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# Antidiabetic and antioxidant properties of *Berberis crataegina* DC.: *In vitro* and *in vivo* studies, and phytochemical analysis

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ABSTRACT

Diabetes mellitus, a chronic metabolic disorder characterized by hyperglycemia, often results in complications that impact vital organs such as the liver and kidneys. Due to the high morbidity and mortality of diabetic complications, there is a need for alternative therapeutic strategies. Therefore, this study evaluates the therapeutic effects of *Berberis crataegina*, which contains bioactive compounds with potential hepatoprotective and nephroprotective benefits, on liver and kidney function in diabetic rats. Diabetes was induced using alloxan, and after a 19-day treatment period, biochemical and histopathological analyses were performed on blood and tissue samples. Antioxidant activity was evaluated by DPPH and FRAP assays, and total phenolic and flavonoid contents were determined, while LC-MS/MS analysis identified the major bioactive compounds. In diabetic rats, liver enzymes significantly increased, and serum insulin levels decreased, indicating hepatic and pancreatic dysfunction. However, treatment improved these parameters, reduced HbA1c levels, and led to improved glycemic control. Histopathological analysis showed severe renal and hepatic damage in diabetic rats, which was significantly mitigated by treatment. Antioxidant assays demonstrated strong free radical scavenging ability, as shown by DPPH and FRAP activities, which correlated with high total phenolic and flavonoid content. In addition, LC-MS/MS analysis identified quinic acid, protocatechuic acid, chlorogenic acid, and caffeic acid as the major bioactive compounds, further supporting the plant's potent antioxidant and antidiabetic properties. These findings suggest that *Berberis crataegina* has significant potential to protect against diabetes-induced liver and kidney damage and improve glycemic control, highlighting its therapeutic potential for diabetes management.

## 1. Introduction

Diabetes mellitus (DM) is a complex metabolic disorder that has become increasingly prevalent in recent decades. This condition is characterized by chronic hyperglycemia resulting from defects in insulin secretion, insulin action, or both. The development and progression of DM are influenced by genetic predisposition, environmental factors, and lifestyle choices [1]. Despite the widespread use of hypoglycemic agents, diabetes and its complications remain a significant global health issue. The increasing incidence and prevalence of diabetes in youth, especially among obese children, is becoming more evident and concerning [2,3]. It is estimated that the global diabetic population will reach 643 million by 2030 and rise to 783 million by 2045 [4].

Diabetes management involves several strategies, including medical nutrition therapy, pharmacological treatments, and physical activity. These approaches regulate blood glucose levels, improve insulin sensitivity, and reduce the risk of diabetes-related complications [5–7]. Clinical management of diabetes typically involves medications such as insulin, sulfonylureas, biguanides (e.g., metformin), and SGLT2 inhibitors. These medications can cause various adverse effects, including hypoglycemia, gastrointestinal discomfort (nausea, diarrhea), weight gain, and urinary tract infections [1,8]. In addition, the financial burden of diabetes is significant and continues to grow. In 2019, global health expenditures related to diabetes were estimated at USD 760 billion, with projections indicating an increase to USD 825 billion by 2030 and USD 845 billion by 2045 [9]. This increase is primarily driven by the growing prevalence of diabetes, which is expected to affect 700 million adults by 2045 [10]. Notably, over 90 % of these cases are anticipated to be type 2

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diabetes, a condition influenced by factors such as urbanization, aging populations, decreased physical activity, and increasing rates of overweight and obesity. In developing nations, especially in rural areas where access to conventional diabetes treatments is limited and costly, there is a growing interest in plant-derived natural α-glucosidase inhibitors to manage postprandial hyperglycemia with minimal side effects. Research indicates that certain natural compounds are increasingly recognized in this context [11].

Plants are significant sources of antioxidants, anti-hyperlipidemic, and anti-hyperglycemic compounds, which may be effective in treating chronic conditions like diabetes [12]. Ethnobotanical literature identifies approximately 800 plant species with potential antidiabetic properties, highlighting the importance of exploring natural sources for new diabetes treatments [13]. Recent research has increasingly focused on the medicinal potential of various plant extracts, highlighting a growing interest in phytochemicals as therapies for diabetes [14]. One such plant is Berberis, known as "barberry," from the family Berberidaceae. The genus Berberis includes about 500 species and is one of the most important, consisting of deciduous or evergreen shrubs that grow 1–5 m tall and have thorny stems. These plants are native to temperate and subtropical regions of Europe, Asia, Africa, and America [15]. The Berberis genus, with a long history in both Eastern and Western medicine, is among the most widely used medicinal plants globally. These species are traditionally used to treat various ailments such as jaundice, rheumatism, lumbago, cardiovascular disorders, fever, gestational emesis, and to dissolve renal and biliary calculi [16,17].

In Turkey, four species of the genus Berberis are found: *Berberis vulgaris* L., *Berberis cretica* L., *Berberis integerrima* B., and *Berberis crataegina* DC. (BC). This study focuses on BC, locally known as 'karamuk' and 'kadın tuzluğu.' This species thrives on rocky terrains and ravines at elevations of 800–1500 m, exhibiting a broad distribution across the Central Anatolian region [18]. The shrub can reach up to 2 m in height and typically has spines shorter than its leaves. The bark of young flowering twigs is dark purplish-brown, glossy, not deeply grooved, and lacks black lenticels. Its leaves are narrowly obovate with coarsely serrated or entire margins. The condensed inflorescence contains 6–15 flowers, and the fruits are black when mature and red when immature [19]. BC is known for its high content of organic acids, vitamin C, tannins, and anthocyanins [20,21], as well as alkaloids like berberine, palmatine, and berbamine, and flavonoids such as quercetin and rutin. The leaves have phenolic compounds like caffeic and chlorogenic acids, while the fruits contain anthocyanins, including malvidol, delphinidol, petunidol, and peonidol [22–25]. These compounds give BC strong antifungal, anti-inflammatory, antipruritic, diuretic, antioxidant, and free radical scavenging properties [26]. In Turkish folk medicine, the roots and root barks treat jaundice, hemorrhoids, and dysuria, while the fruits are used for hypertension, anemia, diarrhea, constipation, and a variety of other ailments, including rheumatism, diabetes, and gynecological issues [27–31].

In the literature, various Berberis species have been studied for their medicinal properties, confirming the traditional use of these plants. However, to the best of our knowledge, the antidiabetic potential, antioxidant activity, and phytochemical profile of BC root have not yet been comprehensively investigated. Furthermore, studies focusing on the biological activities of BC are still limited. This study aims to evaluate the effects of BC in the treatment of diabetes, with the goal of addressing gaps in the current literature. This research is the first comprehensive investigation of the antidiabetic activity of BC root, as well as the analysis of its antioxidant potential and phytochemical composition by LC-MS/MS.

## 2. Material and methods

### 2.1. Plant material

The BC was collected from Günebakan village and Ekmekli village of Erzincan province in June 2021. The plant samples were authenticated by Prof. Dr. Mustafa KORKMAZ (Erzincan Binali Yıldırım University, Faculty of Art and Sciences, Department of Biology). Authentic samples (ID number of BC: 4460) were stored in the Herbarium of Erzincan Binali Yıldırım University (EBYU).

### 2.2. Extraction procedure

In this study, water was selected as the solvent system based on both its traditional application and its widespread use in the literature [32]. The decoction method was used for the extraction of active components from plant materials. The root material was ground in a laboratory-scale blender and mixed with water in a 1:20 (w/w) ratio, then heated to the boiling point. The mixture was heated for 1 h with periodic mixing. After extraction, the mixture was filtered, and the decoction was frozen at −80°C for a day before being lyophilized for 48 h to obtain the crude extract.

### 2.3. In vivo antidiabetic potentials

#### 2.3.1. Animals and housing conditions

A total of 36 adult male Albino Wistar rats, weighing between 200 and 250 g, were used in the study. The animals were procured from the Atatürk University Medical Practice and Research Center (ATADEM). The care and experiments of the rats were conducted at the Pharmacology Department Laboratory of the Faculty of Medicine at Erzincan Binali Yıldırım University. Before the experiments, the animals were assigned to groups. All stages of our work have been approved as ethically compliant by the Atatürk University Local Ethics Committee for Animal Experiments, as per the document dated 31.12.2020 with the number 77040475–641.04-E.2000329275. The animal study was conducted in accordance with the ARRIVE guidelines 2.0 (Animal Research: Reporting of In Vivo Experiments) to promote transparent, comprehensive, and reproducible reporting. The study design included appropriate group allocation and well-defined inclusion and exclusion criteria. Animals were allocated to experimental groups using a randomization method, and all procedures were conducted according to standard protocols. The number of animals per group was determined based on preliminary data to achieve adequate statistical power. All animals were housed under standard laboratory conditions (22 ± 2°C, 12-h light/dark cycle, ad libitum access to food and water).

#### 2.3.2. Alloxan-induced diabetes model

Alloxan was used to induce diabetes in rats. For this purpose, the rats were fasted overnight, and subsequent blood glucose measurements from tail vein samples confirmed that glucose levels were within normal limits. Then, alloxan was dissolved in saline and administered intraperitoneally at a dose of 120 mg/kg to each animal. This procedure was repeated for three days. Following the third day, the rats were fasted overnight again. In the morning, fasting blood glucose levels were measured from tail vein samples. Rats with blood glucose levels over 250 mg/dL were identified as diabetic. Animals were observed daily for any indications of distress, weight loss, or abnormal behaviour during the experimental period.

### 2.4. Experimental design and treatment

The rats were divided into six groups, with six animals in each group. The first and third groups included healthy rats, while the other groups consisted of diabetic rats. Except for the healthy first group and the diabetic second group, the other groups were administered different doses (150, 300, and 600 mg/kg) of BC, which was dissolved in distilled water, via oral gavage for 19 consecutive days. The selected doses of BC extract were based on previous studies reporting pharmacological activity and safety at comparable dose ranges [33]. These doses were chosen to evaluate a potential dose-dependent effect and to remain

within the established non-toxic range reported in the literatüre. Fresh solutions were prepared daily and administered via oral gavage. Following, blood samples were intracardially collected from all rats in normal and ethylenediaminetetraacetic acid (EDTA) containing tubes for biochemical analysis. Additionally, liver and kidney tissue samples were obtained from all groups for histopathological study. The tissue samples were stored in tubes containing formaldehyde. The study design is presented in Table 1. The number of animals used (n = 6 per group) was based on previously published data in comparable experimental models, aiming to achieve a statistical power of ≥ 80 % for detecting differences in biochemical and histological parameters.

### 2.5. Serum and whole blood analyses

To measure insulin, alanine aminotransferase (ALT), alkaline phosphatase (ALP), and aspartate aminotransferase (AST) levels, serum samples were prepared. For these procedures, blood samples were collected from rats and centrifuged at 4000 rpm for 15 min. The resulting serum samples were stored at −80°C. Whole blood was used to measure HbA1c levels.

