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Comparative Studies on Anti-Diabetic Properties of Commonly Consumed *Musa* Cultivars (*M. paradisiaca, M sapientum,* and AAB Group Hybrid) Flour

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ABSTRACT

Musa cultivars are popularly consumed after the peel had been removed. However, various studies had shown that the peels contained vital nutrients, phytochemicals, and antioxidant properties. This study compared the anti-diabetic potentials of *M. paradisiaca* pulp with whole (peel and pulp) flours of three frequently eaten Musa cultivars (*M. paradisiaca, M sapientum, and AAB group hybrid*). The aqueous extracts prepared from pulverized *M. paradisiaca* pulp (PP), whole hybrid (WH), whole *M. sapientum,* (WB), and whole *M. paradisiaca* (WP)were analyzed for Glycemic indices, *in-vitro* antioxidant potentials as well as inhibitory effect on the α-amylase and α-glucosidase enzymes. The three studied flours showed low glycemic index values in the range of (49.33-52.91), similar to (51.60) of PP flour. WH, WB, and WP flours revealed higher total flavonoids, DPPH, FRAP, and α –amylase compared to PP flour. The PP flour conversely exhibited an increase in OH* radical scavenging ability as well as increased α –glucosidase inhibitory abilities compared to the other studied flours. These results indicated that all tested samples were low glycemic index food. However, WH compared most favorably with PP in its total phenols, total flavonoids, FRAP, and α-amylase inhibitory activities compared to the other tested samples and hence could be taken into consideration when managing diabetic issues.

Keywords: Peels, Anti-diabetic, Glycemic indices, Antioxidants, Plantain, Whole flours.

Introduction

Diabetes mellitus (DM) is a chronic metabolic disorder that causes the malfunction of the body from full or partial glucose utilization with the characteristic of excessive accumulation of free blood glucose.[1] This has been attributed to the inability of the pancreatic β-cells to secrete insulin (a hormone secreted to regulate blood sugar level) (Type-1 diabetes) and/or the inability of the body cells to utilize the secreted insulin (type 2 diabetes.[2] WHO estimated global diabetes prevalence come 2030 as high as 10.2%, an indication that millions of people would be diagnosed with the disease.[3] Since a carbohydrate-based diet has been a major consumed diet, its effect has been reported to enhance an individual's likelihood of developing diabetes.[4] The management of this chronic disease necessitates a variety of treatments such as medications, a healthy dietary lifestyle, and nutritional supplements,[5] which are costly and need a lifetime commitment. Therefore, it is necessary to look for different meals that can control and minimize the occurrence of diabetes. Recently, foods with high antioxidant properties, low glycemic indexes (scales that determine how quickly specific carbohydrate foods raise blood glucose levels),[6] and high inhibitory properties on key enzymes related to carbohydrate digestion[7] had been purported as the ideal diet in the management of diabetes. The generic name *Musa* was given to plantain (*Musa paradisiaca*) and other related groups of fruits that are morphologically similar and economically and nutritionally valuable.[8] These fruits are one of the most affordable staple meals which can be found nearly all year round everywhere in the world. The use of plantain flour in its unripe form had since been the preferred diet for diabetes based on the evidence of its low glycemic indices and the high component of resistant starch.[9,10] Flour made from unripe plantain pulp was widely seen as appropriate while the peels which contained 40% of the entire fruit are typically regarded as waste and discarded afterward.[11] Apart from the environmental pollution impact exerted by the generated peel waste, there is alternative usage of the peels in the manufacturing of biodiesel, conventional soaps, and livestock feeds.[12,13] Interestingly, most fruit peels had shown higher antioxidant activities and higher fiber content when compared to their respective pulp.[14-16] This was also proven by a study that reported appreciable levels of nutrients, micronutrients, and low content of antinutrients for plantain peels, thereby suggesting its utilization as a dietary supplement and nutraceuticals.[17] Moreso, the production of flour from whole plantain and banana hybrids has lately gained notice for increasing the economic worth of the crops as well as their nutritional content based on the active compounds contained in the peels.[18] Therefore, it is imperative to investigate the potential health benefits that could be derived from the consumption of whole fruits with a view to creating a disease-preventive diet as well as boosting food options. Hence, this study explores and compares the anti-diabetic effects of whole fruit (peel+pulp) flour derived from three commonly consumed Musa cultivars (plantain, banana, and AAB hybrid group) with plantain pulp and presents them as possible diet in diabetes control.

