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Contents lists available at ScienceDirect

Asian Pacific Journal of Tropical Disease

journal homepage: www.elsevier.com/locate/apjtd

Floral research doi: 10.1016/S2222-1808(16)61102-7 

# Antioxidant and antidiabetic activities of the seed and leaf extracts of *Chrysophyllum albidum*

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ARTICLE INFO

*Article history:*
Received 5 Apr 2016
Received in revised form 22 Apr, 2nd revised from 26 Apr 2016
Accepted 5 Jun 2016
Available online 21 Jul 2016

*Keywords:*
Catalase
*Chrysophyllum albidum*
Diabetes
Glycogen
Malondialdehyde
Oxidative stress

ABSTRACT

**Objective:** To investigate the antioxidant and antidiabetic activities of the seed and leaf extracts of *Chrysophyllum albidum* (*C. albidum*).

**Methods:** After assessing the *in vitro* ferric reducing power and hydrogen peroxide scavenging activities as well as the flavonoid and flavanol contents, the seed and leaf extracts were administered to diabetic rats for 7 days. The animals were sacrificed and serum was obtained for the determination of blood glucose level while liver sample was used for the quantification of glycogen level as well as lipidic peroxidation and catalase activity.

**Results:** Seed and leaf extracts of *C. albidum* showed ferric reducing activity and very high hydrogen peroxide scavenging potential. After the administration of treatment in diabetic rats, there was a significant decrease ($P < 0.05$) in blood sugar level and a significant increase ($P < 0.05$) in liver glycogen level in Groups 3 and 4 animals administered the leaf and seed extracts respectively compared to Group 1 (the negative control). Also, catalase activity and malondialdehyde levels increased in Groups 3 and 4 administered the extracts compared to Group 1 animals (the negative control). Flavonoids and flavanol were present and significantly higher ($P < 0.05$) in the leaf than seed extract. In all, the leaf extract showed the greatest activities.

**Conclusions:** These results suggest that the leaf and seed extracts of *C. albidum* possess both *in vitro* and *in vivo* antioxidant activities in scavenging free radicals as well as antidiabetic activity, and as such, a potentially important compound in antidiabetic drug discovery.

## 1. Introduction

Diabetes is a heterogeneous and multifactorial disorder whose development is contributed by environmental and genetic factors[1]. Globally, diabetes is the 8th leading cause of death with a death rate of about 1.5 million affecting about 4% of the global population[2]. The disease incidence in 2010 was about 285 million people worldwide, and is projected to grow to 439 million by 2030[3]. In Nigeria, about 6 million people have full blown diabetes mellitus with a prevalence of 2.7% of which 2.6% are male adults while 2.8% are female adults[4]. Also, the prevalence of diabetes in isolated regions in Nigeria ranges between 0.9% and 15%[5].

Diabetes is a disorder of carbohydrate, fat and protein metabolism characterized by high sugar level in blood (hyperglycaemia) and in urine (glycosuria). All forms of diabetes may be as a result of decrease in the concentration of circulating insulin (insulin deficiency) and/or decrease in the response of insulin on peripheral tissues (insulin resistance)[6]. Insulin resistant diabetes usually known as type 2 is the most prevalent type accounting for about 90% of all diabetes cases[7]. It develops when the body tissues or cells can no longer recognize insulin for sugar metabolism. As such, glucose is not metabolised and high amount of it remains in circulation (hyperglycaemia). Two major roles of insulin are to promote the breakdown of glucose and synthesis of

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All experimental procedures involving animals were conducted in accordance to the guidelines for ethical conduct in the care and use of non-human animals in research by the American Psychological Association and approved by the Institutional Ethical Review Committee of Godfrey Okoye University Enugu Nigeria.

The journal implements double-blind peer review practiced by specially invited international editorial board members.

glycogen as storage of the excess sugar[8]. However, insulin resistance causes a decrease in the synthesis of glycogen and alters the activity of enzymes involving in glycogen synthesis[9].

The development of diabetes and progression of associated complications are usually associated with oxidative stress which is as a result of over whelming level of reactive oxygen species (ROS) or free radicals[10]. ROS generally contain an unpaired electron in the atomic orbital thus are capable of existing independently and causing damage to cells[11,12]. Under diabetic conditions, glucose oxidation is believe to be the main source of free radicals which are produced mainly through the glycation reaction to generate advanced glycosylation end products (AGEs)[13-15]. AGEs have been shown to be detected in β-cells of the pancreas kept under high concentrations of glucose[16]. Also, lipids are major target of the generated free radicals. Hydroperoxides in the presence of transition metals like copper or iron can react with lipid in the membrane of cells to form stable aldehydes such as malondialdehyde (MDA) thereby damaging cell membranes[17].

