In vitro Assessment of the Potential Antioxidant and Antidiabetic Properties of Edible Parts of *Chrysophyllum albidum* Fruit Extracts

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Abstract *Chrysophyllum albidum* (Linn) belongs to Sapotaceae family and commonly called African star apple. It is traditionally used for the treatment of various ailments. This study aimed at investigating *in vitro* antidiabetic and antioxidant properties of three edible parts of *Chrysophyllum albidum* fruit extracts. Lyophilized *C. albidum* fruit pulp, skin and seed shell pericarp powders were extracted using aqueous and methanol as solvents. *In vitro* antioxidant (2,2-diphenyl-1-picrylhydrazyl: DPPH, Ferrous Ion chelating, Antilipid peroxidation, Hydroxyl radical and Hydrogen peroxide scavenging assay as well as estimation of total phenolic, flavonoid and antioxidant contents) assay and antidiabetic (α-amylase and α-glucosidase inhibitory assay) activities of extracts were evaluated. The study revealed that methanol skin extract of *C. albidum* fruit contained the highest levels of total phenolics (19.0 $\pm$ 0.16mg GAE/g dry weight), flavonoids (41.27 $\pm$ mgQE/g dry weight) and antioxidant (98.51 $\pm$ 0.10mg AAE/g dry weight) and exhibited comparable scavenging property with standards using DPPH, deoxyribose and hydrogen peroxide as substrates. Significant α-amylase (86.45 $\pm$ 0.97) and α-glucosidase (85.07 $\pm$ 0.71) inhibitory activities were also observed in methanol skin extract of *C. albidum* fruit, which was comparable with acarbose standard drug -99.04 $\pm$ 0.04 and 98.99 $\pm$ 0.05 respectively. Results demonstrate the antidiabetic and antioxidant potential of methanol skin extract of *C. albidum* fruit and indicate that *C. albidum* fruit skin could have therapeutic value in diabetes and the related condition of oxidative stress. Hence, further study by *in vivo* model would be required for the plant's potential in the management and/or prevention of diabetes.

Keywords: *Chrysophyllum albidum, fruit skin, lyophilized, antidiabetic, antioxidant*

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1. Introduction

Diabetes mellitus is a chronic metabolic disorder that affects the metabolism of carbohydrate, fat and protein. It is characterized by hyperglycemia resulting from defects in insulin secretion or utilization [1]. The WHO [2] report estimated that 1.7 million people in Nigeria had diabetes with the projection that the number will triple by 2030. People with diabetes is increasing due to population growth, aging, consumption of energy rich diet and increasing prevalence of obesity and physical inactivity [3]. Free radicals are generated during normal metabolic processes but there is an imbalance of oxidants/antioxidants in favour of the former in diabetes. Hyperglycaemia elicits increased production of reactive oxygen species sequel to glucose auto-oxidation and protein glycosylation [4,5].

This may be responsible for increased oxidative stress in the pathogenesis of diabetes. Generally, oxidative stress results from an imbalance between increased free radical production and reduced activity of antioxidant defences [6].

The priority in the management of diabetes is to decrease the postprandial hyperglycaemia [7,8]. Alpha-glucosidase is responsible for the breakdown of oligo- and/or disaccharides to monosaccharides while α-amylase is involved in the breakdown of starch to more simple sugars (dextrin, maltotriose, maltose and glucose) [9,10]. The inhibitory action of these enzymes leads to a decrese in glucose absorption rate resulting in maintaining the serum blood glucose in hyperglycemic individuals [11]. Some inhibitors currently in clinical use are acarbose and miglitol, which inhibit α-glucosidase and α-amylase while voglibose inhibits only α-glucosidase. However, these synthetic hypoglycemic agents have their limitations such

as associated with serious side effects, expensive and ineffective to control diabetic complications [12]. The main side effects of these inhibitors are bloating, abdominal discomfort, diarrhea and flatulence [13].

Fruits and vegetables are sources of abundant natural bioactive phytochemicals with health-promoting activities like antioxidant, antidiabetic, anti-inflammatory, antibacterial, etc. Flavonoids and phenolics compounds are the most widely occurring groups of phytochemicals present in plants. Sunil *et al*. [14] demonstrated that flavonoids, phenols, saponin, etc studied in *Proteus vulgaris, Euphorbia hirta and Cassia glauca* showed potential α-amylase inhibitors. *Chrysophyllum albidum* (Linn) is commonly called African star apple and belongs to Sapotaceae family. It has various ethno-medicinal uses [15,16]. *C. albidum* fruit (Figure 1) is seasonal, found between December and April. It is locally called "agbalumo" in South Western Nigeria and "udara" in South Eastern Nigeria. Different parts of the plant has been studied by several scholars: the leaf [17-22], seed cotyledon [18,23,24], root [18,25] and stem bark [22,26].

However, there is still scarcity of information on the edible parts of the fruit in the available literature. Our earlier studies demonstrated that the edible parts of this fruit contain a great number of active ingredients with potential hypoglycemic effect [27]. This effect has been attributed to the high contents of arabinose, pectin, flavonoids, phenols and saponin in this fruit. Hence, this study was designed to examine the antioxidant and antidiabetic potential of the edible parts of this fruit by *in vitro* assay.

