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Full Length Research Paper

Effect of ripening on the phytochemical constituents and antioxidant properties of plantain (*Musa paradisiaca)*

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The present study sought to investigate the effect of ripening on the antioxidant properties and phytochemical constituents of plantain, a major staple food of global consumption. This was done by measuring the total phenolics and flavonoid content of both ripe and unripe form of plantain. Moreover, the free radical scavenging, ferric reducing and iron chelating properties of both forms of plantain were also investigated. Finally, the ability of both extracts to prevent pro-oxidant induced deoxyribose degradation and lipid peroxidation in both cerebral and hepatic tissues of rat was also assessed. Results revealed that ripening depleted the total phenolics [(unripe, 16.2 ± 0.75 mg/g (GAE); ripe, 8.3 ± 0.55 mg/g (GAE)] and flavonoid [(unripe, 15.2 ± 0.45 mg/g (QE); ripe, 9.5 ± 0.21 mg/g (QE) content of plantain. Moreover, the unripe plantain extract exhibited a significantly higher free radical scavenging, iron (II) chelating and ferric reducing effect than the ripe extract. Finally, the unripe plantain extract offered a marked inhibitory effect against pro-oxidant induced deoxyribose degradation and lipid peroxidation than the ripe extract. Put together, we could suggest that the observed depletion in antioxidant properties of ripe plantain may be due to an irreversible degradation in the phytochemical constituents of the fruit as ripening progresses. This study would create a public awareness on the effect of ripening on the therapeutic potentials of plantain and serve as useful information in the global fight against degenerative diseases whose etiology has been linked to oxidative stress.

Key words: *Musa paradisiaca*, free radical, oxidative stress, phytochemical, ripening.

INTRODUCTION

Plantain (*Musa paradisiaca*) is a staple crop which is consumed both as an energy yielding food and as a dessert providing millions of people in Africa and other developing nations with more than 200 calories per day (John and Marchal, 1995; FAO, 2005). Plantain and banana constitute the fourth most important global food commodity after rice, wheat and maize in terms of the gross value of production (Asiedu et al., 1992; FAO, 2005), and possess immense health-related benefits. Specifically, dried unripe plantains have been shown to exhibit antiulcerogenic activity and have been used as prophylactic treatment of aspirin-induced ulcers due to its ability to stimulate the growth of gastric mucosal and strengthen it against ulcerogens, and promote healing by inducing proliferations (Best et al., 1984; Usha and Vijayammal, 1991). Besides, unripe plantain (*M. paradisiaca*) has been shown to exhibit cholesterol-lowering and antidiabetic properties in experimental animals. In fact, report has shown significant cholesterol-lowering effect and improvement in glucose tolerance in animals treated with unripe plantain flour (Usha and Vijayammal, 1991). Furthermore, there are indications that plantain flakes can be used as a safe and cost-effective treatment for diarrhea, hyperoxaluric urolithiasis and colorectal cancer (Poonguzhali and Chegu, 1994; Emery et al., 1997; Lohsoonthorn and Danvivat, 1995; Deneo-Pellerini et al., 1996; Murakami et al., 1998). Moreover, reports have indicated that treatment with

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plantain significantly decreased the systolic, diastolic and arterial blood pressure during cold stress-induced hypertension (Sarkar et al., 1999) Despite the aforementioned health benefits, plantain undergoes natural or induced ripening which brings about an inevitable alteration in its organoleptic and antioxidant properties. Meanwhile, there is dearth of information on the effect of ripening on the antioxidant properties of plantain. Interestingly, the antioxidant properties of plants are closely linked to its phytochemical content. Keeping the aforementioned views in mind, and considering the vast nutritional and antioxidant potentials of plantain that could be exploited in the management of degenerative diseases coupled, it is pertinent to unravel the effect of ripening on its phytochemical constituents and antioxidant properties which could serve as an impetus in the global fight against degenerative diseases whose etiology has been linked to oxidative stress.

MATERIALS AND METHODS

Chemical reagents

Thiobarbituric acid (TBA), 2-deoxyribose sugar, DPPH (2,2-diphenyl -1- picrylhydrazyl) and 1, 10 phenanthroline were obtained from Sigma (St. Louis, MO). All other chemicals were obtained from standard chemical suppliers and were of analytical grade.

Materials

Plantains (*M. paradisiaca*) were purchase in a local market in Owena metropolis, Ondo State, Nigeria. The fruit was identified botanically at the Department of Crop, Soil and Pest Management of the Federal University of Technology, Akure, Nigeria.

Sample preparation

Plantain fruits were divided into two portions. The first portion (unripe) were peeled and chopped for air drying while the second portion was allowed to ripe. Ripening was effected naturally by keeping the unripe plantains in a black polythene bag airtight and left for three days after which ripening was visually noticeable by a change in colour of the peel from green to yellow. However, we ensured a daily routine inspection of the plantains to ascertain when fully ripe. The ripened plantain were then peeled and dried. The dried fruit was then powdered using a blender. 5 g each of ripe and unripe portions were soaked separately in 100 ml of distilled water and left for 24 h to allow for extraction. The resulting mixture was centrifuged and the filtrate kept in the refrigerator for subsequent analysis. This serves as the stock for all determinations.
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Determination of phenolic content

The total phenol of plantain extracts was determined using the method reported by Singleton et al. (1999). 2.5 ml Folin-ciocalteau's reagent (10%) and 2 ml of sodium carbonate (7.5%) were added to 200 µl of the extracts and the absorbance was measured at 765 nm after incubating at 45°C for 40 min. The amount of phenol in plantain extract was expressed as gallic acid equivalent (GAE).

