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Methanol extracts of *Brachystegia eurycoma* and *Detarium microcarpum* seeds flours inhibit some key enzymes linked to the pathology and complications of type 2 diabetes *in vitro*

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Abstract

The inhibitory effect of methanol extracts of *Brachystegia eurycoma* and *Detarium microcarpum* seeds flours on some key enzymes [α-amylase, α-glucosidase and aldose reductase (AR)] linked to the pathology and complications of type 2 diabetes (T2D); and their antioxidant properties were evaluated. The antioxidant properties evaluated were DPPH$^{\bullet}$ and ABTS$^{\bullet+}$ scavenging abilities, reducing power, and antioxidant phytochemicals (total phenolics, tannins, total flavonoids and total saponins). Extracts of both flours inhibited α-amylase, α-glucosidase and AR in a dose-dependent manner. The half-maximal inhibitory concentrations (IC_{50}) of *B. eurycoma* on α-amylase, α-glucosidase, AR and lipid peroxidation were lower than those of *D. microcarpum*, indicating that it had stronger inhibitory potency than *D. microcarpum*. *B. eurycoma* also had significantly ($P<0.05$) higher DPPH$^{\bullet}$ and ABTS$^{\bullet+}$ scavenging abilities, and reducing power than *D. microcarpum*. The antioxidant phytochemicals (total phenolics, tannins, total flavonoids and total saponins) were also significantly ($P<0.05$) higher in *B. eurycoma* than *D. microcarpum*. The inhibitory effect of *B. eurycoma* and *D. microcarpum* extracts on α-amylase, α-glucosidase and AR activities may be attributed to the combined action of their polyphenols and total saponins, and this may be a possible mechanism of action providing support for their use in managing hyperglycemia and the complications of T2D.
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Keywords: Type 2 diabetes; Enzyme inhibition; Antioxidants; Phytochemical; *Brachystegia eurycoma*; *Detarium microcarpum*

1. Introduction

Diabetes mellitus (DM) is a metabolic disorder characterized by chronic hyperglycemia with disturbances in carbohydrate, lipid and protein metabolism, resulting from defects in insulin secretion, insulin action, or both [1]. It is projected that globally, the total number of diabetic patients will increase to 366 million by the year 2030 [2], with type 2 diabetes (T2D) accounting for 90–95% of the cases. Hence, T2D is a major global health problem.

Control of postprandial hyperglycemia, the main risk factor for the development of T2D, plays a key role in the treatment of DM, and retards chronic complications associated with the disease [3]. Unmitigated diabetic hyperglycemia leads to the stimulation of other factors that accelerate the progression of diabetic complications [4], with the major microvascular complications of diabetes being nephropathy, neuropathy and retinopathy. One of the strategies of controlling postprandial hyperglycemia is by retarding the digestion of carbohydrates and absorption of glucose through the inhibition of α-amylase and α-glucosidase that digest carbohydrate in the digestive tract [3,5]. Thus, some therapeutic approaches aimed at improving the health status of diabetic patients explore this strategy [6]. In addition to this, studies have shown that therapeutic approach targeted at inhibiting AR activity could offer effective means of preventing certain microvascular complications of diabetes [7].

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Phytochemicals are plant secondary metabolites that possess many health benefits [8]. Their health benefits, including antidiabetic activities [9], are attributed to antioxidant activities and the inhibition of enzymes such as α-amylase, α-glucosidase and aldose reductase [10].

Brachystegia eurycoma Harms and *Detarium microcarpum*, both of the family Caesalpinioideae, are two underutilized leguminous tree crops that have both food and medicinal uses. For instance, in South-Eastern part of Nigeria, both seeds are used for thickening of traditional soups [11]. *B. eurycoma* is a very economically valuable tree crop mostly grown in the tropical rain forest of West Africa [12]. The nutrients composition of the seeds shows that they are a good source of nutrient supplement as earlier reported by some studies [11,13]. The seed flour has good water absorption capacity, and so, is useful as functional agent in processed foods such as bakery products and meat formulations [12]. On the other hand, *D. microcarpum* is found mostly in savannah forest of dried type. It is a high yielding tree with large quantities of its fruits being left wasting every year in the field [14]. The seed is known to contain lipids, carbohydrates, proteins, crude fiber and the essential elements: Na, K, Mg, Ca, S, P and Fe [15]. In African ethno-medicine, both plants are used in the treatment of microbial infections such as syphilis, dysentery, bronchitis, leprosy, sore throat, pneumonia, diarrhea, malaria and tuberculosis, and other diseases such as asthma and inflammatory conditions [16,17]. Methanol extract of *D. microcarpum* leaf has also been reported to possess antidiabetic activity [18].

