Evaluation of Phytochemical Composition, Proximate Analysis and Antioxidant Activity of *Justicia Carnea* Leaves on Streptozotocin – Induced Diabetic Wistar Rats

Samuel I. Ojeaburu[1] and Nathan Eimoga[1]

[1]Department of Biochemistry, Faculty of Life Sciences, University of Benin, Benin City, Nigeria

DOI: https://doi.org/10.51244/IJRSI.2024.11110034

Received: 22 October 2024; Accepted: 03 November 2024; Published: 06 December 2024

ABSTRACT

Introduction/Aim: Diabetes mellitus (DM) is a long-term metabolic condition characterized by consistently high blood sugar levels resulting from issues with insulin secretion, function, or both. This disorder, disrupts the normal breakdown and utilization of carbohydrates, lipids, and proteins, leading to a range of complications throughout the body. This study analyzed the proximate and phytochemical compositions of *Justicia carnea* leaves, a commonly used herbal plant in folk medicine and determined the antioxidant potential of the methanol extract of the leaves in streptozotocin (STZ)-induced diabetic wistar rats.

Materials and Methods: Standard methods were employed to determine the proximate and phytochemical composition of the plant. For the antioxidant studies, a total of Thirty-six (36) male wistar rats weighing 180 to 200 g (mean weight = 190 ± 10 g) were divided into six (6) groups of six (6) rats each. Group 1 served as the normal control and received only water and grower's pellet throughout, group 2 was induced with diabetes, but untreated, group 3 animals were induced with diabetes and treated with 50 mg/kg bw of metformin), groups 4, 5 and 6 were induced with diabetes and treated with 100, 200 and 500 mg/kg bw of methanol extract of *Justicia carnea* leaves respectively. Diabetes mellitus was induced in the rats by intraperitoneal injection of 50 mg/kg bw of STZ. After 21 days of the treatment, the animals were sacrificed and the pancreas, liver and kidney tissues were collected for biochemical analyses.

Results: Phytochemical screening revealed the presence of flavonoids, tannins, steroids, phenols, and terpenoids, indicating significant bioactive potential. Proximate analysis showed high carbohydrate (47.88%) and moisture (22.12%) content, with lower levels of crude fat (0.23%) and protein (1.26%). A significant ($p<0.05$) increase in the levels of fasting blood glucose and a significant ($p<0.05$) decrease in enzymic antioxidants, superoxide dismutase (SOD), catalase (CAT), and glutathione peroxidase in the pancreas, liver and kidney of diabetic rats were observed. Oral administration of methanol extract of *Justicia carnea* leaves, significantly ($p<0.05$) decreased fasting blood glucose and improved the antioxidant status of diabetic rats in a dose dependent manner.

Conclusion: This study highlights the significant proximate and phytochemical compositions of *Justicia carnea* leaves, revealing a high content of essential nutrients and bioactive compounds. The notable antioxidant activity observed, supports the plant's use in traditional medicine and underscores its potential in the management of oxidative stress-induced diabetic complications.

Keywords: *Justicia carnea*, phytochemical composition. proximate analysis, antioxidant activity, Diabetes.

INTRODUCTION

Justicia carnea is locally used for its health benefits, including its use in the treatment of diabetes, anemia and hypertension. The leaves of the plant are particularly valued for their high content of bioactive compounds such as flavonoids, alkaloids, and phenolic acids, which are believed to contribute to its therapeutic effects [1]. Research has shown that *Justicia carnea* has a range of pharmacological characteristics and nutraceutical qualities, including anti-inflammatory, hypoglycemic, antimicrobial, antioxidant effects, antiplasmodial, and anti-diabetic properties [2,3]. These characteristics make it a viable option for the creation of herbal treatments