Determination of flavonoid content

The total flavonoid content of both extract was determined using a slightly modified method reported by Meda et al. (2005). Briefly, 0.5 ml of appropriately diluted sample was mixed with 0.5 ml of methanol (80%), 50 µl of $AlCl_3$ (10%), 50 µl of potassium acetate (1 M) and 1.4 ml water, and allowed to incubate at room temperature for 30 min. Thereafter, the absorbance of the reaction mixture was subsequently read at 415 nm. The total flavonoid was calculated using quercetin as standard.

Free radical scavenging ability

The free radical scavenging ability of the plantain extract against DPPH (2,2-diphenyl-1-picrylhydrazyl) free radicals was evaluated according to Gyamfi et al. (1999). Briefly, 600 µl of extract was mixed with 600 µl of methanolic solution containing DPPH (0.3 mM) radicals, the mixture was left in the dark for 30 min and the absorbance was measured at 516 nm.

Ferric reducing activity

The reducing property was determined by assessing the ability of plantain extracts to reduce $FeCl_3$ solution as described by Oyaizu (1986). Briefly, extract (0 to 250 µl of stock) was mixed with 250 µl of sodium phosphate buffer (200 mM; pH 6.6) and 250 µl of potassium ferrocyanide (1%), the mixture was incubated at 50°C for 20 min. Thereafter, 250 µl of trichloroacetic acid [(TCA) 10%] was added, and subsequently centrifuged at 650 rpm for 10 min. l000 µl of the supernatant was mixed with equal volume of water and l00 µl of ferric chloride [0.1% (w/v)]. The absorbance was later measured at 700 nm; a higher absorbance indicates a higher reducing power.

Fe^{2+} - chelating assay

The Fe^{2+} chelating ability of plantain extract was determined using a modified method described by Puntel et al. (2005). 150 µl of freshly prepared $FeSO_4$ (500 µM) was added to a reaction mixture containing 168 µl of Tris - HCl (0.1 M, pH 7.4), 218 µl saline and extract. The reaction mixture was incubated for 5 min, before the addition of 13 µl of 1, 10-phenanthroline (0.25% w/v). The absorbance was subsequently measured at 5l0 nm in a spectrophotometer.

Deoxyribose degradation

Deoxyribose degradation was determined by Halliwell et al. (1987). Deoxyribose (20 mM) was incubated at 37°C for 30 min with potassium phosphate (50 mM, pH 7.4) plus Fe^{2+} (0.1 mM) and H_2O_2 (1 mM) to induce deoxyribose degradation in the presence of plantain extracts. After incubation, 0.4 ml of TBA (0.8 %) and 0.8 ml of TCA (2.8%) were added, and the tubes were heated for 20 min at 100°C. The absorbance was then measured spectrophotometrically at 532 nm.

Animals

Male adult Wistar rats (200 to 250 g) were used. The animals were used according to the standard guidelines of the Committee on Care and Use of Experimental Animal Resources.

Table 1. Antioxidant constituents of *M. paradisiaca* extracts.

Constituent	UP	RP
Total phenol [mg/g (GAE)]	**16.2 ± 0.75***	**8.3 ± 0.55**
Total flavonoid [mg/g (QE)]	**15.2 ± 0.45***	**9.5 ± 0.21**

Each observation is a mean ± SD of 3 to 4 independent experiments.* indicates a statistically significant difference at P < 0.05. GAE, Gallic acid equivalent; QE, Quercetin equivalent. RP and UP represent ripe and unripe extracts, respectively.

Lipid peroxidation

Rats were decapitated via mild cervical dislocation and the cerebral (whole brain) and hepatic (liver); tissues were rapidly dissected, placed on ice and weighed. Tissues were immediately homogenized in cold 50 mM Tris - HCl, pH 7.4 (1/10, w/v). The homogenates were centrifuged for 10 min at 4000 g to yield a pellet that was discarded and a low-speed supernatant (SI). An aliquot of 100 µl of SI was incubated for 1 h at 37°C in the presence of aqueous plantain extracts, with and without the pro-oxidants, iron [final concentration (10 µM)], sodium nitroprusside (SNP) (final concentration 30 µM). This was then used for lipid peroxidation determination. Production of thiobarbituric acid reactive species (TBARS) was determined as described by Ohkawa et al. (1979), excepting that the buffer of the color reaction has a pH of 3.4. The color reaction was developed by adding 200 µl of sodium dodecyl sulfate (SDS 8.1%) to SI, followed by sequential addition of 500 µl acetic acid/HCl (pH 3.4) and 500 µl of TBA (0.8 %). This mixture was incubated at 95°C for 1 h. TBARS produced were measured at 532 nm and the absorbance was compared to that of the control (containing all other assay components except the extract).

