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Comparative Evaluation of Peeled and Unpeeled Unripe Plantain on Liver Function Indices and Oxidative Stress Markers in Wistar Rats

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Abstract
Plantain (Musa paradisiaca) is attracting significant research interest for its medicinal and nutritional properties. However, empirical information on the biochemical effects of a plantain peel-based diet is limited. In the current study, we carried out a comparative evaluation of the biochemical effects of peeled and unpeeled unripe plantain-based diets (UPBD&PPBD) on liver function indices and redox status in Wistar rats. Three groups of six male Wistar rats each were created. Group A was the control diet group, while Group B consisted of a peeled unripe plantain diet group, and Group C consisted of an unpeeled unripe plantain diet group. Proximate analysis of the unripe plantain-based diets showed that the percentage of moisture, ash, carbohydrates (CHO), lipid, crude fiber, and protein were 10.21%, 6.50%, 60.23%, 7.44%, 1.03%, and 14.59%, respectively while UPBD was 12.66%, 4.59%, 59.99%, 7.62%, 4.53% and 11.59%, respectively. The liver function analysis revealed no significant increase in liver enzymes alanine aminotransferase (ALT), aspartate aminotransferase (AST), alkaline phosphatase (ALP), and gamma-glutamyltransferase (GGT) levels among the groups. Malondialdehyde (MDA) levels did, however, significantly rise in the unpeeled unripe plantain group indicating the presence of lipid peroxidation. While catalase activity increased for the group exposed to PPBD, superoxide dismutase (SOD) and glutathione (GSH) activity did not change substantially across the groups. Collectively, the findings suggest that both peeled and unpeeled unripe plantain could serve as functional food or sources of antioxidant nutrients to promote good health.

Keywords: Fruits; Functional foods; Redox balance; Supplementation; Toxicity.

1. Introduction

Plantain is one of the earliest fruits to be cultivated in West and Central Africa (Fakayode et al., 2011). For both large-scale and smallholder farmers in Nigeria, producing plantains has grown to be a substantial source of revenue, especially for those who cultivate them in their own backyards or gardens. Ogede agbagba, ayaba, and Ogadejioke are the names of plantains in the local Yoruba, Hausa, and Igbo languages, respectively. It belongs to the genus Musa, all of whose species are native to tropical Southeast Asia and Oceania, including Indonesia, Malaysia, Brunei, the Philippines, and Northern Australia (Randy et al., 2007). Plantain is a tall, strong herb with an above-ground pseudostem comprised of tightly packed leaves from which the flowering stalk emerges. Underground is where the real stem or rhizome is. Numerous clusters of sterile male flowers are present near the flower stalk's apex, and they are encircled by vivid purple bracts. The fruit is produced by the lower female flower clusters on the meristem. A hand is a single group of 8–12 fingers, and the fruit is referred to as fingers (Pol et al., 2021). In many areas of the world, plantains are a major food source. According to estimates from (Morais-Lino et al., 2016), these crops are produced worldwide in excess of 76 million metric tons yearly, with Africa alone generating an estimated 12 million metric tons. According to (Pereira & Maraschin, 2015), plantains have historically been used to treat inflammatory illnesses, various gastrointestinal disorders, wound healing, and respiratory disorders. Plantain contains tannins and flavonoids, which are stated to contribute to its medicinal properties (Asuquo & Udobi, 2016). Plantain has long been applied topically for the treatment of wounds. Plantain leaves have antibacterial and wound-healing characteristics, which (Amutha &

Selvakumari, 2016) emphasized. They indicated that applying plantain leaves to wounds speeds up healing and avoids infections. Plantain has been used in traditional medicine to treat respiratory diseases and treat symptoms like cough, bronchitis, and asthma. (Agama-Acevedo et al., 2016) highlighted the mucilage content of plantains' calming effects on the respiratory system. Plantain's flavonoids, alkaloids, and phenolic compounds were discovered to be possible bioactive substances that inhibit inflammatory mediators and lessen pain perception (Kapadia et al., 2015).

