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In vivo maltase and sucrase inhibitory activities of five underutilized Nigerian edible fruits

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Abstract.
BACKGROUND: Inhibition of intestinal maltase and sucrase prevents postprandial blood glucose excursions which is beneficial in ameliorating diabetes-associated complications.
OBJECTIVE: In this study, the inhibitory effects of fruit extracts of *Parinari macrophylla, Detarium microcarpum*, *Ziziphus spina-christi, Z. mairei* and *Parkia biglobosa* were investigated against intestinal maltase and sucrase.
METHODS: Rats were given co-administration of the fruit extracts with maltose or sucrose and blood glucose levels were measured at 0, 30, 90 and 120 min.
RESULTS: The glucose-time curves indicated that all the fruits had the most potent inhibitory effects on both maltase and sucrase within the first 30 min. The computed Area Under the Curves (AUC_{0-120}) for all the fruits indicated more potent inhibitory effects against intestinal maltase than sucrase. The ED_{50} range for the fruits extract against maltase and sucrase were 647.15–1118.35 and 942.44–1851.94 mg/kg bw respectively.
CONCLUSION: The data suggests that the fruits could prevent postprandial hyperglycemia via inhibition of intestinal maltase and sucrase.

Keywords: Diabetes mellitus, fruits, α-glucosidases, maltase, sucrase

1. Introduction

Diabetes mellitus is a heterogeneous group of metabolic disorder characterized by high blood glucose levels (hyperglycemia) with alterations in carbohydrate, lipid and protein metabolism resulting from defects in insulin secretion and/or action [1]. According to the International Diabetes Federation (IDF), about 382 million people

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are living with diabetes, a figure projected to increase to 592 million by the year 2035 [2]. Diabetes mellitus is primarily classified in to type 1 diabetes (insulin dependent) and type 2 diabetes (insulin independent) with the latter accounting for more than 90% of all diabetic cases in the world [2]. Type 2 diabetes (T2D) is a heterogeneous disorder characterized by a progressive decline in insulin action (insulin resistance), followed by the inability of pancreatic β-cells to compensate for insulin resistance (β-cell dysfunction) which leads to hyperglycemia [3].

Hyperglycemia is the hallmark of diabetes mellitus that plays vital roles in all aspects of the T2D pathology including macrovascular and microvascular complications associated with the disease [4, 5]. Also, postprandial hyperglycemia is known to immensely contribute to the overall glycemic control in T2D patients [6]. Therefore, the control of postprandial hyperglycemia is considered to be important in the treatment of T2D and prevention of its associated complications. Currently, the major therapeutic strategy for the management of postprandial hyperglycemia is through delaying the release of glucose by inhibiting carbohydrate hydrolyzing enzyme α-glucosidase (EC 3.2.1.20) in the digestive tract. The α-glucosidase is a multifunctional enzyme with distinct maltase and sucrase activities [6]. Inhibition of these enzymatic activities would retard gastrointestinal absorption of dietary carbohydrates by restricting the breakdown of linear or branched oligosaccharide units (like α-limit dextrins, maltose, sucrose, maltotriose) to produce glucose thereby preventing glucose absorption into the blood stream [7]. Consequently, this will ultimately reduce the flow of glucose from dietary carbohydrates into the bloodstream, thereby diminishing the postprandial hyperglycemia. Unfortunately, the leading α-glucosidase inhibitors, acarbose and miglitol, are often reported to produce diarrhea and other intestinal disturbances, with bloating, flatulence, cramping and abdominal pain occurring concurrently [8]. Randomized controlled trials with glucosidase inhibitors report these gastrointestinal side effects as the most common reason for non-compliance and early subject withdrawal [9]. More importantly, these drugs, and indeed all other anti-diabetic drugs, are expensive and not readily available to the majority of the T2D patients living in rural communities. This is also in spite of the high prevalence of the disease in such areas. Luckily, these communities are also naturally endowed with tremendous plant foods, including fruits pulps, which could be exploited for the management of postprandial hyperglycemia.

