

ANTIOXIDANT AND ANTIHYPERGLYCEMIC EFFECTS OF *JUSTICIA CARNEA* LEAVES IN STREPTOZOTOCIN-INDUCED DIABETIC WISTAR RATS

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ABSTRACT

Diabetes mellitus (DM) is a chronic metabolic disorder characterised by persistent hyperglycemia arising from impaired insulin secretion, action, or both. The condition disrupts carbohydrate, lipid, and protein metabolism, resulting in widespread systemic complications. This study evaluated the proximate and phytochemical composition of *Justicia carnea* leaves as well as the antioxidant and antihyperglycemic effects of the methanol leaf extract in streptozotocin (STZ)-induced diabetic Wistar rats. Proximate and phytochemical analyses were conducted using standard procedures. For the in vivo study, thirty-six male Wistar rats (180–200 g) were randomly assigned to six groups. Group 1 served as the normal control, Group 2 was diabetic and untreated, Group 3 received metformin (50 mg/kg body weight (bw)), while Groups 4 to 6 received 100, 200, and 500 mg/kg bw of the methanol extract, respectively. Diabetes was induced using a single intraperitoneal injection of STZ (50 mg/kg bw). After 21 days of treatment, animals were euthanised to measure antioxidant enzyme activity in the pancreas, liver, and kidney. Phytochemical screening indicated the presence of flavonoids, tannins, steroids, phenols, and terpenoids. Proximate analysis revealed high carbohydrate (47.88%) and moisture (22.12%) contents, with low crude fat (0.23%) and protein (1.26%) levels. Untreated diabetic rats exhibited significantly elevated fasting blood glucose and reduced antioxidant enzymes—superoxide dismutase (SOD), catalase (CAT), and glutathione peroxidase (GPx) ($p < 0.05$). Treatment with *J. carnea* extract produced a dose-dependent reduction in fasting blood glucose and significant restoration of antioxidant enzyme activities ($p < 0.05$). These findings demonstrate that *Justicia carnea* leaves possess valuable nutritional and bioactive constituents and exhibit strong antioxidant potential, supporting their traditional use and suggesting therapeutic relevance in managing oxidative stress-associated diabetic complications.

Keywords: Antioxidant activity, Diabetes. *Justicia carnea*, Phytochemical composition, Proximate analysis.

INTRODUCTION

Diabetes mellitus (DM) is a chronic metabolic disorder characterised by persistent hyperglycemia resulting from impaired insulin secretion, insulin action, or both (ADA, 2023). The condition disrupts carbohydrate, lipid, and protein metabolism, culminating in progressive multisystemic complications (Smith *et al.*, 2021). Hyperglycemia induces structural and functional alterations in blood vessels, impairing microvascular and macrovascular integrity and contributing to retinopathy, nephropathy, neuropathy, and cardiovascular diseases (Johnson *et al.*, 2022; Zhao *et al.*, 2023;

Kumar *et al.*, 2024). Globally, the prevalence of diabetes has risen dramatically, driven by rapid urbanisation, lifestyle transitions, and demographic shifts, positioning DM as a significant public health concern (WHO, 2023).

Justicia carnea is widely used in traditional medicine for the management of diabetes, anaemia, and hypertension. The leaves are valued for their richness in bioactive constituents, including flavonoids, alkaloids, and phenolic acids, which are believed to underlie their therapeutic effects (Anyasor *et al.*, 2015). Previous studies have reported diverse pharmacological and nutraceutical properties of *J. carnea*, such as anti-inflammatory, hypoglycemic, antimicrobial, antiplasmodial, antioxidant, and antidiabetic activities (Correa, 2012; Anthonia *et al.*, 2019). These attributes highlight its potential utility in the development of herbal remedies and dietary supplements for chronic diseases, particularly those associated with oxidative stress (Anyasor *et al.*, 2015). Although evidence supporting its traditional use in anaemia management remains limited, the plant's broad phytochemical composition suggests possible additional health benefits (Wood *et al.*, 2020). Phytochemicals are naturally occurring plant compounds that contribute to colour, flavour, and defence mechanisms, and many have been shown to provide significant health benefits (Liu, 2013). These non-nutrient compounds—including flavonoids, carotenoids, alkaloids, and phenolic acids—exert protective effects by modulating detoxification pathways, interacting with cellular signalling cascades, and demonstrating strong antioxidant activity. Proximate analysis, which quantifies moisture, ash, lipids, proteins, and carbohydrates, is essential for determining the nutritional value and energy content of plant materials. Such analyses are critical in food science and nutrition for evaluating dietary quality and ensuring compliance with nutritional standards (AOAC, 2012).

