

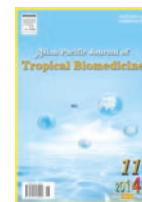
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# Phenolic composition and inhibitory activity of *Mangifera indica* and *Mucuna urens* seeds extracts against key enzymes linked to the pathology and complications of type 2 diabetes

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## PEER REVIEW

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## Comments

This is a valuable research work in which authors have demonstrated the potential inhibitor against the activities of  $\alpha$ -amylase,  $\alpha$ -glucosidase and aldose reductase, as well as lipid peroxidation. Reverse phase chromatographic quantification of the major flavonoids and phenolic acids in the seeds extracts was carried out using HPLC–DAD. The inhibitory activities of the extracts against  $\alpha$ -amylase and  $\alpha$ -glucosidase were estimated using soluble starch and PNPG as their respective substrates.  
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## ABSTRACT

**Objective:** To investigate the phenolic compounds composition and the inhibitory activity of *Mangifera indica* (*M. indica*) and *Mucuna urens* (*M. urens*) seeds extracts against some key enzymes ( $\alpha$ -amylase,  $\alpha$ -glucosidase and aldose reductase) implicated in the pathology and complications of type 2 diabetes *in vitro*.

**Methods:** Reverse phase chromatographic quantification of the major flavonoids and phenolic acids in the seeds extracts was carried out using high performance liquid chromatography coupled with diode array detection. The inhibitory activities of the seeds extracts against  $\alpha$ -amylase and  $\alpha$ -glucosidase were estimated using soluble starch and *o*-nitrophenylglucopyranoside as their respective substrates. Inhibition of aldose reductase activity by the extracts was assayed using partially purified lens homogenate of normal male rat as source of enzyme; inhibition of Fe<sup>2+</sup>-induced lipid peroxidation by extracts was tested in rat pancreas homogenate.

**Results:** The chromatography result revealed that extracts of both seeds had appreciable levels of some major flavonoids and phenolic acids of pharmacological importance, including gallic acid, chlorogenic acid, caffeic acid, ellagic acid, catechin, rutin, quercitrin, quercetin and kaempferol. Extracts of both seeds effectively inhibited  $\alpha$ -amylase,  $\alpha$ -glucosidase and aldose reductase activities in a dose-dependent manner, having inhibitory preference for these enzymes in the order of aldose reductase >  $\alpha$ -glucosidase >  $\alpha$ -amylase. With lower half-maximal inhibitory concentrations (IC<sub>50</sub>) against  $\alpha$ -amylase,  $\alpha$ -glucosidase, and aldose reductase, *M. indica* had stronger inhibitory potency against these enzymes than *M. urens*. Extracts of both seeds also inhibited Fe<sup>2+</sup>-induced lipid peroxidation in a dose-dependent pattern, with *M. indica* being more potent than *M. urens*.

**Conclusions:** The results obtained provide support for a possible use of *M. indica* and *M. urens* seeds in managing hyperglycemia and preventing the complications associated with it in type 2 diabetes.

## KEYWORDS

Hyperglycemia, Diabetic complications, Enzyme inhibition, Lipid peroxidation, *Mangifera indica*, *Mucuna urens*

## 1. Introduction

Diabetes mellitus (DM) is a group of metabolic diseases

characterized by hyperglycemia resulting from defects in insulin secretion, insulin action, or both [1]. With a disturbing global prevalence of 285 million people in

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2010 (that is, 6.4% of the world population), and a projected increase to 439 million people by 2030 (that is, 7.7% of the world population)<sup>[2]</sup>, DM is nearing a pandemic. About 90% to 95% of diabetic cases are diagnosed with type 2 diabetes (T2D)<sup>[3]</sup>.