The fruit of *Parinari macrophylla* Sabine (chrysobalanaceae), commonly called gingerbread plum, is indigenous to northern Nigeria and used in a variety of ways. It is eaten either fresh, boiled or after pounded with water to create a colorful red lemonades-like juice [10]. *Detarium microcarpum* Guill. & Perr. (Leguminosae) fruit pulp is edible and very rich in crude fibre, vitamins C and E, folic acid, mineral elements and carbohydrates [11]. Also, the *D. microcarpum* fruit pulp was reported to affect some hematological indices in rats [12]. *Ziziphus spina-christi* (L.) Desf. And *Ziziphus mairei* Lam. belong to the Rhamnaceae family and are virtually cosmopolitan in warm-temperate and subtropical region. The plants have nutritiously edible fruits with various applications in folk medicine. The fruits are used locally to treat skin cuts and pulmonary ailments [13]. Furthermore, the leaves of *Z. spina-christi* have been shown to possess blood glucose lowering effect in diabetic rats [14]. *Parkia biglobosa* (Jacq.) G. Don (Leguminosae) is widely distributed in West Africa and utilized for both nutritional and medicinal purposes.The fruits of the plant are used as routine flouring and seasoning agent for traditional dishes [15]. Moreover, the seeds of the plant have been shown to possess ameliorative effects on alloxan induced diabetes in experimental animals [16].

All the above mentioned fruits are indigenous to northern Nigeria and other semi arid sub-Saharan Africa which are mostly consumed during period of grain shortage as dietary supplements among rural dwellers [10]. Unfortunately, the health benefits of these fruits, especially with respect to diabetes management, have not been documented. In this study, we report the inhibitory effects of these selected fruits on intestinal maltase and sucrase activities in non-diabetic animals. This is with a view to determine, whether or not, consumption of these fruits would be beneficial to diabetic patients in controlling postprandial hyperglycemia which would go a long way to provide a scientific basis to encourage diabetic patients, especially in rural communities, to supplement their carbohydrate rich meals with these fruits. It is also hoped that this study would serve as a basis for the development of a standardised anti-diabetic supplements or nutraceutical from these fruits.

2. Materials and methods

2.1. Fruit samples

Fresh riped fruit samples of *P. macrophylla*, *D. microcarpum*, *Z. spina-christi*, *Z. mairei* and *P. biglobosa* were purchased at a local market at Wudil local government area of Kano state, Nigeria in September-October, 2014. Subsequently, the fruit samples were immediately washed and shade-dried for three weeks to constant weights. Thereafter, the pulps of the dried fruit samples were carefully excised and pounded to fine powder using laboratory mortar and pestle. The samples were stored in air-tight dry containers until needed.

2.2. Preparation of aqueous extracts

Thirty grams of each of the fine powdered fruit samples were dissolved in 500 ml of distilled water and allowed to stand for 24 h, after which the samples were filtered through cheese cloth. The filtrates were further filtered through Whatmann filter paper (No. 1) and the resultant extracts were evaporated in a water bath at 50°C to obtain the crude aqueous extracts.

2.3. Experimental animals

The protocol employed met the guidelines of the Good Laboratory Practice (GLP) regulations of World Health Organization. Also, the rules and regulations of Ahmadu Bello University, Zaria Ethics Committee were duly followed. Apparently healthy albino rats (Wistar strain) weighing 140–200 g were procured from the animal house of the Nigerian Institute of Trypanosomiasis and Onchorcerciasis Research, Kaduna, Nigeria. The animals were maintained in well ventilated polycarbonated laboratory cages (25 ± 2°C, 12 h light–dark cycle) and fed on a standard rat pellet diet (Vital Feeds, Jos, Nigeria) with drinking water *ad libitum* during the entire experimental period.

