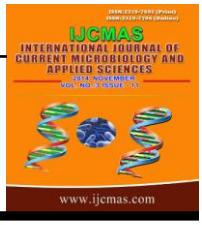
International Journal of Current Microbiology and Applied Sciences
ISSN: 2319-7706 **Volume 3 Number 11 (2014) pp. 964-976**
http://www.ijcmas.com



Original Research Article

Capacity of Boiled Green Plantain (*Musa paradisiaca*) on the enzymes linked with diabetes and hypertension in comparison with synthetic drugs *in Vitro*

S.A.Shodehinde[1]*, G.Oboh[2] and E.E.Nwanna[2]

[1]Department of Biochemistry, Adekunle Ajasin University, P.MB. 01, Akungba Akoko, Ondo State, Nigeria
[2]Functional Foods and Nutraceuticals Unit, Department of Biochemistry, Federal University of Technology, P.M.B. 704, Akure. Ondo State, Nigeria
**Corresponding author*

Keywords

Musa paradisiaca, diabetes, hypertension, angiotensin-I converting enzyme, arcabose**,** captopri

A B S T R A C T

This study was carried out to examine the in vitro interaction of phenolic extracts from boiled 'false horn' unripe plantain on enzymes tightly linked with type-2 diabetes(α-amylase andα-glucosidase) and hypertension [angiotensin-I converting enzyme (ACE)] in comparison with the synthetic drugs. For the extraction of soluble-free phenols, 50 g of the powdered plantain (boiled) was homogenized in 80% acetone (1:2, w/v)while the bound phenol was extracted from the alkaline and acid-hydrolyzed residue with ethyl acetate. The total phenol and total flavonoid were determined, the inhibitory effect of the extracts on sodium nitroprusside induced lipid peroxidation was assessed while the interaction of the phenolic extracts with the enzymes were also determined. The total phenol and total flavonoid contents of the free and bound phenol extracts ranged from (77.03-101.02) mgGAE/100g and (62.02 -87.05) mgQE/100g respectively. The extracts were able to inhibit the sodium nitroprusside induced lipid peroxidationin rat's pancreas in a dose dependent manner with the free phenol exhibiting a higher inhibitory activity. The extracts also displayed a potent inhibition of α-amylase, α-glucosidase and angiotensin-I converting enzyme. Moreover, the IC50 value of each enzyme in comparison with their corresponding standard control indicates that the phenolic extracts of the boiled unripe plantain can compete substantially with synthetic drugs.With these findings, it could be presented here that the revealed inhibitory activities could serve as the background for more specific consideration of the role of boiled green plantain in the diet of those with diabetes.

Introduction

The potentials of free radicals have been revealed to participate in a series of disease states that have degenerative effects on the cardiovascular system through either lipid peroxidation or vasoconstriction (Lachance causes over production of free radical thereby creating oxidative stress (Arango *et al*., 2000). An imbalance between the levels of prooxidants and antioxidants in the biological systems causes the production of

oxidative stress. The prolonged effect of this leads tocellular injury (Villa-Caballero *et al*., 2000). Also, excessive oxidative stress has been reported in the pathology and complications of diabetes mellitus (Wolff, 1993). The prevalence of diabetic complications poses a serious public health problem around the world and at a rate that has been characterized as an epidemic (Mokdad *et al*., 2001). This increase is related to an increased prevalence of type 2 diabetes mellitus, a disorder caused by a combination of insulin resistance and impaired insulin secretion. The condition of type 2 DM is found nearly in all countries, all racial, ethnic groups, across the age range from childhood to adolescence and to the elderly. Although clear risk associated with aging still persists (Kelly, 2003).

Moreover, there have been reported cases of increase in ACE activity in diabetic patients and animal models (Chu and Leung, 2007; Hosseini *et al*., 2007). The increase in ACE activity is believed to influence the development of hypertension in diabetes (Villa-Caballero*etal*., 2000). However, recent explosion of research in the pharmacology of food phytochemicals continues to establish that plant phenolic compounds have potent antioxidative effects (Middleton and Kandaswami, 1994; Rice-Evans *et al*., 1996). Oral hypoglycaemic agents such as the sulphonylureas and biguanides have been commonly used in the management of type 2 diabetes. Many of these available synthetic drugs produce discomforting side effects that include hematological, cutanecous, gastrointestinal reactions and disturbances in both liver and kidney (Arango *et al.,* 2000). In the search for new drugs and cures for human and animal pathological conditions, plants of medicinal properties arenow the most popular targets. Consequently, all parts of the plant body and their derived products have been used in one form or the other either traditionally or conventionally for medicinal purpose (Evans, 2008).

Plantain (*Musa paradisiaca* L) belongs to the Musaceae family which are evergreen tropical giant plants (Nwokocha and William, 2009). *Musa paradisiaca,* also known as plantain (English), ogede agbagba, apanda (Yoruba), ayaba (Hausa) and Ogadejioke (Igbo), is a tropical plant that is native to India (Gill, 1992). Plantains also serve as major food crops in the humid and sub-humid parts of Africa. The annual world production of plantain is estimated at 75 million tones (Asiedu *et al*., 1992). The plant consists of long, overlapping leafstalks and bears a stem which is 1.22 to 6.10 m high. The leaves grow to a length of 1.83 m and 0.61 m wide. The fruits grow in clusters, each separate plantain of the cluster being about 1 inch in diameter and somewhat longer than banana. Plantain fruits can be consumed as ripe or unripe in several forms; it is either boiled, fried, roasted, steamed, baked or grilled. Chips, flour and other products could be derived from plantain (Nwokocha and William, 2009).

