Journal of Scientific and Innovative Research 2014; 3(6): 563-568
Available online at: www.jsirjournal.com

Research Article

ISSN 2320-4818
JSIR 2014; 3(6): 563-568

Received: 29-07-2014
Accepted: 09-09-2014

Fakai I. M.
Department of Biochemistry, Faculty of Science, Kebbi State University of Science and Technology, Aliero, Kebbi State, P.M.B 1144, Nigeria

A.U. Birnin-Yauri
Department of Pure and Applied Chemistry, Faculty of Science, Kebbi State University of Science and Technology, Aliero, Kebbi State, P.M.B 1144, Nigeria

James Jemaima
Department of Biochemistry, Faculty of Science, Kebbi State University of Science and Technology, Aliero, Kebbi State, P.M.B 1144, Nigeria

Correspondence:
Dr. Fakai I. M.
Department of Biochemistry, Faculty of Science, Kebbi State University of Science and Technology, Aliero, Kebbi State, P.M.B 1144, Nigeria
E-mail: imfakai@yahoo.com

In vitro antioxidant properties of *Musa paradisiacal* Peel aqueous extract

Fakai I. M., A.U. Birnin-Yauri, James Jemaima*

Abstract

Plants and plants products are the major sources of natural antioxidant which can be used to ameliorate oxidative stress caused by reactive oxygen species in biological systems. *In vitro* antioxidant activity of *Musa paradisiacapeel* aqueous extract was evaluated. Sodium nitroprusside was used to induce lipid perioxidation in rat pancreas. The total phenol, flavonoid and vitamin C contents were determined using standard methods of analysis. OH radical scavenging ability, Fe^{2+} chelating ability and lipid peroxidation were also analyzed using standard methods. The result revealed substantial amount of total phenol (0.76 ±0.04 mg/g), total flavonoid (0.53 ±0.04 mg/g) and vitamin C contents (24.11 ±0.41 mg/100 g). The result also revealed increased OH radical scavenging ability, Fe^{2+} chelating ability and MDA inhibition with increase in concentration of the extract used. The present study therefore, revealed that *Musa paradisiacapeel* aqueous extract could serve as source of natural antioxidant which can be used to ameliorate some disease associated with oxidative stress.

Keywords: Antioxidants, Phenol, Aqueous extract, *Musa paradisiaca*, Free radicals.

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. When cells use oxygen to generate energy, free radicals are generated. These byproducts 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.[1] 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).[1] 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.[2] 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.[1] ROS cytotoxicity can be used to describe how toxic they can potentially be. Exposure to cytotoxic substances can result in cellular damage or even death. Substances that can be described as cytotoxic can include some chemicals such as Sodium nitroprusside (SNP) or even other types of cells. When it comes to chemicals, some naturally produced ones can come in the form of animal venom, such as in some scorpions and snakes.[3]

Sodium nitroprusside (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.[4]

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.[5] 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.[6] 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.[7] Among plant used for medicinal purposes to cure some ailments is Plantain which belongs to the *Musa parasidiaca* family and is cultivated in many tropics and subtropical countries of the world. It ranks third after yam and cassava for sustainability in Nigeria.[8]

Millions of people in developing nations, including Nigerians, have resorted to the use of plants to treat their ailments; this could be due to the high cost of orthodox health care or as a result of the global shift towards the use of natural sources, rather than synthetic drugs.[9] Plantain (*Musa paradisiaca*) is a major food crops in the humid and sub-humid parts of Africa and a major source of energy for millions of people in these regions.[10] The annual world production of plantain is estimated at 75 million tones.[11] Plantains have been reported to be an important source of vitamin A in parts of Asia, Africa and Latin America.[12]

Plantains are a good source of vitamin A (carotene), vitamin B complex (thiamin, niacin, riboflavin and B6) and vitamin C (ascorbic acid). Plantains provide a better source of vitamin A than most other staples.[13] They are notably high in potassium and low in sodium.[14] Carotenoid-rich foods protect against certain chronic diseases, including diabetes, heart disease and cancer.[15] 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.[16] Unripe plantain meal is usually consumed by Nigerian diabetics to reduce postprandial glucose level.[17] Plantain is employed in the folklore management of diseases such as diabetes, ulcer and wound healing due to its hypoglycaemic, anti-ulcerogenic and analgesic properties.[18] Earlier report on the glycemic indices of green plantain products had been documented.[4, 17]

