



Inhibitory Potential and Antidiabetic Activity of Leaf Extracts of *Justicia carnea*

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Authors' contributions

This work was carried out in collaboration among all authors. Author SCU conceived the work. Author KKA wrote the protocol. Author ONA designed the study and wrote the first draft of the manuscript. Author MOE managed the literature searches. Authors CMO and KSI managed the analyses of the study and performed the statistical analysis. All authors read and approved the final manuscript.

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ABSTRACT

The aim of this study was to evaluate the *in-vitro* inhibitory potential of various leaf extracts of *Justicia carnea* on alpha-amylase and alpha-glucosidase activities and the anti-diabetic activity of the ethanol leaf extract using albino wistar rats. The analyses were carried out using standard biochemical methods. The oral acute toxicity test (LD₅₀) in rats of the ethanol leaf extract was determined using Lorke's method. Diabetes was induced in the rats by a single intraperitoneal dose of 120 mg/kg. b.w of alloxan. Six (6) experimental groups of rats (n=6) were used for the study. Three groups of diabetic rats received oral daily doses of 200 mg/kg, 400 mg/kg and 600 mg/kg ethanol leaf extract of *Justicia carnea* respectively while gilbenclamide (5 mg/ml); a standard diabetic drug was administered to a specific group. Treatment lasted for 14 days. From the results

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of the *in-vitro* inhibitory activity, the different extracts of *Justicia carnea* demonstrated dose dependent strong inhibitory activity against α -amylase but moderate inhibitory activity against α -glucosidase. The ethanol leaf extract was the most potent inhibitor of both α -amylase and α -glucosidase in comparison with other extracts and acarbose; the standard. The ethanol leaf extract exhibited maximum of 82.18% inhibition with IC_{50} of 2.99 ± 1.14 mg/ml for α -amylase and 41.66% with IC_{50} of 9.66 mg/ml ± 0.32 mg/ml for α -glucosidase. From the analysis of the Lineweaver-Burk plot, the ethanol leaf extract exhibited mixed noncompetitive inhibition of α -amylase and competitive inhibition of α -glucosidase activities. The acute toxicity study showed that the extract had an $LD50 > 5000$ mg/kg. From the result of the anti-diabetic study, the fasting blood glucose levels significantly ($p < 0.05$) decreased in the animals treated with the ethanol leaf extract of *Justicia carnea* when compared to the untreated rats. It can be concluded from the results that the leaf extracts of *Justicia carnea* can be used in the management of diabetes.

Keywords: Diabetes; anti-diabetic; inhibition; alpha-amylase; alpha-glucosidase; *Justicia carnea*.

1. INTRODUCTION

Justicia carnea is a flowering plant that belongs to *Acanthaceae* family with common names such as Brazilian plume flower, flamingo flower, Brazilian-plume, and Jacobinia [1]. *Justicia carnea* is a specie of *Justicia*; the largest genus of *Acanthaceae* family [2]. Few species of *Justicia* have been studied (36 species of approximately 600 cataloged species), with fifteen species found in America, thirteen species in Asia, and eight species in Africa [2]. The *Acanthaceae* family is known as an important source of therapeutic drugs [2]. Several of the studied species are used widely in traditional medicine in the treatment of gastrointestinal and respiratory diseases as well as inflammation, rheumatism and arthritis [3]. Other uses include as hallucinogens (for their effects on the central nervous system), sedatives and analgesics in the treatment of headache and fever [4,5], anticancer [6], tooth ache, digestive, weaning agent, laxative [7] and in the management of diabetes [8,9]. Many chemical classes have been isolated from the species of *Justicia*; such as flavonoids, alkaloids, vitamins, essential oils, salicylic acid and fatty acids [10].

Justicia carnea; found Nigeria, is among the plants commonly referred to as "hospital-too-far" owing to local beliefs of offering cure to many illnesses. Even though it is not listed as a treatment for diabetes mellitus in the village investigated. This plant was picked for this study which was as a result of its history of usage in traditional medicine. Herbal preparations containing this plant are usually prepared by boiling in water. Studies on the plant revealed it contains high level of iron to support its usage as a blood-booster in anemic conditions and sickle-cell anemia, treatment of fever and malaria [11].

Diabetes mellitus is the commonest metabolic-disorder of the endocrine system with prolonged high blood glucose with predisposition to macrovascular (heart disease, ischaemic, peripheral vascular disease and stroke) and microvascular (nephropathy, retinopathy and neuropathy) damage [12]. It is a growing health concern and epidemic in the world. Approximately 171 million people in the world have diabetes in 2000 and estimated to reach 366 million people by 2030 [13]. The estimated prevalence of diabetes in Africa is 1% in rural areas, 7% in urban sub-Sahara Africa, and 8-13% in more developed areas [14]. Data from the World Health Organization (WHO) suggests that Nigeria has the greatest number of people living with diabetes in Africa [12]. The prevalence is said to vary from 0.65% in rural North to 11% in urban south [15].

Mammalian α -amylase is a well-known enzyme in the pancreas which catalyzes the catabolism of insoluble and large starch molecules into maltose [16]. While α -glucosidase situated in the mucosal brush border of the small intestine catalyzes the last stage of breakdown of disaccharides and starch [17]. Postprandial hyperglycemia plays important role in development of type 11 diabetes mellitus and the chronic complications associated with the disease. Inhibitors of α -amylase and α -glucosidase retard the rate at which carbohydrate is broken down in the small intestine and reduce the postprandial blood glucose levels in diabetic patients [18]. The inhibition of these two enzymes has been established as an effective and useful strategy to reduce the levels of postprandial hyperglycemia [19]. Oral anti-diabetic agents are first choice of treatment for diabetes as they are more convenient and effective for glycemic control, but

they come with their attendant side effects [20]. Natural alpha- amylase and alpha-glucosidase inhibitors from nutritional and therapeutic plants can be used as a potent therapy for treatment and management of postprandial hyperglycemia with fewer side effects. Screening of α -amylase and α -glucosidase inhibitors from natural sources is increasing and so it is pertinent to examine the ability of *Justicia carnea* to inhibit these enzymes. Therefore, the aim of this study was to screen the various extracts of *Justicia carnea* leaf for inhibitory activity against alpha amylase and alpha glucosidase as well as evaluate the anti-diabetic activity.