### 2.6. Serum insulin measurement in rats

In the study, the serum insulin levels (INS) in rats were measured using an Enzyme-Linked Immunosorbent Assay (ELISA, CK-bio-14821, 96 T) kit. To determine the rats' serum insulin levels, wells pre-coated with objective antibodies were used. Standards and samples were added to these wells, followed by the addition of an HRP-conjugate reagent. The procedure involves the steps of incubation, removal of unbound enzymes through washing, and the addition of substrates A and B.

### 2.7. Measurements of ALP, ALT, and AST

Biochemical analyses were performed using the spectrophotometric method with an automated Beckman Coulter AU5800 analyzer. Each measurement was performed in triplicate, and the mean values were recorded.

### 2.8. Measurement of HbA1c

HbA1c reflects the estimated average glucose levels over the past three months. The HbA1c value is classified as normal if it is below 6 %, and as elevated if it is above 6 %. In the present investigation, HbA1c concentrations were determined by analyzing intracardiac blood samples collected from rats. The HbA1c levels were quantified by analyzing whole blood, collected in vacuum tubes with EDTA as an anticoagulant, to high-performance liquid chromatography (HPLC) analysis. The data were presented as mean ± standard deviation, and differences between groups were statistically compared.

**Table 1**
Experimental study protocol of the antidiabetic effect of BC in alloxan-induced diabetic rats.

| Groups | Day 1 | Day 2 | Day 3 | Day 4 | Day 5 | Day 19 |
|---|---|---|---|---|---|---|
| Group-I (SK-Group) | No treatment Water + feed | | | Blood measurement | No treatment Water + feed | |
| Group-II (DK-Group) | Alloxan dissolved in saline solution (120 mg/kg) Water + feed | | | Blood measurement | No treatment Water + feed | |
| Group-III (BC III) | No treatment Water + feed | | | Blood measurement | *B. crataegina* extract (600 mg/kg) Water + feed | |
| Group-IV (DM+BC I) | Alloxan dissolved in saline solution (120 mg/kg) Water + feed | | | Blood measurement | *B. crataegina* extract (150 mg/kg) Water + feed | |
| Group-V (DM+BC II) | Alloxan dissolved in saline solution (120 mg/kg) Water + feed | | | Blood measurement | *B. crataegina* extract (300 mg/kg) Water + feed | |
| Group-VI (DM+BC III) | Alloxan dissolved in saline solution (120 mg/kg) Water + feed | | | Blood measurement | *B. crataegina* extract (600 mg/kg) Water + feed | |

### 2.9. Measurement of blood glucose

Blood samples were collected from the tails of rats and analyzed for glucose levels. Blood glucose levels were measured using the FreeStyle Optium Neo H blood glucose and ketone monitoring system. Measurements were performed before alloxan injection (day 0), at 72 h post-injection, and on days 5, 10, and 19 following the administration of the plant extract.

### 2.10. Tissue sample collection and evaluation

For the histopathological study, rats were anesthetized with ketamine, and liver and kidney tissue samples were collected from each rat. The obtained tissue samples were incubated in a 10 % formaldehyde solution. Following fixation, the samples were identified and then washed with water for 24 h. The samples were dehydrated using a graded series of alcohols (70 %, 80 %, 90 %, and 100 %) to remove water from the tissues. After dehydration and clearing in xylene, the tissues were embedded in paraffin blocks. From the paraffin-embedded blocks, 4–5 μm-thick tissue sections were obtained using a rotary microtome. These sections were mounted onto glass slides and stained with hematoxylin and eosin (H&E) using a routine staining protocol to visualize general tissue architecture. Histological evaluations were conducted using an Olympus microscope, and representative images were digitally captured using the DP2-SAL imaging system (Olympus® Inc., Tokyo, Japan). All histopathological assessments were performed in a blinded manner by an experienced histopathologist. Renal and hepatic tissue samples were evaluated for structural integrity, signs of inflammation, cellular degeneration, vascular congestion, and any pathological alterations indicative of diabetic complications. Comparative analyses were conducted between groups to assess the degree of tissue protection conferred by the treatment.

### 2.11. DPPH free radical scavenging activity

The free radical scavenging activity of the BC was evaluated using a previously described modified method [34–36]. For DPPH assays, the lyophilized extract was initially dissolved in distilled water to prepare a stock solution at a concentration of 1 mg/mL. Then, the stock solutions of BC extract were prepared in distilled water at different concentrations (20–1000 μg/mL). A 0.26 mM DPPH solution and extract stock solution (1 mg/mL) were prepared. After, each solution was mixed with 1 mL of the DPPH, vortexed, and incubated in the dark at room temperature for 30 min. Absorbance was measured at 517 nm, and the percentage of scavenging activity was calculated. Finally, $IC_{50}$ values (μg/mL) were determined and compared to Trolox, Ascorbic acid, BHT, and BHA to evaluate the antioxidant potential of the extracts.

### 2.12. Ferric-reducing power activity (FRAP)

The reducing power activity of the plant material was evaluated using a previously reported method [37–39]. The BC extract was dissolved in distilled water, and then the extract and standard stock solutions (1 mg/mL) were prepared. For the assay, 100 μL of sample was diluted to 1.25 mL with 0.2 M phosphate buffer (pH 6.6). Then, 1.25 mL of 1 % potassium ferricyanide was added, and the mixture was incubated at 50 °C for 20 min. After incubation, 1.25 mL of 10 %

trichloroacetic acid and 0.25 mL of 0.1 % $FeCl_3$ were added. Absorbance was measured at 700 nm, and results were expressed in mg TE/g extract using a Trolox calibration curve. The antioxidant activity of the BC was compared with that of ascorbic acid, BHT, and BHA.

### 2.13. Total phenolic content (TPC)

The total phenolic content of the BC was determined using the Folin–Ciocalteu method, as previously reported [40–42]. The BC extract was initially dissolved in distilled water. The sample solution (100 μL) was then diluted with 4.5 mL of distilled water, followed by the addition of 100 μL Folin-Ciocalteu reagent and 300 μL of 2 % $Na_2CO_3$ solution. The mixture was incubated for 2 h, and absorbance was measured at 760 nm. Results were expressed as mg gallic acid equivalent phenolic substance/g extract (mg GAE/g extract).

### 2.14. Total flavonoid content (TFC)

The total flavonoid content of the BC was determined using the method as previously described [43–45]. The BC extract was initially dissolved in distilled water, and sample and standard stock solutions (100 μL) were then diluted to 4.8 mL with methanol. Then, 100 μL of 1 M ammonium acetate and 100 μL of 10 % aluminum chloride were added. The mixture was vortexed and incubated for 45 min at room temperature. Absorbance was measured at 415 nm, and results were expressed as mg quercetin equivalent/g extract (mg QE/g extract).

### 2.15. LC-MS/MS-based quantification of phytochemicals

The quantitative screening of phytochemicals in the water extract of *B. crataegina* was performed by a previously developed and validated LC-MS/MS method. Using this modern LC-MS/MS technique, the authors were able to find and measure a total of 53 different phytochemical compounds (Figs. 4 and 5). There were fourteen flavonoid aglycones, thirteen flavonoid glycosides, twenty phenolic acids, and three phenolic aldehydes, as well as one compound each from the benzopyrone, stilbenoid glucoside, and biflavonoid groups. Deuterated internal standards were utilized to make the quantification more accurate and reliable. These standards corrected for matrix effects and analyte losses that happened throughout both the extraction and instrumental measurement operations. In particular, rutin-D3 was used as the internal standard for flavonoid glycosides, quercetin-D3 for flavonoid aglycones, and ferulic acid-D3 for phenolic compounds that are not flavonoids. The methodological validation criteria, which Yilmaz (2020) first proposed, included important performance metrics such as intra-day and inter-day precision (repeatability), linearity across a dynamic concentration range, recovery rates (accuracy), limits of detection and quantification (LOD/LOQ), and relative measurement uncertainty (U%) calculated at a 95 % confidence interval (k = 2) [46].(Table 10).

### 2.16. Instrumentation and analytical conditions for phytochemical quantification

The utilized method, A Shimadzu Nexera ultrahigh-performance liquid chromatograph (UHPLC), coupled with a tandem mass spectrometer (LC-MS/MS), was employed for the quantitative analysis of 53 phytochemicals. The reversed-phase UHPLC system featured an autosampler (SIL-30AC), a column oven (CTO-10ASvp), binary pumps (LC-30AD), and a degasser (DGU-20A3R). Chromatographic separation was performed on a reversed-phase Agilent Poroshell 120 EC-C18 analytical column (150 mm × 2.1 mm, 2.7 μm) maintained at 40°C. The elution process employed a binary solvent system, with eluent A comprising water with 5 mM ammonium formate and 0.1 % formic acid, and eluent B consisting of methanol with 5 mM ammonium formate and 0.1 % formic acid. The gradient elution program was as follows: 20–100 % B (0–25 min), 100 % B (25–35 min), and 20 % B (35–45 min). The flow rate was established at 0.5 mL/min, and the injection volume was 5 μL.

Mass spectrometric detection was performed using a Shimadzu LCMS-8040 tandem mass spectrometer, equipped with an electrospray ionization (ESI) source operating in both positive and negative ionization modes. Data acquisition and processing were conducted using LabSolutions software (Shimadzu). The multiple reaction monitoring (MRM) mode was employed for the selective detection and quantification of phytochemicals, based on optimized precursor-to-product ion transitions. Collision energies (CE) were fine-tuned to achieve optimal fragmentation and maximize the transmission of target product ions. The MS operational parameters were as follows: drying gas ($N_2$) flow rate: 15 L/min, nebulizing gas ($N_2$) flow rate: 3 L/min, desolvation line (DL) temperature: 250°C, heat block temperature: 400°C, and interface temperature: 350°C.

### 2.17. Statistical analysis

Biochemical results were expressed as mean ± standard deviation ($\bar{x} \pm SD$). The assumptions for parametric tests were assessed using the Shapiro-Wilk test for normality and Levene's test for homogeneity of variances. One-way ANOVA was used for data that met these assumptions, while the Kruskal-Wallis test was applied when the assumptions were not satisfied. Where significant differences were detected, appropriate post hoc tests were conducted to determine the source of variation. Results of post hoc comparisons were indicated by superscript letters in the tables. A p-value less than 0.05 was considered statistically significant. All statistical analyses were carried out using SPSS software (version 20.0). For sugar level measurements, the Kolmogorov-Smirnov test was used to evaluate normality, and Levene's test was applied for variance homogeneity.

