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*Full Length Research Paper*

# A Study of the antioxidant activity, proximate composition and lipid peroxidation inhibitory activities of unripe false horn plantain products extracts *in-vitro*

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**This study sought to assess the proximate composition, antioxidant and inhibitory effect of unripe false horn plantain (*Musa paradisiaca*) products on $Fe^{2+}$ and sodium nitroprusside (SNP) induced lipid peroxidation in rat's pancreas. The $EC_{50}$ of $Fe^{2+}$ chelating, 2,2-diphenyl-1picrylhydrazyl (DPPH) and OH free radical scavenging ability were also determined. A portion of the unripe false horn plantain pulps were roasted to form a local meal called 'boli' while the other was boiled in water. Thereafter, each portion was sundried and milled into flour. The aqueous extracts of roasted flour (Rf) and boiled flour (Bf) samples were prepared (1 g/20 mL). Proximate composition showed that the Rf had higher ash and fibre content while the Bf had higher carbohydrate, fat, protein and moisture content. The Bf had higher phenolic contents, reducing power, DPPH and OH free radical scavenging ability. The Rf flour had higher vitamin C content, 2,2 -azino-bis(3-ethylbenzothiazoline-6-sulfonic acid) (ABTS), and $Fe^{2+}$ chelating ability. Bf had higher inhibitory effect on both the $Fe^{2+}$ and SNP induced lipid peroxidation in pancreas at the concentration tested (1.25 to 6.25 mg/mL). Their strong inhibitory activities, coupled with the antioxidant properties suggest that they could be a cheap source of natural antioxidants and also a means of restoring antioxidant status.**

**Key words:** Antioxidant capacity, *Musa paradisiaca*, lipid peroxidation, oxidative stress, 2,2-diphenyl-1picrylhydrazyl (DPPH).

## INTRODUCTION

Oxidative stress plays a major part in the development of chronic and degenerative ailments such as cancer, autoimmune disorders, rheumatoid arthritis, cataract, aging, cardiovascular and neurodegenerative diseases (Pham-Huy et al., 2008). When cells use oxygen to generate energy free radicals are generated. These by-products are generally described as reactive oxygen species (ROS) which include super oxide anion, hydroxyl radical and hydrogen peroxide that result from the cellular redox process. Although, at low concentrations, ROS exert beneficial effects on cellular responses and immune function but at high levels, free radicals and oxidants generate oxidative stress, which is a deleterious process that can damage cell structures, including lipids, proteins, and deoxyribonucleic acid (DNA) (Pham-Huy et al., 2008). In particular, ROS for example, superoxide radical, hydroxyl radical, and hydrogen peroxide, are important factors in the etiology of several pathological conditions such as cellular degeneration related to cardiovascular disease, diabetes, inflammatory diseases, cancer, Alzheimer's disease, and Parkinson's disease (Clayson et al., 1994).

Recent findings have revealed that antioxidants act as free radical scavengers by preventing and repairing damages caused by ROS, and therefore can enhance the immune defense and lower the risk of degenerative diseases (Pham-Huy et al., 2008). SNP is an

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antihypertensive drug, which acts by relaxing smooth vascular muscle; consequently it dilates peripheral arteries and veins (Halliwell and Gutteridge, 1999). However, SNP has been implicated to cause cytotoxicity through the release of cyanide and/or nitric oxide (NO) and that NO, is involved in the pathophysiology of such disorders as stroke, trauma, seizure disorders, etc. NO could act independently or in cooperation with other ROS (Oboh and Rocha, 2007). Iron has also been implicated as the most important pro-oxidant of lipids. Earlier report has revealed that $Fe^{2+}$ accelerates lipid peroxidation by breaking down hydrogen and lipid peroxides formed by the Fenton free radical reaction (Yamaguchi et al., 2000). Peoples of all ages in both developing and undeveloped countries use plants in an attempt to cure various diseases and to get relief from physical sufferings (Edward, 1956). This is because phenolic compounds are an important group of secondary metabolites, which are synthesized by plants because of plant adaptation to biotic and abiotic stress conditions (infection, wounding, water stress, cold stress and high visible light).

Protective phenylpropanoid metabolism in plants has been well documented (Douglas, 1996). Plantain belongs to the Musacace 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 is a staple food crop in West Africa where its starchy fruits are generally cooked or fried before consumption (Lii et al., 1982). 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). The proximate content, functional characteristics and properties of starch of ripe and unripe plantains have been evaluated (Izunfuo and Omuaru, 2006). Since free radicals have been implicated to be responsible for many metabolic disorders and diseases, this study is designed to further explore the antioxidant activity, proximate composition and also assess the lipid peroxidation inhibitory activities of unripe false horn plantain products extracts *in-vitro*.

## MATERIALS AND METHODS

### Plant materials

Fresh mature unripe false horn plantains were bought at a local market at Owena in Ondo State. Authentications of these plantains were carried out at the Department of Biology, Federal University of Technology, Akure, Nigeria.