T2D is characterized by chronic hyperglycemia due to insulin resistance and loss of pancreas  $\beta$ -cell function<sup>[4]</sup>. Hyperglycemia and the complications associated with it are threats to the life of T2D patients. Diabetic hyperglycemia stimulates other factors that facilitate the progression of diabetic complications such as retinopathy and nephropathy<sup>[5]</sup>. For instance, under hyperglycemic condition, the saturation of hexokinase necessitates the channeling of glucose to the polyol pathway in which aldose reductase reduces glucose to sorbitol, which is subsequently reduced to fructose by sorbitol dehydrogenase.

Currently available antidiabetic drugs, such as acarbose, in addition to not being effective in maintaining euglycemia, usually present with some side effects. Hence, there is increasing emphasis on the use of plant products rich in phenolic compounds that could be more effective for the management of T2D with less side effects. In addition to their effectiveness and safety, herbal remedies could be a cheaper alternative to the synthetic antidiabetic drugs. Consequently, the World Health Organization recommended that further research on the antidiabetic effects of medicinal plants should be carried out<sup>[6]</sup>.

Phenolic compounds are part of the secondary metabolites that constitute the active principles in plant products. These active ingredients are responsible for the therapeutic and/or pharmacological activities, such as antidiabetic effects, of medicinal plants<sup>[7]</sup>. Phenolic compounds are known to modulate glucose metabolism by several mechanisms including inhibition of carbohydrate digesting enzymes and aldose reductase<sup>[8,9]</sup>.

The inhibition of carbohydrate metabolizing enzymes such as  $\alpha$ -amylase and  $\alpha$ -glucosidase retards the digestion carbohydrates and the subsequent absorption of glucose, leading to a decrease in postprandial blood glucose level<sup>[8]</sup>. Furthermore, the inhibition of aldose reductase activity has been reported to be an effective pharmacological approach to prevent certain complications of diabetes<sup>[10]</sup>.

*Mangifera indica* L. (*M. indica*) (mango) and *Mucuna urens* L. (*M. urens*) (horse eye bean) seeds both have uses that range from local soup additives as thickeners<sup>[11,12]</sup> to ethnomedicinal applications<sup>[13,14]</sup>. The phytochemical composition and antioxidant activities of seeds extracts of these two plants had earlier been reported<sup>[15]</sup>. To further explore the pharmacological potentials of these two plants, this study investigated the phenolic composition and the *in vitro* inhibitory activity of *M. indica* and *M. urens* seeds extracts against some key enzymes linked to the pathology and complications of T2D.

## 2. Materials and methods

### 2.1. Sample collection and preparation

Samples of *M. indica* and *M. urens* seeds were purchased from a farm settlement in Ibadan, Oyo State, Nigeria. The seeds were later authenticated at the Department of Botany, University of Ibadan, Nigeria. Subsequently, the seeds were sorted, sun-dried, manually shelled and milled to a fine particle size. Milled samples were packed in airtight vials and stored at 4 °C until analysis.

All the chemicals used for analysis were of analytical grade.

### 2.2. Preparation of seed methanol extract

Sample powder (2 g) was extracted by steeping in 100 mL of methanol for 1 h with continuous shaking using a mechanical shaker. The supernatant, subsequently referred to as methanol extract, was collected after centrifuging at 3000 r/min for 10 min, and stored at -4 °C for further analysis. Methanol extract for carbohydrate hydrolyzing enzymes inhibition assays was concentrated to dryness, and redissolved in equal volume of dimethyl sulfoxide.

### 2.3. Handling of experimental animal

The Wister strain albino rats used in this study were handled in accordance with the Guide for the Care and Use of Laboratory Animals, as approved by the Animal Ethics Committee of our institution. Adult male rats weighing 200–250 g were procured from the experimental animal breeding unit of Department of Veterinary Medicine, University of Ibadan, Nigeria.

### 2.4. Quantification of phenolic compounds using high performance liquid chromatography coupled with diode array detection (HPLC-DAD)

The HPLC-DAD instrumentation comprised a Shimadzu Prominence Auto Sampler (SIL-20A) HPLC system (Shimadzu, Kyoto, Japan), equipped with Shimadzu LC-20AT reciprocating pumps connected to a DGU 20A5 degasser with a CBM 20A integrator, SPD-M20A diode array detector and LC solution 1.22 SP1 software.