On the other hand, antioxidants which are molecules stable enough to act as electron donor to free radicals can neutralize or remove free radical thereby preventing it from damaging the cell[18]. In the extracellular and intracellular environment of the body, these antioxidants exist as non-enzymatic and enzymatic molecules capable of removing ROS[19]. The non-enzymatic antioxidants including glutathione, ubiquinol and uric acid produced by the body and vitamin E (α-tocopherol), vitamin C (ascorbic acid), B-carotene, polyphenols, flavonoids, flavonols *etc*. from dietary sources or plants support the enzymatic defence mechanism to remove ROS[20]. On the other hand, the enzymatic antioxidant system is initiated firstly by superoxide dismutase which catalyses the breakdown of superoxide released during oxidative phosphorylation to hydrogen peroxide and oxygen[21]. This is followed by the activity of catalase and glutathione peroxidase which remove hydrogen peroxide by decomposing it to water and oxygen[22-24]. However, the level of these antioxidant enzymes in islet cells under diabetic condition is found to be very low compared to those of other cells and tissues[25].

Diabetes are mostly being managed by keeping the blood sugar level as close to normal with drugs such as metformin, sulfonylurea and thiazolidinediones *etc*. as well as artificial insulin[26,27]. However, some of these oral medications have been shown to have side effects. Metformin causes gastrointestinal side effects and lactic acidosis, sulfonylurea causes hypoglycaemia and weight gain, while thiazolidinediones causes cognitive heart failure, fluid retention and bone fractures and artificial insulin has also been shown to cause weight gain and hypoglycaemia[28-31]. More so, based on the influence of oxidative stress on diabetes complications, more effective methods of management will entail antidiabetic as well as antioxidants treatments[32]. Traditional medicine is gaining so much interest recently due to their multiple modes of actions with minimal adverse effects in humans[33]. Some medicinal plants have been shown to have antidiabetic and antioxidant activities. These includes *Nypa fruticans* Wurmb, *Emblica officinalis*, *Veronica amygdalina*, *Annona muricata*, *Melastoma malabathricum etc*.[34-38].

*Chrysophyllum albidum* Linn. (*C. albidum*), African five-star fruit or locally known as "Udara" in Nigeria belongs to the Sapotaceae family. It is reported to be found in diverse ecozones in Nigeria, Niger Republic, Uganda, Cote d'Ivoire and Cameroon[39]. It is a forest tree that often grows about 36.5 m in height though it can also be smaller in size. This plant is of great agro-economic importance as the fleshy and juicy fruit are commonly consumed and locally sold. The roots, leaves, bark and seed cotyledons have locally been used for various medicinal purposes[40]. The bark is used in treating skin eruptions, diarrhoea and stomach ache while the stem bark extracts has been shown to have antimicrobial and antiplasmodial properties[41]. The leaf has been shown to have antiplatelet and hypoglycaemic properties[42]. More so, *C. albidum* has been reported to have antioxidant activity[43]. However, little or no study has evaluated the combined antioxidant and antidiabetic activities of the seed and leaf extracts of *C. albidum*.

## 2. Materials and methods

### 2.1. Chemicals

Methanol, chloroform, normal saline, dichromate-acetic acid reagent, Hydrochloric acid and hydrogen peroxide were obtained from BDH (Poole, England). Ethanol and phosphate buffer were obtained from Darmstedt, Germany. Aluminum chloride and anthrone reagent were obtained from E-Merek reagent, USA. Ferrous chloride was obtained from Griffin and George, Wembley London, England. Rutin, ascorbic acid and thiobarbituric acid were purchased from JHD, Guangdong Guanghua Chemical Factory Co., China. While trichloroacetic acid, potassium ferricynaide, trichloroacetic acid and sodium acetate were obtained from Vicker Laboratories Ltd, United Kingdom.