### Chemicals and equipment

Folin-Ciocalteu's phenol reagent, gallic acid and anhydrous sodium carbonate used were products of Fluka (Buchs, Switzerland). Quercetin and DPPH, ascorbic acid and starch were products of Merck (Darmstadt, Germany), Iron (II) sulphate, $H_2O_2$, ACE and ABTS were products of Sigma (Aldrich, USA). Iron (III) chloride 6-hydrate and trichloroacetic acid are Fisher products. All other chemicals used were purchased from Rovet Scientific Limited, Benin City, Edo State, Nigeria. The distilled water used was obtained from the Chemistry Department at Federal University of Technology, Akure. Optical absorbance was measured with a ultraviolet (UV)-Visible spectrophotometer (Model 6305; Jenway, Barloworld Scientific, Dunmow, United Kingdom).

### Preparations of samples

#### *Boiled flour (Bf)*

Fresh plantain pulps were washed and peeled. 8 kg quantity was cut (10 mm long) and boiled in 5 L of tap water, for 20 min at a temperature of 100°C.The water was drained off and the boiled plantains 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.

#### *Roasted flour (Rf)*

Fresh plantain pulps were roasted using the Nigerian traditional method. This is done by putting the plantains on wire gauze over red hot charcoal. The roasting was carried out by frequently turning the plantains to maintain even browning. Roasting was done for 10 min. The roasted plantains were also later sun-dried for about 3 weeks to a constant weight, and ground into flour. The sample was also kept in an air tight container for future analysis.

### Proximate composition of unripe false horn plantain flours

The roasted and boiled flour samples were analyzed for moisture content, ash, crude protein, fat and crude fiber by the AOAC (1995) methods. Carbohydrate was determined by difference. The values reported are means of triplicate samples with their standard deviations.

### Aqueous extract preparation

10 g of each milled sample (roasted and boiled flour) was soaked in 100 ml of distilled water for about 24 h. The mixture was filtered. In a situation where the filtrate appeared to be very cloudy, the filtrate was centrifuged to obtain a clear supernatant liquid, which was subsequently used for the various assays (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 of extracts was determined according to the method of Singleton et al. (1999). Each sample extract was oxidized with 2.5 mL 10% Folin-Ciocalteau's reagent (v/v) for 5 min 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 (JENWAY 6305). The total phenol content was subsequently calculated as gallic acid

equivalent.

**Determination of total flavonoid content**

The total flavonoid content of the plantain extracts was determined using a slightly modified method of Meda et al. (2005). Briefly, a volume of 0.5 mL of each sample extract 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 distilled water, 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.

**Determination of vitamin C content**

Vitamin C content of the unripe plantain extracts was determined using the method of Benderitter et al. (1998). A volume of 75 µL DNPH (2 g dinitrophenyl hydrazine, 230 mg thiourea and 270 mg $CuSO_4 \cdot 5H_2O$ in 100 mL of 5 M $H_2SO_4$) was added to 500 µL reaction mixture (300 µL of appropriate dilution of the extracts with 100 µL 13.3% trichloroacetic acid (TCA) and distilled 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 (JENWAY 6305). The vitamin C content of the extracts was subsequently calculated. ABTS radical scavenging ability. The $ABTS^{\cdot+}$ radical scavenging ability of both extracts was determined according to the method described by Re et al. (1999). The $ABTS^{\cdot+}$ radical was generated by reacting an (7 mmol/l) ABTS aqueous solution with $K_2S_2O_8$ (2.45 mmol/l, final concentration) in the dark for 16 h and adjusting the Abs 734 nm to 0.700 with ethanol. 0.2 ml of each sample extract was added to 2.0 ml $ABTS^{\cdot+}$ solution and the absorbance was measured at 734 nm using (JENWAY 6305) after 15 mins The trolox equivalent antioxidant capacity (TEAC) was subsequently calculated. During the $ABTS^{\cdot+}$ reaction, antioxidants transfer an hydrogen atom to radical cation and causes discoloration of the solution (Vincenzo et al.,1999).

**DPPH radical scavenging ability**

The free radical-scavenging ability of the extracts against DPPH free radical was measured by measuring the decrease in absorbance of methanolic DPPH solution at 517 nm in the presence of each sample extract as described by Gyamfi et al. (1999). Briefly, 1 mL of different concentrations (400, 300, 200 and 100 L) of extracts were added to 1 mL of 0.4 mM methanolic solution containing DPPH radicals. The mixture was left in the dark for 30 min and the absorbance was measured at 516 nm in the spectrophotometer using (JENWAY 6305). 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). The ability of the sample to scavenge was calculated relative to the control using the formula (Krings and Berger, 2001):

$$\% \text{ disappearance} = [(A_{control} - A_{sample}) / A_{control}] \times 100\%$$

**$Fe^{2+}$ chelation assay**

The $Fe^{2+}$ chelating ability of the extracts was determined using a modified method of Minotti and Aust (1987) with a slight modification by Puntel et al. (2005). Freshly prepared 500 $molL^{-1}$ $FeSO_4$ (150 L) was added to a reaction mixture containing 168 l of 0.1mol $L^{-1}$ Tris-HCl (pH 7.4), 218 L saline and the extracts (0 to 100 L). The reaction mixture was incubated for 5 min, before the addition of 13 l of 0.25% 1,10-phenanthroline (w/v).The absorbance was subsequently measured at 510 nm in a spectrophotometer (JENWAY 6305). The $Fe^{2+}$ chelating ability was subsequently calculated with respect to the control.

$$\text{Percentage } Fe^{2+} \text{ chelating ability (\%)} = \frac{\text{Absorbance of control} - \text{Absorbance of test sample}}{\text{Absorbance of control}} \times 100$$

$Fe^{2+}$ 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 color reduction allows determination of metal chelating activity as well as the estimation of the chelating activity of the coexisting chelator (Yamaguchi et al., 2000).

