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Antidiabetic Effects of the Ethanolic Leaf Extract of Parinari curatellifolia on Sucrose-Induced Type II Diabetes in Drosophila melanogaster Model

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Abstract: Diabetes mellitus is a pervasive global health challenge, necessitating the exploration of alternative therapies from traditional medicinal plants. *Parinari curatellifolia*, utilized in African folk medicine, has demonstrated preliminary antidiabetic potential. This study investigated the antidiabetic and antioxidant properties of the ethanolic leaf extract of *P. curatellifolia* in a sucrose-induced type II diabetes model in *Drosophila melanogaster*. After a 7-day induction with a high-sucrose diet (2.5 g/10 g diet), diabetic flies were treated with the extract (10, 100, or 200 mg/10 g diet) or metformin (10 mg/10 g diet) for 7 days. The extract significantly reduced circulating glucose levels (up to 45%, $p < 0.001$), enhanced antioxidant enzyme activities (glutathione S-transferase and catalase by up to 60% and 55%, respectively, $p < 0.01$), and improved locomotor performance by 70% ($p < 0.01$) compared to the diabetic control. A preliminary phytochemical screening revealed the presence of flavonoids, tannins, and alkaloids. No significant toxicity was observed at the tested doses. The ethanolic leaf extract of *P. curatellifolia* exhibits notable antidiabetic and antioxidant properties in *D. melanogaster*, suggesting its potential for further investigation as a therapeutic candidate for type II diabetes.

Keywords: *Parinari curatellifolia*; antidiabetic; antioxidant; *Drosophila melanogaster*; type II diabetes; oxidative stress.

1. Introduction

Diabetes mellitus is a metabolic condition defined by persistent elevations in blood glucose levels, which frequently results in significant morbidity and mortality worldwide [1]. The global prevalence of diabetes is projected to rise, underscoring the urgent need for effective and accessible treatments [2]. While conventional pharmacotherapies like metformin are mainstays [3], there is growing interest in medicinal plants as potential sources of novel antidiabetic agents [4].

Parinari curatellifolia (Planch. ex Benth.), a plant from the Chrysobalanaceae family, is used in African traditional medicine for various ailments [5]. Previous studies have reported on the antidiabetic properties of its stem bark and seeds [6,7]. Bioactive compounds such as flavonoids and triterpenoids, isolated from other parts of the plant, have been linked to antioxidant and hepatoprotective activities [8,9].

The fruit fly, *Drosophila melanogaster*, has emerged as a powerful, cost-effective model organism for studying metabolic diseases [10]. Its insulin-signaling pathways are highly conserved with those of humans, and a high-sucrose diet reliably induces a type II diabetic phenotype characterized by hyperglycemia, insulin resistance, and oxidative stress [11,12]. This model allows for rapid *in vivo* screening of therapeutic candidates.

While previous studies have indicated antidiabetic potential in other parts of *P. curatellifolia* [6,13], the effects of its leaf extract in a genetically tractable model organism like *D. melanogaster*, and its concomitant impact on oxidative stress and behavior, remain unexplored. The present research seeks to address this lacuna by comprehensively assessing the antidiabetic and antioxidant potential of the ethanolic leaf extract of *P. curatellifolia* in a sucrose-induced diabetic fly model.

2. Materials and Methods

2.1. Materials and reagents.

Absolute ethanol, sucrose, and methylparaben were purchased from Sigma-Aldrich and Qualikems. The Glucose Assay Kit (GLU-PAP, Cat. No. GL 2613) and Total Protein Assay Kit (Cat. No. TP 2450) were obtained from RANDOX (United Kingdom). Metformin (Glucophage, 500 mg) was sourced from a local pharmacy. All other chemicals were of analytical grade.

2.2. Plant collection, authentication, and extract preparation.

Leaves of *Parinari curatellifolia* were collected in March 2023 from Oiji, Apa LGA, Benue State, Nigeria. The plant was authenticated by Mr. O. Owa, a taxonomist at the Federal College of Forestry, Jos (Voucher specimen number: FCFJ/PC/2317). The air-dried, pulverized leaves (206 g) were macerated in 2.5 L of absolute ethanol for 72 hours. The filtrate was concentrated using a rotary evaporator and subsequently freeze-dried (FreeZone 4.5, Labconco) at -50°C and 0.5 Pa. The extraction yield was calculated as 16.9% (w/w).

2.3. Phytochemical screening.

A qualitative phytochemical analysis was performed on the extract using standard procedures [14,15]. The tests confirmed the presence of flavonoids (alkaline reagent test), tannins (ferric chloride test), alkaloids (Dragendorff's test), saponins (frothing test), and terpenoids (Salkowski test).

