

Alleviation of Oxidative Stress and Modulation of Insulin-Related Genes by *Ficus exasperata* Leaf Fractions in Sucrose-Induced Diabetic *Drosophila melanogaster*

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Oxidative stress plays a central role in the pathogenesis of diabetes mellitus and its complications. *Ficus exasperata* has been widely used in traditional medicine for its potential antioxidant and antidiabetic properties. This study aimed to evaluate and compare the antioxidant and therapeutic effects of n-hexane and n-butanol leaf fractions of *F. exasperata* in sucrose-induced diabetic *Drosophila melanogaster*. Fractions were analyzed for total phenol and flavonoid content, ferric reducing antioxidant power (FRAP), and DPPH radical scavenging activity. In vivo studies assessed survival rate, locomotor activity, glucose concentration, non-protein and total thiol levels, nitric oxide (NO), and hydrogen peroxide (H₂O₂) production. Gene expression analysis was carried out to determine the effects of both fractions on insulin-related genes (ILP-2, Imp-L2, and InR). The n-hexane fraction exhibited higher total phenolic content (152.12 ± 1.95 mg/g) and FRAP activity, whereas the n-butanol fraction showed superior DPPH radical scavenging (86.97 ± 0.92%) and α-amylase inhibition, leading to a greater reduction in glucose concentration. Both fractions enhanced thiol defense systems, reduced oxidative biomarkers (NO and H₂O₂), and improved locomotor activity in diabetic flies, with the n-butanol fraction showing the most pronounced effects. Gene expression analysis revealed dose-dependent upregulation of ILP-2, Imp-L2, and InR genes. The findings demonstrate that *Ficus exasperata* possesses significant antioxidant and antidiabetic activities, mediated through restoration of thiol defenses, reduction of oxidative stress, and modulation of insulin signaling genes. While the n-hexane fraction was richer in phenolic content, the n-butanol fraction exhibited superior antidiabetic potential, making it the recommended fraction for further therapeutic exploration. These results support the ethnomedicinal use of *F. exasperata* and highlight its promise as a natural therapeutic candidate for managing diabetes and oxidative stress-related complications.

Keywords: *Ficus exasperata*, leaf fractions, oxidative stress, insulin signaling, Imp-L2, ILP-2, InR, *Drosophila melanogaster*, sucrose-induced diabetes.

INTRODUCTION

Diabetes mellitus (DM) is a metabolic disorder characterized by elevated blood glucose levels resulting from either insufficient or ineffective insulin. Numerous factors, including heredity and epigenetic propensity, lifestyle choices, and genetics, influence the release and function of insulin (Mobasserri *et al.*, 2020). Diabetes was estimated to be the eighth most common cause of death and disability worldwide in 2019 by the Global Burden of Diseases, Injuries, and Risk Factors Study (GBD), with over 460 million people of all ages and in all countries having the condition (Vos *et al.*, 2020). Diabetes can lead to various complications such as cardiovascular disease, neuropathy, retinopathy, and nephropathy (Izzo *et al.*, 2021). The development of these complications is often caused by oxidative stress triggered by high blood sugar levels (Akinnusi *et al.*, 2023). It is acknowledged that type 2 diabetes is a major public health issue that significantly affects both human life expectancy and medical expenses. In many regions of the world, the prevalence of diabetes is on the rise due to rapid urbanization and economic growth (Onyango *et al.*, 2018).

Drosophila melanogaster, a fruit fly, is a useful model for examining the molecular causes of conditions like metabolic syndrome and type 2 diabetes (Bai *et al.*, 2018; Graham and Pick, 2017). These disorders share genetic and environmental factors that have been retained throughout evolution, and they are typified by metabolic abnormalities (Graham and Pick, 2017). Insulin is essential for anabolism in mammals (Goldfine and Youngren, 1998) and has a major impact on fuel metabolism in *Drosophila* (Álvarez-Rendón *et al.*, 2018). Important elements of the insulin signaling system in fruit flies include the ecdysone-inducible gene L2 (Imp-L2), insulin-like peptide-2 (ILP2), and the insulin-like receptor

(InR). These molecules represent insulin receptors, Insulin-Like Growth Factor-Binding Proteins (IGFBPs), and vertebrate insulin, respectively (Honegger *et al.*, 2008). In *Drosophila*, dietary changes can mimic human diabetes symptoms by causing obesity, metabolic dysfunction, and hyperglycemia (Musselman *et al.*, 2011).

In recent years, the prevention of oxidative stress and exploration of medicinal plants' potentials to combat it has driven extensive research (Bello *et al.*, 2025). The increasing occurrence of diabetes mellitus has spurred extensive research into natural products as potential alternative treatments (Shodehinde *et al.*, 2025). Medicinal plants are widely recognized for their effectiveness in treating various diseases in both humans and animals (Roy *et al.*, 2018). One such plant with a variety of therapeutic uses is the sandpaper leaf, or *Ficus exasperata* Vahl (FEVL) (Adekeye *et al.*, 2020). *Ficus exasperata* leaves extract contains several bioactive compounds including caffeic acid, ferulic acid, maleic acid, p-coumaric acid, tannic acid, salicylic acid, apigenin, and naringenin (Bello *et al.*, 2025) attributed to its wide range of pharmacological effects, such as antiulcer, hypotensive, hypoglycemic, hypolipidemic, anti-inflammatory, anxiolytic, oxytocin-inhibiting, anticonvulsant, antinociceptive, antimicrobial, anticandidal, insecticidal, and pesticidal properties, according to reports from Western Nigeria (Ogunleye *et al.*, 2003). Moreover, *F. exasperata* leaf decoctions and infusions have been used for many years in traditional medicine to treat and manage hypertension, diabetes mellitus, and other cardiovascular conditions (Ayinde *et al.*, 2007). This research explored the therapeutic potential of *Ficus exasperata* on diabetes through in vitro and in vivo studies. N-Hexane and N-Butanol extract fractions of *Ficus exasperata* were assessed for their antioxidant properties using established standard protocols.

MATERIALS AND METHODS

Materials

Plants collection

The leaf of *Ficus exasperata* was obtained from Akungba Akoko, Ondo State, Nigeria. The plant samples were subsequently identified and documented at the Plant Science and Biotechnology Departmental Herbarium (PSBH), Adekunle Ajasin University, Akungba-Akoko, Nigeria.

Fly stock and culture

The *Drosophila melanogaster* used in this study was obtained from the *Drosophila* Laboratory at the Department of Biochemistry, Faculty of Basic Medical Sciences, College of Medicine, University of Ibadan, Oyo State, Nigeria. The flies were maintained at a temperature of 25±2°C in the *Drosophila* Laboratory of the Department of Biochemistry, Adekunle Ajasin University, Akungba Akoko, Nigeria. The diet consisted of water, yeast, corn meal, brewer's yeast, agar-agar, and Nipagin as a preservative, as well as the fly culture.

Preparation and Composition of the feed

The composition of the formulated diet containing the quantity of the ingredients is given below:

Table 1: Composition of feed (250 mL of water)

Ingredients	Amount in diets
Corn meal	8.3 g
Agar	1.0 g
Yeast	3.4 g
Nipargin	1.0 g
Ethanol	3 mL
Water	200-250 mL

During the first 2 weeks, the *Drosophila* were fed with the feed without the addition of extracts. The flies were fed for two weeks so that they could adapt to the new environment (Acclimatization).

Methods

Preparation of extracts

Extraction and solvent-partitioned fractionation of crude extract

The preparation of extracts, extraction and the solvent-partitioned fractionation of crude extract of *Ficus exasperata* was carried out according to the method described by Shodehinde *et al.* (2025). The leaves of *Ficus exasperata* were air-dried in a cool environment for 35 days and subsequently milled into powder using a mechanical grinder. A portion of the powdered sample (148 g) was extracted with ethanol (1000 mL) by soaking for 72 hrs. The ethanol extract was concentrated using a rotary evaporator, and the filtrate was allowed to dry over 2 weeks. The resulting extract (50 g) was dissolved in 500 mL of distilled water and further partitioned with n-hexane and n-butanol, yielding n-hexane and n-butanol

fractions. These fractions were dried and stored for further analysis.

Determination of α -amylase inhibition

Appropriate dilutions of the extracts (500 μ L) in 0.02M sodium phosphate buffer (pH 6.9 with 0.006M NaCl) were added to 0.5 mg/mL of homogenate pancreatic α -Amylase (EC 3.2.1.1) and subsequently incubated at 25°C for 10 min. Then, 500 μ L of 1% starch solution in 0.02M sodium phosphate buffer (pH 6.9 with 0.006M NaCl) was added to each tube. The reaction mixture was incubated at 25°C for 10 min and stopped with 1.0 mL of dinitrosalicylic acid (DNSA) reagent. Therefore, the mixture was incubated in a boiling water bath for 5 min and cooled to room temperature. The reaction mixture was further diluted with distilled water, and the absorbance was read at 540 nm in a spectrophotometer.