**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 to 100 L) 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% TCA; this was followed by the addition of 0.4 mL of 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 (JENWAY 6305):

$$\text{Percentage OH radical scavenging ability (\%)} = \frac{\text{Absorbance of control} - \text{Absorbance of test sample}}{\text{Absorbance of control}} \times 100$$

**Reducing power**

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 volume of 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 1 mL of different concentrations (50, 100, 150 and 200 L) 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 (JENWAY 6305) 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.

**Preparation of pancreas homogenates**

The rats were decapitated under mild diethyl ether anesthesia, and the pancreas was rapidly dissected, placed on ice, and weighed. This tissue was subsequently homogenized in cold saline (1:10 wt/vol) with about 10 up-and-down strokes at approximately 1,200 rpm in a Teflon® (DuPont, Wilmington, DE)-glass homogenizer. The homogenate was centrifuged for 10 min at 3,000 g to yield a pellet that was discarded, and the low-speed supernatant (S1) was collected and kept for lipid peroxidation assay (Belle et al., 2004).

**Table 1.** Total phenolic content, total flavonoid content, vitamin C content, $ABTS^{\cdot+}$, $EC_{50}$ of antioxidant and inhibitory activities of the aqueous extracts of roasted and boiled flour.

| Sample | Total phenol content mg/g | Total flavonoid content mg/g | ABTS mmol /100 g TEAC | Vitamin C mg/g | $EC_{50}$ of antioxidant activities mg/mL | | | |
|---|---|---|---|---|---|---|---|---|
| | | | | | $Fe^{2+}$ chelating ability | OH radical scavenging ability | DPPH radical scavenging ability | Reducing power |
| Rf | 0.89±0.03 | 0.48±0.01 | 0.61±0.04 | 3.79±0.30 | 6.49±0.09 | 7.05±0.04 | 33.31±2.12 | 35.84±2.89 |
| Bf | 0.93±0.01 | 0.61±0.01 | 0.59±0.04 | 3.47±0.04 | 8.55±0.05 | 4.68±0.22 | 27.05±3.14 | 104.98 ±6.27 |

Values represent mean ± standard deviation, n = 3.

#### Lipid peroxidation and thiobarbituric acid reactions

The lipid peroxidation assay carried out using the modified method of Ohkawa et al. (1979). 100 µL of S1 fraction was mixed with a reaction mixture containing 30 µL of 0.1 M (pH 7.4) Tris-HCl buffer, plantain extracts (0 to 100 µL), and 30 µL of the pro-oxidant (70 µM SNP or 250 µM $FeSO_4$), and the volume was made up to 300 µL by water before incubation at 37°C for 1 h. The colour reaction was developed by adding 300 µL of 8.1% sodium dodecyl sulfate to the reaction mixture containing S1. This was subsequently followed by addition of 600 µL of acetic acid/HCl (pH 3.4) buffer and 600 µL of 0.8% thiobarbituric acid. This mixture was incubated at 100°C for 1 h Thiobarbituric acid-reactive species (TBARS) produced were measured at 532 nm in the spectrophotometer (JENWAY 6305) and the absorbance was compared with that of a standard curve using malondialdehyde (MDA).

#### Analysis of data

The results of the replicates were pooled and expressed as mean ± Standard Error (SE). Differences were evaluated by 1-way analysis of variance, followed by the Duncan multiple test (Zar, 1984). $EC_{50}$ (concentration of extract that will cause 50% concentration activity) was determined using linear regression analysis.

## RESULTS

### Proximate analyses

In the study carried out, the proximate composition of the unripe false horn plantain products, that is, Rf and Bf presented in Table 2 revealed that roasting significantly (P<0.05) increased the ash content while it significantly (P<0.05) reduced the protein content than in boiling. There is no significant difference (P<0.05) between moisture, lipids, carbohydrates and fiber contents of both Rf and Bf. However, Bf has higher moisture, fat and carbohydrate content while Rf has higher crude fibre.

### Total phenol, total flavonoid and vitamin C contents

The extractable phenolic content of the aqueous extract of Rf and Bf is presented in Table 1. The result revealed that the total phenol content of Bf extract (0.93±0.01 mg/g) was significantly higher (P<0.05) than that of Rf extract (0.89±0.03) mg/g. The total flavonoid contents of Rf and Bf extracts were (0.48± 0.01 and 0.61±0.01) mg/g, respectively. This result showed that Bf has higher flavonoid content. Furthermore, the vitamin C (in mg/g) content of both the roasted and boiled flour extracts was also assessed (Table 1). The result revealed that the roasted flour extract (3.79±0.30) had higher vitamin C content than the boiled flour extract (3.47±0.04).

