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In-vitro and *In-vivo* Antioxidant Activity of Ethanol Leaf Extract of *Justicia carnea*

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Authors' contributions

This work was carried out in collaboration among all authors. Authors USC and AON conceived the work, wrote the protocol and designed the study. Author AKK managed the analyses of the study and performed the statistical analysis. Authors MFC and OCN managed the literature searches and wrote the first draft of the manuscript. All authors read and approved the final manuscript.

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ABSTRACT

This study investigated the in-vitro antioxidant activity of ethanol leaf extract of *Justicia carnea* and its effect on antioxidant status of alloxan-induced diabetic albino rats. The *in-vitro* antioxidant activity was assayed by determining the total phenol, flavonoids, ascorbic acid, β-carotene and lycopene contents and by using 2,2 diphenyl-1-picrylhydrazyl (DPPH) radical, reducing antioxidant power and inhibition of lipid peroxidation antioxidant systems. Oxidative stress was produced in rats by single intraperitoneal injection of 150 mg/kg alloxan and serum concentration of malonaldehyde (MDA), superoxide dismutase (SOD) and catalase (CAT) were determined. Five experimental groups of rats (n=6) were used for the study. Two groups of diabetic rats received oral daily doses of 100 and 200 mg/kg *Justicia carnea* leaf extract respectively while gilbenclamide (5 mg/ml); a standard diabetic drug was also given to a specific group for 14 days. From the result, the leaf extract contained a higher concentration of flavonoids followed byphenols, ascorbic acid, lycopene and β-carotene. The extract displayed more potent reducing power ability with EC_{50} of 40

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µg/ml compared to BHA (EC_{50} of 400µg/ml). The percentage DPPH radical scavenging activity of the extract was also higher with EC_{50} of 200µg/ml and increased with increase in concentration while BHA had $EC_{50}$of 320µg/ml. The inhibition of lipid peroxidation also increased with increase in concentration with EC_{50} of 58µg/ml and comparable with BHA (EC_{50}=60µg/ml). The effect of the plant extract on antioxidant enzyme activities was concentration-dependent. Administration of 100mg/kg of the plant extract resulted in a significant decrease ($p<0.05$) in serum MDA concentration, while 200 mg/kg of the extract caused a significant ($p<0.05$) increase in superoxide dismutase (SOD) and catalase activities with a non-significant increase ($p>0.05$) in the serum level of MDA when compared with the diabetic untreated group. These findings suggest that ethanol leaf extract of *Justicia carnea* have antioxidant properties and could handle diabetes-induced oxidative stress.

Keywords: Justicia carnea; antioxidant activity; oxidative stress; alloxan-induced; diabetes; superoxide dismutase; catalase; MDA.

1. INTRODUCTION

Oxidative stress is an imbalance between the manifestation of reactive oxygen and biological system's ability to readily detoxify the reactive intermediates or to repair the resulting damage. Oxidative stress is said to be caused by an increase in the production of free radicals or decreased activity of antioxidant defences or both and connected to major causes of diabetes mellitus [1]. Diabetes mellitus is a chronic disease associated with abnormally high levels of glucose in the blood which could be as a result of inadequate production of insulinor insensitivity of cells to the action of insulin. During diabetes, persistent hyperglycaemia causes increased free radical production in the tissues from glucose anti-oxidation, protein glycosylation, impaired glutathione metabolism, alteration in antioxidant enzyme, lipid peroxides formation, increased serum aminotransferases (ALT and AST) and decreased ascorbic acid levels [2]. Antioxidants are chemical compounds that halt the chain reaction known as oxidation which creates free radicals. They are radical scavengers that protect the human body against free radicals by inhibiting the oxidizing chain reaction. Free radicals are atoms, molecules or ions with highly unstable unpaired electrons that can react with other molecules. They are produced under certain environmental conditions and during the normal cellular function in the body. These chemicals are missing an electron, giving them an electric charge. In an attempt to neutralize this charge, free radicals try to extract an electron from or donate an electron to a neighbouring molecule. This processis known as oxidation and creates new free radical from the neighbouring molecule. The newly created free radical in turn searches out another molecule and extracts or donates an electron, setting off a chain reaction that can damage hundreds of molecules [3]. About 5% of the oxygen humans breathe is converted into free radicals called Reactive Oxygen Species which are capable of reacting with membrane lipids, nucleic acids, proteins, enzymes and other small molecules, resulting in cellular damage and enhanced susceptibilityto enzyme inactivation [4]. These damages lead to various diseases which include anaemia, cancer, cardiovascular disorders, arthritis, neurodegenerative diseases, asthma and diabetes mellitus [5]. These diseases can be prevented or hindered by inhibiting the initiation or progression of oxidative chain reaction [6]. Synthetic and natural antioxidants have been reported to handle these effects by terminating their catalytic metal ions [7]. Synthetic antioxidants are effective buthave been associated with various side effects [8]. And so, alternative sources of antioxidants such as natural products which are effective, less expensive and have minimal side effects are sought for. It is generally accepted that natural products are safer compared to synthetic ones [9,10]. Plants have been reported as a large source of essential and structurally different antioxidant compounds with potentials for development into novel therapeutic molecules [11]. Consumption of these natural products with antioxidants such as flavonoid and phenolic acids improves oxidant-antioxidants states and decreases the risk of developing diseases such as diabetes mellitus, cardiovascular disorders, cancer and inflammations.