2.4. Animal grouping

After one week adaptation period, the rats were randomly divided in to twelve groups of five animals each namely; NC: Normal Control, PM100: treated with a low dose (100 mg/kg bw) of *P. macrophylla* fruit extract, PM200: treated with a high dose (200 mg/kg bw) of *P. macrophylla* fruit extract, DM100: treated with a low dose (100 mg/kg bw) of *D. microcarpum* fruit extract, DM200: treated with a high dose (200 mg/kg bw) of *D. microcarpum* fruit extract, ZS100: treated with a low dose (100 mg/kg bw) of *Z. spinachristi* fruit extract, ZS200: treated with a high dose (200 mg/kg bw) of *Z. spinachristi* fruit extract, ZM100: treated with a low dose (100 mg/kg bw) of *Z. mairei* fruit extract, ZM200: treated with a high dose (200 mg/kg bw) of *Z. mairei* fruit extract, PB100: treated with a low dose (100 mg/kg bw) of *P. biglobosa* fruit extract, PB200: treated with a high dose (200 mg/kg bw) of *P. biglobosa* fruit extract, Acarbose: treated with 100 mg/kg bw of a standard drug, acarbose.

2.5. In vivo maltase and sucrase inhibitory activity of the fruit extracts

The rats in all the experimental groups were fasted overnight for 12 h and after which, the animals were orally administered with maltose or sucrose (2 g/kg bw) which was immediately followed by the oral administration of the respective dose of the fruit extract. However, in addition to the similar maltose or sucrose administrations, the animals in NC and acarbose groups were orally administered with distilled water and acarbose respectively, instead of the fruit extracts. The blood glucose concentrations of all animals were measured in the blood collected from the tail vein by using a portable glucometer (Glucoplus Inc., Saint-Laurent, Quebec, Canada) at 0 (just before maltose or sucrose ingestion), 30, 90 and 120 min after the dose of maltose or sucrose. It is noteworthy to state that a seven day wash out period was given between the two experiments for maltase and sucrase

inhibitory effects of the fruit extracts. This was also done to minimize the esophageal as well as overall stress to the animals. The area under the curve (AUC) for all the experimental groups was calculated according to the following formula:

$$AUC_{tk} \sum_{i=1}^{k} \left(\frac{C_{i-1} + C_i}{2} \right) (t_i - t_{i-1})$$

where C_i is the concentration of blood glucose at time t_i.

2.6. Statistical analysis

All data were presented as the mean ± standard deviation of five animals. Data were analyzed by using a statistical software package (SPSS for Windows, version 18, IBM Corporation, NY, USA) using Tukey's-HSD multiple range *post-hoc* test. Values were considered significantly different at $P < 0.05$.

3. Results

The fruit pulps of *P. macrophylla, D. microcarpum*, *Z. spinachristi, Z. mairei* and *P. biglobosa* were found to significantly ($P < 0.05$) inhibit the activity of intestinal maltase in a dose dependent pattern which was observed as significantly lowered ($P < 0.05$) blood glucose level (after maltose loading) in the fruit extracts-treated groups (Fig. 1). However, the extracts of *P. macrophylla* and *P. biglobosa* fruits at 100 mg/kg bw, did not cause a significant ($P > 0.05$) inhibitions of the enzyme at 90 and 120 min (Fig. 1A and E). In fact, all the extracts of the fruits demonstrated more potent inhibitory effects against the intestinal maltase within the first 30 min of the experiment. Moreover, the data from the computed area under the maltose tolerance curves revealed that the 200 mg/kg bw of all fruits extract, except *D. microcarpum,* produced significantly lower AUC values than the normal control and acarbose-treated rats (Table 1). The fruit extracts of *Z. mairei* and *Z. spinachristi* showed lower AUC values in the maltose tolerance curves than other extracts when the data was compared for both 100 and 200 mg/kg bw. Interestingly, the computed ED_{50} of the extracts also demonstrated that the *Z. mairei* fruit had the least ED_{50} value of 647.15 mg/kg bw among the fruits, followed by *Z. spinachristi* fruit with an ED_{50} value of 661.89 mg/kg bw for inhibiting maltase (Table 3).

