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Full Length Research Paper

In vitro antioxidant activities and inhibitory effects of aqueous extracts of unripe plantain pulp (*Musa paradisiaca*) on enzymes linked with type 2 diabetes and hypertension

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The dried plantain pulps were crushed and milled into flour (Raw flour), while a portion of its flour was reconstituted in boiling water to form a thick paste known locally in Western Nigeria as 'amala'. This was dried and milled into 'Amala' flour. The aqueous extracts of raw and 'amala' flour were prepared (1 g/20 mL). The phenolic contents, vitamin C and antioxidant properties (DPPH and OH radical scavenging ability, reducing power and iron-chelating ability) were analyzed. The α-amylase, α-glucosidase, angiotensin-1-converting enzyme (ACE) inhibitory activities and EC_{50} values were also determined. The results showed that 'amala' flour had higher phenolic contents, reducing properties and DPPH radical scavenging ability while the raw flour had higher iron chelating and hydroxyl radical scavenging ability in a dose dependent manner and vitamin C (3.71mg/g) content. The 'amala' flour had higher α-amylase and α-glucosidase inhibitory activities than raw flour. The extracts had stronger inhibitory effects on α-glucosidase than α-amylase activities. The raw flour had higher ACE inhibitory activity (EC_{50}=0.81±1.39 mg/mL). These suggest their potential use in the management of type-2 diabetes and hypertension. This study evaluates the *in vitro* anti-oxidant activities inhibitory effects of aqueous extracts of unripe plantain on enzymes associated with type 2 diabetes.

Key words: *Musa paradisiacal*, type 2 diabetes, α-amylase, α-glucosidase, angiotensin-1-converting enzyme (ACE), hypertension, anti-oxidant.

INTRODUCTION

Hyperglycemia is a condition associated with diabetes mellitus and is linked to most diabetes complications as their primary cause. Hyperglycemia is a condition of abnormal rise in plasma glucose level, and in type-2 diabetes is a result of insulin resistance which may be due to a number of defects in signal transduction ranging from abnormal insulin or insulin receptors to defects in glucose transporters (Oritz et al., 2007). Prolonged hyperglycemia leads to increased generation of reactive oxygen species (ROS) and alteration of endogenous antioxidants (Oritz et al., 2007). An effective strategy for type 2 diabetes management has been through the inhibition of pancreatic α-amylase and limiting the absorption of glucose by inhibiting intestinal α-glucosidase enzyme (Krentz and Bailey, 2005). Many commercially available α-glucosidase inhibitors (acarbose and miglitol) used in the management of the disease have employed this mechanism (Kwon et al., 2006). Intestinal α-glucosidase inhibition, which delays the absorption of glucose following starch and sucrose conversion, moderates the postprandial blood glucose elevation, and thus mimics the effects of dieting on hyperglycemia (Kwon et al., 2006), making natural α-amylase and α-glucosidase inhibitors an attractive therapeutic approach to the treatment of postprandial hyperglycemia by ultimately slowing glucose release from starch (Melo et al., 1999). Chronic amylase inhibition may

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also be useful in the treatment of type-2 diabetes and obesity (Koike, 2005). However, the drugs that are currently used as α-amylase and α-glucosidase inhibitors exhibit side effects such as abdominal distension, bloating, and flatulence (Chakrabarti and Rajagopalan 2002), which are linked to excessive inhibition of the pancreatic α-amylase (Bischoff, 1994). As such, the approach of mild inhibition of α-amylase and strong inhibition of α-glucosidase is potentially better for managing blood glucose than the one of using drugs (Kwon et al., 2006). One of the long-term complications of type 2 diabetes is hypertension or high blood pressure (Bakris et al., 2000). In humans, the renin-angiotensin system (RAS) plays a pivotal role in blood pressure regulation, and in the pathophysiology of cardiovascular diseases such as congestive heart failure and hypertension (Brunner et al., 1972). Renin produces angiotensin I from angiotensinogen, after which it is cleaved by angiotensin-I-converting enzyme (ACE) to release angiotensin II, a potent vasoconstrictor. ACE also inactivates bradykinin, which has depressor action. As such, inhibition of ACE activity may also yield major anti-hypertension benefits. Inhibition of ACE is considered a useful therapeutic approach in the treatment of high blood pressure in both diabetic and non diabetic patients (Erdos and Skidgel, 1987). Previous *in vitro* and *in vivo* animal and clinical studies have also indicated the potential of specific phenolic phytochemicals in hypertension management with direct absorption into the blood (Kwon et al., 2006). Plantain belongs to the Musacae family and is cultivated in many tropics and subtropical countries of the world. It ranks third after yams and cassava for sustainability in Nigeria (Akomolafe and Aborisade, 2007). Plantain (*M. paradisiaca*) is a source of starchy staple for millions of people in Nigeria (Adeniyi et al., 2006). Plantains are a good source of vitamin A (carotenoid), vitamin B complex (thiamin, niacin riboflavin and B_6) and vitamin C (ascorbic acid) and also have been reported as a better source of vitamin A than most other staples (Aurand, 1987). They are notably high in potassium and low in sodium (Marriott et al., 1983). Carotenoid-rich foods protect against certain chronic diseases, including diabetes, heart disease and cancer (Ford et al., 1999). Carotenoids are one of the most important classes of plant pigments and play a crucial role in defining the quality parameters of fruit and vegetables (Demming et al., 1996). Production of plantain flour (one of the forms in which plantain is popularly consumed in Nigeria) from unripe plantain show strong enzymatic browning reactions when cut and exposed to the air (Ozo et al., 1984). This browning is caused by the enzyme tyrosinase which oxidizes the amino acid tyrosine into 3, 4-dihydroxyphenyalanine and finally into brown melanoidin compounds (Zakpaa et al., 2010). In recent studies partial enzymatic oxidation (Manzocco et al., 1998) and non-enzymatic (melanoidins) browning have been reported to have antioxidant properties (Yen and Tsai, 1993). A number of anti-hyperglycemic agents have been found in plants (Jarvill et al., 2001). It is not unusual to find Nigerians who are diabetic consuming unripe plantain meal to reduce postprandial glucose level (Willett et al., 2002; Foster et al., 2003) and in the management of diabetes (Eleazu et al., 2010). Plantain also has a promising potential due to its low sodium (Chandler, 1995), little fat and no cholesterol content. Hence, it is useful in the management of patients with high blood pressure and heart disease (Dzomeku et al., 2007). In view of this, the present study seeks to assess the *in vitro* anti-oxidant activities and inhibitory effects of the water extractable phytochemicals of raw flour and hot water thick paste ('amala') flour on key enzymes linked to type-2 diabetes and hypertension *vis a vis* their phenolic contents and antioxidant properties.