Justicia carnea is a specie of Justicia belonging to the *Acanthaceae* family [12]. It is a plant native to Mexico and South America, Paraguay, and northern America [13] and can now be found in various parts of Africa. *Justicia carnea* is called "hospital too far" in some parts of Nigeria where

the boiled extracts of the leaf are used for the treatment and prevention of anaemia. Pregnant women, nursing mothers, people with sickle cell anaemia, malaria, typhoid and hepatitis also take the boiled extracts of the leaves and stalks. The boiled leaves and stalk extracts are obtained by boiling a mixture of the leaves and stalks in water for a period of 10 to 15 minutes with the resultant appearance of a blood-like colour solution [14]. This extract can be taken when hot or it can be allowed to cool before taking.Several species of Justicia are used in the management of several ailments such as inflammation, gastrointestinal disorders, diarrhoea, liver diseases, rheumatism and arthritis [13].

In our previous work, we investigated the leaves of this plant for their anti-diabetic activity and ability to protect the vital organs in rats from complications due to diabetes. We observed that the plant has hypoglycemic properties and protective ability against cellular damages in diabetes. This it doesby reversing alterations and stabilizing the biochemical indices of organ failure and complications in diabetes [15]. In the present study, to investigate the possible basic mechanisms behind the pharmacological activities of this plant, we determined the bioactive compounds present and evaluated its antioxidant properties presuming that inhibition of oxidative stress may be its mechanism of action. Therefore, this study aimed to evaluate the antioxidant potential of this plant extract and its effect on antioxidant status of alloxan-induced diabetic rats.

2. MATERIALS AND METHODS

2.1 Plant Sample Collection/Identification

Fresh *Justicia carnea* plant leaves were collected from farmlands in Awka, Anambra State, Nigeria. It was identified in Department of forestry, College of Natural Resources and Environmental Management, MichaelOkpara University of Agriculture, Umudike, Abia State, Nigeria.

2.2 Sample Preparation

The harvested fresh leaves of *Justicia carnea* were removed from the stems, sorted, washed, dried under shade and pulverized to powder using an electric grinder. Three hundred grams (300g) of powdered leaves were soaked in70% ethanol and allowed to stand for 48 hours at room temperature with intermittent stirring. The mixture was filtered through Whatman paper No. 4 with the aid of a vacuum filter and the filtrate was evaporated at 60^{o}C using a water bath (Techmel and Techmel, 420. USA). The dried residue was weighed and reconstituted in 70% ethanol at a concentration of 10 mg/ml and stored at 4^{o}C in a refrigerator until further use.

2.3 Test Animals

Thirty (30) albino rats (100-160 g) were used for this study. The animals were obtained from the animal house of the Department of Veterinary Medicine, University of Nigeria, Nsukka. The animals were kept under standard laboratory conditions (12h light, 12h dark cycle and room temperature) and were fed with standard laboratory rat pellets and water for two weeks. They had free access to food and water. The animals were allowed to acclimatize for one week before the start of the experiment.

2.4 Acute Toxicity Study

The LD_{50} of the extract was conducted using the method of Lorke [16].

2.5 Induction of Diabetes

Diabetes mellitus was induced in an overnight fasted albino rats by a single intraperitoneal injection of freshly prepared alloxan monohydrate (150 mg/kg) using distilled water as vehicle. To avoid the alloxan-induced hypoglycemic mortality, glucose solution was given to alloxan-induced animals. Forty-eight (48) hours after induction of diabetes, blood samples were drawn from the tail and the blood glucose level was determined using a One Touch Glucometer and test strips (Life Scan, USA). The rats that exhibited blood glucose levels above 200 mg/dl were regarded as successfully induced and were used to further the experiment.

2.6 Experimental Design

The rats were divided into five (5) groups of six rats each.

Group A - Normal Control, non-alloxan – induced rats, received food and water without treatment.

Group B - Diabetic and treated with glibenclamide (5 mg/kg) daily.

Group C – Diabetic Control (Induced but not treated).

Group D - Diabetic and treated with 100 mg/kg bodyweight of ethanol extract of *Justicia carnea* daily.