Natural antioxidants are powerful substances that rare capable of scavenging ROS or neutralize free radicals before they damage the body's cells. In recent years, phenolic compounds have attracted the interest of researchers because of their antioxidant capacity; to protect the human body from free radicals, whose formation has been associated with the natural metabolism of aerobic cells. The antioxidant activity of phenolics is mainly due to their redox properties, which gives them the capacity to act as reducing agents, hydrogen donors, metal chelators, free radical scavenger and singlet oxygen quenchers.[19] The antiradical activity of flavonoids and phenols is majorly based on the structural relationship between the functional groups on their chemical structure.[20] Various methods have been developed for screening antioxidant activity of various classes of compounds. The reason for this is to search for novel natural antioxidants in plants and vegetables that may be of importance in pathologies involving reactive oxygen species.[21] Recently there has been an upsurge of interest in the therapeutic potentials of medicinal plant as anti-oxidant in reducing free radical induced tissue injury. Besides well known traditionally used anti-oxidants from other source that has been already exploited commercially either as antioxidant additives or as nutritional supplement, Recent investigations suggest that *Musa paradisiaca* fruit has antioxidants effect with free-radical scavenging properties which has a therapeutic importance in free radical mediated diseases like diabetes, cancer, neurodegenerative disease, cardiovascular diseases, aging, gastrointestinal diseases, arthritis, and aging process. Therefore, since there is no or little literature documented on the antioxidant properties of the *Musa paradisiaca* peel, it is imperative to evaluate its antioxidants effect which may have a therapeutic importance in free radical mediated diseases.

Aim and Objectives

The aim of the present study was to evaluate the antioxidant activities of the aqueous extract of *Musa paradisiacal* peel and to assess the inhibitory action of the extract on sodium nitroprusside induced lipid peroxidation in rat pancreas *in vitro* using parameters such as total flavonoid, total phenol, vitamin C, Fe^{2+} chelation assay, OH radicals scavenging ability and Lipid peroxidation assay.

Materials and Methods

Preparation of Samples

The plantain fruits werepeeled and the peels were sliced and shade-dried for about 4 weeks to a constant weight and ground into powder. The powder sample was passed through a local made hand sieve. The fine powder sample was kept in an air tight dry container at room temperature (25 °C) and later used for the analysis.

Aqueous extract preparation

Ten grams (10 g) of the powder sample was soaked in 100 ml distilled water for about 24 hrs. The mixture was filtered using a whattman no.1 filter paper, the filtrate was centrifuged to obtain a clear supernatant liquid, whichwas subsequently used for the various assays.

Experimental animal.

Three (3) male albino rats aged 8-12 weeks weighing between 150±200 g were used for the experiment. The rats were caged in metal cages and were allowed to acclimatise with the

environment after which it was sacrificed and the pancreas was carefully removed and used for lipid peroxidation analysis.

Determination of total phenol content

The total phenol content was determined according to the method as described by Singleton *et al*.[22] Briefly, the aqueous extracts were 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. The total phenol content was subsequently extrapolated from the standard gallic acid curve.

Determination of total flavonoid content

The total flavonoid content of was determined using the method as described by Meda *et al*.[23] Briefly, 0.5 mL of sample was mixed with 0.5 ml methanol, 50 µl of 10% $AlCl_3$, 50 µl of 1 mol/L potassium acetate and 1.4 ml water. The reaction mixture was incubated at room temperature for 30 min. Thereafter, the absorbance of the reaction mixture was measured at 415 nm in the spectrophotometer. Total flavonoid content was extrapolated from the quercetin standard curve.