2.4. Fly culture and experimental design.

The Harwich strain of *Drosophila melanogaster* was obtained from the Africa Centre of Excellence in Phytomedicine Research and Development (ACEPRD), University of Jos, Nigeria. Flies were maintained on a standard cornmeal-yeast-agar diet at 25°C with a 12:12 hour light-dark cycle. For all experiments, 2-3 day-old adult male flies were used. Flies were collected under mild ice anesthesia and randomly assigned to experimental groups to ensure unbiased distribution. The sample size of 50 flies per replicate (with 5 biological replicates per group) was chosen based on established protocols for *Drosophila* metabolic studies [11,16] to ensure sufficient power for statistical analysis.

The experimental design consisted of six groups. Group I served as the normal control, with flies maintained on a standard diet. Group II functioned as the diabetic control, where flies were fed a high-sucrose diet (2.5 g sucrose/10 g diet) to induce diabetes [11]. The treatment groups included Group III, which received the standard drug control consisting of diabetic flies treated with metformin (10 mg/10 g diet). Groups IV, V, and VI comprised the test groups, consisting of diabetic flies treated with P. curatellifolia extract at concentrations of 10 mg, 100 mg, and 200 mg per 10 g of diet, respectively.

Diabetes was established over a seven-day induction period, after which flies received treatment for seven days. The doses chosen for the main investigation were informed by preliminary survival tests, where no significant lethality was observed at concentrations reaching 200 mg per 10 g of diet.

2.5. Survival assay.

Five groups of flies (n=50 per replicate, 5 replicates per group) were fed diets containing 0, 10, 100, 200, or 400 mg of extract per 10 g of diet. Mortality was recorded daily for 28 days.

2.6. Locomotor performance (negative geotaxis assay).

The assay was performed as described by Abolaji et al. [16]. Ten flies per replicate were assessed. The number of flies crossing a 6 cm mark in 6 seconds was recorded. The procedure was repeated three times, and the score was expressed as the mean percentage of successful flies.

2.7. Sample preparation and biochemical assays.

On day 10, flies were anesthetized, rinsed in cold phosphate buffer (PBS, 0.1 M, pH 7.4), and homogenized (10 µL PBS/mg fly weight) on ice. Homogenates were centrifuged at 4,000 × g for 10 min at 4°C, and the supernatants were stored at -18°C for subsequent assays.

Glucose levels were quantified using a glucose oxidase-peroxidase (GOD-POD) kit (RANDOX), with results expressed as mg glucose per mL of homogenate. Total protein concentration was determined via the Biuret method using a RANDOX kit, and the results are presented as mg protein per mL of homogenate. Glutathione S-transferase (GST) activity was assessed by monitoring the conjugation of glutathione with 1-chloro-2,4-dinitrobenzene (CDNB) at 340 nm, following established protocols [17], with activity expressed as U/mg protein. Catalase (CAT) activity was assayed by measuring the decomposition rate of hydrogen peroxide (H_2O_2) at 240 nm [18], and the results are also reported as U/mg protein.

2.8. Statistical analysis.

Data are presented as mean ± standard deviation (SD) of five biological replicates. Statistical analysis was performed using GraphPad Prism 9.0. Data were checked for normality using the Shapiro-Wilk test. One-way analysis of variance (ANOVA) was used, followed by Tukey's post hoc test for multiple comparisons. Statistical significance was set at $p<0.05$.

3. Results and Discussion

3.1. Phytochemical screening and extraction yield.

Analysis of the ethanolic leaf extract revealed the presence of flavonoids through phytochemical screening, tannins, alkaloids, saponins, and terpenoids. The extraction yield was 16.9%.

3.2. Survival rate and extract toxicity.

The 28-day survival assay indicated that the *P. curatellifolia* extract was well-tolerated at concentrations up to 200 mg/10 g diet, with no significant mortality compared to the control group (p < 0.001 for 400 mg/10g diet vs. control; Figure 1).

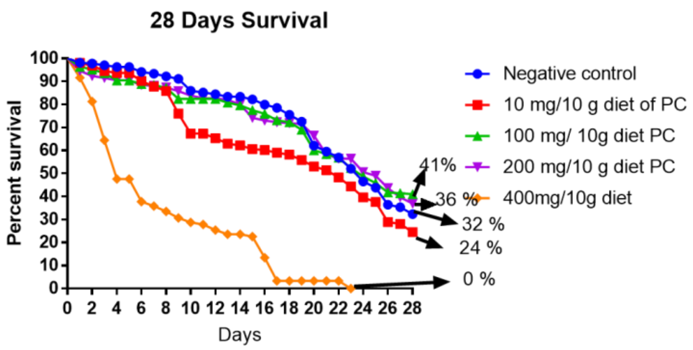

Figure 1. Survival rate of *D. melanogaster* after 28 days of exposure to *P. curatellifolia* extract. Data are presented as mean ± SD (n=5 replicates, each with 50 flies). The group treated with a 400 mg/10g diet is significantly different from the Control group (p < 0.001).