Group E - Diabetic and treated with 200mg/kg bodyweight of ethanol extract of *Justicia carnea* daily.

The administration was done orally for 14 days.

2.7 Blood Sample Collection and Preparation

At the end of the experiment, the rats were anaesthetized with chloroform and then sacrificed. Whole blood samples were obtained by cardiac puncture into well-labelled tubes and were allowed to cloth for about 2 hours and thereafter centrifuged (4000 g for 30minutes) to remove cells and recover serum, which was used for the biochemical assay.

2.8 *In-vitro* Antioxidant Activity Assay

2.8.1 Total flavonoid assay

The flavonoid content was determined by the use of a slightly modified colourimetry method described previously by Barros et al. [17]. A 0.5 ml aliquot of appropriately (2 mg/ml) diluted sample solution was mixed with 2 ml of distilled water and subsequently with 0.15 ml of 5% $NaNO_2$ solution. After 6 min, 0.15 ml of 10% $AlCl_3$ solution was added and allowed to stand for 6 min and then 2 ml of 4% NaOH solution was added to the mixture. Immediately, water was added to bring the final volume to 5 ml and then the mixture was thoroughly mixed and allowed to stand for another 15 min. The absorbance of the mixture was determined at 510 nm versus water blank with reference standard prepared using Catechin concentrations. The analyses were performed in triplicate. The result was expressed as mg Catechin equivalents per 100gram of the sample (mg CE/100 g).

2.8.2 Total phenolic assay

The total phenol content of the samples was determined using the method of Barros *et al*. [17]. The extract solution (1 ml) was mixed with Folin and Ciocalteu's phenol reagent (1 ml). After 3 min, saturated sodium carbonate solution (1 ml) was added to the mixture and adjusted to 10 ml with distilled water. The reaction was kept in the dark for 90 min, after which the absorbance was read at 725 nm (UV-Visible spectrophotometer). Gallic acid was used as standard and the results were expressed as mg of gallic acid equivalents (GAEs) per 100 g of extract.

2.8.3 Determination of ascorbic acid

The ascorbic acid content of the samples was determined according to the method of klein and Perry [18]. Dried leaf powder (20 mg) was extracted with 10ml of 1% metaphosphoric acid. It was allowed to stand for 45 min at laboratory temperature after which it was filtered through Whatman No.4 filter paper. One millilitre (1ml) of the filtrate was mixed with 9ml of 50µM 2,6-dichlorophenolindophenol sodium salt hydrate and the absorbance was measured at 515 nm using a UV-Vis spectrophotometer after 30 min. Ascorbic acid content was calculated from the calibration curve of authentic L-ascorbic acid and the result expressed as mg ascorbic acid equivalent per 100 gram of the dried sample (mgAAE/100g).

2.8.4 Determination of beta carotene and lycopene contents

These were determined by the method of Barros et al. [17]. The quantity of 100 mg of dried ethanol extract was vigorously shaken with 6 ml acetone-hexane mixture in the ratio of (4:6) for one minute and filtered using Whatman No.4 filter paper. The absorbance of the filtrate was read at 453, 505 and 663 nm respectively. The contents of lycopene and β-carotene were calculated according to the following equations:

Lycopene (mg/100 ml) =- $0.0458A_{663}$ $+0.372A_{505}+0.0806A_{453}$

β-carotene (mg/100 ml) = $0.216A_{663}$ +- $0.304A_{505}+0.452A_{453}$.

2.8.5 DPPH scavenging activity assay

The stable 2,2-diphenyl-1-picryl hydrazyl radical (DPPH) was used for the determination of free radical scavenging activity of the ethanolic extract of *Justicia carnea* leaves. This was assayed using the method of Ebrahimzadem, et al. [19]. 0.3 ml of different concentrations of the extract (0-500µg/ml) were mixed with 2.7 ml of methanolic solution of DPPH (100 µM) in test tubes. The mixture was shaken and kept in dark for 60 mins. The absorbance was taken at a wavelength of 517 nm using a spectro-photometer. BHA was used as standard.

The percentage of scavenging activity was calculated thus:

Percentage RSA= [(ADPPH-As)/ADPPH] ×100

Where as is the absorbance of the test solution with the sample and ADPPH is the absorbance of DPPH solution. The EC50 (concentration of sample at 50% RSA) was calculated from the graph of %RSA against the sample concentration.