The numerous nutritional and functional benefits of peeled plantain qualify it as a nutraceutical with potential health advantages. Its presence in a balanced diet can help general well-being by enhancing wound healing, reducing inflammation, and improving digestive health (Pereira & Maraschin, 2015). Usually, Plantain peels are dumped as garbage and are either burned or allowed to decompose by farmers. However, given the availability of this waste material in areas where plantains are cultivated, there is potential to use plantain peels as a supplement in both human and animal feed to lower the cost of feeding, waste, and livestock production. It is essential to look into any potential health implications of unripe plantain peels before adding them to feed.

(Oladiji et al., 2021) asserts that plantain peels can substitute for cornstarch in the food of snails and serve as a source of protein for rats, boosting growth and healthy organ development without having any negative impacts on blood integrity. However, there is currently limited information on the biochemical effects of unripe plantain peel-based diets. Therefore, this study compared the effects of peeled and unpeeled unripe plantain diets on markers of oxidative stress and liver function in Wistar rats.

2. Materials and Methods

Unripe plantain

Unripe plantain (Musa paradisiaca) fruits that are matured were obtained from within Omu-Aran market, Kwara State, Nigeria.

Experimental animals

Eighteen (18) albino rats, about 8 weeks old weighing between 180 and 200 g, were procured from the animal holding facility of the Department of Biochemistry, Landmark University, Omu-Aran, Kwara State, Nigeria.

2.1. Chemicals and reagents

Analytical-grade chemicals and reagents were employed throughout the study, and they were all maintained in optimum laboratory conditions.

2.2. Plantain-based diet formulation

Unripe plantains were screened to remove bad ones. The fruits were divided into two halves and washed. A portion was peeled, sliced, and oven-dried at 50°C for 24 h. The second half was sliced without peeling and oven-dried at 50°C for 24 h. The dried samples of both peeled and unpeeled unripe plantain were milled separately with a local grinding machine and stored in an airtight container for formulation.

2.3. Feed formulation

Three different diets were formulated: a control diet, a diet containing unpeeled unripe plantain, and a diet containing peeled plantain, this is shown in Table 1 below.

Table 1. Components of the formulated diets

Ingredients	Control (g)	Unpeeled (g)	Peeled (g)
Corn meal	59.6	-	-
Fish meal	20.0	20.0	20.0
Groundnut cake	10.0	10.0	10.0
Vitamin/mineral mix	5.0	5.0	5.0
Methionine	0.4	0.4	0.4
Fiber	5.0	5.0	5.0
Plantain flour	-	59.6	59.6
Total	100	100	100

2.4. Animal groupings

All experimental protocols were approved by the Ethics Committee of Landmark University, and animal experiments conducted in this study have been performed in accordance with the National Research Council's Guide for the Care and Use of Laboratory Animals. The rats were kept together in typical laboratory cages for seven (7) days to help them acclimate, during which time they were given access to free water and conventional rat food. They were then assigned into three (3) dietary groups as shown below based on their weight.

Group 1(control) - Fed a control diet

Group 2 (PPBD) - Fed peeled unripe plantain-based diet

Group 3 (UPBD) - Fed an unpeeled unripe plantain-based diet.

Following a 24-hour fast, their separate trial diets and water were introduced. The trial lasted four (4) weeks, and the rats in each group had complete access to their specific meals.

2.5. Blood sampling and serum preparation

After 28 days, the rats were euthanized by putting them to sleep in a jar filled with cotton wool that had been soaked in diethyl ether. Blood samples were then drawn from the rats' jugular veins and placed in unassuming sample vials. One hour was given for the blood in the simple sample bottles to coagulate. To obtain sera, the clotted blood samples were spun in an Eppendorf centrifuge (Hamburg, Germany). The serum samples were thereafter separated into another set of plain sample tubes and stored at a temperature of -20°C pending enzyme assay.