## 3. Results

### 3.1. Findings related to liver enzymes

The study found significant variations in ALP levels across groups ($p = 0.015$). Compared to the control group, significant changes in ALP concentrations were observed in the diabetic control (DK) and groups treated with different doses of BC: BC III (600 mg/kg), DM+BC I (150 mg/kg), DM+BC II (300 mg/kg), and DM+BC III (600 mg/kg). The DM+BC I group had significantly lower ALP levels than the DK group but higher than the healthy control (SK) group. The BC III group showed ALP activity lower than the DK group, similar to SK levels. The DM+BC III group exhibited the most effective ALP activity, similar to SK and BC III groups, suggesting high doses of extract may offer therapeutic benefits. These findings highlight the importance of BC dosage on ALP activity in diabetic conditions. The comprehensive results are presented in Table 2.

The analysis showed notable variations in liver AST values among

**Table 2**
Effect of BC on ALP level in blood plasma (IU/L).

| Group | Average | St D | Median (IQR) | *p |
|---|---|---|---|---|
| SK | 145.50[a] | 6.02 | 148.00 (136.00–155.00) | .015 |
| DK | 652.50[d] | 10.76 | 643.00 (631.00–672.00) | |
| BC III | 150.75[a] | 2.02 | 152.00 (148.00–153.50) | |
| DM+BC I | 511.00[c] | 2.38 | 511.50 (507.00–515.00) | |
| DM+BC II | 443.00[b] | 0.91 | 443.00 (441.50–444.50) | |
| DM+BC III | 153.50[a] | 2.33 | 153.50 (149.50–157.50) | |

**St D:** Standard deviation; **IQR:** Interquartile range; *$p < 0.05$ considered significant. Significant differences between groups were denoted by lowercase letters. Groups denoted by the same lowercase letters did not show significant differences, while groups denoted by different letters exhibited significant differences. **SK:** Healthy control group; **DK:** Diabetic control group; **BC III:** Water III. dose control group; **DM+BC I:** Water I. dose group; **DM+BC II:** Water II. dose; **DM+BC III:** Water III. dose group.

the groups (p = 0.000). Significant changes were observed in the DK, BC III, DM+BC I, DM+BC II, and DM+BC III groups compared to the control group. According to the data in Table 3, the DM+BC I group had lower AST levels than the DK group but higher than the SK group. The BC III group showed lower AST levels than both the DK and SK groups. The DM+BC III group was more effective than the DM, DM+BC I, and DM+BC II groups, with AST levels close to the SK and BC III groups. High doses of BC brought AST activity similar to that of the SK group, indicating potential therapeutic benefits.

The study found significant differences in liver ALT levels among the groups (p = 0.000). The DK, BC III, DM+BC I, DM+BC II, and DM+BC III groups showed significant deviations in ALT values compared to the control group. According to Table 4, the DM+BC I group had reduced ALT levels compared to the DK group but higher than the SK group. The BC III, DM+BC II, and DM+BC III groups had lower ALT levels than the DK group. The BC III group also had lower ALT activity than the SK group. The DM+BC III group showed more effective results than the SK group. A high dose of the BC brought ALT activity comparable to that of the SK group.

### 3.2. Findings related to serum insulin values

Insulin measurements using the ELISA method demonstrated significant differences in serum insulin levels among the groups (p = 0.002). Compared to the control group, the DK, BC III, DM+BC I, DM+BC II, and DM+BC III groups displayed significant changes. The DM+BC I (150 mg/kg) and DM+BC II (300 mg/kg) groups had lower serum insulin levels than the DK group. The BC III and DM+BC III (600 mg/kg) groups also showed reduced levels compared to the DK group. The DM+BC III group was more effective than the SK group. High doses of the extract brought serum insulin levels comparable to those of the SK group, as shown in Table 5.

### 3.3. Findings related to blood glucose

Table 6 presents the blood glucose levels in rats, expressed as Mean ± SEM. The SK group (control) had significantly lower glucose levels (80.50*±4.75, p < .001) compared to the DK group (diabetic control) with elevated levels (303.37$^{\#}$±21.95). The BC III group, treated with plant extract, showed glucose levels (83.54*±3.11) similar to the control. Diabetic groups treated with BC (DM+BC I, DM+BC II, DM+BC III) exhibited higher glucose levels (369.59$^{\#}$±57.01, 304.56$^{\#}$±42.22, 348.01$^{\#}$±22.03), with DM+BC I being the highest. These results indicate a potential dose-dependent effect of the BC on glucose regulation in diabetic rats.

**Table 3**
Effect of BC on AST level in blood plasma (IU/L).

| Group | Average | St D | Median (IQR) | *p |
|---|---|---|---|---|
| SK | 141.75$^{a}$ | 3.97 | 141.50 (135.00–148.50) | .000 |
| DK | 222.81$^{d}$ | 1.05 | 222.92 (222.00–223.00) | |
| BC III | 139.00$^{a}$ | 1.08 | 138.50 (137.50–140.50) | |
| DM+BC I | 184.56$^{c}$ | 1.54 | 83.63 182.60–186.50) | |
| DM+BC II | 161.21$^{b}$ | 0.45 | 161.43 (160.55–161.88) | |
| DM+BC III | 144.53$^{a}$ | 0.66 | 144.63 (143.55–145.50) | |

**St D:** Standard Deviation; **IQR:** Interquartile Range; *p < 0.05 was considered significant. Significant intergroup differences were indicated with lowercase letters. No significant difference was found between groups denoted by the same letter, whereas a significant difference was observed between groups denoted by different letters. **SK:** Healthy Control Group; **DK:** Diabetic Control Group; **BC III:** Water III. Dose Control Group; **DM+BC I:** Diabetic Group with Water I. Dose; **DM+BC II:** Diabetic Group with Water II. Dose; **DM+B III:** Diabetic Group with Water III. Dose.

**Table 4**
Effect of BC on ALT level in blood plasma (IU/L).

| Group | Average | St D | Median (IQR) | *p |
|---|---|---|---|---|
| SK | 44.75$^{a}$ | 1.75 | 44.50 (42.00–47.50) | .000 |
| DK | 98.20$^{d}$ | 1.76 | 100.09 (95.00–101.00) | |
| BC III | 42.50$^{a}$ | 1.04 | 42.50 (41.00–44.00) | |
| DM+BC I | 85.50$^{c}$ | 1.19 | 83.50 (81.50–85.50) | |
| DM+BC II | 73.75$^{b}$ | 1.25 | 73.50 (72.00–75.50) | |
| DM+BC III | 43.73$^{a}$ | 1.10 | 45.95 (44.00–47.45) | |

**St D:** Standard Deviation; **IQR:** Interquartile Range; *p < 0.05 was considered significant. Significant intergroup differences were indicated with lowercase letters. No significant difference was found between groups denoted by the same letter, whereas a significant difference was observed between groups denoted by different letters. **SK:** Healthy Control Group; **DK:** Diabetic Control Group; **BC III:** Water III. Dose Control Group; **DM+BC I:** Diabetic Group with Water I. Dose; **DM+BC II:** Diabetic Group with Water II. Dose; **DM+BC III:** Diabetic Group with Water III. Dose.

**Table 5**
Effect of BC on serum insulin level (mg/dL).

| Group | Average | St D | Median (IQR) | *p |
|---|---|---|---|---|
| SK | .4466$^{a}$ | .0184 | .4490 (.4325–.4590) | .002 |
| DK | .5347$^{d}$ | .0027 | .5363 (.5340–.5370) | |
| BC III | .4360$^{a}$ | .0365 | .4345 (.4030–.4680) | |
| DM+BC I | .4770$^{b}$ | .0006 | .4775 (.4756–.4783) | |
| DM+BC II | .4494$^{a}$ | .0002 | .4495 (.4490–.4498) | |
| DM+BC III | .4459$^{a}$ | .0002 | .4459 (.4456–.4464) | |

**St D:** Standard Deviation; **IQR:** Interquartile Range; *p < 0.05 was deemed significant. Intergroup differences were signified by lowercase letters. Groups marked with the same letter did not exhibit significant differences, whereas those marked with different letters did show significant differences. **SK:** Healthy Control Group; **DK:** Diabetic Control Group; **BC III:** Water III. Dose Control Group; **DM+BC I:** Diabetic Group with Water I. Dose; **DM+BC II:** Diabetic Group with Water II. Dose; **DM+BC III:** Diabetic Group with Water III. Dose.

**Table 6**
Effect of BC on blood glucose level.

| Group | Mean ± SEM | *p |
|---|---|---|
| SK | 80.50*±4.75 | .000 |
| DK | 303.37$^{\#}$±21.95 | |
| BC III | 83.54*±3.11 | |
| DM+BC I | 369.59$^{\#}$±57.01 | |
| DM+BC II | 304.56$^{\#}$±42.22 | |
| DM+BC III | 348.01$^{\#}$±22.03 | |

**Mean:** Average; **SEM:** Standard Error of the Mean; *p < 0.05, considered significant. Intergroup differences in means were denoted by symbols. No statistically significant difference was found between the mean blood sugar levels of groups denoted by the same symbol, whereas a significant difference was observed between groups denoted by different symbols. **SK:** Healthy control group; **DK:** Diabetic control group; **BC III:** Water III. dose control group; **DM+BC I:** Water I. dose group; **DM+BC II:** Water II. dose group; **DM+BC III:** Water III. dose group.

### 3.4. Findings related to HbA1c

HbA1c levels in rat groups were measured using HPLC on whole blood samples (Perkin Elmer). Table 7 shows significant differences among groups (p = 0.000). Compared to controls, DK, BC III, DM+BC I, DM+BC II, and DM+BC III groups had altered HbA1c levels. DM+BC I and DM+BC II groups had HbA1c values closer to DK, while BC III and DM+BC III groups had lower values. DM+BC III was the most effective, showing results comparable to the SK group. High doses of BC resulted in HbA1c levels similar to those of the healthy control group.

**Table 7**
Effect of BC on glycated hemoglobin (HbA1c) level (%).

| Group | Average | St D | Median (IQR) | *p |
|---|---|---|---|---|
| SK | 4.78[a] | .09 | 4.75 (4.65–4.90) | .000 |
| DK | 13.19[d] | .26 | 13.15 (12.64–13.80) | |
| BC III | 4.93[a] | .05 | 4.95 (4.85–5.00) | |
| DM+BC I | 9.79[c] | .21 | 9.88 (9.53–10.05) | |
| DM+BC II | 8.39[b] | .04 | 8.38 (8.33–8.45) | |
| DM+BC III | 5.13[a] | .03 | 5.12 (5.09–5.17) | |

**St D:** Standard Deviation; **IQR:** Interquartile Range; *p < 0.05 was considered significant. Intergroup differences were indicated with lowercase letters. No statistically significant difference was found between the mean blood sugar levels of groups denoted by the same symbol, whereas a significant difference was observed between groups denoted by different symbols. **SK:** Healthy Control Group; **DK:** Diabetic Control Group; **BC III:** Water III. Dose Control Group; **DM+BC I:** Water I. Dose Group; **DM+BC II:** Water II. Dose Group; **DM+BC III:** Water III. Dose Group.