MATERIALS AND METHODS

Collection of sample

Fresh pulps of unripe plantain (*M. paradisiaca*) were obtained from a local market at Owena, Ondo State, Nigeria. Authentication of the unripe plantains was carried out at the Department of Biology, Federal University of Technology, Akure, Nigeria.

Sample preparation

The unripe plantain pulps were sliced and sun-dried for about 3 weeks to a constant weight, and ground into flour which was termed 'raw flour'. The flour was passed through a sieve. The plantain flour was later divided into two (A portion of the raw flour was stored in an airtight container at room temperature (25°C) for future analysis) while the other portion was prepared to make the thick paste 'amala'. This was achieved by stirring the flour continuously in a pot of boiling water until it was well cooked to form a thick, smooth brown paste. The thick paste was later sun-dried for about 4 weeks to constant weight, ground into flour and also kept for future analysis.

Chemicals and equipment

Folin-Ciocalteu's phenol reagent, gallic acid and anhydrous sodium carbonate used were products of Fluka (Buchs, Switzerland). Quercetin and DPPH (2, 2-diphenyl-1picrylhydrazyl), Iron (II) sulphate, H_2O_2 and ACE were products of sigma (Aldrich, USA). Ascorbic acid and starch were products of Merck (Darmstadt, Germany), iron (III) chloride 6-hydrate and trichloroacetic acid Fisher products. All other chemicals used were purchased from Associated Laboratories, Aba, Abia State, Nigeria. The water used was obtained from the Chemistry Department at Federal University of Technology, Akure. Optical absorbance was measured with a UV-Visible spectrophotometer (Model 6305; Jenway, Barloworld Scientific, Dunmow, United Kingdom).

Extraction procedure

10 g each (of the raw flour and flour from thick paste 'amala') was soaked in 100 ml of distilled water for about 24 h. The mixture was filtered and the filtrate was centrifuged for 10 min to obtain a clear supernatant liquid, which was then used for subsequent analysis (Oboh et al., 2007). All antioxidant tests and analyses were

performed in triplicate, and results were averaged.

Determination of total phenol content

The total phenol content was determined according to the method of Singleton et al. (1999). Briefly, appropriate dilutions of the extracts were oxidized with 2.5 mL 10% Folin-Ciocalteau's reagent (v/v) and neutralized by 2.0 mL of 7.5% sodium carbonate. The reaction mixture was incubated for 40 min at 45°C and the absorbance was measured at 765 nm in the spectrophotometer. The total phenol content was subsequently calculated as gallic acid equivalent.

Determination of total flavonoid content

The total flavonoid content of the unripe plantain extracts was determined using method of Meda et al. (2005). 0.5 mL of appropriate volume of the samples/standard quercetin was mixed with 0.5 mL methanol, 50 µl of 10% $AlCl_3$, 50 µL of 1 mol/L potassium acetate and 1.4 mL water. The reaction mixture was incubated at room temperature for 30 min. Thereafter, the absorbance of the reaction mixture was measured at 415 nm in the spectrophotometer. Total flavonoid content was calculated using quercetin as a standard.

Determination of vitamin C content

Vitamin C content of the unripe plantain extracts was determined using the method of Benderitter et al. (1998). 75µL DNPH (2g dinitrophenyl hydrazine, 230 mg thiourea and 270 mg $CuSO_4 \cdot 5H_2O$ in 100 mL of 5M H_2SO_4) was added to 500 µL reaction mixture (300 µL of appropriate dilution of the extracts with 100 µL 13.3% trichloroacetic acid (TCA) and water). The reaction mixture was subsequently incubated for 3 h at 37°C, then 0.5 mL of 65% H_2SO_4 (v/v) was added to the medium and the absorbance was measured at 520 nm using a spectrophotometer. The vitamin C content of the extracts was subsequently calculated.