In vitro antioxidant assays

Determination of total phenol content (TPC)

Folin-Ciocalteu (FC) reagent (1:10v/v) of 2.5 mL was mixed with 0.5 mL of the fraction (1 mg/mL). After 5 min of incubation at 25°C, sodium carbonate in the amount of 2 mL (7.5%) was added. The mixture was measured at 765 nm after incubation at 40°C for 30 min. The standard used was gallic acid (0.02-0.1 mg/mL), and the fraction's phenol was calculated as mg of gallic acid equivalent (mg GAE/g extract) using the standard curve (Singleton *et al.*, 1999). The experiment was carried out in triplicate (n = 3).

Total flavonoid content

The total flavonoid content of each sample extract was determined according to the method of Meda *et al.* (2005). The appropriate volume (0.1 mL) of the fraction was mixed with 0.5 mL methanol, 50 μ L of 10% Aluminum Chloride ($AlCl_3$), 50 μ L of 1 M potassium acetate, and 1.4 mL of water. The reaction mixture was incubated at room temperature for 30 min. Thereafter, the absorbance of the reaction mixture was measured at 415 nm in the spectrophotometer.

Ferric reducing property (FRAP)

The reducing property of the extracts will be determined according to the method described by Oyaizu (1986). A 2.5 mL aliquot was mixed with 2.5 mL of 200 mM sodium phosphate buffer (pH 6.6) and 2.5 mL of 1% potassium ferricyanide. The mixture was incubated at 50°C for 20 min. Then, 2.5 mL of 10% trichloroacetic acid was added. The mixture was centrifuged at 650 rpm for 10 min. As 5 mL of the supernatant was measured with an equal volume of water, and 1 mL of 0.1% ferric chloride. The absorbance was measured at 700 nm. The ferric-reducing antioxidant property was subsequently calculated.

DPPH free radical scavenging ability

The free radical-scavenging ability of each sample fraction against DPPH (1,1-diphenyl-2-picrylhydrazyl) free radical was evaluated according to the method of Gyamfi *et al.* (1999) with modifications. To 1 mL of 0.4 mM methanol solution of DPPH radicals, 0.1 mL of the sample extracts was added. The mixture was left in the dark for 30 min, and the absorbance was measured at 516 nm in the spectrophotometer.

Survival assay

Flies that are one to three days old were divided into 5 groups of 25 flies each. They were transferred under mild anesthesia using ice from culture vials into new diet vials for 45 days to avoid contamination and ensure good quality of diet throughout the experiment (Shodehinde *et al.*, 2025). The effects of the n-hexane and n-butanol fractions on fly survival were investigated with a pilot study to determine the concentration of the fractions used. The experiment was done in triplicate:

Group I: Basal diet (1 mL ethanol/10 g diet)

Group II: 2 mg n-hxe/10 g diet

Group III: 4 mg n-hex/10 g diet

Group IV: 2 mg n-bute/10 g diet

Group V: 4 mg bute/10 g diet.

The n-hxe and n-bute concentrations were added to the fly diet every 5 days for 45 days, and fly mortality was documented daily for 45 days.

Locomotor assay

Locomotor assay was adopted to measure negative geotaxis assay according to the method of Adedara *et al.* (2022). Ten normal flies and experimental flies were briefly anesthetized under ice and transferred to a 15 x 15 cm glass column. After recovery, the flies were gently tapped down the column. The number of flies that were able to climb up to the 6 cm mark was recorded. The percentage negative geotaxis was estimated using the following formula:

$$\% \text{ negative geotaxis} = \frac{\text{Total No of flies} - \text{No of flies that climb above 6 cm}}{\text{Total No of flies}} \times 100$$

Induction of flies with diabetes using sucrose

The fruit flies were induced with type 2 diabetes according to the method of Omoboyowa et al. (2023) with slight modifications. The 2.5 g sucrose/10 g diet was added to the normal diet of the fly with other diet components remaining constant (1% agar, 3.4% yeast, 8.3% cornmeal, and 1% nipagin) to induce diabetes in the Harwich strain of *D. melanogaster*. The flies were exposed to sucrose incorporated in the diet for 10 days and observed for diabetes symptoms such as low rate of L3 larvae emergence, decreased body size, and decreased locomotive activities.

Grouping of flies and treatment

The antidiabetic activities of n-hexane (n-hxe) and n-butanol (n-bute) fractions were investigated by incorporating them in the diet prepared for the flies, and metformin served as reference drug for 10 days. The experiment was carried out in triplicate with 25 flies in each vial and arranged according to the design given below:

Control groups:

Group 1: *Drosophila* flies (DF) fed with basal diet and 1ml of ethanol/10g of diet

Group 2: Diabetic *Drosophila* flies (DF) with no treatment

Group 3: Diabetic *Drosophila* flies (DF) plus administered 16 mg metformin/10 g diet

Plant treated groups

Group 4: Diabetic *Drosophila* flies (DF) plus administered 2.0 g n-hxe/10g diet

Group 5: Diabetic *Drosophila* flies (DF) plus administered 4.0g n-hxe/10g diet

Group 6: Diabetic *Drosophila* flies (DF) plus administered 2.0g n-bute/10g diet

Group 7: Diabetic *Drosophila* flies (DF) plus administered 4.0g n-bute/10g diet

After the treatment of flies was carried out for 10 days, the flies were anesthetized using ethanol. The weight of flies was taken at a ratio of 1 mg of flies/ 10 mL of buffer and were homogenized using 0.1 M phosphate buffer (pH 7.0). The homogenates were centrifuged for 10 min at 4000 x g and the supernatants were separated from the pellets. The supernatants were kept and used for glucose and *in vivo* antioxidant assays.

Evaluation of glucose concentration

The assay for determining the concentration of glucose in the fly homogenate was conducted according to the procedure of Trinder (1969) using the Agappe LiquiCHEK Kit.

Estimation of nitric oxide level

The nitric oxide (NO) concentration was estimated by the Griess reaction procedure as reported by Green et al. (1982) (24). The mixture of homogenate and Griess solution was allowed to stand for 20 min at 27 °C. Thereafter, the absorbance was read at 550 nm. The level of NO in the mixture was extrapolated from the NaNO₂ calibration curve.

Determination of total thiols and non-protein thiol content

Total thiol content was assayed by the method reported by Adedara et al. (2022) with slight modification. Briefly, the reaction mixture contained 1700 µL of 0.1 M potassium phosphate buffer (pH 7.4), 200 µL of sample, and 100 µL of 5,5'-dithiobis-(2-nitrobenzoic acid (DTNB). After incubation for 30 min at room temperature, the absorbance was measured at 412 nm. For non-protein thiol, the sample was precipitated with 4% sulphosalicylic acid in the ratio of 1:1. The samples were kept at 4°C for 1 h and then subjected to centrifugation at 5000 rpm for 10 min at 4 °C. The assay mixture consisted of 1700 µL of 0.1 M phosphate buffer, 200 µL of supernatant and 100 µL of DTNB. The reaction was allowed to incubate for 30 min at room temperature, and the absorbance was read at 412 nm. For both total thiols and non-protein thiols, reduced Glutathione (GSH) was used as standard, and the data were expressed as in µmol/mg of protein.

Hydrogen peroxide generation

Hydrogen peroxide level was determined according to the method of Wolff, (1994) with slight modification. Briefly, the assay mixture contained 2950 µL of Ferrous Oxidation-Xylenol orange (FOX) reagent and 50 µL of sample. This was followed by 30 min incubation at room temperature, and the absorbance measured at 560 nm.

Gene expression Study

Isolation of Total RNA

Total RNA was isolated from whole *Drosophila* samples with Quick-RNA MiniPrep™ Kit (Zymo Research). The DNA contaminant was removed following DNase I (NEB, Cat: M0303S) treatment. The RNA was quantified at 260 nm and the purity confirmed at 260 nm and 280 nm using A&E Spectrophotometer (A&E Lab. UK).

cDNA conversion

One (1 µg) of DNA-free RNA was converted to cDNA by reverse transcriptase reaction with the aid of cDNA synthesis kit based on ProtoScript II first-strand technology (New England BioLabs) in a condition of 3-step reaction: 65 °C for 5 min, 42 °C for 1 h, and 80 °C for 5 min (Elekofehinti *et al.*, 2020).