ISSN No. 2321-2705 | DOI: 10.51244/IJRSI |Volume XI Issue XI November 2024

and dietary supplements meant to treat chronic diseases including diabetes and disorders associated with oxidative stress [1]. The use of *Justicia carnea* leaf as a nutraceutical to treat physiological difficulties and prevent the development of chronic illnesses may have additional health benefits, despite the paucity of evidence supporting its use to treat anemia [4]. Phytochemicals are naturally occurring compounds found in plants that enhance their color, flavor, and ability to withstand disease [5]. These substances have been demonstrated to offer health benefits, including the prevention of chronic diseases like cancer, heart disease, and diabetes, even though they are not necessary nutrients like vitamins and minerals. They include a wide range of compounds such as flavonoids, carotenoids, alkaloids, and phenolic acids. Their mechanisms of action involve modulating detoxifying enzymes, interacting with cellular signaling networks, and exhibiting antioxidant activity [5]. Proximate analysis refers to the quantitative examination of food's or biological samples' main constituents, such as moisture, ash (minerals), lipids (fats), proteins, and carbohydrates. Proximate analysis provides valuable information about the nutritional content and energy value of the sample. This form of analysis is crucial in food science and nutrition for developing diet plans, assessing food quality, and ensuring compliance with nutritional labeling standards [6]. Antioxidants are molecules that inhibit the oxidation of other molecules, thereby protecting cells from damage caused by free radicals. A variety of chronic diseases such as cancer, neurological problems, and cardiovascular diseases, are linked to oxidative stress, which is caused by an imbalance between free radicals and antioxidants. There are two categories of antioxidants: enzymatic (such as catalase and superoxide dismutase) and non-enzymatic (like vitamin C, vitamin E, and flavonoids). Antioxidants found in food, especially those from fruits and vegetables, are important in scavenging free radicals and lowering the chance of illness [7]. Additionally, as dietary supplements are regarded as nutraceuticals when they are utilized for health-related rather than nutritional objectives, there is a need for continued and additional research or characterization of the bioactive component profile of *Justicia carnea* leaf. Nutraceutical supplements are defined as formulations that contain a minimum of one vitamin, one amino acid, one mineral, one medicinal plant, one concentrate metabolite, extracts, or a combination of these constituents [8]. Diabetes mellitus (DM) is a long-term metabolic condition defined by persistently high blood sugar levels caused by problems with insulin secretion, function, or both. This disorder, known simply as diabetes, interferes with the regular breakdown and utilization of carbohydrates, lipids, and proteins, resulting in various consequences throughout the body [9]. Diabetes develops gradually because of various complicated and interconnected physiological processes, leading to a wide range of symptoms that worsen over time [10]. Hyperglycaemia and its related metabolic disruptions have a negative impact on the functioning of several organs, compromising the integrity of both small and large blood vessels. The development of vascular problems plays a crucial role in the development of organ damage in individuals with diabetes. This can result in retinopathy, nephropathy, and cardiovascular diseases [11,12]. Prolonged elevation of blood sugar levels, known as chronic hyperglycaemia, initiates alterations in the structure and function of the blood vessels. These changes lead to impaired organ function and failure, with notable impact on organs such as the eyes, kidneys, heart, and peripheral nerves [13]. The global prevalence of diabetes has escalated, positioning it as one of the most pressing public health challenges of the 21st century. This rise has been driven by rapid urbanization, economic development, and lifestyle changes, which have contributed to increased incidence rates worldwide [14]. As such, addressing diabetes and its complications remains a critical focus for health research and intervention strategies. The current diabetes treatment regimen that focuses on insulin secretion and insulin sensitization results in undesirable side effects in patients. In addition, gene therapy as well as induced β-cells regeneration are not widely used in Nigeria as most patients cannot afford the cost associated with such treatment [15]. As a result of this predicament, a sizeable number of households in Nigeria rely heavily on herbal medicines for the management of diabetic disease conditions. Thus, the objective of this research is to assess the phytochemical makeup and the proximate analysis of *Justicia carnea* leaves as well as determine the antihyperglycemic and antioxidant potential of the methanol extract of the leaves of *Justicia carnea* in STZ-induced diabetic wistar rats.

MATERIALS AND METHODS

Chemicals and Reagents

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

ISSN No. 2321-2705 | DOI: 10.51244/IJRSI |Volume XI Issue XI November 2024

Plant collection and identification

The plant sample, *Justicia carnea* fresh leaves were obtained from a botanical garden in Ovbiogie community along Benin – Lagos express road, Ugbowo, Benin city, Edo state, Nigeria. The identification and authentication of the plant material was done by Dr. H.A. Akinnibosun, a taxonomist, in the Department of Plant Biology and Biotechnology, Faculty of Life Sciences, University of Benin, Benin City, Nigeria and a specimen voucher (UBH-J386) was deposited in the Department's Herbarium for reference.

Preparation of plant sample

Fresh samples of *Justicia carnea* leaves were washed and air-dried. The dried leaves were pulverized using a mechanical grinder. The powdered sample was then soaked in methanol for 72 hours for extraction, during this period the solution was stirred at intervals using a stirring rod for proper extraction. The solution was filtered with a muslin cloth and the filtrate was concentrated using a rotary evaporator at 60℃ and the moisture was removed by freeze drying to finally obtain the *Justicia carnea* methanol extract which was stored in the refrigerator at -4°C until required for use.