2.8.6 Reducing power capacity assay

The reducing power was determined according to the method of Barros et al. [17]. This method is based on the principle of increase in the absorbance of the reaction mixture. 2.5 ml of various concentration of ethanolic extract of *Justicia carnea* leaves (0-500µg/ml) was mixed with 2.5 ml of 0.2 M sodium phosphate buffer (pH 6.6) and 2.5 ml of 1% potassium ferricyanide. The mixture was incubated at 50°C for 20mins. 2.5 ml of 10% Trichloroacetic acid was added and the mixture centrifuged at 1000rpm for 8min. The upper layer (5 ml) was mixed with 5ml of deionized water followed by the addition of 1ml of 0.1% ferric chloride. The absorbance was measured at 700nm. The graph of absorbance at 700nm against the extract concentrations was plotted. Butylated Hydroxyanisole (BHA) was used as a standard antioxidant.

2.8.7 Inhibition of lipid peroxidation assay

This was determined by the method of Barros et al. [20]. Determination of the extent of inhibition of lipid peroxidation was carried out using homogenate of the brain of a goat. The brain was dissected and homogenized with pestle and mortar in an ice-cold Tris-HCl buffer (pH 7.4, 20 mM) to produce 50% w/v brain homogenate which was centrifuged at 3000g for 10mins. An aliquot (0.1 ml) of the supernatant was incubated with 0.2 ml of the sample extract at various concentrations (0-500 µg/ml), in the presence of 0.1 ml of 10uM ferrosulphate and 0.1 ml of 0.1 nm ascorbic acid at 37^{o}C for 1hr. The reaction was stopped by the addition of 0.5 ml of 28% TCA followed by the addition of 0.38 ml of 2% TBA. The mixture was then heated at 80^{o}C for 20mins. After centrifugation at 3000rpm for 10mins to remove the precipitated protein, the colour intensity of the malondialdehyde (MDA)-TBA complex in the supernatant was measured by its absorbance at 532 nm. The inhibition ratio (%) was calculated using the following formula:

Inhibition ratio (%) = [(A-B)/A]×100%

Where A and B were the absorbance of the control and the compound solution respectively. The extract concentration providing 50% lipid peroxidation inhibition (IC_{50}) was calculated from the graph of antioxidant activity percentage against the extract concentrations. BHA was used as the standard.

2.9 *In-vivo* Antioxidant Assay

2.9.1 Assay of Superoxide Dismutase (SOD) activity

Superoxide Dismutase activity was determined by its ability to inhibit the auto-oxidation of epinephrine determined by the increase in absorbance at 480 nm as described by Sun and Zigma [21]. The reaction mixture(3 ml) contained 2.95 ml 0.05 m sodium carbonate buffer pH 10.2, 0.02 ml of the serum and 0.03 ml of 0.3 mM adrenaline in 0.005N HCL was used to initiate the reaction. The reference cuvette contained 2.95 ml buffer, 0.03 ml of the substrate (epinephrine) and 0.02 ml of water. Enzyme activity was calculated by measuring the change in absorbance at 480 nm for 3 min. Molar extinction coefficient (Σ) = 4020$M^{-1}Cm^{-1}$.

2.9.2 Assay of catalase activity

Catalase activity was determined according to the method of Sinha [22]. It was assayed calorimetrically at 620nm and expressed as µmoles of H_2O_2 consumed/min/mg protein at 25^{o}C. The reaction mixture (1.5 ml) contained 1.0ml of 0.01M phosphate buffer (pH7.0), 0.1ml of the serum and 0.4 ml of 2M H_2O_2. The reaction was stopped by the addition of 2.0 ml of dichromate-acetic acid reagent (5% potassium dichromate and glacial acetic acid were mixed in 1:3 ratio). The absorbance was recorded at 620nm using UV-Vis spectrophotometer and the catalase activity was calculated using Σ=40 $M^{-1}Cm^{-1}$.

2.9.3 Assay of malondialdehyde (MDA)

Malondialdehyde (MDA) an index of lipid peroxidation was determined using the method ofBuege and Aust [23]. 0.1 ml of the serum was diluted to 1ml using physiological saline, this was followed by the addition of 2 ml of (1:1:1 ratio) TCA - TBA -HCL reagent (thiobarbituric acid 0.37%, 0.24N HCL and 15% TCA) trichloroacetic acid- thiobarbituric acid –hydrochloric acid reagent. The mixture was boiled at 100^{o}C for 20

min and allowed to cool. Flocculent materials were removed by centrifuging at 3000rpm for 10mins. The supernatant was removed and the absorbance read at 532 nm against a blank. MDA was calculated using the molar extinction coefficient for MDA-TBA-Complex of 1.56 x $10^5M^{-1}CM^{-1}$.

2.10 Statistical Analysis

Values were expressed as mean ± SD. Data were analyzed using one-way analysis of variance (ANOVA). Statistical significance of the results between groups was determined using differences between means and values were considered significant at $P<0.05$.