In the sucrase inhibition studies, there was a sharp and significant ($P < 0.05$) rise in blood glucose concentrations in the normal and acarbose-treated rats at 30 min after the sucrose loading (Fig. 2). However, all the fruits extract significantly ($P < 0.05$) inhibit the intestinal sucrase activities within the first 30 min which manifested as significantly ($P < 0.05$) lowered blood glucose levels in the extracts-treated groups compared with normal control group within the time period. There were no significant differences ($P > 0.05$) in the blood glucose concentrations of all experimental groups at 90 and 120 min. The computed area under the sucrose tolerance curves did not show significant ($P > 0.05$) differences between the normal control and the extracts-treated groups (Table 2). However, the *Z. spinachristi* had the least ED_{50} value of 982.44 mg/kg bw followed by *Z. mairei* with a value of 1512.76 mg/kg bw (Table 3). In fact, the ED_{50} value for inhibiting sucrase is in the order *Z. spinachristi* < *Z. mairei* < *P. biglobosa* < *D. microcarpum* < *P. macrophylla*.

4. Discussion

Targeting of postprandial hyperglycemia has been suggested to be an important control strategy especially in preventing cardiovascular mortality in diabetics [17]. The rise in plasma glucose in normoglycemic individuals after consumption of carbohydrate begins at about 10 min and reaches maximum in ∼60 min [18]. In this study,