Figure 1. *Chrysophyllum albidum* fruits (Source: Orwa *et al.* [28])

2. Materials and Methods

2.1. Collection and Identification of Plant Materials

The fresh fruits of *C. albidum* were purchased in Moniya market, Akinyele local government area of Oyo State, South-Western Nigeria. The fruit was identified and authenticated by the taxonomist, Esimekhuai, D.P.O. in the herbarium unit of Botany department, University of Ibadan, Oyo State, Nigeria where a voucher specimen was deposited with the voucher specimen registration No. UIH/20I6/22502.

2.2. Preparation of Plant Materials

The fresh riped fruit of *C. albidum* was separated, washed, weighed and its seed-shell pericarp, fruit-pulp and fruit-skin (samples) removed and cut into small pieces. The samples were lyophilized for 54 h using Lyophilizer Millorock Bench-Top Freeze Dryer, Germany. Lyophilized samples were stored at -20°C until further use.

2.3. Preparation of the Plant Extract

The lyophilized samples (seed-shell pericarp, fruit-pulp and fruit- skin) of *C. albidum* fruit was pulverized into powder before the extraction. Each of the pulverized samples was suspended in different solvents (distilled water for 24 h and pure methanol for 48 h) in a ratio of 1: 8 (w/v) using cold maceration methods [29]. The crude extracts was filtered and concentrated in a rotary evaporator and dried to completion in a hot air- oven at 40°C. The dry extracts were refrigerated at 2-4°C for further use.

2.4. *In vitro* Anti-oxidant Assessment of the Extracts

2.4.1. Total Phenol Content

The total phenolic content of crude extracts was determined according to Singleton and Rossi, [30] and as described by Djilani et al. [31] with little modification. The method is based on Folin-Ciocalteu (FC) reducing capacity. When phenolic compounds in the extract react, they are oxidized and the FC reagent is reduced in the alkaline solution to form a blue color. The absorbance of the blue colored solution is detected spectrophotometrically at 750 nm. The quantification of the phenolic contents is expressed as mg gallic acid equivalent (mgGAE) per gram of the extract, using gallic acid as a reference. Folin-ciocalteu's phenol reagent (0.2 ml) was added into a mixture of 0.1 ml of each extract and 0.9 ml of water. The resulting mixture voltexed and allowed to stand for 5 min. Then, 1.0 ml of 7% Na_2CO_3 solution was added and the solution distilled to 2.5 ml. The resulting distillate was incubated for 90 min at room temperature and absorbance measured.

2.4.2. Total Flavonoid Content

Total flavonoid content was estimated according to Zhilen *et al.* [32] and as described by Miliauskas *et al.* [33]. The extract/standard (0.1 ml) was added to 0.4 ml of distilled water and followed by 0.1 ml of 5% sodium nitrite. After 5 min, 0.1 ml of 10% aluminum chloride and 0.2 ml of sodium hydroxide solution were added. The volume was made up to 2.5 ml with distilled water. The absorbance was measured against the blank at 510 nm. The quercetin with varying concentrations (0.1, 0.2, 0.3, 0.4 and 0.5 mg/ml) was used as standard and the total flavonoid content in the extract, expressed as mg quercetin equivalents (mgQE) per gram of the extract.

2.4.3. Total Antioxidant Capacity

Total antioxidant capacity of the extracts was carried out according to the method of Prieto *et al.* [34] and as reported by Oyesola *et al*. [35] with little modification. The method is based on the reduction of molybdenum (VI) to molybdenum (V) by the extract and the subsequent formation of a green phosphate/molybdenum (V) complex

at an acidic pH. The sample extracts / standard solutions (0.1 ml) of varying concentrations (20, 40, 60, 80, 100 µg/ml) was mixed with 1.0 ml of the reagent solution (0.6 M sulphuric acid, 28 mM sodium phosphate and 4 mM ammonium molybdate). The tubes were capped and incubated in a boiling water bath at 95°C for 90 min, cooled to room temperature and the absorbance measured at 695 nm against a blank; consist of the reacting mixture containing distilled water in place of the extract. The antioxidant activities of the extracts were expressed as an ascorbic acid equivalent (AAE).

2.4.4. DPPH Radical Scavenging Assay

The free radical scavenging ability of the extracts and ascorbic acid standard were determined according to the method of Brand-Williams *et al.* [36] and as described by Kavitha *et al.* [37]. 2, 2-diphenyl-1-picrylhydrazyl (in powder form), is a stable free radical (DPPH•) with purple-color, which is reduced to DPPH-H by antioxidant compounds and consequently discoloration occurs. The degree of discoloration indicates the scavenging potential of the antioxidant compounds or extracts in terms of hydrogen donating ability [38]. The colour change from deep violet to light yellow was measured at 517 nm.

