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PHENOLIC EXTRACTS OF 'AMALA' FROM UNRIPE PLANTAIN (*MUSA PARADISIACA*) PULPS INHIBIT KEY ENZYMES LINKED TO TYPE-2-DIABETES AND HYPERTENSION

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ABSTRACT

This study sought to characterize the interaction of phenolic extract from 'amala' with α-amylase, α-glucosidase and angiotensin-I-converting enzyme (ACE) activities. 'Amala' is a local meal in western part of Nigeria that is prepared by reconstituting dried unripe plantain flour in boiling water to form a thick paste. The ready to eat 'amala' was later dried to a constant weight and milled into flour and kept as a sample. The free phenolic of 'amala' was extracted with 80% acetone, while the bound phenolic was extracted from the alkaline and acid-hydrolyzed residue with ethyl acetate; and their interactions with the enzymes were assessed. The phenolic extracts inhibited α-amylase, α-glucosidase and ACE enzyme activities; however, free phenolic had significantly ($P< 0.05$) higher α-amylase, α-glucosidase and ACE inhibitory activities than bound phenolic. Nevertheless, the extracts were strong inhibitor of α-glucosidase when compared to α-amylase. The phenolics inhibited Fe^{2+} and sodium nitroprusside-induced lipid peroxidation in pancreas in a dose-dependent manner in which the free phenolic also had higher inhibition. The results of this study suggest that the free and bound phenolic extracts from 'amala may be adjudged to be beneficial as anti-diabetes and anti-hypertension.

Keywords: 'Amala', Free phenol, bound phenol, α-amylase, α-glucosidase.

INTRODUCTION

Diabetes mellitus is a chronic metabolic disorder that is characterized by hyperglycemia which results from insufficient or inefficient insulin secretion and causes alterations in carbohydrate, protein, and lipid metabolism (Shobana *et al.*, 2009). However, NIDDM (non insulin dependent diabetes mellitus) is the most common form of diabetes, accounting for 90% of all cases, and is a metabolic disorder primarily characterized by insulin resistance, relative insulin deficiency, and an abnormal rise in blood sugar, right after a meal, called postprandial hyperglycemia (Kwon *et al.*, 2007). Recent reports have revealed that hyperglycemia could induce non enzymatic glycosylation of various macromolecules, generation of reactive oxygen species, and alteration of endogenous antioxidants, which could lead to chronic complications (Lebovitz, 2001). Pancreatic α-amylase breaks down large polysaccharides (starch) into disaccharides and oligosaccharides, before the action of α-glucosidase break down disaccharides into monosaccharides (glucose) which is readily absorbed into the blood stream. Inhibition of pancreatic α-amylase and α-glucosidase is the mechanism adopted by many commercially available drugs for the

management of NIDDM (Krentz and Bailey, 2005). The drugs that are currently used as α-amylase and α-glucosidase inhibitors exhibit side effects such as abdominal distension, bloating, flatulence and diarrhea (Chakrabarti and Rajagopalan, 2002., Kimmel and Inzucchi, 2005) which are linked to excessive inhibition of the pancreatic α-amylase (Bischoff, 1994). However, isolated natural inhibitors of the enzymes have been reported to be effective in decreasing postprandial hyperglycaemia with minimal side effects (Kwon *et al.*, 2006). Hence, natural α-amylase and α-glucosidase inhibitors offer an attractive therapeutic approach to the treatment of NIDDM by ultimately slowing the degradation of starch to glucose. This necessitates the search for natural inhibitors with strong α-glucosidase, but mild α-amylase activities.

Hypertension is a firsthand complication of NIDDM. Angiotensin I converting enzyme (ACE) (EC 3.4.15.1) plays an important physiological role in regulating blood pressure.1 ACE belongs to the class of zinc proteases which is expressed in the vascular endothelial lining of human lungs. ACE is a dipeptidyl carboxypeptidase that catalyzes the conversion of angiotensin I (decapeptide) to angiotensin II (octapeptide), it inactivates the antihypertensive vasodilator bradykinin, thereby increasing blood pressure. Inhibition of ACE activity leads to a decrease in the concentration of angiotensin II and consequently reduces blood pressure (Skeggs and Khan, 1957). In addition, inhibition of ACE is considered a useful therapeutic approach in the treatment of high blood pressure in both diabetic and non diabetic patients, (Johnston and Franz, 1992) and dietary intervention with the use of could be a readily available means of preventing and/or managing diabetes and hypertension. Previous in vitro and in vivo animal and clinical studies have also demonstrated the potency of specific phenolic phytochemicals in hypertension management with direct absorption into the blood (Kwon *et al*., 2006).