### 3.5. Histopathological findings

The histopathological examination findings for renal and liver tissues are shown in Figs. 1 and 2, respectively. In the control group, the glomeruli, proximal, and distal tubules are structurally intact (Figs. 1–1). Group 2 exhibited significant renal damage, including glomerular atrophy, dilation, degenerative changes in the tubular epithelium, vascular dilation, and congestion (Figs. 1–2). The histology of Group 3 resembles that of the control group (Figs. 1–3). Groups 4 and 5 showed degenerative changes akin to Group 2 (Fig. 1–4, 1–5). Notably, Group 6 demonstrated significant improvements in glomerular and tubular structures, vascular architecture, and reduced glomerular dilation (Fig. 1-6).

Histological evaluations indicate that the liver tissue of the control group maintains a normal architecture, with hepatocytes organized in orderly cell cords and intact vascular structures (Figure 2–1). In Group 2, hepatocytes exhibit degeneration, vacuolization, and lipid accumulation, accompanied by an increase in phagocytic Kupffer cells and a disrupted hepatic lobule structure. Additionally, congestion and dilation are observed in the central veins and sinusoidal capillaries, along with polymorphonuclear cell infiltration (Figs. 2–2). Group 3 shows no significant histological differences compared to the control group (Figs. 2–3). Groups 4 and 5 display similar but less severe alterations as seen in Group 2 (Figs. 2–4, 2–5). In Group 6, hepatic cords appeared normal, hepatocyte degeneration was significantly reduced, and mild congestion and dilation were observed in central veins and sinusoidal capillaries, thus preserving the overall hepatic structure (Fig. 2–6)

### 3.6. DPPH free radical scavenging activity

The antioxidant capacity of BC was evaluated using the DPPH assay, a widely employed method for determining the antioxidant capacity of both natural and synthetic compounds due to their crucial roles in neutralizing free radicals within biological systems. The dose-dependent DPPH radical scavenging activity of the extract and standard antioxidants is shown in Fig. 2, while the comparison of their $IC_{50}$ values is presented in Table 8. The DPPH radical scavenging activity of the BC extract was determined to be 14.44 ± 0.95 μg/mL. Although this value is higher than those of synthetic antioxidants such as Trolox (8.50 ± 0.77 μg/mL), BHA (8.04 ± 0.69 μg/mL), and ascorbic acid (9.91 ± 0.87 μg/mL), it is comparable to standard antioxidants, indicating a significant potential for free radical scavenging.

### 3.7. Ferric-reducing power activity (FRAP)

In this study, the FRAP of the sample to be analyzed was quantified as trolox equivalents (TE) and is presented in Table 8. The FRAP activity of the BC extract was determined to be 125.90 ± 0.88 μg/mL. When compared to standard antioxidants, the reducing power of BC was found to be lower than that of BHA (338.57 ± 0.31 mg TE $g^{-1}$), BHT (257.80 ± 1.24 mg TE $g^{-1}$), and ascorbic acid (394.17 ± 0.98 mg TE $g^{-1}$). This suggests that although the reducing capacity of BC is both measurable and significant, it does not reach the levels exhibited by these commonly

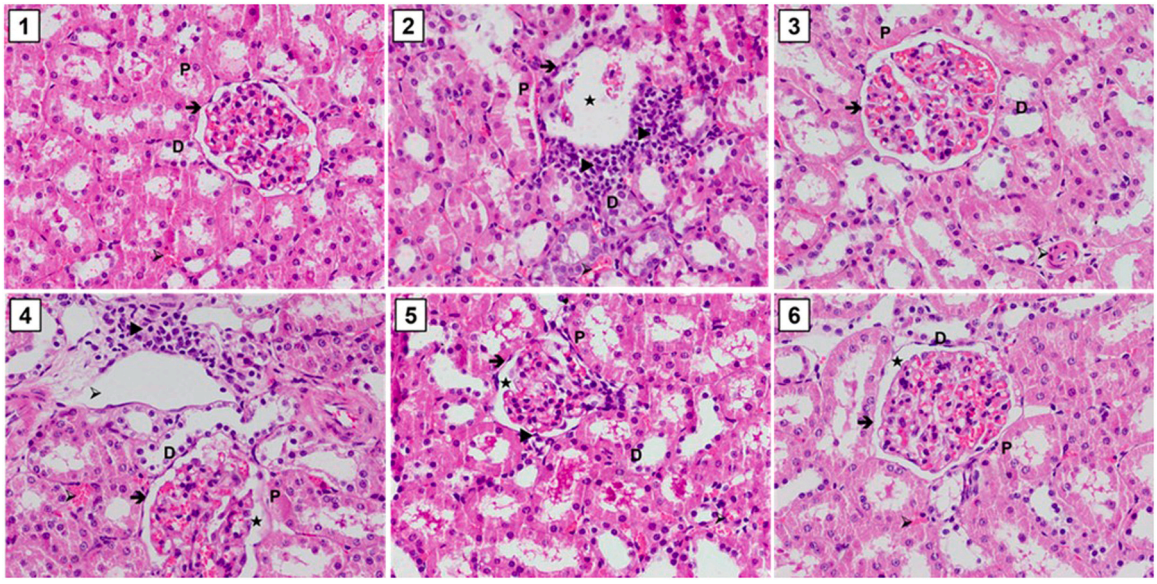



**Fig. 1.** Hematoxylin and eosin staining in renal tissue (H&E x200). ***(1) Group 1 (Control)***: *Normal renal histology with intact glomerulus (➡), proximal (P) and distal (D) tubules, and blood vessels (➢).* ***(2) Group 2:*** *Marked renal damage characterized by glomerular atrophy (➡), degeneration of proximal (P) and distal (D) tubules, dilation and obstruction in blood vessels (➢),and prominent glomerular dilation (★).Intense polymorphonuclear cell infiltration is observed (▶).* ***(3) Group 3:*** *Renal structures including glomerulus (➡), proximal (P) and distal (D) tubules, and blood vessels (➢)appear similar to control.* ***(4) Group 4:*** *Severe pathological changes including atrophic glomerulus (➡),degeneration of P and D tubules, marked vascular dilation and obstruction (➢),glomerular dilation (★),and dense polymorphonuclear leukocyte infiltration (▶).* ***(5) Group 5:*** *Normal glomerular morphology (➡),with degenerative changes in P and D tubules, mild vascular dilation (➢),and moderate glomerular dilation (★). Inflammatory cell infiltration is present (▶).* ***(6) Group 6:*** *Preserved renal structure with normal glomerulus (➡),proximal (P) and distal (D) tubules, and slightly dilated blood vessels (➢). Mild cellular infiltration may be present (★).*

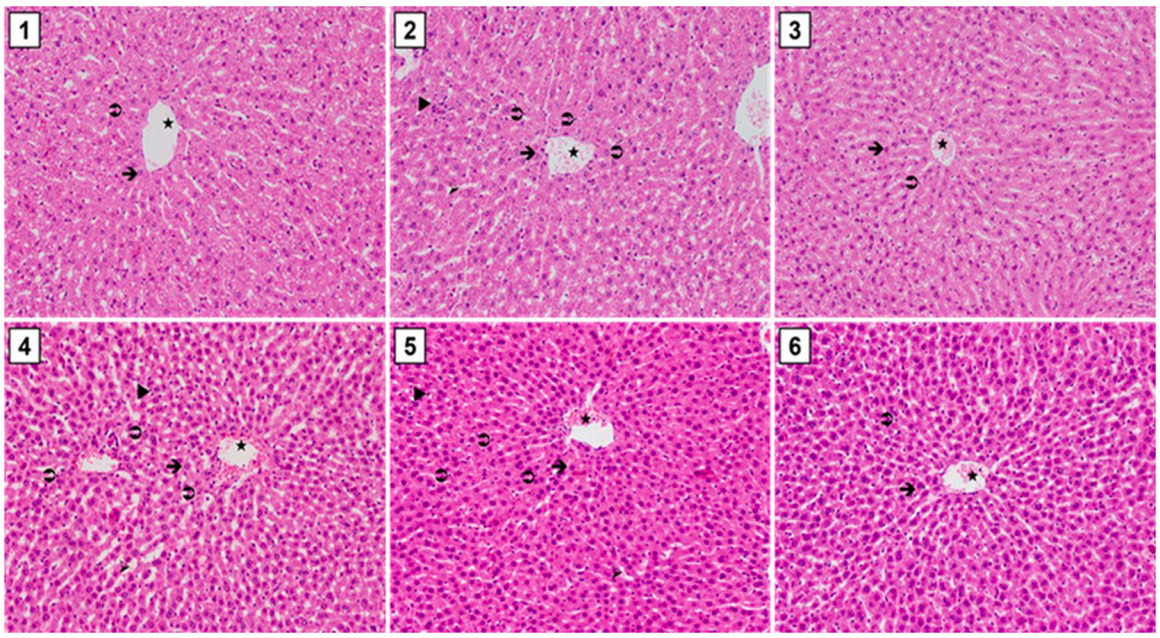



**Fig. 2.** Histological evaluation of liver tissues stained with hematoxylin and eosin (H&E, ×200). ***Group 1 (Control)***: *Normal hepatic architecture with regular hepatocyte arrangement (➡),normal Kupffer cells (➲),and centrally located, non-dilated central vein (★).* ***(2) Group 2***: *Marked hepatic injury showing vacuolated and degenerated hepatocytes (➡), increased number of Kupffer cells (➲),dilated and obstructed central vein (★), dilated sinusoidal capillaries (➢), and dense polymorphonuclear cell infiltration (▶).* ***(3) Group 3:*** *Hepatic structure is comparable to the control, with normal hepatocyte morphology (➡), Kupffer cell (➲), and central vein (★).* ***(4) Group 4:*** *Mild to moderate hepatic degeneration with vacuolated hepatocytes (➡),increased Kupffer cells (➲), dilated central vein (★),sinusoidal dilation (➢), and infiltration of polymorphonuclear cells (▶).* ***(5) Groups 5:*** *Slight hepatocellular vacuolization (➡),elevated Kupffer cell presence (➲),dilated and partially obstructed central vein (★), sinusoidal dilation (➢), and mild inflammatory cell infiltration (▶).* ***(6) Group 6:*** *Normal hepatic tissue with well-preserved hepatocytes (➡), central vein structure (★),and Slightly increased Kupffer cells may be noted (➲).*

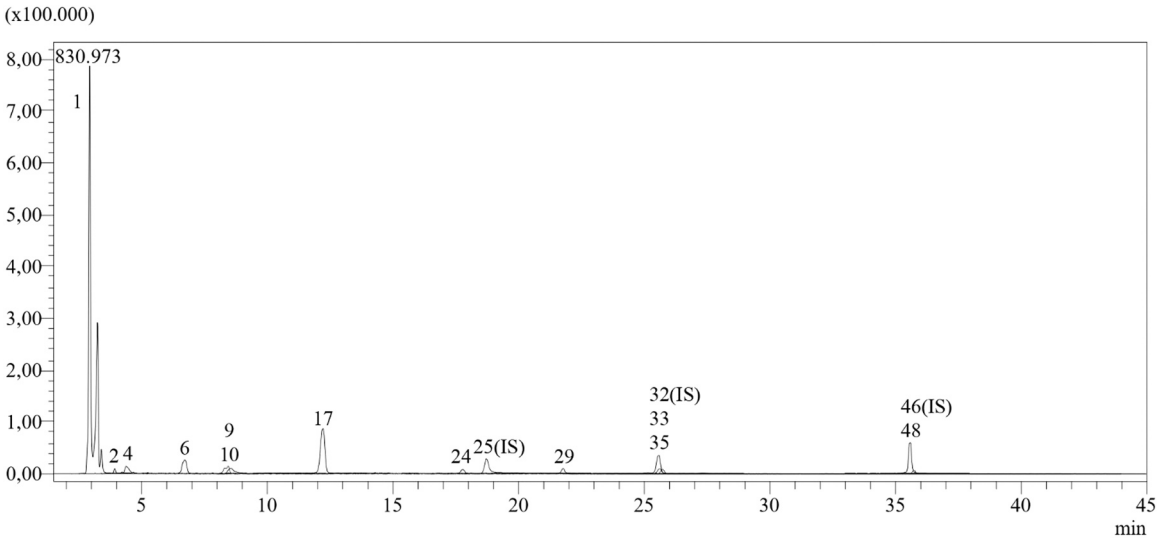



**Fig. 3.** Liquid chromatography–tandem mass spectrometry total ion current chromatograms of BC.

used synthetic antioxidants.