2. MATERIALS AND METHODS

2.1 Plant Material

The leaves of *Justicia carnea* were obtained from Umunta village, Obi Ngwobi community in Isiala Ngwa South local government area of Abia State, Nigeria. It was identified in the Department of Forestry, Michael Okpara University of Agriculture, Umudike, Abia State, Nigeria.

2.2 Preparation of Plant Extracts

The leaves of *Justicia carnea* were shade-dried, crushed using a mechanical grinder into a coarse powder and stored in air tight containers. The powder was then divided into three parts (100 g each) and was extracted in various (500 ml) solvents (methanol, ethanol and aqueous).

2.3 Plant Material Extraction

2.3.1 Aqueous extract

One part of the powdered sample was macerated in distilled water. The extract was centrifuged at 10,000 rpm for ten minutes to get the supernatant from which the aqueous extract of *Justicia carnea* was prepared at a concentration of 10 mg/ml.

2.3.2 Methanol extract

One part of the ground sample was extracted by stirring with methanol at 25°C, soaked for 72 h and filtered using Whatman No.4 filter paper. The residue was then evaporated at 40°C to dryness. Dried extracts were weighed and dissolved in dimethylsulphoxide (DMSO) to obtain methanol extract of *Justicia carnea* at a concentration of 10 mg/ml, and stored at 4°C for further use.

2.3.3 Ethanol extract

One part of the ground sample was extracted by stirring with ethanol at 25°C, soaked for 72 h and filtered through Whatman No.4 paper. The residue was then evaporated at 40°C to dryness. Dried extracts were weighed and dissolved in dimethylsulphoxide (DMSO) to obtain ethanol extract of *Justicia carnea* at a concentration of 10 mg/ml, and stored at 4°C for further use.

2.4 Alpha-amylase Inhibitory Assay

Alpha-amylase inhibitory activity of the extracts was carried out using starch-iodine method as described by Mahomoodally et al. [21] and Kotowaroo et al. [22] with slight modifications. Alpha-amylase activity can be measured in-vitro by degradation of starch by α -amylase. This process was quantified using iodine, which gives blue-black colour with starch. The reduced intensity of blue-black colour shows the enzyme-induced hydrolysis of starch into monosaccharides. If the substance/extract possesses α -amylase inhibitory activity, the intensity of blue-black colour will be more. That is, the intensity of blue-black colour in test sample is directly proportional to α -amylase inhibitory activity [23].

Thus, using graded concentrations of plant extracts (2-10 mg/ml); 100 μ l of extract was pre-incubated with 100 μ l of enzyme solution at room temperature for 15 min. 3 ml soluble starch solution (1% starch) and 2 ml phosphate buffer were added to the reaction mixture. The reaction mixture was incubated at room temperature for 1 h. At timed interval aliquot (0.1 ml) from the reaction mixture was put into 10 ml iodine solution and mixed. The absorbance of the starch-iodine solution was measured at 565 nm. Alpha-amylase inhibitory activity was calculated as percentage inhibition thus;

$$\% \text{ inhibition} = [(A_0 - A_t)/A_0] \times 100;$$

A_0 and A_t are the absorbance of starch-iodine solution at $t = 0$ min and $t = 60$ min, respectively [21]. Controls without plant extracts were considered to contain 100% enzyme activity. Acarbose was used as positive reference control. Extract concentrations at 50% inhibition of enzyme activity (IC_{50}) were determined graphically. Plant extracts were compared based on their IC_{50} values.

2.5 Mode of α -amylase Inhibition

This was conducted using the extract with the least IC_{50} according to the method of Ali et al. [24]; with some modifications. Two hundred and fifty microliters (250 μ l) of the ethanol leaf extract (2 mg/ml) was pre-incubated with 250 μ l of α -amylase solution at 25°C for 10 mins in a set of test tubes. In another set of test tubes, α -amylase was also incubated with 250 μ l of phosphate buffer (pH 6.9). At increasing concentrations (0.30 – 5.0 mg/mL), 250 μ l of starch solution was added to both sets of reaction mixtures to start the reaction followed by incubation for 10mins at 25°C. Five hundred microliters (500 μ l) of dinitrosalicylic acid (DNS) was added to stop the reaction and boiled for 5mins. The absorbance was read at 575 nm and the amount of reducing sugars released was determined using and maltose standard curve and converted to reaction velocities. Lineweaver-Burk plot of $1/V$ versus $1/[S]$ was plotted; v is the reaction velocity and $[S]$ is substrate concentration. The mode of inhibition of the extract on α -amylase activity was determined by analysis of the Lineweaver-Burk plot. Kinetic parameters namely, the Michaelis-Menten constant affinity (K_m) and maximum velocity (V_{max}), were derived.