Plantain (*Musa paradisiaca*) is known as plantain (English), ogede agbagba (Yoruba), ayaba (Hausa) and Ogadejioke (Igbo). It is a perennial tropical plant that is native to India. The plant consists of long, overlapping leafstalks with a stem which is 1.22 to 6.10 m high while the leaves grow to a length of 1.83 m and 0.61 m wide. The fruits grow in clusters and each separate plantain is about 1 inch in diameter but somewhat longer than a banana (Gill, 1992). The fruit of unripe plantain is used in folklore medicine for treating diarrhoea, dysentery, intestinal lesions in ulcerative colitis, diabetes, uremia, nephritis, gout, hypertension and cardiac disease (Ghani, 2003). In Nigeria, it is very common to find Nigerian diabetics consuming unripe plantain meal to reduce postprandial glucose level. This is because the propensity of individuals to develop diabetes and obesity is due to the increased consumption of carbohydrate rich foods with a high Glycemic index (Willet *et al.,* 2002).

Plantain fruits are good sources of plant phytochemicals, (Ghani, 2003) which promote health and well-being. Phenol rich foods could protect against certain chronic degenerative diseases (Dykes and Rooney, 2007). Recently, plant phytochemicals such as phenols have been reported to inhibit key enzymes linked to type 2 diabetes (Kwon *et al*., 2007). Plantain fruits can be consumed as ripe or unripe in several forms; it is either boiled, fried, roasted, steamed, baked or grilled. Other products derived from plantain could be in form of chips and flour (Nwokocha and Williams, 2009). When processed into flour, it is used traditionally for preparation of gruel which is made by stirring the flour with appropriate quantities of boiling water to form a brown thick paste (Idowu *et al*., 1996) which is known locally as 'amala'. Beside these forms of use; Plantain flour is now used as a functional agent in bakery products (Akubor, 1998). During the production of plantain flour, plantain shows strong enzymatic browning reactions when cut and exposed to the air (Ozo et a., 1984). This browning is caused by enzyme tyrosinase which oxidizes the amino acid tyrosine into 3,4-dihydroxyphenyalanine and further application of heat (i.e reconstitution of plantain flour in boiling water) eventually leads to the formation brown melanoidin compounds (Zakpaa *et al*., 2010). Recent studies have revealed that partial enzymatic oxidation (Manzocco *et al*., 1998) and non-enzymatic (melanoidins) browning

exhibit antioxidant properties (Yen and Tsai, 1993).

The consumption of the plantain and its product is now on the increase even though it could be eaten singly. Nigerians have adopted various means of including it in their various meals as part of their diet. As mentioned above, it could be processed into various products to facilitate its diverse use. Various analyses has been carried out on plantain but as at the time of this study there is no information on the antioxidant and enzymatic activities of free and bound phenolics from unripe plantain and its products. Hence, this study was carried out to investigate the antioxidant and enzymatic activities of free and bound phenols in the inhibition of the key enzymes linked to type 2 diabetes and hypertension.

MATERIALS AND METHODS

Materials

Fresh matured 'false horn' unripe plantain was bought at Oja-Oba in Akure. Ondo State. The unripe plantain was identified at the Department of Biology, Federal University of Technology, Akure, Nigeria.

Chemicals and equipment

Porcine pancreatic α-amylase (EC 3.2.1.1), α-glucosidase (EC 3.2.1.20), p-nitrophenyl- α-D-glucopyranoside, gallic acid, quercetin and Folin–Ciocalteu reagent, were procured from Sigma Aldrich, Inc. (St. Louis, MO, USA). Thiobarbituric acid (TBA) was purchased from Sigma-Aldrich Laborchemikaliem GmbH (Seelze, Germany). Trichloroacetic acid, malondialdehyde
(MDA), and 3,5-dinitrosalicylic acid were obtained from Sigma-Aldrich Chemie GmbH (Steinheim, Germany). All other chemicals used were of analytical grade, and the water used was glass distilled. Optical absorbance was measured with a UV-Visible spectrophotometer (Model 6305; Jenway, Barloworld Scientific, Dunmow, United Kingdom).

Sample preparation

Unripe plantain pulps (6kg) were sliced, sun-dried for about 3 weeks to a constant weight, and ground into flour. The flour was passed through a local made hand sieve and was processed into 'amala'. This was achieved by stirring the flour continuously in a pot of boiling water until it is 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 kept in an air tight container for future analysis.