Reducing activity

The reducing activity of the plantain extracts was determined by assessing the ability to reduce $FeCl_3$ solution as described by Pulido et al. (2000). A 2.5 mL aliquot was mixed with 2.5 mL of 200 mM sodium phosphate buffer (pH 6.6) and 2.5 mL of 1% potassium ferricyanide. The mixture was incubated at 50°C for 20 min and then 2.5 mL of 10% trichloroacetic acid was added. This mixture was then centrifuged at 805 *g* for 10 min. A volume of 5 mL of the supernatant was mixed with an equal volume of water and 1 mL of 0.1% ferric chloride. The absorbance was measured at 700 nm in the spectrophotometer after allowing the solution to stand for 30 min. A graph of absorbance vs. concentration of extract was plotted to observe the reducing power where a higher absorbance values indicates a higher reducing power.

α-Amylase inhibition assay

The aqueous extracts dilution (500 µL) and 500 µL of 0.02 M sodium phosphate buffer (pH 6.9 with 0.006 M NaCl) containing Hog pancreatic α-amylase (EC 3.2.1.1) (0.5 mg/mL) were incubated at 25°C for 10 min. Then, 500 µL of 1% starch solution in 0.02 M sodium phosphate buffer (pH 6.9 with 0.006M NaCl) was added to each tube. The reaction mixtures was incubated at 25°C for 10 min and stopped with 1.0 mL of dinitrosalicylic acid colour reagent. Thereafter, the mixture was incubated in a boiling water bath for 5 min, and cooled to room temperature. The reaction mixture was then diluted by adding 10 mL of distilled water, and absorbance measured at 540 nm. The percentage (%) enzyme inhibitory activity of the phenolic extracts was calculated (Worthington, 1993).

α-Glucosidase inhibition assay

Appropriate dilution of the aqueous extracts (50 µL) and 100 µL of α-glucosidase solution (1.0 U/ml) in 0.1 M phosphate buffer (pH 6.9) was incubated at 25°C for 10 min. Then, 50 µL of 5 mM p-nitrophenyl-α-D-glucopyranoside solution in 0.1 M phosphate buffer (pH 6.9) was added. The mixtures were incubated at 25°C for 5 min, before reading the absorbance at 405 nm in the spectrophotometer. The α-glucosidase inhibitory activity was expressed as percentage inhibition. The percentage (%) enzyme inhibitory activity of the phenolic extracts was calculated (Apostolidis, 2007).

DPPH free radical-scavenging

The free radical-scavenging ability of the extracts against DPPH (1, 1-diphenyl–2-picrylhydrazyl) free radical was evaluated as described by Gyamfi et al. (1999) with modifications. To 1mL of 0.4 mM methanolic solution of DPPH radicals, 0.05 ml of test extracts/standard quercetin was added. The mixture was left in the dark for 30 min and the absorbance was measured at 516 nm in the spectrophotometer. The DPPH free radical scavenging ability was subsequently calculated by comparing the results of the test with those of the control (not treated with the extract) using the formula (Shirwaikar et al., 2004):

$$\text{Percentage inhibition} = \frac{(\text{Absorbance of control} - \text{Absorbance. of test})}{\text{Absorbance of control}} \times 100$$

The bleaching of the DPPH solution increases regularly with increasing amount of extract in a given volume of solution. The bleaching action is mainly attributed to the presence of polyphenols and ascorbic acid extracted into the solution

Fe^{2+} chelation assay

The Fe^{2+} chelating ability of the extracts were determined using a modified method of Minotti and Aust (1987) with a slight modification by Puntel et al. (2005). Freshly prepared 500 µmolL^{-1} $FeSO_4$ (150 µL) was added to a reaction mixture containing 168 µl of 0.1mol L^{-1}Tris-HCl (pH 7.4), 218 µL saline and the extracts (0 – 100 µL). The reaction mixture was incubated for 5 min, before the addition of 13 µl of 0.25% 1; 10-phenanthroline (w/v).The absorbance was subsequently measured at 510 nm in a spectrophotometer. The Fe^{2+} chelating ability was subsequently calculated with respect to the control:

Percentage Fe^{2+} chelating ability (%) = [(Absorbance of control – Absorbance of test sample) / Abs of control] × 100

Fe^{2+} ion can form complexes with ferrozine but its complex formation can be prevented in the presence of a chelating agent which will result in a decrease in the red color of the complex. The measurement of this colour reduction allows determination of metal chelating activity as well as the estimation of the chelating activity of the coexisting chelator (Yamaguchi et al., 2000).

Table 1. Total phenol, total flavonoid and vitamin C contents of the aqueous extracts of raw flour and 'amala' flour.