Oboh and Erema, 2010 had earlier given reports on the glycemic indices of green plantain products.In support of what was reported above by Evans 2008, aside the fruits, other parts of the plant such as the leaves and roots have been used for medicinal purposes. Again, the leaf juice is used in the treatment of fresh wounds, cuts and insect bites while the leaves act as an arbortifacient. The sap of the plant is used as a remedy for diarrhea, dysentry, hysteria and epilepsy. A cold infusion of the root is used to treat veneral diseases and anaemia. In addition, plantain fruit has been reportedly used as antiscorbutic, aphrodisisac and diuretic (Gill, 1992).The first parts of this work explored the antidiabetic properties of

the aqueous extract and characterized boiled unripe plantain (Shodehinde and Oboh, 2012; Shodehinde and Oboh, 2013). The researched work on boiled unripe plantain was also extended to examining the effect of free and bound phenolic extracts on free radicals (Shodehinde and Oboh, 2014).

At the time of carrying out this present study, there arestill scarceinformation on the potential activities of free and bound phenolics of boiled unripe plantain vis-à-vis their interactions with the enzymes linked with type 2 diabetes (α-amylase and α-glucosidase) and hypertension(angiotensin-I converting enzyme). Expediency of this research has redirected our aim by comparing the inhibitory potentials of these enzymes with the synthetic drugs as well as its inhibitory effect on sodium nitroprusside induced lipid peroxidation.

Materials and Methods

Materials

Sample collection

Fresh samples of matured green plantain (*Musa paradisiaca*) were collected at Akure South Local Area. Ondo State. The plantain fruits were washed, peeled (8kg) and boiled in 5L of tap water, for twenty minutes at a temperature of 100^0C. The water was drained off and the boiled pulps were later sun-dried for about 3 weeks to a constant weight and ground into flour. The sample was kept in an air tight container 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, Iron (II) sulphate, H_2O_2,porcine pancreatic α-amylase (EC 3.2.1.1), α-glucosidase (EC 3.2.1.20), pnitrophenyl- α-D-glucopyranoside and ACE were products of sigma (Aldrich, USA). 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 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 boiled plantain flour was extracted with 80% acetone (1:5w/v) and filtered (filterpaper Whatmanno.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 extract was frozen, while the residues were kept for the extraction of bound phenols.

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 h with shaking. Then, the pH of the mixture adjusted to pH 2 with concentrated HCl and the bound phytochemicals were extracted with ethyl acetate (six times). The ethyl acetate fractions were then evaporated at 45^0C (Chu et al., 2002).

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–Ciocalteu's reagent (v/v) and neutralized by 2.0 ml of 7.5% sodium carbonate. The reaction mixture was incubated for 40 min at 45C and the absorbance was measured at 765 nm 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 method of Meda et al. (2005). The volume of 0.5 ml of sample/standard quercetin was mixed with 0.5 ml methanol, 50µl of 10% $AlCl_3$, 50µl 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 (JENWAY 6305). Total flavonoid content was calculated using quercetin as a standard.

Lipid peroxidation assay

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

Lipid peroxidation assay

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 approximately1200 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 3000 g to yield a pellet that was discarded and a low-speedsupernatant, which was kept for lipid peroxidation assay (Belle et al., 2004).

Lipid Peroxidation and Thiobarbituric Acid Reactions

The lipid peroxidation assay was carried out using the modified method of Ohkawa et al. (1979); 100ml S1 fraction was mixed with a reaction mixture containing 30ml of 0.1 M pH 7.4 Tris–HCl buffer, extract (0–100ml) and 30ml of 7mM freshly prepared sodium nitroprusside. The volume was made up to 300ml by water before incubation at 37^0C for 1hour. The color reaction was developed by adding 300ml 8.1% Sodium dodecyl sulfate to the reaction mixture containing S1;this was subsequently followed by the addition of 600ml of acetic acid/HCl (pH 3.4) mixture and 600ml 0.8% thiobarbituric acid. This mixture was incubated at 100^0C for 1 hour. Thiobarbituric acid reactive species produced were measured at 532 nm and the absorbance was compared with that of standard curve using malondialdehyde.

α-Amylase inhibition assay

Different concentrations of the plantain phenolic extracts(3.62 – 14.48 mg/mL) and

500 µl of 0.02Msodium phosphate buffer (pH 6.9 with 0.006 MNaCl) containing Hog pancreatic α-amylase (EC 3.2.1.1) (0.5mg/ml) were incubated at 25°C for 10min. Thereafter, 500µl of starch (1%) solution in 0.02Msodium phosphate buffer was added and the mixture was incubated at 25°C for 10min. The reaction mixture was stopped with 1.0 ml of dinitrosalicylic acid (DNSA) reagent and incubated thereafter at 100^{o}C for 5 minutes in water bath. After allowed to cool at room temperature, 10ml of distilled water was added and the absorbance was measured at 540nm. The α-amylase inhibitory activity of the plantain phenolic extracts was expressed as percentage inhibition while acarbose at different concentration (10, 20, 30 and 40 µg/mL) was used as standard control in line with experiment of Worthington (1993).