2.6 Alpha Glucosidase Inhibition Assay

The α -glucosidase inhibitory activity was assessed by measuring the release of 4-nitrophenol from paranitrophenyl α -D glucopyranoside [25]. paranitrophenyl α -D glucopyranoside (5 mM, 0.3 ml) was added to 1.0 ml of sodium phosphate buffer (0.1 M, pH: 6.9), 0.2 ml of enzyme solution (0.5 mg/ml) and 0.2 ml of extract (varying concentrations of 2-10 mg/ml), all in a final volume of 1.7 ml. Following an incubation period of 30 minutes at room temperature, the reaction was terminated by the addition of 2.0 ml of 0.2 M sodium carbonate. The liberated p-nitrophenol was determined at 400 nm using spectrophotometer. The % inhibition rates were calculated thus;

$$\% \text{ Inhibition} = \left(\frac{\text{Absorbance Control} - \text{Absorbance Test}}{\text{Absorbance Control}} \right) \times 100.$$

Suitable reagent blank and inhibitor controls were also carried out and subtracted. Control tubes contained only buffer, enzyme and substrate, while in positive controls acarbose replaced the plant extracts. Concentrations of extracts resulting in 50% inhibition of enzyme

activity (IC_{50} values) were determined graphically. Different plant extracts were compared on the basis of their IC_{50} values estimated from the dose response curves.

2.7 Mode of α -glucosidase Inhibition

The type of inhibition of plant extracts on α -glucosidase action was determined by increasing PNPG concentration following the modified method of Ali et al. [24]. The extract with the lowest IC_{50} was used. Briefly, 50 μ l of the (2 mg/ml) extract was pre-incubated with 100 μ l of α -glucosidase solution for 10 mins at 25°C in one set of tubes. In another set of tubes α -glucosidase was pre-incubated with 50 μ l of phosphate buffer (pH 6.9). 50 μ l of PNPG at increasing concentrations (0.63 – 2.0 mg/mL) was added to both sets of reaction mixtures to start the reaction. The mixture was then incubated for 10 minutes at 25 °C, and 100 μ L of Na_2CO_3 was added to stop the reaction. The amount of reducing sugars released was determined spectrophotometrically using a paranitrophenol standard curve and converted to reaction velocities. A double reciprocal plot ($1/V$ versus $1/[S]$) where V is reaction velocity and $[S]$ is substrate concentration was plotted. The type (mode) of inhibition of the extract on α -glucosidase activity was determined by analysis of the double reciprocal (Lineweaver-Burk) plot using Michealis-Menten kinetics.

2.8 Animal Study

The animal studies were carried out according to the method of Chandira and Jayakar [26] with slight modification.

2.8.1 Experimental animals

A total of 36 Wistar albino rats were purchased from Chris Farm at Mgbakwu, Awka North LGA, Anambra State and acclimatized for a week during which they were fed with standard feed and distilled water ad libitum. At the end of the one week, the animals were weighed and their initial blood glucose was recorded using finestest glucometer.

2.8.2 Plant material extraction

One kilogram (1 kg) of the ground sample was soaked in 10 L of 70% ethanol. This was allowed to stay for 48hours after which it was filtered using three layer muslin cloth first, then with Whatman No. 4 filter paper with the aid of a vacuum pump. The filtrate was concentrated on

a water bath at 60 degrees Celsius and redissolved at a concentration of 600 mg/ml and stored in refrigerator at 4 degrees Celsius until further use.

2.8.3 Acute toxicity test

The 24 hours oral acute toxicity test (LD₅₀) was carried out using the method of Lorke [27]. The study was done in two phases. In the first phase, 9 animals were grouped into three (n=3) and the ethanol leaf extract of *Justicia carnea* was administered at low doses of 10 mg/kg, 100 mg/kg and 1000 mg/kg respectively to each group. They were observed for 48 hrs for signs of gross change in behavior, toxicity and death. Then in the second phase, another set of animals were grouped into three groups (n=1) and were given three different high doses of the extract at 1600 mg/kg, 2900 mg/kg and 5000 mg/kg respectively. They were also observed for any sign of toxicity or death.

2.8.4 Study design

Six (6) animals were randomly selected, placed in a separate cage and labeled as normal group. The remaining 30 animals were administered intraperitoneally with 120 mg/kg body weight of alloxan dissolved in ice-cold normal saline. This was allowed to stay for 48 hours after which the blood glucose was checked again and recorded as post-induction blood glucose level. Animals with blood glucose level above 200 mg/dl were taken as diabetic and were grouped into 5 groups of six animals each. Thus:

Group A: This group was given 200 mg/kg of *Justicia carnea* extract orally, the standard feed and distilled water.

Group B: This group was given 400 mg/kg of *Justicia carnea* extract orally, the standard feed and distilled water.

Group C: This group was given 600 mg/kg of *Justicia carnea* extract orally, the standard feed and distilled water.

Group D: This group was given 5 mg/kg of glibenclamide orally, the standard feed and distilled water.

Group E: This group was the positive control, they were diabetic and were given standard feed and distilled water.

Group F: The normal control group.

The blood glucose level of the animals in each group was taken daily by drawing blood from the tip of their tail and measured using Finetest glucometer for 14 days. Their weights were also measured using electronic balance.

2.9 Determination of Body Weight

The weights of the rats were checked before and after induction of diabetes as well as during the course of the treatment on weekly basis using an electronic weighing balance.

2.10 Statistical Analysis

Statistical analysis was performed using GraphPad Prism 7 statistical package (GraphPad Software, USA). Values were expressed as mean \pm SD. Statistical significance of the results between groups was determined using one way analysis of variance (ANOVA). Differences between means were considered significant at $P < 0.05$.

3. RESULTS

3.1 α -amylase Inhibition Assay

The result of the inhibitory activity of different extracts of *Justicia carnea* on α -amylase activity are shown in Table 1. The α -amylase inhibition was found to be dose-dependent. The three extracts demonstrated strong activity against α -amylase. However, the ethanol extract showed stronger inhibitory activity of 82.18% inhibition at 10mg/ml with IC₅₀ of 2.99 \pm 1.14 mg/ml when compared to other extracts and acarbose.