### 2,2'-azinobis (3-ethylbenzothiazoline-6-sulfonic-acid ($ABTS^{\cdot+}$)

The total antioxidant activity of the aqueous extract of the Rf and Bf was determined and reported as TEAC. The result is presented in Table 1. The result revealed that Rf has higher $ABTS^{\cdot+}$ (0.61±0.04 mmol/100 gTEAC) radical scavenging ability.

### DPPH radical scavenging ability

The roasted and boiled flour extracts were examined for their radical scavenging ability toward the stable free radical DPPH. The result is presented in Figure 1 and its $EC_{50}$ in Table 1. The result revealed that the radical-scavenging activities of both extracts increased with increasing concentration. However, the boiled flour extract has higher DPPH radical scavenging ability than the roasted flour extract. However, as shown by the $EC_{50}$ values, the boiled flour extract had significantly higher (P<0.05) radical scavenging ability that islower $EC_{50}$ value (27.05 mg/mL) than roasted flour extract (33.31 mg/mL).

### $Fe^{2+}$ chelating activity

The plot of chelating % vs. mg/ml was shown in Figure 2 and its $EC_{50}$ (Table 1). The result revealed that the extracts chelated iron in a dose dependent manner and judging by the $EC_{50}$, the roasted flour extract exhibited higher $Fe^{2+}$ chelating ability.

**Table 2.** Proximate composition of the plantain (roasted and boiled) products on dry weight percent (%).

| Proximate analyzed | Roasted flour (Rf) | Boiled flour (Bf) |
|---|---|---|
| Moisture content | 53.38±1.22 | 54.68±0.09 |
| Ash | 9.65±0.03 | 5.11±1.24 |
| Protein | 3.09±0.05 | 5.48±0.18 |
| Fat | 1.63±0.15 | 1.81±1.09 |
| Carbohydrate | 31.43±0.01 | 32.17±0.33 |
| Crude fibre | 0.82±0.02 | 0.75±0.12 |

Values represent mean ± standard deviation, n = 3.

### OH radical scavenging ability

Figure 3 depicts the OH radical scavenging ability. The extracts (roasted and boiled flour) exhibited OH radical scavenging activity in a dose dependent manner. However, the higher activity was found in the boiled flour extract. The $EC_{50}$ value of OH radical scavenging ability is presented on Table 1 which revealed that $EC_{50}$ of the boiled flour extract lower than that of the roasted flour extract.

### Reducing power

The antioxidant activity of the aqueous extracts of roasted and boiled flour was assessed for their reducing power in a dose dependent manner. The result is presented in Figure 4. Considering its $EC_{50}$ (Table 1), the boiled flour extract has significantly higher ($P<0.05$) reducing power than the roasted flour extract.

### Inhibition of $Fe^{2+}$- induced pancreatic lipid peroxidation

The interaction of the roasted and boiled flour extracts with Fe (II) induced lipid peroxidation in isolated rat's pancreas homogenates is presented in Figure 5. As shown, increased MDA (131.58%) produced due to incubation of the pancreas tissues in the presence of 250 M Fe (II) was decreased by both plantain products extracts at the concentration range tested (1.25 to 6.25 mg/ml). However, roasted flour extract had higher inhibition in the Fe (II)-induced lipid peroxidation in the rat's pancreas than boiled flour extract.

### Inhibition of SNP-induced pancreatic lipid peroxidation

The extracts of roasted and boiled flour inhibited SNP induced lipid peroxidation in cultured rats' pancreas in a dose dependent manner (Figure 6). The roasted flour extract had significantly higher ($P<0.05$) inhibitory activity on SNP than boiled flour extract.

## DISCUSSION

The proximate composition of the unripe false horn plantain studied (Table 2) revealed that processing increased the proximate compositions (except in a few cases where processing had reduced effect). Roasting process reduced the protein content in Rf while it increased the ash content in Rf than the Bf. The reduction in the protein content of the roasted flour could be attributed to the denaturation and loss of protein due to participation of amino acids in Maillard reactions as a result of heating (Onyango et al., 2004). The ash content of the roasted and Boiled flour are higher compared to some Nigerian indigenous seeds such as *Sphenostylis stenocarpa, Pentaclethra macrophylla* and *Citrullus colocynthis* (Ojiako et al., 2010).The percentage of fat (1.63±0.15 and 1.81±1.09) %, moisture (53.38±1.22 and 54.68±0.09) % content obtained in roasted and boiled flour, respectively agree with previous study (Oboh and Erema, 2010) in which the boiled flour has a higher fat and moisture content. It is worth noting that the increase in moisture content in the boiled flour could be attributed to the fact that boiling allowed the starch to absorb water (Oboh and Ogbebor, 2010).