α-Glucosidase inhibition assay

The unripe plantain phenolic extracts at different concentration (3.62 – 14.48 mg/mL) and 100µl of α-glucosidase (EC 3.2.1.20) solution (1.0 U/ml) in 0.1M phosphate buffer (pH 6.9) was incubated at 25°C for 10min. Then, 50µl of 5 mM p-nitrophenyl-α-D-glucopyranoside solution in 0.1M phosphate buffer (pH 6.9) was added. The mixture was incubated at 25°C for 5minutes after which the absorbance was read in a spectrophotometer at 405nm. The assay was carried out in triplicate. The α-glucosidase inhibitory activity of the plantain phenolic extracts and acarbose (Standard control) at different concentration (10, 20, 30 and 40 µg/mL) were subsequently calculated and expressed as percentage inhibition (Apostolidis et al., 2007).

$$\% \text{ Inhibition} = [(Abs_{Control} - Abs_{Samples}) / Abs_{Control}] \times 100$$

Angiotensin I converting enzyme (ACE) inhibition assay

The inhibition of ACE activity by the unripe plantain phenolic extracts was measured as reported by Cushman and Cheung (1971). Different concentrations (0.8, 1.6, 3.2, and 4.0 mg/mL) of the extracts samples and 50 µl of rabbit lungs ACE (EC 3.4.15.1) solution (4 mU/ml) were preincubated at 37^{o}C for 15 min. Thereafter, enzymatic reaction was initiated by adding 150 µl of 8.33 mM of ACE substrate [hippuryl-L-histidyl-L-leucine (HHL)] in 125 mM Tris–HCl buffer (pH 8.3) to the reaction mixture and incubated at 37°C for 30 min. The reaction was halt by adding 250µL of 1M HCl. The hippuric acid (Bz-Gly) produced by the reaction was extracted with 1.5 mL ethyl acetate. The mixture was then centrifuged to separate the ethyl acetate layer, after which the 1 mL of the ethyl acetate layer was transferred to a clean test tube and evaporated to dryness. The residue was re-dissolved in distilled water and its absorbance was measured at 228 nm. The average value from three determinations of each concentration was used to calculate the ACE inhibition while Captopril (an ACE synthetic inhibitor) at concentration between 1.25 – 6.30 µg/mL was used as control. The calculation was expressed as percentage inhibition as follows:

$$\% \text{ Inhibition} = [(Abs_{Control} - Abs_{Samples})/Abs_{Control}] \times 100$$

Statistical Analysis

All experiments were performed in triplicate. Data are expressed as mean–standard deviation. Differences were evaluated by 1-way analysis of variance, followed by the Duncan multiple test (Zar, 1984). Findings were considered statistically significant when the P value was less than .05 (Microsoft Excel 2010; Redmond, WA, USA).

Results and Discussion

The results of the evaluation of free soluble and bound phenolic extracts of the boiled unripe plantain are presented in Table 1. The free soluble extract contained 101.02 mg/100g of total phenol while the bound phenol extract contained 87.05mg/100g. The total phenol content of the free phenol was observed to be significantly higher ($P<0.05$) than the bound phenol. The total flavonoid contents of the free and bound phenolic extracts of the boiled unripe plantain are also presented in Table 1. The free soluble extract contained 77.03mg/100g while the bound phenol contained 62.02 mg/100g. Following the trend in the total phenol content, the free soluble phenol extract also have significantly higher ($P<0.05$) total flavonoid content than the bound phenol extract.

The ability of the boiled unripe plantain to protect against lipid peroxidation was carried out. The study revealed that there was a significant increase in the level of lipid peroxidation in the rat's pancreas tissue when it was incubated in the presence of 7μM of sodium nitroprusside. Conversely, the free soluble and bound phenol extracts of boiled unripe plantain caused a significant ($p<0.05$) reduction in the SNP-induced lipid peroxidation in rat pancreas tissues in a dose dependent manner (Fig. 1). However, free soluble phenolic had higher inhibitory potentials than bound phenol.

Figure 2 revealed the interaction of boiled unripe plantain with α-amylase. As shown, both free and bound phenol extracts caused a marked inhibition of pancreatic α-amylase activity in vitro. The same pattern of result was also exhibited by the α-glucosidase activity (Figure 3)in which both the free and bound phenol extracts inhibited the glucosidase activities. However, the free soluble phenol had a higher ($p<0.05$) inhibitory effect than the bound phenol taking into account the IC_{50} values of the phenolic extracts (Table 2). Also, the results show that the inhibitory activities displayed by the phenolic extracts are very close to that of the arcabose (a commercial drug).

The interaction of the free soluble and bound phenolics with ACE which revealed the antihypertensive potentials of the boiled unripe plantain phenolic extracts was presented inFigure 4. The result also revealed that both extracts had high inhibitory effect on ACE activity in a dose dependent manner; however, the free soluble phenol had higher ($p < 0.05$) activity than the bound phenol. Considering the IC_{50} values (Table 2), the inhibitory activities of the phenolic extracts are also following that of the captopril (blood pressure regulating) drug.