2.6. Preparation of tissue homogenate

Rats from each group had their liver, heart, kidney, and stomach carefully removed and preserved for histological purposes in a sample container containing formalin. The liver was transferred to a beaker filled with ice-cold 0.25 M sucrose solution. The liver tissue was homogenized in a pestle and mortar that had been pre-cooled and placed in a bowl of ice cubes. Following homogenization, the resultant homogenates were diluted using a 1 in 5 dilution ratio by adding 0.25 M sucrose solution. The diluted homogenates were then stored at a temperature of -20°C until they were ready to be used for further analysis or experimentation.

2.7. Biochemical assays

Liver Function Markers – ALT and AST Randox laboratories Ltd. assay kits were used. The GGT activity was measured using Szasz, 1969 techniques. The ALP activity was assessed using the technique developed by (Wright et al., 1972). Malondialdehyde (MDA) concentration was measured using the Satoh, (1978) method for identifying oxidative stress indicators. The (Misra &Fridovich, 1972) approach was used to describe the activity of superoxide dismutase (SOD). Beers & Sizer, (1952) method for analyzing catalase activity and Ellman, (1959) method for determining reduced glutathione (GSH) level activity were both used.

2.8. Statistical analysis and data presentation

Data are expressed as means ± standard deviation of three replicate measurements. One-way analysis of variance and Dunnett's multiple comparison tests were used to evaluate the data; differences between means were considered significant at $p< 0.05$.

3. Results

3.1. Proximate analysis

Table 2 shows the moisture content of the PPBD sample was 10.21%, which was lower than that of the UPBD sample. This difference may be attributable to the unripe plantain's peeling, which can expose a larger surface area to the air and cause moisture to evaporate. The presence of inorganic components is indicated by a value of 6.50% ash content. The sample had a significant carbohydrate composition, with a carbohydrate content of 60.23 %. The PPBD sample's calorific value, which measures the amount of energy released after burning, was found to be 1530.04 Kj/100g. Crude fiber and protein content were found to be 1.03% and 14.59%, respectively, while the lipid content was 7.44%. The moisture percentage of the UPBD sample was greater than the PPBD sample at 12.66%, indicating a little higher water content which may be a result of its protective skin that reduces the exposed surface area. With a reduced ash percentage of 4.59%, there may be fewer inorganic components present. Similar to the PPBD sample, the amount of carbs was determined to be 59.99%. With a greater calorific value of 1632.88 kj/100g, the UPBD sample showed more energy. Crude fiber and protein compositions were 4.53% and 11.59%, respectively, while the lipid content was 7.62%.

Table 2. Proximate analysis of peeled and unpeeled unripe plantain-based diet

SAMPLE	Moisture (%)	Ash (%)	CHO (%)	Calorific Value (Kj/100g)	Lipid (%)	Crude Fiber (%)	Protein (%)
PBD	10.21	6.49	60.23	1530.04	7.44	1.03	14.59
UPBD	12.66	4.59	59.99	1632.88	7.61	4.53	11.59

(PBD- Peeled plantain-based diet, UPBD- Unpeeled plantain-based diet)

3.2. Liver function indices

The liver enzyme ALT is frequently used as a measure of liver health. According to McGill, (2016), elevated ALT levels in the blood may be a sign of liver illness or injury. Based on Figure 1A, there is a slight increase in the peeled group compared to the control and a slight increase in the unpeeled group compared to both the peeled and control groups, suggesting that there may be some variation in the ALT levels between the groups. However, these differences are not statistically significant, suggesting that the diet may have little to no impact on the liver health of albino rats when compared to the control diet, which is primarily composed of corn. Aspartate aminotransferase, often known as AST, is an enzyme that is mostly found in the liver, heart, and muscles. It's vital to remember that AST activity can also be influenced by characteristics including age, sex, and exercise. Elevated levels of AST activity in the blood can signal liver or muscle injury (Khattab et al., 2015). The lack of substantial changes in AST activity levels between the groups, as shown in Figure 1B, may indicate that the albino rats' liver or muscle function was not significantly affected by the diets, at least in terms of AST activity. Increased activity of one particular enzyme, which is present in the liver, bones, and intestines among other body tissues, may signal liver or bone disease, whereas decreased activity may signify improved liver and bone function.