Although there are these reports on the health benefits of *B. eurycoma* and *D. microcarpum*, information on the ability of their seeds flours extracts to inhibit the activities of some key enzymes linked to the pathology and complications of T2D is scarce. Therefore, this study was designed to evaluate the inhibitory activity of the methanol extracts of *B. eurycoma* and *D. microcarpum* seeds flours against α-amylase, α-glucosidase, aldose reductase (AR) and lipid peroxidation, as well as determine their antioxidant properties *in vitro*.

2. Materials and methods

2.1. Sample collection and preparation

Samples of *B. eurycoma* and *D. microcarpum* seeds were purchased from a local farm settlement in Ibadan, Oyo State, Nigeria, in September 2013. The seeds were later authenticated by Professor A.E. Ayodele of the Department of Botany, University of Ibadan, Nigeria. Subsequently, they were sorted, sun-dried and manually shelled. Seed flours were obtained by grinding the shelled samples into fine particle size powder using a laboratory grinder. Then the flour samples were packed in airtight vials and stored at −4 °C until analysis.

All the chemicals used for analysis were of analytical grade.

2.1.1. Preparation of seed flour methanol extract

Flour sample (2 g) was extracted by steeping in 100 mL of methanol with continuous shaking using a mechanical shaker. The mixture was centrifuged at 3000 × *g* for 10 min, and the supernatant (subsequently referred to as extract) was collected and stored at −4 °C for further analysis. The extracts for carbohydrate hydrolyzing enzymes inhibition assays were concentrated to dryness, and redissolved in equal volume of dimethylsulfoxide (DMSO) to give a final concentration of 80 mg/mL.

2.2. Handling of experimental animal

Adult male Wister strain albino rats weighing 200–250 g were procured from the experimental animal breeding unit of Department of Veterinary Medicine, University of Ibadan, Nigeria. The rats were handled in accordance with the guidelines for the care and use of laboratory animals approved by the Animal Ethics Committee of our institution. The guidelines were followed to ensure the protection of the animals' welfare during the experiment. The experiment license number is BCH/09/9395. The rats were kept in a cage to acclimatize for 7 days, during which they maintained on a 12 h light/12 h dark cycle at 25 °C, with free access to food and water.

2.3. α-Amylase inhibition assay

α-Amylase inhibition assay was conducted following the protocol described by Kwon et al. [19]. The porcine pancreas α-amylase (EC 3.2.1.1) used for this assay was procured from Sigma (Cat. No. A3176). Appropriate dilutions of methanol extract amounting to 500 μL, and 500 μL of 0.02 mol/L sodium phosphate buffer (pH 6.9 with 0.006 mol/L sodium chloride) containing 0.5 mg/mL α-amylase solution were incubated at 37 °C for 10 min. After pre-incubation, 500 μL of 1% starch solution in 0.02 mol/L sodium phosphate buffer was added. The reaction mixture was then incubated at 37 °C for 15 min and the reaction was terminated with 1.0 mL of DNSA color reagent (1% 3,5-dinitrosalicylic acid and 12% sodium potassium tartrate in 0.4 mol/L NaOH). The reaction mixture was then incubated in a boiling water bath for 5 min, cooled to room temperature, and diluted with 10 mL distilled water. The absorbance was measured at 540 nm. Percentage α-amylase inhibition was calculated using the formula:

$$\%\ \text{Inhibition} = [(A540_{\text{reference}} - A540_{\text{sample}}) \div A540_{\text{reference}}] \times 100.$$