One milliliter each of different concentrations (1.0, 0.5, 0.25, 0.125, 0.0625, 0.03125 mg/ml) of extract/ascorbic acid standard was pipetted into a test tube and followed by the addition of 1.0 ml of 0.3 mM (freshly prepared) DPPH solution in methanol. The mixture was shaken and incubated in the dark for 30 min. The absorbance was read at 517 nm against a DPPH control containing only 1 ml methanol in place of the extract and the percentage inhibition (I%) was calculated as in equation 1.

$$I\% = \left[\left(Ac. - As. \right) / Ac. \right] x100 \qquad (1)$$

Where Ac. is the absorbance of the control reaction (containing all reagents except the test extract), and As. is the absorbance of the test extract.

2.4.5. Ferrous Ion-chelating Ability Assay

The ferrous ion chelating (FIC) ability was carried out according to the method of Singh and Rajini [39] with some modifications. Ferrous ion chelating (FIC) ability is measured by the decrease in the absorbance at 562 nm of the iron (II) and ferrozine complex. Ferrozine can quantitatively chelate with Fe^{2+} and form a red coloured complex. This reaction is limited in the presence of other chelating agents and results in a decrease of the red colour of the ferrozine- Fe^{2+} complex. Measurement of the color reduction estimates the chelating ability of the extracts to compete with ferrozine for the ferrous ions. The antioxidants present in plant extract/EDTA standard forms a coordinate complex with the metal ions (chelating ability) and inhibit the transfer of electrons. Thus, oxidation reaction is arrested and no free radicals are produced.

Solutions of 2 mM FeCl$_2$•4H$_2$O and 5 mM ferrozine were diluted 20 times. An aliquot (1 ml) of different concentrations of each extract was mixed with 1ml FeCl$_2$•4H$_2$O, incubated for 5 min and the reaction was initiated by the addition of ferrozine (1.0 ml). The mixture was properly mixed, incubated for 10 min and the

absorbance measured. The percentage inhibition of ferrozine–Fe^{+2} complex formation was calculated as in equation 2.

$$Chelating\ effect\ \% = \left[\left(Ac. - As. \right) / Ac. \right] x100 \qquad (2)$$

Where Ac. = absorbance of control sample (the control contains FeCl$_2$ and ferrozine, complex formation molecules) and As. = absorbance of the test extract.

2.4.6. Hydroxyl Radical Scavenging Assay

The hydroxyl radical scavenging property was measured according to the method of Halliwell *et al.* [40]. Hydroxyl radical is one of the potent reactive oxygen species in the biological system. It reacts with polyunsaturated fatty acid moieties of cell membrane phospholipids and causes damage to cell. This method is based on the competition between deoxyribose and the extracts for hydroxyl radicals generated from the Fe^{3+}/ascorbate /EDTA/H$_2$O$_2$ system.

The reaction mixture contained 1.0 ml of reagent (3.0 mM deoxyribose, 0.1 mM EDTA, 2 mM H$_2$O$_2$, 0.1 mM L-Ascorbic acid, 0.1 mM FeCl$_3$.6H$_2$O in 10 mM phosphate buffer, pH 7.4) and various concentrations of the extracts (50-350 µg/ml). The reaction mixtures were incubated at 37°C for 1 h, followed by the addition of 1.0 ml of 1 % (w/v) thiobarbituric acid (TBA) in 0.25 N HCl and 1.0 ml 10 % (w/v) trichloroacetic acid (TCA). The reaction mixtures were heated in boiling water bath at 100°C for 20 min. The pink chromogen [malondialdehyde-(TBA) adduct] was extracted into 1.0 ml of butan-1-ol and absorbance read at 532 nm against reagent blank.

The percentage inhibition (I%) of the extracts and BHT standard were calculated using the expression in equation 3.

$$\left(I\% \right) = \left[\left(Ac. - As. \right) / Ac. \right] x100 \qquad (3)$$

Where Ac. is the absorbance of the control reaction (containing all reagents except the test extract), and As. is the absorbance of the test extract.

2.4.7. Hydrogen Peroxide (H$_2$O$_2$) Radical Scavenging Assay

The hydrogen peroxide content was determined according to the method of Ruch *et al.* [41]. The method is based on the ability of antioxidant compounds (extracts)/ascorbic acid standard to scavenge hydrogen peroxide before its decomposition through Fenton reaction in the presence of redox metals. Hydrogen peroxide solution (2 mM) was prepared in 50 mM phosphate buffer (pH 7.4). Different concentrations (µg/ml) of extracts/ascorbic acid standard were transferred into the test tubes and their volumes made up to 0.4 ml with 50 mM phosphate buffer (pH 7.4). After addition of 0.6 ml (2 mM) hydrogen peroxide solution, tubes were vortexed and absorbance of hydrogen peroxide was taken at 230 nm against a blank solution containing phosphate buffer without H$_2$O$_2$ [42,43]. The percentage inhibition of extract and standard was calculated as in equation 4.

$$\left(I\% \right) = \left[\left(Ac. - As. \right) / Ac. \right] x\ 100 \qquad (4)$$

Where Ac. is the absorbance of the control reaction (containing all reagents except the test extract), and As. is the absorbance of the test extract.