Determination of vitamin C content

Vitamin C content was determined using the method as described by Benderitter *et al*.[24] Briefly, 75 µl DNPH (2 g dinitrophenyl hydrazine, 230 mg thiourea and 270 mg $CuSO_4 \cdot 5H_2O$ in 100 ml of 5 mol/L H_2SO_4) was added to 500 µl reaction mixture (300 µl of the extracts with 100 µl 13.3% trichloroacetic acid and water). The reaction mixture was subsequently incubated for 3 h at 37 °C, then 0.5 ml of 65% H_2SO_4 (v/v) was added to the medium and the absorbance was measured at 520nm using a spectrophotometer. The vitamin C content of the extracts was subsequently calculated.

$$\text{Vit. C conc.} = \frac{Absorbance\ \ of\ test}{Absorbance\ \ of\ std} \times conc\ of\ std$$

Fe^{2+} Chelating assay

The Fe^{2+} chelating ability of the extract was determined using a modified method as described by Puntel *et al*.[25] Freshly prepared 500 µmol/L $FeSO_4$ (150 µl) was added to a reaction mixture containing 168 µl of 0.1 mol/L Tris-HCl (pH 7.4), 218 µL saline and the extracts (0-100 µl). The reaction mixture was incubated for 5 min, before the addition of 13 µl of 0.25% 1,10-phenanthroline (w/v). The absorbance was subsequently measured at 510 nm in a spectrophotometer. The Fe^{2+} chelating ability was subsequently calculated with respect to the control using the expression below.

$$\%\ Fe^{2+}\ \text{chelating ability} = \frac{A_{control} - A_{sample}}{A_{control}} \times 100$$

OH radicals scavenging ability

The ability of the extract to prevent $Fe^{2+}H_2O_2$ induced decomposition of deoxyribose was carried out using the method as described by Halliwell and Gutteridge.[26] Briefly, freshly prepared aqueous extract (0-100 ml) was added to a reaction mixture containing 120 ml 20 mmol/L deoxyribose, 400 ml 0.1 mol/L phosphate buffer, 40 ml 20 mol/L hydrogen peroxide and 40 ml 500 mmol/L FeSO4, and the volume was made to 800 ml with distilled water. The reaction mixture was incubated at 37 °C for 30 min, and the reaction was stopped by the addition of 0.5 ml of 2.8% trichloroacetic acid; this was followed by the addition of 0.4 ml of 0.6% thiobarbituric acid solution. The tubes were subsequently incubated in boiling water for 20 min. The absorbance was measured at 532 nm in spectrophotometer. The OH radicals scavenging ability was the determined using the expression below.

$$\%\ \text{OH radical scavenging ability} = \frac{A_{control} - A_{sample}}{A_{control}} \times 100$$

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 mortar and pastle. 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.

Lipid peroxidation and thiobarbituric acid reactions

The lipid peroxidation assay was carried out using the modified method as discibed by Ohkawa *et al*.[27] 100 µl of S1 fraction was mixed with a reaction mixture containing 30 µl of 0.1 mol/L Tris-HCl buffer (pH 7.4), sample extract (0-100 µl), and 30 µl of the pro-oxidant solution (7 µmol/L sodium nitroprusside). The volume was made up to 300 µl with water before incubation at 37 °C for 1 h. The colour reaction was developed by adding 300 µl 8.1% sodium dodecyl sulfate to the reaction mixture containing S1; this was subsequently followed by the addition of 600 µl of acetic acid/HCl (pH 3.4) and 600 µl 0.8% TBA. This mixture was incubated at 100 °C for 1 h. The thiobarbituric acid reactive species produced were measured at 532 nm. A graph of MDA produced was ploted against extract concentration.

Results and Discussion

Table 1: Total phenolic contents, total flavonoid contents, vitamin C content of aqueous extract of *Musa paradisiacapeel*

Parameters	Result
Total phenol (mg/g)	0.76 ±0.04
Total flavonoid (mg/g)	0.53 ±0.04
Vitamin C (mg/100g)	24.11 ±0.41

Data are represented as mean±SD, *n*=3

Table 2: EC_{50} antioxidant activities of theaqueous extract of *Musa paradisiacapeel*

Parameters	EC_{50} of antioxidant activities (mg/ml)
OH radical scavenging ability	6.66±0.77
Fe^{2+} chelating ability	10.67±1.23

Data are represented as mean±SD of 3 replicate.