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Materials and Methods

Materials

Fresh matured unripe plantain (*M. paradisiaca*), banana (*M. sapientum*), and AAB hybrid group fruits were bought on 25th January 2021 from Owena, a local market in Ondo East local government of Ondo State. The samples were validated at the Department of Crop,

Soil, and Pest Management where the sample herbarium was preserved with voucher code number FUTA/CSP/1327, and their authenticity was subsequently confirmed at the Center for Research and Development, Federal University of Technology, Akure. The fruits were divided into whole and pulp portions; the whole portion contained the peel and pulp of the samples while the pulp portion contained plantain pulp only. The pulp portion was prepared by separating the pulp from the whole fruit. One kilogram of each sample was thinly sliced separately and then sun-dried until a constant weight was achieved. Thereafter, the dried samples were pulverized and stored in an airtight container for analysis.

Preparation of sample
Each sample's aqueous extract was prepared by dissolving two grams of flour in 20 mL of distilled water and shaking vigorously in an orbital shaker for 8 hours. Thereafter, each mixture was filtered with Whatman no1 filter papers and the filtrate was collected and refrigerated at 4^{o}c for subsequent analysis.

Tissue preparation
The small intestine of a Pig was isolated and homogenized in 0.1 M, pH 6.9 phosphate buffers. The homogenized tissue was centrifuged at 2000 rpm for 15 mins while the supernatant was collected and used for the estimated glycemic index and enzyme inhibitory analysis according to Adebayo's method.[19]

Total phenolics
The content of total phenolic extracts was evaluated by using 2.5 mL of 10% Folin-Ciocalteau reagents (v/v) to oxidize each sample extract for 5 minutes, according to Singleton's method.[20] The mixture was further neutralized with 2.0 mL 7.5% sodium carbonate and incubated for 40 mins at 45°C with the absorbance measured using a spectrophotometer at 765 nm. Thereafter, the amount of total phenol was determined and expressed as gallic acid equivalent (GAE).

Total flavonoids
A technique described by Meda[21] with slight modification was adapted in determining the total flavonoid content of the extracts. 0.5 mL of each sample extract was first mixed with 0.5 mL methanol, and further addition of 50 mL of 10% $AlCl_3$, 50 mL of 1 M potassium acetate, and 1.4 mL of distilled water. The sample mixture was incubated for 30 minutes at 25°C. Afterward, the reaction mixture's absorbance was assessed at 415 nm, while the total flavonoid concentration was determined and represented as quercetin equivalent (QUE).

Antioxidant assays
DPPH radical scavenging ability
The ability to scavenge DPPH (1, 1-diphenyl– 2 picrylhydrazyl) free radical by each extract was investigated according to the method described by Gyamfi.[22] 1 mL of different sample concentrations (50, 100, 150, 200 µL) was added to 1 mL of 0.4 mM methanolic solution of DDPH radicals. The reaction mixture was incubated for 30 mins in the dark and thereafter, the absorbance was measured at 516 nm.

*ABTS and OH*radical scavenging ability*
The 2,2-azino- bis (3-ethylbenzthiazoline- 6- sulphonic acid) (ABTS) radical of each extract was evaluated using the method of Re.[23] 0.2 mL of each extract was added to 1.8 mL ABTS reagent and the reaction mixture was incubated for 15 minutes in the dark, while the absorbance at 734 nm was measured Spectrophotometrically. Furthermore, the extract's capacity to neutralize the hydroxyl radical caused by the Fe^{2+}/H_2O_2-induced degradation of deoxyribose was also assessed using the Halliwell and Gutteridge[24] techniques.