Phytochemical Analysis

Glycoside

The method of Sofowora, 1996 [16] was used to determine the presence of glycosides. Here, 0.5 g portion of the sample was mixed with 2 mL of glacial acetate and a drop of ferric chloride solution, after which 1 mL of concentrated sulphuric acid were added. The reaction was observed for a brown ring formation.

Flavonoids

The method of Harborne, 1973 [17] was used to determine the presence of flavonoids. A portion (0.5 g) of powdered plant was heated with 10 mL of ethyl acetate in a test tube over a steam bath for 3 minutes. The mixture was filtered, and 4 mL of the filtrate was shaken with 1 mL of dilute ammonia solution. Yellow coloration was observed that indicated the presence of Flavonoids.

Tannins

The method of Harborne, 1973 [17] was used to determine the presence of tannins. Here, 0.5 g of the dried powdered sample was boiled in 20 mL of distilled water in a test tube and filtered. 0.1% ferric chloride ($FeCl_3$) solution was added to the filtrate. The appearance of brownish green or a blue-black colouration indicates the presence of tannins in the test samples.

Saponins

The presence Saponins was determined using the method of Obadoni and Ochuko, 2001 [18]. About 2.0 g portion of the powdered sample was boiled in 20 mL of distilled water in a test tube in boiling water bath and filtered. 10 mL of the filtrate was mixed with 5 mL of distilled water and shaken vigorously to form a stable persistent froth. About 3 drops of olive oil was added to the frothing and shaken vigorously for the formation of emulsion characteristic of saponins.

Alkaloids

The presence of alkaloids was determined using the method of Harborne, 1976 and Trease and Evans 1989 [19,20]. Here, 0.5 g of the extract was stirred with 5 mL of 1% aqueous HCl on a steam bath. Few drops of picric acid solution were added to 2 mL of the extract. The formation of a reddish-brown precipitate was taken as preliminary evidence for the presence of alkaloids.

ISSN No. 2321-2705 | DOI: 10.51244/IJRSI |Volume XI Issue XI November 2024

Steroid

The presence of steroids was determined using the method of Finar, 1986 [21]. About 2 mL of Acetic anhydride was added to 0.5 g of the sample with 2mL H_2SO_4. Positive tests result in distinct color changes (e.g., pink, blue, green, or reddish-brown), which indicate the presence of steroidal compounds.

Terpenoids

The presence of terpenoids was determined using the method of Edeoga *et al*., 2005 [22]. The extract (5 mL) was mixed in 2 mL of chloroform and 3 mL of concentrated H_2SO_4 was carefully added to form a layer. The formation of a reddish brown colouration of the interface is an indication of the presence of terpenoids.

Proximate analysis

Proximate analysis conducted includes Moisture content, Ash content, Crude Protein, Crude Fiber, Crude Fat, Carbohydrate. The moisture content was determined, and the dried samples were then analyzed to determine crude protein, crude fiber, ash content and crude fat were determined using the micro – Kjeldahl method described by A.O.A.C (1990) [23] while the carbohydrate determination was done using AOAC (2000) [24].

Antioxidant Assays

The determination of catalase was done using the method of Cohen et al., (1970) [25]. The determination of MDA was done by the method of Buege and Aust (1978) [26]. SOD was determined by the method of Misra and Fridovich (1972) [27]. Glutathione peroxidase was determined by the method of Nyman (1959) [28].

Animals

A total number of thirty-six (36) male Wistar rats (8 weeks old) weighing between 180g – 200g (mean weight = 190±10) 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. The animals were acclimatized for two weeks under healthy and hygienic condition. The rats were housed in metal cages under standard laboratory conditions: room temperature, 55 – 65 % humidity and 12-h light/12-h dark cycle. They were allowed free access to pelletized growers mash and clean drinking water. All the experiments were carried out in accordance with the National Health's Guide for the Care and Use of Laboratory Animals (NIH Publication No. 85–23) revised 1996. All the experimental procedures and protocols used in this study were reviewed and approved by the Research Ethics Committee of the Faculty of Life Sciences, University of Benin, Benin City Nigeria with an approval reference number (FLSRE-2023-018).