3.3. Locomotor performance is restored by extract treatment.

The negative geotaxis assay revealed a significant impairment in climbing ability in diabetic flies compared with normal controls (p < 0.001). Treatment with all concentrations of the extract significantly improved locomotor performance (p < 0.01 for 10 mg; p < 0.001 for 100 and 200 mg; Figure 2).

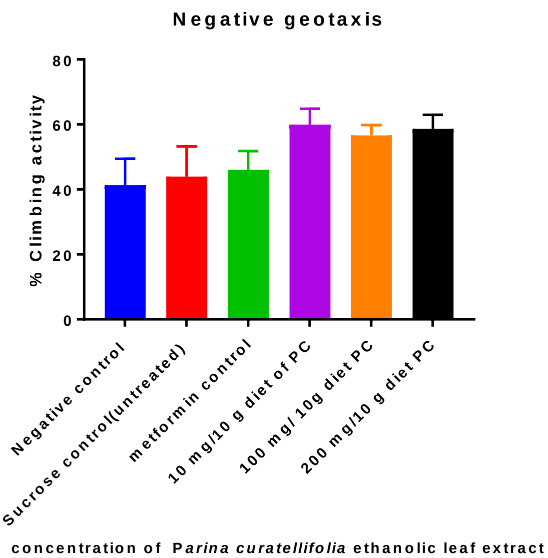

Figure 2. Locomotor performance of diabetic flies after 7-day treatment. Data are presented as mean ± SD (n=5 replicates, each with 10 flies assessed). **a** p < 0.001 vs. Normal Control; **b** p < 0.01; **c** p < 0.001 vs. Diabetic Control.

3.4. P. curatellifolia extract ameliorates hyperglycemia.

Induction of diabetes led to a significant increase ($p < 0.001$) in circulating glucose levels (Figure 3). Post-treatment with the extract resulted in a dose-dependent and significant reduction in glucose levels ($p < 0.05$ for 10 mg; $p < 0.001$ for 100 and 200 mg; Figure 4, Table 1).

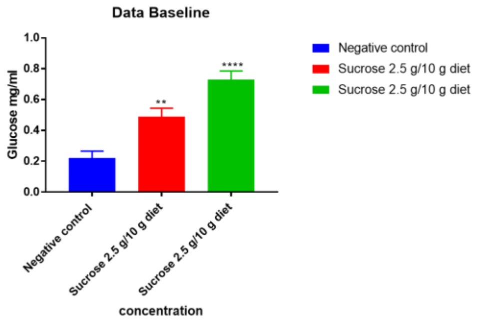

Figure 3. Circulating glucose levels post-induction of diabetes. Data are presented as mean ± SD (n=5 homogenate samples). *** $p < 0.001$ vs. Normal Control.

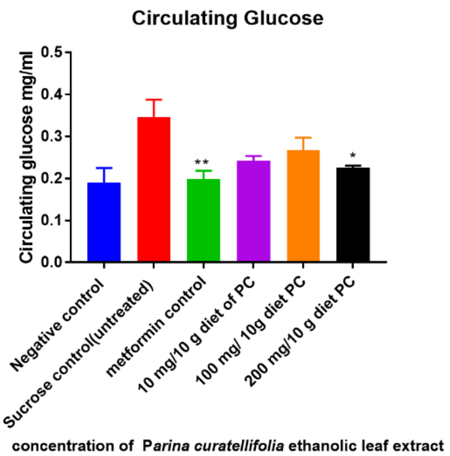

Figure 4. Glucose levels in diabetic flies after 7-day treatment. Data are presented as mean ± SD (n=5 homogenate samples). **a** $p < 0.001$ vs. Normal Control; **b** $p < 0.05$; **c** $p < 0.001$ vs. Diabetic Control.

3.5. Extract treatment enhances antioxidant defense systems.

Diabetic flies exhibited a significant decline in antioxidant markers. Treatment with the *P. curatellifolia* extract significantly reversed these effects (Table 1).