2.4.8. Anti-lipid Peroxidation Assay

The anti-lipid peroxidative property of the extracts/butylated hydroxyl toluene (BHT) standard were determined according to the method of Ohkawa *et al*. [44] and as described by Nabasree and Bratati [45]. The method is based on a modified thiobarbituric acid reactive species (TBARS) assay. In this assay, the product of lipid peroxidation using egg yolk homogenate as lipid rich media [46] was quantified by determining the MDA formed, which react with the TBA under acidic condition to form an MDA-TBA adduct. The pink coloured product was measured at 532 nm.

About 0.5 ml of egg yolk homogenate was added to 0.1 ml of varying concentration of the extract and followed by the addition of 1.0 ml distilled water. Lipid peroxidation was induced by adding 50 µL of $FeSO_4$ (0.07M) together with 50 µL of ascorbate to the reaction mixture. The mixture was incubated in water bath at 37°C for 1 h followed by the addition of 100 µL of BHT, 1.5 ml of 20% acetic acid and 1.5 ml of 0.67 % (w/v) TBA. The resulting mixture was added to each tube and centrifuged at 3,000x g for 10 min. The absorbance of the organic upper layer was then measured. The TBARS values were calculated using the extinction coefficient 1.53×10^5 M^{-1}cm^{-1} [47]. Inhibition of lipid peroxidation (%) by the extract was calculated as in equation 5.

$$(I\%) = \left[(Ac. - As.)/Ac.\right] x 100 \qquad (5)$$

Where Ac. is the absorbance of the control reaction (containing all reagents except the test extract), and As. is the absorbance of the test extract.

2.5. *In vitro* Antidiabetic Assay

2.5.1. *α*-Amylase Inhibitory Assay

In vitro α-amylase inhibitory effect of the extracts were determined according to the method of Bernfield [48] using the chromogenic method and as described by Sindhu *et al*. [49] with slight modification. The method is based on quantifying the reducing sugar (maltose equivalent) liberated under the assay conditions as in equation 6.
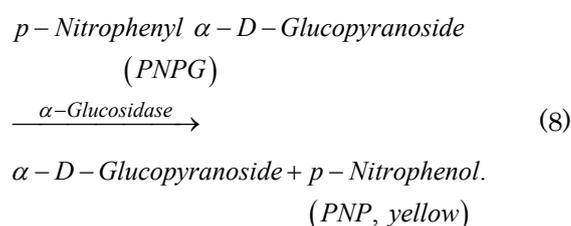
$$Starch \xrightarrow[pH\ 6.9]{\alpha - Amylase} Maltose\ (reducing\ sugar) \quad (6)$$
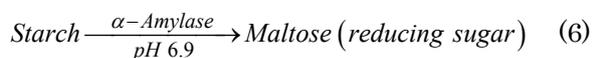
The enzyme inhibitory activity was expressed as a decrease in units of maltose liberated. Acarbose was used as positive control of α-amylase inhibitor. A modified dinitrosalicylic acid (DNSA) method was adopted to estimate the maltose equivalent. Appropriate dilutions of the extracts (500 µl) and 500 µl of 0.02 mol/L sodium phosphate buffer (pH 6.9 with 0.006 mol/L NaCl) containing Hog pancreatic α-amylase (EC 3.2.1.1) (0.5 mg/ml) were incubated at 25°C for 10 min. Thereafter, addition of 500µl starch solution (1%) in 0.02 mol/L sodium phosphate buffer (pH 6.9 with 0.006 mol/L NaCl) was made to the reacting mixture. The reaction mixture was incubated at 25°C for 10 min and stopped with 1.0 ml DNSA colour reagent. The mixture was further incubated in a boiling water bath for 5 min, cooled to room temperature and diluted with 10 ml distilled water. The absorbance was read at 540 nm. Control represent 100% enzyme activity and were conducted in a similar way by replacing extract with vehicle, 0.02 mol/L sodium phosphate buffer (pH 6.9 with 0.006 mol/L NaCl). Inhibition of starch hydrolysis by α-amylase inhibitor results in a diminished absorbance at 540 nm in comparison with the controls. The percentage inhibition of α-amylase activities (I%) at different concentrations of the extracts was calculated as in equation 7.

$$(I\%) = \left[(Ac. - As.)/Ac.\right] x 100 \qquad (7)$$

2.5.2. α-Glucosidase Inhibitory Assay

The α-glucosidase inhibitory activity was determined as described by Apostolidis *et al*. [50]. The method is based on the following reaction:

$$p - Nitrophenyl\ \alpha - D - Glucopyranoside$$
$$(PNPG)$$
$$\xrightarrow{\alpha - Glucosidase} \qquad (8)$$
$$\alpha - D - Glucopyranoside + p - Nitrophenol.$$
$$(PNP,\ yellow)$$

Acarbose was used as positive control of α-glucosidase inhibitor. Appropriate dilution of the extracts (50 µl) and 100 µl of α-glucosidase solution were incubated at 25°C for 10 min. Thereafter, addition of 50 µl *p*-nitrophenyl-α-D-glucopyranoside solution (5 mmol/L) in 0.1 mol/L phosphate buffer (pH 6.9) was made. The reacting mixture was incubated at 25°C for 5 min and the absorbance read at 405 nm. The percentage inhibition of α-glucosidase activities (I %) at different concentrations of the extracts was calculated as in equation 9.

$$(I\%) = \left[(Ac. - As.)/Ac.\right] x 100 \qquad (9)$$

Where Ac. is the absorbance of the control and As. is the absorbance of the sample.