2.4. α-Glucosidase inhibition assay

The inhibitory effect of extracts against α-glucosidase activity was determined according to the method described by Kim et al. [20], using *Bacillus stearothermophilus* α-glucosidase (EC 3.2.1.20) procured from (Sigma, Cat. No. G 3651). Briefly, 5 units of α-glucosidase were pre-incubated with 20 μg/mL of the different seeds methanol extracts for 15 min. 3 mmol/L para-nitrophenylglucopyranoside (PNPG) dissolved in 20 mmol/L phosphate buffer, pH 6.9 was added as a substrate to start the reaction. The reaction mixture was further incubated at 37 °C for 20 min and stopped by addition of 2 mL of 0.1 mol/L Na_2CO_3.

The α-glucosidase activity was determined by measuring the yellow colored p-nitrophenol released from PNPG at 400 nm. Each test was performed in triplicates and the mean absorption was used to calculate percentage α-glucosidase inhibition as follows:

$$\%\ \text{Inhibition} = [(A400_{\text{reference}} - A400_{\text{sample}}) \div A400_{\text{reference}}] \times 100.$$

2.5. Aldose reductase (AR) inhibition assay

Partially purified rat lens aldose reductase (EC 1.1.1.21) was prepared following a procedure adapted from Hayman and Kinoshita [21]. Briefly, lenses were quickly removed from rats following euthanasia with ether, and homogenized in a glass homogenizer with a Teflon pestle in 5 volume of ice-cold distilled water. The homogenate was centrifuged at 10,000 × *g* at 0–4 °C for 20 min. The supernatant was precipitated with saturated ammonium sulfate at 40, 50% and then at 75% salt saturation. The supernatant was retained after the first two precipitations. The pellet from the last step, possessing AR activity, was dispersed in 75% ammonium sulfate and subsequently used for the inhibition assay.

Inhibition of AR activity was assayed by estimating the consumption of NADPH at 340 nm as described by Da Settimo et al. [22]. The reaction mixture contained 4.67 mmol/L D,L-glyceraldehyde as a substrate, 0.11 mmol/L NADPH, 0.067 mol/L phosphate buffer, pH 6.2 and 0.05 mL of the enzyme preparation in a total volume of 1.5 mL. The reference blank contained all the above reagents except the substrate D,L-glyceraldehyde to correct for the oxidation of NADPH not associated with reduction of the substrate. The enzyme reaction was initiated by addition of D,L-glyceraldehyde and was monitored for 4 min after an initial period of 1 min at 30 °C. A decrease in absorbance of sample test relative to the reference test at 340 nm is a function amount of NADPH consumed. Hence:

$$\%\ \text{Inhibition} = [(A340_{\text{reference}} - A340_{\text{sample}}) \div A340_{\text{reference}}] \times 100.$$

2.6. Inhibition of lipid peroxidation assay

The ability of the extracts to inhibit Fe^{2+}-induced lipid peroxidation in rat pancreas homogenate was assayed according to the modified method of Ohkawa et al. [23]. Low-speed rat pancreas supernatant used for this assay was prepared by mildly anaesthetizing the rats in ether, and rapidly excising and placing the pancreas in ice. Then 10% (w/v) pancreas homogenate prepared by homogenizing the pancreas in cold saline, was centrifuged for 10 min at 1400 × *g* to yield a pellet that was discarded and the low-speed supernatant (S1). To a reaction mixture containing 100 μL of S1 fraction, 30 μL of 0.1 mol/L Tris–HCl buffer (pH 7.4) and different concentrations of plants methanol extracts, 30 μL of freshly prepared 25 μmol/L ferric sulfate solution was added to initiate lipid peroxidation. The volume was made up to 300 μL with deionized water before incubation at 37 °C for 1 h. The color reaction was initiated by adding 300 μL of 81 g/L sodium dodecyl sulphate to the reaction mixture containing the S1, followed by the addition of 600 μL of acetic acid/HCl (pH 3.4) and 600 μL of 0.8% (v/v) TBA (thiobarbituric acid). This mixture was incubated at 100 °C for 1 h. The absorbance of thiobarbituric acid reactive species (TBARS) produced was measured at 532 nm in an UV–Visible spectrophotometer. The decrease in the absorbance of test sample in relation to the reference test was used to calculate the % inhibition as follows.