PCR amplification and agarose gel electrophoresis

Polymerase chain reaction (PCR) for the amplification of gene of interest was carried out with OneTaqR2X Master Mix (NEB) using the following primers (Inqaba Biotec, Hatfield, South Africa): set: >NM_079288.3 *Drosophila melanogaster* Insulin-like peptide 2 (Ilp2), mRNA Insulin-like peptide-2 Forward primer: AGGTGCTGAGTATGGTGTGC Reverse primer: TGTCGGCACCGGGCAT, >NM_001274480.1 *Drosophila melanogaster* Ecdysone-inducible gene L2 (ImpL2), transcript variant D, mRNA Forward primer TGCCGGCTTAGACTAATTT Reverse primer GCTGCCTGAATCGCTAGGAA, >NM_001144622.2 *Drosophila melanogaster* Insulin-like receptor (InR), transcript variant C, mRNA Forward primer TTTGTGCCTCGCACTTTGC Reverse primer ATACGCTACCAACACATGC, >NM_001273184.2 *Drosophila melanogaster* Glycerol-3-phosphate dehydrogenase (Gapdh), transcript variant G, mRNA, mRNA Forward primer TCGGACTGCGTAGACTAGA Reverse primer AGCGCCATCTATGTAAGGATGT. PCR amplification was performed in a total of 25 µl volume reaction mixture containing cDNA, primer (forward and reverse) and Ready Mix Taq PCR master mix. Under the following condition: Initial denaturation at 95 °C for 5 min, followed by 30 cycles of amplification (denaturation at 95 °C for 30 s, annealing for 30 s and extension at 72 °C for 60 s) and ending with final extension at 72 °C for 10 min. The amplicons were resolved on 1.0% agarose gel. The GAPDH gene was used to normalize the relative level of expression of each gene, and quantification of band intensity was done using "image J" software (Elekofehinti *et al.*, 2020).

Statistical analysis

All antioxidant studies were performed in triplicate. The data was statistically analyzed using GraphPad Prism. The data was presented as a Mean Standard Error of Mean (SEM). The statistical analyses employed One-way Analysis of Variance (ANOVA) with multiple comparisons, and Duncan's multiple range tests to compare the means. The $p < 0.05$ was used to evaluate statistical significance.

RESULTS

Table 2 shows that, the n-hexane fraction has a significantly higher total phenol content (152.12 ± 1.95 mg/g) compared to the n-butanol fraction (79.80 ± 4.31 mg/g). The total flavonoid content is similar for both the n-hexane (2.52 ± 0.18 mg/g) and n-butanol (2.49 ± 0.02 mg/g) fractions, indicating that both fractions contain similar amounts of flavonoids. The n-hexane fraction has a significantly higher FRAP value (1.11 ± 0.05 mg/g) compared to the n-butanol fraction (0.69 ± 0.01 mg/g). However, the n-butanol fraction has a significantly higher DPPH radical scavenging ability ($86.97 \pm 0.92\%$) compared to the n-hexane fraction ($70.90 \pm 4.83\%$). The n-butanol fraction showed a strongest inhibitory activity in a dose-dependent manner compared to its n-hexane fraction counterpart at different concentrations against alpha amylase, one of the key enzymes involved in carbohydrate digestion (Figure 1). The survival rates of the different groups were recorded. Statistical analysis (Figure 2) revealed non-significant difference in the survival rates of the different groups ($p > 0.05$). This suggests that both n-hexane and n-butanol fractions of *F. exasperata* had a significant effect on the survival of the flies. However, despite the statistical insignificance, the n-butanol fractions still offer the best effect. The n-butanol fraction showed a notable increase in climbing (locomotor) activity (Figure 3), more pronounced reduction in glucose concentration (Figure 4), amelioration of non-protein thiol level (Figure 5), restoration of total thiol level (Figure 6) and significant reduction of NO concentration (Figure 7) was recorded in diabetic flies treated with diets containing n-hexane and n-butanol fractions compared to untreated diabetic flies. Treatment with n-hexane and n-butanol fractions of *Ficus exasperata* reduced hydrogen peroxide concentration compared to the induced and not treated group (II) particularly at 2 mg/10g diet n-hexane fraction (Figure 8).

The results of the gene expression analysis of the n-Hexane and n-Butanol fractions of *Ficus exasperata* on the ILP-2 gene showed that the plant extract upregulated the expression of the gene, indicating that both fractions of *Ficus exasperata* leaf could have a stimulatory effect on the expression of the ILP-2 gene particularly at low dose of 2 mg/kg but could be diminished at high dose of 4 mg/kg (Figure 9A and Figure 9B).

Interestingly, the groups that received the both fractions of *Ficus exasperata* leaf extract (groups 4 and 5) showed significantly higher relative expression values of the Imp-L2 gene (Figure 10A and Figure 10B) compared to the control groups, suggesting their therapeutic effects on glucose metabolism by regulating insulin signaling, which could make it a useful tool in the management of diabetes.

Both fractions of *Ficus exasperata* potentially enhanced the expression of the InR gene, which could be beneficial in improving glucose uptake and regulating insulin signaling in the body. However, this potential effect on the regulation of glucose homeostasis is more pronounced at low dose of 2 mg/kg (Figure 11A and Figure 11B).

Table 2: Polyphenolic content and antioxidant activity of *F. exasperata* leaf

Parameter	Total phenol (mg/g)	Total flavonoid (mg/g)	FRAP (mg/g)	DPPH (%)
n-hexane	152.12 ± 1.95	2.52 ± 0.18	1.11 ± 0.05	70.90 ± 4.83
n-butanol	79.80 ± 4.31	2.49 ± 0.02	0.69 ± 0.01	86.97 ± 0.92

Result shows Mean ± SEM, where, n = 3, FRAP: Ferric reducing antioxidant property and DPPH: 2,2-diphenyl-1-picrylhydrazyl

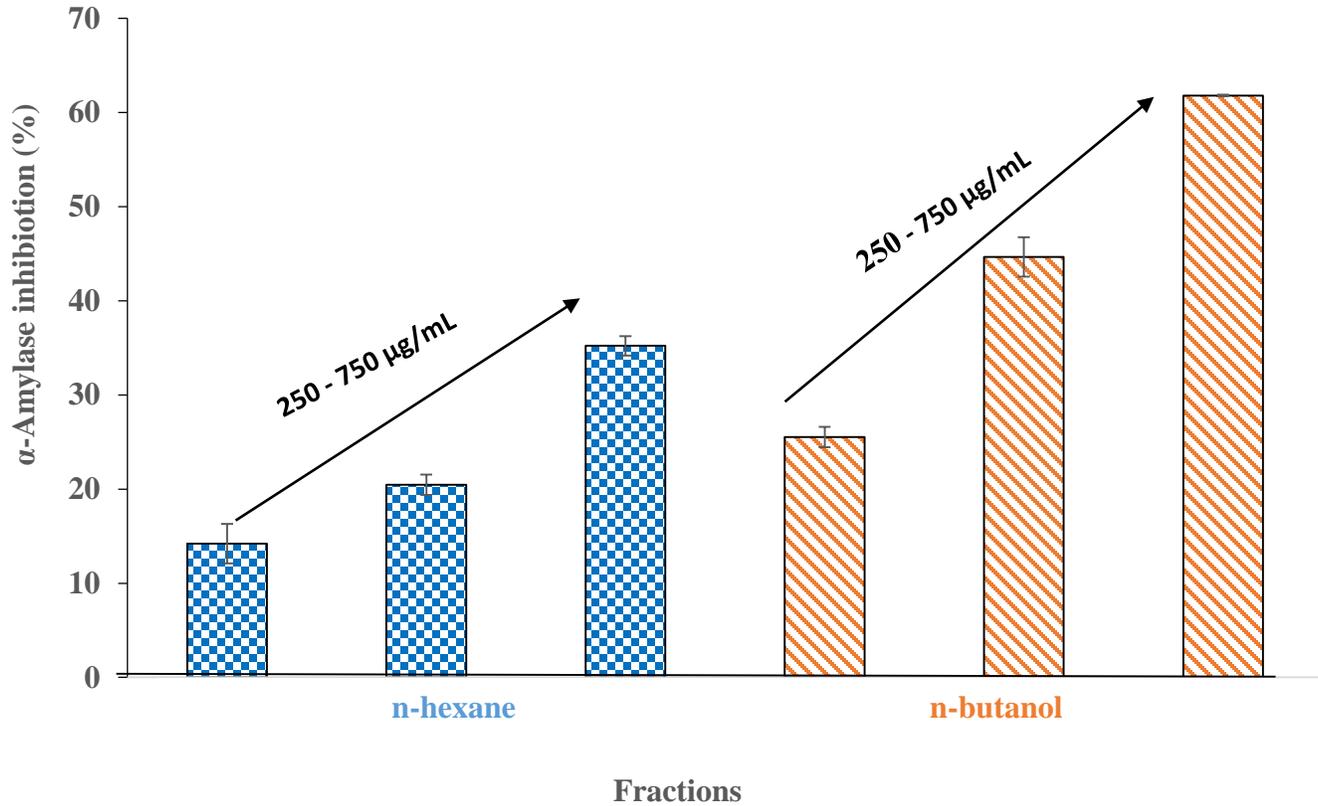


Figure 1: The effect of diets incorporated with n-hexane and n-butanol fractions of *Ficus exasperata* on α -amylase activity in sucrose induced diabetic *D. melanogaster*.