Statistical analysis

The results were expressed as mean ± SD of three-four independent experiments performed in triplicate and were analyzed by one-way analysis of variance, followed by Duncan's multiple-range test. Differences between groups were considered significant when $p < 0.05$.

RESULTS

Antioxidant constituents of ripe and unripe plantain extracts

Table 1 showed the effect of ripening on the total phenolics and flavonoid content of plantain extract. It shows that ripening depletes the total phenolics and flavonoids content. Perhaps, this observation seems to suggest that unripe plantains are better antioxidant candidate than ripened ones since they contain higher phenolic and flavonoid content.

Free radical scavenging activity

Figure 1 displayed the effect of ripening on the free radical scavenging activity of plantain extracts. Apparently, it shows that ripening causes a significant depletion in the free radical scavenging ability of plantain against DPPH radical. One - way ANOVA also suggested that unripe plantain sample exhibited a significantly higher free radical scavenging property than the ripe sample probably due to a higher phenolic content.

Iron (II)–chelating activity

The iron (II)-chelating effect of plantain extracts is as shown in Figure 2. Obviously, the unripe sample displayed a markedly higher Fe (II)–chelating effect than the riped plantain sample. Infact, one – way ANOVA showed that the unripe plantain sample exhibited a potent chelating effect which was significant even at the least volume of extract used.

Ferric reducing properties

Figure 3 shows the ferric reducing ability of plantain extracts. It clearly showed that the ferric reducing power of unripe plantain extract was significantly ($P < 0.05$) higher than ripe extract. However, both extracts showed a concentration-dependent ferric reducing power which was potent even at the least volume of extract used for the assay.

Hydroxyl radical scavenging properties

The hydroxyl radical scavenging activity of plantain extracts is as presented in Figure 4. Although both extracts exhibited hydroxyl scavenging ability, one – way ANOVA showed that unripe plantain extract demonstrated a markedly higher and concentration-dependent protective effect against deoxyribose degradation than ripe plantain extract.

Effect of plantain extracts on pro-oxidant-induced lipid peroxidation

Figure 5a and b and Figure 6a and b showed the effect of ripening on the protective ability of plantain extracts on iron (II) and SNP – induced lipid peroxidation respectively in both hepatic and cerebral tissues of rat. One – way ANOVA revealed that both extracts inhibited lipid peroxidation. However, unripe plantain sample demonstrated a significantly ($P < 0.05$) higher inhibitory effect against both cerebral and hepatic lipid peroxidation regardless of the pro-oxidant employed for oxidative assault.

DISCUSSION

In recent times, research has been directed at identifying and exploiting plants with potent antioxidant properties

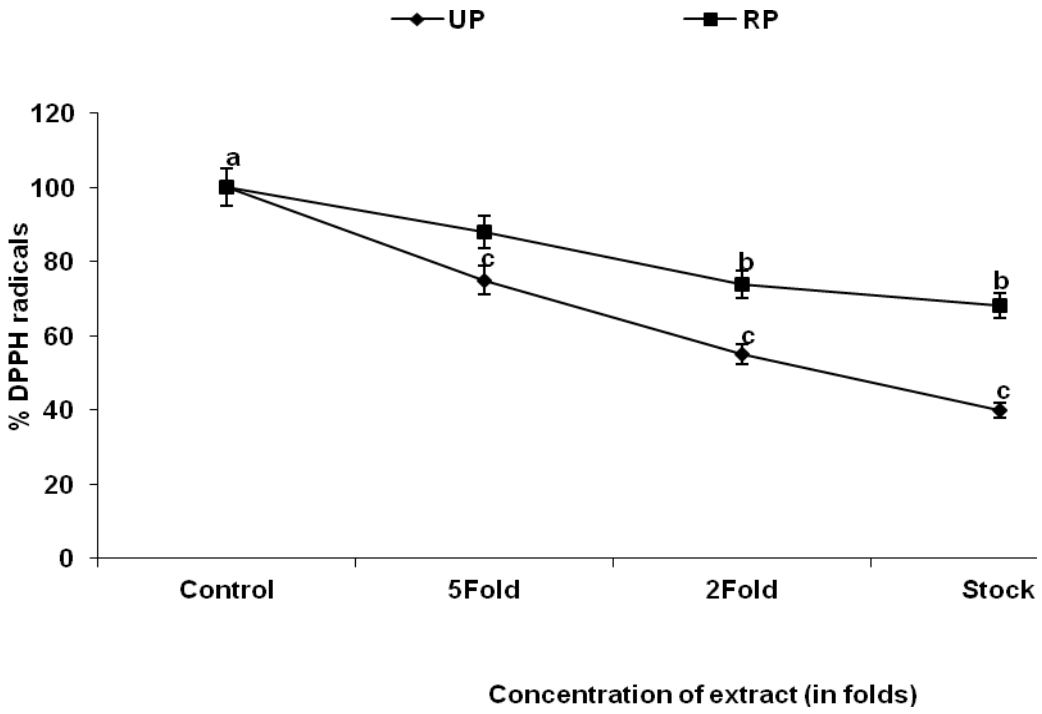


Figure 1. Free radical scavenging ability of plantain extracts. Data show means ± SEM values averages from 3 to 4 independent experiments performed in triplicate. 'b' and 'c' indicate a significant difference from the control 'a' (containing other assay components without plantain extract) at $p < 0.05$. RP and UP represent ripe and unripe plantain extracts, respectively.