Table 1. Summary of biochemical parameters and locomotor performance in control and induced diabetic *D. melanogaster.*

Group	Glucose (mg/mL)	GST (U/mg protein)	Catalase (U/mg protein)	Total protein (mg/mL)	Locomotor performance (%)
Normal Control	0.08 ± 0.01	45.2 ± 3.5	25.8 ± 2.1	8.5 ± 0.6	92.0 ± 4.2
Diabetic Control	0.21 ± 0.02[a]	28.1 ± 2.8[a]	12.3 ± 1.5[a]	5.1 ± 0.4[a]	45.3 ± 5.1[a]
Metformin (10 mg)	0.09 ± 0.01[b]	40.5 ± 3.1[b]	22.1 ± 1.8[b]	7.8 ± 0.5[b]	85.7 ± 3.8[b]
PC (10 mg)	0.16 ± 0.02[b,c]	36.8 ± 2.9[b]	17.5 ± 1.6[b]	6.9 ± 0.5[b]	70.2 ± 4.5[b]
PC (100 mg)	0.12 ± 0.01[b]	39.1 ± 3.0[b]	20.8 ± 1.7[b]	7.2 ± 0.6[b]	78.9 ± 4.0[b]
PC (200 mg)	0.10 ± 0.01[b]	42.3 ± 3.2[b]	23.5 ± 1.9[b]	7.6 ± 0.5[b]	84.5 ± 3.9[b]

Data are presented as mean ± SD (n = 5). PC = *Parinari curatellifolia* extract; [a] Significantly different from the Normal Control group ($p < 0.001$); [b] Significantly different from the Diabetic Control group ($p < 0.05$). Specific p-values for comparisons with the Diabetic Control are as follows: *$p < 0.05$, **$p < 0.01$, ***$p < 0.001$ (see figures for detailed breakdown); [c] The 10 mg PC group for glucose was significantly different from the Diabetic Control at $p < 0.05$. All other [b] annotations for PC and Metformin groups indicate $p < 0.01$ or $p < 0.001$ versus Diabetic Control.

Treatment with the extract resulted in a significant increase in GST activity across all doses compared with the diabetic control group, with $p < 0.01$ for the 10 mg and 100 mg doses and $p < 0.001$ for the 200 mg dose, as illustrated in Figure 5. Similarly, total protein content was significantly elevated in all treatment groups relative to the diabetic control ($p < 0.01$; Figure 6). Furthermore, the extract restored catalase activity to near-normal levels; this enzyme, which was significantly reduced in the diabetic control flies, showed significant improvement following treatment ($p < 0.01$ for the 10 mg dose; $p < 0.001$ for the 100 mg and 200 mg doses; Figure 7).

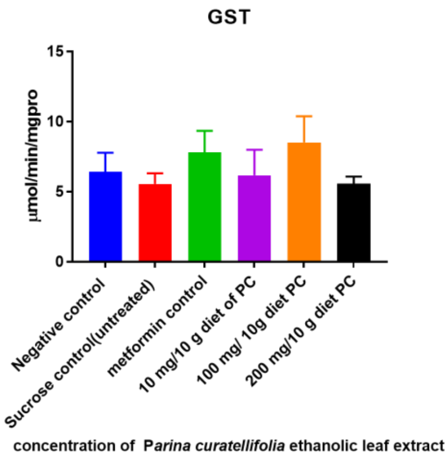

Figure 5. Glutathione S-transferase (GST) activity after treatment. Data are presented as mean ± SD (n=5). **a** $p < 0.001$ vs. Normal Control; **b** $p < 0.01$; **c** $p < 0.001$ vs. Diabetic Control.

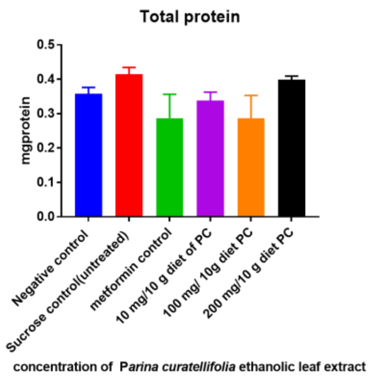

Figure 6. Total protein content after treatment. Data are presented as mean ± SD (n=5). **a** $p < 0.001$ vs. Normal Control; **b** $p < 0.01$ vs. Diabetic Control.

