematically elucidates a coordinated "local-systemic" regulatory mechanism by which LBLBFs ameliorate DR. It simultaneously targets the VEGF/MAPK/NF- κ B signaling axis in ocular tissues and modulates gut microbial homeostasis to alleviate systemic inflammation. This integrative approach distinguishes this work from prior studies focusing on isolated pathways [60, 61], thereby advancing the understanding of DR-related metabolic-ocular crosstalk. Moreover, the identification of flavonoids and phenolic acids as core bioactive components and discovery of the gut-eye axis regulatory pathway provide a scientific basis for the development of LBL as a functional food or dietary supplement for DR prevention and intervention, while also opening new avenues for the research and development of natural product-based anti-DR agents.

5. Conclusion

In summary, the result of this study confirmed that LBLBFs exerted an improving effect on DR by directly inhibiting the MAPK/NF- κ B/VEGF signaling axis and regulating gut microbial homeostasis to suppress systemic inflammation. Moreover, phenolic acids and flavonoids were identified as the principal bioactive constituents. These findings systematically elucidate the protective role of edible LBL in supporting ocular health under diabetic conditions and provide a mechanistic basis for its development as a functional food or dietary supplement suitable for long-term use.

Declaration of competing interest

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

Data availability

Data will be made available on request.

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References

- [1] Y. Zheng, S.H. Ley, F.B. Hu, Global aetiology and epidemiology of type 2 diabetes mellitus and its complications, *Nature Reviews Endocrinology*. 14 (2018) 88-98. <https://doi.org/10.1038/nrendo.2017.151>.
- [2] Z.L. Teo, Y.C. Tham, M. Yu, et al., Global Prevalence of Diabetic Retinopathy and Projection of Burden through 2045: Systematic Review and Meta-analysis, *Ophthalmology*. 128 (2021) 1580-1591. <https://doi.org/10.1016/j.ophtha.2021.04.027>.
- [3] S. Vujosevic, S.J. Aldington, P. Silva, et al., Screening for diabetic retinopathy: new perspectives and challenges, *The Lancet Diabetes & Endocrinology*. 8 (2020) 337-347. [https://doi.org/10.1016/s2213-8587\(19\)30411-5](https://doi.org/10.1016/s2213-8587(19)30411-5).
- [4] D.A. Antonetti, P.S. Silva, A.W. Stitt, Current understanding of the molecular and cellular pathology of diabetic retinopathy, *Nature Reviews Endocrinology*. 17 (2021) 195-206. <https://doi.org/10.1038/s41574-020-00451-4>.
- [5] F. Binet, G. Cagnone, S. Crespo-Garcia, et al., Neutrophil extracellular traps target senescent vasculature for tissue remodeling in retinopathy, *Science*. 369 (2020) 1-13. <https://doi.org/10.1126/science.aay5356>.
- [6] H.D. Li, M.X. Li, W.H. Zhang, et al., Effectiveness and safety of traditional Chinese medicine for diabetic retinopathy: A systematic review and network meta-analysis of randomized clinical trials, *World Journal of Diabetes*. 14 (2023) 1422-1449. <https://doi.org/10.4239/wjd.v14.i9.1422>.
- [7] Y. Huang, J. Wang, Y. Wang, et al., Pharmacological mechanism and clinical study of Qiming granules in treating diabetic retinopathy based on network pharmacology and literature review, *Journal of Ethnopharmacology*. 302 (2023) 115861. <https://doi.org/10.1016/j.jep.2022.115861>.