Extraction of Free Soluble Phenols

The extraction of free soluble phenolics was carried out according to the method reported by Chu *et al*., (2002) Ten grams of the 'amala' flour was extracted with 80% acetone (1:5w/v) and filtered (filter paper Whatman no.2, Whatman International Ltd., Maidstone, England) under vacuum. The filtrate was then evaporated using a rotary evaporator under vacuum at 45^0C until about 90% of the filtrate had been evaporated. The phenolic extracts were frozen, while the residues were kept for the extraction of bound phenolics.

Extraction of Bound Phenols

The residue from free soluble extraction above was flushed with nitrogen and hydrolyzed with about 20 mL of 4 M NaOH solution at room temperature for 1 hour with shaking. Then, the pH of the mixture adjusted to pH 2 with concentrated HCl and the bound phytochemicals were extracted with ethylacetate (sixtimes). The ethylacetate fractions were then evaporated at 45^0C (Chu *et al*., 2002).

Determination of Total Phenol Content

The phenol content of the unripe plantain extracts was determined by using the method reported by Singleton *et al*., (1999). Briefly, 0.5 mL of the extracts were oxidized with 2.5mL 10% Folin–Ciocalteu reagent (v/v) and neutralized by 2.0mL of 7.5% sodium carbonate. The reaction mixture was incubated for 40 minutes at 45^0C, and the absorbance was measured at 765nm in the spectrophotometer (JENWAY 6305). 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 a slightly modified method of Meda *et al*., (2005). The volume of 0.5mL of sample extracts was mixed with 0.5 mL methanol, 50µl of 10% $AlCl_3$, 50µL of 1 mol/L potassium acetate and 1.4 mL water, and was incubated at room temperature for 30 minutes. Thereafter, the absorbance of the

reaction mixture was measured at 415 nm in the spectrophotometer (JENWAY 6305). Total flavonoid content was calculated using quercetin as a standard.

Lipid peroxidation assay

Experimental animals.

Male Wistar albino rats weighing 190–250g were purchased from the Central Animal House, Department of Biochemistry, University of Ibadan, Ibadan, Nigeria. They were housed in stainless steel cages under controlled conditions with a 12-hour/12-hour light/ dark cycle, 50% humidity, and temperature of 28^{0}C. The rats were allowed ad libitum access to food and water. The animals were handled in accordance with the procedure approved by the Animal Ethics Committee of the Federal University of Technology, Akure, Nigeria.

Preparation of tissue homogenates.

The rats were decapitated under mild diethyl ether anaesthesia, and the pancreas tissue was rapidly dissected and placed on ice and weighed. This tissue was subsequently homogenized in cold saline (1/10w/v) with about 10 up-and-down strokes at approximately 1200 revolutions/min in a Teflon glass homogenizer (Mexxcare, mc14 362, Aayushi Design Pvt. Ltd., New Delhi, India). The homogenate was centrifuged (KX3400C Kenxin International Co., Hong Kong) for 10 minutes at 3000g to yield a pellet that was discarded and a low-speed supernatant, which was kept for lipid peroxidation assay (Belle *et al*., 2004).

Lipid peroxidation and TBA reactions.

The lipid peroxidation assay was carried out by using the modified method of Ohkawa *et al*., (1979). Briefly, 100µL of the supernatant fraction was mixed with a reaction mixture containing 30µL of 0.1M Tris-HCl buffer (pH, 7.4), extract (100µL), and 30µL of 25µM freshly prepared $FeSO_4$. A volume of 300µL was achieved by adding water before incubation at 37^{0}C for 2 hours. The colour reaction was developed by adding 300µL 8.1% sodium dodecyl sulfate) to the reaction mixture containing supernatant; this was subsequently followed by the addition of 500µL of acetic acid/HCl (pH, 3.4) mixture and 500µL of 0.8% TBA. This mixture was incubated at 100^{0}C for 1 hour. The TBA reactive species produced were measured at 532nm and expressed as MDA produced (% control) by using the malondialdehyde (MDA) standard curve.