3. RESULTS

3.1 Acute Toxicity Test

The result of the acute toxicity (LD_{50}) test showed that the extract had an acute oral LD_{50} greater than 5000 mg/kg in rats. There was no sign of toxicity observed and no death was recorded.

3.2 Bioactive Compounds Composition of *J. carnea*

The result of the composition of bioactive components of ethanol leaf extract of *Justicia carnea* is illustrated in Fig. 1 with flavonoid as the highest (65.65mgCE/100g), followed by phenol (57.8mgGAE/100g) and 40mgAAE/100gfor ascorbic acid with moderate amounts of lycopene and β-carotene which are 2.3533 mg/100g and 0.7mg/100g of the sample respectively.

3.3 DPPH Free Radical Scavenging Activity

Fig. 2 shows the DPPH radical scavenging activity of the ethanol leaf extract of *Justicia carnea*. The extract exhibited higher DPPH scavenging activity when compared to the standard; BHA, indicating higher antioxidant activity. The percentage of radical scavenging activity increased with increase in concentration but had a drop at a concentration of 125µg/ml. Different concentrations of the ethanol extract of *Justicia carnea* were plotted against %RSA. The plant extract had more potent DPPH radical scavenging activity with effective concentration at 50% inhibition; EC_{50} of 200µg/ml while the standard BHA had an EC_{50} value of 320 µg/ml.

3.4 Reducing Power Activity

Fig. 3 illustrates the reducing power activity of the plant extract and BHA. Both displayed a concentration-dependent increase in absorbance. However, the plant extract displayed higher activity indicated by a higher increase in absorbance which remained constant from 250-1000µg/ml as an additional increase in concentration did not produce any further increase in absorbance as shown in Fig. 3. More so, the plant extract produced higher reducing capacity with EC_{50} of 40µg/ml compared to BHA (400µg/ml).

3.5 Inhibition of Lipid Peroxidation

The ability of the plant extract to inhibit the process of lipid peroxidation is shown in Fig. 4 and compared with BHA. The percentage inhibition increased with increase in concentration and comparable with the standard; BHA. However, the percentage of inhibition for BHA was higher in all concentrations tested. At the highest concentration tested (100µg/ml), BHA produced 80.32% inhibition while the plant extract produced 60% inhibition.But the plant extract exhibited higher inhibition efficiency with EC_{50} value of 58µg/ml compared to BHA (60µg/ml).

3.6 *In-vivo* Antioxidant Activity

The result of the serum concentration of the antioxidant enzymes (SOD and Catalase) and MDA of the control and diabetic rats are shown in Table 1. Induction of diabetes produced oxidative stress which is shown from the significant reduction ($p<0.05$) in enzyme activities of all the diabetic groups when compared with the normal control group (group A). At lower extract dose of 100 mg/kg (group D), the enzyme activities were significantly reduced ($p<0.05$) but significantly increased at 200 mg/kg extract dose when compared with the diabetic untreated group (group C). MDA; a product of lipid peroxidation was non-significantly reduced ($p>0.05$) in group D and non-significantly increased in Group E when compared with group C (untreated diabetic rats) but significantly reduced ($p<0.05$) when compared with the normal control group (A).

4. DISCUSSION

The acute toxicity test result showed that the plant had an LD_{50} greater than 5000 mg/kg. This plant is therefore safe within this range and

shows that the plant has a high margin of safety at an acute level.

The bioactive constituents of *Justicia carnea* determined in this study as shown in Fig. 1 include total phenol, flavonoids, ascorbic acid, beta carotene and lycopene. The result revealed that the plant extract is rich in these bioactive compounds. This has confirmed the reports on phytochemical screening of *Justicia carnea* by other authors who also reported high contents of these bioactive compounds [24,25]. Phenolic compounds constitute the major group of compounds that take part in the antioxidant activity of natural products such as fruits, vegetables and all plant materials [26]. Specifically, flavonoids in plants have therapeutic benefits which include among others anti-inflammatory and antioxidant activities [27,28]. They possess the capacity to scavenge free radicals such as superoxide anions, lipid peroxy radicals and hydroxyl radicals [29,30]. Flavonoids are also modifiers, enhances and increases the ability of the body to react to allergens and other disease-causing agents such as viruses and cancer agents [31]. In this study, flavonoid was the highest bioactive compound present in the plant extract and with a high content of phenol and moderate content of the carotenoids, this plant can serve therapeutic purposes and employed as a source of antioxidants. The decrease in oxidative stress plays an important role in the maintenance of good health [32]. Natural anti-oxidants are capable of scavenging free radicals which include nitrogen and reactive oxygen species majorly implicated in oxidative stress [33].