Antioxidants play a fundamental role in neutralising free radicals and reducing oxidative stress, a major contributor to chronic diseases such as cancer, cardiovascular disorders, and neurodegenerative conditions. These comprise enzymatic antioxidants, including catalase (CAT) and superoxide dismutase (SOD), as well as non-enzymatic antioxidants such as vitamins C and E, and polyphenols. Dietary antioxidants, especially from plant sources, are recognised for their ability to scavenge reactive oxygen species and mitigate disease risk (Halliwell & Gutteridge, 2015). In light of the increasing utilisation of medicinal plants as nutraceuticals, further comprehensive analysis of the bioactive constituents of *J. carnea* is justified. Nutraceuticals are defined as formulations that contain vitamins, amino acids, minerals, medicinal plants, metabolites, extracts, or combinations thereof and are used for therapeutic rather than solely nutritional purposes (Nasri *et al.*, 2014). Conventional therapies for diabetes management, such as insulin secretagogues and insulin

sensitisers, are commonly prescribed to control blood glucose levels. However, these pharmacological interventions are frequently associated with undesirable side effects, which can impact patient adherence and overall quality of life. Furthermore, advanced medical approaches, including gene therapy and β -cell regeneration, offer promise for more definitive treatment but are largely inaccessible to the majority of patients in Nigeria due to their high cost and limited availability. Consequently, many households have turned to the use of medicinal plants as alternative or complementary therapies for the treatment and management of diabetes and its related complications. This practice reflects the need for affordable, accessible, and culturally accepted solutions in resource-limited settings (Osemwenkhae & Ojeaburu, 2024).

Given the growing interest in plant-based therapies, this study aims to evaluate the phytochemical composition and proximate profile of *Justicia carnea* leaves and to investigate the antihyperglycemic and antioxidant effects of the methanol extract in streptozotocin (STZ)-induced diabetic Wistar rats.

MATERIALS AND METHODS

Chemicals and Reagents

All chemicals and reagents used in this study were of analytical grade and were products of British Drug House (BDH), England, or Sigma-Aldrich, USA.

Plant Collection and Identification

Fresh leaves of *Justicia carnea* were collected from a botanical garden in Ovbiogie Community along the Benin-Lagos Expressway, Ugbowo, Benin City, Edo State, Nigeria. The plant was identified and authenticated in the Department of Plant Biology and Biotechnology, Faculty of Life Sciences, University of Benin, Benin City. A voucher specimen (UBH-J386) was deposited in the Departmental Herbarium for reference.

Preparation of Plant Sample

Fresh leaves of *Justicia carnea* were washed thoroughly and air-dried at room temperature. The dried leaves were pulverised using a mechanical grinder. The resulting powder was macerated in methanol for 72 hours with intermittent stirring to enhance extraction efficiency. The mixture was filtered through muslin cloth, and the filtrate was concentrated using a rotary evaporator at 50°C. Final drying was achieved using a freeze dryer to obtain the methanol extract of *Justicia carnea*, which was stored at -4°C until required for analysis.

Phytochemical Analysis

Glycosides

Glycosides were tested following the method of Sofowora (1996). A 0.5 g portion of the sample was mixed with 2 mL glacial acetic acid and one drop of ferric chloride solution. Thereafter, 1 mL concentrated sulphuric acid was added. The appearance of a brown ring indicated the presence of glycosides.

Flavonoids

Flavonoids were identified using the method of Harborne (1973). Exactly 0.5 g of powdered plant material was heated with 10 mL of ethyl acetate over a steam bath for 3 minutes. After filtration, 4 mL of the filtrate was mixed with 1 mL of dilute ammonia. A yellow coloration indicated flavonoids.

Tannins

Tannins were analyzed using the method of Harborne (1973). A 0.5 g portion of the powdered sample was boiled in 20 mL of distilled water, cooled, and filtered. Addition of 0.1% FeCl₃ to the filtrate produced a brownish-green or blue-black coloration, confirming the presence of tannins.