α-Amylase inhibition assay

The α-amylase inhibitory assay was performed as reported by Oboh *et al*., (2010). Briefly, sample extracts (50µL) and 500µL of 20mM sodium phosphate buffer (pH, 6.9, with 6mM NaCl) containing porcine pancreatic α amylase (0.5mg/mL) were incubated at 25^{0}C for 10 minutes. (One unit of the enzyme will liberate 1.0mg of maltose from starch in 3 minutes.) Then, 500µL of 1% starch solution in 20mM sodium phosphate buffer (pH, 6.9, with 6mM NaCl) was added to each tube. The reaction mixtures was incubated at 25^{0}C for 10 minutes and stopped with 1.0mL of 3,5-dinitrosalicylic acid colour reagent. Thereafter, the mixture was incubated in a boiling water bath for 5 minutes and cooled to room temperature. The reaction mixture was then diluted by adding 10mL of distilled water, and absorbance was measured at 540nm using spectrophotometer (JENWAY 6305).

α-Glucosidase inhibition assay

The assay was performed as described by Apostolidis *et al*., (2007). Briefly, 50µL of sample extracts and 100µL of α-glucosidase solution (1.0U/mL) in 0.1M phosphate buffer (pH, 6.9) was incubated at 25^{0}C for 10 minutes. (One unit of the enzyme will liberate 1.0µmol of D-glucose from p-nitrophenyl- α-D-glucoside per minute) Then, 50µL of 5mM p-nitrophenyl-α-D-glucopyranoside solution in 0.1M phosphate buffer (pH 6.9) was added. The mixtures were incubated at 25^{0}C for 5 minutes, and the absorbance was read at 405nm using spectrophotometer (JENWAY 6305).

Data Analysis

All experiments were performed in triplicate. The results were pooled and expressed as mean ± standard error. Student's t-test was carried out. one-way analysis of variance (ANOVA) and the least significance difference (LSD) were carried out. Significance was accepted at $P < 0.05$ (Zar, 1984). EC_{50} (concentration of extract that will

cause 50% enzyme inhibitory activity) was determined using linear regression analysis.

RESULTS AND DISCUSSION

Phytochemicals, especially phenolics, in fruits, n uts and vegetables are suggested to be the major bioactive compounds for health benefits (Rocha-Ghzman *et al*., 2007). In the present study, we observed that the residue obtai ned after the extraction of free soluble phenolic compound produced red color upon treatment with HCl acid. The red solution is a characteristic of anthocyanidins produced from the acid hydrolysis of procyanidins and this confirms that glycoside bound phenolic compound shows the presence of anthocyanidins (Mohd *et al*., 2009) and a wide spectrum of beneficial activity for human health has been attributed to procyanidins (Shi *et al*., 2003). This indicates that unripe plantain is an adjudged good source of procyanidins. However, the results of the total phenol (Tab. 1.) revealed that free soluble phenolic content (104.07 mg/g) of the 'amala' extract was significantly ($P< 0.05$) higher than the bound phenolic (91.03mg/g) content. These phenolic distributions agree with earlier reports on some plant foods such as commonly consumed vegetables (Chu *et al*., 2002), fruits (Sun *et al*., 2002) and mushrooms (Oboh and Shodehinde, 2009) in that their free soluble phenolic content are significantly higher than the bound phenolic but in contrast to what was reported by Shela *et al*., (2004) that the bound phenolic content of jaffa sweetie and grape fruits are higher than the free soluble phenolic. Moreover, Phenolic compounds can protect the human body from the generation of free radicals whose formation is associated with the normal metabolism that takes place in aerobic cells. However, free soluble phenolics are more readily absorbed in early digestion to exert its beneficial biological function. Bound phenols on the other hand, exert its potency possibly due to the different plant foods with different amounts of bound phytochemicals that can be digested and absorbed at different sites of the gastrointestinal tract where they render their unique role in health benefits. Bound phytochemicals, mainly in β-glycosides, are not digestible by human enzymes, thus could survive stomach and small intestine digestion to reach the colon where they are digested by bacteria flora to release phytochemicals which have been widely reported to have health benefits (Sun *et al*., 2002; Chu *et al*., 2002).

Table 1. Total phenolic contents, flavonoid contents and EC50 of the enzyme inhibitory activities of free and bound phenolic extracts of 'amala' flour.

Samples	Total Phenol (mg/100g)	Total flavonoid (mg/100g)	EC50 of the enzyme inhibitory activity (mg/ml)		
			α-amylase	α-glucosidase	Angiotensin- 1 converting enzyme
Free	104.07 ± 0.01^{a}	80.07 ± 0.05^{a}	0.48 ± 0.21^{b}	0.42 ± 0.10^{b}	0.09 ± 0.12^{b}
Bound	91.03 ± 0.04^{b}	67.22 ± 0.01^{b}	0.57 ± 0.10^{a}	0.51 ± 0.03^{a}	0.11 ± 0.08^{a}

Values represent mean ± standard deviation, n = 3.Values with the same letter along the same row are not significantly different ($P < 0.05$).