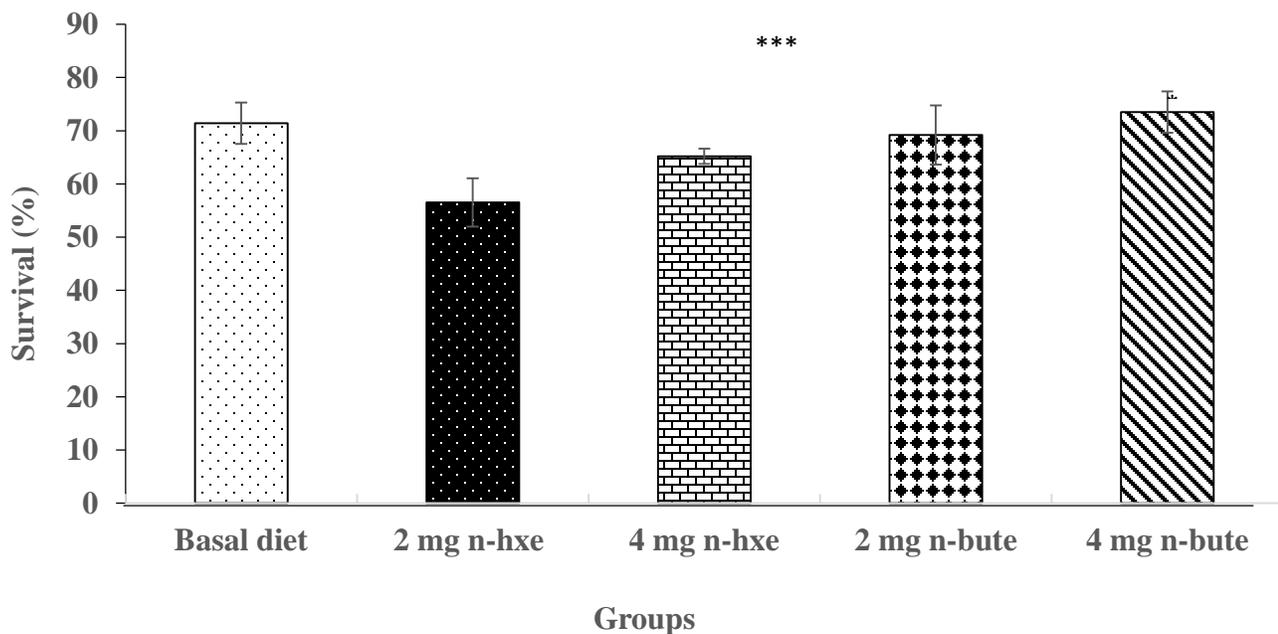


Figure 2: Evaluation of the survival of *D. melanogaster* that are fed with varied concentrations of n-hexane and n-butanol fractions of *Ficus exasperata*. *** ($P < 0.05$) significantly compared with the basal diet fed group.

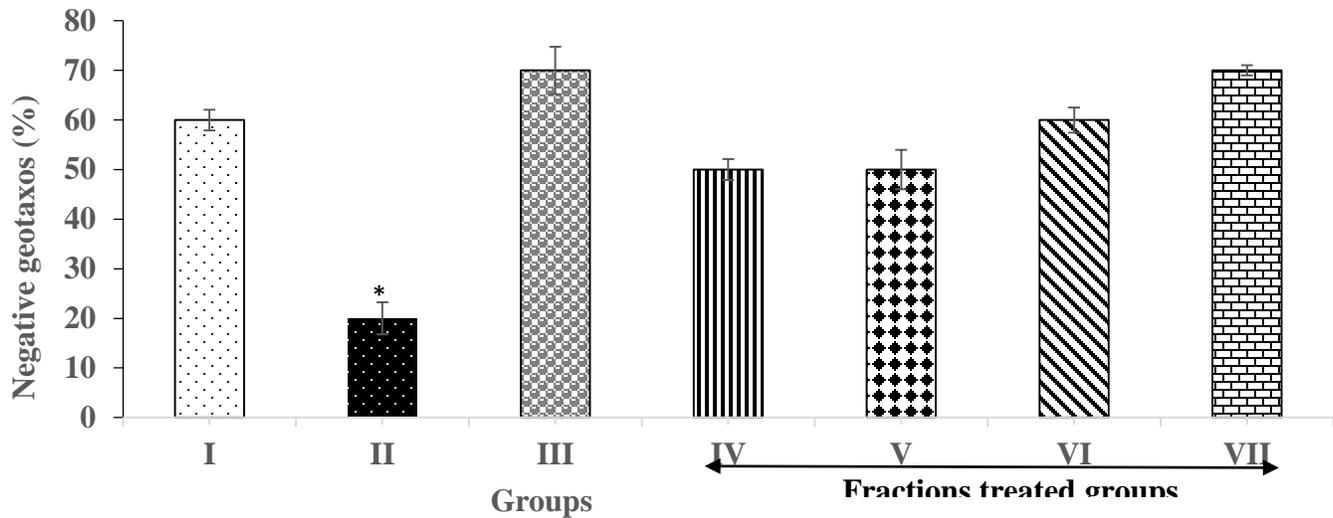


Figure 3: Effect of n-hexane and n-butanol fractions of *Ficus exasperata* leaves on climbing (locomotion) activity in sucrose-induced and normal *Drosophila melanogaster*. *($P < 0.05$) significantly different from the normal and treated *D. melanogaster*.

- ▤ I: DF (Basal)
- II: DF + No Treatment
- ▨ III: DF + Met. (10mg/10g Diet)
- ▩ IV: DF + n-hxe (2mg/10g Diet)
- ▧ V: DF + n-hxe (4mg/10g Diet)
- ▦ VI: DF + n-bute (2mg/10g Diet)
- ▥ VII: DF+ n-bute (4mg/10g Diet)

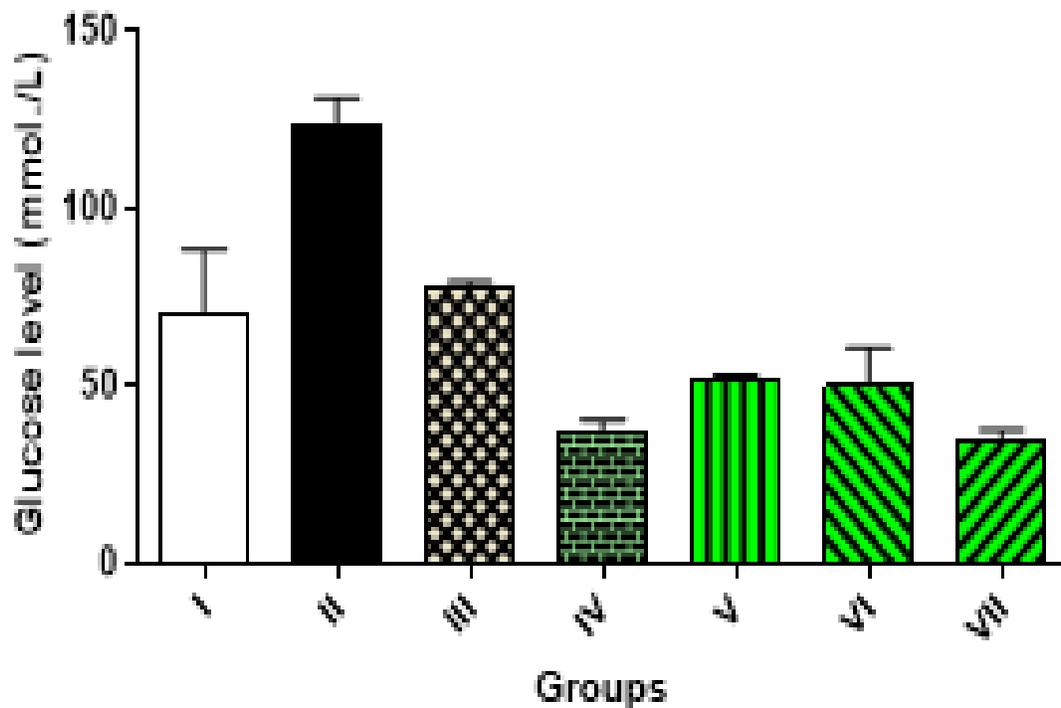


Figure 4: Effects of *Ficus exasperata* leaf fractions on sucrose induced diabetes in diabetic *D.*