Saponins

Saponins were detected using the method of Obadoni and Ochuko (2001). Two grams of plant powder were boiled in 20 mL of distilled water, filtered, and 10 mL of the filtrate was mixed with 5 mL of distilled water and shaken vigorously. Persistent frothing followed by emulsion formation upon the addition of 3 drops of olive oil confirmed the presence of saponins.

Alkaloids

Alkaloids were tested using the methods of Harborne (1976) and Trease & Evans (1989). A 0.5 g sample was stirred with 5 mL of 1% aqueous HCl on a steam bath. A few drops of picric acid (1% w/v solution) were added to 2 mL of the extract. A reddish-brown precipitate indicated alkaloids.

Steroids

Steroids were identified using the Finar method (1986). To 0.5 g of the sample, 2 mL of acetic anhydride and 2 mL of concentrated H₂SO₄ were added. Distinct colour changes (pink, blue, green, or reddish-brown) indicated the presence of steroids.

Terpenoids

Terpenoids were screened using the method of Edeoga *et al.* (2005). Five millilitres of extract were mixed with 2 mL chloroform, followed by careful addition of 3 mL concentrated H₂SO₄. A reddish-brown interface indicated terpenoids.

Proximate Analysis

Proximate parameters assessed included moisture content, ash content, crude protein, crude fibre, crude fat, and carbohydrate. Moisture content was determined before further analysis. Crude protein, crude fibre, ash content, and crude fat were analysed using the micro-Kjeldahl and related procedures described by AOAC (1990), while carbohydrate content was determined according to AOAC (2000).

Antioxidant Assays

Antioxidant enzyme assays were performed as follows:

- **Catalase:** Determined by the method of Cohen *et al.* (1970).
- **Malondialdehyde (MDA):** Assessed using the method of Buege and Aust (1978).
- **Superoxide Dismutase (SOD):** Measured by the method of Misra and Fridovich (1972)
- **Glutathione Peroxidase (GPx):** Determined using the method of Nyman (1959).

Experimental Animals

A total of thirty-six (36) male Wistar rats (8 weeks old) weighing 180–200 g (mean weight = 190 ± 10 g) were used in this study. The animals were obtained from the animal house of the Department of Biochemistry, University of Benin, Benin City, Edo State, Nigeria. Rats were acclimatised for two weeks under hygienic conditions, housed in metal cages, and maintained under

standard laboratory conditions (room temperature, 55–65% humidity, 12-h light/12-h dark cycle). They had ad libitum access to pelletized grower's mash and clean drinking water. All experimental procedures conformed to the National Research Council (US) Institute for Laboratory Animal Research (Guide for the Care and Use of Laboratory Animals, Washington (DC):1996). Protocols were reviewed and approved by the Research Ethics Committee of the Faculty of Life Sciences, University of Benin, Benin City, Nigeria (Approval reference: FLSRE-2023-018).

Experimental Design

The rats were randomised into six groups of six animals each.

- Group 1: Normal control received standard feed and water.
- Groups 2–6: Diabetic groups induced via a single intraperitoneal injection of streptozotocin (50 mg/kg body weight).
 - Group 2: Diabetic, untreated.
 - Group 3: Diabetic, treated with metformin (25 mg/kg bw).
 - Groups 4–6: Diabetic, treated with methanol extract of *Justicia carnea* leaves at doses of 100, 200, and 500 mg/kg bw, respectively.

Treatments were administered for 28 days. At the end of the study, rats were fasted overnight, sacrificed, and the pancreas, liver, and kidney were harvested, blotted dry, and weighed. Portions of the tissues were homogenised for biochemical assays. Tissue homogenates were prepared by homogenising 1 g of each tissue in 5 mL ice-cold saline using a ceramic pestle and mortar on ice. The homogenates were centrifuged at 3500 rpm for 20 minutes, and the supernatants were collected and stored at -4°C until analysis.

Acute Toxicity Test

An oral acute toxicity study of the methanol extract of *Justicia carnea* leaves was performed according to Lorke (1983). Eighteen (18) Wistar rats (180–200 g, mean 190 ± 10 g) were used in a two-phase study.

- **Phase 1:** Rats were randomized into three groups ($n = 3$) and given single oral doses of 10, 100, or 1000 mg/kg bw via gavage. Animals were observed for signs of toxicity at 60 minutes post-administration and continuously monitored for 24 hours.
- **Phase 2:** Based on the absence of mortality in Phase 1, three rats per group received higher single doses (1500, 2500, and 5000 mg/kg bw). Animals were monitored for 24 hours, with extended observation for 48 hours to assess delayed toxicity or mortality.