2.6. Statistical Analysis

Data was analyzed by using Statistical Software Package of Social Science (SPSS) for Window XP Software Programme (version 13.0). All the data were expressed as mean ± standard error of mean (SEM) of triplicate determination. Significant differences at $p < 0.05$ among means were determined using one-way analysis of variance (one-way ANOVA) and followed by LSD (least significant difference).

3. Results and Discussion

3.1. *In vitro* Antioxidant Effects of the Extracts

3.1.1. Total Phenol, Total Flavonoid and Total Antioxidant Contents

Table 1 showed that the total phenol, total flavonoids and total antioxidant contents of the three edible parts of

C. albidum extracts studied are significantly different (p< 0.05). The total phenol content of *C. albidum* aqueous and methanol extracts expressed as GAE ranged from 1.28 ± 0.12 to 12.66 ± 0.37 mgGAE/g dry wt (Table 1) and between 9.10 ± 0.12 and 19.06 ± 0.16 mg/GAE/g dry wt (Table 2) respectively. The highest content was found in the fruit-skin methanol extract while the lowest content was observed in the fruit-pulp aqueous extract. The total flavonoid contents of different extracts, which was expressed as quercetin equivalent per gram of the fruit extracts showed a range of 2.69 ± 0.78 to 11.66 ± 1.19 mgQE/g dry wt for aqueous (Table 1) and 13.91 ± 1.19 to 41.27 ± 1.62 mgQE/g dry wt for methanol (Table 2). The highest and lowest flavonoid contents were also found in the methanolic extract of the fruit- skin and aqueous extract of fruit-pulp respectively. The total antioxidant capacity (TAC) expressed as ascorbic acid equivalents (AAE), followed the same trend as total phenol and total flavonoid contents. The highest (98.51 ± 0.10 mgAAE/g dry wt) contents was observed in the methanolic extract (Table 2) of fruit- skin while the lowest contents was found in the aqueous extract of the fruit-pulp (6.19 ± 0.40 mgAAE/g dry wt) (Table 1).

Table 1. Total- Phenol, Flavonoid and Antioxidant contents Expressed as Standards Equivalent of *C. albidum* Fruit Aqueous Extract

Groups/Parameters (mg/g)	Seed Shell Pericarp	Fruit-Pulp	Fruit-Skin
Total phenol (mg/g GAE)	6.72 ± 0.14[b]	1.28 ± 0.12[c]	12.66 ± 0.37[a]
Total flavonoid (mg/g QE)	9.42 ± 0.78[b]	2.69 ± 0.78[c]	11.66 ± 1.19[a]
Total antioxidant (mg/g AAE)	31.16 ± 0.41[b]	6.19 ± 0.40[c]	52.35 ± 0.23[a]

Means followed by different alphabet within the row are significantly (p<0.05) different.

Table 2. Total- Phenol, Flavonoid and Antioxidant Expressed as Standards Equivalent of *C. albidum* Fruit Methanolic Extract

Groups/Parameters (mg/g)	Seed Shell Pericarp	Fruit-Pulp	Fruit-Skin
Total phenol (mg/g GAE)	12.89 ± 0.21[b]	9.10 ± 0.12[c]	19.06 ± 0.16[a]
Total flavonoid (mg/g QE)	23.78 ± 1.62[b]	13.91 ± 1.19[c]	41.27 ± 1.62[a]
Total antioxidant (mg/g AAE)	57.73 ± 0.40[b]	39.52 ± 0.40[c]	98.51 ± 1.00[a]

Means followed by different alphabet within the row are significantly (p<0.05) different.

The results of these findings demonstrated that the fruit-skin methanol extract of *C. albidum* is richer in phenolic and flavonoid contents than the seed shell pericarp and fruit-pulp extracts of *C. albidum*. Phenolic compounds are important dietary antioxidants because they have ideal structural chemistry for free radical scavenging activities, and have been shown to be more effective antioxidants *in vitro* than vitamins E and C on a molar basis [51].

3.1.2. Antiradical and anti-lipid Peroxidation Effects

The antiradical and anti-lipid peroxidation assays studied on different test extracts showed that the activities were concentration dependent as the effects proportionally increased with the increasing concentration of the extract.

The methanolic extract of the fruit-skin exhibited comparable radical scavenging effects with Ascorbic acid-standard in DPPH and hydrogen peroxide assays. The decreasing order of radical scavenging effects of the extracts in DPPH and hydrogen peroxide assays were in the order Asc. (standard) > SM > SA> EM > PA > PM > EA (Figure 2A) and Asc. (Standard) > SM > EA> SA > PM > PA > EM (Figure 2D) respectively. The aqueous and methanolic extracts of seed shell pericarp showed the least antiradical effects in DPPH and hydrogen peroxide assays respectively.