Reverse phase chromatographic quantification of the major phenolic compounds in *M. indica* and *M. urens* seeds extracts was carried out at a concentration of 20 mg/mL under gradient conditions using  $C_{18}$  column (4.6 mm  $\times$  150 mm) packed with 5  $\mu$ m diameter particles; the mobile phase was water containing 1% formic acid (A) and acetonitrile (B), and the composition gradient was: 13% of B until 10 min and changed to obtain 20%, 30%, 50%,

60%, 70%, 20% and 10% B at 20, 30, 40, 50, 60, 70 and 80 min, respectively, following the method described by Kamdem *et al.* with slight modifications<sup>[16]</sup>. Samples extracts and mobile phase were filtered through 0.45 µm membrane filter (Millipore) and then degassed by ultrasonic bath prior to use. The flow rate was 0.7 mL/min, injection volume 50 µL and the wavelength were 257 nm for gallic acid, 281 nm for catechin, 325 nm for chlorogenic, caffeic and ellagic acids, and 366 nm for quercetin, quercitrin, rutin and kaempferol. Stock solutions of standards references were prepared in the HPLC mobile phase at a concentration range of 0.030–0.250 mg/mL for kaempferol, quercetin, quercitrin, rutin and catechin, and 0.050–0.450 mg/mL for ellagic, gallic, caffeic and chlorogenic acids. Chromatography peaks were confirmed by comparing its retention time with those of reference standards and by DAD spectra (200 to 600 nm). Both the sample extracts and the standards were analyzed in triplicates. Calibration curves obtained for the standards were as follows: gallic acid:  $Y=13\,569x+1\,276.2$  ( $r=0.999\,6$ ); chlorogenic acid:  $Y=13\,054x+1\,267.5$  ( $r=0.999\,8$ ); ellagic acid:  $Y=12\,832x+1\,246.3$  ( $r=0.999\,5$ ); catechin:  $Y=12\,783x+1\,257.9$  ( $r=0.999\,7$ ); caffeic acid:  $Y=12\,480x+1\,275.2$  ( $r=0.999\,3$ ); quercitrin:  $Y=13\,405x+1\,264.7$  ( $r=0.999\,9$ ); rutin:  $Y=11\,983x+1\,271.3$  ( $r=0.999\,8$ ); quercetin:  $Y=12\,683x+1\,292.5$  ( $r=0.999\,6$ ) and kaempferol:  $Y=12\,539x+1\,183.0$  ( $r=0.999\,7$ ).

### 2.5. $\alpha$ -amylase inhibition assay

$\alpha$ -amylase inhibition assay was carried out according to the method described by Kwon *et al.*<sup>[17]</sup>. Appropriate dilutions of the 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  $\alpha$ -amylase solution (0.5 mg/mL) 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 dinitrosalicylic acid 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  $\alpha$ -amylase inhibition was calculated using the formula:

$$\text{Inhibition (\%)} = [(A_{540_{\text{control}}} - A_{540_{\text{sample}}}) / A_{540_{\text{control}}}] \times 100$$

The IC<sub>50</sub> was calculated from the dose–inhibition non-linear regression curve of each extract.

### 2.6. $\alpha$ -glucosidase inhibition assay

The inhibitory effect of extracts against  $\alpha$ -glucosidase activity was determined according to the method described by Kim *et al.*<sup>[18]</sup>, using  $\alpha$ -glucosidase from *Bacillus stearothermophilus*. Briefly, 5 units of  $\alpha$ -glucosidase were pre-incubated with 20 µg/mL of the different seeds

methanol extracts for 15 min. About 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<sub>2</sub>CO<sub>3</sub>. The  $\alpha$ -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  $\alpha$ -glucosidase inhibition as follows:

$$\text{Inhibition (\%)} = [(A_{400_{\text{control}}} - A_{400_{\text{sample}}}) / A_{400_{\text{control}}}] \times 100$$

The IC<sub>50</sub> was calculated from the dose–inhibition non-linear regression curve of each extract.