Experimental Design

The Wistar rats were randomized into 6 groups of 6 animals each. Group 1 was the control group which received only food and water throughout the duration of the experiment. Groups 2 to 6 animals were earlier induced with diabetes following intraperitoneal injection of streptozotocin (50 mg/kg bw). Group 2 was the diabetic untreated group, group 3 was the diabetic group treated with Metformin (25 mg/kg bw), while groups 4, 5 and 6 were diabetic groups treated with 100 mg/kg bw, 200 mg/kg bw and 500 mg/kg bw respectively of methanol extract of *Justicia carnea* leaves. The treatments lasted for 21 days after which the rats were sacrificed after an overnight fast. The pancreas, liver and kidney of the rats were harvested at once, after sacrificing the rats, blotted dry and weighed. Portions of the tissues were homogenized for biochemical assays. The tissue homogenates were prepared as follows: exactly 1g of the respective tissues (pancreas, liver and kidney) were separately homogenized in 5 ml of ice-cold saline solution using a ceramic pestle and mortar placed in ice park. The homogenates were centrifuged at 3500 rpm for 20 minutes. The supernatant of each tissue was decanted into plain bottles and stored in the refrigerator at -4°C until required for use.

ISSN No. 2321-2705 | DOI: 10.51244/IJRSI |Volume XI Issue XI November 2024

Acute Toxicity Test

An oral acute toxicity study was conducted on the methanol extract of Justicia carnea leaves employing the Lorke, 1983 method [29]. A total of eighteen (18) Wistar rats weighing from 180 g – 200 g (mean weight = 190 ± 10 g) were used in this two-phase study. In phase 1, the rats were randomized into 3 groups of 3 animals each. Each group received a designated oral dose of the extracts (10, 100, and 1000 mg/kg body weight) respectively by gavage. The animals were observed initially for signs of toxicity 60 minutes after administration and were continuously monitored for 24 hours. The absence of mortality in phase I necessitated a second phase. In phase 2, three rats were allocated to separate groups, with each group receiving a single, high-dose oral gavage of the extracts (1500, 2500, and 5000 mg/kg body weight) respectively. The animals were observed for signs of toxicity within 24 hours, with extended monitoring for an additional 48 hours to assess for delayed mortality.

Statistical Analysis

Count data are expressed as mean ± standard error of mean. The statistical analysis was performed using SPSS (version 20). The various treatment groups were compared using Duncan multiple range test. Statistical significance was assumed at $p < 0.05$.

RESULTS

Phytochemical constituent, proximate analysis and antioxidant activity of *Justicia carnea* leaves

Justicia carnea leaves contain flavonoids, tannins, steroids, phenols, and terpenoids, according to a qualitative analysis of their phytochemical composition. Notably, there is absence of other phytochemicals like cardiac glycosides, saponins, phlobatannins, coumarin, alkaloids, and anthraquinone. (Table 1)

Table 1: Phytochemical constituent of *Justicia carnea* leaves

Phytochemical Components	Relative composition
Flavonoids	++
Tannins	++
Cardiac Glycosides	-
Saponin	-
Steroids	++
Phenols	++
Phlobatannins	-
Coumarin	-
Alkaloids	-
Anthraquinone	-
Terpenoids	++

KEY:
- Negative (Absent)

+ Positive (Present) but low

++ (High)

ISSN No. 2321-2705 | DOI: 10.51244/IJRSI |Volume XI Issue XI November 2024

Proximate Analysis of *Justicia carnea* Leaves

Table 2 shows the proximate composition of the leaves of *J. carnea***.** The results indicated that while crude fat and crude protein were low (0.23% and 1.26%), the percentage of carbohydrates and moisture content was high (47.88% and 22.12%, respectively). The leaves of *Justicia carnea* are rich in carbohydrates and fiber, with a substantial mineral (ash) content, while being low in fat and protein. These attributes suggest that *Justicia carnea* leaves may offer energy and aid digestion but are not a major source of protein or fats. Their mineral content could make them valuable for contributing to dietary micronutrient intake.

Table 2: Proximate Composition of *Justicia carnea* Leaves

Proximate composition	**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 means ± SEM of three determinations.