Similarly, the methanolic and aqueous extracts of the fruit-skin exhibited comparable radical scavenging effects with butylated hydroxyl toluene (BHT)-standard in hydroxyl radical and antilipid peroxidation assays respectively. The decreasing order of radical scavenging effects of the extracts in hydroxyl radical and antilipid peroxidation assays were in the order BHT (standard) > SM > EM> PM > SA > EA > PA (Figure 2C) and BHT (standard) > SA > EM> SM > EA > PA > PM (Figure 3A) respectively. However, the aqueous and methanolic extracts of fruit-pulp has the least hydroxyl radical scavenging and antilipid peroxidation effects. The ferrous-ion chelating ability (Figure 2B) followed the trend of EDTA-standard > EM > EA > SM > PM > SA > PA. The methanolic extract of the fruit-skin has a moderate ferrous-ion chelating effect while the aqueous extract of fruit-pulp has the least effect.

The experimental data obtained revealed that of the six extracts studied, the methanol extract of *C. albidum* fruit-skin exhibited comparable radical scavenging properties with the standards in dose dependent manner. These findings are in agreement with the observed phenolic and flavonoids contents of *C. albidum* fruit-skin methanol extract. Polyphenol compounds such as phenol and flavonoid are the major contributors that scavenge the free radicals in oxidation pathways as they exhibit a wide range of biological effects such as protection of LDL and DNA from oxidative damage with significant consequences in atherosclerosis and age-related development of some cancers respectively [52].

DPPH radical scavenging model is a widely used method to evaluate antioxidant property of natural compound and plant extracts. The degree of discoloration indicates the scavenging potential of the antioxidant extract, which is due to the hydrogen donating ability [53]. The experimental data on DPPH radical, hydroxyl radical and hydrogen peroxide scavenging assays revealed that the fruit skin methanol extract have the potential to scavenge free radicals, which are comparable with ascorbic acid, butylated hydroxyl toluene (BHT) and ascorbic acid standards respectively. The increased productions of free radicals lead to the development of diabetes complications such as cardiovascular diseases. Thus, *C. albidum* fruit skin methanol extract could be beneficial in preventing oxidative stress related chronic diseases such as diabetes and its complications. The lipid peroxidation effect of aqueous extract of *C. albidum* fruit skin also showed a comparable effect with BHT standard. This finding suggests that the possible mechanisms of antidiabetic property of *C. albidum* fruit-skin could be through the scavenging of free radicals induced by hyperglycemia [54,55,56,57] and inhibition of lipid peroxidation.
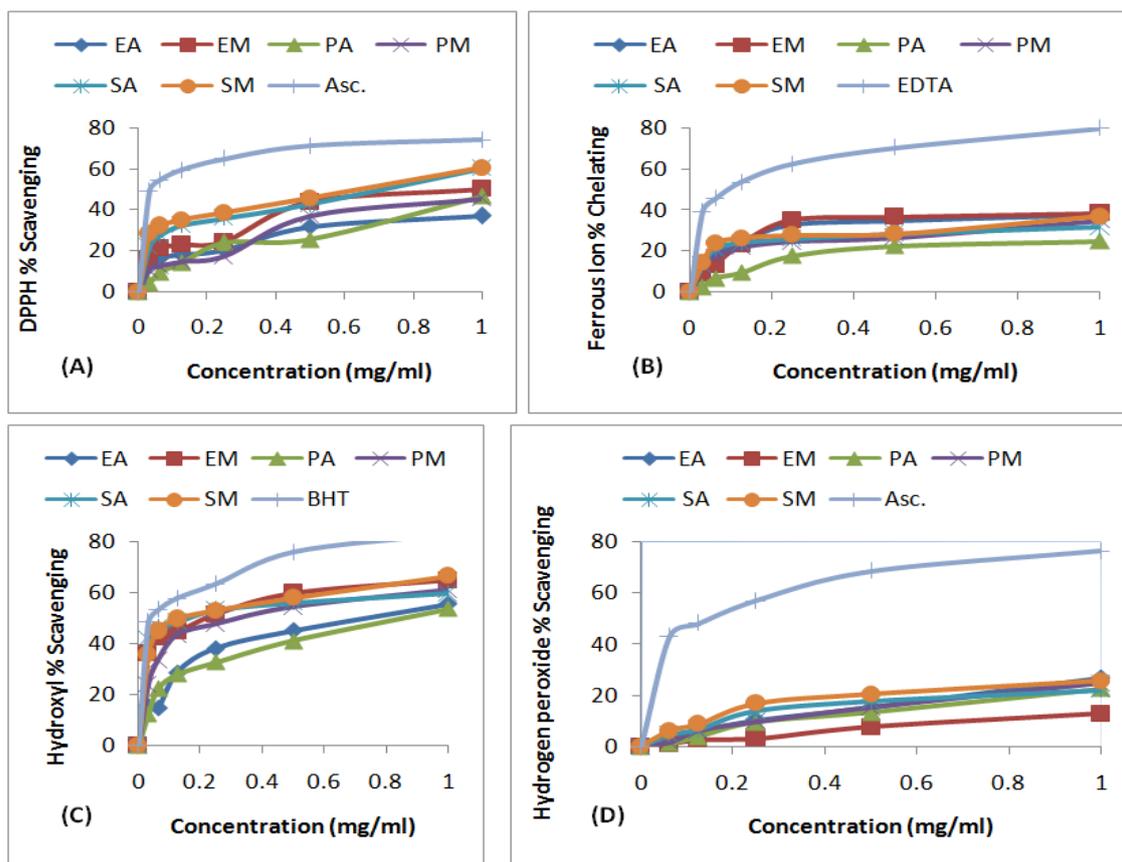
Figure 2. Antiradical Effects of the Edible parts of C. albidum Fruit extracts at various concentrations (Each value represents a mean ± SEM (n = 3): (A) DPPH radical scavenging, (B) Ferrous ion chelating, (C) Hydroxyl radical scavenging, (D) Hydrogen peroxide radical scavenging, EA., seed shell pericarp (aqueous extract) , EM., seed shell pericarp (methanol extract), PA., fruit-pulp (aqueous extract, PM., fruit-pulp (methanol extract), SA., fruit-skin (aqueous extract), SM., fruit-skin (methanol extract), Asc., ascorbic acid (standards), EDTA., Ethylenediamine tetraacetic acid (metal chelating standard), BHT., butylated hydroxyl toluene (standards))