$$\%\ \text{Inhibition} = [(A532_{\text{reference}} - A532_{\text{sample}}) \div A532_{\text{reference}}] \times 100.$$

2.7. Estimation of DPPH free radical scavenging ability

The $DPPH^{\bullet}$ scavenging ability of the extracts was determined as described by Cervato et al. [24]. Briefly, appropriate dilution of the extracts (1 mL) was mixed with 3 mL of 60 μmol/L $DPPH^{\bullet}$; the mixture was left in the dark for 30 min before the absorbance was taken at 517 nm. The decrease in absorbance of DPPH on the addition of extract in relation to the reference test was used to calculate the percentage scavenging ability following the equation:

$$\%\ \text{Scavenging ability} = [(A517_{\text{reference}} - A517_{\text{sample}}) \div A517_{\text{control}}] \times 100.$$

2.8. Estimation of $ABTS^{\bullet+}$ scavenging ability

The $ABTS^{\bullet+}$ scavenging ability of the extracts was determined according to the method described by Re et al. [25]. The $ABTS^{\bullet+}$ was generated by incubating equal volume of 7 mmol/L $ABTS^{\bullet+}$ aqueous solution with $K_2S_2O_8$ (2.45 mmol/L) in the dark for 16 h at room temperature and adjusting the absorbance at 734 nm to 0.7 ± 0.02 with 95% ethanol. Then 0.2 mL appropriate dilution of the extract was added to 2.0 mL $ABTS^{\bullet+}$ solution and the absorbance was measured at 734 nm after 15 min. The $ABTS^{\bullet+}$ scavenging ability of the extracts was subsequently calculated and expressed in Trolox equivalent (TE).

2.9. Determination of reducing power

The reducing power of the extracts was determined by assessing their ability to reduce $FeCl_3$ solution as described by Oyaizu [26]. 2.5 mL aliquot of extract was mixed with 2.5 mL of 200 mmol/L sodium phosphate buffer (pH 6.6) and 2.5 mL of 1% potassium ferricyanide. The reaction mixture was incubated

Table 1
Half-maximal inhibitory concentration (IC_{50}) of *B. eurycoma* and *D. microcarpum* seeds flour extracts on α-amylase, α-glucosidase, AR activities and lipid peroxidation.

Parameters	*B. eurycoma* IC_{50} (mg/mL)	*D. microcarpum* IC_{50} (mg/mL)
α-Amylase	55.64 ± 1.02[b]	69.35 ± 1.32[a]
α-Glucosidase	26.31 ± 0.98[b]	31.14 ± 1.02[a]
AR	17.84 ± 0.84[b]	25.79 ± 1.15[a]
Lipid peroxidation	11.47 ± 0.75[b]	30.17 ± 1.24[a]

Data represent the mean ± standard deviation of replicate readings. Values with the different lowercase superscript letters along the same row are significantly different ($P < 0.05$).

at 50 °C for 20 min and then 2.5 mL of 10% trichloroacetic acid was added. This mixture was centrifuged at 650 × *g* for 10 min. Then 5 mL supernatant was mixed with an equal volume of water and 1 mL of 0.1% ferric chloride. The absorbance was measured at 700 nm. The reducing power of the extracts was subsequently calculated.

2.10. Quantitative determination of phytochemicals (total phenolics, tannins, total flavonoids and total saponins)

Phytochemicals were quantitatively determined in methanol extracts of *B. eurycoma* and *D. microcarpum* seeds according to the following standard procedures. Total phenolics content was to the Folin–Ciocalteu method reported by Chan et al. [27], and the result was expressed as mg gallic acid equivalent/g (mg GAE/g) sample. Total tannins content was determined following the method reported by Amorim et al. [28], and the result was expressed as mg tannic acid equivalent/g (mg TAE/g) sample. Total flavonoids content was determined using aluminum chloride method as reported by Kale et al. [29], and the result was expressed as mg quercetin equivalent/g (mg QE/g) sample. Total saponins content was determined by the method described by Makkar et al. [30], and the result was expressed as mg diosgenin equivalent/g (mg DE/g) sample.