### 3.8. Total phenolic content (TPC)

The TPC of BC was measured to evaluate the presence of phenolic compounds, which are well-known for their antioxidant properties due to their ability to donate hydrogen atoms and neutralize free radicals. The results are presented in Table 8. The results of the total phenolic content of the sample analyzed were 85.43 ± 0.42 mg GAE $g^{-1}$ extract, indicating a significant concentration of phenolic compounds. The high phenolic content aligns with the significant antioxidant activity observed in the DPPH assay, supporting the hypothesis that phenolics significantly contribute to its radical scavenging properties.

### 3.9. Total flavonoid content (TFC)

Flavonoids, a significant class of plant secondary metabolites, are well-known for their potent antioxidant activities, primarily attributed to their capacity to neutralize free radicals and chelate metal ions. In this study, the BC extract was analyzed, and the TFC was determined to be 40.60 ± 0.55 mg QE $g^{-1}$ extract (Table 8). The considerable flavonoid content of the extract suggests its potential role in radical scavenging

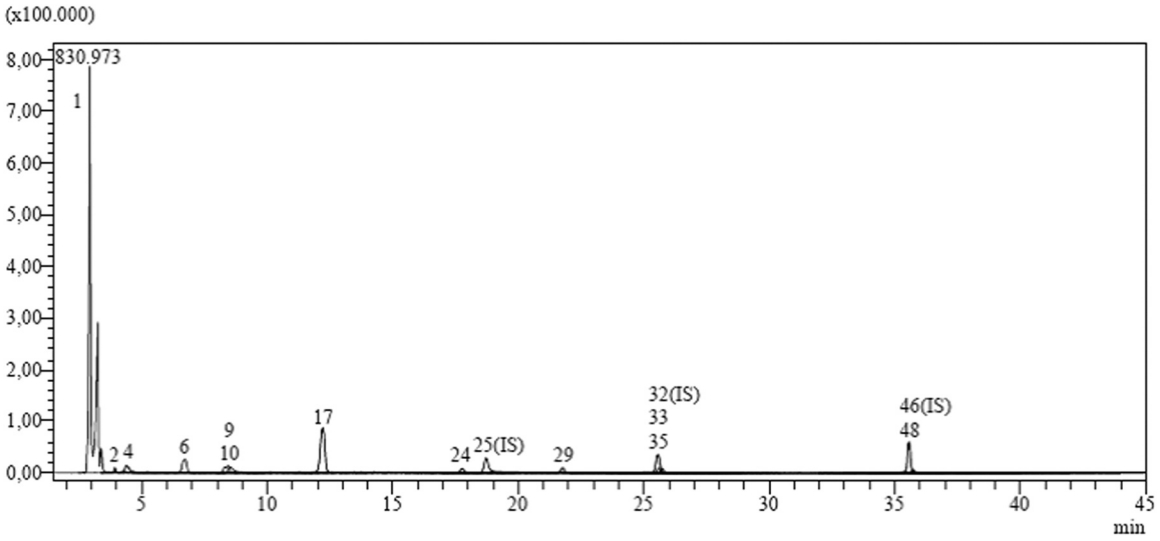



**Fig. 4.** LC-MS/MS Total Ion Chromatogram (TIC) of the BC extract.

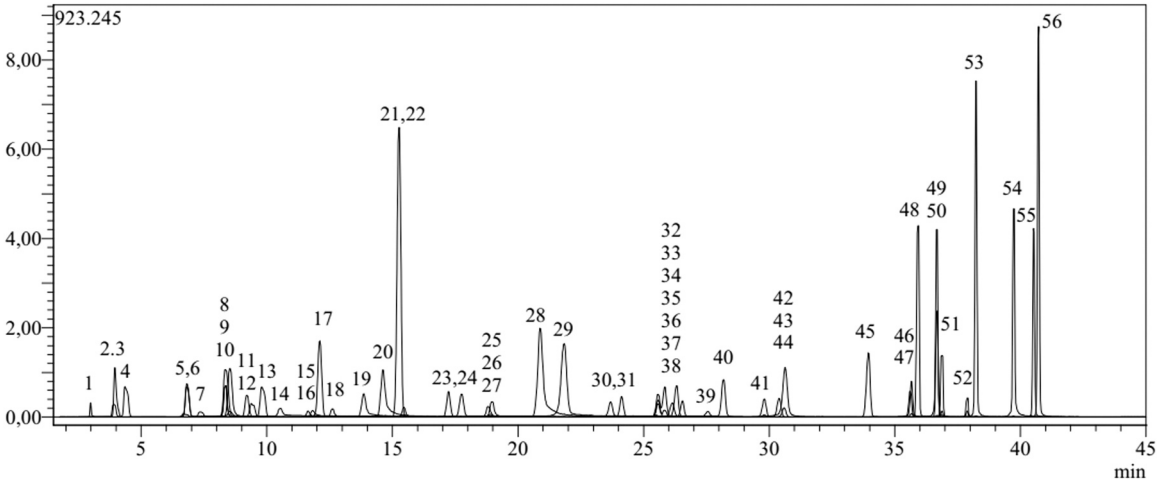



**Fig. 5.** LC-MS/MS Total Ion Chromatogram (TIC) of the standard phytochemicals present in the method.

**Table 8**
The in vitro evaluation of TPC, TFC, FRAP, and DPPH• activities of BC.

| Plant Species | DPPH $IC_{50}$ (μg $mL^{-1}$) | Total phenolics mg GAE $g^{-1}$ Extract | Total flavonoids mg QE $g^{-1}$ Extract | Reducing power mg TE $g^{-1}$ Extract |
|---|---|---|---|---|
| BC | 14.44 ± 0.95[b] | 85.43 ± 0.42 | 40.60 ± 0.55 | 125.90 ± 0.88[d] |
| Trolox | 8.50 ± 0.77[a] | - | - | - |
| BHA | 8.04 ± 0.69[a] | - | - | 338.57 ± 0.31[b] |
| BHT | 10.70 ± 0.73[c] | - | - | 257.80 ± 1.24[c] |
| Askorbic acid | 9.91 ± 0.87[d] | - | - | 394.17 ± 0.98[a] |

**Differences were considered significant for $p < 0.05$

activity.

### 3.10. Identification and quantification of phenolic compounds

The phytochemical screening of the BC extract was performed using a previously developed and validated LC-MS/MS method [47]. The extract was analyzed for a total of 53 bioactive phytochemicals, including 50 phenolic and 3 non-phenolic compounds. The quantitative results of this investigation are presented in Table 9, highlighting the presence of various phenolic acids such as quinic acid, protocatechuic acid, caffeic acid, chlorogenic acid, protocatechuic aldehyde, salicylic acid, and naringenin. However, most flavonoids were not detected. Fig. 3 shows the LC-MS/MS total ion chromatogram of the BC extract.

Quinic acid was identified as the most prevalent compound in the extract of BC, with a concentration of 57.413 mg analyte/g extract. Other detected phenolic acids included protocatechuic acid (1.03 mg analyte/g extract), caffeic acid (0.647 mg analyte/g extract), chlorogenic acid (0.439 mg analyte/g extract), and protocatechuic aldehyde (0.319 mg analyte/g extract) whereas salicylic acid, gallic acid, rutin, hesperidin and naringenin were present in lower amounts (0.069, 0.065, 0.064, 0.059, and 0.003 mg analyte/g extract, respectively). Additionally, fumaric acid and p-coumaric acid were detected at concentrations of 0.661 and 0.006 mg analyte/g extract, respectively. Interestingly, the majority of flavonoids, such as quercetin, kaempferol, luteolin, and apigenin, were not detected in the extract. According to the LC-MS/MS analysis, quinic acid was identified as the predominant component.