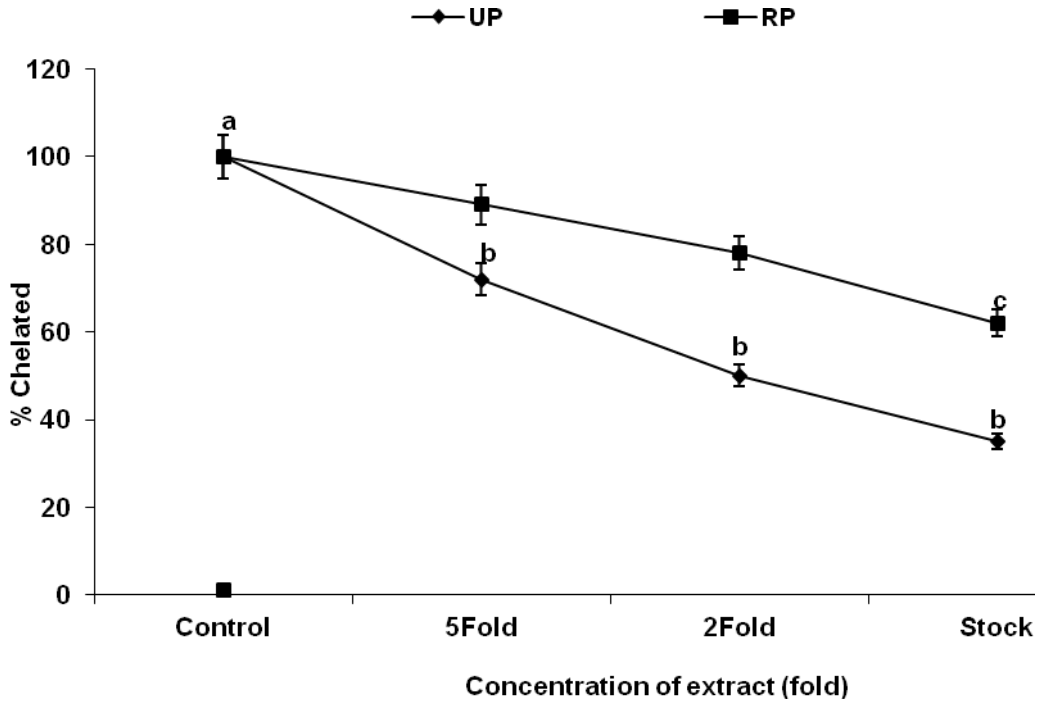


Figure 2. Fe (II) - chelating properties of plantain extracts. Data show means ± SEM values averages from 3 to 4 independent experiments performed in triplicate. 'b' and 'c' indicate a significant difference from the control 'a' (containing other assay components without plantain extract) at $p < 0.05$. RP and UP represent ripe and unripe plantain extracts, respectively.