STATISTICAL ANALYSIS

Data are expressed as mean \pm standard error of the mean (SEM). Statistical analysis was performed using SPSS version 20. Treatment groups were compared using Duncan's multiple range test, and differences were considered statistically significant at $p < 0.05$.

RESULTS

Phytochemical Constituents of *Justicia carnea* Leaves

Qualitative phytochemical analysis of *Justicia carnea* leaves revealed the presence of flavonoids, tannins, steroids, phenols, and terpenoids. Other tested phytochemicals, including cardiac

glycosides, saponins, phlobatannins, coumarin, alkaloids, and anthraquinones, were absent (Table 1).

Table 1. Phytochemical Constituents of *Justicia carnea* Leaves

Phytochemical Component	Relative Composition
Flavonoids	++
Tannins	++
Cardiac Glycosides	–
Saponin	–
Steroids	++
Phenols	++
Phlobatannins	–
Coumarin	–
Alkaloids	–
Anthraquinone	–
Terpenoids	++

Key: – Absent/Not detected; + Present (low); ++ Present (high)

Proximate Composition of *Justicia carnea* Leaves

The proximate analysis (Table 2) showed that *J. carnea* leaves are rich in carbohydrates (47.88%) and moisture (22.12%), with moderate fiber and mineral content (11.53% and 16.97%, respectively). Crude protein (1.26%) and crude fat (0.23%) were low. These findings indicate that the leaves could provide a source of dietary energy and fiber, while contributing to essential minerals, but are not significant sources of protein or fat.

Table 2. Proximate Composition of *Justicia carnea* Leaves

Proximate Component	Amount (g% dry weight)
Moisture Content	22.12 ± 0.32
Ash Content	16.97 ± 0.09
Crude Fibre	11.53 ± 1.07
Crude Fat	0.23 ± 0.03
Crude Protein	1.26 ± 0.03
Carbohydrate	47.88 ± 1.31

Values are expressed as mean \pm SEM of three determinations.

Acute Toxicity Test of Methanol Extract of *J. carnea* Leaves

Oral administration of the methanol extract at doses up to 5000 mg/kg did not cause mortality or observable signs of toxicity in rats during the 72-hour observation period, indicating that the extract is relatively safe at the tested doses (Tables 3 and 4).

Table 3. Phase 1 Acute Toxicity Test

Dose (mg/kg bw)	Mortality
10	0/3
100	0/3
1000	0/3

Table 4. Phase 2 Acute Toxicity Test

Dose (mg/kg bw)	Mortality
1500	0/3
2500	0/3
5000	0/3

Effect on Fasting Blood Glucose in STZ-Induced Diabetic Rats

As shown in Table 5, STZ administration resulted in a significant increase in fasting blood glucose (FBS) levels in diabetic groups compared to the normal control ($p < 0.05$), confirming successful induction of diabetes. Untreated diabetic rats (Group 2) maintained elevated glucose levels throughout the study (final FBS: $369.33 \pm$

28.01 mg/dL). Treatment with the methanol extract of *J. carnea* leaves significantly reduced FBS levels in diabetic rats in a dose-dependent manner. Notably, Group 6 (500 mg/kg) showed a marked reduction from 297.67 ± 43.31 mg/dL post-induction to 53.00 ± 12.00 mg/dL after 21 days of treatment. The metformin-

treated group (Group 3) and other extract-treated groups (Groups 4 and 5) also exhibited significant reductions ($p < 0.05$). The normal control group (Group 1) maintained stable FBS throughout the study.