- I: DF (Basal)
- II: DF + No Treatment
- III: DF + Met. (10mg/10g Diet)
- IV: DF + n-hxe (2mg/10g Diet)
- V: DF + n-hxe (4mg/10g Diet)
- VI: DF + n-bute (2mg/10g Diet)
- VII: DF+ n-bute (4mg/10g Diet)

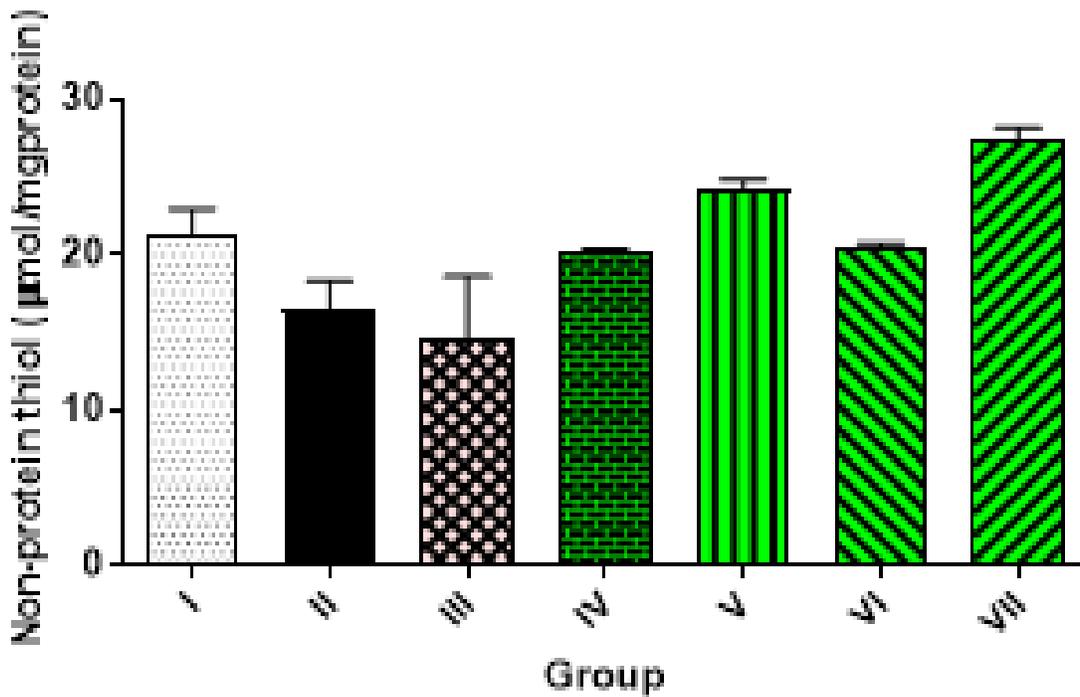


Figure 5: Treatments of sucrose-induced diabetes in diabetic *D. melanogaster* with *Ficus exasperata* leaf fractions ameliorated nonprotein thiol.

- I: DF (Basal)
- II: DF + No Treatment
- III: DF + Met. (10mg/10g Diet)
- IV: DF + n-hxe (2mg/10g Diet)
- V: DF + n-hxe (4mg/10g Diet)
- VI: DF + n-bute (2mg/10g Diet)
- VII: DF+ n-bute (4mg/10g Diet)

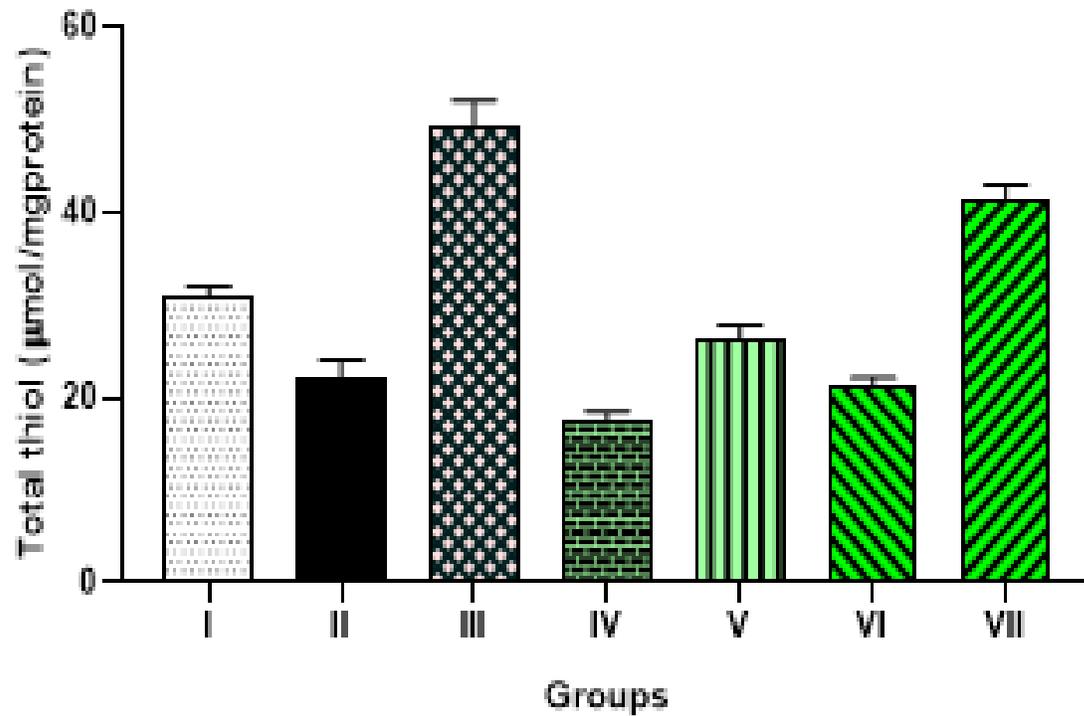


Figure 6: Treatments of sucrose-induced diabetes in diabetic *D. melanogaster* with *Ficus exasperata* leaf fractions ameliorated total thiol concentration.

- ▤ I: DF (Basal)
- II: DF + No Treatment
- ▨ III: DF + Met. (10mg/10g Diet)
- ▩ IV: DF + n-hxe (2mg/10g Diet)
- ▧ V: DF + n-hxe (4mg/10g Diet)
- ▦ VI: DF + n-bute (2mg/10g Diet)
- ▥ VII: DF+ n-bute (4mg/10g Diet)

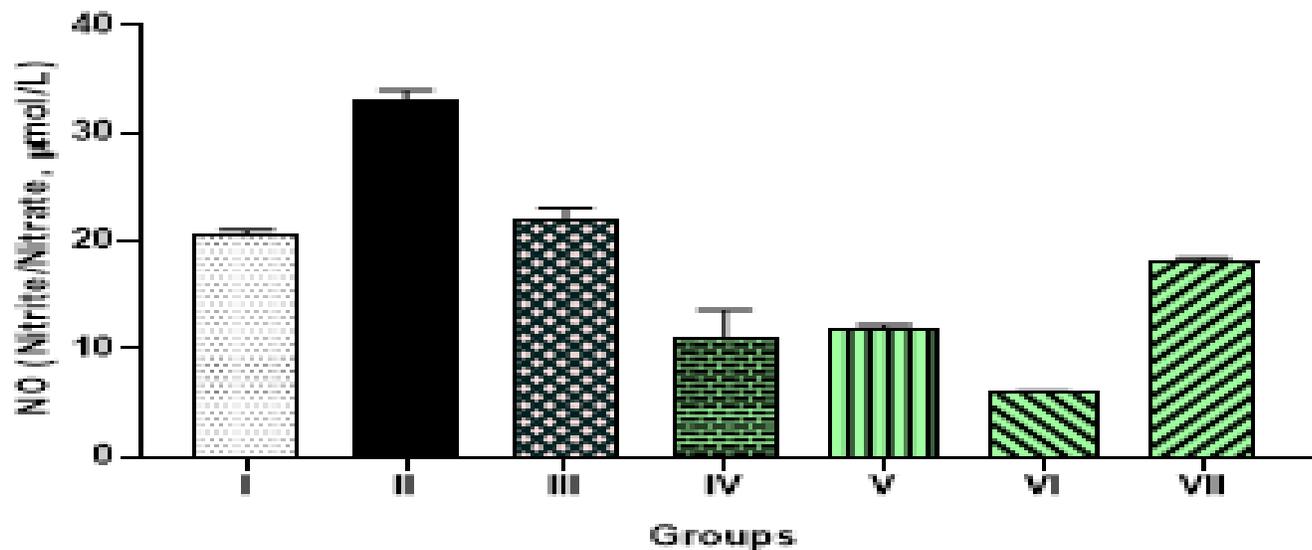


Figure 7: Treatments of sucrose-induced diabetes in diabetic *D. melanogaster* with *Ficus exasperata* leaf fractions alleviated nitrite production.