### 2.7. Aldose reductase inhibition assay

Normal male rats weighing 200–250 g were used for the preparation of partially purified rat lens aldose reductase following a procedure adapted from Hayman and Kinoshita<sup>[19]</sup>. 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 10000 r/min 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 aldose reductase activity, was dispersed in 75% ammonium sulfate and subsequently used for the inhibition assay.

Inhibition of aldose reductase activity was assayed by estimating the consumption of NADPH at 340 nm as described by Da Settimo *et al.*<sup>[20]</sup>. 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 test relative to the control at 340 nm is a function amount of NADPH consumed. Hence:

$$\text{Inhibition (\%)} = [(A_{340_{\text{control}}} - A_{340_{\text{sample}}}) / A_{340_{\text{control}}}] \times 100$$

The IC<sub>50</sub> was estimated from the dose–inhibition non-linear regression curve of each extract.

### 2.8. Lipid peroxidation inhibition assay

The ability of the methanol extracts to inhibit Fe<sup>2+</sup>-induced lipid peroxidation in rat pancreas homogenate was assayed according to the modified method of Ohkawa *et al.*<sup>[21]</sup>. To prepare the low-speed pancreas supernatant used for the assay, normal male rats weighing 200–250 g were mildly

anaesthetized in ether; the pancreas were rapidly excised and placed in ice. Then 10% (w/v) pancreas homogenate prepared by homogenizing the pancreas in cold saline, was centrifuged for 10 min at 1400 r/min to yield a pellet that was discarded and the low-speed supernatant (S1). To a reaction mixture containing 100  $\mu$ L of S1 fraction, 30  $\mu$ L of 0.1 mol/L Tris-HCl buffer (pH 7.4) and different concentrations of plants methanol extracts, 30  $\mu$ L of freshly prepared 25  $\mu$ mol/L  $\text{Fe}^{2+}$  solution was added to initiate lipid peroxidation. The volume was made up to 300  $\mu$ L with deionized water before incubation at 37 °C for 1 h. The color reaction was initiated by adding 300  $\mu$ L of 81 g/L sodium duodecyl sulphate to the reaction mixture containing the S1, followed by the addition of 600  $\mu$ L of acetic acid/HCl (pH 3.4) and 600  $\mu$ L of 0.8% (v/v) thiobarbituric acid. This mixture was incubated at 100 °C for 1 h. The absorbance of thiobarbituric acid reactive species produced were measured at 532 nm in an UV-visible spectrophotometer. Appropriate controls of each plant extract and the positive control were carried out.

### 2.9. Statistical analysis

Results of triplicate experiments were expressed as mean  $\pm$ SD. Analysis of variance (ANOVA) and least significance difference (LSD) were carried out on the result data at 95% confidence level using SPSS statistical software package, version 17.