Table 5. Effect of Methanol Extract of *J. carnea* Leaves on Fasting Blood Glucose in STZ-Induced Diabetic Rats

Group	Initial FBS	Day 3 FBS	Day 7 FBS	Day 14 FBS	Day 21 FBS
1	76.00 ± 4.15	76.00 ± 4.15	93.67 ± 2.53 ^b	92.33 ± 2.37 ^b	65.50 ± 3.42 ^b
2	94.67 ± 14.45	305.00 ± 27.40 ^a	475.00 ± 41.02 ^a	368.00 ± 54.08 ^a	369.33 ± 28.01 ^a
3	62.20 ± 3.88	310.80 ± 56.81 ^a	286.40 ± 55.00 ^{ab}	204.60 ± 1.17 ^{ab}	168.00 ± 76.00 ^{ab}
4	58.80 ± 4.11	283.20 ± 50.71 ^a	223.00 ± 58.15 ^{ab}	222.00 ± 59.05 ^{ab}	171.20 ± 67.12 ^{ab}
5	56.80 ± 1.65	370.20 ± 49.42 ^a	321.75 ± 53.10 ^{ab}	295.00 ± 112.16	222.33 ± 62.22 ^{ab}
6	59.17 ± 6.32	297.67 ± 43.31 ^a	240.20 ± 70.83 ^{ab}	190.00 ± 55.08 ^{ab}	53.00 ± 12.00 ^b

Values are expressed as Mean ± SEM (n=3). Values with superscript "a" are significantly different from the normal control, while values with superscript "b" are significantly different from the diabetic control ($p < 0.05$). Values with superscripts "ab" are significantly different from both normal and diabetic controls ($p < 0.05$) but not significantly different when compared to the Metformin-treated groups.

Effect on Antioxidant Activities in the Pancreas

The methanol extract significantly enhanced antioxidant enzyme activities (SOD, CAT, and GPx) in the pancreas of diabetic rats (Groups 3 to 6) compared with the diabetic control group ($p < 0.05$), indicating restoration of antioxidant defence systems (Table 6). Malondialdehyde (MDA), a marker of lipid peroxidation, was significantly reduced in Groups 3–5. Group 6 exhibited the highest antioxidant enzyme activity but also a comparatively higher MDA level, suggesting a complex interaction between antioxidant defences and oxidative stress. Overall, the extract demonstrated substantial *in vivo* antioxidant activity, supporting its potential to mitigate oxidative damage associated with diabetes.

Effect of Methanol Extract of *Justicia carnea* Leaves on Antioxidant Activities Pancreas

The methanol extract of *J. carnea* leaves significantly improved antioxidant enzyme activities (SOD, CAT, GPx) in the pancreas of STZ-induced diabetic rats (Groups 3–6) compared to the diabetic control (Group 2) (Table 6). Malondialdehyde (MDA) levels, a marker of lipid peroxidation, were significantly reduced in Groups 3–5. Group 6 displayed the highest SOD, CAT, and GPx activities but also elevated MDA levels, suggesting partial protection against oxidative stress at this dose. These results indicate that the extract enhances pancreatic antioxidant defences in diabetic conditions.

Table 6. Antioxidant Activities in the Pancreas of STZ-Induced Diabetic Rats Treated with Methanol Extract of *J. carnea* Leaves

Parameter	Control	Group 2	Group 3	Group 4	Group 5	Group 6
SOD (unit/mg protein)	0.980 ± 0.08	1.107 ± 0.00	1.273 ± 0.00	1.137 ± 0.08	1.651 ± 0.00 ^{ab}	2.659 ± 0.00 ^{ab}
CAT (unit/mg protein)	0.370 ± 0.06 ^b	0.072 ± 0.00	0.466 ± 0.0 ^b	0.485 ± 0.05 ^b	0.820 ± 0.00 ^{ab}	0.948 ± 0.00 ^{ab}
GPx (u/L)	2.589 ± 0.349	1.774 ± 0.00	3.320 ± 0.5 ^b	3.319 ± 0.41 ^b	5.711 ± 0.00 ^{ab}	6.536 ± 0.00 ^{ab}
MDA (unit/mg protein)	1.503 ± 0.146	1.333 ± 0.00	1.659 ± 0.42	1.863 ± 0.14 ^b	1.235 ± 0.00	4.179 ± 0.00 ^{ab}

Values are expressed as Mean ± SEM (n=6). Values with superscript "a" are significantly different from the normal control, while values with superscript "b" are significantly different from the diabetic control ($p < 0.05$). Values with superscripts "ab" are significantly different from both normal and diabetic controls ($p < 0.05$) but not significantly different when compared to the Metformin-treated groups.

Kidney

In the kidney, SOD and CAT activities were significantly reduced in Groups 2–4 compared to the normal control ($p < 0.05$), while GPx activity showed no significant difference across treated groups

relative to controls. MDA levels were significantly higher in all diabetic groups compared to the normal control, indicating ongoing oxidative stress. These findings suggest that the extract partially restores antioxidant activity in the kidney of diabetic rats (Table 7).