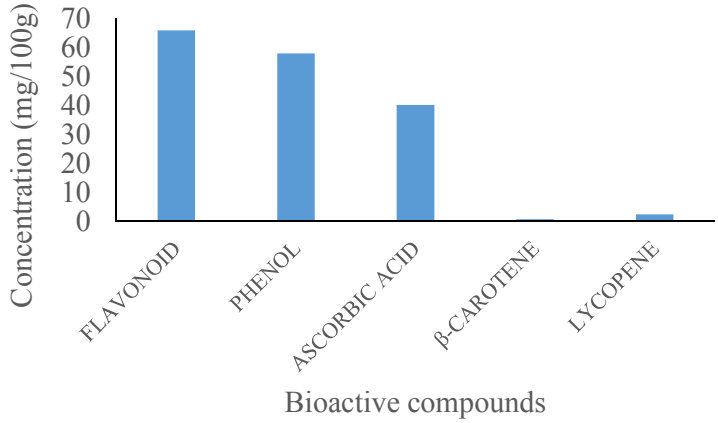

Fig. 1. Bioactive compounds composition of ethanol leaf extract of *Justicia carnea*

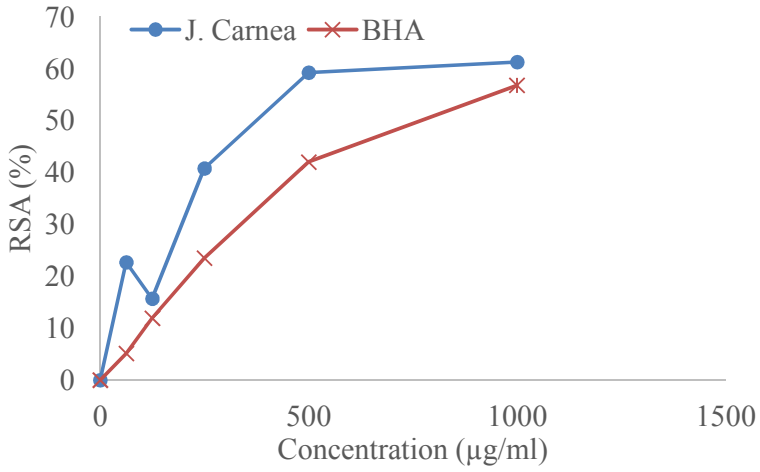

Fig. 2. DPPH radical scavenging activity of ethanol leaf extract of *Justicia carnea* and BHA

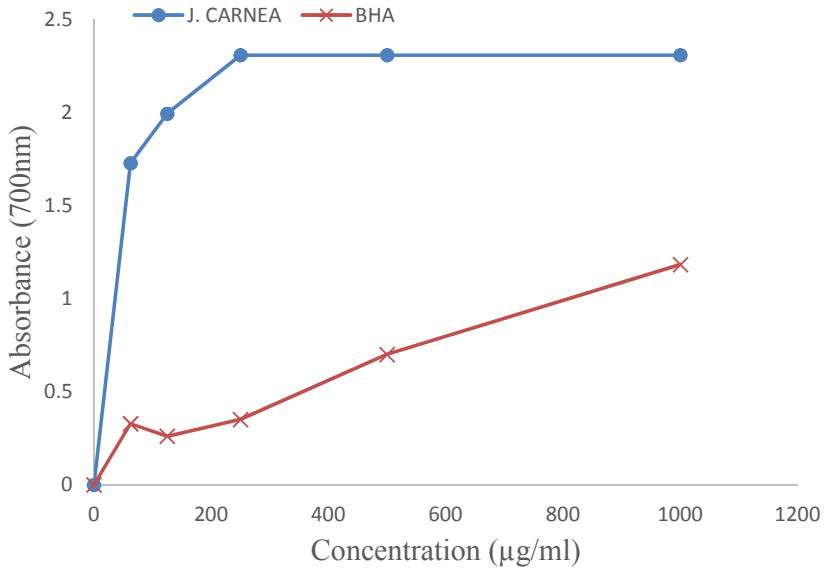

Fig. 3. Reducing power abilities of ethanol leaf extract of *Justicia carnea* and BHA