- [8] W.-J. Guo, Y. Zhang, H. Elsayed, et al., Iridoid terpenoids: a class of promising compounds to regulate blood glucose, *Food Med. Homol.* 2 (2025) 9420074. <https://doi.org/10.26599/FMH.2025.9420074>.
- [9] P.-D. Chen, J.-J. Li, S. Zhang, et al., Research progress on the regulatory effects of Chinese food and medicine homology on type 1 diabetes mellitus, *Food Med. Homol.* 3 (2026) 9420085. <https://doi.org/10.26599/FMH.2026.9420085>.
- [10] R. Yao, An in-silico assessment suggests the potential effects of goji (fruit of *Lycium barbarum* L.) against aging-related diseases, *Food Med. Homol.* 2 (2025) 9420036. <https://doi.org/10.26599/FMH.2025.9420036>.
- [11] M.K. Song, N.K. Salam, B.D. Roufogalis, et al., *Lycium barbarum* (Goji Berry) extracts and its taurine component inhibit PPAR- γ -dependent gene transcription in human retinal pigment epithelial cells: Possible implications for diabetic retinopathy treatment, *Biochemical Pharmacology*. 82 (2011) 1209-1218. <https://doi.org/10.1016/j.bcp.2011.07.089>.
- [12] Y. Niu, G. Zhang, X. Sun, et al. Distinct Role of *Lycium barbarum* L. Polysaccharides in Oxidative Stress-Related Ocular Diseases. *Pharmaceuticals*. 16. 2023:215.
- [13] L. Liu, X.-Y. Sha, Y.-N. Wu, et al., *Lycium barbarum* polysaccharides protects retinal ganglion cells against oxidative stress injury, *Neural Regeneration Research*. 15 (2020), https://journals.lww.com/nrronline/fulltext/2020/15080/lycium_barbarum_polysaccharides_protects_retinal.24.aspx
- [14] X.Q. Zhao, S. Guo, H. Yan, et al., Analysis of phenolic acids and flavonoids in leaves of *Lycium barbarum* from different habitats by ultra-high-performance liquid chromatography coupled with triple quadrupole tandem mass spectrometry, *Biomedical Chromatography*. 33 (2019) e4552. <https://doi.org/10.1002/bmc.4552>.
- [15] X. Xiao, W. Ren, N. Zhang, et al. Comparative Study of the Chemical Constituents and Bioactivities of the Extracts from Fruits, Leaves and Root Barks of *Lycium barbarum*. *Molecules*. 24. 2019:1585.
- [16] A. Mocan, G. Zengin, M. Simiriotis, et al., Functional constituents of wild and cultivated Goji (*L. barbarum* L.) leaves: phytochemical characterization, biological profile, and computational studies, *Journal of Enzyme Inhibition and Medicinal Chemistry*. 32 (2017) 153-168. <https://doi.org/10.1080/14756366.2016.1243535>.
- [17] O.J. Olatunji, H. Chen, Y. Zhou, *Lycium chinense* leaves extract ameliorates diabetic nephropathy by suppressing hyperglycemia mediated renal oxidative stress and inflammation, *Biomedicine & Pharmacotherapy*. 102 (2018) 1145-1151. <https://doi.org/10.1016/j.biopha.2018.03.037>.
- [18] C. Wen, C. Liu, Y. Li, et al., Ameliorative potentials of the ethanolic extract from *Lycium chinense* leaf extract against diabetic cardiomyopathy. Insight into oxido-inflammatory and apoptosis modulation, *Biomedicine & Pharmacotherapy*. 154 (2022) 113583. <https://doi.org/10.1016/j.biopha.2022.113583>.
- [19] E.H. Choi, Y.S. Chun, J. Kim, et al., Modulating lipid and glucose metabolism by glycosylated kaempferol rich roasted leaves of *Lycium chinense* via upregulating adiponectin and AMPK activation in obese mice-induced type 2 diabetes, *Journal of Functional Foods*. 72 (2020) 104072. <https://doi.org/10.1016/j.jff.2020.104072>.