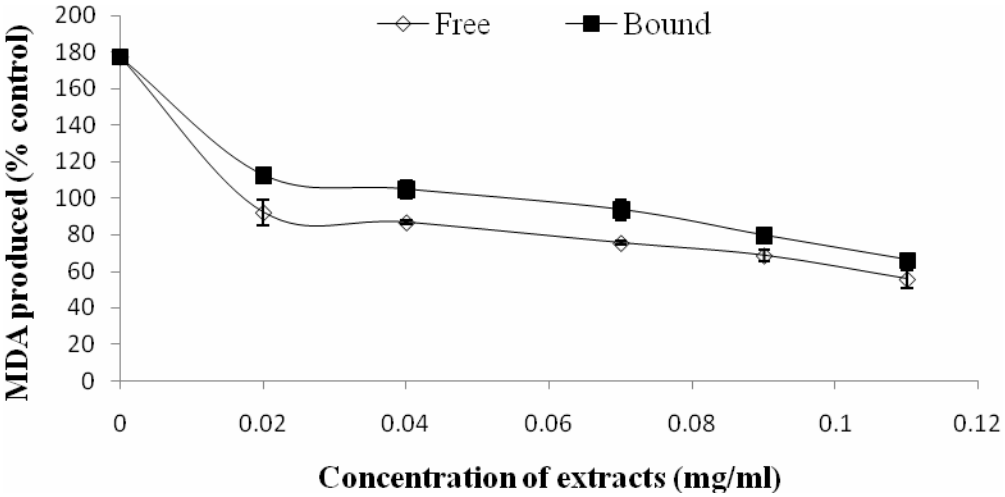

Figure 1. Inhibition of sodium nitroprusside induced lipid peroxidation by free and bound phenolics from 'amala'

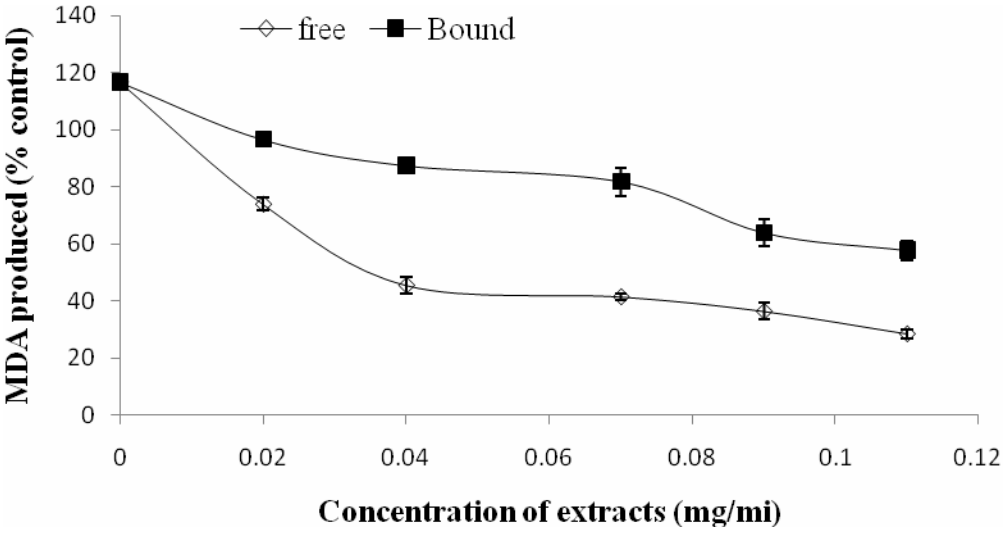

Figure 2. Inhibition of Fe^{2+} induced lipid peroxidation by free and bound phenolics from 'amala'