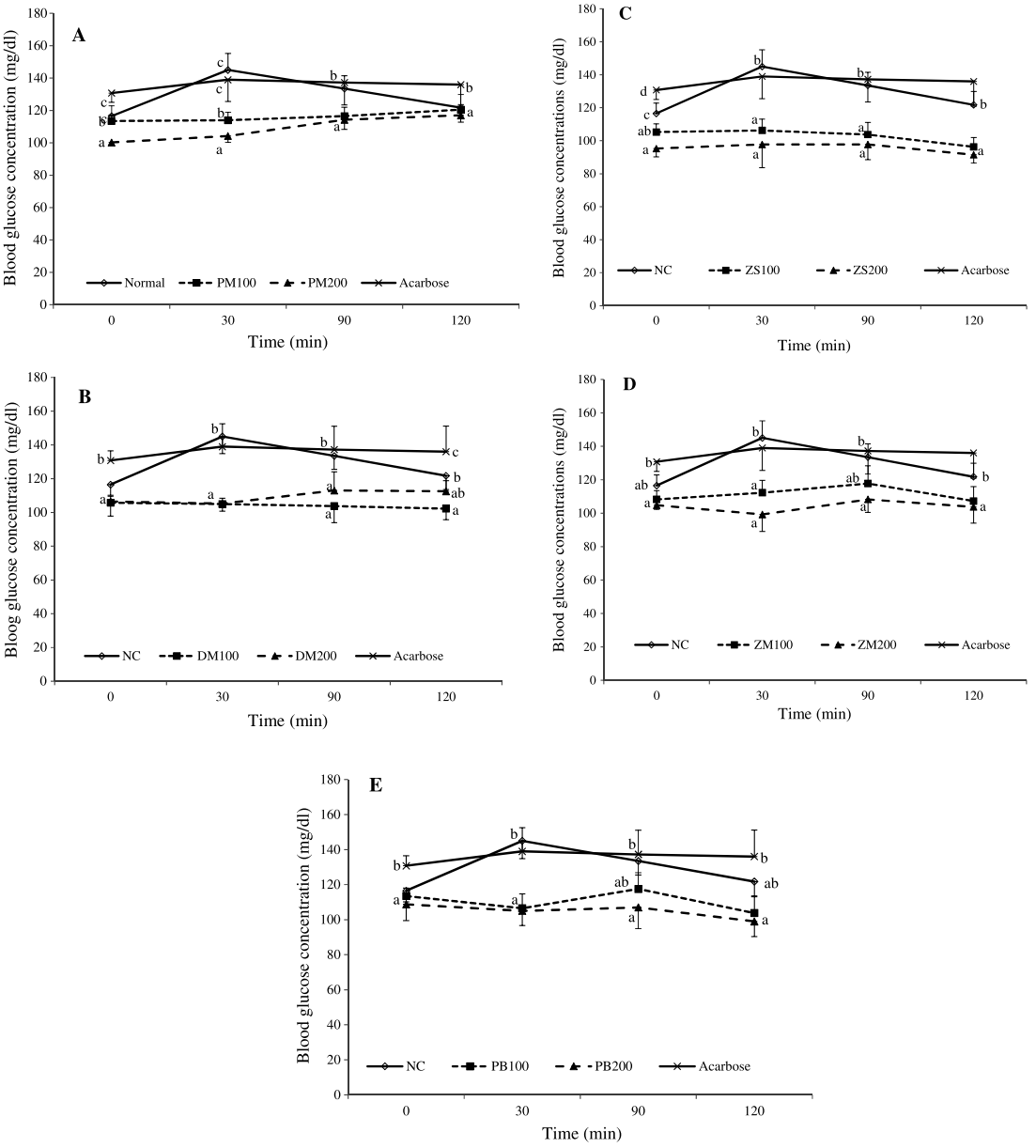

Fig. 1. *In vivo* inhibitory effects of *P. macrophylla* (A), *D. microcarpum* (B), *Z. spinachristi* (C), *Z. mairei* (D) and *P. biglobosa* (E) fruits on intestinal maltase activity. Data are presented as mean ± SD of five animals. $^{a-c}$Values with different letters for a given time are significantly different from each other group of animals (Tukey's-HSD multiple range *posthoc* test, $P < 0.05$). NC is normal control; PM, DM, ZS, ZM and PB are group of rats given co-administration of maltose with *P. macrophylla*, *D. microcarpum, Z. spinachristi, Z. mairei* and *P. biglobosa* fruit extract respectively. The 100 and 200 merged with the above-mentioned abbreviations refer to the dose of 100 and 200 mg/kg bw respectively, of the fruit given to the animals.