Table 7. Antioxidant Activities in the Kidney of STZ-Induced Diabetic Rats Treated with Methanol Extract of *J. carnea* Leaves

Parameter	Control	Group 2	Group 3	Group 4	Group 5	Group 6
SOD (unit/mg protein)	1.33 ± 0.07 ^b	0.93 ± 0.20 ^a	0.83 ± 0.07 ^a	0.73 ± 0.04 ^a	1.03 ± 0.62	1.11 ± 0.20
CAT (unit/mg protein)	0.55 ± 0.02 ^a	0.34 ± 0.08 ^a	0.29 ± 0.02 ^a	0.26 ± 0.02 ^a	0.37 ± 0.02 ^a	0.39 ± 0.06 ^a
GPx (u/L)	2.65 ± 0.07	2.36 ± 0.56	1.89 ± 0.19	1.74 ± 0.10	2.50 ± 0.15	2.64 ± 0.45
MDA (unit/mg protein)	0.86 ± 0.02 ^b	0.28 ± 0.02 ^a	0.54 ± 0.05 ^{ab}	0.67 ± 0.02 ^{ab}	0.59 ± 0.08 ^{ab}	0.38 ± 0.06 ^a

Values are expressed as Mean ± SEM (n=6). Values with superscript "a" are significantly different from the normal control, while values with superscript "b" are significantly different from the diabetic control ($p < 0.05$). Values with superscripts "ab" are significantly different from both normal and diabetic controls ($p < 0.05$) but not significantly different when compared to the Metformin-treated groups.

Liver

In the liver, SOD activity was largely maintained across groups, except for reductions in Groups 4 and 6 relative to the diabetic control group. CAT activity and MDA levels were significantly altered in Group 6, suggesting increased oxidative stress at this

dose. GPx activity decreased in Groups 5 and 6, indicating limited protection against oxidative stress at higher extract concentrations. Overall, the methanol extract modulated liver antioxidant enzymes and partially mitigated oxidative damage in diabetic rats (Table 8).

Table 8. Antioxidant Activities in the Liver of STZ-Induced Diabetic Rats Treated with Methanol Extract of *J. carnea* Leaves

Parameter	Control	Group 2	Group 3	Group 4	Group 5	Group 6
SOD (unit/mg protein)	1.09± 0.02	1.30±0.00	1.12±0.14	1.05±0.04 ^b	1.10±0.11	0.89±0.00 ^b
CAT (unit/mg protein)	0.47±0.03	0.46±0.00	0.40±0.05	0.45 ± 0.03	0.40±0.04	0.33±0.00 ^{ab}
GPx (u/L)	2.43± 0.06	2.85±0.00	2.55±0.30	2.40 ± 0.10	2.21±0.22 ^a	1.90±0.05 ^{ab}
MDA (unit/mg protein)	0.33±0.03 ^b	0.70±0.0 ^a	0.33±0.00 ^b	0.37±0.03 ^b	0.45±0.00 ^{a,b}	0.40±0.02 ^{ab}

Values are expressed as Mean ± SEM (n= 6). Values with superscript “a” are significantly different from the normal control, while values with superscript “b” are significantly different from the diabetic control (p < 0.05). Values with superscripts “ab” are significantly different from both normal and diabetic controls (p < 0.05) but not significantly different when compared to the Metformin-treated groups.

DISCUSSION

Diabetes mellitus is a chronic metabolic disorder characterised by persistent hyperglycemia due to inadequate insulin secretion, insulin resistance, or both (Kangraikar *et al.*, 2010). In this study, induction of diabetes with 50 mg/kg body weight of streptozotocin (STZ) resulted in a sharp elevation of blood glucose levels, which were significantly reduced after 28 days of treatment with various doses of *Justicia carnea* leaf extract. This indicates that the combined effects of the plant’s phytochemical composition, nutritional profile, and its capacity to enhance antioxidant defences can ameliorate the deleterious effects of diabetes on various organs.