- [20] J. Liao, J. Guo, Y. Niu, et al., Flavonoids from *Lycium barbarum* leaves attenuate obesity through modulating glycolipid levels, oxidative stress, and gut bacterial composition in high-fat diet-fed mice, *Frontiers In Nutrition*. 9 (2022) 972794. <https://doi.org/10.3389/fnut.2022.972794>.
- [21] M.-Y. Li, G.-F. Wu, F. Lu, et al., Food and Medicine Homology Focus in 2026, *Food Med. Homol.* 3 (2026) 9420133. <https://doi.org/10.26599/FMH.2026.9420133>.
- [22] Q. Kang, C. Yang, Oxidative stress and diabetic retinopathy: Molecular mechanisms, pathogenetic role and therapeutic implications, *Redox Biology*. 37 (2020) 101799. <https://doi.org/10.1016/j.redox.2020.101799>.
- [23] J.M. Kyriakis, J. Avruch, Mammalian MAPK signal transduction pathways activated by stress and inflammation: a 10-year update, *Physiological. Reviews*. 92 (2012) 689-737. <https://doi.org/10.1152/physrev.00028.2011>.
- [24] M. Rahimifard, F. Maqbool, S. Moeini-Nodeh, et al., Targeting the TLR4 signaling pathway by polyphenols: A novel therapeutic strategy for neuroinflammation, *Ageing Res Rev.* 36 (2017) 11-19. <https://doi.org/10.1016/j.arr.2017.02.004>.
- [25] T. Behl, A. Kotwani, Exploring the various aspects of the pathological role of vascular endothelial growth factor (VEGF) in diabetic retinopathy, *Pharmacological Research*. 99 (2015) 137-148. <https://doi.org/10.1016/j.phrs.2015.05.013>.
- [26] R. Simó, J.M. Sundstrom, D.A. Antonetti, Ocular Anti-VEGF Therapy for Diabetic Retinopathy: The Role of VEGF in the Pathogenesis of Diabetic Retinopathy, *Diabetes Care*. 37 (2014) 893-899. <https://doi.org/10.2337/dc13-2002>.
- [27] S. Wei, D. Yang, Z. Shou, et al., Proanthocyanidin capsules remodel the ROS microenvironment via regulating MAPK signaling for accelerating diabetic wound healing, *Materials Today Bio.* 31 (2025) 101467. <https://doi.org/10.1016/j.mtbio.2025.101467>.
- [28] T.Y. Wong, C.M.G. Cheung, M. Larsen, et al., Diabetic retinopathy, *Nature Reviews Disease Primers*. 2 (2016) 16012. <https://doi.org/10.1038/nrdp.2016.12>.
- [29] D. Li, P. Wang, P. Wang, et al., The gut microbiota: A treasure for human health, *Biotechnol Adv.* 34 (2016) 1210-1224. <https://doi.org/10.1016/j.biotechadv.2016.08.003>.
- [30] W. Feng, H. Ao, C. Peng, et al., Gut microbiota, a new frontier to understand traditional Chinese medicines, *Pharmacol Res.* 142 (2019) 176-191. <https://doi.org/10.1016/j.phrs.2019.02.024>.
- [31] F.Z. Marques, C.R. Mackay, D.M. Kaye, Beyond gut feelings: how the gut microbiota regulates blood pressure, *Nat Rev Cardiol.* 15 (2018) 20-32. <https://doi.org/10.1038/nrcardio.2017.120>.
- [32] K. Agrawal, A.S.Y. Hong, C. Cifuentes-González, et al., Microbiome signatures and their role in uveitis: Pathogenesis, diagnostics, and therapeutic perspectives, *Progress In Retinal And Eye Research*. 109 (2025) 101409. <https://doi.org/10.1016/j.preteyeres.2025.101409>.
- [33] R. Shen, W. Zhao, Y. Wang, et al., Multi-omics analysis reveals Angelica sinensis-Carthusus tinctorius herb pair ameliorates diabetic retinopathy comorbid with depressive symptoms via the gut-eye-brain axis, *Phytomedicine*. 143 (2025) 156874. <https://doi.org/10.1016/j.phymed.2025.156874>.
- [34] R. Wang, Y. Bi, Y. Xie, et al., Dendrobium nobile Lindl. Polysaccharides ameliorate the inflammatory microenvironment in the retina of diabetic rats: a multi-omics study of the gut-blood-retina axis, *Int J Biol Macromol.* 316 (2025) 144732. <https://doi.org/10.1016/j.ijbiomac.2025.144732>.
- [35] Y. Shi, F. Chen, Z. Wang, et al., Effect and mechanism of functional compound fruit drink on gut microbiota in constipation mice, *Food Chemistry*. 401 (2023) 134210. <https://doi.org/10.1016/j.foodchem.2022.134210>.
- [36] B. Hu, W. Zhou, X. Deng, et al., Structural analysis of polysaccharide from Inonotus obliquus and investigate combined impact on the sex hormones, intestinal microbiota and metabolism in SPF male mice, *International Journal of Biological Macromolecules*. 262 (2024) 129686. <https://doi.org/10.1016/j.ijbiomac.2024.129686>.
- [37] X. Liu, Y. Zhang, W. Li, et al., Fucoidan Ameliorated Dextran Sulfate Sodium-Induced Ulcerative Colitis by Modulating Gut Microbiota and Bile Acid Metabolism, *Journal of Agricultural and Food Chemistry*. 70 (2022) 14864-14876. <https://doi.org/10.1021/acs.jafc.2c06417>.
- [38] S.I. Miller, R.K. Ernst, M.W. Bader, LPS, TLR4 and infectious disease diversity, *Nature Reviews Microbiology*. 3 (2005) 36-46. <https://doi.org/10.1038/nrmicro1068>.
- [39] Y. Fu, H. Kim, D.S. Lee, et al., Structural insight into TLR4/MD-2 activation by synthetic LPS mimetics with distinct binding modes, *Nature Communications*. 16 (2025) 4164. <https://doi.org/10.1038/s41467-025-59550-3>.
- [40] M. Shah, N. Kirthivasan, S. Chakraborty, et al., Phagosome Maturation in Macrophages is Enhanced by p38 α MAPK Signaling,