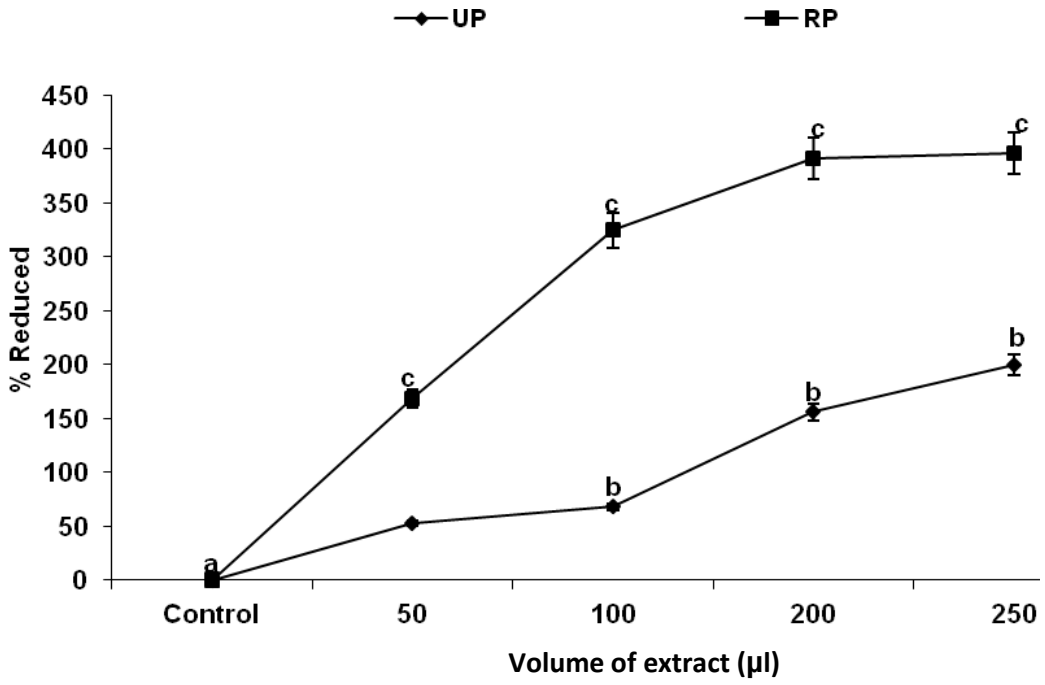


Figure 3. Ferric reducing antioxidant properties of plantain extracts. Data show means ± SEM values averages from 3 to 4 independent experiments performed in triplicate. 'b' and 'c' indicate a significant difference from the control (containing other assay components without plantain extract) 'a' at $p < 0.05$. RP and UP represent ripe and unripe plantain extracts, respectively.

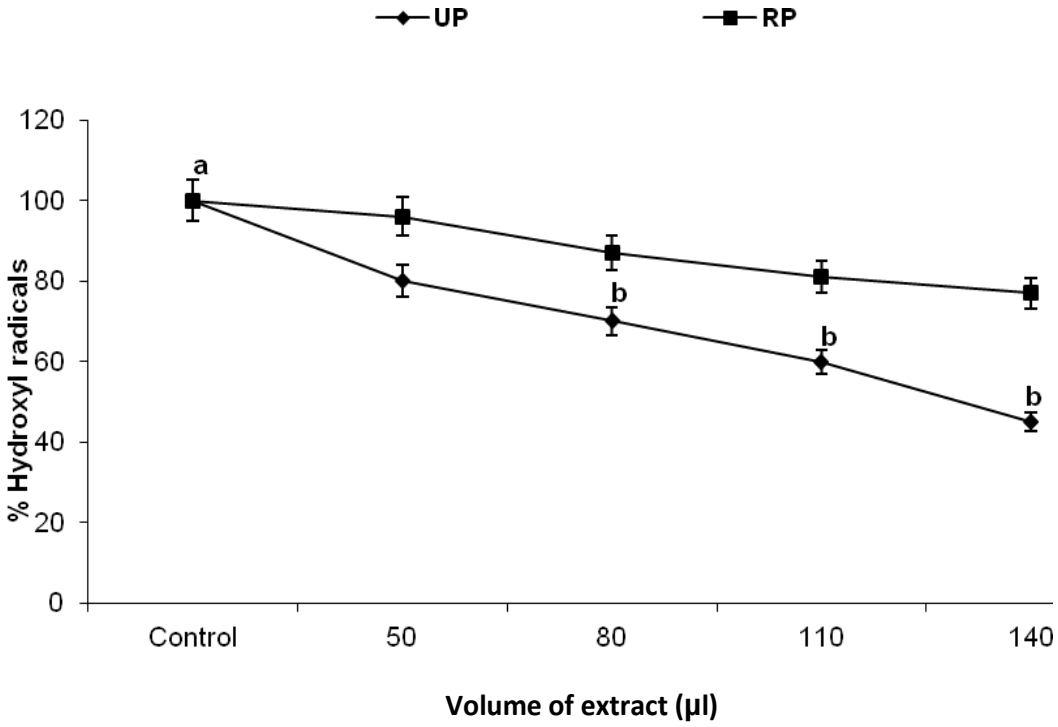


Figure 4. Hydroxyl radical scavenging ability of plantain extracts. Data show means ± SEM values averages from 3 to 4 independent experiments performed in triplicate. 'b' indicate a significant difference from the control 'a' (containing other assay components without plantain extract) at $p < 0.05$. RP and UP represent ripe and unripe plantain extracts, respectively.