3.2 Mode of Inhibition of α -amylase

The Lineweaver-Burk plot was generated to determine the mode of inhibition of the enzyme and the result showed that the ethanol leaf extract of *Justicia carnea* inhibited α -amylase in a mixed non-competitive manner as shown in Fig. 1. There was an increase in Km and a reduction in Vmax. In the presence of the extract the Km was increased from 0.85 mg/ml to 0.89 mg/ml while the Vmax was reduced from 0.44 mg/ml.min⁻¹ to 0.30 mg/ml.min⁻¹.

3.3 α -glucosidase Inhibition Assay

In the study, the three extracts were found to possess moderate α -glucosidase inhibitory effects (Table 2). The α -glucosidase effectiveness of the extracts was compared on

the basis of their resulting IC₅₀ values. At a concentration of 8mg/ml, the ethanol extract showed a maximum inhibition activity of 41.66% with an IC₅₀ value of 9.66 mg/ml. The aqueous extract was the second most potent and showed inhibitory activity of 20.40% with IC₅₀ value of 20.03mg/ml. Acarbose; the reference inhibitor showed inhibition activity of 40.35% with an IC₅₀ value of 9.0 mg/ml.

3.4 Mode of α-glucosidase Inhibition

The Lineweaver-Burk plot as shown in Fig. 2 depicts that the mode of inhibition of α-glucosidase by the ethanol extract (the most potent extract) of *Justicia carnea* is a near competitive type. The Vmax values were 0.54 and 0.53 mg/ml.min⁻¹ in the presence and absence of the extract while the Km values were 0.70 and 0.48 mg/ml in the presence and absence of the extract respectively.

3.5 Animal Experiment

3.5.1 Weight of the animals

The weights of the animals taken on weekly basis are presented in Table 3. There was a significant improvement (p<0.05) in the weight of the animals treated with the ethanol leaf extract in the second week of the experiment compared to the weight in the first week and at the commencement of the experiment. The weight of the untreated diabetic group reduced when compared with the extract treated groups.

3.5.2 Acute toxicity test

Acute toxicity studies showed that the ethanol leaf extract of *Justicia carnea* had an oral LD₅₀ > 5000 mg/kg. No physical signs of gross behavioral changes were observed and no death was recorded.

Table 1. The percentage inhibition of alpha-amylase by different extracts of *Justicia carnea* and acarbose (the reference inhibitor) at varying concentrations

Concentration (mg/ml)	Percentage inhibition			
	Acarbose	<i>Justicia carnea</i> ethanol	<i>Justicia carnea</i> methanol	<i>Justicia carnea</i> aqueous
2	59.51±10.1	68.84±13.38	61.60±13.11	61.77±9.63
4	62.89±9.01	70.99±11.75	65.44±8.81	65.18±8.68
6	66.75±3.11	75.66±7.83	70.97±4.57	69.82±5.91
8	69.00±3.31	79.67±6.57	73.17±8.15	73.66±3.51
10	72.45±3.26	82.18±6.48	76.89±2.85	79.51±1.14
IC ₅₀	4.07±0.83	2.99±1.14	3.66±0.83	3.63±0.81

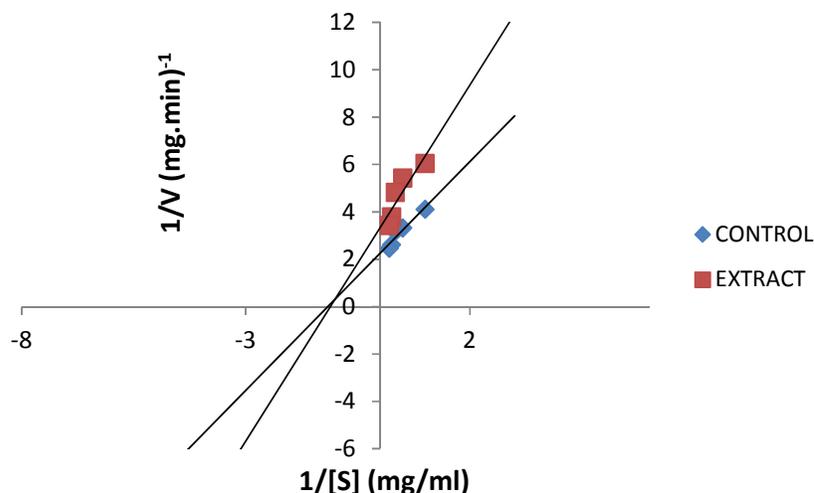


Fig. 1. Mixed inhibition of α-amylase by ethanol extract of *Justicia carnea*

Table 2. Percentage inhibition of alpha-glucosidase by different extracts of *Justicia carnea* and acarbose (the reference inhibitor) at varying concentrations

Concentration (mg/ml)	Percentage inhibition			
	Acarbose	<i>Justicia carnea</i> ethanol	<i>Justicia carnea</i> methanol	<i>Justicia carnea</i> aqueous
2	5.95±0.14	14.57±0.09	2.91±0.15	6.74±0.04
4	32.23±0.05	18.99±0.015	4.59±0.10	6.84±0.05
6	34.66±0.09	32.00±0.04	9.40±5.27	16.04±0.03
8	40.35±0.13	41.66±0.03	12.31±0.34	20.40±1.84
10	37.43 ± 0.10	22.96±0.05	11.30±0.24	15.33±0.20
IC ₅₀	9.0±0.03	9.66±0.32	33.14±6.09	20.03±1.23