### 2.2. Plant material and extraction

The leaves of *C. albidum* were obtained from Akpugo in Nkanu, Enugu State while the seeds were purchased from New Haven market in Enugu, Enugu State of Nigeria. The leaves were dried for 2 weeks under room temperature [(25 ± 2) ℃] in the Chemistry Laboratory of Godfrey Okoye University, Enugu after which they were ground to powder. Extraction was done with slight modifications using the method of Redfern *et al*.[44]. About 100 g of the powdered leaves was extracted using 500 mL ethanol in a soxhlet extractor. Using a filter paper, 100 g of the sample was put in a filter bag and loaded into a flask containing the extracting solvent (500 mL of ethanol) in the main chamber of the Soxhlet extractor. The solvent was then heated to reflux that the evaporated extracted compounds condensed and dissolved in the warm solvent. The extract was then evaporated in a water bath at 45 ℃ to obtain 25.15 g with a percentage yield of 25.15%. The seeds were dried for 5 weeks under room temperature [(25 ± 2) ℃] and ground to powder. About 100 g of the powdered seed was macerated in hexane for 30 min to defat and was extracted using 300 mL of ethanol in Soxhlet extractor. The extract was evaporated in a water bath at 45 ℃ to obtain 14.65 g giving a percentage yield of 14.65%.

### 2.3. Estimation of polyphenol compounds

#### 2.3.1. Total flavonoids

Total flavonoids content in the extract solution was determined by the method of Ordoñez *et al*. which was based on the formation of an aluminium-flavonoids complex[45]. An amount of 0.5 mL of 2% aluminum chloride-ethanol solution was added to 0.5 mL solution of plant extract. The mixture was incubated at room temperature for 1 h and the absorbance was measured at 420 nm using a UV spectrophotometer by Medifield Equipment and Scientific Ltd, England. All experiments

were done in triplicate and the values were calculated from the calibration curve of rutin (Figure 1) using the equation:

$Y = 0.001X + 0.030$, $R^2 = 0.910$

$$X = \frac{Y - 0.030}{0.001}$$

where, $Y$ was absorbance and $X$ was concentration of rutin (mg/L).

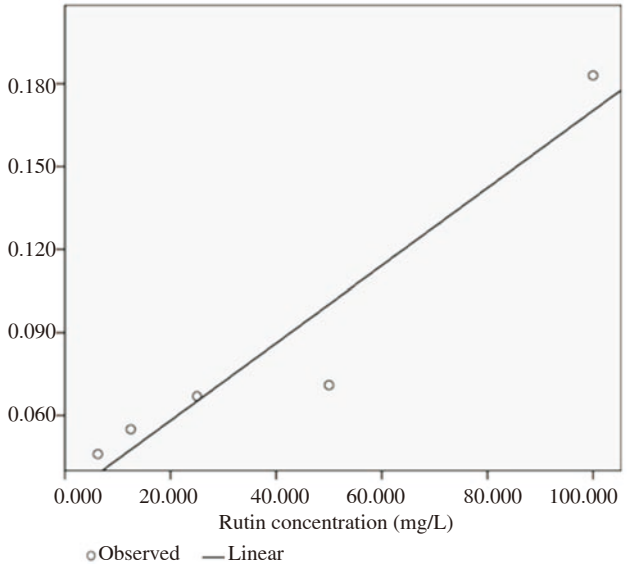


**Figure 1.** Calibration curve of total flavonoid of rutin.

### 2.3.2. Total flavonols

The method of Kumaran and Karunakaran was adopted for the determination of total flavonol[46]. An amount of 2.0 mL of the sample, 2.0 mL of aluminum chloride-ethanol solution and 3.0 mL of (50 g/L) sodium acetate solution were all added together in a test tube. After incubation at 20 °C for 150 min, the absorbance was read at 440 nm. All experiments were done in triplicate and the value for total flavonol was calculated as rutin (mg/L) equivalent from the calibration curve of rutin (Figure 2) using the equation:

$Y = 0.004X + 0.129$, $R^2 = 0.973$

$$X = \frac{Y - 0.129}{0.004}$$

where, $Y$ was absorbance and $X$ was concentration of rutin (mg/L).