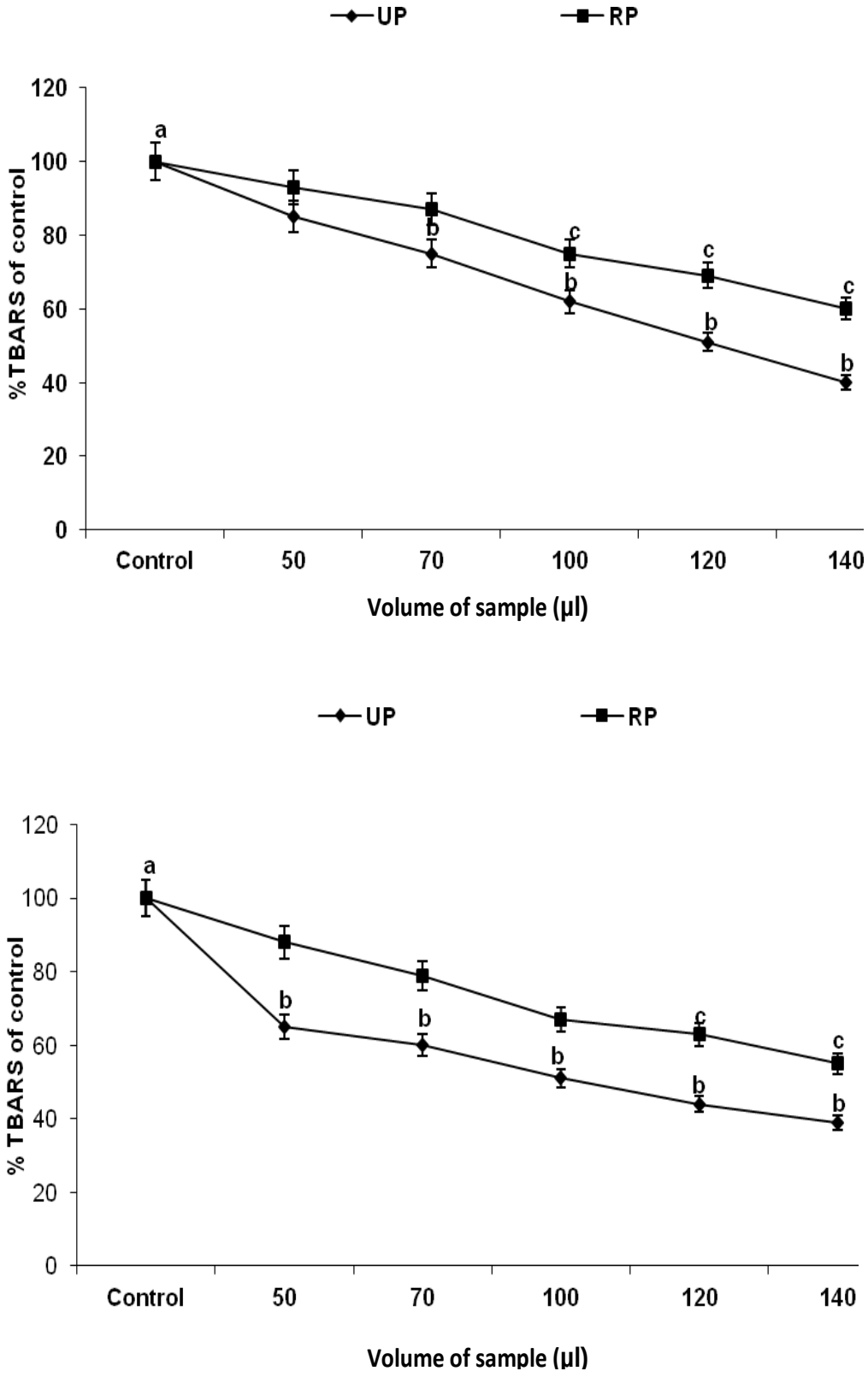
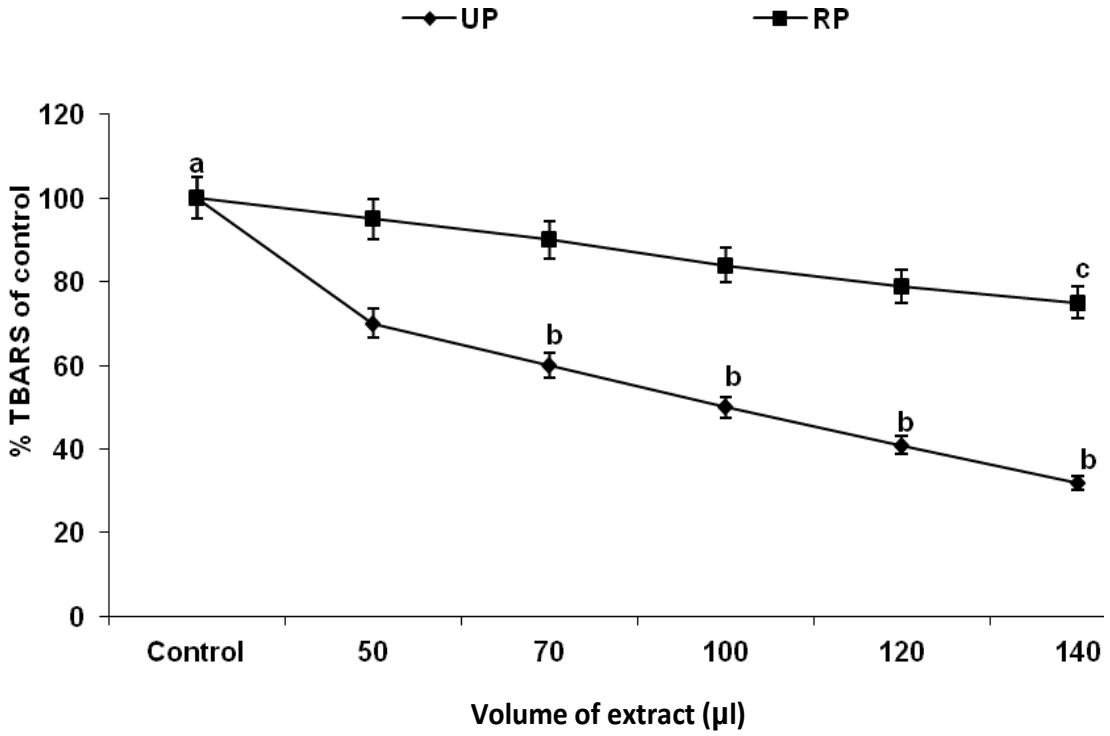


Figure 5. (a) Inhibitory effect of plantain extracts on Fe (II)- induced lipid peroxidation in rat liver. (b) Inhibitory effect of plantain extracts on Fe (II)- induced lipid peroxidation in rat brain. Data show means ± SEM values averages from 3 to 4 independent experiments performed in triplicate. 'b' and 'c' indicate a significant difference from the control 'a' (containing other assay components without plantain extract) at $p < 0.05$. RP and UP represent ripe and unripe plantain extracts, respectively.