## 3. Results

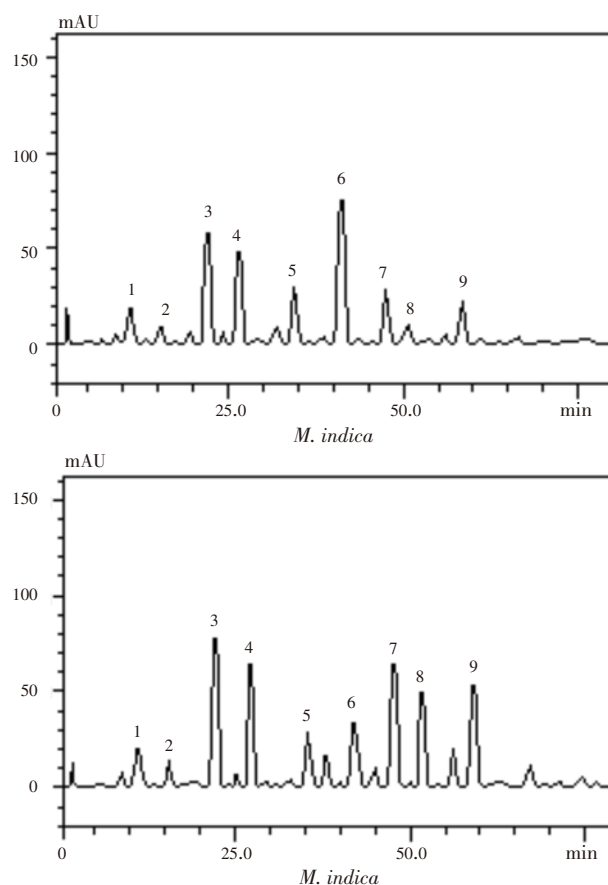
The phenolics (flavonoids and phenolic acids) composition of *M. indica* and *M. urens* seeds as quantified using HPLC-DAD is presented in Table 1. The result showed that both seeds had high levels of some major flavonoids and phenolic acids of pharmacological importance, including gallic acid, catechin, chlorogenic acid, caffeic acid, ellagic acid, rutin, quercitrin, quercetin and kaempferol (Figure 1). Gallic acid, chlorogenic acid, caffeic acid, quercitrin, quercetin and kaempferol were significantly ( $P < 0.05$ ) higher in *M. urens* than in *M. indica*. In contrast, *M. indica* had significantly ( $P < 0.05$ ) higher level of rutin than *M. urens*. The levels of catechin and ellagic acid were comparable ( $P > 0.05$ ) in both seeds.

**Table 1**

Major phenolics composition of *M. indica* and *M. urens* by HPLC-DAD.

Phenolic compounds (mg/g)	<i>M. indica</i>	<i>M. urens</i>
Gallic acid	3.81 $\pm$ 0.02 <sup>b</sup>	3.95 $\pm$ 0.01 <sup>a</sup>
Catechin	1.09 $\pm$ 0.01 <sup>a</sup>	1.12 $\pm$ 0.01 <sup>a</sup>
Chlorogenic acid	12.45 $\pm$ 0.03 <sup>b</sup>	18.05 $\pm$ 0.03 <sup>a</sup>
Caffeic acid	9.97 $\pm$ 0.03 <sup>b</sup>	15.73 $\pm$ 0.01 <sup>a</sup>
Ellagic acid	5.36 $\pm$ 0.01 <sup>a</sup>	5.26 $\pm$ 0.03 <sup>a</sup>
Rutin	17.72 $\pm$ 0.03 <sup>a</sup>	7.31 $\pm$ 0.01 <sup>b</sup>
Quercitrin	5.29 $\pm$ 0.03 <sup>b</sup>	15.82 $\pm$ 0.02 <sup>a</sup>
Quercetin	1.18 $\pm$ 0.01 <sup>b</sup>	10.17 $\pm$ 0.01 <sup>a</sup>
Kaempferol	3.87 $\pm$ 0.02 <sup>b</sup>	11.06 $\pm$ 0.03 <sup>a</sup>

Results are expressed as mean $\pm$ SD of triplicate determinations. Values with different letters along the same row differ ( $P < 0.05$ ).



**Figure 1.** Representative HPLC chromatograms of *M. indica* and *M. urens* seed extracts.

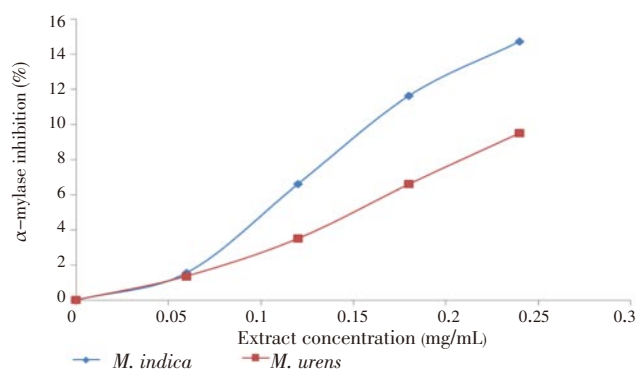
Gallic acid (peak 1), catechin (peak 2), chlorogenic acid (peak 3), caffeic acid (peak 4), ellagic acid (peak 5), rutin (peak 6), quercitrin (peak 7), quercetin (peak 8) and kaempferol (peak 9).