**Table 9**
LC-MS/MS quantitative results of extract of BC (μg analyte/g extract).

| No | Compound Name | *RT* | *BC* | No | Compound Name | *RT* | *BC* |
|---|---|---|---|---|---|---|---|
| **1** | Quinic acid | 3.0 | **57413.12** | **29** | Salicylic acid | 21.8 | 69. 36 |
| **2** | Fumaric acid | 3.9 | 661.13 | **30** | Cyranoside | 23.7 | N.D. |
| **3** | Aconitic acid | 4.0 | N.D. | **31** | Miquelianin | 24.1 | N.D. |
| **4** | Gallic acid | 4.4 | 65. 06 | **32** | Rutin-D3-IS | 25.5 | N.A. |
| **5** | Epigallocatechin | 6.7 | N.D. | **33** | Rutin | 25.6 | 64. 95 |
| **6** | Protocatechuic acid | 6.8 | **1030. 51** | **34** | isoquercitrin | 25.6 | N.D. |
| **7** | Catechin | 7.4 | N.D. | **35** | Hesperidin | 25.8 | 59.63 |
| **8** | Gentisic acid | 8.3 | N.D. | **36** | o-Coumaric acid | 26.1 | N.D. |
| **9** | Chlorogenic acid | 8.4 | **439. 18** | **37** | Genistin | 26.3 | N.D. |
| **10** | Protocatechuic aldehyde | 8.5 | **319. 25** | **38** | Rosmarinic acid | 26.6 | N.D. |
| **11** | Tannic acid | 9.2 | N.D. | **39** | Ellagic acid | 27.6 | N.D. |
| **12** | Epigallocatechin gallate | 9.4 | N.D. | **40** | Cosmosiin | 28.2 | N.D. |
| **13** | Cynarin | 9.8 | N.D. | **41** | Quercitrin | 29.8 | N.D. |
| **14** | 4-OH Benzoic acid | 10.5 | N.D. | **42** | Astragalin | 30.4 | N.D. |
| **15** | Epicatechin | 11.6 | N.D. | **43** | Nicotiflorin | 30.6 | N.D. |
| **16** | Vanillic acid | 11.8 | N.D. | **44** | Fisetin | 30.6 | N.D. |
| **17** | Caffeic acid | 12.1 | **647. 61** | **45** | Daidzein | 34.0 | N.D. |
| **18** | Syringic acid | 12.6 | N.D. | **46** | Quercetin-D3-IS | 35.6 | N.A. |
| **19** | Vanillin | 13.9 | N.D. | **47** | Quercetin | 35.7 | N.D. |
| **20** | Syringic aldehyde | 14.6 | N.D. | **48** | Naringenin | 35.9 | 3. 45 |
| **21** | Daidzin | 15.2 | N.D. | **49** | Hesperetin | 36.7 | N.D. |
| **22** | Epicatechin gallate | 15.5 | N.D. | **50** | Luteolin | 36.7 | N.D. |
| **23** | Piceid | 17.2 | N.D. | **51** | Genistein | 36.9 | N.D. |
| **24** | p-Coumaric acid | 17.8 | 6. 99 | **52** | Kaempferol | 37.9 | N.D. |
| **25** | Ferulic acid-D3-ISh | 18.8 | N.A. | **53** | Apigenin | 38.2 | N.D. |
| **26** | Ferulic acid | 18.8 | N.D. | **54** | Amentoflavone | 39.7 | N.D. |
| **27** | Sinapic acid | 18.9 | N.D. | **55** | Chrysin | 40.5 | N.D. |
| **28** | Coumarin | 20.9 | N.D. | **56** | Acacetin | 40.7 | N.D. |

N.D.: Not Detected. N.A.: Not Applicable. IS: Internal Standard

## 4. Discussion

In this comprehensive study, the antidiabetic properties of BC were evaluated using both biochemical and histopathological analyses in an alloxan-induced diabetic rat model. The experimental results demonstrated that the administration of BC led to significant improvements in several critical biomarkers associated with diabetes. There was a significant reduction in blood glucose levels and HbA1c, as well as a considerable increase in serum insulin levels. Furthermore, the extract demonstrated a significant effect on liver enzymes (ALP, ALT, and AST), indicating improved liver function. Histopathological examinations supported these findings, showing reduced damage in both liver and kidney tissues. These findings are in agreement with the existing literature on the therapeutic potential of plant-based extracts in diabetes management, providing evidence of the antidiabetic efficacy of BC.

The efficacy of plant-based treatments in controlling diabetes has been widely documented in several studies. In the present investigation, BC demonstrated a dose-dependent effect, with the highest dose being the most effective in reducing blood glucose levels to those comparable to the healthy control group. This glucose-lowering effect of BC is consistent with previous research, which has frequently demonstrated the ability of plant-based compounds to improve glucose metabolism and reduce hyperglycemia [48–51].

The analysis of serum insulin levels showed a significant improvement in insulin secretion in the BC-treated groups, particularly at the highest administered dose. This suggests that BC may improve insulin sensitivity and support pancreatic β-cell function, which are crucial factors in diabetes management. Improved insulin sensitivity and β-cell function are vital for regulating glucose homeostasis and reducing diabetes progression [52–54]. Similar studies have shown that plant-based treatments improve insulin sensitivity and reduce insulin resistance in diabetic models [55–57]. The increase in serum insulin levels in the DM+BC III group indicates that BC could modulate insulin release, aligning with findings from Edek et al. (2023) and Shahid et al. (2024), who used similar ELISA-based techniques [58,59]. These results support the hypothesis that BC may act as an insulin sensitizer, improving glucose utilization in peripheral tissues.

The observed decrease in liver enzyme activities (ALP, ALT, and AST) highlights the hepatoprotective potential of BC. ALT is an intracellular enzyme released into the blood by hepatocytes in the event of necrosis. This is a particular enzyme of the liver, making it a very sensitive marker of hepatotoxicity. Similarly, AST is a predictor parameter of hepatocyte damage [60], and elevated levels of these enzymes are common in diabetic patients, indicating hepatic damage resulting from chronic hyperglycaemia [61–63]. This study found a significant decrease in ALT and AST levels in high-dose BC groups, suggesting BC's protective effects on liver function by reducing oxidative stress and inflammation. These findings are consistent with previous studies [64–66] that reported similar improvements in liver enzyme levels with plant-based treatments in diabetic models. The improvement in liver enzyme levels in BC-treated groups highlights its potential to reduce diabetes-induced hepatotoxicity, possibly due to its antioxidant properties. These results support the traditional use of BC for liver health and its potential as a therapeutic agent in diabetes management.

Histopathological analysis demonstrated that BC significantly protected liver and kidney tissues in diabetic models. The diabetic control group showed notable glomerular and tubular damage in the kidneys and hepatocellular degeneration, typical of hyperglycemia's effects. High-dose BC treatment significantly improved these conditions. Similar findings by Offor et al. [68] and Sundus et al. [67] support the efficacy of plant-based compounds in reducing oxidative damage and inflammation in diabetic nephropathy [67,68]. Additionally, BC treatment improved hepatic cord structure and reduced liver lipid accumulation, consistent with previous studies on plant-derived hepatoprotective treatments [69, 70]. These results suggest that BC may reduce diabetes-induced organ damage by lowering oxidative stress and inflammation, highlighting its potential as a therapeutic agent in diabetes management.

The DPPH assay, known for its ease of use and reliability, is widely used to evaluate the antioxidant properties of various plant species, including those from the Berberis genus [71–73]. This method has provided a reliable basis for understanding the mechanisms of antioxidant activity in these plants. Recent investigations have demonstrated