- I: DF (Basal)
- II: DF + No Treatment
- III: DF + Met. (10mg/10g Diet)
- IV: DF + n-hxe (2mg/10g Diet)
- V: DF + n-hxe (4mg/10g Diet)
- VI: DF + n-bute (2mg/10g Diet)
- VII: DF+ n-bute (4mg/10g Diet)

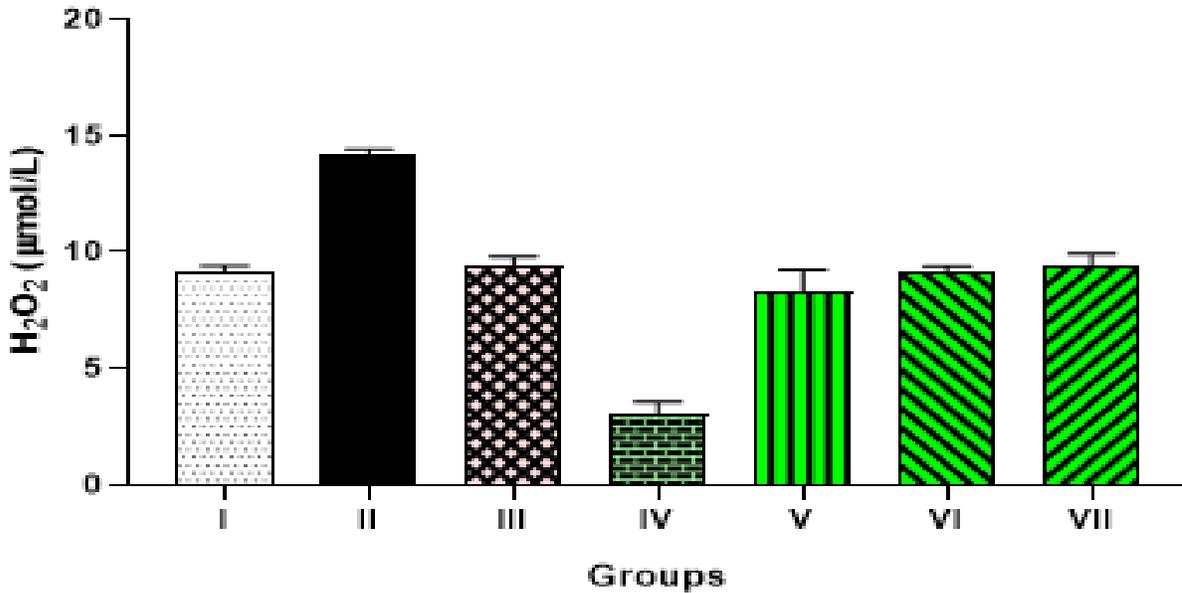


Figure 8: Treatments of sucrose-induced diabetes in diabetic *D. melanogaster* with *Ficus exasperata* leaf fractions alleviated hydrogen peroxide.

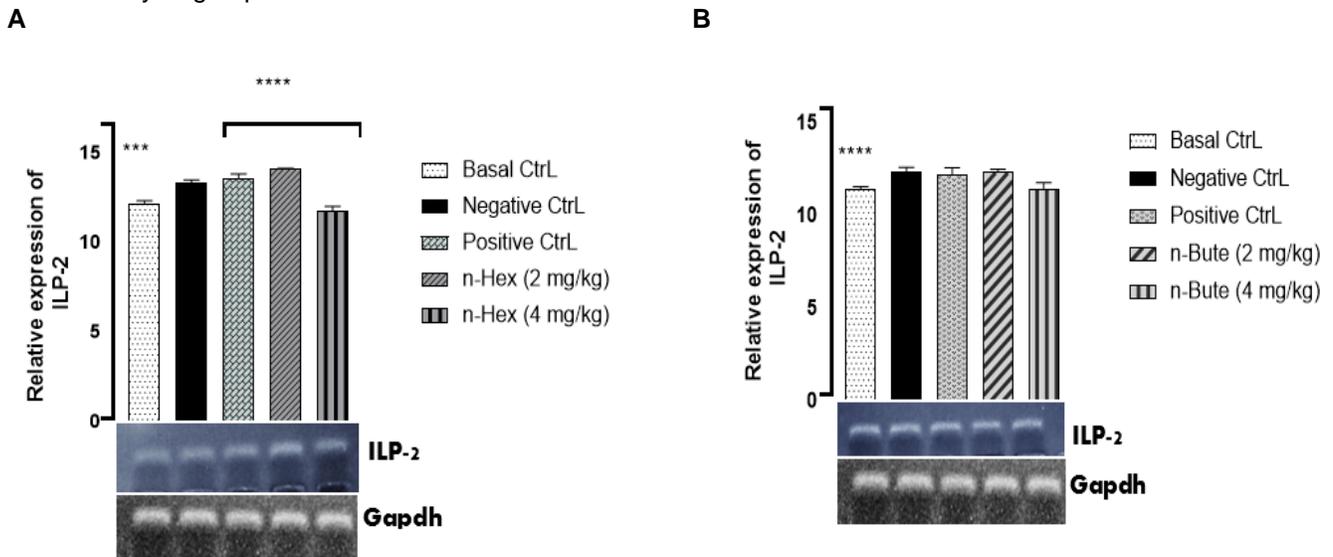
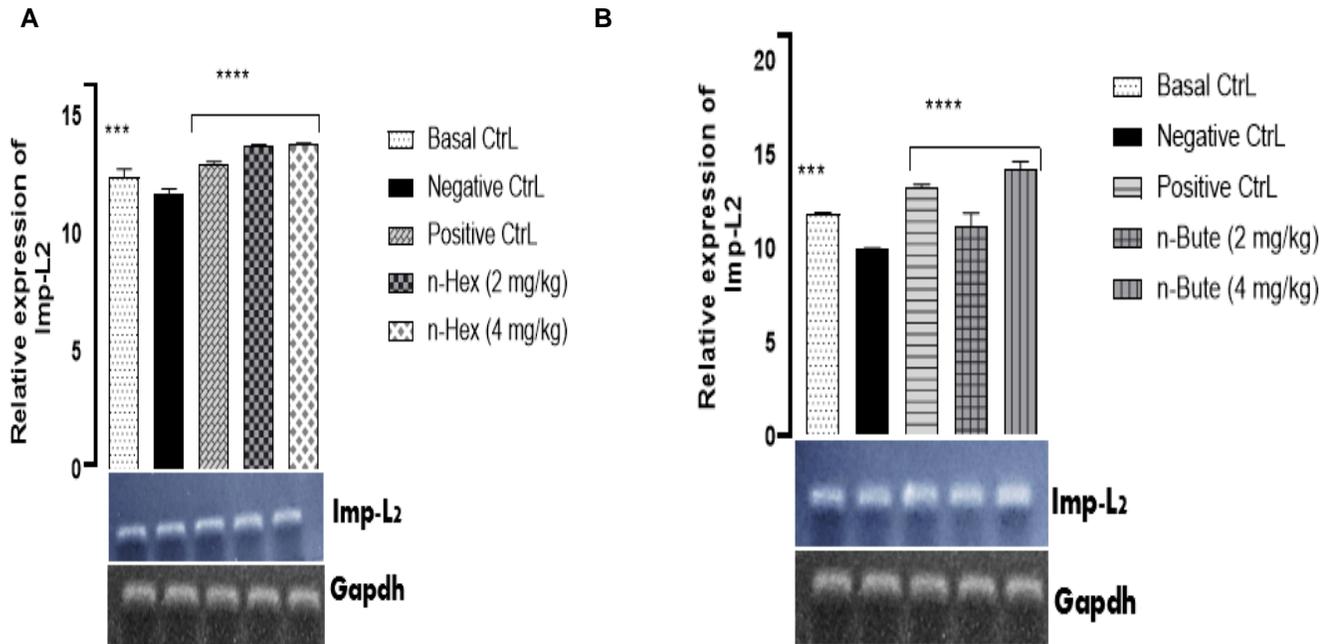