Plants of medicinal value have been playing a vital roleon the health and healing of man since the beginning ofhuman civilization. Despite the huge success recorded in the area of allopathic medicinesin the last century, the use of plants still remains oneof the major sources of drugs in modern aswell as in traditional system of medicine (Khalil *et al*., 2007). This eventually continues to increase the upsurge in demand of plant materials containing phenolicsas they retard oxidative degradation of lipidsand thereby improving quality and nutritionalvalue of food (Khalil *et al*., 2007). This results of total phenol of the free and bound phenolic extracts of the boiled unripe plantain go in line with the earlier reports by Chu *et al*., (2002) on the phenolic content in some common vegetables and with that of Sun *et al*., (2002) reported for the phenolic content in some commonly consumed fruits, in that they have more free soluble phenolic contents than the bound phenolic.

The existence of free radicals and oxidants have so far been reported to form part of normal human metabolism but when produced in excess they can cause tissue injury. Based on research, tissue injury can itself cause ROS generation by causing activation of phagocytes or releasing transition metal ions from damaged cell which may (or may not under normal situation) contribute to the worsening of the injury (Sun *et al*., 2002).

Phenols are antioxidants with the strong capability to remove free radicals, chelate metal catalysts, activate antioxidant enzymes, reduce α-tocopherol radicals and inhibit oxidases (Amic *et al*., 2003). However, free soluble phenolics are more readily absorbed and can easily render their beneficial bioactivities upon early digestion. On the other hand, the significance of bound phytochemicals to human health is still understudy (Sun *et al*., 2002), there is possibility that different plant foods with different amounts of bound phytochemicals 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 making it possible for them to survive stomach and small intestine digestion to reach the colon where they are digested by bacteria flora to release phytochemicals locally to exert health benefits (Sun *et al*., 2002; Chu *et al*., 2002).

Furthermore, the result of the boiled unripe plantain,is in agreement with the findings of Ademiluyi *et al*., (2009) on clove bud. Long before now has flavonoidscontinuously been identified as polyphenolic compounds with strong antioxidant properties, which occur ubiquitously in plants (Nair and Nagar, 1997). Hence, it could be suggested that their profound beneficial existence can be continuously tapped to enlarge the horizon that could provide a means of reducing the high number of degenerating diseases that stalks the world.

Lipid peroxidation is an important parameter of oxidative stress. It is also regarded as important in vivo and for the stability of the processed food. This is because they contribute to the development of cardiovascular diseases such as pre-eclampsia and atherosclerosis. The end products of lipid peroxidation lead to the production of cytotoxic aldehydes such as malondialdehyde (MDA) and 4-hydroxynonenal (HNE). These end products cause damage to proteins and to DNA. The incubation of the pancreas tissuesin the presence of 7μM SNP caused a significant($p < 0.05$) increase of lipid peroxidation in pancreas but was conversely decreased in the presence of free soluble phenolic and bound phenolic. The phenolic content of boiled unripe plantain can be claimed to compare favourably with those found in other plants (Chu *et al*., 2002). This was revealed in one of our published work in which some phenolic contents in unripe plantain products such as raw, boiled, roasted and elastic pastry were quantified using gas chromatography (Shodehinde and Oboh, 2013).

The inhibition of enzymes linked with the metabolism of carbohydrates such as α-amylase and α-glucosidase (Figure 2 and 3 respectively) is one of the therapeutic approaches for the management and control of hyperglycemia (Shim *et al*., 2003). Although the mechanisms involved in the hypoglycemic effects of many plant foods have not been fully documented. However, there are speculations that phenolics might be responsible for the inhibitory activities of α-amylase and α-glucosidase. These activities of α-amylase and α-glucosidase have been linked to chemical structures such as the unsaturated C ring, 3-OH, 4-CO, the

linkage of the B ring at the hydroxyl substitution on the B ring of flavonoid (Li *et al*., 2009). Further results have also revealed that flavonoid could exert a better protection by preventing progressive impairment of pancreatic beta-cell function due to oxidative stress thus forestalling the occurrence of non insulin dependence diabetes mellitus (NIDDM). This could be achieved by reducing the level of lipid, glycosylated hemoglobin and postprandial blood glucose as well as increase insulin sensitivity when compare with the effect of some commercial drugs like arcabose and viglibose (Li *et al*., 2009).

Angiotensin –I converting enzyme (ACE, E.C.3.4.15.1 carboxyl terminal dipeptidyl exopeptidase) is synthesized in epithelial and endothelial cells that are found in organs such as kidney and lung and blood vessels. Reported work on ACE has revealed that ACE has fundamental roles in blood pressure regulation and hypertension. Angiotensin-I converting enzyme (ACE) continues to attract attention as an important enzyme that maintains vascular tension by two different catalyzed reactions which are conversion of the inactive angiotensin I into angiotensin II (a vasoconstrictor) and inactivation of the bradykinin (a vasodilator) which participates in the lowering blood pressure (Johnston and Franz, 1992).Observations on changes in ACE activity levels have been reported to have effects on actions of the renin-angiotensin-aldosterone system (Welsch *et al*., 1989) while the effects of the renin-angiotensin system involvement have been demonstrated at both local and systemic level in diabetic complications which are derived from events taking place in blood vessels (Wautier*et al*., 2001).Although it is well documented by several studies that diabetic complications may rise due to several factors. Recent studies indicate that most diabetic complications are vascular-originated which consequentially graduated to a more serious complications that is responsible for high morbidity andmortality in diabetic patients. Recent morphological studies in patients with Type 2 diabetes have been verified that the presence of serum ACE activity increases with diabetes even in streptozotocin-induced diabetic rats. On the other hand Clinical trials have shown that antihypertensive treatment that include the use of ACE inhibitor (captopril) or beta-blocker (antenolol) decrease blood pressure in type II DM patients, both drugs were shown to be similarly effective in reducing diabetic complications (Heidenreichet al., 1997).Aside this information, it also supplies that there is an interrelation between diabetes and hypertension.