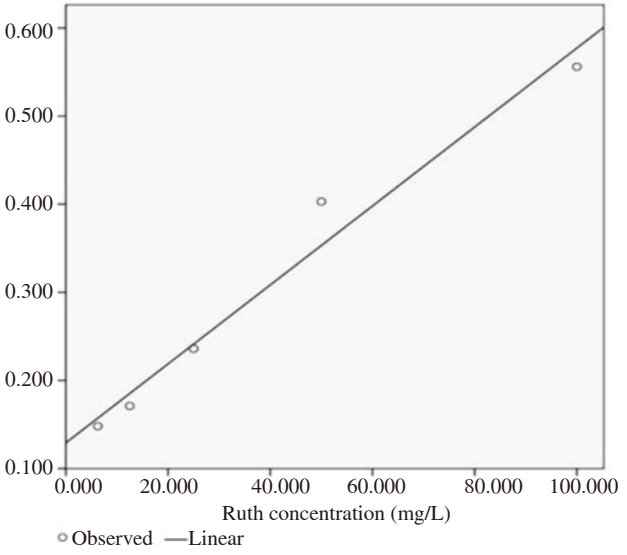


**Figure 2.** Calibration curve of total flavonol of rutin.

## 2.4. In vitro antioxidant activity

### 2.4.1. Determination of reducing power

The method of Yen and Chen was used to determine the reducing power of the extract[47]. The concentration range between 0.125 and 1.0 mg/mL each for the leaf and seed extracts and ascorbic acid were separately prepared, then 1 mL each of the concentrations was mixed with a mixture containing 2.5 mL of potassium ferricyanide (1% w/v) and 2.5 mL of 0.2 mol/L phosphate buffer (pH 7.0). The resulting mixture was incubated at 50 °C for 20 min. About 2.5 L of trichloroacetic acid (10% w/v) was further added to the mixture and then centrifuged at 3 000 r/min for 10 min. The upper layer of the solution (2.5 mL) was collected and mixed with 0.5 mL of ferrous chloride (0.1% w/v) and 2.5 mL of distilled water. The absorbance was taken at 700 nm against a blank sample. Higher reducing power of the plant extract was indicated by an increased absorbance of the reaction mixture

### 2.4.2. Hydrogen peroxide scavenging activity

The method of Ruch *et al*. was adopted to determine the hydrogen peroxide scavenging activity of the plant extracts[48]. About 4 L of seed and leaf extracts and ascorbic acid at various concentrations of 0.125–1.0 mg/mL each was mixed with 0.6 mL of 4 mmol/L hydrogen peroxide solution prepared in phosphate buffer (0.1 mol/L, pH 7.0). The mixture was incubated at room temperature for 10 min. The absorbance of the solution mixture was read at 230 nm against a blank solution containing only the plant extract without hydrogen peroxide. The amount of hydrogen peroxide radical inhibited by the extract was calculated using the equation:

Hydrogen peroxide radical scavenging activity = [(Absorbance control – Absorbance sample)/(Absorbance control)] × 100

where, Absorbance control was the absorbance of hydrogen peroxide radical + solvent; Absorbance sample was the absorbance of hydrogen peroxide radical + sample extract or standard.

## 2.5. Experimental animals and handling

Male albino rats weighing between 70 and 150 g were obtained from the Veterinary Department of Medicine, University of Nigeria Nsukka, Enugu State and transported to the animal house of Godfrey Okoye University, Enugu Nigeria. The animals were acclimatized under laboratory conditions for 2 weeks prior to the experiments based on the guidelines of proper care and use of laboratory animals. The rats were maintained at ambient room temperature [(25 ± 2) °C] under a light/ dark cycle of 12 h and fed with rat chow with water *ad libitum*.

## 2.6. Experimental design

A total of 25 rats were randomized into five groups each consisting of five animals. Groups 2–5 animals were administered 20 mg/mL of alloxan monohydrate dissolved in distilled water at a dose of 80 mg/

kg intraperitoneally after fasting for 16 h to develop diabetes. After 2 days, the animals were administered various treatments. Group 1 as the negative control consisting of diabetic rats were treated with 0.5 mL of distilled water. Group 2 as the positive control consisting of the diabetic rats were given 120 mg/mL metformin dissolved in distilled water at a dose of 500 mg/kg. Group 3 consisting of diabetic animals were given 50 mg/mL of the leaf extract dissolved in distilled water at a dose of 200 mg/kg. Group 4 consisting of diabetic animals were given 50 mg/mL of the seed extract dissolved in vegetable oil at a dose of 200 mg/kg. Group 5 as the normal control consisting of non-diabetic animals were given 0.5 mL of distilled water only. The treatments were administered orally between 0.3 and 0.7 mL relative to the weight of the animals in respect to the various doses using a 1 mL syringe for 7 days. The blood glucose was measured before and after induction of diabetes and after 7 days of treatment.

### 2.7. Collection of samples from animals

Following an overnight fasting for 24 h after 7 days of treatment, the animals were anaesthetized using chloroform and sacrificed. Blood was collected by cardiac puncture to quantify fasting blood glucose using Accu-Chek glucometer. The liver from each animal was excised after dissecting the animals and rinsed with normal saline. A volume of 10% liver homogenate was prepared using 0.25 mol/L sucrose in phosphate buffer (0.2 mol/L, pH 7.0) and centrifuged at 12 000 r/min for 5 min. The supernatant obtained was used for the estimation of catalase and lipid peroxidation.