**Table 10**
Analytical method validation parameters that belong to the LC-MS/MS method.

| No | Analytes | RT[a] | M.I. (*m/z*)[b] | F.I. (*m/z*)[c] | Ion. mode | Equation | $r^{2d}$ | *RSD*%[e] | | Linearity Range (mg/L) | *LOD/LOQ* (μg/L)[f] | Recovery (%) | | $U^{g}$ | Gr. No[i] |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | | | | | | | | Interday | Intraday | | | Interday | Intraday | | |
| 1 | Quinic acid | 3.0 | 190.8 | 93.0 | Neg | $y = -0.0129989 + 2.97989\times$ | 0.996 | 0.69 | 0.51 | 0.1–5 | 25.7/33.3 | 1.0011 | 1.0083 | 0.0372 | 1 |
| 2 | Fumaric aid | 3.9 | 115.2 | 40.9 | Neg | $y = -0.0817862 + 1.03467\times$ | 0.995 | 1.05 | 1.02 | 1–50 | 135.7/167.9 | 0.9963 | 1.0016 | 0.0091 | 1 |
| 3 | Aconitic acid | 4.0 | 172.8 | 129.0 | Neg | $y = -0.7014530 + 32.9994\times$ | 0.971 | 2.07 | 0.93 | 0.1–5 | 16.4/31.4 | 0.9968 | 1.0068 | 0.0247 | 1 |
| 4 | Gallic acid | 4.4 | 168.8 | 79.0 | Neg | $y = 0.0547697 + 20.8152\times$ | 0.999 | 1.60 | 0.81 | 0.1–5 | 13.2/17.0 | 1.0010 | 0.9947 | 0.0112 | 1 |
| 5 | Epigallocatechin | 6.7 | 304.8 | 219.0 | Neg | $y = -0.00494986 + 0.0483704\times$ | 0.998 | 1.22 | 0.73 | 1–50 | 237.5/265.9 | 0.9969 | 1.0040 | 0.0184 | 3 |
| 6 | Protocatechuic acid | 6.8 | 152.8 | 108.0 | Neg | $y = 0.211373 + 12.8622\times$ | 0.957 | 1.43 | 0.76 | 0.1–5 | 21.9/38.6 | 0.9972 | 1.0055 | 0.0350 | 1 |
| 7 | Catechin | 7.4 | 288.8 | 203.1 | Neg | $y = -0.00370053 + 0.431369\times$ | 0.999 | 2.14 | 1.08 | 0.2–10 | 55.0/78.0 | 1.0024 | 1.0045 | 0.0221 | 3 |
| 8 | Gentisic acid | 8.3 | 152.8 | 109.0 | Neg | $y = -0.0238983 + 12.1494\times$ | 0.997 | 1.81 | 1.22 | 0.1–5 | 18.5/28.2 | 0.9963 | 1.0077 | 0.0167 | 1 |
| 9 | Chlorogenic acid | 8.4 | 353.0 | 85.0 | Neg | $y = 0.289983 + 36.3926\times$ | 0.995 | 2.15 | 1.52 | 0.1–5 | 13.1/17.6 | 1.0000 | 1.0023 | 0.0213 | 1 |
| 10 | Protocatechuic aldehyde | 8.5 | 137.2 | 92.0 | Neg | $y = 0.257085 + 25.4657\times$ | 0.996 | 2.08 | 0.57 | 0.1–5 | 15.4/22.2 | 1.0002 | 0.9988 | 0.0396 | 1 |
| 11 | Tannic acid | 9.2 | 182.8 | 78.0 | Neg | $y = 0.0126307 + 26.9263\times$ | 0.999 | 2.40 | 1.16 | 0.05–2.5 | 15.3/22.7 | 0.9970 | 0.9950 | 0.0190 | 1 |
| 12 | Epigallocatechin gallate | 9.4 | 457.0 | 305.1 | Neg | $y = -0.0380744 + 1.61233\times$ | 0.999 | 1.30 | 0.63 | 0.2–10 | 61.0/86.0 | 0.9981 | 1.0079 | 0.0147 | 3 |
| 13 | 1,5-dicaffeoylquinic acid | 9.8 | 515.0 | 191.0 | Neg | $y = -0.0164044 + 16.6535\times$ | 0.999 | 2.42 | 1.48 | 0.1–5 | 5.8/9.4 | 0.9983 | 0.9997 | 0.0306 | 1 |
| 14 | 4-OH Benzoic acid | 10.5 | 137,2 | 65.0 | Neg | $y = -0.0240747 + 5.06492\times$ | 0.999 | 1.24 | 0.97 | 0.2–10 | 68.4/88.1 | 1.0032 | 1.0068 | 0.0237 | 1 |
| 15 | Epicatechin | 11.6 | 289.0 | 203.0 | Neg | $y = -0.0172078 + 0.0833424\times$ | 0.996 | 1.47 | 0.62 | 1–50 | 139.6/161.6 | 1.0013 | 1.0012 | 0.0221 | 3 |
| 16 | Vanilic acid | 11.8 | 166.8 | 108.0 | Neg | $y = -0.0480183 + 0.779564\times$ | 0.999 | 1.92 | 0.76 | 1–50 | 141.9/164.9 | 1.0022 | 0.9998 | 0.0145 | 1 |
| 17 | Caffeic acid | 12.1 | 179.0 | 134.0 | Neg | $y = 0.120319 + 95.4610\times$ | 0.999 | 1.11 | 1.25 | 0.05–2.5 | 7.7/9.5 | 1.0015 | 1.0042 | 0.0152 | 1 |
| 18 | Syringic acid | 12.6 | 196.8 | 166.9 | Neg | $y = -0.0458599 + 0.663948\times$ | 0.998 | 1.18 | 1.09 | 1–50 | 82.3/104.5 | 1.0006 | 1.0072 | 0.0129 | 1 |
| 19 | Vanillin | 13.9 | 153.1 | 125.0 | Poz | $y = 0.00185898 + 20.7382\times$ | 0.996 | 1.10 | 0.85 | 0.1–5 | 24.5/30.4 | 1.0009 | 0.9967 | 0.0122 | 1 |
| 20 | Syringic aldehyde | 14.6 | 181.0 | 151.1 | Neg | $y = -0.0128684 + 7.90153\times$ | 0.999 | 2.51 | 0.77 | 0.4–20 | 19.7/28.0 | 1.0001 | 0.9964 | 0.0215 | 1 |
| 21 | Daidzin | 15.2 | 417.1 | 199.0 | Poz | $y = 9.45747 + 152.338\times$ | 0.996 | 2.25 | 1.32 | 0.05–2.5 | 7.0/9.5 | 0.9955 | 1.0017 | 0.0202 | 2 |
| 22 | Epicatechin gallate | 15.5 | 441.0 | 289.0 | Neg | $y = -0.0142216 + 1.06768\times$ | 0.997 | 1.63 | 1.28 | 0.1–5 | 19.5/28.5 | 0.9984 | 0.9946 | 0.0229 | 3 |
| 23 | Piceid | 17.2 | 391.0 | 135/106.9 | Poz | $y = 0.00772525 + 25.4181\times$ | 0.999 | 1.94 | 1.16 | 0.05–2.5 | 13.8/17.8 | 1.0042 | 0.9979 | 0.0199 | 1 |
| 24 | *p*-Coumaric acid | 17.8 | 163.0 | 93.0 | Neg | $y = 0.0249034 + 18.5180\times$ | 0.999 | 1.92 | 1.43 | 0.1–5 | 25.9/34.9 | 1.0049 | 1.0001 | 0.0194 | 1 |
| 25 | Ferulic acid-D3-IS[h] | 18.8 | 196.2 | 152.1 | Neg | N.A. | N.A. | N.A. | N.A. | N.A. | N.A. | N.A. | N.A. | 0.0170 | 1 |
| 26 | Ferulic acid | 18.8 | 192.8 | 149.0 | Neg | $y = -0.0735254 + 1.34476\times$ | 0.999 | 1.44 | 0.53 | 1–50 | 11.8/15.6 | 0.9951 | 0.9976 | 0.0181 | 1 |
| 27 | Sinapic acid | 18.9 | 222.8 | 193.0 | Neg | $y = -0.0929932 + 0.836324\times$ | 0.999 | 1.45 | 0.52 | 0.2–10 | 65.2/82.3 | 1.0031 | 1.0037 | 0.0317 | 1 |
| 28 | Coumarin | 20.9 | 146.9 | 103.1 | Poz | $y = 0.0633397 + 136.508\times$ | 0.999 | 2.11 | 1.54 | 0.05–2.5 | 214.2/247.3 | 0.9950 | 0.9958 | 0.0383 | 1 |
| 29 | Salicylic acid | 21.8 | 137.2 | 65.0 | Neg | $y = 0.239287 + 153.659\times$ | 0.999 | 1.48 | 1.18 | 0.05–2.5 | 6.0/8.3 | 0.9950 | 0.9998 | 0.0158 | 1 |
| 30 | Cynaroside | 23.7 | 447.0 | 284.0 | Neg | $y = 0.280246 + 6.13360\times$ | 0.997 | 1.56 | 1.12 | 0.05–2.5 | 12.1/16.0 | 1.0072 | 1.0002 | 0.0366 | 2 |
| 31 | Miquelianin | 24.1 | 477.0 | 150.9 | Neg | $y = -0.00991585 + 5.50334\times$ | 0.999 | 1.31 | 0.95 | 0.1–5 | 10.6/14.7 | 0.9934 | 0.9965 | 0.0220 | 2 |
| 32 | Rutin-D3-IS[h] | 25.5 | 612.2 | 304.1 | Neg | N.A. | N.A. | N.A. | N.A. | N.A. | N.A. | N.A. | N.A. | N.A. | 2 |
| 33 | Rutin | 25.6 | 608.9 | 301.0 | Neg | $y = -0.0771907 + 2.89868\times$ | 0.999 | 1.38 | 1.09 | 0.1–5 | 15.7/22.7 | 0.9977 | 1.0033 | 0.0247 | 2 |
| 34 | isoquercitrin | 25.6 | 463.0 | 271.0 | Neg | $y = -0.111120 + 4.10546\times$ | 0.998 | 2.13 | 0.78 | 0.1–5 | 8.7/13.5 | 1.0057 | 0.9963 | 0.0220 | 2 |
| 35 | Hesperidin | 25.8 | 611.2 | 449.0 | Poz | $y = 0.139055 + 13.2785\times$ | 0.999 | 1.84 | 1.35 | 0.1–5 | 19.0/26.0 | 0.9967 | 1.0043 | 0.0335 | 2 |
| 36 | *o*-Coumaric acid | 26.1 | 162.8 | 93.0 | Neg | $y = 0.00837193 + 11.2147\times$ | 0.999 | 2.11 | 1.46 | 0.1–5 | 31.8/40.4 | 1.0044 | 0.9986 | 0.0147 | 1 |
| 37 | Genistin | 26.3 | 431.0 | 239.0 | Neg | $y = 1.65808 + 7.57459\times$ | 0.991 | 2.01 | 1.28 | 0.1–5 | 14.9/21.7 | 1.0062 | 1.0047 | 0.0083 | 2 |
| 38 | Rosmarinic acid | 26.6 | 359.0 | 197.0 | Neg | $y = -0.0117238 + 8.04377\times$ | 0.999 | 1.24 | 0.86 | 0.1–5 | 16.2/21.2 | 1.0056 | 1.0002 | 0.0130 | 1 |
| 39 | Ellagic acid | 27.6 | 301.0 | 284.0 | Neg | $y = 0.00877034 + 0.663741\times$ | 0.999 | 1.57 | 1.23 | 0.4–20 | 56.9/71.0 | 1.0005 | 1.0048 | 0.0364 | 1 |
| 40 | Cosmosiin | 28.2 | 431.0 | 269.0 | Neg | $y = -0.708662 + 8.62498\times$ | 0.998 | 1.65 | 1.30 | 0.1–5 | 6.3/9.2 | 0.9940 | 0.9973 | 0.0083 | 2 |
| 41 | Quercitrin | 29.8 | 447.0 | 301.0 | Neg | $y = -0.00153274 + 3.20368\times$ | 0.999 | 2.24 | 1.16 | 0.1–5 | 4.8/6.4 | 0.9960 | 0.9978 | 0.0268 | 2 |
| 42 | Astragalin | 30.4 | 447.0 | 255.0 | Neg | $y = 0.00825333 + 3.51189\times$ | 0.999 | 2.08 | 1.72 | 0.1–5 | 6.6/8.2 | 0.9968 | 0.9957 | 0.0114 | 2 |
| 43 | Nicotiflorin | 30.6 | 592.9 | 255.0/284.0 | Neg | $y = 0.00499333 + 2.62351\times$ | 0.999 | 1.48 | 1.23 | 0.05–2.5 | 11.9/16.7 | 0.9954 | 1.0044 | 0.0108 | 2 |
| 44 | Fisetin | 30.6 | 285.0 | 163.0 | Neg | $y = 0.0365705 + 8.09472\times$ | 0.999 | 1.75 | 1.19 | 0.1–5 | 10.1/12.7 | 0.9980 | 1.0042 | 0.0231 | 3 |

(*continued on next page*)

**Table 10** (*continued*)

| No | Analytes | RT[a] | M.I. (*m/z*)[b] | F.I. (*m/z*)[c] | Ion. mode | Equation | $r^{2[d]}$ | RSD%[e] | | Linearity Range (mg/L) | LOD/LOQ (μg/L)[f] | Recovery (%) | | $U^{g}$ | Gr. No |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | | | | | | | | Interday | Intraday | | | Interday | Intraday | | |
| 45 | Daidzein | 34.0 | 253.0 | 223.0 | Neg | $y = -0.0329252 + 6.23004\times$ | 0.999 | 2.18 | 1.73 | 0.1–5 | 9.8/11.6 | 0.9926 | 0.9963 | 0.0370 | 3 |
| 46 | Quercetin-D3-IS[h] | 35.6 | 304.0 | 275.9 | Neg | N.A. | N.A. | N.A. | N.A. | N.A. | N.A. | N.A. | N.A. | N.A. | 3 |
| 47 | Quercetin | 35.7 | 301.0 | 272.9 | Neg | $y = +0.00597342 + 3.39417\times$ | 0.999 | 1.89 | 1.38 | 0.1–5 | 15.5/19.0 | 0.9967 | 0.9971 | 0.0175 | 3 |
| 48 | Naringenin | 35.9 | 270.9 | 119.0 | Neg | $y = -0.00393403 + 14.6424\times$ | 0.999 | 2.34 | 1.69 | 0.1–5 | 2.6/3.9 | 1.0062 | 1.0020 | 0.0392 | 3 |
| 49 | Hesperetin | 36.7 | 301.0 | 136.0/286.0 | Neg | $y = +0.0442350 + 6.07160\times$ | 0.999 | 2.47 | 2.13 | 0.1–5 | 7.1/9.1 | 0.9998 | 0.9963 | 0.0321 | 3 |
| 50 | Luteolin | 36.7 | 284.8 | 151.0/175.0 | Neg | $y = -0.0541723 + 30.7422\times$ | 0.999 | 1.67 | 1.28 | 0.05–2.5 | 2.6/4.1 | 0.9952 | 1.0029 | 0.0313 | 3 |
| 51 | Genistein | 36.9 | 269.0 | 135.0 | Neg | $y = -0.00507501 + 12.1933\times$ | 0.999 | 1.48 | 1.19 | 0.05–2.5 | 3.7/5.3 | 1.0069 | 1.0012 | 0.0337 | 3 |
| 52 | Kaempferol | 37.9 | 285.0 | 239.0 | Neg | $y = -0.00459557 + 3.13754\times$ | 0.999 | 1.49 | 1.26 | 0.05–2.5 | 10.2/15.4 | 0.9992 | 0.9990 | 0.0212 | 3 |
| 53 | Apigenin | 38.2 | 268.8 | 151.0/149.0 | Neg | $y = 0.119018 + 34.8730\times$ | 0.998 | 1.17 | 0.96 | 0.05–2.5 | 1.3/2.0 | 0.9985 | 1.0003 | 0.0178 | 3 |
| 54 | Amentoflavone | 39.7 | 537.0 | 417.0 | Neg | $y = 0.727280 + 33.3658\times$ | 0.992 | 1.35 | 1.12 | 0.05–2.5 | 2.8/5.1 | 0.9991 | 1.0044 | 0.0340 | 3 |
| 55 | Chrysin | 40.5 | 252.8 | 145.0/119.0 | Neg | $y = -0.0777300 + 18.8873\times$ | 0.999 | 1.46 | 1.21 | 0.05–2.5 | 1.5/2.8 | 0.9922 | 1.0050 | 0.0323 | 3 |
| 56 | Acacetin | 40.7 | 283.0 | 239.0 | Neg | $y = -0.559818 + 163.062\times$ | 0.997 | 1.67 | 1.28 | 0.02–1 | 1.5/2.5 | 0.9949 | 1.0011 | 0.0363 | 3 |