2.11. Statistical analysis

Results of triplicate experiments were expressed as mean ± standard deviation (SD), and independent samples *t*-test was carried out on the result data at 95% confidence level using SPSS statistical software package, version 17. Half-maximal inhibitory concentration (IC_{50}) was calculated from the % inhibition versus extract concentration non-linear regression curve of each extract.

3. Results

The IC_{50} of *B. eurycoma* and *D. microcarpum* seeds flour extracts on α-amylase, α-glucosidase, aldose reductase (AR) and Fe^{2+}-induced lipid peroxidation are presented in Table 1. The results revealed that both plants were able to inhibit α-amylase, α-glucosidase and AR. Fig. 1a–c further depicted that their inhibitory pattern was dose-dependent.

Table 2
DPPH IC_{50}, $ABTS^{\bullet+}$ scavenging ability and reducing power of *B. eurycoma* and *D. microcarpum* seeds flour extracts.

Antioxidant activity	*B. eurycoma*	*D. microcarpum*
DPPH IC_{50} (mg/mL)	5.05 ± 0.64[b]	12.63 ± 0.87[a]
$ABTS^{\bullet+}$ scavenging ability (μmol TE/g)	29.78 ± 0.07[a]	21.17 ± 0.91[b]
Reducing power (mg GAE/g)	8.79 ± 0.24[a]	2.15 ± 0.01[b]

Data represent the mean ± standard deviation of replicate readings. Values with the different lowercase superscript letters along the same row are significantly different ($P < 0.05$).

Consistently, the IC_{50} values of *B. eurycoma* on these three carbohydrate-metabolizing enzymes were lower than those of *D. microcarpum*; hence, *B. eurycoma* had stronger inhibitory activity than *D. microcarpum*. Similarly, both extracts inhibited Fe^{2+}-induced lipid peroxidation in rat pancreas homogenate in a dose-dependent manner (Fig. 1d), with *B. eurycoma* being more effective than *D. microcarpum*, as indicated by its lower IC_{50} value.

The results of the antioxidant activities of both extracts, as tested using $DPPH^{\bullet}$ and $ABTS^{\bullet+}$ scavenging, and reducing power assays, are presented in Table 2. Both extracts exhibited $DPPH^{\bullet}$ scavenging activity in a dose-dependent manner. However, based on their IC_{50} values, *B. eurycoma* (5.05 mg/mL) possessed a stronger $DPPH^{\bullet}$ scavenging ability than *D. microcarpum* (12.63 mg/mL). Similarly, *B. eurycoma* had significantly ($P < 0.05$) higher $ABTS^{\bullet+}$ scavenging ability and reducing power than *D. microcarpum.*

The antioxidant phytochemical composition of *B. eurycoma* and *D. microcarpum* on dry weight basis are presented in Table 3. The result showed that *B. eurycoma* had significantly ($P < 0.05$) higher total phenolics, tannins, total flavonoids and total saponins contents than *D. microcarpum*.

4. Discussion

In view of the attendant clinical side effects and high cost of synthetic oral hypoglycemic drugs available for the management of T2D, research focus has been on affordable natural products that can be used to manage postprandial hyperglycemia and diabetic complications with minimal side effects. The results obtained in this study (Table 1) revealed that extracts of *B. eurycoma* and *D. microcarpum* seeds flours were potent in inhibiting the activities of α-amylase, α-glucosidase and AR. Plant-derived inhibitors of carbohydrate-hydrolyzing enzymes

Table 3
Antioxidant phytochemical composition of *B. eurycoma* and *D. microcarpum* seeds flour extracts.