Table 3. Effect of peeled and unpeeled unripe plantain-based diet on the AST-ALT ratio of Wistar rats

Groups	ALT/AST ratio (IU/ml)
Control	0.54
Peeled plantain-based diet	0.52
Unpeeled plantain-based diet	0.61

A diagnostic technique for evaluating the health of the liver is the ALT/AST ratio. If the ratio is noticeably more than 1, it can indicate liver disease, such as cirrhosis or hepatitis (Goddard & Warnes, 1992). On the other hand, according to [20], if the ratio is lower than 1, it may indicate other conditions, such as muscle injury or alcoholism. In this study, as shown in Table 3, the ALT/AST ratio between the three groups was 0.7, and the difference between the groups revealed no statistically significant difference ($p > 0.05$), suggesting a reasonably balanced ratio between the liver enzymes ALT (alanine aminotransferase) and AST (aspartate aminotransferase).

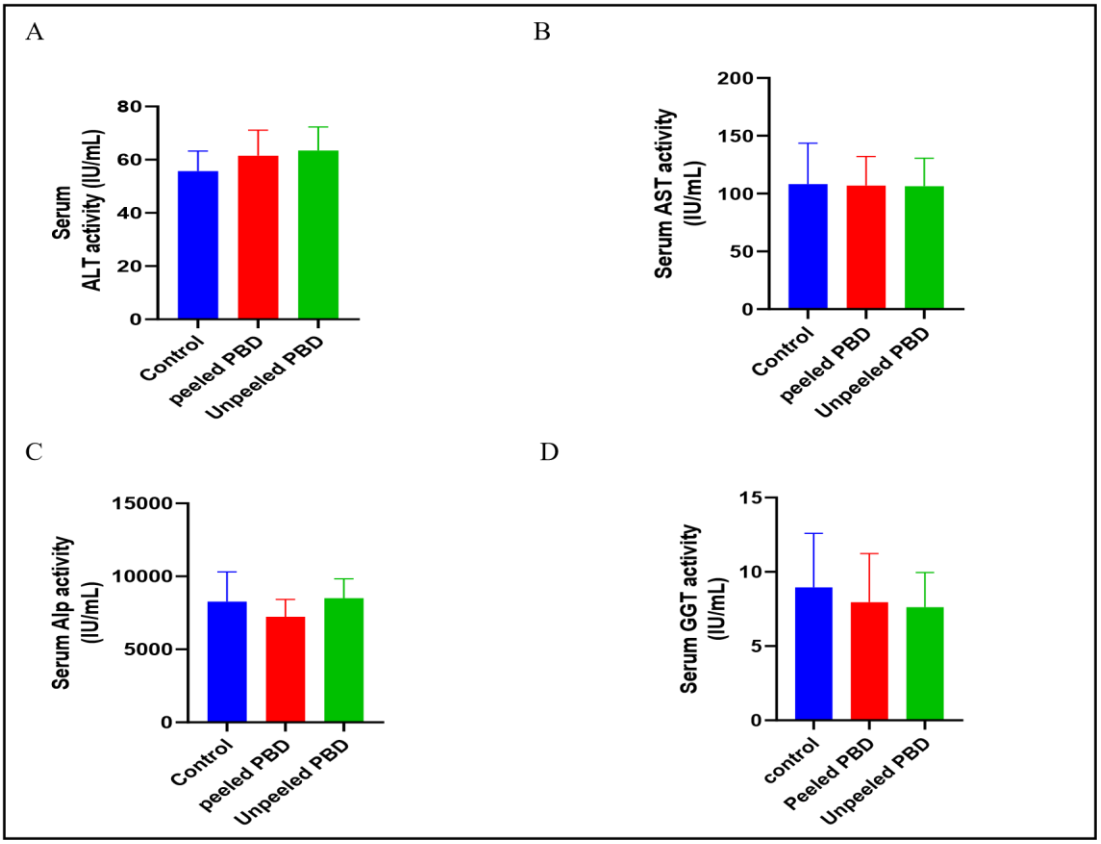

Figure 1: Effects of peeled and unpeeled unripe plantain-based diets (PBD) on alanine aminotransferase A), aspartate aminotransferase B), alkaline phosphatase C), and gamma-glutamyl transferase D) activity of Wistar rats. Values are presented as mean ± SD, n=6. Differences were considered significant at p< 0.05.

Analyzing Figure 1C, it can be seen that compared to the control group, the peeled group's enzyme activity was slightly lower, whereas that of the unpeeled group was slightly higher. However, these variations in enzyme activity between the groups did not reach statistical significance, indicating that the Wistar rats' diets, which included both peeled and unpeeled unripe plantains, had no appreciable effect on the ALP enzyme levels relevant to liver and bone function. GGT is a liver-specific enzyme, and increased levels of GGT activity in the blood can signify liver disease or damage. On the other hand, reduced GGT activity might point to improved liver health (Sugiura et al., 2005). The levels of GGT between the groups are not significantly different, according to Figure 1D. This suggests that the albino rats' GGT activity was not significantly affected by their ingestion of either peeled or unpeeled plantain diets.