### 2.8. In vivo antioxidant activity

#### 2.8.1. Determination of catalase activity

The method described by Pari and Latha was adopted for the determination of catalase activity[49]. Liver tissue was obtained and homogenized in 0.01 mol/L phosphate buffer (pH 7.0) and the homogenate was centrifuged at 5 000 r/min. A volume of 0.1 mL of liver homogenate (10% w/v) was added to a reaction mixture containing 0.4 mL of hydrogen peroxide (0.2 mol/L) and 1 mL of 0.01 mol/L phosphate buffer (pH 7.0). Finally, 2 mL of dichromate-acetic acid reagent (5% potassium dichromate prepared in glacial acetic acid) was added to the mixture to stop the reaction. The absorbance was taken at 620 nm and the percentage inhibition was calculated using the equation:

Catalase inhibition (%) = [(Normal activity – Inhibited activity)/(Normal activity)] × 100%

where, Normal activity was hydrogen peroxide + phosphate buffer; Inhibited activity was hydrogen peroxide + phosphate buffer + liver homogenate.

#### 2.8.2. Estimation of lipid peroxidation

A modified method of Niehaus and Samuelsson was adopted for the estimation of lipid peroxidation in the liver[50]. This involved a colorimetric assay for the determination of thiobarbituric acid reactive substances. About 100 μL of liver homogenate (10% w/v) was added into a mixture of 2 mL (1:1:1 ratio) of 0.37% thiobarbituric acid, 15% trichloroacetic acid and 0.25 mol/L hydrogen chloride reagent. The resultant mixture was incubated at 100 °C in a water bath for 30 min after which it was then cooled. A clear supernatant was collected and the absorbance was taken at 535 nm against the blank to determine the quantity of MDA formed. Concentration of MDA was calculated using the equation:

$$C = \frac{A}{E \times L}$$

where, A was the absorbance of the sample; E was the extinction coefficient ($1.56 \times 10^5$ mol/L/cm); L was the length of the light path (1 cm).

### 2.9. Determination of liver glycogen content

The liver glycogen content of the samples was measured based on the method of Carroll *et al.*[51]. About 50 mL of liver was added to 2 mL of 30% potassium hydroxide and the mixture was incubated in a water bath at 100 °C for 15 min to hydrolyse it. The liver hydrolysate was cooled and 2.4 mL of 95% ethanol was added. The resultant mixture was incubated overnight at 4 °C and then centrifuged at 3 000 r/min for 15 min. The supernatant was discarded and the tubes were kept in an inverted position for 10 min to allow them to drain and obtain dry glycogen pellet. The glycogen pellet was dissolved in 1 mL of distilled water via vigorous shaking and added 5 mL of anthrone reagent (0.05% anthrone, 1% thiourea, 72% (v/v) $H_2SO_4$) with the tubes placed in cold water to prevent overheating. After cooling, the reaction mixture was incubated in a water bath at 100 °C for 15 min and then cooled. The absorbance was read at 620 nm using a spectrophotometer by Medifield Equipment and Scientific Ltd, England. Glucose (0.25 mg) was used as standard and glycogen content was expressed as milligram of glycogen per gram of wet liver tissue.

Glycogen content = Absorbance of glycogen/Absorbance of glucose × 0.25 mg

### 2.10. Ethical considerations

All experimental procedures involving animals were conducted in accordance to the guidelines for ethical conduct in the care and use of non-human animals in research by the American Psychological Association and approved by the Institutional Ethical Review Committee of Godfrey Okoye University Enugu Nigeria.

### 2.11. Statistical analysis

The data obtained was analyzed using SPSS version 16.0 and the results were expressed as mean ± SE. Significant differences were established by One-way ANOVA and *post-hoc* test of multiple comparisons was used to determine between group differences. A difference was considered significant at $P \leqslant 0.05$.

## 3. Results

### 3.1. In vitro antioxidant activity

The seed and leaf extracts showed ferric reducing power for the various concentrations ranging between 0.128 and 0.479 mg/mL, with a greater activity observed in the leaf extract. The ferric reducing power was lower than that of ascorbic acid, the standard. The ferric reducing power for both extracts as well as ascorbic acid increased (0.637 to 1.363 mg/mL) with increasing concentration, thus was dose dependent (Figure 3).