Phytochemical	*B. eurycoma*	*D. microcarpum*
Total phenolics (mg GAE/g)	4.13 ± 0.27[a]	1.56 ± 0.03[b]
Tannins (mg TAE/g)	4.25 ± 0.09[a]	1.74 ± 0.03[b]
Total flavonoids (mg QE/g)	1.70 ± 0.03[a]	0.81 ± 0.04[b]
Total saponins ((mg DE/g)	10.99 ± 0.11[a]	9.76 ± 0.03[b]

Data represent the mean ± standard deviation of triplicate readings. Values with different lowercase superscript letters along the same row are significantly different ($P < 0.05$).

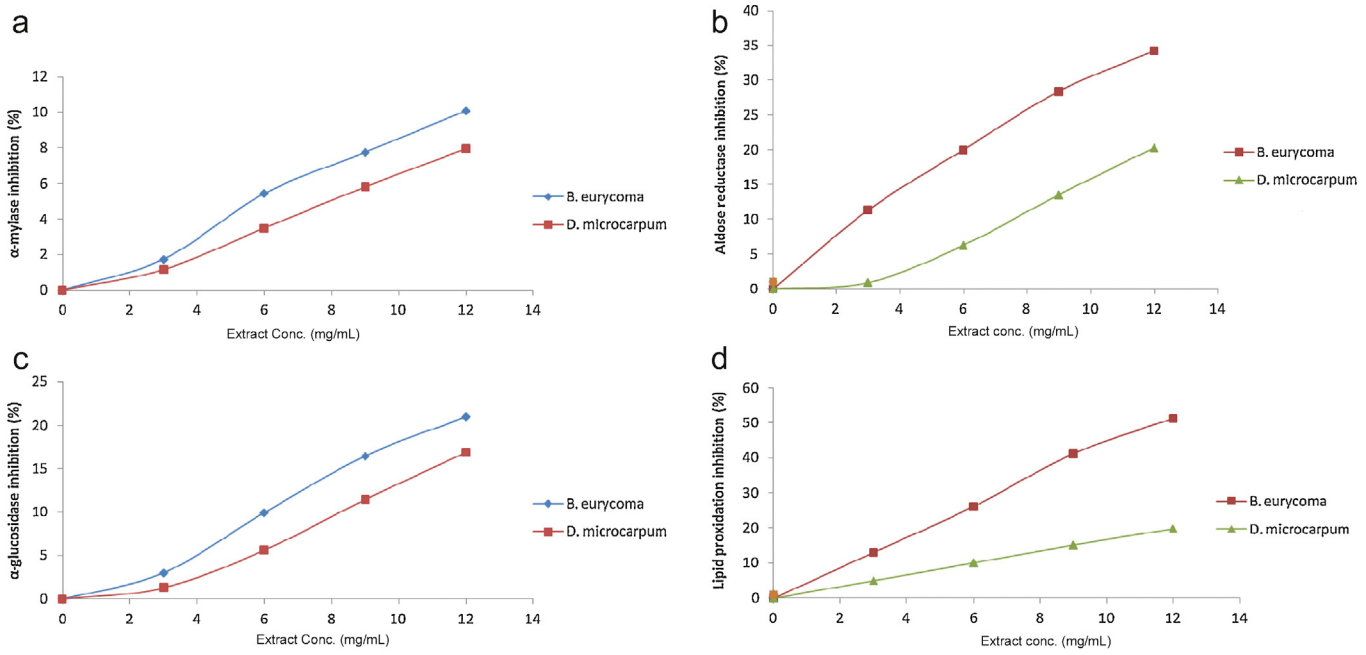


Fig. 1. (a) α-Amylase activity % inhibition versus extract concentration curve of *B. eurycoma* and *D. microcarpum* seeds flour. (b) α-Glucosidase activity % inhibition versus extract concentration curve of *B. eurycoma* and *D. microcarpum* seeds flour. (c) AR activity % inhibition versus extract concentration curve of *B. eurycoma* and *D. microcarpum* seed flour. (d) Fe^{2+}-induced lipid peroxidation % inhibition versus extract concentration curve of *B. eurycoma* and *D. microcarpum* seed flour.