[a]R.T.: Retention time, [b]MI (*m/z*): Molecular ions of the standard analytes (*m/z* ratio), [c]FI (*m/z*): Fragment ions [d]$r^2$: Coefficient of determination, [e]*RSD*: Relative standard deviation, [f]*LOD/LOQ* (μg/L): Limit of detection/quantification, [g]*U* (%): percent relative uncertainty at 95 % confidence level ($k = 2$), [h]IS: Internal standard, [i]Gr. No: Represents grouping of internal standards, these numbers indicate which IS stands for which phenolic compound.

the significant DPPH radical scavenging activity of Berberis extracts, indicating their potential as natural antioxidants [74]. This finding is consistent with existing literature indicating that phenolic compounds, including chlorogenic acid, play a crucial role in the antioxidant properties of various plant extracts [75]. The findings of the present study support these reports, as the DPPH assay results demonstrated a potent radical scavenging capacity for BC extract. The observed antioxidant activity is consistent with previous research emphasizing the high radical scavenging potential of Berberis species [72,76,77], further supporting their role as valuable natural antioxidants. This observation suggests that BC exhibits bioactive properties similar to those of other Berberis species, supporting its potential as a source of natural compounds with notable antioxidant effects.

In the context of evaluating the antioxidant properties of BC, it is essential to compare its FRAP value with those of synthetic antioxidants such as BHA, BHT, and ascorbic acid, as well as with findings from other studies on various natural extracts. The study found that BC's FRAP value is lower than that of these synthetic antioxidants, consistent with literature indicating that plant-derived antioxidants often exhibit lower ferric-reducing power [78–80]. This recurring trend observed in multiple studies suggests that, although natural antioxidants can be beneficial, they often do not achieve the efficacy of synthetic alternatives regarding ferric ion reduction.

Phenolic compounds play a crucial role in the antioxidant activity of plant extracts due to their redox properties, allowing them to act as reducing agents, hydrogen donors, and singlet oxygen scavengers. This finding is supported by numerous studies [81,82]. The high TPC in the BC sample indicates a considerable presence of these compounds, consistent with other *Berberis* species like *Berberis vulgaris*, *Berberis nummularia*, and *Berberis atrocarpa* [83–85]. This high phenolic content likely contributes to BC's potent antioxidant activity by neutralizing free radicals and chelating metal ions [86]. Comparative analyses have confirmed that phenolic content significantly contributes to the antioxidant potential of Berberis species [87,88], highlighting their relevance in supporting health and reducing the risk of oxidative stress-related diseases [74].

Flavonoids, a group of phenolic compounds, are widely recognized for their potent antioxidant properties and contribute significantly to the total antioxidant capacity of various plant extracts [89,90]. In the sample of BC, the TFC was found to be relatively high, consistent with findings from other Berberis species, such as Berberis vulgaris, Berberis thunbergii, Berberis atrocarpa, and Berberis nummularia, which also contain considerable levels of flavonoids [74,84]. This indicates a general pattern of high flavonoid content among Berberis species, supporting their role in antioxidant activity. The analysis of different Berberis species, including BC, suggests their potential as sources of antioxidants due to their high flavonoid content. The similar trend observed among species suggests a shared flavonoid composition and antioxidant potential within the genus, supporting the role of flavonoids in contributing to antioxidant activity.

The LC-MS/MS analysis of BC extracts identified a specific phytochemical profile, with quinic acid (57.413) as the predominant compound. This non-phenolic organic acid is commonly found in various plant species and has been reported to have pharmacological potential, acting as a starting material for the development of several pharmaceuticals, including those used in the treatment of influenza [91]. The significant presence of quinic acid in BC suggests that it may play a crucial role in the bioactivity of the extracts, potentially influencing metabolic pathways and modulating cellular responses to oxidative stress [92,93]. Additionally, quinic acid has been reported to exhibit potential antidiabetic effects by modulating glucose metabolism and insulin sensitivity [94,95]. This is consistent with findings that highlight the importance of various phytochemicals in regulating metabolic functions and their potential therapeutic applications in diabetes management [94,96].

Protocatechuic acid was identified at a measurable concentration

(1.03), suggesting its potential role in antioxidant, anti-inflammatory, anticancer, and neuroprotective activities [97,98]. The pharmacological significance of protocatechuic acid has been well-documented, particularly in its ability to influence cellular oxidative status and affect signaling pathways associated with inflammation and carcinogenesis [99–101]. Several studies have also indicated its antidiabetic potential by improving insulin secretion and reducing oxidative stress-induced pancreatic damage [102,103]. Similarly, chlorogenic acid (0.439) and caffeic acid (0.647), both recognized for their potent antioxidant and hepatoprotective effects, were detected at moderate levels [104]. Chlorogenic acid has been shown to exhibit glucose-lowering properties by inhibiting glucose absorption in the intestine and improving insulin sensitivity, suggesting that BC may have beneficial effects on glucose homeostasis [105,106].

Comparative analysis with previous LC-MS studies on Berberis species shows both similarities and observable differences. Several Berberis species, including *B. vulgaris* and *B. lycium*, have been reported to contain significant levels of protocatechuic acid, chlorogenic acid, and caffeic acid, supporting our findings [107,108]. However, in contrast to earlier reports on B. vulgaris, which highlighted high concentrations of vanillic acid and syringic acid, these compounds were not detected in the BC extract [83,107]. This finding is further supported by the research of Gidik, which shows that BC does not contain the same phenolic constituents as *B. vulgaris* [109]. This supports the hypothesis that these species may have developed different biochemical characteristics or adaptations. Such variation may be attributed to genetic differences, environmental conditions, and variations in extraction methodologies.

Interestingly, other well-documented phenolic compounds, including vanillic acid, gentisic acid, syringic acid, tannic acid, epigallocatechin, catechin, and ellagic acid, were not detected. The non-detection of these bioactive compounds contrasts with previously published data on Berberis species and may be associated with factors such as soil composition, elevation, and climatic conditions that influence phytochemical biosynthesis [87,110]. Additionally, compounds such as p-coumaric acid (0.006) and salicylic acid (0.069) were detected at relatively low concentrations, suggesting limited contributions to the overall antioxidant capacity of the extract. Given that oxidative stress plays a significant role in diabetes pathogenesis, the presence of these antioxidants, even in low amounts, may contribute to the protective effects of BC against diabetes-related complications [111].

Flavonoids such as rutin (0.064) and hesperidin (0.059) were also present in minimal amounts. These flavonoids are known for their vascular protective properties, contributing to anti-inflammatory and cardioprotective effects [112–114]. Rutin, in particular, has been shown to have antidiabetic effects by improving insulin resistance and reducing oxidative stress in pancreatic β-cells [115,116]. However, their relatively low concentrations in BC suggest that other compounds may play a more dominant role in its biological activity. Flavonoids such as kaempferol, apigenin, chrysin, acacetin, epicatechin gallate, sinapic acid, and genistein were not detected. This observation is significant, as several of these compounds have been reported in other Berberis species, suggesting species-specific variations in phytochemical composition [117].

## 5. Conclusion

The study comprehensively examines the antidiabetic, hepatoprotective, nephroprotective, and antioxidant properties of BC using an alloxan-induced diabetic rat model. The findings clearly reveal that BC root extract exerts significant therapeutic benefits on diabetes-associated metabolic disturbances, particularly regarding liver and kidney dysfunction. BC treatment resulted in a significant improvement in glycemic control, as indicated by decreased blood glucose and HbA1c levels, as well as increased serum insulin secretion. These results suggest that BC may play a crucial role in influencing pancreatic function, potentially improving insulin sensitivity and supporting β-cell regeneration.

Histopathological analyses have further supported the protective effects of BC against diabetes-induced hepatic and renal damage. In diabetic rats, significant structural alterations were observed in liver and kidney tissues, including hepatocellular degeneration, vacuolization, glomerular atrophy, and tubular necrosis. High-dose BC treatment significantly reduced these pathological changes, improving tissue architecture to near-normal conditions. These findings suggest that BC not only exerts a hypoglycemic effect but also functions as a potent organoprotective agent against diabetes-related complications.

The antioxidant potential of BC was demonstrated via various in vitro assays, including DPPH and FRAP, which highlighted its potent free radical scavenging activity. The high total phenolic and flavonoid content of BC further validates its antioxidant capacity, with LC-MS/MS analysis identifying significant bioactive compounds such as quinic acid, protocatechuic acid, chlorogenic acid, and caffeic acid. These phytochemicals are well-documented for their potential in reducing oxidative stress, a major factor in diabetes-induced cellular damage. The correlation between BC's potent antioxidant activity and its beneficial effects on metabolic parameters suggests that its therapeutic effects may be primarily attributed to its ability to reduce oxidative stress and inflammation.

Overall, these findings position BC as a promising natural therapeutic candidate for diabetes and its associated complications. The dose-dependent efficacy observed in this study highlights the need for further pharmacokinetic and mechanistic investigations to optimize its therapeutic application. Future research should focus on clinical trials to evaluate BC's effectiveness and safety in human populations and investigate its molecular mechanisms of action. Furthermore, comparative studies with other Berberis species may provide a more comprehensive understanding of the genus-wide pharmacological potential and contribute to the development of standardized herbal formulations.

## CRediT authorship contribution statement

**Yilmaz Rabia:** Writing – review & editing, Writing – original draft, Methodology, Investigation. **Aktaş Mehmet:** Writing – review & editing, Writing – original draft, Supervision. **Şimşek Samed:** Writing – review & editing, Methodology, Investigation.

## Ethics statement

All in vivo experimental assays were conducted in accordance with international guidelines for the care and use of laboratory animals.

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## 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.

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## Data availability

The authors are unable or have chosen not to specify which data has been used.

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