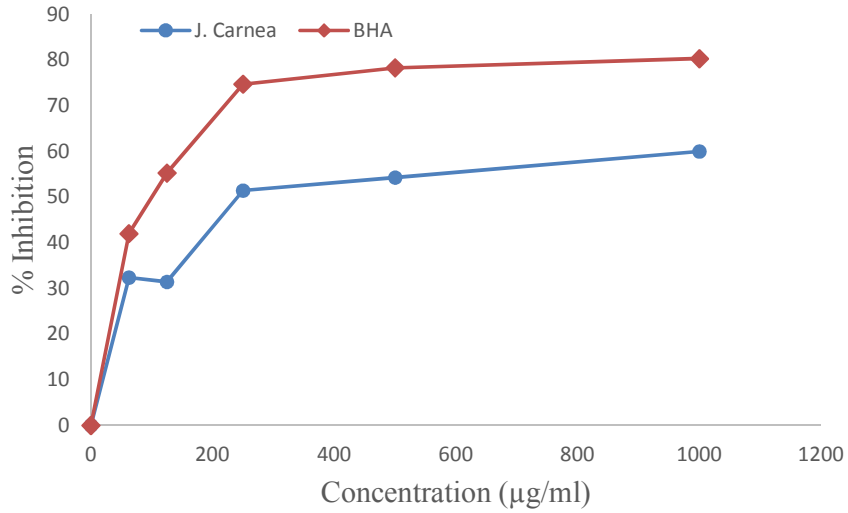

Fig. 4. Percentage inhibition of lipid peroxidation by ethanol extract of *Justicia carnea* and BHA

Table 1. Effects of ethanol leaf extract of *Justicia carnea* on serum SOD, catalase and MDA

Groups	SOD	Catalase	MDA
A (normal control)	6.39 ± 0.02^{b}	0.27 ± 0.02^{c}	4.59 ± 1.26^{c}
B (diabetic;5mg/kg glibenclamide)	7.68 ± 0.12^{b}	0.12 ± 0.01^{b}	4.97 ± 0.00^{c}
C (diabetic untreated)	2.21 ± 0.00^{c}	0.22 ± 0.00^{c}	3.21 ± 0.00^{a}
D (diabetic; 100mg/kg extract)	0.98 ± 0.09^{d}	0.09 ± 0.01^{b}	2.24 ± 1.31^{b}
E (diabetic; 200mg/kg extract)	3.21 ± 0.05^{a}	0.37 ± 0.10^{a}	3.53 ± 1.28^{a}

Values represent mean ± SD. Means with different letter are significantly different from each other (P<0.05)

The radical scavenging activity of the extract of *Justicia carnea* was tested using an ethanol solution of the stable free radical DPPH. The change in colour of DPPH from violet to pale yellow indicates the presence of free radical scavengers. In the radical form, DPPH molecule

has an absorbance at 517nm, which disappears after the acceptance of an electron or hydrogen radical from anantioxidant in the solution to become a stable diamagnetic molecule [34]. From studies, it was shown that the reduction in theabsorbance of DPPH radical caused by the phenolic compound is because of the reaction between radicals and antioxidant molecules, resulting in the scavenging of the radical by hydrogen or electron donation and seen as discolouration from violet to yellow [35]. The high DPPH scavenging activity of the plant extract recorded in this study would be attributed to the high phytochemical constituents. This is in line with various studies on scavenging abilities of flavonoids [36,37] from medicinal plants. Particularly important in the mechanism of action of flavonoids is its hydrogen donating ability in scavenging reactive species [38]. From this study, the EC_{50} of the plant extract was 200µg/ml while that of the BHA (Standard) was 320µg/ml. This implies that the ethanol leaf extract of *Justicia carnea* is a better and more efficient free radical scavenger than BHA and could be useful in handling and treatment of free radical-mediated cellular and pathological damages.

The results obtained in reducing power activity indicated that ethanol leaf extract of *Justicia carnea* had effective reducing power significantly higher ($p<0.05$) than the standard BHA. This is also evident from the EC_{50} value of the plant extract (40µg/ml) which is ten times lower than that of the standard (400µg/ml), indicating higher reductive efficiency. The reducing power of the plant extract was concentration-dependent similarly with BHA. In reducing power assay, the change in colour depends on the reducing power of the sample. The reducing power was evaluated by measuring absorbance at 700nm after mixing the samples with ferric compounds. In principle, the yellow colour of the test solution changes to various shades of green depending upon the reducing power of the extract. The presence of antioxidant in the extract causes the reduction of Fe3+/ Ferric cyanide complex to the ferrous form. Higher absorbance indicates increasing reducing power capacity. Reducing power assay mechanism is single electron transfer [39] and so from this study, it could be inferred that the plant extract are electron donors and thus possibly could reduce intermediates of lipid peroxidation by reacting with free radicals to stabilize and blockchain reactions [40,41]. This result suggests that the plant possesses high antioxidant potential asreducing power can be associated with the antioxidant activity of plant extract [42]. The reduction power indicates that the antioxidant compounds present in the sample are electron donors.