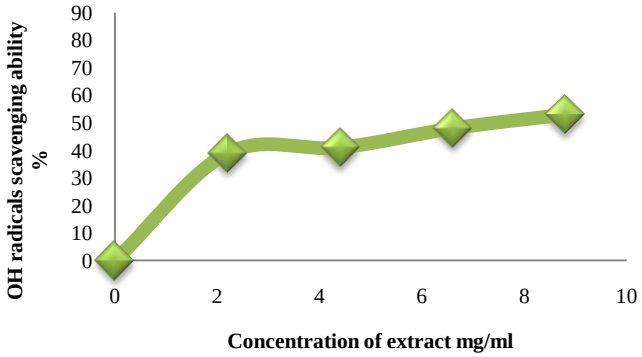

Figure 1: OH radical scavenging ability of aqueous extracts of *Musa paradisiacapeel*

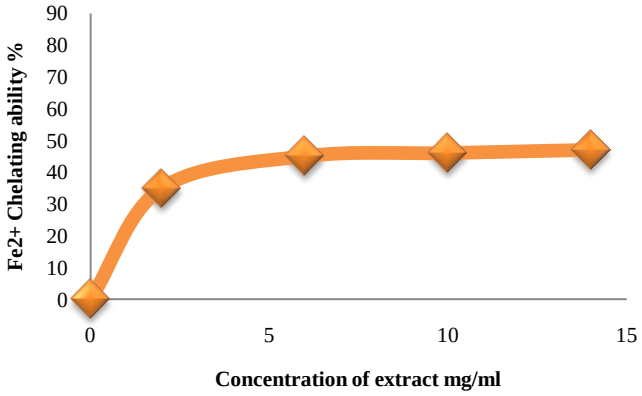

Figure 2: Fe^{2+} chelating ability of aqueous extracts of *Musa paradisiacapeel*

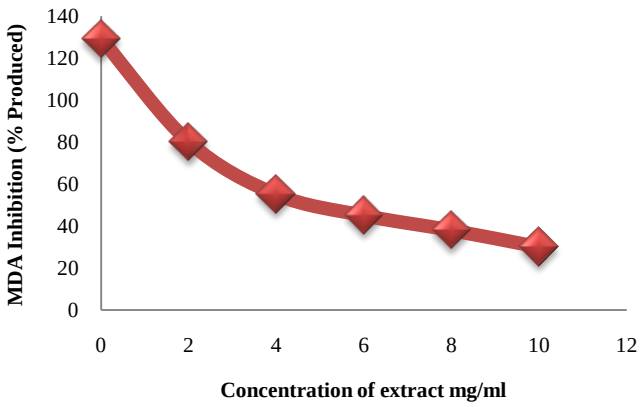

Figure 3: Inhibition of sodium nitroprusside induced lipid peroxidation of pancreas by aqueous extract of *Musa paradisiacapeel*

Antioxidant activity in higher plants has often been associated with phenolic compounds.[28] In addition to their roles in plants, phenolic compounds in our diet may provide health benefits associated with reduced risk of degenerative diseases.[19] The mechanism of action of antioxidants chelate and deactivate transition metals thereby prevent such metals from participating in the initiation of lipid peroxidation and oxidative stress through metal catalysed reaction.[29] Overproduction of reactive oxygen species (ROS) can also have a direct attack on the polyunsatureated fatty acids of the cell membrane to induce lipid peroxidation.[30] The deleterious effect caused by iron is done by reacting with hydrogen peroxide to produce hydroxyl radical (OH.) through Fenton reaction. Superoxide can also react with Fe^{3+} to regenerate Fe^{2+} which can participate in the Fenton reaction. Up till this present time, there are consistent suggestions that ROS induce lipid peroxidation in cell membranes and all effort is concentrated on terminating the deleterious effects of free radicals. The measurement of H_2O_2 radical scavenging activity is one of the designed methods of estimating the ability of anti-oxidants to reduce the level of pro-oxidants. Hydrogen peroxide is not very reactive all by its self, but could be toxic to cells when there is an increase in its concentration in the cells.[31] In this study, there is a clear indication that the aqueous extracts of plantain peels were able to chelate and/or scavenge the Fe and OH and this adds to the fact that the plantain peels have Fe^{2+} chelating ability.