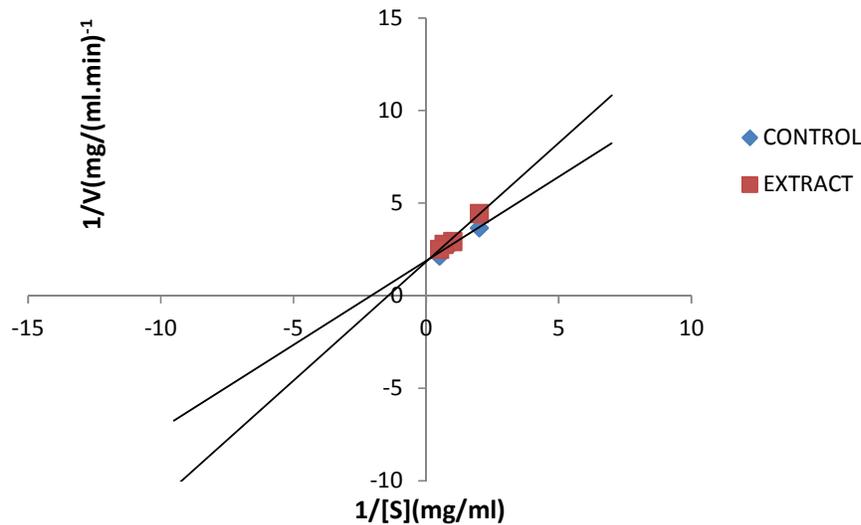


Fig. 2. Competitive inhibition of α-glucosidase by ethanol extract of *Justicia carnea*

Table 3. Effect of ethanol leaf extract of *Justicia carnea* on body weight of diabetic rats

Groups	Initial weight (g)	Post induction weight (g)	1 st week (g)	2 nd week (g)
200mg/kg extract	184.89±4.17	178.00±4.34	195.00 ±2.73	204.00 ±0.18
400mg/kg extract	212.16±5.86	220.67±4.36	195.00±2.18	210.00±4.14
600mg/kg extract	230.97±3.45	232.00±2.41	226.00±2.21	229.00±0.00
5mg/kg Glibenclamide	175.00±5.01	172.00±2.43	165.00±1.19	176.00±3.89
Diabetic untreated	189.50±1.28	187.00±0.12	182.00±3.56	178.50±0.00
Normal Control	165.81±3.24	171.75±2.16	170.00±4.13	186.00±4.87

Values are presented as mean ±SD

3.5.3 Determination of anti-diabetic activity

The result obtained in the inhibitory potential of the ethanol leaf extract of *Justicia carnea* on α-amylase and α-glucosidase activities prompted us to perform an in-vivo anti-diabetic study with the ethanol leaf extract in view to establish its anti-diabetic activity. The results of the anti-diabetic activity which are expressed as change

in daily blood glucose levels are presented in Table 4. The diabetic groups treated with ethanol leaf extract of *Justicia carnea* showed significant (p<0.05) reduction in blood glucose levels. The blood glucose levels were reduced considerably within 14 days of extract administration. At all the doses tested, there was a significant difference (α=0.05) between the treated groups and the untreated group but there was no significant

Table 4. Fasting blood glucose levels of the extract treated rats groups and the control groups measured daily for 14 days

	200 mg	400 mg	600 mg	Normal	Glibenclamide	Untreated
Initial	62±4.24 ^a	62±0.89	69±2.12	86±3.01	77±2.32	82±1.14 ^d
Post Induction	470±11.21 ^a	600±13.21	430±6.99	124±2.10	600±14.12	600±13.15 ^d
Day 1	370±3.46 ^a	600±10.56 ^b	430±4.45 ^c	107±1.0	600±12.23 ^{ab}	523±7.81 ^d
Day2	296±0.00 ^a	590±3.21 ^b	373±5.12 ^c	89±2.00	600±8.67 ^{ab}	523±9.13 ^d
Day3	290±4.21 ^a	463±3.30 ^b	295±5.10 ^c	93±3.10	523±11.1 ^{ab}	489±12.31 ^d
Day4	290±12.1 ^a	382±0.00 ^b	274±4.10 ^c	101±2.21	368±4.31 ^{ab}	490±6.12 ^d
Day5	238±2.30 ^a	317±3.10 ^b	243±3.32 ^c	89±2.20	364±3.12 ^{ab}	490±4.10 ^d
Day6	230±5.22 ^a	280±0.91 ^b	225±10.21 ^c	76±0.89	361±3.10 ^{ab}	471±3.11 ^d
Day 7	200±3.25 ^a	255±2.30 ^b	223±2.10 ^c	81±2.10	282±5.19 ^{ab}	461±5.02 ^d
Day 8	200±0.00 ^a	255±0.00 ^b	220±1.00 ^c	87±4.21	276±4.21 ^{ab}	410±3.15 ^d
Day 9	195±9.10 ^a	232±5.11 ^b	220±0.00 ^c	98±2.20	160±2.20 ^{ab}	400±1.09 ^d
Day 10	181±1.32 ^a	200±4.21 ^b	212±3.23 ^c	100±3.12	151±3.7 ^{ab}	398±3.98 ^d
Day 11	171±0.00 ^a	162±1.89 ^b	198±2.10 ^c	90±3.30	111±3.11 ^{ab}	390±2.20 ^d
Day 12	168±3.10 ^a	141±2.12 ^b	182±4.12 ^c	85±2.10	90±3.21 ^{ab}	390±0.00 ^d
Day 13	161±1.12 ^a	132±3.78 ^b	180±0.00 ^c	89±2.20	101±1.13 ^{ab}	388±0.00 ^d
Day 14	140±4.45 ^a	98±2.22 ^b	180±0.00 ^c	90±1.90	90±1.10 ^{ab}	282±0.00 ^d

Values are presented as mean ± SD. Means with the same letter(s) are not significantly different at p<0.05

difference between the group treated with the ethanol leaf extract of *Justicia carnea* and that treated with glibenclamide, a standard anti-diabetic drug. However, at 600 mg/kg dose, there was a significant difference between the extract treated groups and that treated with glibenclamide. The standard drug showed a better glucose lowering capacity compared to the extract at this dose.