Sample	Total phenol content (mg/g)	Total flavonoid content (mg/g)	Vitamin C content (mg/g)
Raw flour	0.94±0.02	0.71±0.02	3.71±0.38
'Amala' flour	1.09±0.04	0.73±0.01	2.73±0.07

OH radical-scavenging ability

The ability of plantain extracts to prevent Fe^{2+}/H_2O_2-induced decomposition of deoxyribose was carried out using the method of Halliwell and Gutteridge (1981). Briefly, freshly prepared aqueous extract (0–100 mL) was added to a reaction mixture containing 120 mL 20-mM deoxyribose, 400 mL 0.1-M phosphate buffer, 40 mL 20-mM hydrogen peroxide and 40 mL 500-mM $FeSO_4$, and the volume was made to 800 mL with distilled water. The reaction mixture was incubated at 37°C for 30 min, and the reaction was stopped by the addition of 0.5 mL of 2.8% trichloroacetic acid (TCA); this was followed by the addition of 0.4 mLof 0.6% thiobarbituric acid (TBA) solution. The tubes were subsequently incubated in boiling water for 20 min. The absorbance was measured at 532 nm in spectrophotometer:

Percentage OH radical scavenging ability (%) = [(Absorbance of control – Absorbance of test sample)/ Abs of control] × 100

Angiotensin-I-converting enzyme (ACE) inhibition assay

Aqueous extract dilution (50 µL) and ACE solution (50µl, 4 mU) were incubated at 37°C for 15 min. The enzymatic reaction was initiated by adding 150 µl of 8.33 mM of the substrate Bz–Gly–His–Leu in 125 mM Tris– HCl buffer (pH 8.3) to the mixture. After incubation for 30 min at 37°C, the reaction was arrested by adding 250µl of 1M HCl. The Gly–His bond was then cleaved and the Bz–Gly produced by the reaction was extracted with 1.5 mL ethyl acetate. Thereafter the mixture was centrifuged to separate the ethyl acetate layer; then 1 mL of the ethyl acetate layer was transferred to a clean test tube and evaporated. The residue was redissolved in distilled water and its absorbance was measured at 228 nm. The percentage (%) enzyme inhibitory activity of the phenolic extracts was calculated (Cushman and Cheung ,1981).

Data analysis

The results of the three replicates were pooled and expressed as mean ± standard error (S.E.). Student t-test was carried out (Zar, 1984).

RESULTS AND DISCUSSION

Total phenol, total flavonoid and vitamin C contents

The results for total phenol, total flavonoid and vitamin C contents of the raw flour and flour from the thick paste ('amala' flour) are presented in Table 1. The results revealed that the total phenol content of the 'amala' flour extract was higher than that of the raw flour extract. The total phenolic contents of raw flour and 'amala' flour were 0.94 mg/g and 1.09 mg/g respectively. The reason for this wide range of values in the total phenolic contents cannot be categorically stated; however the difference may be attributed to the liberation of some phenolic compounds as a result of the heat process associated with the making of 'amala'. Phenolic compounds can protect the human body from free radicals, whose formation is associated with the normal metabolism of aerobic cells and are strong antioxidants capable of removing free radicals, they may chelate metalic catalysts, activate antioxidant enzymes, reduce α-tocopherol radicals and inhibit oxidases (Amic et al., 2003). For the flavonoid content of raw flour and 'amala' flour; 0.71 and 0.73 mg/g respectively (Table 1), it was observed that 'amala' flour had higher content. Phenolics and Flavonoids have been widely reported to possess remarkable antioxidant and medicinal properties (Liu, 2004). Vitamin C has been reported to contribute to the antioxidant activities of plant food. Ascorbic acid is a good reducing agent and exhibits its antioxidant activities by electron donation (Oboh, 2005). The vitamin C (in mg/g) content of raw flour extract was higher than that of 'amala' flour. This wide range of value between them could be attributed to polyphenols, ascorbic acid and other carbonyl compounds taking part in the Maillard reaction (Rizzi, 1994) and as such, some vitamin C could have been lost during the further heat processing of 'amala'.

Reducing activity

Reducing power is a novel antioxidation defence mechanism; the two mechanisms available to affect this property are: electron transfer and hydrogen atom transfer (Dastmalchi et al., 2007). This is because the ferric-to-ferrous iron reduction occurs rapidly with all reductants with half reaction reduction potentials above that of Fe^{3+}/Fe^{2+}, the values in the Ferric reducing antioxidant property (FRAP) assay expresses the corresponding concentration of electron-donating antioxidants (Halvorsen et al., 2002). The reducing powers of the aqueous extracts of the raw flour and the 'amala' flour were assessed based on their ability to reduce Fe^{3+} to Fe^{2+} and the results are presented in Figure 6. The results revealed that both extracts exhibited reducing power in a dose dependent manner. However, the reducing power of the 'amala' flour extract was higher