The introduction of a functional group containing two catenated oxygen atoms O-O in a free radical reaction lead to the generation of lipid peroxidation which have both direct and indirect effects in organisms. Its binding process has been connected with malonaldehyde formation which has been designated a biomarker of free radical-mediated damage and oxidative stress.[20] The release of cyanide and/or nitric oxide (NO) in sodium nitroprusside can cause cytotoxicity.[31] NO is a free radical with a short half-life (<30 s). Although, the independent action of NO may cause neuronal damage in cooperation with other reactive oxygen species such as superoxide radical to form peroxynitrite radical. owever, the result revealed that extract had MDA inhibitory activity. Hence, plantain peels may be suggested to partake in carrying out inhibitory activity by preventing cellular peroxidation of lipids.

Vitamin C contributes majorly to the antioxidant activities of plant food. Ascorbic acid is a good reducing agent and exhibits its antioxidant activities by electron donation. This is done by oxidizing tocopheroxyl free radical into dehydroascorbate. Vitamin C (non-enzymatic) antioxidant reaction scavenges the free radical pathway by destroying both the initiation and propagation reactions that promotes lipid peroxidation. Eventually, this mechanism has been tagged a harmless one.[32]

However, the result of the vitamin C for the plantain peels is higher when compared with some natural fresh fruit juices.[19]

Phenolics are one of the largest and the most widely studied groups of phytochemicals. They are widely reported to possess remarkable antioxidant and medicinal properties. Flavonoids are very large in number. Most of the antioxidant and medicinal properties credited to phenolics have been attributed to the function of flavonoids. In addition, each group of flavonoid has the capacity to exert antioxidant properties.[33] The Total phenolic content measures the total amount of phenolics, which include flavonoids. Total flavonoid content is designed to quantify the amount of flavonoids. The total phenol and total flavonoid content of extracts have been often reported as promising medicinal and nutritional ingredients.[34] The total phenol content of the plantain peels product is higher than some commonly consumed tropical plants.[35] Furthermore, the trend of the total phenol content and total flavonoid content results are in contrast to the earlier report which revealed that there is a direct relationship between the total phenol content and the antioxidant activity in some plant foods. The major ingredients of nutraceuticals have been identified as flavonoids.

Flavonoids such as quercetin, kaempferol, morin, myricetin and rutin act by exerting antioxidant effects such as anti-inflammatory, antiallergic, antiviral and anticancer activity. They have also been reported to protect against liver diseases, cataracts, and cardiovascular diseases. Quercetin for example have been revealed to also protect against liver reperfusion ischemic tissue damage.[33] As well as a good inhibitor of α-amylase activity.[36] The chemical structures such as the unsaturated C ring, 3-OH, 4-CO, the linkage of the B ring at the 3 position and the hydroxyl substitution on the B ring of flavonoids have also been reported to be responsible for α-amylase and α-glucosidase activity. As observed, the presence of polyphenol constituents could have contributed immensely to the antioxidant properties of this product. Reports have shown that flavonoids could exert a better protection by preventing the progressive impairment of pancreatic beta-cell function due to oxidative stress and may thus reduce the occurrence of type 2 diabetes. This could be achieved by lowering the level of lipid, glycosylated hemoglobin and postprandial blood glucose as well as increasing the insulin sensitivity when compared with the effect of some commercial drugs like arcabose and viglibose.[37] The result of the extract which exhibited a high Fe^{2+} chelating ability agreed with the earlier report where phytochemicals of the functional groups such as OH and C=O present in apigenin and naringenin may be responsible for the Fe^{2+} chelating ability of the phenolic-rich extract.[33]

Conclusion

The results of the present work highlighted antioxidant activities present in the plantain peels. The results also indicated that plantain peels is protective against reactive oxygen species. On the basis of the studied data on antioxidant activities identified, it could be suggested that the consumption of plantain peels or use as a supplement could render a multifaceted action that will terminate the instant generation of free radicals thereby setting the human system free from accumulation of radicals.

Considering the antioxidant activities and ability to inhibit lipid peroxidation of plantain peel, this could justify their traditional use in the management/prevention of diseases related to stress.

Recommendation

The screening carried out on the *Musa pradisiaca*peel in the research was focused on the invitro antioxidant activity.

The above findings recommend the following:

- There is need for further research on the *in vivo* antioxidant activity of the *Musa pradisiaca*peel to ascertain its effectiveness
- Further research could be carried out on *Musa pradisiaca*peel with the view to making it suitable for pharmaceutical, cosmetics, nutraceutical and food & beverage industries.