4. DISCUSSION

Diabetes is considered as one of the world's greatest health problems that affects about 171 million people and most dominated by those suffering from type II diabetes [28]. Management of the blood glucose level is a critical strategy in the control of diabetes and its complications [29]. One of the approaches used in therapy for reducing postprandial hyperglycemia is the inhibition of the enzymes involved in the metabolism of carbohydrates [30]. Inhibitors of these enzymes have been effective oral hypoglycemic drugs for the control of hyperglycemia in Type II diabetic patients [31]. They do this by retarding breakdown of carbohydrate and thereby prolonging the overall carbohydrate breakdown time leading to decrease in the rate of glucose absorption and decreasing the postprandial blood glucose rise consequently [32]. Therefore, natural products are good sources of such inhibitors and many have been reported for their anti-diabetic activities in traditional medicine for the treatment of diabetes.

In the present study, the leaf extracts of *Justicia carnea* were investigated for their α -amylase and α -glucosidase inhibitory potential. The extracts showed appreciable inhibition of α -amylase with moderate inhibition of α -glucosidase. The ethanol leaf extract was the most potent inhibitor of the two enzymes. From documented phytochemical analysis, the plant was shown to contain bioactive compounds such as phenol and flavonoids which are known to inhibit enzyme activity [33,34,35]. From the curve of Lineweaver-Burk plot, the ethanol leaf extract displayed a mixed non-competitive mode of inhibition of α -amylase. This can be seen from the increase in Km value from 0.85 mg/ml in the absence of extract to 0.89 mg/ml in the presence of extract while the value of Vmax was reduced from 0.44 mg/ml.min⁻¹ to 0.30 mg/ml.min⁻¹ in the absence and presence of the extract respectively. From this result, it implies that the active components in the ethanol leaf extract do

not compete with the substrate for binding to the active site rather the inhibitors bind to a separate site on the enzyme to delay the breakdown of carbohydrates [36]. The ethanol leaf extract also displayed a near competitive mode of inhibition of α -glucosidase. The effect of a competitive inhibitor is to decrease the apparent affinity (Km) of the enzyme for its substrate without any effect on the reactivity (Vmax) of the enzyme-substrate complex once formed [37]. Thus, competitive inhibition causes an increase in the Km value but does not affect Vmax. This was confirmed by the approximately the same Vmax values of 0.53 mg/ml.min⁻¹ and 0.54 mg/ml.min⁻¹ obtained in the absence and presence of the extract respectively. Further, the Km value was increased from 0.48 mg/ml in the absence of the extract to 0.70 mg/ml in the presence of the extract thereby decreasing the apparent affinity of the enzyme. The result implies that the active components of the extract compete with the substrate for binding to the active site of the enzyme, thereby preventing or delaying the breakdown of carbohydrates [38].

From the result of the animal study, the acute toxicity test indicated that the extract had LD₅₀ greater than 5000 mg/kg. This shows a wide margin of safety and the extract is not toxic at acute level. Alloxan produced loss of body weight in all the diabetic groups as seen 48 h post induction when compared to the normal control in Table 3. The result of the second week of extract treatment revealed that all the three extract doses of 200, 400 and 600 mg/kg groups showed significant improvement in body weight compared to the untreated diabetic control.

The result of the anti-diabetic study conducted for 14 days showed that there was a significant ($p < 0.05$) reduction in the fasting blood glucose level of the groups of animals treated with the ethanol leaf extract of *Justicia carnea* compared to the untreated diabetic group. In this study it was observed that on administration of alloxan, diabetes was induced which caused significant increase in blood glucose levels as shown in Table 4 at post-induction. Alloxan-induced hyperglycemia has been a useful experimental model to study the effect of anti-diabetic agents against diabetes mellitus [39]. Alloxan enters the pancreatic beta cells through the GLUT2 glucose transporter. In the presence of glutathione an intracellular thiol, alloxan produces reactive oxygen radicals in a cyclic redox reaction further producing dialuric acid as its reduction product [40]. Through autoxidation, dialuric acid

generates hydroxyl radicals, superoxide radicals and hydrogen peroxide. These hydroxyl radicals cause death of the beta cells, which have a low anti-oxidative defense capacity and the consequent insulin-dependent 'alloxan diabetes'. Alloxan as a thiol reagent also inhibits glucokinase; the beta cell glucose sensor by selectively preventing glucose-induced insulin secretion. As seen in Table 4 of this study, blood glucose levels were higher in alloxan induced groups than in the normal group. Following the administration of ethanol leaf extract of *Justicia carnea* with doses of 200 mg/kg b.w., 400 mg/kg b.w and 600 mg/kg.b.w there was significant ($p < 0.05$) decrease in blood glucose levels when compared to the untreated diabetic control group within the days of administration. It was also observed that as the blood glucose level reduces, the body weight also increases. The reduction in blood glucose level may be due to regeneration of β cells in pancreas which is in agreement with the view that regeneration of β cells causes reduction in blood glucose levels [41].

The ethanol leaf extract of *Justicia carnea* reduced the elevated fasting blood glucose level in rats. The mechanism of the anti-diabetic effects of the extracts as shown was through inhibition of the two key carbohydrate metabolizing enzymes; alpha-amylase and alpha glucosidase. This ability might be due to presence of antioxidant phytochemicals like flavonoids, polyphenols, and tannins, which acts as a free radical scavengers [33,42,43]. Polyphenols are known to interact with proteins and can inhibit enzyme activity [44]. The polyphenols have been reported to inhibit alpha-amylase and alpha- glucosidase associated with lipid peroxidation and type II diabetes [45].