There is no significant difference between the percentage fibre content of both the roasted and boiled flour samples. However, roasted has higher fibre content. This finding is higher than that reported for fried plantain (Oboh and Erema, 2010). Fibre has a physiological effect on the gastrointestinal function of promoting the reduction of tracolonic pressure which is beneficial in diverticular disease. Fibre also has a biochemical effect on the absorption and re-absorption of bile acids and consequently the absorption of dietary fats and cholesterol (Edeoga et al., 2006). The total carbohydrate of both the roasted and boiled flour is lower than that reported for boiled and pounded plantain (Oboh and Erema, 2010). The total phenol content of the plantain products (Table 1) revealed that boiled flour extract has higher phenolic

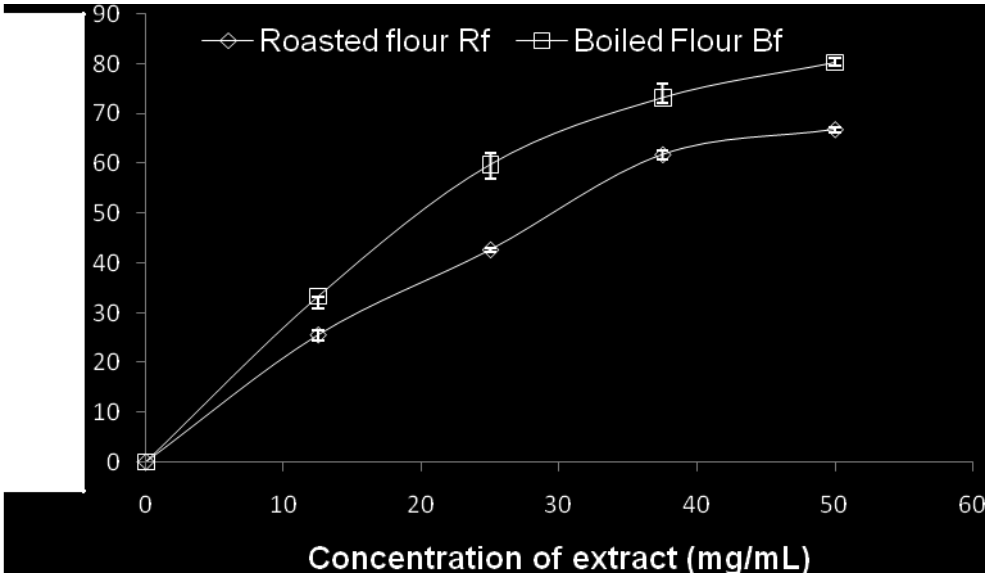



**Figure 1.** DPPH free radical-scavenging ability of aqueous extracts of roasted and boiled flour.

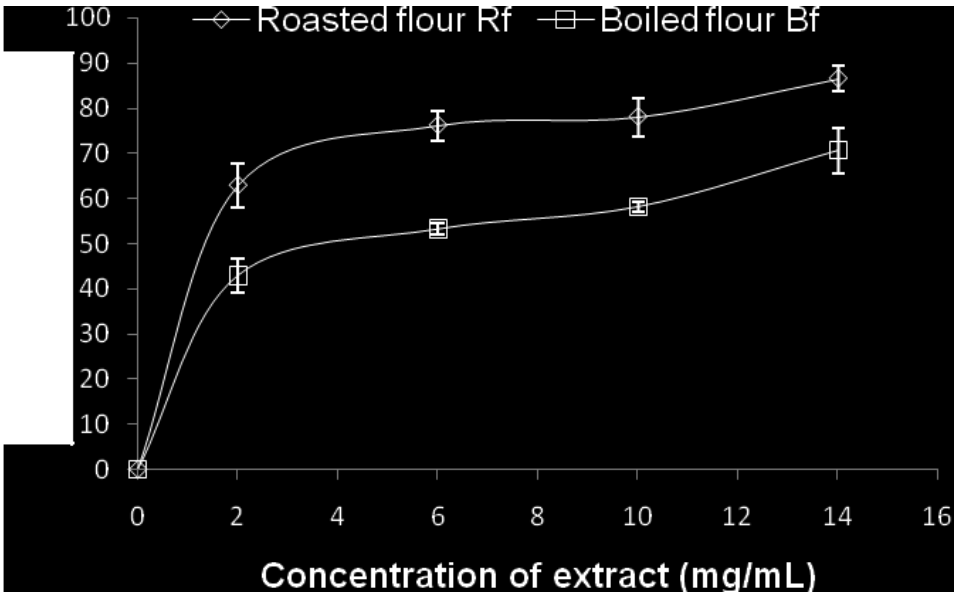



**Figure 2.** $Fe^{2+}$ chelating ability of aqueous extracts of roasted and boiled flour.

content. This is higher than that reported for white ginger (Oboh et al., 2010). Phenolics have been shown to be highly effective in scavenging most types of oxidizing molecules, including singlet oxygen and other free radicals produced by lipid peroxidation (Kuchukashvili et al., 2006). Phenolics have also been reported to be the most important antioxidative components of plant materials, as well as good correlation between concentration of plant phenolics and the total antioxidant capacity (Madsen et al., 1996).