References

1. Pham-Huy, L.A., He, H. and Pham-Huyc, C. Free Radicals, Antioxidants in Disease and Health. Int. J. Biomed. Sci., 2008; 4: 89-96.

2. Clayson, D.B., Mehta, R. and Iversin, F. Oxidative DNA damage the effects of certain genotoxic and operationally non-genotoxic carcinogens. Mutat. Res., 1994; 317: 25-42.

3. McCord, J.M. and Fridovich I. The Reduction of Cytochrome C by Milk Xanthinse Oxidase. J. Biol. Chem. 1968; 243:5733-5760.

4. Oboh, H.A. and Erema, V.G. Glycemic indices of processed unripe plantain (*Musa paradisiaca*) meals.Afr J Food Sci., 2010; 4:514-521.

5. Yamaguchi, F., Ariga, T., Yoshimira, Y. and Nakazawa, H. Antioxidant and antilycation of carcinol from *Garcinia indica* fruit rind. J. Agric. Food Chem., 2000; 48: 180-185.

6. Edward, P.C. Leaf and Febiger Philadelphia, 1956, p. 731.

7. Douglas, C.J. Phenylpropanoid metabolism and lignin biosynthesis: From weeds to trees. Trends Plant Sci., 1996; 1: 171-178.

8. Akomolafe, O.M. and Aborisade, A.T. Effects of stimulated storage conditions on the quality of plantain (*Musa paradisiaca*) fruits.Int. J. Agric. Res., 2007; 2(12): 1037-1042.

9. Omonkhua, A.A. and Onoagbe, I.O. Evaluation of the long-term effects of Urenalobataroot extracts on blood glucose and hepatic function of normal rabbits. J of Toxicol Environ Health Sci, 2011; 3:204-213.

10. Asiedu. R., Ng, S.Y.C., Vuylsteke. D., Terauchi, R. and Hahn, S.K. Analysis of the need for biotechnology research on cassava, yam and plantain. In: Thottappilly, G., Monti, L., Mohan-Raj, D.R. and Moore, A.W. (Eds.): Biotechnology: Enhancing research on tropical crops in Africa. CTA/IITA co-publication. IITA, Ibadan, Nigeria, 1992, pp. 376.

11. John, P. and Marchal, J. Ripening and biochemistry of the fruit. In: Gowen SR. (Eds): Bananas and Plantains, Chapman and Hall, London, UK. 1995; 15 (6): 238

12. Sommer, A. New imperatives for an old vitamin A. J of Nutrition, 1989; 119: 96-100.

13. Aurand, W.L. Food Composition and Analysis of Food, Von Nostrand Reinhold, New York, 1987, pp. 135-138.

14. Marriott, J., Robinson, M. and Karikari, S.K. Starch and sugar transformations during ripening of plantains and bananas.J Sci of Food and Agr., 1983; 32: 1021-1026.

15. Ford, E.S., Will, J.C., Bowman, B.A. and Narayan, K.M. Diabetes mellitus and serum carotenoids: findings from the third national health and nutrition examination survey. Am J Epidemiol. 1993; 149:168-176.

16. Dadzie, B.K. and Orchard, J.E. Routine Post Harvest Screening of Banana/Plantain Hybrids: Criteria and Methods, INIBAP Technical Guidelines 2. International Plant Genetic Resources Institute, Rome, Italy; International Network for the Improvement of Banana and Plantain, Montpellier, France; ACP-EU Technical Centre for Agricultural and rural Cooperation,Wageningen, The Netherlands. 1997.

17. Willett, W., Manson, J.and Liu, S. Glycemic index, glycemic load, and risk of type 2 diabetes.Am J Clin Nutrition.2002; 76:274S-80S.

18. Bischoff, H. Pharmacology of alpha-glucosidase inhibition.Eur J Clin Invest., 1994; 24:3-10.

19. Mahdavi, R., Nikniaz, Z., Rafraf, M. and Jouyban, A. Determination and comparison of total polyphenol and vitamin C contents of natural fresh and commercial fruit juices.Pak J Nutr. 2010; 2: 968–972.

20. Dauqan, E.M.A., Abdullah, A. and Sani, H.A. Natural antioxidants: Lipid profile, lipid peroxidation, antioxidant enzymes of different vegetable oils. Adv J Food SciTechnol 2011; 3(4):308–316.