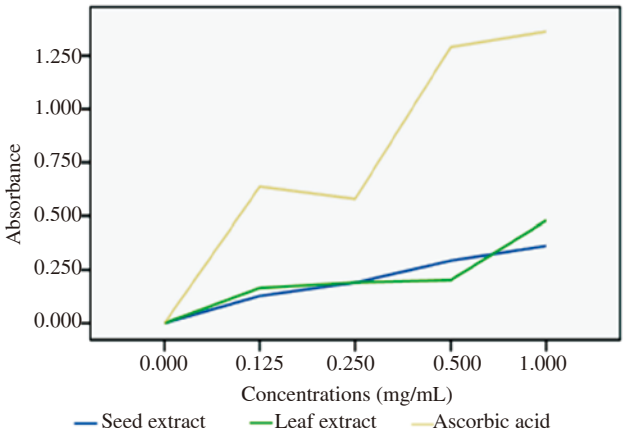


**Figure 3.** Reducing power activity of the seed and leaf extracts of *C. albidum* compared to ascorbic acid.

The seed and leaf extracts showed extremely high hydrogen peroxide scavenging activity close to 99.9% and were comparable to ascorbic acid. The hydrogen peroxide scavenging activity was very high for both extracts at the lowest concentration of 0.125 mg/mL (Table 1).

**Table 1**

Hydrogen peroxide scavenging activity of the seed and leaf extracts of *C. albidum* compared to ascorbic acid.

| Concentrations (mg/mL) | Inhibition of hydrogen peroxide (%) | | |
|---|---|---|---|
| | Seed extract | Leaf extract | Ascorbic acid |
| 0.125 | 99.59 | 99.64 | 99.55 |
| 0.250 | 99.99 | 99.89 | 99.89 |
| 0.500 | 99.99 | 99.99 | 99.96 |
| 1.000 | 99.99 | 99.99 | 99.99 |

### 3.2. Estimation of total phenolic compounds

Total flavonoids and flavonols were present in different amounts in the aqueous and ethanol extracts. The concentration of flavonoid was significantly higher ($P > 0.05$) in the leaf extract (704.00 ± 23.06) mg/g than in the seed extract (27.33 ± 18.56) mg/g. Also, the flavonol content was significantly higher ($P > 0.05$) in the leaf extract (606.09 ± 58.07) mg/g than in the seed extract (244.96 ± 11.12) mg/g. Overall, the leaf extract contained more flavonoid and flavanol than the seed extract (Table 2).

**Table 2**

Total flavonoid and flavonol content in plant extract.

| Compounds | Concentrations of rutin (mg/g) | |
|---|---|---|
| | Seed extract | Leaf extract |
| Flavonoid[a] | 64.00 | 730.00 |
| | 4.00 | 686.00 |
| | 14.00 | 696.00 |
| Flavonol[b] | 254.50 | 539.88 |
| | 232.75 | 648.38 |
| | 247.62 | 630.00 |

[a]: $P = 0.000$; [b]: $P = 0.000$

### 3.3. In vivo antioxidant activity

The percentage of catalase inhibition and MDA levels were shown in Table 3. Catalase activity was significantly higher ($P < 0.05$) in Group 5 animals (normal control) which were not administered alloxan compared to Groups 1, 2, 3 and 4. The catalase activity was lower in Group 1 animals which were not treated with distilled water compared to Groups 2, 3 and 4 (the treated group). The catalase activity of Group 1 was significantly lower ($P < 0.05$) compared to Group 3 animals which were administered leaf extract.

MDA was lower in Group 5 animals (normal control) compared to all the other groups (1, 2, 3 and 4). The MDA level was the highest in Group 4 animals administered seed extract while the lowest level was recorded in Group 5 animals.

### 3.4. Blood glucose and glycogen level

The blood glucose and glycogen levels were summarized in Table 4. Prior to administration of alloxan, the blood glucose level was not

**Table 3**

Catalase activity and MDA level in various animal groups.

| Parameters | Group 1 | Group 2 | Group 3 | Group 4 | Group 5 | *P* value |
|---|---|---|---|---|---|---|
| Catalase inhibition (%) | 10.15 ± 0.00[a] | 25.87 ± 4.72[ab] | 27.39 ± 0.27[a] | 22.88 ± 0.00[a] | 58.61 ± 6.11[b] | 0.001 |
| MDA (μmol/L) | 0.78 ± 0.00 | 1.91 ± 0.75 | 1.57 ± 1.28 | 8.70 ± 0.00[a] | 0.44 ± 0.07 | 0.000 |

All values were expressed as mean ± SE; Group 1: Negative control; Group 2: Positive control; Group 3: Leaf extract; Group 4: Seed extract; Group 5: Normal control; [a]: Significantly different from Group 5; [b]: Significantly different from Group 1.