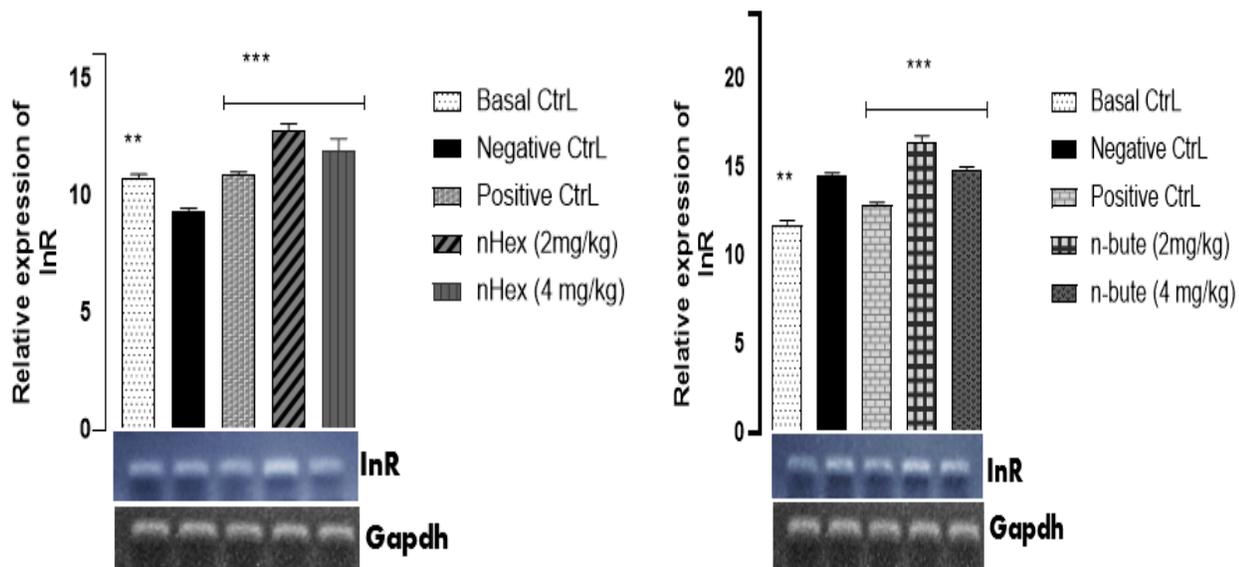
Figure 9: Gene expression of insulin-like peptide-2 (ILP-2). The values are mean \pm SEM, value with the asterisks (***) is significantly different from negative ctrl (without asterisk), (****) is significantly different from treated flies. The experimental n = 15 species of wild type *D. melanogaster* (Harwich strain). Diets of groups I-V in (A) comprised of I = DF (Basal Diet), II = DF (Basal Diet +2.5 g sucrose/100 mL distilled water; negative ctrl), III = DF (Basal diet + metformin), IV = DF (Basal Diet + 2 mg/kg n-hex *Ficus exasperata* leaf) V = DF (Basal Diet + 4 mg/kg n-hex *Ficus exasperata* leaf). (B) Comprised of I = DF (Basal Diet), II = DF (Basal Diet +2.5 g sucrose/100 mL distilled water; negative ctrl), III = DF (Basal diet + metformin), IV = DF (Basal Diet + 2 mg/kg n-bute *Ficus exasperata* leaf) V = DF (Basal Diet + 4 mg/kg n-bute *Ficus exasperata* leaf). Ctrl= control, DF = *Drosophila* flies.



A

Figure 10: Gene expression of Imp-L2 (ecdysone inducible L-2).

The values are mean \pm SEM triplicate, (***) is significantly different from negative ctrl and (****) is significantly different from treated groups while the experimental $n = 15$ species of wild type *D. melanogaster* (Harwich strain). Diets of groups I-V in (A) comprised of I = DF (Basal Diet), II = DF (Basal Diet +2.5 g sucrose/100 mL distilled water; negative ctrl), III = DF (Basal diet + metformin), IV = DF (Basal Diet + 2 mg/kg n-hex *Ficus exasperata* leaf) V = DF (Basal Diet + 4 mg/kg n-hex *Ficus exasperata* leaf). (B) Comprised of I = DF (Basal Diet), II = DF (Basal Diet +2.5 g sucrose/100 mL distilled water; negative ctrl), III = DF (Basal diet + metformin), IV = DF (Basal Diet + 2 mg/kg n-bute *Ficus exasperata* leaf) V = DF (Basal Diet + 4 mg/kg n-bute *Ficus exasperata* leaf). Ctrl= control, DF = *Drosophila* flies.



A

B

Figure 11: Gene expression of InR (insulin-like receptor).

The values are mean \pm SEM triplicate, (**) is significantly different from negative ctrl and (***) is significantly different from treated groups while the experimental $n = 15$ species of wild type *D. melanogaster* (Harwich strain). Diets of groups I-V in (A) comprised of I = DF (Basal Diet), II = DF (Basal Diet +2.5 g sucrose/100 mL distilled water; negative ctrl), III = DF (Basal diet + metformin), IV = DF (Basal Diet + 2 mg/kg n-hex *Ficus exasperata* leaf) V = DF (Basal Diet + 4 mg/kg n-hex *Ficus exasperata* leaf). (B) Comprised of I = DF (Basal Diet), II = DF (Basal Diet +2.5 g sucrose/100 mL distilled water; negative ctrl), III = DF (Basal diet + metformin), IV = DF (Basal Diet + 2 mg/kg n-bute *Ficus exasperata* leaf) V = DF (Basal Diet + 4 mg/kg n-bute *Ficus exasperata* leaf). Ctrl= control, DF = *Drosophila* flies.

DISCUSSION

To evaluate the antioxidant properties of *F. exasperata*, two different leaf fractions using n-hexane and n-butanol solvents were prepared, then several parameters to assess the antioxidant activity of each fraction were measured which include total phenol content, total flavonoid content, ferric reducing antioxidant property, and DPPH radical scavenging ability.

Total phenol content is a measure of the amount of phenolic compounds in a sample (Noreen *et al.*, 2017). Phenolic compounds are known to have antioxidant properties, so a higher total phenol content is generally considered to be an indicator of stronger antioxidant activity. Phenolic compounds, known for their electron-donating properties, are key contributors to the neutralization of free radicals and oxidative stress (Bello *et al.*, 2025). In Table 2, the n-hexane fraction has a significantly higher total phenol content (152.12 ± 1.95 mg/g) compared to the n-butanol fraction (79.80 ± 4.31 mg/g). This suggests that the n-hexane fraction had stronger antioxidant activity than the n-butanol fraction.

Total flavonoid content is another measure of the amount of bioactive compounds in a sample that have antioxidant properties. Flavonoids are a type of phenolic compound that are known to have strong antioxidant activity. In Table 2, the total flavonoid content is similar for both the n-hexane (2.52 ± 0.18 mg/g) and n-butanol (2.49 ± 0.02 mg/g) fractions, indicating that both fractions contain similar amounts of flavonoids.

The ferric reducing antioxidant property (FRAP) assay measures the ability of a sample to reduce ferric ions (Fe^{3+}) to ferrous ions (Fe^{2+}). This reduction process is associated with antioxidant activity, as it can help to neutralize free radicals and prevent oxidative damage. In Table 2, the n-hexane fraction has a significantly higher FRAP value (1.11 ± 0.05 mg/g) compared to the n-butanol fraction (0.69 ± 0.01 mg/g), suggesting stronger antioxidant activity of the n-hexane fraction.

The DPPH radical scavenging ability assay measures the ability of a sample to scavenge free radicals, specifically the DPPH radical. A higher percentage of DPPH radical scavenging indicates stronger antioxidant activity. In Table 2, the n-butanol fraction has a significantly higher DPPH radical scavenging ability ($86.97 \pm 0.92\%$) compared to the n-hexane fraction ($70.90 \pm 4.83\%$). This suggests that the n-butanol fraction could have stronger antioxidant activity than the n-hexane fraction in terms of scavenging DPPH radicals.

One of the therapeutic strategies for managing type 2 diabetes involves controlling glucose absorption by reducing starch hydrolysis through the inhibition of pancreatic α -amylase, thereby limiting glucose uptake (Kaurinovic and Vastag, 2019; Krentz and Bailey, 2005). As shown in Figure 1 and Figure 4, the n-butanol fraction showed reduction of glucose level and stronger inhibitory activity at different concentrations against alpha amylase, one of the key enzymes involved in carbohydrate digestion indicating that the n-butanol fractions have stronger potential to inhibit carbohydrate absorption and reduction of glucose concentration than the n-hexane fractions, reinforcing that *F. exasperata* possesses medicinal properties with a promising therapeutic value (Shodehinde *et al.*, 2025).