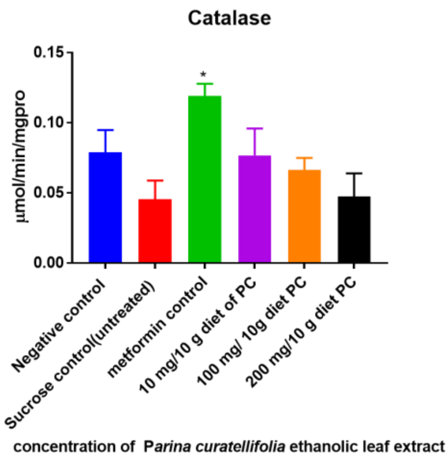

Figure 7. Catalase activity after treatment. Data are presented as mean ± SD (n=5). **a** $p < 0.001$ vs. Normal Control; **b** $p < 0.01$; **c** $p < 0.001$ vs. Diabetic Control.

3.6. Discussion.

This study provides compelling evidence that the ethanolic leaf extract of *Parinari curatellifolia* possesses significant antidiabetic and antioxidant properties in a sucrose-induced *Drosophila melanogaster* model of type II diabetes.

The most pronounced effect was the dose-dependent reduction in circulating glucose levels, with the highest dose performing comparably to metformin. This aligns with a previous study on the stem bark extract in flies [6]. The mechanism may involve enhanced insulin sensitivity or inhibition of carbohydrate-digesting enzymes, pathways conserved in *Drosophila* [12]. The presence of flavonoids and alkaloids, as identified in our phytochemical screen, is significant, as such compounds are known to influence glucose homeostasis through these mechanisms [4,8].

A key finding is the robust enhancement of the antioxidant defense system. The significant upregulation of GST and catalase activities suggests the extract mitigates diabetes-associated oxidative stress by both scavenging reactive oxygen species (ROS) and bolstering endogenous enzymatic defenses [19]. The increase in total protein content in treated flies may indicate a reduction in protein glycation and oxidative damage, common features of diabetic pathology [20]. The observed antioxidant activity can be attributed to the flavonoids and tannins present in the extract, which are well-documented for their free radical-scavenging properties [8,21]. These findings are consistent with studies on other plant extracts improving biochemical parameters in diabetic models [22,23].

The restoration of locomotor performance in treated flies is a functionally relevant outcome. Diabetes-induced oxidative stress is known to impair neuronal function and muscle integrity. The improvement in negative geotaxis suggests that the extract's antioxidant activity confers neuroprotective benefits, mitigating the motor deficits associated with the diabetic state [16,24].

While these results are promising, several limitations must be acknowledged. The study relied on a crude extract, and the specific bioactive compound(s) responsible for the effects are unknown. The use of a single fly strain and the lack of blinding during assessments are also limitations. Furthermore, the exclusive use of an invertebrate model means the translational relevance to mammals remains unverified.

4. Conclusions

In conclusion, the ethanolic leaf extract of *Parinari curatellifolia* effectively ameliorates key hallmarks of type II diabetes in *D. melanogaster*, including hyperglycemia, oxidative stress, and locomotor dysfunction. The bioactivity is likely mediated by the flavonoid and tannin constituents of the extract. These findings provide a strong scientific basis for the traditional use of *P. curatellifolia* and suggest its potential for further investigation as a therapeutic candidate. However, the therapeutic potential must be validated in mammalian models to assess bioavailability, pharmacokinetics, and safety before any clinical relevance can be inferred. Future studies should focus on the bioassay-guided fractionation of the extract to identify the active compound(s) and elucidate the precise molecular mechanisms involved.

Author Contributions

Conceptualization, S.S.B., N.P., and D.A.P.; methodology, E.Z.W.; software, P.I.K.; validation, S.S.B., E.Z.W., and P.I.K.; formal analysis, D.A.P.; investigation, S.S.B.; resources,

E.Z.W.; data curation, P.I.K.; writing original draft preparation, S.S.B.; writingrev iew and editing, E.Z.W.; visualization, P.I.K. and N.P.; supervision, E.Z.W.; project administration, S.S.B. All authors have read and agreed to the published version of the manuscript.

Institutional Review Board Statement

The study was conducted according to the guidelines for the care and use of invertebrates and was approved by the Institutional Review Board of the University of Jos (protocol code UJ/2023/001, date of approval 15 March 2023).

Informed Consent Statement

Not applicable.

Data Availability Statement

The data presented in this study are available within the article. Raw data are available on request from the corresponding author.

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Conflicts of Interest

The authors declare no conflict of interest.

Abbreviations

Abbreviation	Meaning
PC	*Parinari curatellifolia*
DM	Diabetes Mellitus
ROS	Reactive Oxygen Species
GST	Glutathione S-Transferase
CAT	Catalase
HSD	High-Sucrose Diet
PBS	Phosphate Buffer Solution
SD	Standard Deviation
ANOVA	Analysis of Variance

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