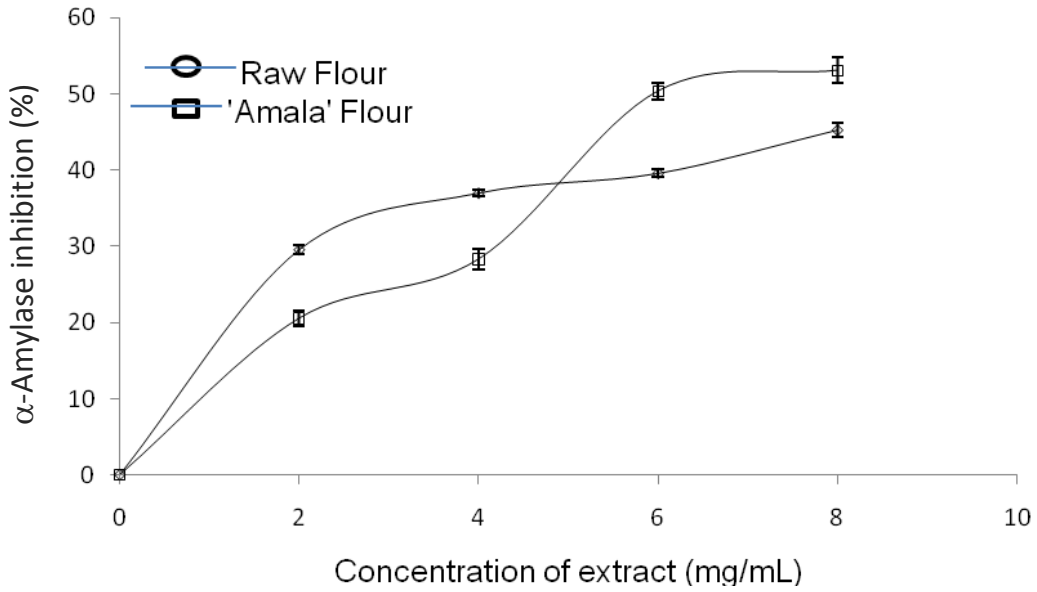

Figure 1. α-Amylase inhibitory activities of aqueous extracts of raw flour and 'amala' flour. Values represent mean ± standard deviation, *n* = 3.

than that of the raw flour extract. This trend is in agreement with the total phenolic and flavonoid contents of raw and 'amala' flour studied. Since the antioxidant activity of phenolics is mainly due to their redox activities, this allows them to act as reducing agents, hydrogen donors and singlet oxygen quenchers (Rice et al., 1996).

Inhibition of α-amylase activity

The control of postprandial plasma glucose levels is critical in the early treatment of diabetes mellitus and in reducing chronic vascular complications (Oritz et al., 2007) and the therapeutic approach available for managing type 2diabetes is by controlling the absorption of glucose through the reduction of starch hydrolysis by inhibiting pancreatic α-amylase and limiting the absorption of glucose by inhibiting intestinal α-glucosidase enzymes (Krentz and Bailey, 2005). The interaction of aqueous extracts (Raw flour and 'amala' flour) with α-amylase is presented in Figure 1; both aqueous extracts inhibited the enzyme in a dose-dependent manner (in the range of 0–8 mg/mL). However, judging by the EC_{50} (extract concentration causing 50% enzyme inhibition) values (Table 2), the 'amala' flour had higher inhibitory activity than the raw flour extract. The determined α-amylase inhibitory activity agreed with some earlier reports where plant phytochemicals from green and black tea inhibited saliva α-amylase activity (Zhang and Kashket, 1998) and inhibitory effects of *Allium* spp. on α-amylase activity (Nickavar and Yousefian 2009). These inhibitions of α-amylase by both aqueous extracts could contribute to the anti-diabetic potentials of unripe plantains, by slowing down the breakdown of starch to simple sugar.

Inhibition of α-glucosidase activity

The interaction of the aqueous (raw flour and 'amala' flour) extracts with α-glucosidase is presented in Figure 2. Both aqueous extracts inhibited α-glucosidase activity *in vitro*; this inhibition of the enzyme could slow down the breakdown of disaccharide to simple glucose, hence reducing the amount of glucose absorbed in the blood (Kwon et al., 2006). However, both extracts exhibited a dose-dependent enzyme inhibitory activity in the range of 0–8 $mg \cdot ml^{-1}$. Extracts of the 'amala' flour exhibited higher inhibitory activity than the raw flour extracts, when taking into account the EC_{50} values of the aqueous extracts (Table 2). Both extracts had higher inhibitory effect on α-glucosidase than their corresponding α–amylase inhibitory activity; this mild inhibition of α-amylase and strong inhibition of α-glucosidase is of great pharmacological importance in addressing some of the side effects associated with the drugs (acarbose and voglibose) such as abdominal distention, flatulence, meteorism and possibly diarrhea (Bischoff, 1994). These adverse effects have been suggested to be caused by the excessive pancreatic α-amylase inhibition resulting in the abnormal bacterial fermentation of undigested saccharides in the colon (Bischoff, 1994).

DPPH radical-scavenging ability

The prevention of the chain initiation step by scavenging

Table 2. EC_{50} of raw flour and 'amala' flour extracts on different parameters.

	Assays/samples	EC_{50}
	DPPH Scavenging ability (mg/mL)	
a	Raw flour	28.85±2.82
	'Amala' flour	25.89±0.94
	OH Scavenging ability (mg/mL)	
b	Raw flour	4.92±1.03
	'Amala' flour	6.00±1.17
	Fe^{2+}Chelating ability (mg/mL)	
c	Raw flour	6.10±0.30
	'Amala' flour	7.05±0.21
	inhibition of α-amylase (mg/mL)	
d	Raw flour	**7**.44±0.60
	'Amala' flour	6.81±0.95
	inhibition of α-glucosidase (mg/mL)	
e	Raw flour	6.55±4.09
	'Amala' flour	4.10±7.17
	inhibition of Angiotensin-1- converting enzyme (mg/mL)	
f	Raw flour	0.81±1.39
	'Amala' flour	0.87±2.77