can delay carbohydrate digestion, thereby leading to a reduction in the rate of glucose absorption, and consequently, reduce the rise in postprandial blood glucose [31]. Thus, the level of postprandial blood glucose depends partly on the activities of these enzymes mainly, α-amylase and α-glucosidase, that breakdown dietary starch and sugars into glucose [32]. α-Amylase, found in the saliva and pancreatic juice, is responsible for cleaving the α-1,4 bonds of starch releasing dextrin, maltose, and maltotriose [33]. Subsequently, the glycosidic linkages of these oligosaccharides are hydrolyzed by α-glucosidase present in the ciliary membrane of small intestine to produce glucose molecules which are then absorbed [34]. Hence, the inhibition of α-amylase and α-glucosidase activities represents one of the most effective approaches to control hyperglycaemia in type 2 diabetic patients [20].

The inhibitory preference of *B. eurycoma* and *D. microcarpum* extracts for α-glucosidase over α-amylase observed in this study is of interest as it may give the extracts pharmacological advantage over acarbose, a widely used synthetic oral hypoglycemic drug. Acarbose inhibits both α-amylase and α-glucosidase, but has some clinical side effects such as diarrhea, flatulence and abdominal discomfort which have been attributed to its excessive inhibition of pancreatic α-amylase [20]. Hence, the stronger inhibitory effect of the extracts on α-glucosidase than α-amylase may suggest lesser clinical side effects than acarbose. This finding is consonant with an earlier report that unlike acarbose, α-amylase and α-glucosidase inhibitors of plant origin have a stronger inhibitory effect on α-glucosidase activity than α-amylase activity [20].

The inhibition of AR activity by plant extracts has been reported as a possible therapeutic approach to ameliorating diabetic complications [7]. AR belongs to the aldo–keto reductase super family that reduces excess D-glucose into D-sorbitol with concomitant conversion of NADPH into $NADP^+$ [35], in the polyol pathway where it serves as the first and rate-limiting enzyme. This AR-catalyzed reaction that is favored in hyperglycemic condition has been implicated in the complications of DM, as previous studies provided evidence for the involvement of AR in diabetic neuropathy, retinopathy, nephropathy and cataract [36]. Abnormal activation of the polyol pathway during diabetes leads to accumulation of osmotically active sorbitol, which results in osmotic and oxidative stress that culminate in tissue injury [37]. In this study, both *B. eurycoma* and *D. microcarpum* seeds flour extracts inhibited AR in a dose-dependent manner, suggesting that they have the potential to mitigate the complications of T2D. However, the lower IC_{50} value of *B. eurycoma* relative to that of *D. microcarpum* indicates that *B. eurycoma* could be more potent than *D. microcarpum*.

The result further showed that both extracts inhibited Fe^{2+}-induced lipid peroxidation in rat pancreas homogenate in a dose-dependent pattern. Lipid peroxidation is an oxidative deterioration of polyunsaturated lipids, involving reactive oxygen species and transition metal ions, which yield diverse cytotoxic products, most of which are aldehydes [38]. These products, such as malondialdehyde (MDA), can impair membrane function; inactivate membrane-bound receptors and enzymes, and increase tissue permeability [39]. If not controlled, lipid peroxidation will in turn result in increased production of free radicals that can cause oxidative damage to the body cells, including the β-cells of the pancreas [40]. Hence, lipid peroxide-mediated tissue damages have been observed in type 1 and type 2 DM [41]. The ability of *B. eurycoma* and *D. microcarpum* extracts to inhibit Fe^{2+}-induced lipid peroxidation in rat pancreas, therefore suggests that both seeds might be helpful in alleviating the

vulnerability of the beta-cells of the pancreas to oxidative damage in T2D. However, as with the carbohydrate-metabolizing enzymes inhibition results *B. eurycoma* had a stronger inhibitory effect on lipid peroxidation than *D. microcarpum*.