Summarily, the comparative evaluation of unpeeled and peeled unripe plantain on liver function indices in rats showed no statistically significant differences in ALT, AST, ALP, and GGT levels between the groups. Collectively, the data suggest that the peeled and the unpeeled unripe plantain-based diets might not have affected the liver functions adversely. The results on rat liver function indices in this study are consistent with an earlier study that demonstrated that the usage of plantain peels had no damaging effects on rat blood (Idoko et al., 2011).

3.3. Antioxidant Biomarkers

Oxidative stress refers to a conflict between the body's antioxidant defense mechanisms and the production of reactive oxygen species (ROS). This imbalance can cause cellular harm and aid in the emergence of several disorders. The unripe plantain-based diet's ability to modulate redox was revealed by measuring the levels of glutathione (GSH), catalase, superoxide dismutase (SOD), and malondialdehyde (MDA). One of the main lipid peroxidation by products is MDA, which is employed as a biomarker of cell membrane damage (Grotto et al., 2009). The unpeeled unripe plantain group had the highest serum MDA levels, followed by the peeled unripe plantain group and then the control group, as shown in Figure 2G. However, there was no statistically significant difference between the control

and peeled unripe plantain groups. The unpeeled unripe plantain group had significantly higher MDA levels than the control group, however, there was little or no difference in MDA levels in the liver between the control and peeled unripe plantain groups. These results suggest that the consumption of unpeeled unripe plantain may predispose to oxidative stress in rat liver and serum. SOD plays an important role in protecting the body against oxidative stress (Van Deel et al., 2008). Figures 2A and B showed no significant difference in serum and liver among the three groups (control, PPBD, and UPBD). There is no significant decrease in the SOD activity for either peeled or unpeeled unripe plantain when compared to the control group.