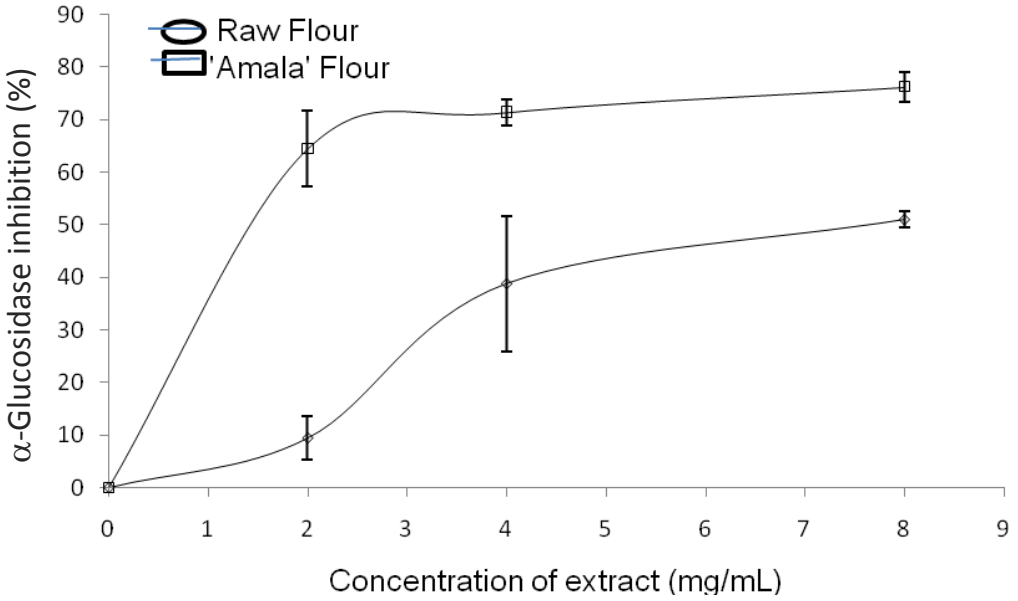

Figure 2. α-Glucosidase inhibitory activities of aqueous extract of raw flour and 'amala' flour. Values represent mean ± standard deviation, *n* = 3.

various reactive species such as free radicals is considered to be an important antioxidant mode of action (Dastmalchi et al., 2007). The DPPH free radical scavenging ability of the raw flour and 'amala' flour

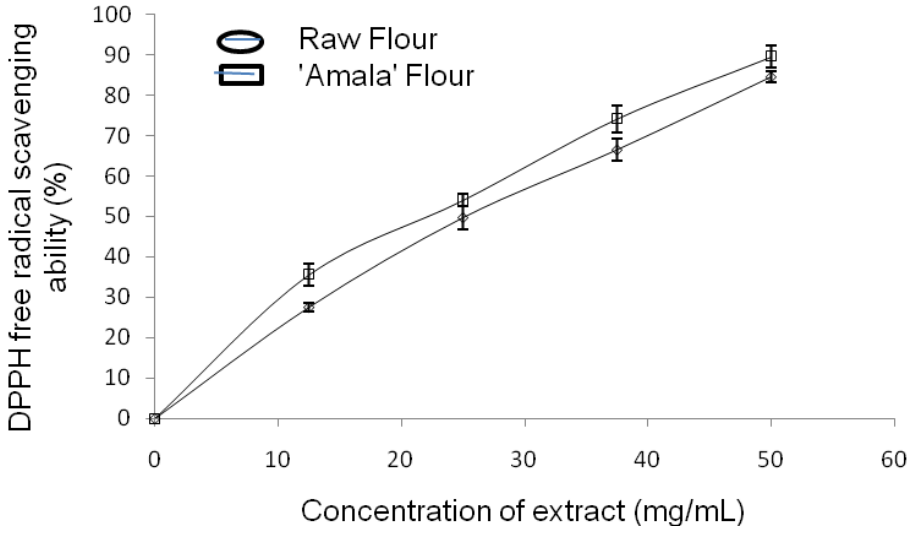

Figure 3.DPPH free radical-scavenging ability of aqueous extract of raw flour and 'amala' flour. Values represent mean ± standard deviation, *n* = 3.

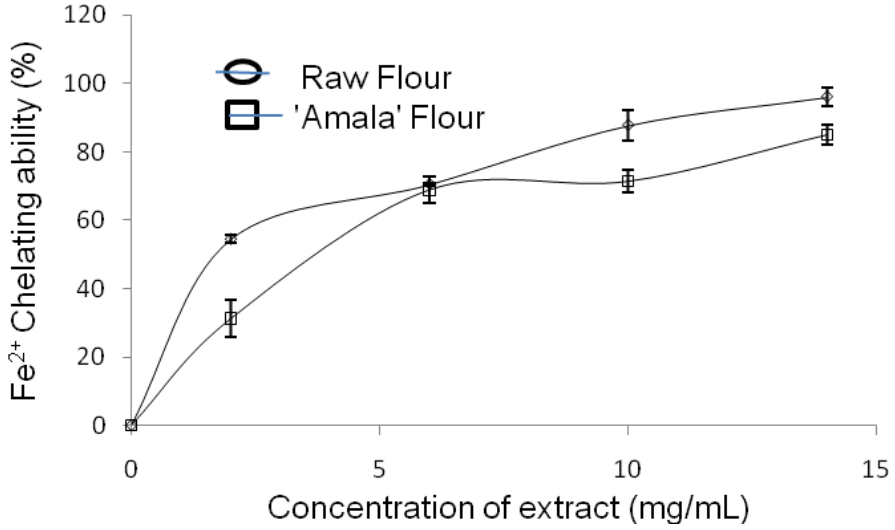

Figure 4. Fe^{2+} chelating ability of aqueous extracts of raw flour and 'amala' flour. Values represent mean ± standard deviation, *n* = 3.