Phytochemical analysis of *J. carnea* leaves revealed the presence of flavonoids, tannins, phenols, steroids, and terpenoids, consistent with previous reports (Onyeabo *et al.*, 2017). These bioactive compounds have been documented to exert synergistic antihyperglycemic effects (Gaikwad *et al.*, 2014). Proximate analysis demonstrated that the leaves are rich in carbohydrates (47.88 %), fibre (11.53 %), and ash (16.97 %), but low in protein (1.26 %) and fat (0.23 %). Dietary fibre plays a crucial role in slowing glucose absorption, thereby reducing postprandial hyperglycemia and mitigating the risk of complications (Boutwell, 1988).

Oxidative stress is a key factor in the pathophysiology of diabetes. The antioxidant enzymes superoxide dismutase (SOD), glutathione peroxidase (GPx), and catalase (CAT) serve as primary defences against reactive oxygen species (ROS). In this study, the methanol extract of *J. carnea* significantly enhanced the activity of these enzymes in the pancreas, as evidenced by the reduction of malondialdehyde (MDA) levels compared with the diabetic control group (Table 6). Similar improvements were observed in the kidney and liver (Tables 7 and 8), particularly at higher extract doses. These results suggest that *J. carnea* mitigates oxidative damage induced by STZ, supporting its traditional use in the management of diabetes and its associated oxidative stress.

Collectively, the findings indicate that *J. carnea* leaves exhibit potent antihyperglycemic and antioxidant activities, likely attributable to their rich phytochemical composition and nutritional content. The enhancement of endogenous antioxidant defences demonstrates the plant’s potential to protect against diabetes-induced oxidative damage in multiple organs.

Conclusion

This study demonstrates that *Justicia carnea* leaves possess significant phytochemical and nutritional properties, alongside notable antioxidant activity. These findings provide a scientific basis for its traditional and medicinal use, particularly in the management of diabetes and its complications. Further

investigations are warranted to isolate and characterise the bioactive compounds, standardise dosing regimens, and elucidate the precise mechanisms underlying the therapeutic effects of *J. carnea* leaf extract.

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Declaration of Competing Interest

The authors declare no conflicts of interest related to this study.

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REFERENCES

- ADA (American Diabetes Association). (2023). Classification and diagnosis of diabetes: Standards of Medical Care in Diabetes—2023. *Diabetes Care*, 46(Supplement 1), S19–S40. <https://doi.org/10.2337/dc23-S002>
- Anthonia, O. C., Ikechukwu, W. R., Uzoma, N. O., & Sunday, E. L. U. (2019). Nutritive properties of aqueous extract of *Justicia carnea* leaves and its effects on haematological and some biochemical indices of anaemia-induced male Wistar albino rats. *Biomedical Research*, 30(4), 645–654.
- Anyasor, G. N., Ogunwenmo, K. O., Ajayi, D., & Obinna, M. (2015). Mechanisms of hypoglycemic and antidiabetic actions of *Justicia carnea* leaf extract. *Journal of Ethnopharmacology*, 171, 10–20.
- AOAC. (1990). *Official methods of analysis of the Association of Official Analytical Chemists* (15th ed., Vol. II, Sec. 985.29). AOAC.
- AOAC. (2000). *Official methods of analysis of the Association of Official Analytical Chemists* (14th ed.). AOAC.
- AOAC International. (2012). *Official methods of analysis of AOAC International* (19th ed.). AOAC International.
- Boutwell, R. J. (1988). Dietary fiber and glucose metabolism. *American Journal of Clinical Nutrition*, 48(4), 1078–1084.
- Buege, J. A., & Aust, S. D. (1978). Microsomal lipid peroxidation. In S. Fleischer & L. Packer (Eds.), *Methods in enzymology* (Vol. 52, pp. 302–310). Academic Press.
- Cohen, G., Dembiec, D., & Marcus, J. (1970). Measurement of catalase activity in tissue extracts. *Analytical Biochemistry*, 34(1), 30–38.
- Correa, G. M. (2012). Chemical constituents and biological