The result of the assay of lipid peroxidation revealed that the plant extract has very potent lipid peroxidation inhibition activity when compared with BHA. The $EC_{50}$of the plant extract was 58µg/ml while that of BHA was 60µg/ml showing that the plant extract has higher inhibition potency than BHA. Unsaturated lipids undergo oxidative degradation which is one of the deteriorating outcomes of free radicals that brings about lipid peroxidation [43]. The high amount of unsaturated fatty acids in bio-membranes renders them most susceptible to oxidative stress induced by free radicals [44] and is associated with cellular damages [45]. These damages have been reported to produce various life-threatening illnesses such as diabetes, cancer and cardiovascular diseases [46,47]. The plant extract of *Justicia carnea* displayed an effective inhibition of lipid peroxidation and this could also be attributed to the presence of phenolic compounds. This is true as phenolic compounds in addition to their capability to scavenge and mop up oxidizing radicals also act as potent chain-breaking antioxidants [48]. This distinguishing ability explains the role of phenolic compounds in mediating membrane lipid peroxidation arising from free radical species [49]. Their capacity to interact with membrane phospholipids and penetrate lipid bilayers coupled with their structural features make them efficient membrane stabilizers and inhibitors of lipid peroxidation [49,50]. Electron and/or hydrogen donation contributes to the chain breaking process, an essential step in the inhibition of lipid peroxidation [51]. This is also shown from DPPH scavenging and reducing power abilities displayed by the plant extract.

Induction of diabetes using alloxan to experimental animals has been shown to cause various pathological changes which affect marker enzymes [15]. Alloxan being structurally similar to glucose enters the pancreatic beta cells through the GLUT2 glucose transporter [52,53]. In the presence of intracellular thiols, especially glutathione, alloxan generates reactive oxygen species (ROS) in a cyclic redox reaction, giving dialuric acid as its reduction product [54]. Autoxidation of dialuric acid generates superoxide radicals, hydrogen peroxide and, in a final iron-catalysed reaction step, hydroxyl radicals [55,56] leading to oxidative stress. Oxidative injury and lipid peroxidation can be

monitored by a measure of malondialdehyde (MDA) levels. Lipid peroxidation of cellular membrane structures arising from the free radical activity is implicated in ageing and complications of diabetes mellitus [57]. MDA; the end product of lipid peroxidation is considered to be a significant marker of the oxidative process in body cells [58]. The results of this study (Table 1) showed a significant reduction ($p<0.05$) in serum MDA concentration following administration of the ethanol leaf extract of *Justicia carnea* at 100mg/kg when compared with the untreated diabetic rats. This reduction suggests that the extract prevented the excessive formation of free radicals and caused a reduction in the rate of lipid peroxidation. This correlates with the result of the high *in-vitro* inhibition of lipid peroxidation observed in Fig. 4. Increased lipid peroxidation in the blood and organs can lead to various degenerating diseases. The decreased level of MDA shows that *Justicia carnea* leaf extract can improve the pathological condition of lipid peroxidation in diabetes.

SOD and CAT are the two scavenging enzymes that eliminate toxic free radicals [59] and are considered primary enzymes, since they are involved in direct elimination of reactive oxygen species [60]. SOD is an important defence enzyme which catalyzesthe conversion of superoxide radical to hydrogen peroxideand CAT is a heme protein which catalyzes the detoxification and reduction of$H_2O_2$to water and protects tissue from highly reactive hydroxyl radicals [61,62,63]. The reduced activity of SOD and CAT observed during diabetes may be due to the deleterious effect of the accumulation of free radicals such as superoxide anion radicals and hydrogen peroxide produced by alloxan. High levels of free radicalsand reduced levels of antioxidant enzyme activity lead to cellular damages, inactivation of enzymes and lipid peroxidation. The results of the present study as presented in Table 1 showed that diabetes induction by alloxan reduced the activities of these two enzymes in all the diabetic groups. But following the administration of the plant extract at 200mg/kg there was a significant increase ($p<0.05$) in serum SOD and CAT activities when compared to the diabetic untreated rats (group C). The extract could not reverse the effect at lower dose implying that the extract works better at a higher dose. This increase and restoration of the activities of the enzymes following treatment with the extract could be attributed to the presence of phenolic compounds. It has been reported that phenol antioxidant activity prevents oxidation and free radical change [64]. Enzyme induction by phenolic compounds has also been demonstrated [65].

5. CONCLUSION

The leaf extract of *Justicia carnea* has been widely used in traditional medicine for the treatment of various illnesses. This study serves as scientific proof of its pharmacological activities and linked to its potent and rich content of bioactive compounds. The strong antioxidant capacity exhibited by the plant extract in both in-vitro and in-vivo experiment shows that the plant could be used in the treatment and management of debilitating diseases emanating from oxidative damage. It has also shown that the plant extract possesses protective ability against cellular damages arising from free radical-mediated complications. Although the plant has proven to possess antioxidant properties in both in-vitro and in-vivo, further study is needed on possible toxic effect on chronic usage.

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.

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COMPETING INTERESTS

Authors have declared that no competing interests exist.

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