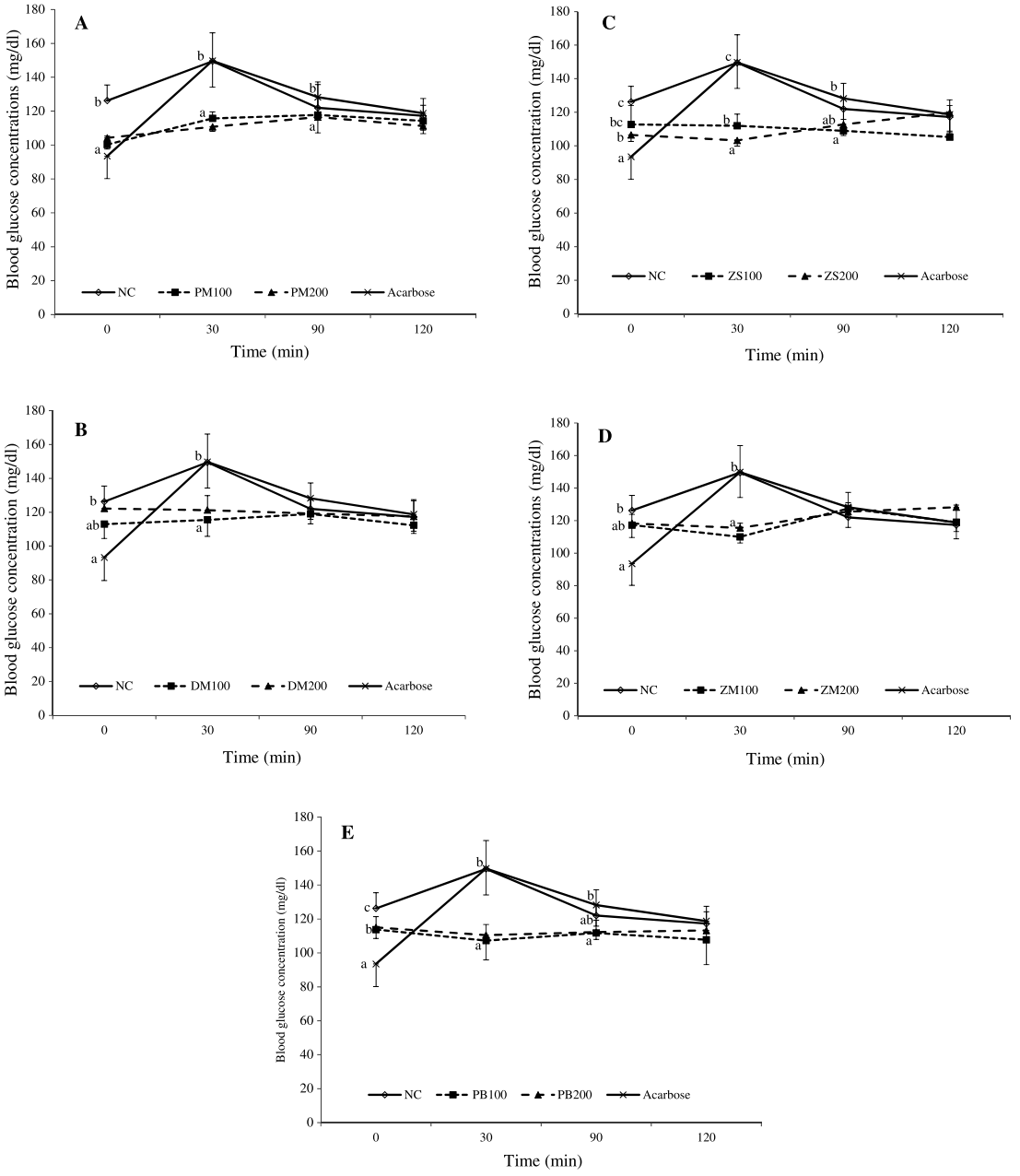

Fig. 2. *In vivo* inhibitory effects of *P. macrophylla* (A), *D. microcarpum* (B), *Z. spinachristi* (C), *Z. mairei* (D) and *P. biglobosa* (E) fruits on intestinal sucrase activity. Data are presented as mean ± SD of five animals. $^{a-c}$Values with different letters for a given time are significantly different from each other group of animals (Tukey's-HSD multiple range *posthoc* test, $P < 0.05$). NC is normal control; PM, DM, ZS, ZM and PB are group of rats given co-administration of sucrose with *P. macrophylla*, *D. microcarpum, Z. spinachristi, Z. mairei* and *P. biglobosa* fruit extract respectively. The 100 and 200 merged with the above-mentioned abbreviations refer to the dose of 100 and 200 mg/kg bw respectively, of the fruit given to the animals.

Table 1
Computed Area Under the Curve (AUC) values for the maltose and sucrose tolerance tests over the 2 hour period

Groups	$AUC_{0-120\ min}$ (Minutes·mg/dl)	
	Maltose	Sucrose
NC	16106.25 ± 2028.10^{c}	$15870.00 \pm 2034.39^{ab}$
PM100	13882.50 ± 404.36^{bc}	13875.00 ± 914.14^{ab}
PM200	13166.25 ± 347.86^{b}	13458.75 ± 449.50^{a}
DM100	$12513.75 \pm 1141.04^{ab}$	$13931.25 \pm 1904.77^{ab}$
DM200	$13106.25 \pm 1377.22^{abc}$	$14418.75 \pm 1182.69^{ab}$
ZS100	12472.50 ± 728.69^{ab}	$13215.00 \pm 1555.75^{ab}$
ZS200	11576.25 ± 625.01^{a}	13113.75 ± 956.42^{ab}
ZM100	$13582.50 \pm 976.38^{abc}$	14231.25 ± 464.60^{b}
ZM200	12565.00 ± 911.02^{ab}	14542.50 ± 263.13^{b}
PB100	$13338.75 \pm 1185.19^{abc}$	13177.50 ± 891.25^{ab}
PB200	$12656.25 \pm 1682.43^{ab}$	13447.50 ± 654.75^{a}
Acarbose	16432.50 ± 1358.53^{c}	$15693.75 \pm 1316.26^{ab}$