3.2. *In vitro* Antidiabetic

Figure 3B showed the percentage inhibition of different extracts of the edible parts of *C. albidum* fruit on α- amylase activities, which ranged from 38.10 ± 0.97 to 68.86 ± 0.37 (aqueous) and 47.61 ± 0.97 to 86.45 ± 0.97 (methanol). Similarly, percentage inhibition of alpha glucosidase activities (Figure 3C) ranged from 48.53 ± 0.71 to 74.13 ± 0.71 (aqueous) and 53.33 ± 0.53 to 85.07 ± 0.71 (methanol) at a concentration of 0.04 mg/ml. The methanol extract of *C. albidum* fruit-skin has comparable inhibitory effect with Acarbose–standard. The percentange inhibition at 0.04 mg/ml of the extract was 86.45 ± 0.97 (*α*-amylase) and 85.07 ± 0.71 (*α*- glucosidase) while that of standard was 99.04 ± 0.04 and 98.99 ± 0.05 on *α*-amylase and *α*-glucosidase activities respectively. The aqueous extract of *C. albidum* fruit-pulp however, has the least activities.

Many bioactive compounds from different plants have been reported for their hypoglycemic effect [58,59]. Our earlier studies demonstrated that *C. albidum* fruit skin possesses a number of active ingredients (flavonoids, pectin, saponin, arabinose, etc.) with potential hypoglycemic effect [27]. Polyphenolic compounds have been shown to have positive correlation with antidiabetic agents. Flavonoids inhibit the activity of pancreatic amylase [14,60], intestinal *α*-glucosidase [61,62], and glucose transporter (GLUT2) [63] by binding to protein molecules and alter their functionalities. Pectin binds to cholesterol

in the gastrointestinal tract and delays glucose absorption by trapping ingested carbohydrates in the gel matrix [64] and L-arabinose selectively inhibits intestinal sucrase activity in an uncompetitive manner by forming an enzyme-inhibitor-substrate (EIS) complex, which suppresses the glycemic response [65,66,67,68].