Ferric-reducing antioxidant property
The sample extract's capacity to reduce $FeCl_3$ solution was studied according to the method of Oboh.[25] (0-0.1 mL) of each sample extracts were mixed with 0.168 mL Tris-HCl (pH 7.4) and 0.218 mL of normal saline while the reaction mixture was incubated at 37°C for 5 minutes After that, freshly prepared $FeSO_4$ (0.15 mL) and (0.013 mL) 1,10-phenanthroline were added to the reaction mixture, and the absorbance was measured spectrophotometrically at 510 nm.

Determination of total starch, sugar, amylose, and amylopectin
The Sugar and starch content was investigated according to Onitilo's[26] method. 0.02 g of each sample extract was treated with 10mL (80%) hot ethanol and thereafter centrifuged at 2000 rpm for 10 mins. 0.2 mL of aliquots from the supernatant was added to 0.5 mL (5%) phenol with further addition of 2.5 mL concentrated H_2SO_4, while the reaction mixture was read at 490 nm spectrophotometrically. The recovered residue was then tested for starch content using Adedayo's[27] methods. The residue was treated with 7.5 mL of Perchloric acid for an hour and filtered. Following that, 0.05 mL aliquots was treated with 2.5 mL concentrated H_2SO_4 and 0.5 mL (5%) phenol. The reaction mixture was cooled, and the absorbance was measured spectrophotometrically at 490 nm. The amylose content was determined by mixing 0.1 g of each sample extract with 1 mL of 95% ethanol and 9.0 mL of 1 N NaOH. Afterward, 0.1 mL of 1 N acetic acid solution and 0.2 mL of iodine solution (0.2% I_2 in 2% KI) were added to the reaction mixture and incubated at 25^{o}c for 20 mins. Finally, the absorbance of the sample mixture was measured at 620 nm, while the real amylose obtained was used to calculate Amylopectin content.

Estimated Glycemic Index (eGI)
The estimated glycemic index was determined according to the method described by Oboh.[28] 0.05 g of each sample extract was measured and added to 5 mL porcine stomach solution (KCl-HCl buffer pH 1.5). Samples were incubated with porcine tissue at varying time intervals for adequate digestion and the aliquots were subjected to further analysis while the absorbance was measured spectrophotometrically at 540 nm.

Alpha amylase and glucosidase inhibitory assay
The sample extract's inhibitory effects on *α*-amylase and *α*-glucosidase activities were investigated according to the method of Adefegha[29] respectively. 0.5 mL of each sample extract was diluted with 0.5 mg/mL porcine pancreatic *α*-amylase prepared in 0.02 M pH 6.9 Sodium phosphate buffer with 0.5 mg/mL and 2.5 mL (0.02 M pH 6.9) Sodium Phosphate buffers. Afterward, the mixture was incubated at 25^{o}C for 10 mins. Thereafter, 0.5 mL 1% starch was added to the reaction mixture and the reaction was terminated by the addition of 2.0 mL dinitrosalicylic acid reagent while the absorbance was measured at 540 nm. Likewise, 0.05 mL of each sample extract was subjected to dilution by the addition of 1.0 mL porcine intestinal *α*-glucosidase with 4.45 mL (0.02 M) phosphate buffer pH 6.9. The reaction mixture was incubated at 37^{o}c for 20 mins after which 0.4 mL p-nitrophenyl-α-D-glucopyranoside solution with 2 mL (1%) sodium bicarbonate was added and the absorbance was measured at 400 nm while the enzyme inhibitory percentage was expressed as percentage inhibition for the α-glucosidase inhibitory assay.

Statistical analysis
The mean and standard deviation were calculated using descriptive statistics, and their significant differences ($p<0.05$) were evaluated using analysis of variance (ANOVA) and Tukey's test for multiple comparisons. Analysis and graphs design were also generated using the software GraphPad Prism version 5.00 (GraphPad Prism Software, Inc).