An extended research into the area of phytomedicine has further widened the scope that brings remedy to the cure and management of diabetes as well as its complications. Suggestions that oxidative stress play an important role in human disease have led to the proposal that health might be improved by increased dietary intake of antioxidants (Li *et al*., 2009).

Many studies have reported the beneficial effects of polyphenol-rich extracts on the starch hydrolyzing enzymes (by ultimately slowing glucose release from starch) (Li *et al*., 2009) and hypertension (by retarding the development of hypertension and 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). Our finding eventually corresponds with earlier reports in which plants that are rich in polyphenols can be used in the management of diabetes (Kwon et al., 2006) and vascular oriented diseases (Dzomeku *et al*., 2007).

Table.1 Total phenol and flavonoid contents of free and bound phenolic extracts from boiled green plantain

Samples	Total phenol (mgGAE/100g)	Total flavonoid (mgQE/100g)
Free	101.02±1.1[a]	87.05±0.01[a]
Bound	77.03±2.1[b]	62.02±0.02[b]

Values represent mean ± standard deviation (n = 3).

Values with the same superscript number on the same column are not significantly (P<0.05) different

Table.2 Values for theIC_{50} of enzymes inhibitory activities in comparison with the standard drugs (mg/ml)

	Free	Bound	Standard drugs
α-Amylase/Arcabose	0.41±0.01[c]	0.48±0.03[b]	1.86±0.33[a]
α-Glucosidase/Arcabose	0.44±0.02[c]	0.53±0.03[b]	2.07±0.09[a]
ACE/Captopril	0.08±0.01[c]	0.13±0.02[b]	1.73±0.05[a]

Values with the same superscript number on the same row are not significantly (P<0.05) different

*ACE= Angiotensin-1- Converting enzyme

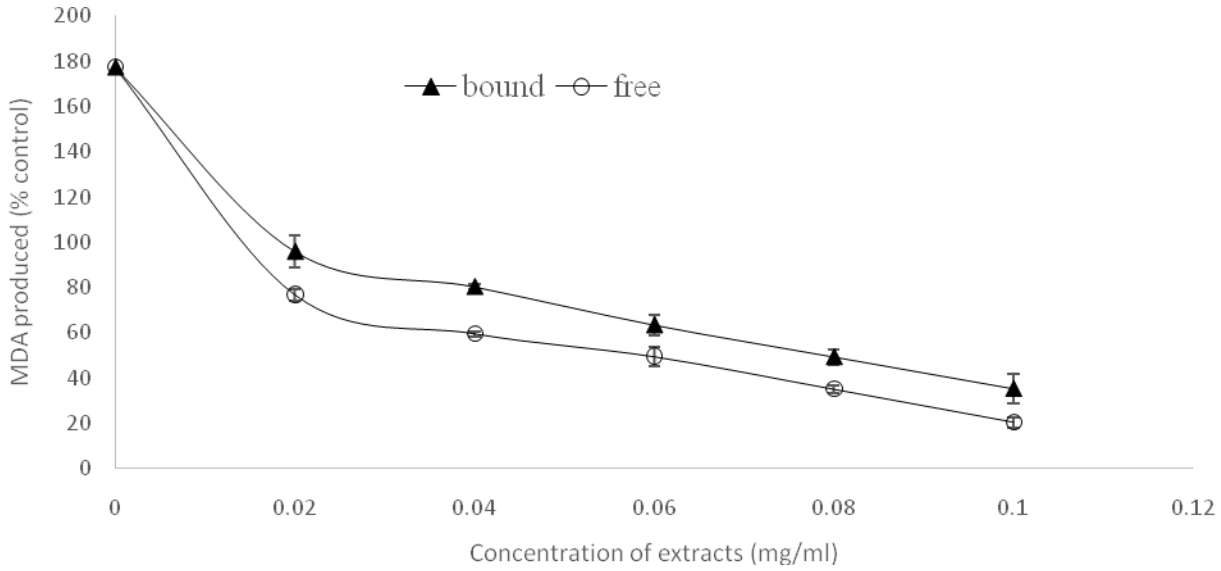


Figure.1 Inhibition of Sodium Nitroprusside induced lipid peroxidation of pancreas by free and bound phenols of boiled green plantain.
Values represent mean ± standard deviation, n = 3.