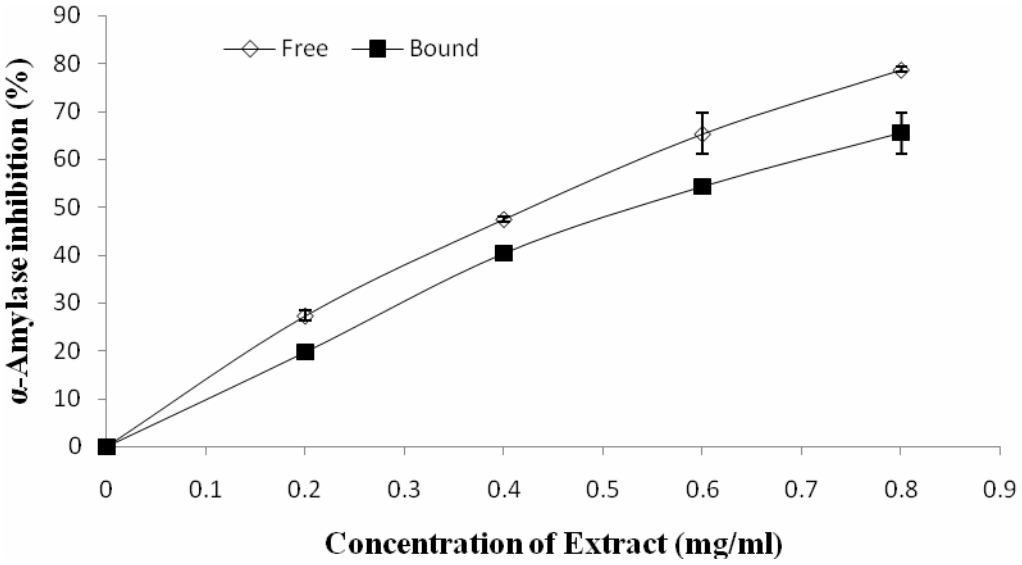

Figure 3. α-Amylase inhibitory activity of free and bound phenolics from 'amala'

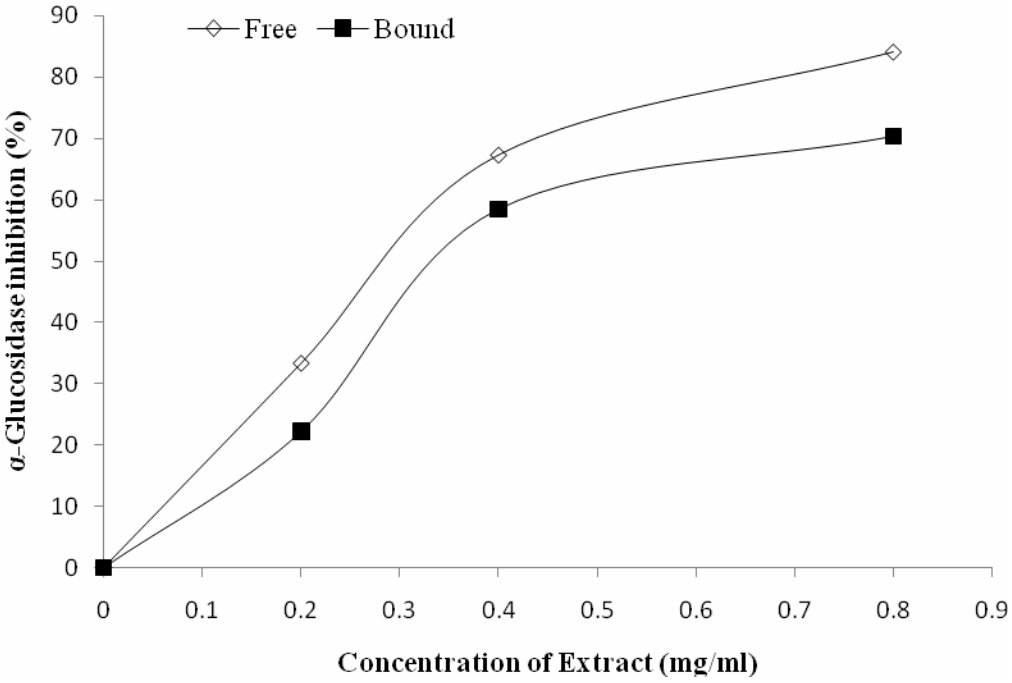

Figure 4. α-Glucosidase inhibitory activity of free and bound phenolics from 'amala'.