21. Sarvajeet, S.G. and Narendra, T. Reactive oxygen species and antioxidant machinery in abiotic stress tolerance in crop plants.Plant PhysiolBioch. 2010; 48:909–930.

22. Singleton, V.L., Orthofer, R.And Lamuela-Raventos, R.M. Analysis of total phenols and other oxidation substrates and antioxidants by means of Folin-Cioalteau reagent.Methods Enzymol. 1999; 299:152–178.

23. Meda, A., Lamien, C.E., Romito, M., Millogo, J. and Nacoulma, O.G. (2005).Determination of the total phenolic, flavonoid and proline contents in Burkina Faso honey, as well as their radical scavenging activity.Food Chem.91:571–577.

24. Benderitter, M., Maupoil, V., Vergely, C., Dalloz, F., Briot, F. and Rochette, L. Studies by electron paramagnetic resonance of the importance of iron in hydroxyl scavenging properties of ascorbic acid in plasma: Effects of iron chelators. FundamClin Pharmacol.1998; 12:510–516.

25. Puntel, R.L., Nogueira, C.W. and Rocha, J.B.T. Krebs cycle intermediates modulate thiobarbituric acid reactive species (TBARS) production in rat brain in vitro. Neurochem Res. 2005; 30:225–235.

26. Halliwell, B. And Gutteridge, J.M.C. Formation of thiobarbituric-acid-reactive substance from deoxyribose in the presence of iron salts: The role of superoxide and hydroxyl radicals. FEBS Lett.1981; 128:347–352.

27. Ohkawa, H., Ohishi, N. and Yagi, K. Assay for lipid peroxides in animal tissues by thiobarbituric acid reaction. Anal Biochem. 1979; 95:351–358.

28. Adefegha, S.A. and Oboh, G. In vitro inhibition activity of polyphenol-rich extracts from *Syzygium aromaticum* (L.) Merr.& Perry (Clove) buds against carbohydrate hydrolyzing enzymes linked to type 2 diabetes and Fe2+-induced lipid peroxidation in rat pancreas. Asian Pac J Trop Biomed. 2012; 2(10):774–781.

29. Oboh, G, Puntel R.L. and Rocha J.B.T. Hot pepper (*Capsicum annuum*, Tepin and *Capsicum chinese*, Habanero) prevents Fe2+-induced lipid peroxidation in brain-in vitro. Food Chem. 2007; 102:178–185.

30. Oboh, G. and Rocha, J.B.T. Polyphenols in red pepper (*Capsicum annuum* var. aviculare (Tepin)) and their protective effect on some pro-oxidants induced lipid peroxidation in brain and liver. Eur. Food Res. Tech., 2007; 225: 239-247.

31. Oboh, G. and Rocha, J.B.T. Hot pepper (*Capsicum* spp.) protects brain from sodium nitroprusside- and quinolinic acid-induced oxidative stress in vitro. J Med Food. 2008; 225:349–355.

32. Moreira, D., Silva, F., Marques, A. and Chaveiro A. Reactive oxygen species: a double-edged sword in reproduction. The Open Vet Sci 2010; J:127–133.

33. Tapas, A.R., Sakarkar, D.M. and Kakde, R.B. Flavonoids as nutraceuticals.Trop J Pharm Res. 2008; 1089–1099.

34. Akinmoladun, A.C., Obuotor, E.M. and Farombi, E.O. Evaluation of antioxidant and free radical scavenging capacities of some Nigerian indigenous medicinal plants. J Med Food. 2010; 13:444–451.

35. Iyawe, H.O.T. and Azih, M.C. Total phenolic contents and lipid peroxidation potentials of some tropical antimalarial plants.Eur J Med Plants. 2011; 4:33–39.

36. Li, Y.Q., Gao, F., Gao, F., Shan, F., Bian, J.S. and Zhao, C.G. Study on the Interaction between 3 flavonoid compounds and r-amylase by fluorescence spectroscopy and enzymatic kinetics. J Food Sci. 2009; 6: C199–C203.

37. Li, Y.Q., Zhou, F.C., Gao, F., Bian, J.S and Shan F. Comparative evaluation of quercetin and rutin as inhibitors of α-glucosidase.J Agric Food Chem. 2009; 15:11463–11468.