All values are presented as mean ± SD of five animals. $^{a-c}$Values with different letters along a column are significantly different from each other (Tukey's-HSD multiple range *posthoc* test, $P < 0.05$). NC is normal control; PM, DM, ZS, ZM and PB are group of rats given co-administration of the disaccharide (maltose or sucrose) with *P. macrophylla*, *D. microcarpum, Z. spinachristi, Z. mairei* and *P. biglobosa* fruit extract respectively. The 100 and 200 merged with the above-mentioned abbreviations refer to the dose of 100 and 200 mg/kg bw respectively, of the fruit given to the animals.

Table 2
The ED_{50} values for inhibiting intestinal maltase and sucrase by the selected Nigerian fruits

	ED_{50} (mg/kg bw)	
	Maltase	Sucrase
PM	1118.35	1851.94
DM	742.55	1677.57
ZS	661.89	982.44
ZM	647.15	1512.76
PB	858.35	1643.62

PM, DM, ZS, ZM and PB refers to *P. macrophylla*, *D. microcarpum, Z. spinachristi, Z. mairei* and *P. biglobosa* fruit extract respectively.

the peak in plasma glucose level is observed within the first 30 min of maltose or sucrose dose, after which a decrease was observed until the level returns to normal. Moreover, the blood glucose lowering effect of the extracts appears to be higher with increasing dose (except *D. microcarpum*). The observed effects of the fruits extract within the initial 30 min suggest a fast inhibitory activity against intestinal maltase and sucrase. From a therapeutic point of view, this fast action is desirable in preventing postprandial rise in blood glucose for the reason aforementioned. It was also evident from the computed AUC data that all the extracts (at 200 mg/kg bw) have better inhibitory activity on maltase than sucrase. This could possibly be due to the presence of additional phytochemicals in the extracts that specifically target maltase in addition to non-specific α-glucosidase inhibitors. It is possible that the α-glucosidase inhibitors in the fruits have more preference towards inhibiting the hydrolysis of α (1–4) than α (1-2) glycosidic bonds.

On a general note, the fruits of either of the two *Ziziphus* species have the lowest ED_{50} values for inhibiting both maltase and sucrase among the fruits. Indeed, various studies have reported on the anti-diabetic properties of different parts of *Ziziphus* species [14, 19] with the exception of the fruit which is the one commonly consumed. Among the frequently cited phytochemicals responsible for the antihyperglycemic properties are phenolics, alkaloids, flavonoids, terpenoids and glycosides [20]. It is interesting to note that a phloretin derivative has been identified in the fruits of *Z. spina-christi* as well as other *Ziziphus* species [21]. Phloretin is a known hexose transport inhibitor across the basolateral membrane of the small intestine which has been employed for experimental purposes [22]. Hence, it is logical to hypothesize the involvement of inhibition of sugar transport in addition to enzyme inhibition in the mechanism of action of these fruits.

5. Conclusion

In conclusion, the fruits of *Z. spinachristi*, *Z. mairei*, *P. biglobosa, D. microcarpum,* and *P. macrophylla* possess intestinal maltase and sucrase inhibitory effects in rats. Because these fruits are edible and safe, frequent consumption would be a rational control measure in preventing postprandial hyperglycemia especially in diabetics. The data also suggested that the development of a standardised anti-hyperglycemic supplements or nutraceuticals from these fruits could be possible. On the other hand, isolation of the specific phytochemicals responsible for the inhibition of sugar hydrolysis and/or transport should be a theme for future research. Moreover, the findings of this study are a preliminary proof of concept that need to be further validated with higher number of animals and eventually in clinically relevant human-based studies. This would be in the interest of the global effort to provide a broad spectrum of blood glucose lowering agents in order to curtail the menace of hyperglycemia-related complications.

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Conflict of Interest

Authors declare no conflict of interest for the study conducted.

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