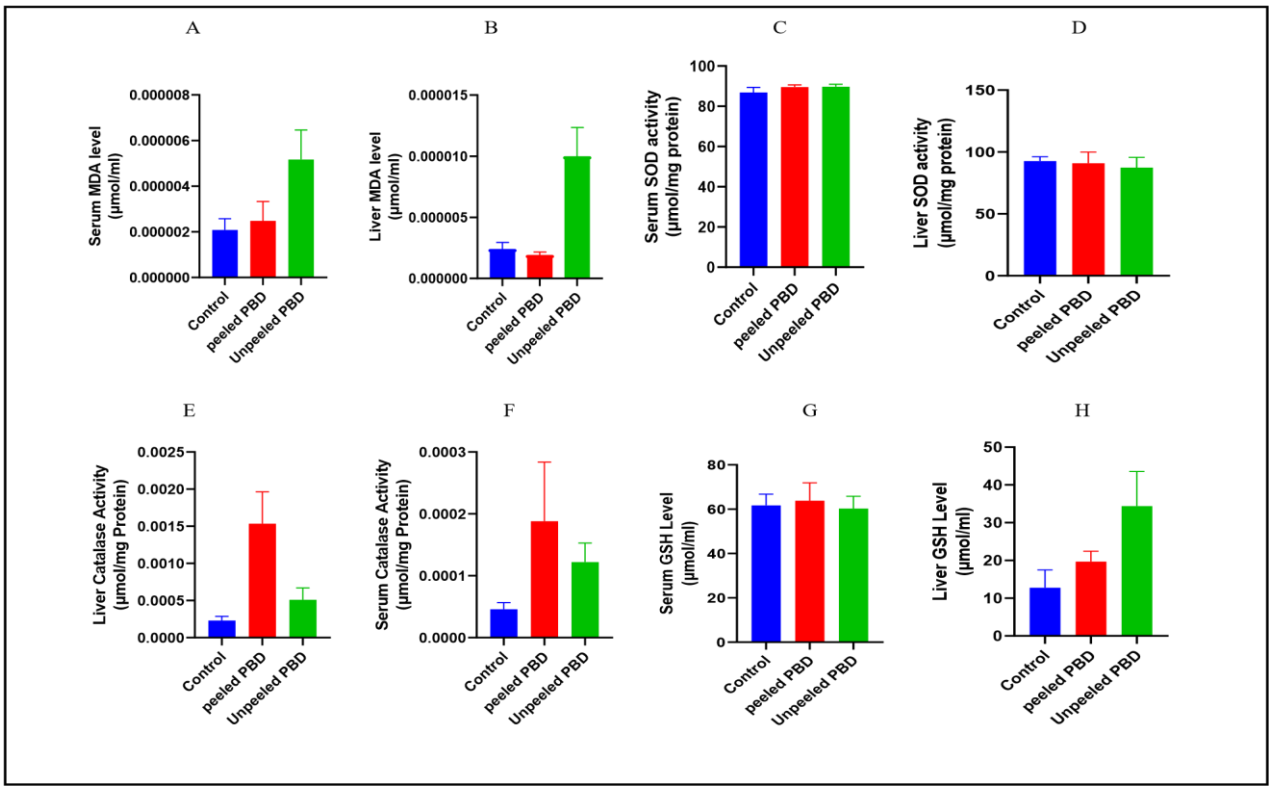

Figure 2: Effects of peeled and unpeeled unripe plantain-based diets (PBD) on plasma A), and liver B) malondialdehyde level, plasma C), and liver D), sodium dismutase level, plasma E), and liver F), Catalase level, plasma G), and liver H) glutathione level of Wistar rats. Values are presented as mean ± SD, n=6. Differences were considered significant at p< 0.05.

Overall, the findings imply that unripe plantains may function as an alternative dietary supply of important antioxidant elements and that peeled and unpeeled unripe plantains may have equivalent effects on SOD activity in rats. The essential antioxidant enzyme catalase shields cells from reactive oxygen species (ROS) damage by converting toxic hydrogen peroxide into molecular oxygen and water (Chelikani et al., 2008). It can be found in high concentrations in the liver and blood, among other tissues where it is essential for preserving cellular health. According to Figs. 2E and F, both the peeled and unpeeled unripe plantain groups had higher levels of catalase activity in the rat serum and liver when compared to the control. The results suggest that the consumption of both peeled and unpeeled unripe plantains may have a positive effect on catalase activity in rats. Therefore, incorporating peeled or unpeeled unripe plantain into the diet may boost antioxidant status to mitigate oxidative stress in rats.