Phenols and polyphenolic compounds, such as flavonoids, are widely found in food products derived from plant sources, and they have been reported to possess significant antioxidant activities (Nabavi et al., 2009a). The antiradical activity of flavonoids and phenols is principally based on the structural relationship between different parts of their chemical structure (Rice-Evans et al., 1996). The boiled flour extract has higher flavonoid content (Table 1.) and this follows the same trend with the phenolic content. This finding agrees with the earlier

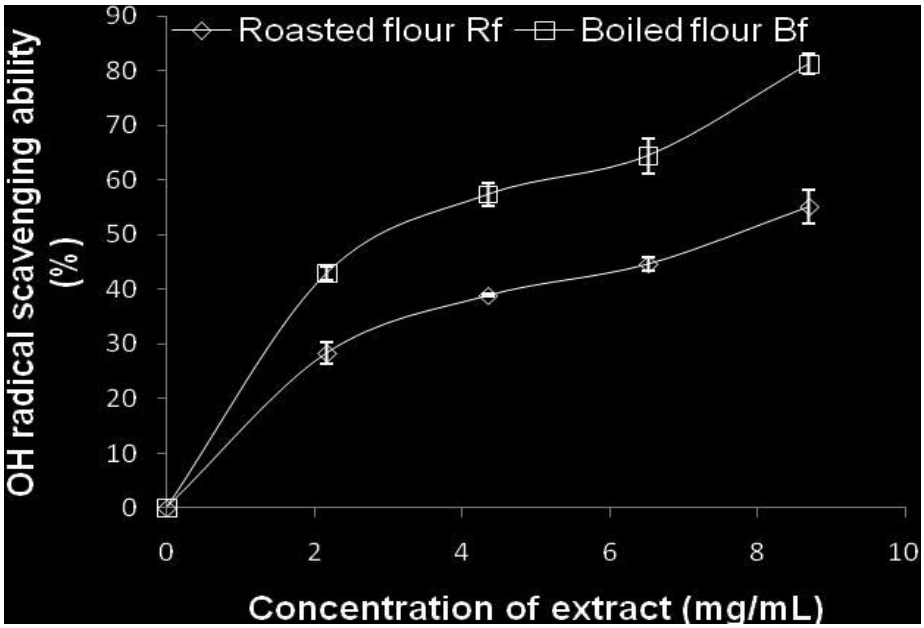



**Figure 3.** OH radical scavenging ability of aqueous extracts of roasted and boiled flour.

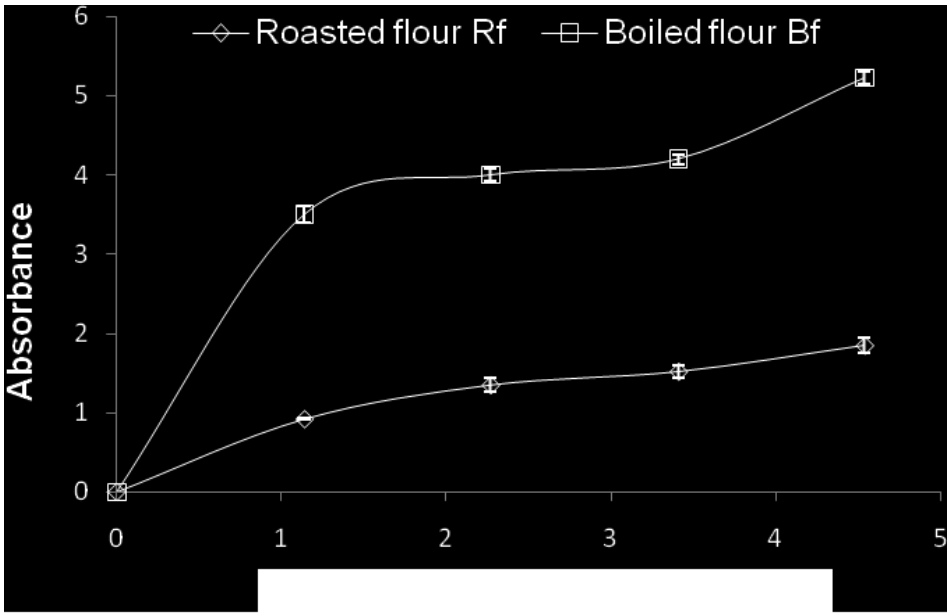



**Figure 4.** Reducing power of aqueous extracts of roasted and boiled flour.

report (Oboh et al., 2010) in which the sample with higher total phenol content exhibited higher flavonoid content. Ascorbic acid is a good reducing agent and exhibits its antioxidant activities by electron donation (Oboh, 2005). As a water-soluble antioxidant, vitamin C is in a unique position to "scavenge" aqueous peroxyl radicals before these destructive substances damage the lipids (Kronhausen et al., 1989). The roasted flour extract has higher vitamin C content (Table 1). The vitamin C content of Rf is lower than that of some reported spices such as basil, bird pepper, black pepper and cinnamon (Calucci et al., 2003) but higher than those reported for sorrel drinks