The methanol extract of *C. albidum* fruit-skin possessed substantial inhibitory activities on α-amylase and α-glucosidase, which compare favourably well with Acarbose standards. This finding also correlates with the flavonoid contents of *C. albidum* fruit-skin. Flavonoids may act against diabetes mellitus either through the inhibition of α-amylase and α-glucosidase activities in the intestine [69,70] or through competitive inhibition of sodium-dependent glucose transporter-1 to improve glucose tolerance [71]. The inhibition of α-amylase and α-glucosidase activities has been suggested as practical therapeutic approaches for reducing postprandial hyperglycaemia [72], which induces the non-enzymatic glycosylation of various proteins and biomolecules that causes the development of chronic diabetes complications. Thus, management of postprandial glucose is critical in prevention and treatment of type 2 diabetes (T2D) and reduction of chronic vascular complications [73,74]. It can therefore be hypothesized that the significant enzyme inhibitory activity of methanolic extract of *C. albidum* fruit-skin may interfere or delay the absorption of dietary carbohydrates as well as disaccharides in the small intestine, leading to the suppression of meal-induced increase of plasma glucose [75].
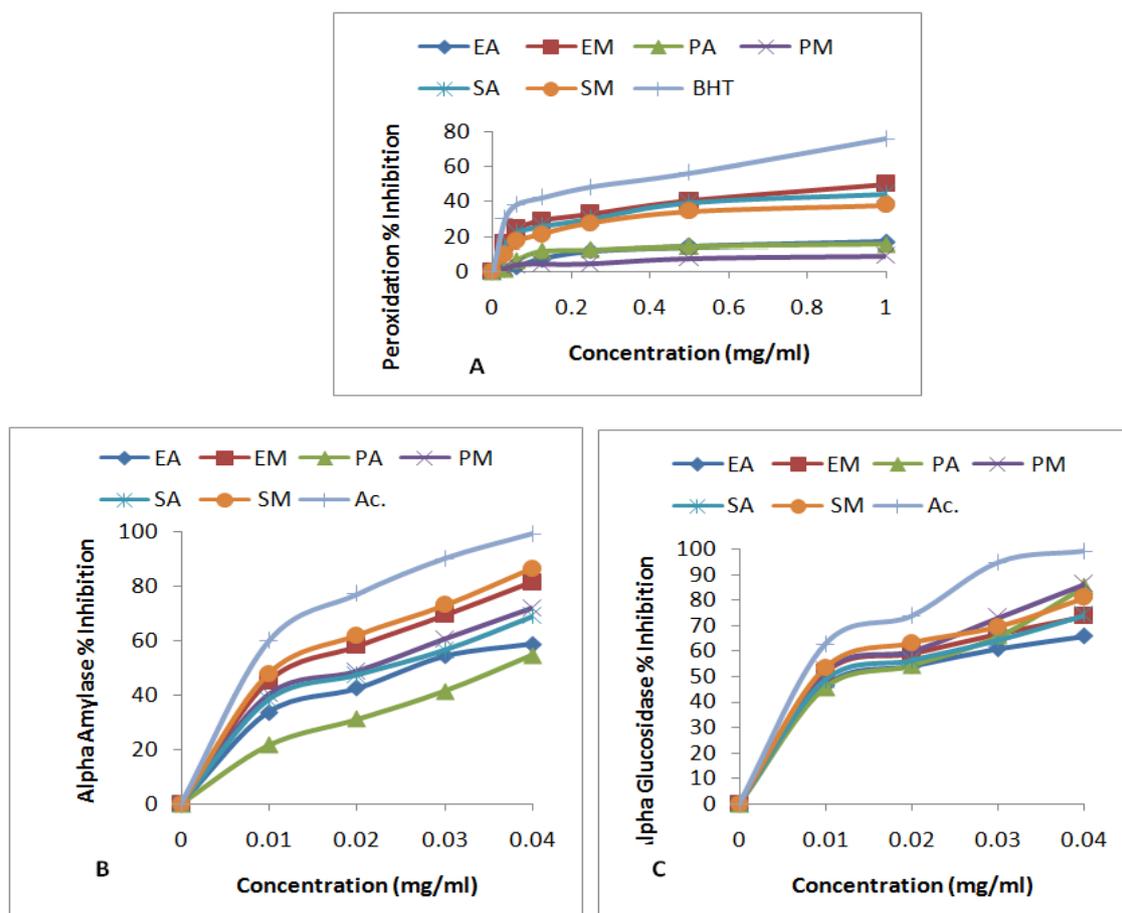
Figure 3. Inhibitory Effects on Peroxidation, α- Amylase) and α- Glucosidase activties of the Edible parts of *C. albidum* Fruit extracts at various concentrations (Each value represents a mean ± SEM (n = 3): (A) peroxidation inhibition; (B) Inhibition of **α-** amylase activity; (C) Inhibition of **α-**glucosidase activity. EA., aqueous extract of seed shell pericarp , EM., methanol extract of seed shell pericarp , PA., aqueous extract of fruit-pulp, PM., methanol extract of fruit-pulp, SA., aqueous extract of fruit-skin, SM., methanol extract of fruit-skin, BHT., butylated hydroxyl toluene (standards), Ac., acarbose (standards))

4. Conclusions

In conclusion, the fruit-skin methanolic extract of *C. albidum* exhibited comparable radical scavenging effects with standards among the extracts studied. In addition, the methanol extract of *C. albidum* fruit-skin, also showed comparable α-amylase and α-glucosidase inhibitory activities with standards. This suggests that the fruit-skin of *C. albidum* possesses antidiabetic properties possibly due to a combination of mechanisms.

The mechanisms include (a) scavenging free radicals induced by hyperglycemia, (b) inhibition of lipid peroxidation and (c) inhibition of α-amylase and α-glucosidase activities in the intestine leading to delaying of dietary carbohydrates absorption through the high contents of flavonoids and phenols in the fruit skin. Thus, *C. albidum* fruit-skin may be good sources of antioxidants and inhibitors of carbohydrate digestive enzymes used in the treatment of diabetes and its complication.

5. Recommendation

Further experimental studies by different *in vivo* models and clinical trials are required to elucidate the antioxidant and antidiabetic potentials of *C. albidum* fruit-skin for its effective utilization as therapeutic agent.

Competing Interests

The authors declare that they have no competing interests.

Acknowledgments

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