Acute Toxicity Studies of Methanol Extract of *Justicia carnea* Leaves

The methanol extract of the leaves of *J. carnea* did not produce any mortality in the rats across the doses utilized. (Tables 3 & 4)

Table 3: Phase 1 Acute toxicity Test

Dose (mg/kg body weight) of methanol extract of *J. carnea* leaves	Mortality
10	0/3
100	0/3
1000	0/3

Table 4: Phase 2 Acute toxicity Test

Dose (mg/kg body weight) of methanol extract of *J. carnea* leaves.	Mortality
1500	0/3
2500	0/3
5000	0/3

Effect of Methanol Extract of *J. carnea leaves* on Fasting Blood Glucose

Table 5 shows the effects of the methanol extract of *J. carnea* leaves on fasting blood glucose levels in STZ-induced diabetic rats. The result indicates that the blood glucose level of the diabetic groups increased significantly ($p < 0.05$) three days after the administration of streptozotocin when compared to the normal

ISSN No. 2321-2705 | DOI: 10.51244/IJRSI |Volume XI Issue XI November 2024

control. The glucose level of the diabetic control remained significantly increased ($p < 0.05$) when compared with the normal control and diabetic treated groups after the 21 days period of study. In the normal control group (Group 1), the fasting blood sugar (FBS) levels remained stable and within normal range throughout the experiment. For all the diabetic groups (Groups 2 to 6), there was a sharp increase in blood glucose levels 72 hours after STZ administration**,** confirming the successful induction of diabetes. For example, in Group 2, FBS increased to 305.00 ± 27.40 mg/dL (from an initial value of 94.67 ± 14.45 mg/dL), indicating severe hyperglycemia. The untreated diabetic control (Group 2) maintained high glucose levels throughout the experiment, with little to no reduction (e.g., final FBS: 369.33 ± 28.01 mg/dL). This indicates that without treatment, the hyperglycemia induced by STZ persisted. The treated groups (Groups 3 to 6) showed a significant reduction in blood glucose levels ($p<0.05$) after receiving treatment, especially after 21 days. For example, Group 6 showed a dramatic decrease in glucose levels from 297.67 ± 43.31 mg/dL (after induction) to 53.00 ± 12.00 mg/dL after treatment.

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

	INITIAL FBS	**FBS ON DAY 3**	**FBS AFTER 7 DAYS TREATMENT**	**FBS AFTER 14 DAYS TREATMENT**	**FBS AFTER 21 DAYS TREATMENT**
GROUP 1	76.00±4.15	76.00±4.15	93.67±2.53[b]	92.33±2.37[b]	65.5±3.42[b]
GROUP 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]
GROUP 3	62.20±3.88	310.80±56.81[a]	286.40±55.00[a,b]	204.60±51.17[a,b]	168.00±76.00[a,b]
GROUP 4	58.80±4.11	283.20±50.71[a]	223.00±58.15[a,b]	222.00±59.05[a,b]	171.20±67.12[a,b]
GROUP 5	56.80±1.65	370.20±49.42[a]	321.75±53.10[a,b]	295±112.155	222.33±62.22 [a,b]
GROUP 6	59.17±6.32	297.67±43.31[a]	240.20±70.83[a,b]	190.00±55.08[a,b]	53.00±12.00[b]

Values are expressed as Mean ± SEM (n = 3). [a] represent significant difference from control ($p<0.05$). [b] represents significant difference from diabetic control ($p<0.05$)

Effect of Methanol Extract of *Justicia carnea* Leaves on Antioxidant Activities in the

Pancreas of STZ-induced Diabetic Wistar Rats.

The effect of methanol extract of *J. carnea* leaves on antioxidant status in the pancreas of STZ-induced diabetic rats is shown in table 6. Groups 3, 4, 5, and 6 all showed significant improvements in antioxidant enzymes, SOD, CAT, and GPx activities compared to the diabetic control group. These results indicate that the methanol extract of *J. carnea* leaves helped to restore and boost the antioxidant defense system, protecting against oxidative stress in diabetic conditions. MDA, a marker of oxidative stress, was significantly lower in Groups 3 to 5 compared to Group 6, which had a high MDA level. This suggests that while antioxidant enzymes were restored in Group 6, the extract might not have been fully effective in preventing against lipid peroxidation in this group. The methanol extract of *J. carnea* leaves demonstrated significant in vivo antioxidant activities, especially by enhancing the activities of key antioxidant enzymes, SOD, CAT, and GPx in treated groups (Groups 3 to 6). These results suggest that the extract could help mitigate oxidative stress caused by diabetic conditions, although lipid peroxidation (MDA) results indicate varying levels of oxidative damage across the groups. Group 6 displayed the most remarkable improvement in enzyme activity, but also had the highest lipid peroxidation, suggesting a complex interaction between the antioxidant defense and the extent of oxidative stress.