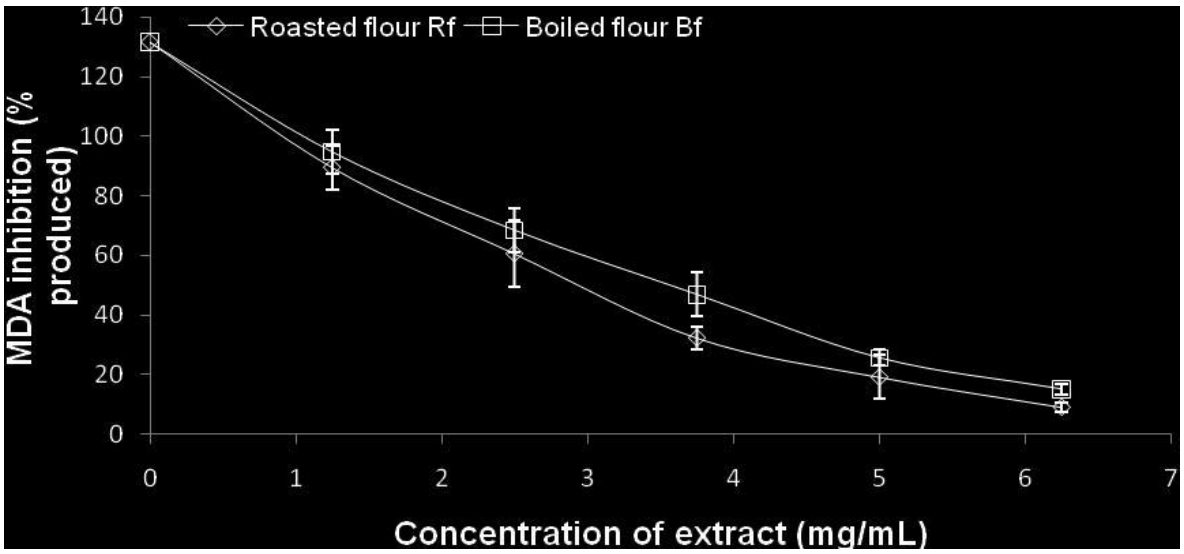



**Figure 5.** Inhibition of $Fe^{2+}$- induced lipid peroxidation in pancreas by aqueous extracts of roasted and boiled flour.

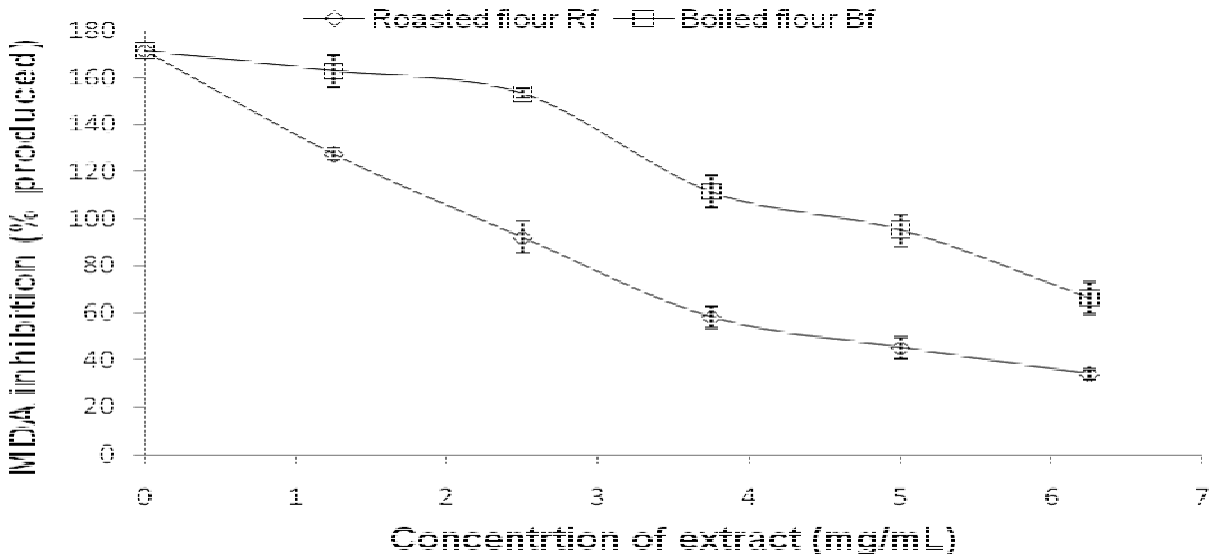
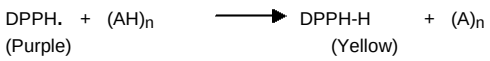



**Figure 6.** Inhibition of SNP-induced lipid peroxidation in pancreas by aqueous extracts of roasted and boiled flour.

(Oboh and Elusiyan, 2004). Antioxidants carry out their protective properties on cells either by preventing the production of free radicals or by neutralizing/scavenging free radicals produced in the body (Oboh et al., 2007).