5. CONCLUSION

From this study, it can be concluded that the leaf extracts of *Justicia carnea* are potent inhibitors of α -amylase and α -glucosidase. It was observed that they competed favorably with the reference inhibitor (acarbose). Moreover, the ethanol extract was the most potent of all the extracts tested, and this could be that the ethanol extract has higher concentration of inhibitory phytochemicals as several scientific reports highlight the inhibitory action of plant phytochemicals on α -amylase. The ethanol leaf extract with doses of 200, 400 and 600 mg/kg body weight had anti-diabetic effects on alloxan-induced albino rats. The result of the *in vivo*

study has validated its glycemic control ability. Therefore, extracts of *Justicia carnea* may be useful in drug formulations for the treatment and management of diabetes mellitus.

ETHICAL APPROVAL

All authors hereby declare that "Principles of laboratory animal care" were followed and all experiments have been examined and approved by the ethics committee of Enugu State University of Science and Technology, Enugu, Nigeria.

COMPETING INTERESTS

Authors have declared that no competing interests exist.

REFERENCES

1. USDA. *Justicia carnea*. Natural Resources Conservation Service Plants Database. Available: <https://plants.usda.gov/java/ClassificationServlet?source=display&classid=JUCA17> (Retrieved January 2020)
2. Corrêa GM, Alcântara AFC. Chemical constituents and biological activities of species of *Justicia* - A review. Brazilian Journal of Pharmacognosy. 2012;22(1): 220-238.
3. Kanchanapoom T, Noiarsa P, Ruchirawat S, Kasai R, Otsuka H. Triterpenoidal glycosides from *Justicia betonica*. Phytochemistry. 2004;65:2613-2618.
4. Day SH, Lin YC, Tsai M, Tsao LT, Ko HH, Chung MI, Lee JC, Wang JP, Won SJ, Lin CN. Potent cytotoxic lignans from *Justicia procumbens* and their effects on nitric oxide and tumor necrosis factor-production in mouse macrophages. Journal of Natural Products. 2002;65:379-381.
5. Ratnasooriya WD, Deraniyagala SA, Dehigaspitiya DC. Antinoceptive activity and toxicological study of aqueous leaf extract of *Justicia gendarussa* Burn F. in rats. Pharmacognosy Magazine. 2007;3: 145-155.
6. Rajasekhar D, Subbaraju GV. Jsmicranthin, a new aryl naphthalide lignin from *Justicia neesii*. Fitoterapia. 2000;71:598-599.
7. Ajibesin KK, Ekpo BA, Bala DN, Essien EE, Adesanya SA. Ethnobotanical survey of Akwa Ibom State of Nigeria. Journal of Ethnopharmacology. 2008;115:387-408.

8. Lizcano LJ, Bakkali F, Ruiz-Larrea MB, Ruiz-Sanz JI. Antioxidant activity and polyphenol content of aqueous extracts from Colombian Amazonian plants with medicinal use. *Food Chemistry*. 2010;119: 1566-1570.
9. Andrade-Cetto A, Heinrich M. Mexican plants with hypoglycaemic effect used in the treatment of diabetes. *Journal of Ethnopharmacology*. 2005;99:325-348.
10. Al-Juaid SS, Abdel-Mojib MA. A novel podophyllotoxin lignin from *Justicia heterocarpa*. *Chemical and Pharmaceutical Bulletin*. 2005;52:507-509.
11. Orjiakor CA, Uroko RI, Njoku OU, Ezeanyika LUS. 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*. 2019;30(4):645-654.
12. Wild S, Roglic G, Green A, Sicree R, King H. Global prevalence of Diabetes: Estimates for the year 2000 and projections for 2030. *Diabetes Care*. 2004; 27:1047-1053.
13. WHO. Launch of Diabetes Action Now. Available:<http://www.who.int> (Assessed on 10/05/2020)
14. Motala AA. Diabetes Trends in Africa. *Diabetes/Metabolism Research and Reviews*. 2002;18:514-520.
15. Akinkingbe OO. Non-communicable diseases in Nigeria: National survey (Final Report) on Hypertension, Coronary Heart Disease, Diabetes mellitus, Haemoglobinopathy, G6PD Deficiency and Anaemia. National Expert Committee on Non-Communication Disease. Federal Ministry of Health and Social Services; 1997.
16. Gupta R, Gigras P, Mohapatra H, Goswami VK, Chauhan B. Microbial α -amylases: A biotechnological perspective. *Process Biochemistry*. 2003;38:1599-1616.
17. Anam K, Wodharna RM, Kusriani D. Alpha-glucosidase inhibitor of Terminalia species. *International Journal of Pharmacology*. 2009;5:277-280.
18. Rhabasa-Lhoret R, Chiasson JL. Alpha-glucosidase inhibitors (3rd edition). In RA Defronzo, E Ferrannini, H Keen, P Zimmet. *International textbook of diabetes mellitus*; 1. UK: John Wiley; 2004.
19. Fred-Jaiyesimi A, Kio A, Richard W. α -Amylase inhibitory effect of 3,-olean-12-en-3-yl (9Z)-hexadec-9-enoate isolated from *Spondias mombin* leaf. *Food Chemistry*. 2009;116:285-288.
20. Kwon YI, Apostolidis E, Shetty K. Evaluation of pepper (*Capsicum annuum*) for management of diabetes and hypertension. *Journal of Food Biochemistry*. 2007;31(3):370-385.
21. Mahomoodally MF, Subratty AH, Gurib-Fakim A, Choudary MI, Khan SN. Traditional medicinal herbs and food plants have the potential to inhibit key carbohydrates hydrolyzing enzymes in vitro and reduce postprandial blood glucose peaks in vivo. *The Scientific World Journal*. 2012;1-9.
22. Kotowaroo MI, Mahomoodally MF, Gurib-Fakim A, Subratty AH. Screening of traditional anti-diabetic medicinal plants of Mauritius for possible α -amylase inhibitory effects in vitro. *Phytotherapy Research*. 2006;20(3):228-231.
23. Sheikh JH, Iyo TMT, Ashabul MD, Rajat SB, Hitoshi A. Total Phenolic Content, Anti-oxidative, anti-Amylase, Anti-Glucosidase and Anti-Histamine release activities of Bangladeshi fruits. *Food Science and Technology Research*. 2008; 14:261-68.
24. Ali H, Houghton PJ, Soumyanath A. Alpha-amylase inhibitory activity of some Malaysian plants used to treat diabetes; with particular reference to *Phyllanthus amarus*. *Journal of Ethnopharmacology*. 2006;107(3):449-455.
25. Kim SD, Nho HJ. Isolation and characterization of α -glucosidase inhibitor from the fungus *Ganoderma lucidum*. *Journal of Microbiology*. 2004;42:223-227.
26. Chandira M, Jayakar B. Formulation and evaluation of herbal tablets containing ipomoea digitata linn. Extract. *International Journal of Pharmaceutical Sciences Review and Research*. 2010;3(1):101-110.
27. Lorke D. A new approach to practical acute toxicity testing. *Archives of Toxicology*. 1983;53:275-289.
28. Gershell L. Type 2 diabetes market. *Nature Review and Drug Discovery*. 2005;4:367-68.
29. Dicarli MF, Janisse J, Grunberger G, Ager J. Role of chronic hyperglycemia in the pathogenesis of coronary microvascular dysfunction in diabetes. *Journal of the American College of Cardiology*. 2003;41: 1387-1393.
30. Oritz-Andrade RR, Garcia-Jimenez S, Castillo-Espana R, Ramirez-Avila