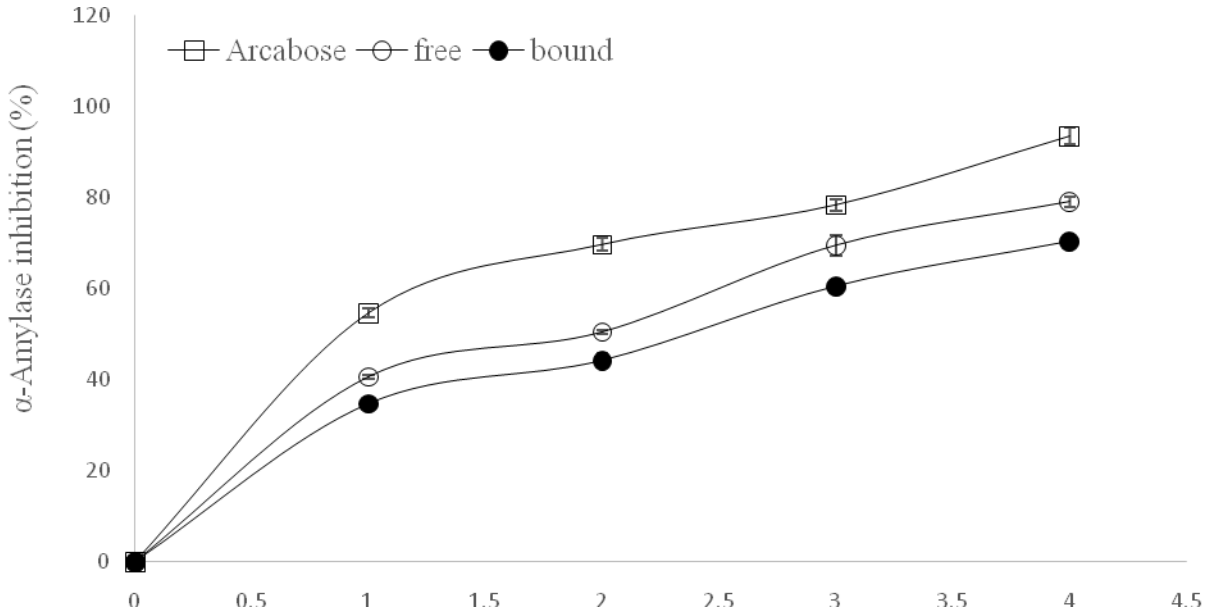


Figure.2 α-Amylase inhibitory activities of Free and bound phenolic extracts of boiled unripe Plantain in comparison with arcabose drug as the standard control
Values represent mean ± standard deviation, *n* = 3.

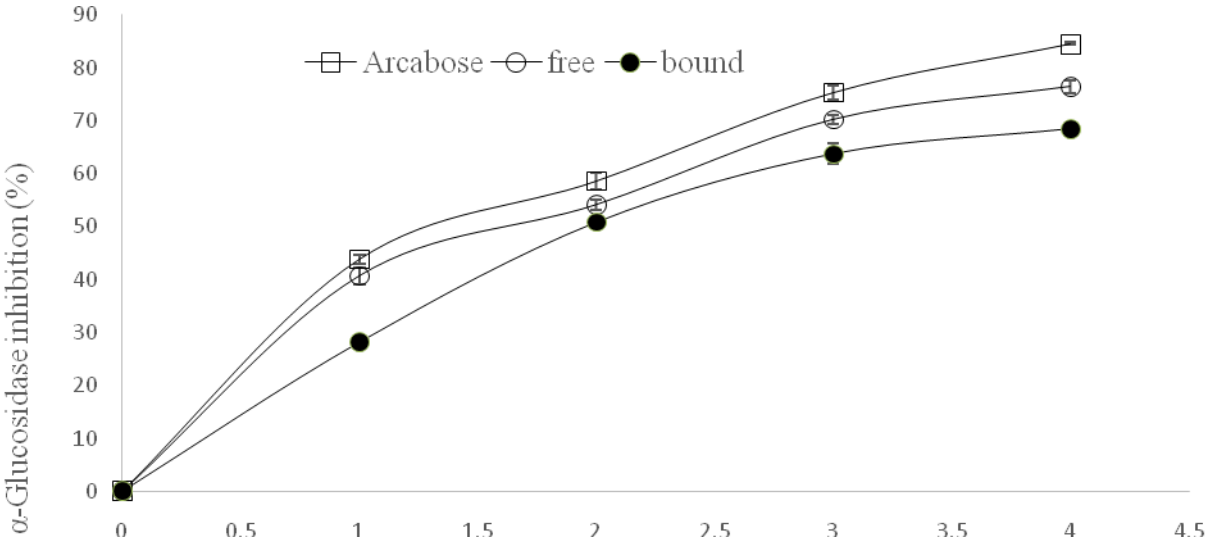


Figure.3 α-Glucosidase inhibitory activities of Free and bound phenolic extracts of boiled unripe Plantain in comparison with arcabose drug as the standard control
Values represent mean ± standard deviation, *n* = 3