GSH (glutathione), a tripeptide molecule made up of glutamate, cysteine, and glycine functions as an antioxidant and is essential for cellular redox processes. Based on the present results (fig 2C), there was a slight increase in the GSH levels in rat serum for the group fed peeled unripe plantain-based diet compared with the control or the unpeeled unripe plantain groups. Similarly, there was no significant difference in the serum GSH levels between the control and unpeeled

plantain-based diet groups. This implies that the consumption of both peeled and unpeeled unripe plantain diets might not have a significant impact on GSH activity in rats. In contrast, in the liver, the GSH levels were significantly different among the three groups in the following order; control < peeled unripe plantain < unpeeled unripe plantain. These findings imply that unpeeled unripe plantain eating may increase the levels of GSH, a crucial antioxidant, and detoxifier, in the rat liver. Collectively, the findings on SOD, catalase, and GSH are consistent with previous research that established that the peel of different fruits such as mango, and apples possessed antioxidant properties (Idoko et al., 2011).

4. Conclusion

In the current investigation, the results demonstrated that the consumption of both unripe plantain diets did not exert any detrimental effects on rat liver indices. However, the unpeeled plantain diet might have caused oxidative stress in rat serum and liver. Nevertheless, the peeled or unpeeled plantain diets seem to improve the antioxidant status, particularly catalase activity in rat serum and liver. Taken together, the findings support the prospects of unripe plantain (peeled or unpeeled) as functional foods or as an alternative source of antioxidant nutrients to promote good health**.**

Declarations

Ethical approval Handling of experimental animals was in accordance with international guidelines, and the procedure was approved by the by Landmark University Ethical Committee (Approval number: LUAC/BCH/2023/0001A).

Competing interests The authors declare no competing interests.

Conflict of interest The authors declare no competing interests.

Authors' contributions Project design: E.T.O., and O.M.O.
Experimental: E.T.O.
Data analysis: E.T.O.
Writing of original draft: E.T.O., and O.S.A.
Writing review and editing: E.T.O., A.G.O., and O.S.A.

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References

Agama-Acevedo E., Sañudo-Barajas J.A., Vélez De La Rocha R., González-Aguilar G.A., Bello-Peréz L.A., Potential of plantain peels four (Musa paradisiaca L.) as a source of dietary fiber and antioxidant compound, CyTA- Journal of Food Science, 14 (2016)117–123.

[1] Amutha K., Selvakumari U., Wound healing activity of methanolic stem extract of Musa paradisiaca Linn. (Banana) in wistar albino rats, International Wound Journal, 13(5) (2016) 763-767.

[2] Asuquo E.G., Udobi C.E., Antibacterial and toxicity studies of the ethanol extract of Musa paradisiaca leaf, Cogent Biology, 2(1) (2016)1219248.

[3] Beers R.F., Sizer I.W., A spectrophotometric method for measuring the breakdown of hydrogen peroxide by catalase, Journal of Biology chemistry, 195(1) (1952)133-140.

[4] Chelikani P., Fita I., Loewen P.C., Diversity of structures and properties among catalases, Cellular and Molecular Life Sciences, 61(2008)192–208.

[5] Ellman G.L., Tissue sulfhydryl groups: Archives of Biochemistry and Biophysics, 82(1) (1959)70-77.

[6] Fakayode B.S., Rahji M.A, Ayinde O., Nnom G.O., An economic assessment of plantain production in Rivers State, Nigeria, International Journal of Agricultural Economics & Rural Development, 4(2) (2011) 28-36.

[7] Goddard C.J., Warnes T.W., Raised liver enzymes in asymptomatic patients: investigation and outcome, Digestive Diseases, 10(1992) 218 – 226.