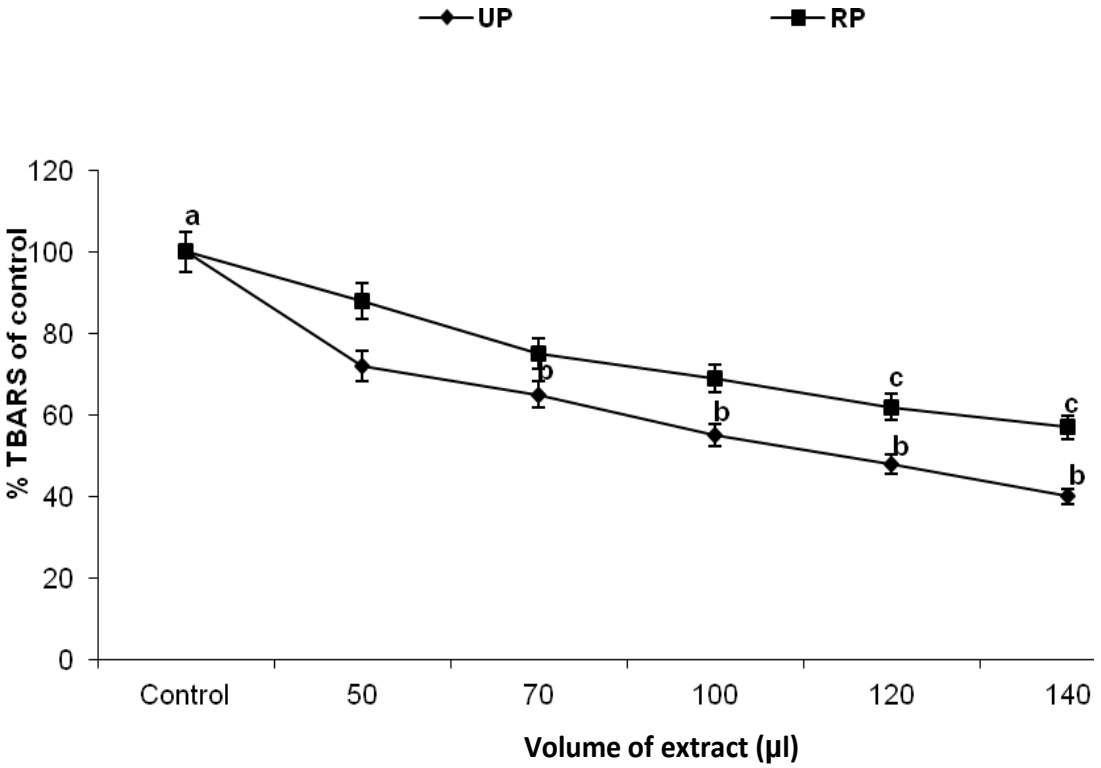


Figure 6. (a) Inhibitory effect of plantain extracts on SNP- induced lipid peroxidation in rat liver. (b) Inhibitory effect of plantain extracts on SNP- induced lipid peroxidation in rat brain. Data show means ± SEM values averages from 3 to 4 independent experiments performed in triplicate. 'b' and 'c' indicate a significant difference from the control 'a' (containing other assay components without plantain extract) at $p < 0.05$. RP and UP represent ripe and unripe plantain extracts, respectively.

that could be harnessed in the management of degenerative diseases. One widely reported fruit with considerable nutritional relevance is Plantain *M. paradisiacal* L. (Stover and Simmonds, 1987) which has been identified with antiulcerogenic, antidiabetic, antihypertensive, and anticancer activities (Vaaler et al., 1982; Block et al., 1992; Botting et al., 1999). However, the mechanisms behind these pharmacological potentials are far from being understood. Meanwhile, the antioxidant properties of medicinal plants are intrinsically linked with their phytochemical constituents. Hence, the effect of ripening on the total phenolics and flavonoid content of plantain extracts was investigated.

Interestingly, Table 1 revealed that ripening depletes the total phenolics and flavonoid content of plantain. In recent times, research interest has been paid to polyphenols and flavonoids as a result of their antioxidant capacity which is principally based on the redox properties of their hydroxyl groups and the structural relationship between different functional groups in their structure which allow them to act as reducing agents, hydrogen donors, free radical scavengers, singlet oxygen quenchers and metal chelators (Rice-Evans et al., 1996; Amic et al., 2003; Materska and Peruka, 2005; Oboh and Rocha, 2008a; Oboh et al., 2008; Gomez- Alonso et al., 2003). Hence, the fact that unripe plantain extract has a higher phenolic and flavonoid content could partly explain why unripe plantain is recommended to diabetic and hypertensive patients as dietary intervention in the management of their pathological conditions. However, since antioxidants could elicit their effects through several antioxidant mechanisms, some *in vitro* antioxidant mechanisms of plantain extract were investigated.