aqueous extracts of the unripe plantains as presented in Figure 3 and their EC_{50} in Table 2 revealed that both aqueous extracts scavenged DPPH radicals in a dose-dependent manner in the range of 0–50 mg/mL. As observed, the 'amala' flour extract had higher DPPH scavenging ability than the raw flour extract. The observed trend in the results agree with the phenolic contents and reducing activities (Table 1), where the 'amala' flour with higher phenolic content had higher antioxidant activity, as well as many earlier research articles, where correlations were reported between phenolic content and antioxidant capacity of some plant foods (Oboh and Shodehinde, 2009) .

Fe^{2+} chelating ability

Iron has been implicated as the most important pro-oxidant of lipids. It is also known that the Fe^{2+} accelerates lipid peroxidation by breaking down hydrogen and lipid peroxides formed by the Fenton free radical reaction: $Fe^{2+}+H_2O_2 \quad Fe^{3+}+ OH^{+} OH^{\bullet}$ (Yamaguchi et al., 2000). The Fe^{2+} chelating ability of the aqueous extracts is presented in Figure 4 and their EC_{50} in Table 2. Both extracts were able to chelate Fe^{2+} in a dose-dependent manner; however, the raw flour extract (EC_{50}=6.10±0.30 mg/mL) had higher Fe^{2+} chelating ability except at the lower concentration of the extract tested (6 mg/mL)

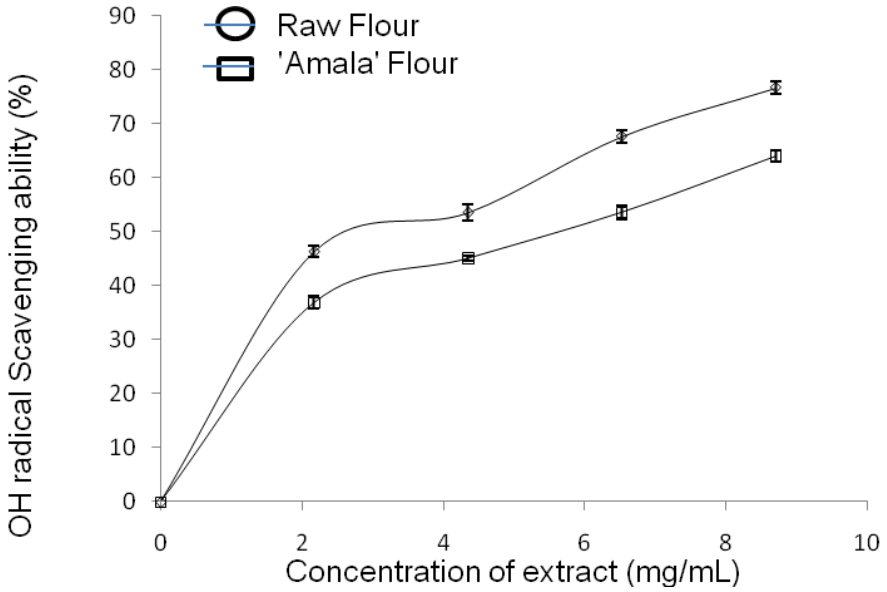

Figure 5. OH radical scavenging ability of aqueous extracts of raw flour and 'amala' flour. Values represent mean ± standard deviation, *n* = 3.

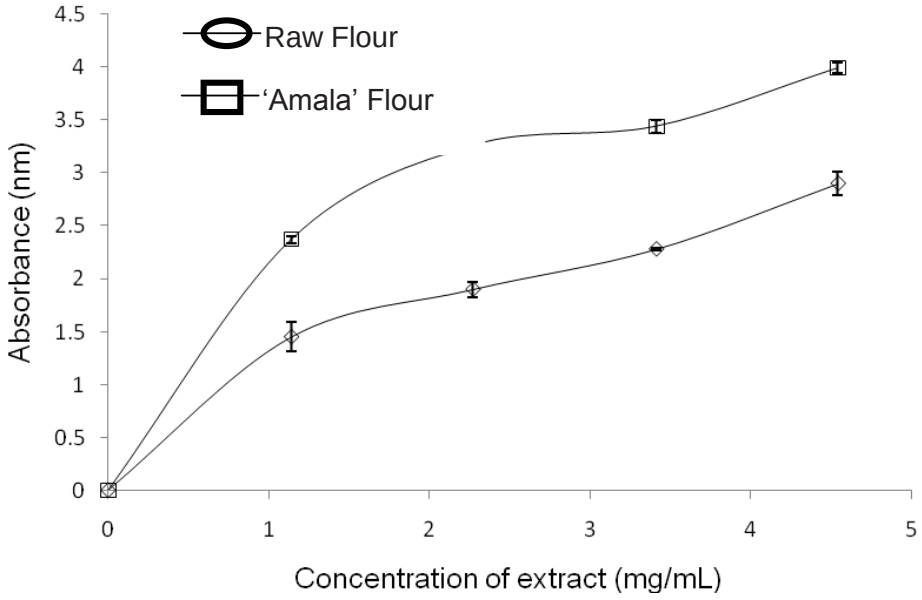

Figure 6. Reducing activity of aqueous extracts of raw flour and 'amala' flour. Values represent mean ± standard deviation, *n* = 3.

where the 'amala' flour had higher inhibitory effect.