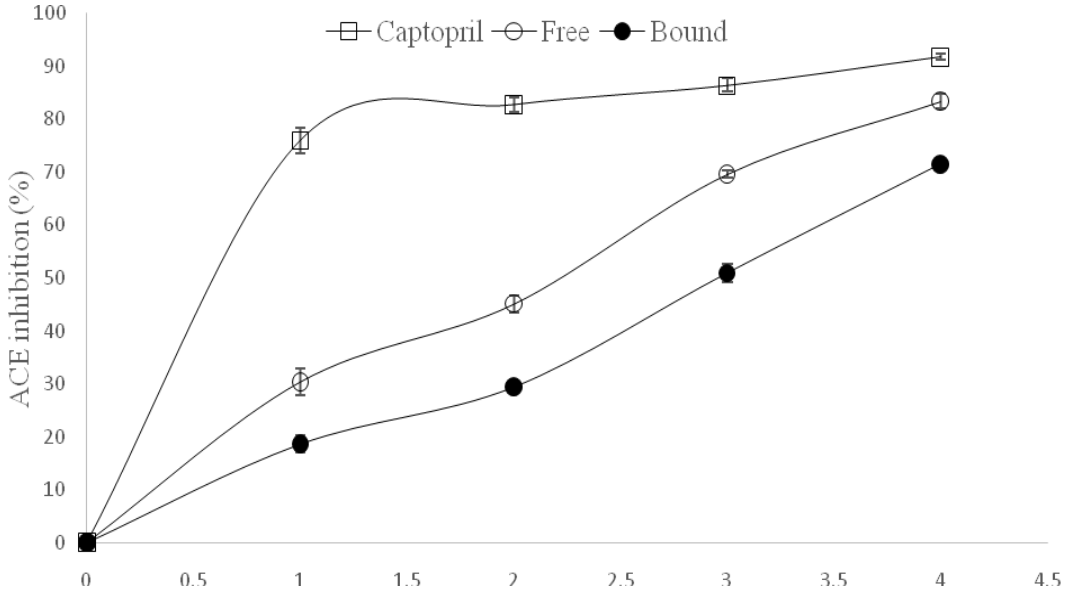


Figure.4 Angiotensin- I converting enzyme ACE) inhibitory activities of Free and bound phenolic extracts of boiled unripe plantain in comparison with captopril drug as the standard control.
Values represent mean ± standard deviation, *n* = 3.

References

AI-Haroni M and Skaug N. (2007) Incidence of antibiotic prescribing in dental practice in Norway and its contribution to national consumption. J. Antimicrob. Chemother., 59: 1161-1166,

Aqueveque P, Becerra J, Palfner G, Silva M, Alarcon J, Anke T, Sterner O. (2006) Antimicrobial activity of metabolites from mycelial cultures of Chilean basidiomycetes. J. Chil. Chi. Soc., 51: 1057-1060.

Autio, JT. (2002) Effect of xylitol chewing gum on salivary *Streptococcus mutans* in preschool children. ASDC J Dent Child; 69(1):81–6.

Bakri, I. M and Dougles, C. W (2005) Inhibition effect of garlic on oral bacteria, Arch. Oral. Biol. 50: 645-51.

Bauer AW, Kirby WMM, Sherris JC and Turck M(1966) Antibiotic susceptibility testing by a standardized single disk method. Am. *J. Clin. Pathol*., 45, 493-496.

Bratthall D. (1997) Discovery! A *Streptococcus mutans* safari! J Dent Res 76: 1332-1336.

Buchannan RE and Gibbon ME (1974) Bergey's Manual of Determinative Bacteriology (8th ed) Williams and Wilkins Baltimore, USA, pp. 450-860

Chany Y.C, Huang F. M, Tai K.W and Chou, M. Y (2001) The effect of sodium hypochlorite and chlorhexidine on cultured human periodontal ligament cells. Oral Surg. Oral Med. Oral Patho. Oral Radial Endod. 92: 446-4-80.

Cheesbrough M (2000) District Laboratory Practice in Tropical country (PT 2). University Press, Cambridge, pp. 157-255.

Chowdhury AK, Ahsan M, Islam S N and Ahmed Z U (1991) Efficacy of aqueous extract of garlic and allicin in experimental shigellosis in rabbits. Indian J. Med. Res. 93: 33-36.

David B. U, Linda O. O and Charles O. E. (2011) Isolation, Characterization and Antibiotic Susceptibility Studies of Clinical Isolates of *teptocococcus mutans* Obtained from Patients Visiting Major Dental Clinics in Nsukka, Nigeria . AJPSP 2:1, 1-15.

Ellmore G. S and Feldberg R. S (1994) Alliin lyase localization in bundle sheaths of garlic cloves (*Allium sativum*) Am. J. Bot. 81: 89- 94.

Fani M.M, Kohanteb J. and Dayaghi M. (2007) Inhibitory activity of garlic (*Allium sativum*) extract on muiltdrug resistant Streptococcus mutans. J. Indian Soc. Pedod Prevent Dent. 164-168.

Fang F, Li H, Cui W and Dong Y (1999) Treatment of hepatitis caused by cytomegalovirus with allitridin injection, an experimental study. J. Tougji Med. Univ, 19: 271-274.

Farzaneh H; , Afsoon A. and Fariba S. (2013) Antibacterial Activity of *Pistacia atlantica* extracts on *Streptococcus mutans* biofilm. *International Research Journal of Biological Sciences*. 2 (2): 1-7

Featherstone JDB (2008) Dental caries: a dynamic disease process. Aust Dent J 53: 286-291.

Fitzgerald RJ, Keyes PH. (1960) Demonstration of the etiologic role of streptococci in experimental caries in the hamster. J Am Dent Assoc 61: 9-19.

Forbes BA, Sahm DF and Weissfeld AS (1998)Laboratory methods for detection of antibacterial resistance. In: Roche J, Parker SJ, McAdam L, editors Baily and Scott's diagnostic microbiology, 10th Ed, Mosby Inc., St Louis, 250-272.