Results and Discussion

The results of the anti-diabetic effect of aqueous extract of the whole hybrid (WH), whole banana (WB), and whole plantain (WP) flours compared with plantain pulp (PP) are presented in Table 1. The reference PP is significantly different ($P<0.05$) from other studied cultivars with reduced starch content (22.44) and increased amylose (40.50) content. Whereas the result of the sugar (7.30) content exhibited by the PP flour differed significantly ($P<0.05$) from other studied cultivars while WH flour revealed reduced sugar content (5.7). The result from the Glycemic index (GI) showed no significant difference ($P<0.05$) between the PP, WH, and WB flour (51.60, 52.91, and 51.58) respectively. However, a lower GI (49.33) data was revealed in WP flour compared to other studied flour.

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Table1: The starch, sugar, amylose, and amylopectin contents, the glycemic index of Musa cultivars flour

SAMPLE	Plantain Pulp (PP)	Whole hybrid (WH)	Whole banana (WB)	Whole plantain (WP)
GLYCEMIC INDICES				
Sugar (g/100g)	7.30 ± 0.11	5.7 ± 0.07	8.25 ± 0.2	9.68 ± 0.11
Starch (g/100g)	22.44 ± 0.7	43.82 ± 0.4	35.49 ± 0.5	42.15 ± 0.07
Amylose content (g/100g)	40.50	29.44	33.75	31.75
Amylopectin (g/100)	24.78	28.99	23.79	28.76
eGlycemic index (%)	51.60	52.91	51.58	49.33

Values represented mean± standard deviation of triplicate readings plantain pulp (PP), whole hybrid (WH), whole banana (WB), and whole plantain (WP).

The result from the total phenolic content of aqueous extracts of PP, WH, WB, and WP flour as represented in Fig. 1a revealed that the reference sample PP was significantly different (P<0.05) from WH flour with higher phenolic content compared to WB and WP. The total flavonoid level of the investigated samples revealed that PP flour was significantly different (P<0.05) from WH and WB, whereas WH exhibited the highest flavonoid content. From the results presented in Fig. 2a and b, it was also shown that the ABTS and DPPH radical scavenging ability of the PP flour differed significantly (P<0.05) from WH and WP. While increased scavenging ability on ABTS and DPPH was revealed in WP compared to other studied samples. The result of the reducing power (FRAP) of the studied samples WH, WB, and WP compared to PP flour as presented in Fig. 3a revealed that PP was significantly different from WH and WP with reduced power in PP flour. However, the OH radical scavenging ability shown in Fig. 3b for the studied sample flours revealed that PP flour differed significantly (P<0.05) from WH and WB as both samples exhibited reduced scavenging abilities compared to PP flour but no significant difference (P<0.05) was observed in WP flour. Fig.4 revealed that PP differed significantly (P<0.05) from the other studied sample flours (WH, WB, and WP) in Fe^{2+}-chelating ability. Moreso, no significant difference was observed in PP flour compared to other studied samples in the inhibition of the *α*–Amylase enzyme as presented in Fig. 5a except in WH flour with increased inhibitory ability. Contrastingly, the result of *α*-glucosidase inhibitory ability shown in fig.5b revealed that PP flour differed significantly (P<0.05) from other studied samples with increased PP inhibitory power compared to WH, WB, and WP.

Amylose is essential in preserving starch structure; a higher amylose concentration may result in a more compact starch granule. The present study showed a considerable amount of amylose and amylopectin than the one presented by Adefegha's[30] studies on cassava flour. Although there was no significant difference (P<0.05) in the amylose content of the three studied *Musa* cultivars flour, yet the PP revealed higher contents of amylose with reduced starch content compared to other studied flour. This could be attributed to the slightly lower susceptibility of the whole flour to enzyme hydrolysis as postulated by Alonso-Gomez[31] findings, which justifies its role as a diabetes diet. Artkinson[32] described glycemic index (GI) as a tool that determines the degree of rise in blood glucose level after the consumption of a carbohydrate-containing meal. Ingesting a high-GI diet rapidly raises blood glucose concentrations over the physiological range of ≥ 70 (a hyperglycemic state). To avoid a fast rise in postprandial blood glucose levels, a concept of the glycemic index is employed to serve as a guide in planning meals and for the selection of foods. All three unripe whole (WP, WB, and WH) flours showed low glycemic index values. These values were lower than the GI reported by Oboh[33] for unripe plantains. This could be attributed to higher fermentable sugars determined in the whole plant compared to pulp according to Alonzo-Gomez.[31] Effects of peel inclusion on the studied flour had accounted for high starch content[34] as well as high fiber content. Since, increased fiber content leads to decrease GI, this in turn decreases the release of glucose in the blood.[35] Phenolics and their derivatives-rich foods had been proven to play a major role in the prevention of chronic diseases via the direct scavenging of free radicals.