- activities of species of *Justicia*: A review. *Brazilian Journal of Pharmacognosy*, 22, 220–238.
- Edeoga, H. O., Okwu, D. E., & Mbaebie, B. O. (2005). Phytochemical constituents of some Nigerian medicinal plants. *African Journal of Biotechnology*, 4(7), 685–688.
- Finar, G. (1986). *Plants of economic importance: Medicinal plants and medicine in Africa*. Spectrum Books Ltd.
- Gaikwad, S. B., Mohan, G. K., & Rani, M. S. (2014). Phytochemicals for diabetes management. *Pharmaceutical Crops*, 5(1), 11–28.
- Halliwell, B., & Gutteridge, J. M. C. (2015). *Free radicals in biology and medicine* (5th ed.). Oxford University Press.
- Harborne, J. B. (1973). Phenolic compounds. In *Phytochemical methods: A guide to modern techniques of plant analysis* (pp. 33–88).
- Harborne, J. B. (1976). A unique pattern of anthocyanins in *Daucus carota* and other Umbelliferae. *Biochemical Systematics and Ecology*, 4(1), 31–35.
- Johnson, R. J., Sánchez-Lozada, L. G., Andrews, P., & Devaraj, S. (2022). Hyperglycemia and vascular complications: A comprehensive review. *Journal of Vascular Research*, 59(3), 112–123.
- Kangralkar, V. A., Patil, S. D., & Bandivadekar, R. M. (2010). Oxidative stress and diabetes: A review. *International Journal of Pharmaceutical Applications*, 1(1), 38–45.
- Kumar, S., Singh, R., & Gupta, R. (2024). Cardiovascular complications in diabetes mellitus: A review. *Cardiovascular Diabetology*, 23(1), 50. <https://doi.org/10.1186/s12933-024-02050-y>
- Liu, R. H. (2013). Health-promoting components of fruits and vegetables in the diet. *Advances in Nutrition*, 4(3), 384–392.
- Lorke, D. (1983). A new approach to practical acute toxicity testing. *Archives of Toxicology*, 54, 275–287.
- Misra, H. P., & Fridovich, I. (1972). The role of superoxide anion in the autoxidation of epinephrine and a simple assay for superoxide dismutase. *Journal of Biological Chemistry*, 247(10), 3170–3175.
- Nasri, H., Baradaran, A., Shirzad, H., & Rafieian-Kopaei, M. (2014). New concepts in nutraceuticals as an alternative to pharmaceuticals. *International Journal of Preventive Medicine*, 5(12), 1487–1499.
- National Research Council. (1996). *Guide for the care and use of laboratory animals*. National Academies Press. <https://doi.org/10.17226/5140>
- Nyman, M. (1959). Glutathione peroxidase in erythrocytes: Method for determination and some biochemical aspects. *Scandinavian Journal of Clinical and Laboratory Investigation*, 11(4), 353–358.
- Obadoni, B. O., & Ochuko, P. O. (2001). Phytochemical studies and comparative efficacy of crude extracts of some homeostatic plants in Edo and Delta States of Nigeria. *Global Journal of Pure and Applied Sciences*, 8(1), 203–208.
- Onyeabo, C., Ekechi, M. O., Ojinnaka, M. C., & Omeke, N. O. (2017). Phytochemical composition, mineral content, and in vitro antioxidant activity of *Justicia carnea* leaves. *Biokemistri*, 29(1), 1–8.
- Osemwenkhae, P. O., & Ojeaburu, S. I. (2024). Hypoglycemic and hepatoprotective potentials of dichloromethane (DCM) fraction of *Gongronema latifolium* extract in streptozotocin-induced diabetic Wistar rats. *Journal of Medical and Health Studies*, 5(2), 76–84. <https://doi.org/10.32996/jmhs.2024.5.2.10>
- Smith, A., Lee, J., & Miller, H. (2022). Pathophysiology and management of diabetes mellitus: Recent advances. *Annual Review of Medicine*, 73, 21–37.
- Sofowora, A. (1996). Research on medicinal plants and traditional medicine in Africa. *The Journal of Alternative and Complementary Medicine*, 2(3), 365–372.
- Trease, G. E., & Evans, W. C. (1978). *Pharmacology* (11th ed.). Bailliere Tindall.
- Wood, J., Yasmin-Karim, S., Moreau, M., Kumar, R., Akwanwi, J., Derek, A., Atoneche, F., Kress, J., & Ngwa, W. (2020). Characterization of isolated extracts from *Justicia* plant leaves used as a remedy for anemia. *Molecules*, 25(3), 534.
- World Health Organization. (2023). *Global report on diabetes*. WHO <https://www.who.int/publications/i/item/9789240068526>
- Zhao, Y., Zhang, H., & Chen, J. (2023). Microvascular and macrovascular complications in diabetes: Pathophysiology and clinical implications. *Frontiers in Endocrinology*, 14, 931018. <https://doi.org/10.3389/fendo.2023.931018>