Grönroos L, Mättö J, Saarela M, Luoma A-R, Luoma H, Jousimies-Somer H, Pyhälä L, Asikainen S, Alaluusua S. (1995) Chlorhexidine susceptibilities of mutans streptococcal serotypes and ribotypes. Antimicrob Agents Chemother, 39: 894-898.

Hasan S, Singh K, Danisuddin M, Verma PK, Khan AU (2014) Inhibition of Major Virulence Pathways of *Streptococcus mutans* by Quercitrin and Deoxynojirimycin: A Synergistic Approach of Infection Control. j. pone.0091736

Jain R.(1998) antitubercular activity of garlic oil. Indian J. Pathol. Microbial. 43: 3045.

Johnson NW. (1991) Introduction: the nature of the caries process and the need for markers of risk. In: Risk markers for oral diseases. vol. I. Dental caries. Markers of high and low risk groups and individuals. Johnson NW, ed. Cambridge University Press: Cambridge ,1-12.

Knuuttila ML and Makinen K. (1975) Effect of xylitol on the growth and metabolism of Streptococcus mutans. Caries Res, 9(3):177–89.

Kuramitsu HK (1993) Virulence factors of mutans streptococci: role of molecular genetics. Crit Rev Oral Biol Med 4: 159–76.

Lee SS, Zhang W and Li Y. (2004)The antimicrobial potential of 14 natural herbal dentrifices : Results of an *in vitro* diffusion method study. J. Am. Dent. Assoc., 135, 1133- 1141.

Loesche WJ.(1986) Role of *Streptococcus mutans* in human dental decay. Microbiol Rev 50: 353-380.

Makinen KK and Isokangas, P.(1988) Relationship between carbohydrate sweeteners and oral diseases. Prog Food NutrSci 12(1):73–109.

Makinen K.K.(1985) New biochemical aspects of sweeteners. Int Dent J. 35(1):23–35.

Miller-Torbert TA, Sharma S, Holt RG

(2008) Inactivation of a gene for a fibronectin-binding protein of the oral bacterium *Streptococcus mutans* partially impairs its adherence to fibronectin. Microb Pathog 45: 53–9. doi: 10.1016/j.micpath.2008.02.001

Nakano K, Nomura R, Nemoto H, Mukai T, Yoshioka H, *et al.* (2007) Detection of novel serotype k *Streptococcus mutans* in infective endocarditis patients. J Med Microbiol56: 1413–5. doi: 10.1099/jmm.0.47335-0

Natagta E, Okayama H, Ito H, Inoue M. and Oha T. (2006) Serotype- specific polysaccharide of Sterptococcus mutans contributes to infectivity in endocarditis. Oral Microbial Immunol. 21: 420- 423.

National Committee for clinical Laboratory Standard (1995) methods for dilution antimicrobial tests for bacteria that grow aerobically. Standard M7-A3. NCCLS .Villanova.

Ross Z. M, O'Gara E. A, Hill D. J, Sleigholme H. V and Maslin D. J. (2001) Antimicrobial properties of garlic oil against human enteric bacteria. Evaluation of methodologies and comparisons with garlic oil sulfides and garlic powder. Appl. Environ. Microbial. 67: 475-480.

Scheinin A and Makinen KK.(1976) Turku sugar studies. An overview. Acta Odontol Scand;34(6):405–8.

Selwitz RH, Ismail A and Pitts NB. (2007) Dental caries. Lancet 369: 51- 59.

Sina K, Markus A, Bettina S, Annette A, Elmar H, and Ali A (2014) Antimicrobial Effects of Dental Luting Glass Ionomer Cements on Streptococcus mutans. The Scientific World J. Volume 2014, Article ID 807086, 7.

Sivam G. P, Lampe J. W, Ulness B, Swanzy S.R and Potter J. D. (1997) Helicobacter pylori in vitro susceptibility to garlic (Allium sativum) extract. Nutr. Cancer. 27: 118-121.

Slots J.(1992) Contemporary Oral Microbiology and Immunology Robert. W.R. and Emma, C. eds London

Sung H L , Bong K. C and Young J. K (2012) The cariogenic characters of xylitol-resistant and xylitol-sensitive Streptococcus mutans in biofilm formation with salivary bacteria, arch of oral boil. 57: 697-703.

Tanzer J. M, Livingston J. and Thompson A.M (2001) The microbiology of primary dental caries in human. J. Dent. Educ. 65: 1028-37.

Tsao SM, Hsu CC and Yin MC (2003) Gralic extract and two dially sulfide inhibit methicillin resistant *Staphylococcus aureus* infections in BALB/cA mice. J. Antimicrobial chemother. 52: 974-980.

Tsao S. M. and Yin M. C. (2001) In vitro antimicrobial activity of four daily sulfides occurring naturally in garlic and Chinese leek oil. J. Med. Microbial. 50: 646-649.

Twetman S and Stecksen-Blicks C.(2003) Effect of xylitol-containing chewing gums on lactic acid production in dental plaque from caries active pre-school children. Oral Health Prev. Dent;1(3):195–9.

Vadeboncoeur C, Trahan L, Mouton C, Mayrand D. (1983)Effect of xylitol on the growth and glycolysis of acidogenic oral bacteria. J Dent Res, 62(8):882–4.

Weber ND, Aderson DO, North JA, Murray BK, Lawson, LD and Hughes BG (1992) In vitro virucidal effects of Allium sativum garlic extract and compound. Planta Med. 58: 417-23.