[8] Grotto D., Maria L.S., Valentini J., Paniz C., Schmitt G., Garcia. S.C.., Importance of the lipid peroxidation biomarkers and methodological aspects for malondialdehyde quantification. Quimica Nova, 32 (2009)16974.

[9] Idoko A.S., Oladiji A., Aska A., Growth and Hematological Parameters of Rats Fed Diets with or without Ripe Plantain Peel (Musa paradisiaca), Journal of Environment, Technology & Sustainable Agriculture, 2(1)(2011) 82-86.

[10] Kapadia S.P., Pudakalkatti P.S., Shivanaikar S., Detection of antimicrobial activity of banana peel (Musa paradisiaca L.) on Porphyromonas gingivalis and Aggregatibacter actinomycetemcomitans, an in vitro study, Contemporary Clinical Dentistry, 6 (2015)496–499.

[11] Khattab H., Fouad A., Hamza M., Mohey M.A., El-Akel W., Ghoneim H., Esmat G., Relation of ALT and AST levels to the histopathological changes in liver biopsies of patients with chronic hepatitis C genotype 4, Arab Journal of Gastroenterology, 16(2) (2015)50-53.

[12] Majhi S., Baral N., Lamsal M., Mehta K.D., De Ritis ratio as diagnostic marker of alcoholic liver disease. Nepal Medical College Journal, 8(1) (2006)40-2.

[13] McGill M.R., The past and present of serum aminotransferases and the future of liver injury biomarkers, EXCLI journal, 15 (2016)817.

[14] Misra, H. P., & Fridovich, I. (1972). The role of superoxide anion in the autoxidation of epinephrine and a simple assay for superoxide dismutase. Journal of Biological Chemistry, 247(10), 3170–3175.

[15] Morais-Lino L.S., Santos-Serejo J.A., Amorim E.P., Santana J.R., M. Pasqual, S.S. Oliveira, Somatic embryogenesis, cell suspension, and genetic stability of banana cultivars, In Vitro Cellular & Developmental Biology-Plant, 52 (2016) 99-106.

[16] Oladiji A.T., Yakubu M.T., Idoko A.S., Adeyemi O., Salawu M.O., Studies on the physicochemical properties and fatty acid composition of the oil from ripe plantain peel (Musa paradisiaca), African Scientist, 11(1) (2021).

[17] Pereira A., Maraschin M., Banana (Musa spp) from peel to pulp: ethnopharmacology, source of bioactive compounds and its relevance for human health, Journal of ethnopharmacology, 160 (2015)149-163.

[18] Pol M., Schmidtke K., Lewandowska S., Plantago lanceolata- An overview of it's agronomically and healing valuable features, Open Agriculture, 6(1) (2021) 479-488.

[19] Randy C., Ploetz A., Jeff D., Scot C.N., Banana and plantain – an overview with emphasis on the Pacific island cultivars, Species Profiles for Pacific Island Agroforestry, 1 (2007) 21-32.

[20] Satoh K., Serum lipid peroxide in cerebovascular disorder determined by a new colometric method, Clinica Chimica Acta, 90 (1978)37-43.

[21] Sugiura M., Nakamura M., Ikoma Y., High serum carotenoids are inversely associated with serum gammaglutamyl transferase in alcohol drinkers within normal liver function, Journal of epidemiology, 15 (2005)180186.

[22] Szasz G., A kinetic photometric method for serum γ-glutamyl transpeptidase, Clinical chemistry, 15(2) (1969)124-136.

[23] Van Deel E.D., Lu Z., Xu X., Zhu G., Hu X., Oury T.D., Chen Y., Extracellular superoxide dismutase protects the heart against oxidative stress and hypertrophy after myocardial infarction. Free Radical Biology and Medicine, 44(7) (2008) 1305-1313.

[24] Wright P.J., Leathwood P.D., Plummer D.T., Enzymes in rat urine: alkaline phosphatase, Enzymologia, 42(4) (1972)317-327.