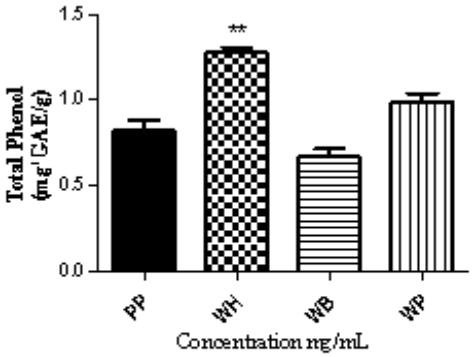

Fig. 1a Total phenol content of whole hybrid (WH), whole Banana (WB) and whole plantain (WP) compared with Plantain pulp (PP), value represent means ±SEM(n=4).* bars are statistically different (p<0.05) compared to PP

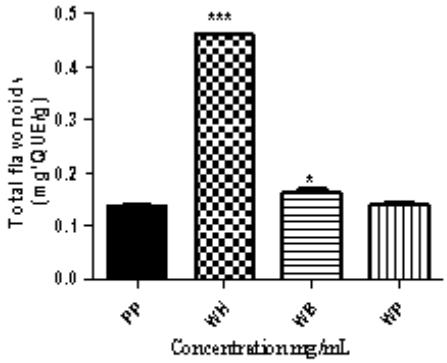

Fig. 1b Total flavonoids content of whole hybrid (WH), whole Banana (WB) and whole plantain (WP) compared with Plantain pulp (PP), value represent means ±SEM(n=4).*bars are statistically different (p<0.05) compared to PP

The total phenol and flavonoid contents of the tested samples as presented in figures 1a and 1b, showed WH to possess the highest values of total phenol and flavonoid contents when compared to other tested samples. The difference between WH and others was significant (p<0.05), according to Akin-Idowu.[36] However, hybrid cultivars had been proven to possess superiority over plantain in nutrient constituents. Although this data is lower than the result reported by Eleazu[37] and the discrepancy in values could result from the use of different sample preparation techniques. Antioxidants are the bioactive component in plant and animal tissues that prevents the oxidation of target cells or susceptible molecules, such as membrane lipids, from oxidation to preserve their functional and structural integrity.[38] Antioxidants fulfilled these functions in three ways: blocking the processes that produce free radicals, scavenging reactive species, and chelating or sequestering metals that can function as catalysts.

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Unhealthy eating patterns and lifestyle choices have been theorized as contributing factors to the production of free radicals that resulted in oxidative stress. As a result, epidemiological studies have revealed the biological importance of an antioxidant-rich diet[39] and how it can function in conjunction with other bioactive components of the sample to prevent and treat illnesses.[40] The ABTS and DPPH radical scavenging ability as expressed in terms of Trolox equivalent antioxidant capacity (TEAC) of the studied flour as well as FRAP and metal chelating ability as presented in Fig. 2a, b, 3a, and 4 respectively, revealed WH, WB, and WP to possessed most ability than PP flour. The presence and positioning of numerous hydroxyl groups on polyphenols, which have been proposed as powerful metal chelators are responsible for this metal chelating activity[41] expressed by these flours. However, the findings support the earlier report from Oboh's[28] research that validates the consumption of peels as food due to their high antioxidant activity and presence of phytochemicals.