Routinely, antioxidants are determined by their free radical scavenging ability against DPPH which is an unstable free radical that attains stable configuration on interaction with any proton - rich species. Figure 1 showed that ripening significantly diminishes the free radical scavenging activity of plantain extracts. Although the reason behind this observation is not completely understood, it could be attributed to some irreversible biochemical changes associated with ripening which might have depleted the phytochemical content of plantain needed for scavenging free radical.

Moreover, antioxidants could chelate transition metals, specifically iron, preventing it from being oxidized since it is physiologically useful in the Fe^{2+} state. Hence, the antioxidant activity of compounds can be measured by assessing their ability to chelate transition metals of physiological relevance. Interestingly, Figure 2 shows that ripening depletes the iron chelating ability of plantain extract. Speculatively, we could attribute this observation to the diminishing effect of ripening on the polyphenolic content of plantain (Table 1).

Another parameter used for assessing a good antioxidant is the ability to reduce Fe^{3+} to Fe^{2+}. Rationally, Fe^{2+} is the oxidation state of iron that would enhance the ability of haemoglobin to transport oxygen in the blood. Figure 3 shows that ripening diminished the reductive power of plantain extract on Fe^{3+} which is traceable to the decrease in phenolics and flavonoid content which are required for reduction of Fe^{3+} to Fe^{2+}.

Furthermore, the hydroxyl radical ($^{\bullet}OH$) majorly produced from the interaction of Fe^{2+} and H_2O_2 in cells via the Fenton reaction can easily cross cell membranes and react with critical macromolecules including DNA leading to mutations with its attendant hazardous effects (Aruoma et al., 2002). Thus, removal of $^{\bullet}OH$ would offer good protecttion by preventing the oxidation of critical biomolecules. Hence, antioxidants could be assessed based on their ability to scavenge $^{\bullet}OH$ radical. Figure 4 shows that ripening has a diminishing effect on the $^{\bullet}OH$ scavenging ability of plantain extract. Although the reason for this observation is not completely understood, it could be speculated that hydroxyl radical scavenging is a component of the antioxidant properties of plantain. Further still, we could suggest that the phenols and flavonoids are responsible for scavenging hydroxyl radical.

Apart from nucleic acids, lipid is another macromolecule prone to oxidative damage in the biological system (Markesbery and Lovell, 2007). When oxidatively assaulted, lipid produces aldehydic compounds that react with thiobarbituric acid (TBA) to generate a colored product. Hence, antioxidants could be assessed based on their ability to offer protection to lipids preventing them from oxidative attack of free radicals. Interestingly, Figures 5a and b and 6a and b show that unripe plantain extract demonstrated potent and concentration dependent inhibitory effect against proxidant – induced lipid peroxidation irrespective of the lipid source and pro-oxidant employed for oxidative assault. However, in order to ascertain the antioxidant effect of plantain on lipid peroxidation, two pro-oxidants were employed since they differ in their mechanisms of causing oxidative assaults on lipids.

Iron as a proxidant have been said to be involved in the generation of hydroxyl radicals via Fenton reaction (Graf et al., 1984), which ultimately leads to the formation of other reactive oxygen species (ROS) (Klebanoff et al., 1992). An overproduction of ROS attack membrane lipids abstracting protons thereby causing oxidation of lipid otherwise called lipid peroxidation. Interestingly, Figure 5a and b showed that unripe plantain extract exhibited a markedly higher inhibitory effect against lipid peroxidation than the ripe extract probably due to its higher phytochemical constituents.

Moreover, sodium nitroprusside (SNP), one of the pro-oxidants employed for assault has been suggested to exhibit cytotoxic effect via the release of cyanide and/or nitric oxide (NO) (Rauhala et al., 1998) and NO has been implicated in the pathophysiology of strokes, traumas, seizures and Alzheimer's and Parkinson's diseases (Castill et al., 2000; Prast and Philippou, 2001). Reports have shown that NO could be degraded photolytically

(Arnold et al., 1984; Singh et al., 1995) causing its cleavage from SNP. Besides, literature data have shown that after the release of $NO^{\bullet}$, SNP or $[NO\text{-}Fe\text{-}(CN)5]^{2-}$ is converted to iron containing $[(CN)5\text{-}Fe]^{3-}$ and $[(CN)4\text{-}Fe]^{2-}$ species (Loiacono and Beart, 1992). Consequently, the iron moiety may go on to attack macromolecules causing damage which could lead to oxidative stress. Figure 6a and b showed that unripe plantain extract significantly ($P < 0.05$) inhibited SNP - induced lipid peroxidation in both hepatic and cerebral tissues than the ripe plantain extract. We may presumably attribute the observed difference in activity to their phytochemical constituents.

From the foregoing, it is evident that the widely reported health beneficial effect of plantain is intrinsically linked with its phytochemical constituents. Unfortunately, ripening depletes these phytochemicals which are responsible for the pharmacopotency of the fruit.

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