- Small. 21 (2025) e06883. <https://doi.org/https://doi.org/10.1002/sml.202506883>.
- [41] O.J. Olatunji, H. Chen, Y. Zhou, *Lycium chinense* leaves extract ameliorates diabetic nephropathy by suppressing hyperglycemia mediated renal oxidative stress and inflammation, *Biomedicine & Pharmacotherapy*. 102 (2018) 1145-1151. <https://doi.org/10.1016/j.biopha.2018.03.037>.
- [42] Z. Wang, X. Zhou, Z. Shu, et al., Regulation strategy, bioactivity, and physical property of plant and microbial polysaccharides based on molecular weight, *Int J Biol Macromol*. 244 (2023) 125360. <https://doi.org/10.1016/j.ijbiomac.2023.125360>.
- [43] S.E. Quaggin, A half-century of VEGFA: from theory to practice, *Journal of Clinical Investigation*. 134 (2024) <https://doi.org/10.1172/jci184205>.
- [44] C.H. Su, Vascular endothelial dysfunction induced by 3 bromofluoranthene via MAPK mediated NF B proinflammatory pathway and intracellular ROS generation, *European Heart Journal*. 44 (2023) ehad655.3292. <https://doi.org/10.1093/eurheartj/ehad655.3292>.
- [45] J. Chen, W. Pan, H. Yu, et al., GW27-e1067 Resveratrol Protects against TNF- α -Induced Injury in Human Umbilical Endothelial Cells through Promoting Sirtuin-1-Induced Repression of NF-KB and p38 MAPK, *Journal of the American College of Cardiology*. 68 (2016) C38-C38. <https://doi.org/10.1016/j.jacc.2016.07.141>.
- [46] Y. Ling, D. Nie, Y. Huang, et al., Antioxidant Cascade Nanoenzyme Antagonize Inflammatory Pain by Modulating MAPK/p-65 Signaling Pathway, *Adv. Sci. (Weinh)*. 10 (2023) e2206934. <https://doi.org/10.1002/adv.202206934>.
- [47] J. Guan, X. Wang, Z. Huang, et al., PML+ exosomes derived from microglia attenuate retinal inflammation and neovascularization in diabetic retinopathy, *Chemical Engineering Journal*. 516 (2025) 163857. <https://doi.org/https://doi.org/10.1016/j.cej.2025.163857>.
- [48] G. Yang, C.-C. Chang, Y. Yang, et al., Resveratrol Alleviates Rheumatoid Arthritis via Reducing ROS and Inflammation, Inhibiting MAPK Signaling Pathways, and Suppressing Angiogenesis, *Journal of Agricultural and Food Chemistry*. 66 (2018) 12953-12960. <https://doi.org/10.1021/acs.jafc.8b05047>.
- [49] D.G. Binion, M.F. Otterson, P. Rafiee, Curcumin inhibits VEGF-mediated angiogenesis in human intestinal microvascular endothelial cells through COX-2 and MAPK inhibition, *Gut*. 57 (2008) 1509-1517. <https://doi.org/10.1136/gut.2008.152496>.
- [50] H. Han, T. Lin, Z. Wang, et al., RNA-binding motif 4 promotes angiogenesis in HCC by selectively activating VEGF-A expression, *Pharmacological Research*. 187 (2023) 106593. <https://doi.org/https://doi.org/10.1016/j.phrs.2022.106593>.