ISSN No. 2321-2705 | DOI: 10.51244/IJRSI |Volume XI Issue XI November 2024

Table 6: Effect of Methanol Extract of *Justicia carnea* Leaves on Antioxidant Activities in the Pancreas of STZ-induced Diabetic Wistar Rats.

PARAMETERS	CONTROL	GROUP 2	GROUP 3	GROUP 4	GROUP 5	GROUP 6
SOD (unit/mg protein)	0.980±0.098	1.107±0.00	1.273±0.00	1.137±0.080	1.651±0.00[a,b]	2.659±0.00[a,b]
CAT (unit/mg protein)	0.370±0.068[b]	0.072±0.00	0.466±0.057[b]	0.485±0.056[b]	0.820±0.00[a,b]	0.948±0.00[a,b]
GPx (u/L)	2.589±0.349	1.774±0.00	3.320±0.597[b]	3.319±0.411[b]	5.711±0.00[a,b]	6.536±0.00[a,b]
MDA (unit/mg protein)	1.503±0.146	1.333±0.00	1.659±0.421	1.863±0.144[b]	1.235±0.00	4.179±0.00[a,b]

Values are expressed as Mean ± SEM (n=3). [a] Represent significant difference from control (P<0.05). [b] Represent significant difference from diabetic control (P<0.05)

Effect of Methanol Extract of *Justicia carnea* Leaves on Antioxidant Activities in the Kidney of STZ-induced Diabetic Wistar Rats

Table 7 shows the effect of methanol extract of *J. carnea* leaf on the *in vivo* antioxidant status in the kidneys of STZ-induced diabetic wistar rats. The activity of SOD significantly decreased ($p<0.05$) in groups 2, 3, and 4 when compared to the normal control whilst there was a significant decrease ($p<0.05$) in the activity of CAT in groups 2,3 and 4 compared to the normal control. There were no significant differences in the activity of GPx in groups 3, 4, 5, and 6 compared to the normal control and diabetic control groups respectively. The level of MDA was significantly lower ($p<0.05$) in the normal control group than in any other group, and there was a significant difference ($p<0.05$) in the diabetic group compared to the other groups.

Table 7: Effect of Methanol Extract of *Justicia carnea* Leaves on Antioxidant Activities in the Kidney of STZ-induced Diabetic Wistar Rats

PARAMETERS	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±0.2
CAT (unit/mg protein)	0.55±0.02[b]	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[a,b]	0.67±0.02[a,b]	0.59±0.08[a,b]	0.38±0.06[a]

Values are expressed as Mean ± SEM (n=3). [a] Represent significant difference from control (P<0.05). [b] Represent significant difference from diabetic control (P<0.05)

Effect of Methanol Extract of *Justicia carnea* Leaves on Antioxidant Activities in the Liver of STZ-induced Diabetic Wistar Rats.

Table 8 shows the effects of the methanol extract of *Justicia carnea* leaves, on the *in vivo* antioxidant status in the liver of STZ-induced diabetic rats. The results indicate that the methanol extract of *Justicia carnea* leaves affects antioxidant enzyme activities and oxidative stress markers in diabetic conditions. Although SOD activity did not significantly differ from the normal control across the groups, both group 4 and group 6

ISSN No. 2321-2705 | DOI: 10.51244/IJRSI |Volume XI Issue XI November 2024

exhibited reduced SOD activity compared to the diabetic control ($p<0.05$), suggesting a potential impairment of the antioxidant defense system in these groups. Group 6 showed significant changes in both CAT activity and MDA levels, implying that this group may have experienced increased oxidative stress, which could be linked to the treatment's effects. The significant decrease in GPX activity in Groups 5 and 6 indicates that the extract at the respective doses may be less effective in managing oxidative stress compared to the normal control group.

Table 8: Effect of Methanol Extract of *Justicia carnea* Leaves on Antioxidant Activities in the Liver of STZ-induced Diabetic Wistar Rats.