The survival rates of the different groups were recorded and analyzed using statistical methods. The statistical analysis (Figure 2) revealed no significant difference in the survival rates of the different groups ($p > 0.05$). This suggests that the n-hexane and n-butanol fractions of *F. exasperata* had a significant effect on the survival of the flies. However, despite the non-significance statistical differences the n-butanol fractions still offers the best effect.

The negative geotaxis results observed in the flies validated the concentrations of fractions utilized in this study (Figure 3). A notable ($p < 0.05$) increase in climbing (locomotor) activity was recorded in both normal flies and diabetic flies treated with diets containing n-hexane and n-butanol extract fractions compared to untreated diabetic flies with the n-butanol fractions offering the best effect.

Non-protein thiols play an important role in protecting cells from oxidative stress (Salbitani *et al.*, 2023), which is a major contributor to diabetes and its complications. Non-protein thiol levels are often reduced in diabetic patients, which can lead to further damage from oxidative stress. In this study, the estimation of non-protein thiol concentration in the fly homogenates was carried out to investigate the effect of the n-hexane and n-butanol fractions of *F. exasperata* on the levels of non-protein thiol in diabetic flies. The results showed that treatment with these fractions led to an increase in non-protein thiol levels in the diabetic flies compared to the untreated diabetic flies with the protective effect more pronounced in the n-butanol fractions (Figure 5). The increase in non-protein thiol levels suggests that the plant extract could have a protective effect against oxidative stress in diabetic flies. This protective effect could potentially translate to humans with diabetes as well, indicating the potential of *F. exasperata* as a natural therapy for diabetes and its complications (Shodehinde *et al.*, 2025).

The total thiol concentration is an important parameter for assessing the antioxidant status of a biological system. Thiols are important components of the antioxidant defense system as they can directly scavenge free radicals or regenerate other antioxidants such as glutathione (Prakash *et al.*, 2009). Similar study was reported by Shodehinde *et al.* (2025), that the decrease in total thiol concentration in diabetic flies compared to the control (Figure 6), suggests that diabetes is associated with a decrease in the antioxidant defense system, which may contribute to the development of diabetic complications. The augmented total thiol concentration in the diabetic flies treated with n-hexane and n-butanol fractions compared to the untreated diabetic flies (group II) and compared with the drug-treated flies in group III suggests that these fractions have antioxidant activity and may help to restore the antioxidant defense system. The decrease in total thiol concentration of diabetic flies is in agreement with the results of Prakash *et al.* (2009). Interestingly, treatment with n-butanol fraction showed the best restoration of total thiol level.

Nitric oxide (NO) is an essential regulatory molecule with significant cellular and metabolic functions. Managing NO metabolism is crucial in type 2 diabetes, as insulin plays a role in activating NO synthase (Chowański *et al.*, 2021). An increase in NO concentration, along with hydrogen peroxide production, as shown in Figure 7 and Figure 8, indicates oxidative damage. The significant reduction of NO and hydrogen peroxide levels in sucrose-induced diabetic flies by n-hexane and n-butanol fractions further supports their antioxidant potential. Interestingly, at low dose (2 mg/10g diet), n-butanol fraction reduce the level of Nitric oxide (NO) and n-hexane fraction reducing hydrogen peroxide production

suggesting that high doses of the both fractions could reduce impact in managing NO concentration and inhibiting hydrogen peroxide production respectively.

The gene expression analysis conducted in this study aimed to evaluate the effect of the n-hexane and n-Butanol fraction of *Ficus exasperata* leaf extract on the expression of the ILP-2, Imp-L2 and InR genes in *Drosophila Melanogaster*. The ILP-2 is an important insulin-like peptide that plays a critical role in regulating energy homeostasis, glucose metabolism, and lifespan in insects (Chowański *et al.*, 2021). Ecdysone (Imp-L2) plays a regulatory role by suppressing hunger in individuals with diabetes (Honegger *et al.*, 2008). Diabetes and β -cell failure are the outcomes of peripheral insulin resistance brought on by systemic malfunction of insulin-like growth factor receptors (InR). On the other hand, the progression happens more quickly than in human type 2 diabetes mellitus (White, 2002). Insulin receptor (InR) is a transmembrane receptor that plays a crucial role in insulin signaling in the body (Chen *et al.*, 2022). The receptor binds to insulin and initiates a cascade of signaling events that regulate glucose uptake and metabolism in various organs, such as the liver, muscle, and adipose tissue.

The results of the gene expression analysis of the n-Hexane and n-Butanol fractions of *Ficus exasperata* on the ILP-2 gene showed that the plant extract upregulated the expression of the gene in a dose-dependent manner. The expression level was moderate in the positive control group treated with metformin. However, Treatment with the n-Hexane and n-Butanol fractions at both 2 mg/kg and 4 mg/kg (groups 4 and 5, respectively) resulted in a slightly higher expression level of the ILP-2 gene compared to the control group particularly at low dose of 2 mg/kg (Figure 9A and Figure 9B). This suggests that the both fractions of *Ficus exasperata* leaf could have a stimulatory effect on the expression of the ILP-2 gene but could be diminished at high dose of 4 mg/kg.

The Imp-L2 gene is a critical gene involved in insulin signaling and glucose metabolism. Therefore, any changes in its expression could indicate a potential therapeutic benefit for diabetes management. The results of the study showed that the relative expression of the Imp-L2 gene was higher in groups 4 and 5, which received different doses of the n-hexane and n-butanol fractions of *Ficus exasperata* leaf extract, compared to the control groups. This suggests that the both fractions of the plant extract could have a positive effect on glucose metabolism, possibly through the regulation of insulin signaling. The basal control group (group 1) showed a relative expression value, which indicates that the expression of the Imp-L2 gene was normal under basal conditions. The negative control group (group 2) received a high sucrose diet, which is known to cause insulin resistance and impaired glucose metabolism. As expected, the relative expression value of the Imp-L2 gene in this group was slightly lower than that of the basal control group. The positive control group (group 3), which received metformin, a known anti-diabetic drug, showed a slightly higher relative expression value than the basal control group. Interestingly, the groups that received the both fractions of *Ficus exasperata* leaf extract (groups 4 and 5) showed significantly higher relative expression values of the Imp-L2 gene (Figure 10A and Figure 10B) compared to the control groups, suggesting their therapeutic effects on glucose metabolism by regulating insulin signaling, which could make it a useful tool in the management of diabetes.

The results of the gene expression analysis of the n-hexane and n-butanol fractions of *Ficus exasperata* on the InR gene showed that the plant extract upregulated the expression of the gene in a dose-dependent manner. The relative expression values of group 4 and group 5 were higher than that of the negative control (group 2). This suggests that both fractions of *Ficus exasperata* has the potential to enhance the expression of the InR gene, which could be beneficial in improving glucose uptake and regulating insulin signaling in the body. However, this potential effect on the regulation of glucose homeostasis is more pronounced at low dose of 2 mg/kg (Figure 11A and Figure 11B). The upregulation of InR gene expression by *Ficus exasperata* suggests that the plant extract has the potential to improve insulin signaling, glucose uptake, and metabolism. This is in line with previous studies that have reported the antidiabetic properties of *Ficus exasperata* on InR gene expression (Shodehinde *et al.*, 2025).

CONCLUSION

This study demonstrates that *Ficus exasperata* leaf fractions possess significant antioxidant and antidiabetic properties, with n-hexane and n-butanol extracts exhibiting complementary bioactivities. Both fractions improved non-protein and total thiol concentrations, reduced oxidative biomarkers such as nitric oxide and hydrogen peroxide, and enhanced locomotor activity in diabetic flies. The gene expression analysis revealed that both fractions upregulated ILP-2, Imp-L2, and InR genes, with effects most pronounced at lower doses, suggesting a potential role in improving insulin signaling and glucose metabolism. While the n-hexane fraction showed higher phenolic content, stronger ferric-reducing antioxidant capacity, and greater hydrogen peroxide inhibition, the n-butanol fraction excelled in DPPH radical scavenging, glucose-lowering effects, α -amylase inhibition, and restoration of thiol defense systems, making it the recommended fraction for further therapeutic exploration. These findings highlight the therapeutic potential of *Ficus exasperata* in mitigating oxidative stress, regulating glucose homeostasis, and managing diabetes-related complications. Importantly, the dose-dependent outcomes indicate that while low to moderate doses confer protective effects, higher concentrations may attenuate benefits, underscoring the need for careful dosage optimization. Overall, the study provides strong evidence that *Ficus exasperata* could serve as a natural source of antioxidant and antidiabetic agents, supporting its ethnomedicinal use and paving the way for future pharmacological and clinical investigations.

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