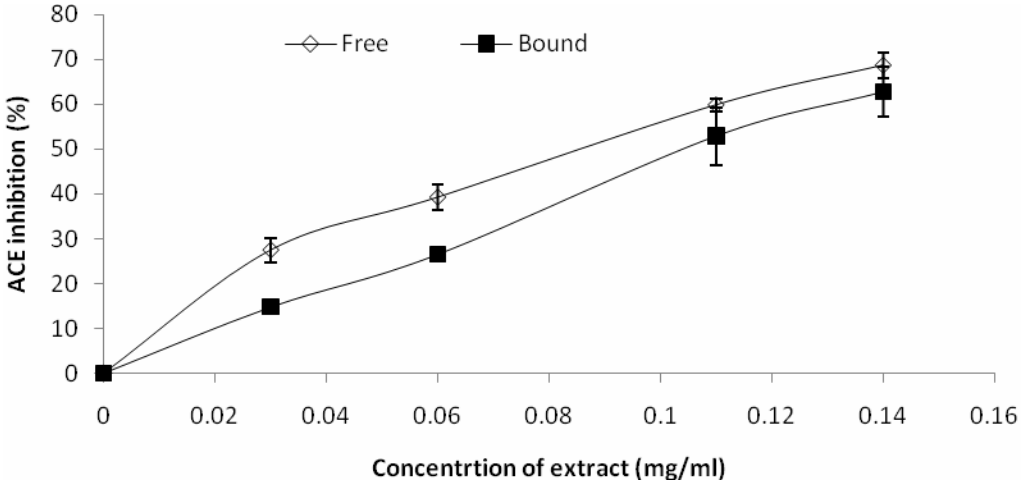

Figure 5. Angiotensin 1 converting enzyme inhibitory activity of free and bound phenolics from 'amala'

Furthermore, results of the flavonoid content of the 'amala' (Tab.1.) revealed that, the free soluble flavonoid content (80.07mg/100g) was higher than bound flavonoid content. The trend in the total flavonoid contents agreed with the total phenolic contents. This suggests that flavonoids in plantain fruits are more in their aglycone form rather than the esterified (bound) form. The presence of flavonoids such as quercetin and its 3-Ogalactoside, 3-O-glucoside, and 3-O-rhamnosyl glucoside has been found in unripe plantain (Ragasa *et al*., 2007) moreover, numerous studies have conclusively shown that the majority of the antioxidant activity may be from compounds such as flavonoids, anthocyanins, catechins and isocatechins, rather than from vitamins C, E and β-carotene (Oboh *et al*., 2007). Flavonoids have antioxidant activity and could therefore lower cellular oxidative stress (Oboh *et al*., 2007). Polyphenols are considered to be strong antioxidants due to the redox properties of their hydroxyl groups (Materska *et al*., 2005). It is also well known that phenolic compounds contribute to quality of food by modifying the colour, taste, aroma and flavor (Memmune *et al*., 2009). in addition, other constituent phenols in *Musa spp* are principally doparnine, catechin, chlorogenic acid, cinnamic acid, hydroxylbenzoic, Resorcinol, progallic acid, salicylic acid, ferulic acid, vanillin, coumurin, P-coumaric acid, phenols (Khalil *et al*., 2007) and interestingly, these phenolic compounds are also actively responsible for certain browning reactions that take place in the unripe plantain pulp on exposure to air (Ko *et al*., 2008) which had been revealed to have antioxidant properties (Yen and Tsai, 1993). Diabetogenic process is caused by immune destruction of the beta cells process and is also mediated by white cell production of reactive oxygen species (ROS). Alloxan and Streptozotocin induced animal models result in the production of active oxygen species which leads to diabetes even though the mechanism of action of these two drugs is different. However, scavengers of oxygen radicals are effective in preventing diabetes in these animal models (Oberley, 1988).

The protective ability of 'amala' against SNP and Fe^{2+} induced lipid peroxidation in rat pancreas in vitro is addressed in this study. As shown in Fig. 1. incubating the pancreas tissues in the presence of 7μM SNP caused a significant ($P < 0.05$) increase the lipid peroxidation (177.42%) in pancreas. Conversely, the 'amala' phenolic extracts caused a significant decrease ($P < 0.05$) in the SNP-induced lipid peroxidation in rat pancreas tissues in a dose dependent manner (Fig. 1) at the concentration range of 0.02–0.12 mg/mL. However, free soluble phenolic had higher inhibitory activities than bound phenolic. Sodium nitroprusside is an