PARAMETERS	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±0.0[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[a,b]
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[a,b]
MDA (unit/mg protein)	0.33±0.03[b]	0.70±0.00[a]	0.33±0.0[b]	0.37±0.03[b]	0.45±0.00[a,b]	0.40±0.02[a,b]

Values are expressed as Mean ± SEM (n=3). [a]Represent significant difference from control ($P<0.05$). [b] Represent significant difference from diabetic control ($P<0.05$)

DISCUSSION

Diabetes mellitus is a metabolic disorder that is characterized by an abnormally high level of blood glucose for a prolonged period. This could be because of inadequate insulin synthesis, insulin resistance or even both [30]. Induction of diabetes with 50mg/kg body weight of streptozotocin led to a sharp rise in the blood glucose level which drastically reduced after 21 days of treatment with various doses of *Justicia carnea* leave extract. This suggest that the combined effort of the phytochemical content, nutritional composition and the plant's ability to improve antioxidant response to the oxidative stress induced by diabetes can help in the amelioration of the effects of diabetes mellitus on various organs. This study revealed the presence of various important phytochemical in *Justicia carnea* leaves, this includes flavonoids, tannins, phenols, steroids and terpenoids. This agrees with previous report on the phytochemical composition of *Justicia carnea* leaves [31]. Previous studies have shown that the combined action of these phytochemicals has the potential of decreasing high blood sugar levels [32]. The analysis of the proximate composition of *Justicia carnea* leaves reveals 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) and carbohydrate (47.88±1.31). The body requires adequate amount of these nutrients for proper physiological functioning, for instance the presence of fibre in diet helps to slow down the rate of absorption of glucose into the bloodstream thereby reducing the risk of hyperglycemia [33]. Superoxide dismutase, glutathione peroxidase, and catalase are antioxidant enzymes that remove free radicals *in vivo*. The results obtained shows that methanol extract of *Justicia carnea* had significant influence on the activity of the various *in vivo* antioxidants in the rat's pancreas by enhancing the rate of disappearance of reactive oxygen species (ROS) as indicated in table 6 where the diabetic control group exhibited significant increase ($p<0.05$) in MDA which decrease significantly when the experimental animals were treated with various doses of *Justicia carnea* leave extract. The antioxidant activities in the kidney (table 7) also showed a significant increase ($P<0.05$) in the activities of SOD, CAT and GPx in group 5 and 6 which were treated with higher doses of the extract and table 4 also showed a significant increase ($p<0.05$) the activities of SOD, CAT and GPx. The increased antioxidant activities induced by the introduction of the *Justicia carnea* shows the plants ability to decrease the effect of reactive oxygen species that were released because of the effect of diabetes induced in the experimental animals.

ISSN No. 2321-2705 | DOI: 10.51244/IJRSI |Volume XI Issue XI November 2024

CONCLUSION

This study highlights the significant phytochemical composition and proximate analysis of *Justicia carnea* leaves, revealing a high content of essential nutrients and bioactive compounds. The notable antioxidant activity observed supports the plant's traditional use in medicine and underscores its potential as a natural source of antioxidants. These findings provide a scientific basis for the therapeutic applications of *Justicia carnea* in the management of diabetic complications. Further research is warranted to isolate and characterize the bioactive components, establish standardized dosing regimens for therapeutic applications and determine the mechanism of action of the leave extract.

Funding

This research project did not receive any funds from any agency. It was self-funding

Declaration of Competing Interest

The authors declare that they have no conflict of interest related to the publication of this paper

ACKNOWLEDGEMENTS

The authors appreciate the Natural Product Research and Disease Control Laboratory (NPRDC), Department of Biochemistry, University of Benin for providing the facilities needed to carry out this study. The authors also thank the following persons: Mr Shuaibu Braimah, Mr Kingsley Akpeh and Mr Emmanuael Nwemuche for their technical support

REFERENCES

1. Anyasor, G. N., Ogunwenmo, K. O., Ajayi, D. and Obinna, M. (2015). Mechanisms of hypoglycemic and antidiabetic actions of *Justicia carnea* leaf extract. Journal of Ethnopharmacology. 171: 10–20.
2. Correa G. M. (2012). Chemical constituents and biological activities of species of Justicia: A review. Braz J Pharmacogn. 22**:** 220-238.
3. Anthonia, O. C., Ikechukwu, W. R., Uzoma, N. O. and Sunday, E.L.U. (2019). Nutritive properties of aqueous extract *Justicia carnea* leaves and its effects on haematological and some biochemical indices of anaemia induced male wistar albino rats. Biomed Res**.** 30(4): 645-654.
4. Wood, J., Yasmin-Karim, S., Moreau, M., Kumar, R., Akwanwi, J., Derek, A., Atoneche, F., Kress, J. and Ngwa, W. (2020). Characterization of isolated extracts from Justicia plant leaves used as remedy for anemia. Molecules. 25(3): 534.
5. Liu, R. H. (2013). Health-promoting components of fruits and vegetables in the diet. Advances in Nutrition. 4(3), 384-392.
6. AOAC International. (2012). Official Methods of Analysis of AOAC International (19th ed.). Gaithersburg, MD, USA: AOAC International.
7. Halliwell, B. and Gutteridge, J. M. C. (2015). Free Radicals in Biology and Medicine (5th ed.). Oxford University Press
8. Nasri, H., Baradaran, A., Shirzad, H. and Rafieian-Kopaei, M. (2014). New concepts in nutraceuticals as alternative for pharmaceuticals. Int J Prev Med. 5(12): 1487-1499.
9. American Diabetes Association. (2023). Classification and diagnosis of diabetes: Standards of Medical Care in Diabetes—2023. *Diabetes Care*, 46(Supplement 1), S19-S40.
10. Smith, A., Lee, J., & Miller, H. (2022). Pathophysiology and management of diabetes mellitus: Recent advances. *Annual Review of Medicine*, 73, 21-37.
11. Zhao, Y., Zhang, H., & Chen, J. (2023). Microvascular and macrovascular complications in diabetes: Pathophysiology and clinical implications. *Frontiers in Endocrinology*, 14, 931018.
12. Kumar, S., Singh, R., & Gupta, R. (2024). Cardiovascular complications in diabetes mellitus: A review. *Cardiovascular Diabetology*, 23(1), 50.
13. 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.