Increase in the free radicals and reactive oxygen species burden of the body without a corresponding robust antioxidant defense system leads to oxidative stress, which plays an important role in the pathogenesis of degenerative diseases such as diabetes [42]. Consequently, the ability of the extracts to scavenge $DPPH^{\bullet}$ and $ABTS^{\bullet+}$ was tested (Table 2). The results revealed that both extracts scavenged both $DPPH^{\bullet}$ and $ABTS^{\bullet+}$ to various extents. DPPH is a stable free radical with characteristic deep purple color in solution; antioxidants react with converting it to α,α-diphenyl-β-picryl hydrazine. DPPH solution loses its characteristic deep purple color, on accepting proton from antioxidants, leading to absorption decrease (λ_{max} 515–517 nm). Thus, the degree of discoloration is an indication of the scavenging ability of the antioxidant extract. Evidently, *B. eurycoma* exhibited a stronger DPPH scavenging ability than *D. microcarpum*, due to its lower IC_{50} value. The $ABTS^{\bullet+}$ scavenging result of the extracts followed the same trend as the DPPH result, with *B. eurycoma* being significantly ($P < 0.05$) higher than *D. microcarpum*. Unlike $DPPH^{\bullet}$, $ABTS^{\bullet+}$ is a moderately stable nitrogen-centered species, and the assay involves electron transfer process [43]. The reaction of $ABTS^{\bullet+}$ with antioxidants results in its discoloration, and the level of discoloration reflects the amount of $ABTS^{\bullet+}$ that is scavenged within a given time period in relation to that of Trolox (6-hydroxy-2,5,7,8-tetramethylchroman-2-carboxylic acid). Both extracts were able to reduce Fe^{3+} to Fe^{2+}, although *B. eurycoma* had a significantly ($P < 0.05$) higher reducing power than *D. microcarpum*.

Phytochemicals including polyphenols and saponins are prominent for their diverse health benefits including antioxidant and antidiabetic activities [44]. In this study, polyphenolics (total phenolics, tannins and total flavonoids) and total saponins were quantified in *B. eurycoma* and *D. microcarpum* seeds flour. The pharmacological activities of polyphenols have been demonstrated in several studies. They are well-known for their antidiabetic activity [45], and are regarded as the most important plant antioxidants [46]. Polyphenolic compounds exhibit antioxidant activity through a variety of mechanisms, including scavenging of free radicals, lipid peroxidation and chelating of metal ions [47]. Their ability to inhibit α-amylase and α-glucosidase is considered to be among the mechanisms of their antidiabetic activity [6,10], and this makes them natural pharmacological agents for the management of postprandial hyperglycaemia and its associated complications. Recently, Irondi et al. [10] demonstrated that *Mangifera indica* and *Mucuna urens* seeds extracts rich in phenolic compounds are potent inhibitors of α-amylase, α-glucosidase and aldose reductase which are linked to the pathology and complications of T2D *in vitro*. Similarly, saponins have been reported to exhibit health-benefitting effects including antioxidant properties [48] and reduction in blood glucose response [49]. Yang et al. [50] also reported that two new saponins from *Gynostemma pentaphyllum* inhibited α-glucosidase activity.

Thus, the inhibition of α-amylase, α-glucosidase, AR and lipid peroxidation; as well as the antioxidant activities observed in this study could be attributed to the total phenolics, tannins, total flavonoids and total saponins in the flours of the *B. eurycoma* and *D. microcarpum* seeds. A similar action between polyphenols and saponins was suggested by Michel et al. [51]. The predominance of these phytochemicals in *B. eurycoma* than *D. microcarpum* could be responsible for its stronger inhibitory potency.

5. Conclusion

The inhibition of α-amylase, α-glucosidase, AR activities by the extracts of *B. eurycoma* and *D. microcarpum* seeds flours observed in this study could be a possible mechanism of action supporting their use for the management of hyperglycemia and its associated complications in T2D. This inhibitory effect may be attributed to the action of their inherent polyphenols and total saponins. Comparatively, *B. eurycoma* proved to be more potent than *D. microcarpum*. The finding of this study therefore provides support for the exploitation of these two underutilized leguminous tree crops for nutraceutical purposes.

Conflict of interest statement

There are no conflicts of interest regarding the design, execution and publication of the study.

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