The extracts of unripe WH, WB, and WP flours were also found to have a high inhibitory activity as seen from the value obtained for α – Amylase (Fig. 5a), the result contrasts the recent findings of Odubanjo[42] in which the hydrolyzing enzyme activities were highly inhibited by Plantain flour. Increased inhibition was revealed in WH compared to the PP flour, while α–Glucosidase in (Fig.5b) indicated higher inhibitory properties for PP flour compared to whole studied samples and this was also validated by recent findings of Odubanjo.[42] This could be a result of the presence of fermentable starches that are readily available as well as phenols and antioxidants with free radical scavenging activities in the unripe whole studied flour. High amylase inhibitory properties as revealed from the result indicate a reduction in the hydrolysis of starch to monosaccharides which can further prolong overall carbohydrate digestion time and thus prevent postprandial plasma glucose increase.

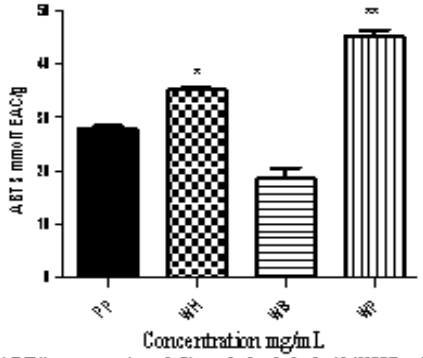

Fig. 2a ABTS scavenging ability of whole hybrid (WH), whole Banana (WB) and whole plantain (WP) compared with Plantain pulp (PP), value represent means ±SEM (n=4).* bars are statistically different ($p<0.05$) compared to PP

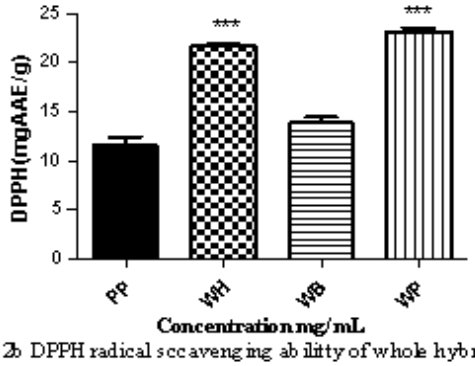

Fig. 2b DPPH radical scavenging ability of whole hybrid (WH), whole Banana (WB) and whole plantain (WP) compared with Plantain pulp (PP), value represent means ±SEM(n=4).
* bars are statistically different ($p<0.05$) compared to PP

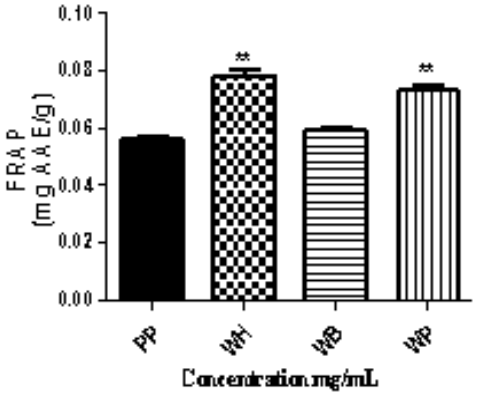

Fig. 3a FRAP radical scavenging ability of whole hybrid (WH), whole Banana (WB) and whole plantain (WP) compared with Plantain pulp (PP), value represent means ±SEM(n=4).* bars are statistically different ($p<0.05$) compared to PP

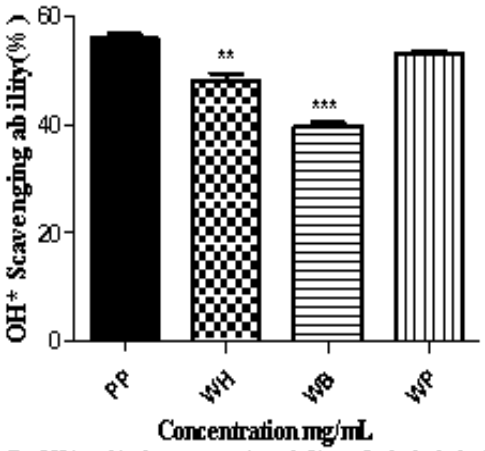

Fig. 3b OH* radical scavenging ability of whole hybrid (WH), whole Banana (WB) and whole plantain (WP) compared with Plantain pulp (PP), value represent means ±SEM(n=4).
* bars are statistically different ($p<0.05$) compared to PP