Hydroxyl radical (OH) radical scavenging ability

The ability of aqueous extracts of raw flour and 'amala flour to scavenge H_2O_2 is shown in Figure 5 and their EC_{50} in Table 2. The extracts were capable of scavenging H_2O_2 in a dose-dependent manner. However, raw flour extract had higher $OH^{\bullet}$ radical scavenging ability than the 'amala' flour (EC_{50}= 4.92±1.30 mg/mL) (Table 2). The $OH^{\bullet}$ radical scavenging ability of the unripe plantain extracts agree with the Fe^{2+} chelating ability. This high Fe^{2+} chelating ability is of immense importance in the protective ability of antioxidant phytochemicals against oxidative stress, because it is usually too late to attempt

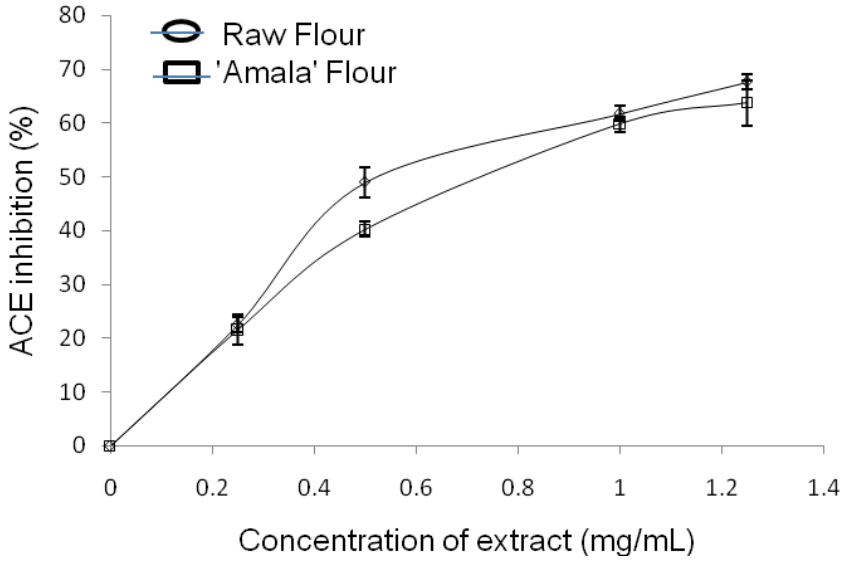

Figure 7. Angiotensin-1- converting enzyme (ACE) inhibitory activity of aqueous extracts of raw flour and 'amala' flour. Values represent mean ± standard deviation, *n* = 3.

to use OH radical scavengers for therapeutic purposes. The reason for this is that extraordinarily high reactivity of hydroxyl radicals towards most biomolecules would require unreasonably high concentrations of intercepting scavengers to outcompete the biomolecules of interest (Bayýr et al., 2006). Hence, the removal of H_2O_2 as well as O_2 is very important for the protection of food and body systems.

Angiotensin-I-converting enzyme (ACE) inhibition assay

The antihypertensive potentials of the unripe plantain extracts were also assessed by characterizing the interaction of the aqueous with ACE. This is shown in Figure 7 and their EC_{50} in Table 2. Both extracts had high inhibitory effect on ACE activity in a dose-dependent manner. The raw flour extract had higher inhibitory effect (EC_{50}= 0.81±1.39 mg/mL) (Table 2). The raw flour extract with lower phenolic contents (total phenol and total flavonoid) and reducing power (Table 1) had higher Fe^{2+} chelating ability, OH radical scavenging ability and ACE inhibitory activity. Their exhibited inhibitory activities could be attributed to the presence of some non-phenolic phytochemicals and heterogeneous nature of the aqueous extracts thereby giving an additive or synergistic effect. ACE is an important enzyme involved in maintaining vascular tension by two different reactions that it catalyzes: conversion of the inactive angiotensin I into a angiotensin II (a vasoconstrictor) and inactivation of the bradykinin (a vasodilator) which is conducive in lowering blood pressure (Johnston and Franz, 1992). Many studies have reported the beneficial effects of polyphenol-rich extracts on hypertension by not only retarding the development of hypertension but also by normalizing the blood pressure (Taubert et al., 2003). Furthermore, some foods and herbs have been reported to have the potential to treat hypertension, especially for patients with borderline to mild high blood pressure (Chen et al., 2009). This eventually corresponds with the earlier reports in which plantain has been suggested as a useful diet due to its low sodium (Chandler, 1995), little fat and no cholesterol, in the management of high blood pressure and heart disease (Dzomeku et al., 2007).

Conclusion

The high inhibitory effect of extracts (raw flour and thick paste also known as 'amala') from unripe plantains on key enzymes linked to type 2 diabetes and hypertension could make unripe plantains cheap and good sources of nutraceutical for the management of diabetes and hypertension and as discussed earlier there is a need to continue to explore the relationship between free radicals, diabetes and hypertension, and its complications, and to elucidate the mechanisms by which increased oxidative stress accelerates the development of diabetic complications, in an effort to expand treatment options.

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