**Table 4**

Blood glucose and glycogen level in various animal groups (mg/dL).

| Parameters | Group 1 | Group 2 | Group 3 | Group 4 | Group 5 | *P* value |
|---|---|---|---|---|---|---|
| Blood glucose level before diabetes induction | 83.80 ± 8.48 | 112.00 ± 12.65 | 88.60 ± 9.64 | 72.00 ± 4.53 | 109.00 ± 13.89 | 0.079 |
| Blood glucose level after diabetes induction | 314.00 ± 67.04[a] | 315.00 ± 61.82[a] | 182.00 ± 75.52[a] | 186.00 ± 59.04[a] | 110.00 ± 13.59 | 0.192 |
| Blood glucose level after treatment | ND | 129.50 ± 20.50 | 70.50 ± 4.50[b] | 119.00 ± 1.00 | 133.30 ± 14.75 | 0.128 |
| Glycogen content (mg/g) | 0.48 ± 0.00 | 0.76 ± 0.06 | 0.83 ± 0.25[b] | 0.82 ± 0.00[b] | 0.68 ± 0.01 | 0.018 |

All values were expressed as mean ± SE; Group 1: Negative control; Group 2: Positive control; Group 3: Leaf extract; Group 4: Seed extract; Group 5: Normal control; [a]: Significant difference with Group 5; [b]: Significantly different from Group 1; ND: Not determined because animals died due to alloxan intoxication.

significantly different ($P > 0.05$) between all the animal groups. After diabetes induction, there was a significant increase ($P < 0.05$) in the blood glucose level in all the groups (1–4) administered with alloxan compared to Group 5 (normal control) administered with distilled water. After treatment was administered, the blood sugar level significantly decreased ($P < 0.05$) in Groups 2, 3 and 4 animals administered with metformin, leaf and seed extracts respectively compared to Group 5, the normal control administered with distilled water.

Glycogen level was increased in the treated groups (2, 3 and 4) compared to the untreated groups (1 and 5). The glycogen level significantly increased ($P < 0.05$) in Groups 3 and 4 animals administered with the leaf and seed extracts respectively compared to Group 1, the negative control.

## 4. Discussion

Oxidative stress, a condition characterized by an increase in ROS in the body is known to be partly responsible for diabetes and its complications[52]. The genesis of oxidative stress in both diabetic patients as well as experimental diabetic animals may be due to glucose protein glycation, formation of AGEs, autooxidation and the polyol pathway[53]. It has also been shown that higher concentrations of glucose impair or depress the natural antioxidant defence system thus promoting oxidative stress and eventually diabetes[54]. Therefore, restoring the antioxidant defence system may prevent diabetes and its complications. So, many medicinal plants have been known for their therapeutic activities with minimal side effects, a limitation of currently available oral pharmacological agents[55]. *C. albidum* is a plant with numerous medicinal properties, but most importantly, it has been reported to have antioxidant activity[41,43]. Hence, we hypothesize a possible antioxidant as well as antidiabetic activities of *C. albidum.*

Antioxidants are capable of scavenging free radicals *in vitro* by reducing $Fe^{3+}$ to $Fe^{2+}$ through electron transfer ability[56]. In this study, the plant extracts showed ferric reducing activity with the leaf extract having a higher activity than the seed extract. However, the ferric reducing power was lower in the plant extract than in ascorbic acid. More so, *C. albidum* was shown to scavenge hydrogen peroxide in the reaction mixture and the activity of seed and leaf extracts was very high and similar to that of ascorbic activity. Hydrogen peroxide is an ROS of great health implication because of its ability to attack, react with and penetrate biological membranes[57]. If converted to hydroxyl radical by $Cu^{2+}$ and $Fe^{2+}$ ions, hydrogen peroxide may also be toxic in the cell[58]. Therefore, the ability of *C. albidum* to scavenge free radicals *in vitro*, prompted *in vivo* analysis to confirm its antioxidant activity.