- GVillalobos-Molina R, Estrada-Soto S. Alpha-glucosidase inhibitory activity of methanolic extract from *Tournefortia hartwegiana*: an antihyperglycemic agent. *Journal of Ethnopharmacology*. 2007;109(1):48-53.
31. Gin H, Rigalleau V. Post-prandial hyperglycemia and Diabetes. *Diabetes Metabolism*. 2000;26:265-272.
 32. Bhandari MR, Jong-Amurakkum N, Hong G, Kawabata J. Alpha glucosidase and alpha amylase inhibitory activities of a Nepalese medicinal herb *Pakhanbhed* (*Bergenia ciliate* Haw). *Food Chemistry*. 2008;106:247-252.
 33. Udedi SC, Ani ON, Asogwa KK, Maduji FC, Okafor CN. *In-vitro* and *In-vivo* antioxidant activity of ethanol leaf extract of *Justicia carnea*. *International Journal of Biochemistry Research and Review*. 2020; 29(4):48-60.
 34. Sama K, Murugesan K, Sivaraj R. "In vitro alpha amylase and alpha glucosidase inhibition activity of crude ethanol extract of *Cissus arnotiana*. *Asian Journal of Plant Science and Research*. 2012;2(4):550–553.
 35. Matsumoto M, Ishigaki F, Ishigaki A, Iwashina H, Hara Y. Reduction of blood glucose levels by tea catechin. *Bioscience, Biotechnology and Biochemistry*. 1993;57: 525–527.
 36. Mogale AM, Lebelo LS, Thovhogi N, De Freitas AN, Shai LJ. "α- amylase and α-glucosidase inhibitory effects of *sclerocarya birrea* [(A. Rich.)Hochst.] Sub-species *caffra* (Sond) Kokwaro (*Anacardiaceae*) stem-bark extracts. *African Journal of Biotechnology*. 2011;10(66):15033–15039.
 37. Storey KB. *Functional Metabolism: Regulation and Adaptation*. John Wiley and sons, New York. 2004;12.
 38. Shai LJ, Masoko P, Mokogetho MP, Magano SR, Mogale AM, Boaduo N, Ellof JN. Yeast alpha-glucosidase inhibitory and antioxidant activities of six medicinal plants collected in Phalaborwa, South Africa. *South African Journal of Botany*. 2010;76: 465-470.
 39. Rohilla A, Ali S. Alloxan Induced Diabetes: Mechanisms and Effects. *International Journal of Research in Pharmaceutical and Biomedical Sciences*. 2012;3:819-823.
 40. Lenzen S. The mechanisms of alloxan- and streptozotocin-induced Diabetes. *Diabetologia*. 2008;51:216-226.
 41. Sabu MC, Kuttan R. Anti-diabetic activity of medicinal plants and its relationship with their antioxidant property. *Journal of Ethnopharmacol*. 2002;81:155-160.
 42. Muhtadi Primarianti AU, Sujono TA. Antidiabetic Activity of Durian (*Durio zibethinus* Murr.) and rambutan (*Nephelium lappaceum* L.) Fruit peels in alloxan diabetic rats. *Procedia Food Science*. 2015;3:255–261.
 43. Sharma B, Balomajumder C, Roy P. Hypoglycemic and hypolipidemic effects of flavonoid rich extract from *Eugenia jambolana* seeds on streptozotocin induced diabetic rats. *Food Chemistry and Toxicology*. 2008;46:2376–2383.
 44. Oboh G, Ademiluyi AO, Akinyemi AJ, Henle T, Saliu JA, Schwarzenbolz U. Inhibitory effect of polyphenol-rich extracts of jute leaf (*Corchorusolitorius*) on key enzyme linked to type 2 diabetes (α-amylase and α-glucosidase) and hypertension (angiotensin I converting) in vitro. *Journal of Functional Foods*. 2012;4: 450-458.
 45. Reddy NVLS, Anarthe SJ, Raghavendra NM. *In vitro* antioxidant and alpha-amylase inhibitory activity of isolated fractions from methanolic extract of *Asystasia gangetica* (Chinese Violet) Linn. (*Acanthaceae*). *Journal of Research in Biomedical Science*. 2010;1(2):72-75.

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