- [51] K. Oliphant, M. Ali, M. D'Souza, et al., Bacteroidota and Lachnospiraceae integration into the gut microbiome at key time points in early life are linked to infant neurodevelopment, *Gut Microbes*. 13 (2021) 1997560. <https://doi.org/10.1080/19490976.2021.1997560>.
- [52] D.-C. Shi, P.-Y. Wang, L. Xu, et al., Potential of *Dendrobium officinale* oligosaccharides to alleviate chronic colitis by modulating inflammation and gut microbiota, *Food Med. Homol*. 2 (2025) 9420077. <https://doi.org/10.26599/FMH.2025.9420077>.
- [53] K. Ray, Intestinal tract: A breach in the intestinal barrier during hyperglycaemia, *Nat Rev Gastroenterol Hepatol*. 15 (2018) 255. <https://doi.org/10.1038/nrgastro.2018.26>.
- [54] L.Y. Chen, C.N. Huang, C.K. Liao, et al., Effects of Rutin on Wound Healing in Hyperglycemic Rats, *Antioxidants (Basel)*. 9 (2020) <https://doi.org/10.3390/antiox9111122>.
- [55] R.F.A. Abdelhameed, A.K. Ibrahim, M.A. Elfaky, et al., Antioxidant and Anti-Inflammatory Activity of *Cynanchum acutum* L. Isolated Flavonoids Using Experimentally Induced Type 2 Diabetes Mellitus: Biological and In Silico Investigation for NF- κ B Pathway/miR-146a Expression Modulation, *Antioxidants (Basel)*. 10 (2021) <https://doi.org/10.3390/antiox10111713>.
- [56] J. Huang, M. Xie, L. He, et al., Chlorogenic acid: a review on its mechanisms of anti-inflammation, disease treatment, and related delivery systems, *Front Pharmacol*. 14 (2023) 1218015. <https://doi.org/10.3389/fphar.2023.1218015>.
- [57] S.A. Amin, N. Adhikari, S. Gayen, et al., Homoisoflavonoids as potential antiangiogenic agents for retinal neovascularization, *Biomed Pharmacother*. 95 (2017) 818-827. <https://doi.org/10.1016/j.biopha.2017.09.004>.
- [58] Y. Lin, J.Z. Wang, Y. Niu, et al., Potential Therapeutic Actions of Flavonoids Present in Propolis That Modulate Vascular Endothelial Growth Factor Signaling to Regulate Angiogenesis, *Phytother Res*. 39 (2025) 4230-4246. <https://doi.org/10.1002/ptr.70029>.
- [59] J. Xie, S. Xiong, Y. Li, et al., Phenolic acids from medicinal and edible homologous plants: a potential anti-inflammatory agent

- for inflammatory diseases, *Front Immunol.* 15 (2024) 1345002. <https://doi.org/10.3389/fimmu.2024.1345002>.
- [60] Y. Huang, Z. Wang, B. Ye, et al., Sodium butyrate ameliorates diabetic retinopathy in mice via the regulation of gut microbiota and related short-chain fatty acids, *Journal of Translational Medicine.* 21 (2023) 451. <https://doi.org/10.1186/s12967-023-04259-4>.
- [61] W. Shi, Y. Dong, S. Liu, et al., Corilagin alleviates ferroptosis in diabetic retinopathy by activating the Nrf2 signaling pathway, *Biomedicine & Pharmacotherapy.* 179 (2024) 117409. <https://doi.org/https://doi.org/10.1016/j.biopha.2024.117409>.