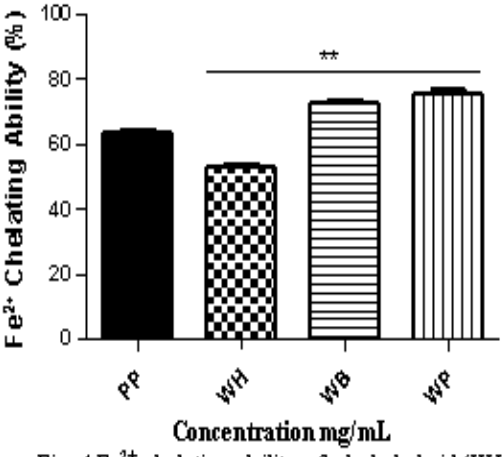

Fig. 4 Fe^{2+} chelating ability of whole hybrid (WH), whole Banana (WB) and whole plantain (WP) compared with Plantain pulp (PP), value represent means ±SEM (n=4).
* bars are statistically different ($p<0.05$) compared to P

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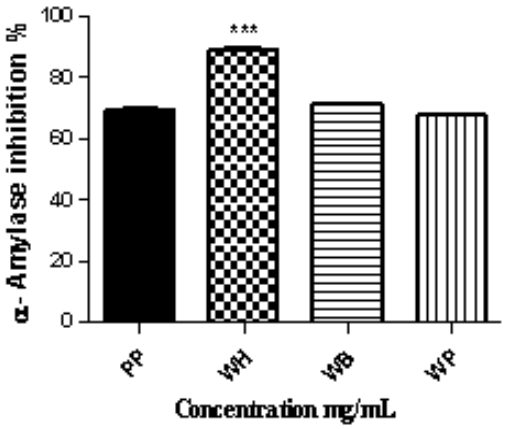

Fig. 5a α-Amylase inhibition activity of whole hybrid (WH), whole Banana (WB) and whole plantain (WP) compared with Plantain pulp (PP), value represent means ±SEM (n=4). * bars are statistically different ($p<0.05$) compared to PP

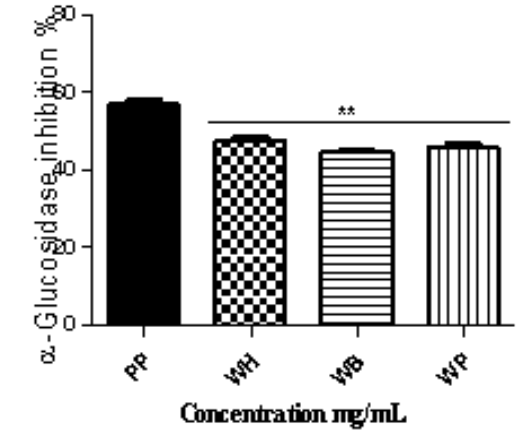

Fig. 5b α-Glucosidase ability of whole hybrid (WH), whole Banana (WB) and whole plantain (WP) compared with Plantain pulp (PP), value represent means ±SEM (n=4). * bars are statistically different ($p<0.05$) compared to PP

Conclusion

The overall studies suggested the studied flour (whole fruits of plantain, banana, and hybrid group) to be a rich source of antioxidants, low glycemic index, and high enzymes inhibitory capacity which can be attributed to the synergistic influence of the peel inclusion in the flour. The influence of peel on the anti-diabetic properties of the whole studied flours had been proven positive from this study. Hence, this study suggested unripe whole (pulp + peel) plantain, hybrid, and banana flour as suitable functional foods for the control and management of diabetes, thereby creating more food options as well as maximizing health potentials that could be derived from the waste.

Conflict of Interest

The authors declare no conflict of interest.

Authors' Declaration

The authors hereby declare that the work presented in this article is original and that any liability for claims relating to the content of this article will be borne by them.

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