14. World Health Organization. (2023). Global report on diabetes. WHO. Retrieved from https://www.who.int/publications/i/item/9789240068526
15. Osemwenkhae, P.O. and Ojeaburu SI (2024). Hypoglyceamic 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. doi: 10.32996/jmhs.2024.5.2.10
16. Sofowora, A. (1996). Research on medicinal plants and traditional medicine in Africa. The Journal of Alternative and Complementary Medicine. 2(3): 365-372.
17. Harborne, J. B. (1973). Phenolic compounds. Phytochemical methods*:* A guide to modern techniques of plant analysis. pp 33-88.
18. Obadoni, B. O. and Ochuko, P. O. (2001). Phytochemical studies and Comparative efficacy of the crude extracts of some homeostatic plants in Edo and Delta States of Nigeria. Global J Pure Appl Sci. 8:203-208.
19. Harborne, J. B. (1976). A unique pattern of anthocyanins in Daucus carota and other Umbelliferae. Biochemical Systematics and Ecology. 4(1): 31-35.
20. Trease, G. E. and Evans, W. C. (1978). Pharmacology 11th Ed. Bailliere Tindall Ltd, London. pp 60-75
21. Finar, G. (1986). Plants of economic importance. Medicinal Plants and Medicine in Africa. Spectrum Books Ltd. Ibadan. 78: 150-153.
22. Edeoga, H. O., Okwu, D. E. and Mbaebie, B. O. (2005). Phytochemical constituents of some Nigerian medicinal plants. African journal of biotechnology. 4(7): 685-688.
23. AOAC. (1990). Official Methods of Analysis of the Association of Official Analytical Chemists, Vol. II, 15th ed. Sec.985.29. The Association: Arlington, VA.
24. AOAC, (2000). Official Methods of Analysis of the Association of Official Analytical Chemists, 14th Ed. Washington D.C. USA.
25. Cohen, G., Dembiec, D. and Marcus, J. (1970). Measurement of catalase activity in tissue extracts. Analytical Biochemistry. 34(1): 30-38.
26. Buege, J. A. and Aust, S. D. (1978). "Microsomal lipid peroxidation." In S. Fleischer & L. Packer (Eds.), Methods in Enzymology (Vol. 52, pp. 302-310). Academic Press.
27. Misra, H. P. and 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.
28. 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.
29. Lorke, D. (1983). A New Approach to Practical Acute Toxicity Testing. Arch Toxicol. 54:275-287
30. Kangralkar, V. A., Patil, S. D. and Bandivadekar, R. M. (2010). "Oxidative stress and diabetes: A review." International Journal of Pharmaceutical Applications. 1(1): 38-45.
31. Onyeabo, C., Ekechi, M. O., Ojinnaka, M. C. and Omeke, N. O. (2017). "Phytochemical composition, mineral content and in vitro antioxidant activity of *Justicia carnea* leaves." Biokemistri. 29(1): 1-8.
32. Gaikwad, S. B., Mohan, G. K. and Rani, M. S. (2014). "Phytochemicals for diabetes management." Pharmaceutical Crops. 5(1): 11-28.
33. Bouttwell, R. J. (1988). "Dietary fiber and glucose metabolism." American Journal of Clinical Nutrition. 48(4): 1078-1084.