Catalase is an endogenous enzyme that mediates the breakdown of hydrogen peroxide to water and oxygen[59]. Increase in catalase activity may suggest potential *in vivo* antioxidant activity. In this study, there was significant increase ($P < 0.05$) in the catalase activity of the experimental groups administered the seed and leaf extracts as well as the positive control compared to the normal control whereas this activity was significantly lower ($P < 0.05$) in the non-treated diabetic group compared to the normal control. This confirms the ability of the plant extract to scavenge free radicals *in vivo* by promoting catalase activity. In oxidative stress conditions, cell membranes are usually damaged due to lipid peroxidation caused by ROS and by-products for example thiobarbituric acid reactive substances are formed[60]. However, because ROS species have short live span, they are difficult to be measured directly. Instead, the by-products such as MDA are measured as markers of lipid peroxidation[61]. The MDA level was higher in animals administered with leaf extract compared to the other groups, thus suggesting no protective role of extract in preventing lipid damage.

Flavonoid and flavonol are compounds of plant origin highly found in many fruits and vegetables with powerful antioxidant properties[62]. This antioxidant activity could be due to their ability to absorb, neutralize and quench free radicals[63]. Their ability to scavenge free radical could also be attributed to the presence of conjugated ring structures, their redox properties and carboxylic group which have been reported to inhibit lipid peroxidation[25]. In order to evaluate the contributing factors of the antioxidant activity, flavonoid and flavanol levels in the plant extracts were assessed. In this study, the leaf and seed extracts contained flavonoid and flavonol. The presence of these phytochemicals may be accountable for the antioxidant activity of *C. albidum*. More so, the level of flavonoid and flavanol was significantly higher ($P < 0.05$) in the leaf than the seed extract. This may justify why the antioxidant activity was higher in the leaf extract than in the seed.

To support the hypothesis of antioxidants as possible contributor to the antidiabetic activity of *C. albidum*, the glucose lowering activity and glycogen content were investigated. The seed and leaf extracts of *C. albidum* were shown to lower blood sugar level in diabetic rats. This effect was significant ($P < 0.05$) in the animal group administered with the leaf extract compared to the normal control. This result suggests that *C. albidum* possesses hypoglycaemic activity, thereby promoting cellular parameters involving in the breakdown of glucose. Also, the hypoglycaemic activity was the highest in the leaf extract than the standard antidiabetic drug, metformin. This may be that metformin is a drug involved in reducing hepatic glucose production rather than promoting glucose catabolism[55].

In the body, excess glucose is not immediately metabolized which is usually converted to glycogen which is either stored in the liver or muscle until need arises for its metabolism to generate energy. The essence of this process is to prevent hyperglycaemic condition in the body. Hence, promoting glycogen formation may be a good strategy in preventing or controlling diabetes. The result obtained from the oral administration of the seed and leaf extracts showed an increase in the liver glycogen level compared to the normal group. This increase in liver glycogen was significant ($P < 0.05$) in the plant extract treated groups compared to the non-treated diabetic group. This suggests that the plant extract has the ability to stimulate parameters involved in glycogenesis (the synthesis of glycogen). This is in accordance with some previous studies which have shown that plant extracts possess glyconeogenic effect[64,65].

Insulin is a hormone which is principal in glucose metabolism and known to promote glycogenesis[66]. Thus, in diabetes, liver glycogen can abnormally accumulate because of abnormal amounts of insulin. More so, insulin is generally known to promote glycolysis; glucose break down by promoting the transportation of glucose into the cell and stimulates metabolic enzymes to metabolise glucose and lower blood sugar level to normal[67]. As such, the ability of the seed and leaf extract

can lower both glucose and glycogen synthesis activities which may suggest taht this plant can promote the synthesis of insulin. Thus, this hypothesize will be perspective for future studies.

The leaf and seed extracts of *C. albidum* possess antioxidant and antidiabetic activities. The antioxidant property may partly be due to the presence of flavonoid and flavonol in the plant. The ability of the plant extracts can scavenge free radicals which are responsible for oxidative stress, a condition which has been shown to promote diabetes and its complication may be a contributory factor to its antidiabetic activity. This may be due to the ability of antioxidants to remove free radicals thereby preventing glucose oxidation and allowing glucose in its natural state without modifications to ease its metabolism. However, further studies may be needed to confirm this hypothesis. More so, as a result of the combined antidiabetic effects in lowering blood glucose and increasing liver glycogen, it will be of interest to further investigate the effect of *C. albidum* extracts on insulin which promotes these activities.

## Conflict of interest statement

We declare that we have no conflict of interest.

## Acknowledgments

We are thankful to the management of Godfrey Okoye University, Enugu Nigeria for financial assistance in carrying out this research.

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