antihypertensive drugs that has been implicated to cause cytotoxicity through the release of cyanide and/or nitric oxide (NO). NO is active universal neuronal messenger in the central nervous system that could cause neuronal damage in cooperation with other ROS. It could also be independent in action (Bates *et al*., 1990). Nevertheless, the interaction of 'amala' phenolics exhibited an inhibition of the prooxidant (SNP) which is an added fact to its inhibitory activity in the prevention or delay of peroxidation in pancreas tissues. Also, the incubation of rat pancreas in the presence of 25µM Fe^{2+} (induced) caused a significant increase ($P < 0.05$) in the MDA content (116.58%) of the pancreas (Fig.2). The transition metals, such as Fe, are major components of physiologically important enzymes and proteins; however, free Fe in the extracellular circulation has been shown to participate in the initiation of lipid peroxidation and oxidative stress (Oboh *et al*., 2007). The mechanism through which iron causes this deleterious effect is through Fe^{2+}-catalyzed hydrogen peroxide (H_2O_2) decomposition to produce OH via the Fenton reaction (Oboh *et al*., 2007). The introduction of the 'amala' phenolic extracts caused a significant ($P<.05$) inhibition in the MDA produced in the pancreas in vitro. However, soluble free phenolic caused a higher inhibition. The trend of these results followed that of the total phenol and total flavonoid contents. The possible mechanism through which the extracts protect the pancreas could be by Fe^{2+} chelation and the scavenging of OH (Oboh *et al*., 2007).

The interaction of 'amala' extracts with α-amylase, as shown in Fig. 3, revealed that both free and bound phenolic caused marked inhibition of pancreatic α-amylase activity in vitro. However, the free phenolic had higher inhibitory activities at the concentration range 0.2-0.8mg/mL tested when taking into account the EC_{50} values of the phenolic extracts (Tab. 1). Postprandial hyperglycemia could induce the nonenzymatic glycosylation of various proteins and biomolecules; resulting in the development of chronic complications. Therefore, control of postprandial plasma glucose levels is very fundermental in the early treatment of diabetes mellitus and in reducing chronic vascular complications (Oritz-Andrade *et al*., 2007). Inhibition of enzymes that take part in the degradation of carbohydrates such as α-amylase and α-glucosidase is one of the therapeutic approaches for managing and controlling hyperglycemia (Shim *et al*., 2003). The result of the inhibition of α-amylase by the free and bound phenolic also agreed with our earlier reports on the inhibitory effect of aqueous extracts of 'amala' flour on salivary α-amylase activity (Shodehinde and Oboh, 2009).

Fig. 4 depicts the interaction of the 'amala' phenolic with α-glucosidase activity. Both the free and bound phenolic exhibited strong α-glucosidase inhibitory ability at the concentration range of 0.2-0.8 mg/mL tested. However, free phenolic of the 'amala' exhibited stronger α-glucosidase inhibitory actions than the bound phenolic which is in agreement with the results of the EC_{50} value where its lower value indicates a stronger inhibitory activity (Tab. 1.). This is also in agreement with the results of the total phenol and total flavonoid of the 'amala' where the free soluble phenolic has higher antioxidants than the bound phenolic. Therefore, it could be suggested that the inhibition of α-amylase by unripe plantain product phenolic ('amala') could have justified the reason for its use in folklore for the management of diabetes.

In addition to the explanations of the activities of the α-amylase and α-glucosidase, the α-glucosidase inhibitory activities of both the free and bound phenolic extracts were significantly
($P < 0.05$) higher than the α-amylase inhibitory activities. This mild inhibition of α-amylase when compared to α-glucosidase is of great pharmaceutical importance in addressing some of the side effects associated with the antidiabetic drugs (such as Acarbose and Voglibose) presently used for the management of diabetes.

The interaction of the phenolics with ACE which revealed the antihypertensive potentials of the 'amala' phenolic extracts was presented in fig. 5. The result revealed that both extracts had high inhibitory effect on ACE activity in a dose-dependent manner; however, the free soluble phenolic had higher activity than the bound

phenolic. ACE cleaves angiotensin I to produce angiotensin II, which is a powerful vasoconstrictor that has been related to increase in blood pressure (Ahnfelt-Ronne, 1991). As a result of this, ACE inhibitors have been widely developed to prevent angiotensin II production in cardiovascular diseases, and utilized in clinical applications since its discovery in snake venom (Villar *et al*., 1986).

CONCLUSION

The high inhibitory effects of the free soluble phenolic, bound phenolic, the enzymatic and non enzymatic properties which had been revealed to possess antioxidant activities could have contributed in no small measure to the potency of the unripe plantain and its product ('amala') in the prevention and/or management of diabetes and hypertension. As a result of these findings their traditional use is valid. Besides, studies would still be carried out to further provide evidences of efficacy of the plant in human.

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