The free radical scavenging ability of the aqueous extract of roasted and boiled flour extract was studied using a moderately stable nitrogen-centred radical species – ABTS radical (Re et al., 1999). As shown in Table 1, the roasted flour extract with the higher vitamin C content has higher $ABTS^{\cdot+}$ antioxidant activity. It can be noted that the $ABTS^{\cdot+}$ value is lower than its vitamin C content and total phenol but higher than its total flavonoid content. DPPH is also relatively stable nitrogen centered free radical that easily accepts an electron or hydrogen radical to become a stable diamagnetic molecule. DPPH radicals react with suitable reducing agents as a result of which the electrons become paired off forming the corresponding hydrazine (Blois, 2001) and the color of the reaction changes from violet to yellow upon reduction by either the process of hydrogen- or electron- donation and substances which are able to perform this reaction can be considered as antioxidants and therefore radical

scavengers (Dehpour et al., 2009). The mechanism of scavenging DPPH by radical scavenger (Epsin et al., 2000) can be briefly expressed as:




In DPPH assay the lower the extract concentration causing 50% concentration inhibition ($EC_{50}$) the better it is able to scavenge the radicals, particularly peroxy radicals which are the propagators of the autoxidation of lipid molecules and thereby break the free radical chain reaction (Frankel, 1991). The boiled flour extract has higher radical scavenging ability (Figure 1) than the roasted flour extract considering the $EC_{50}$ (F 1).

The trend of this result followed the pattern observed with its total phenol and flavonoid. This agreed with our earlier report that antioxidant properties of plant food correlate with the phenolic contents (Oboh and Shodehinde, 2009). The $Fe^{2+}$ chelating ability of an extract measures how e ective the compounds in it can compete with ferrozine to form ferrous ion. By forming a stable iron (II) chelate, an extract with high chelating power reduces the free ferrous ion concentration thus decreasing the extent of Fenton reaction which is implicated in many diseases (Halliwell and Gutteridge, 1990). The roasted flour extract which has higher Vitamin C content and $ABTS^{\cdot+}$ (Table 1) antioxidant activity exhibited higher $Fe^{2+}$ chelating ability (Figure 2). The measurement of $H_2O_2$ scavenging activity is one of the useful methods for determining the ability of anti-oxidants to decrease the level of pro-oxidants such as $H_2O_2$ (Oboh and Ademosun, 2011). Hydrogen peroxide itself is not very reactive, but sometimes it can be toxic to cells because of rise in the hydroxyl radicals in the cells (Rey et al., 2010). The higher OH radical scavenging was exhibited by boiled flour extract (Figure 3). Furthermore, it was obvious from the study that there was an agreement between the phenolic contents DPPH and OH radical scavenging ability.

Reducing power is a potent antioxidant defense mechanism; the two mechanisms that are available to affect this property are electron transfer and hydrogen atom transfer (Dastmalchi et al., 2007). The higher reducing power was displayed by boiled flour extract (Figure 4). The trend of this result is in agreement with the phenolic contents, DPPH and OH radical scavenging ability. The increased lipid peroxidation in the presence of $Fe^{2+}$ could be attributed to the fact that $Fe^{2+}$ can catalyze one-electron transfer reactions that generate ROS, such as the reactive $OH^{\bullet}$, which is formed from $H_2O_2$ through the Fenton reaction. Iron also decomposes lipid peroxides, thus generating peroxyl and alkoxyl radicals, which favours the propagation of lipid oxidation (Zago, 2000). However, roasted flour extract exhibited higher inhibition in the Fe (II)-induced lipid peroxidation in the rat's pancreas than boiled flour extract (Figure 5); the reason for the higher inhibition of the lipid peroxidation in the pancreas by the roasted flour extract cannot be categorically stated; however, it could be attributed to the unique radical scavenging ability of vitamin C (Kronhausen et al., 1989) which could have formed complexes with the Fe (II), thereby preventing them from catalyzing the initiation of lipid peroxidation, as higher iron chelating ability of roasted flour extract has been demonstrated in Figure 2 as well as its higher vitamin C content (Table 1).

In humans, the diabetogenic process appears to be caused by immune destruction of the beta cells; part of this process is apparently mediated by white cell production of ROS and scavengers of oxygen radicals are effective in preventing diabetes in animal models (Oberley, 1988). Diabetes can be initiated in animals by the drugs alloxan and streptozotocin; the mechanism of action of these two drugs is different, but both result in the production of active oxygen species. Scavengers of oxygen radicals are effective in preventing diabetes in these animal models (Oberley, 1988). SNP is a component of antihypertensive drugs; it causes cytotoxicity through the release of cyanide and/or NO. NO is a universal neuronal messenger in the central nervous system which can independently; cause neuronal damage in cooperation with other ROS (Oboh and Rocha, 2007). Both extracts exhibited inhibitory activity. However, the roasted flour extract has higher inhibitory activity of SNP (Figure 6). This also follows the same pattern of inhibitory effect on the $Fe^{2+}$ induced lipid peroxidation (Figure 5) as well as that of its higher $ABTS^{\cdot+}$ antioxidant activity and vitamin C content (Table 1).

## Conclusion

Oxidative stress is generated when there is an excessive production of free radicals. The most likely means of fighting oxidative stress could be achieved by consuming food rich in antioxidants. The results of the present study indicate that extracts of unripe false horn plantain products (roasted and boiled) are protective against ROS. On the basis of this study, there is a high likelihood that they may provide the types of nutritional and health benefits associated with consumption of various unripe plantain varieties in general.

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