Table 2 shows the  $\text{IC}_{50}$  values of *M. indica* and *M. urens* extracts against  $\alpha$ -amylase,  $\alpha$ -glucosidase, aldose reductase and  $\text{Fe}^{2+}$ -induced lipid peroxidation. The results revealed that both extracts inhibited  $\alpha$ -amylase,  $\alpha$ -glucosidase and aldose reductase, in a dose-dependent pattern as shown in Figures 2–4. The  $\text{IC}_{50}$  values of *M. indica* extract against each of these three enzymes were consistently lower than those of *M. urens*. This was an indication that *M. indica* had a more potent inhibitory ability against  $\alpha$ -amylase,  $\alpha$ -glucosidase and aldose reductase activity than *M. urens*. The result further showed that both extracts displayed inhibitory preference against the activities of these three enzymes in the order of aldose reductase  $>$   $\alpha$ -glucosidase  $>$   $\alpha$ -amylase. Similarly, extracts of both seeds inhibited lipid peroxidation induced by iron (II) in rat pancreas homogenate in a dose-dependent manner (Figure 5); however, *M. indica* was more effective than *M. urens* as indicated by its lower  $\text{IC}_{50}$  value.

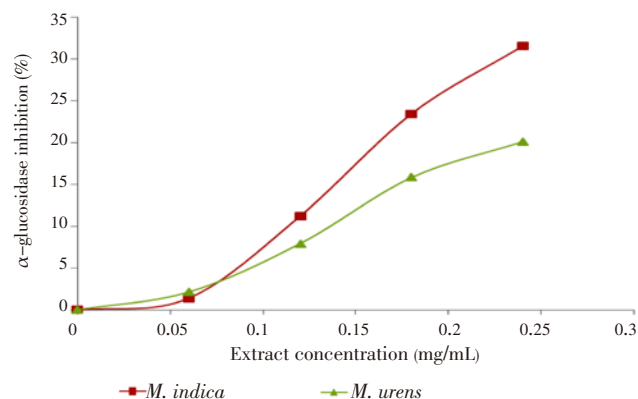
**Table 2**

Half-maximal inhibitory concentration ( $\text{IC}_{50}$ ) of *M. indica* and *M. urens* seeds extracts against  $\alpha$ -amylase,  $\alpha$ -glucosidase, aldose reductase activities and lipid peroxidation.

$\text{IC}_{50}$ (mg/mL)	<i>M. indica</i>	<i>M. urens</i>
$\alpha$ -amylase	0.71	1.13
$\alpha$ -glucosidase	0.34	0.52
Aldose reductase	0.27	0.42
Lipid peroxidation	0.10	0.16



**Figure 2.** Concentration–inhibition curve of *M. indica* and *M. urens* seed extracts against  $\alpha$ -amylase activity.

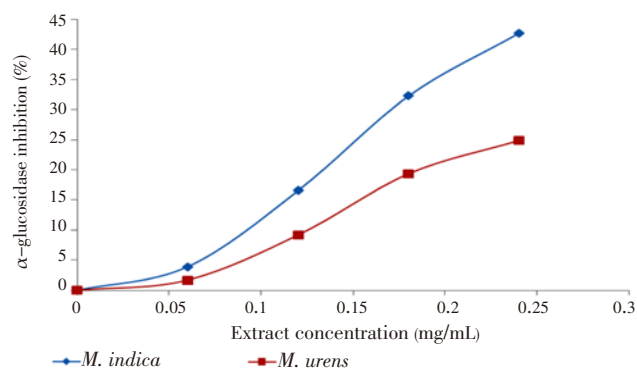


**Figure 3.** Concentration–inhibition curve of *M. indica* and *M. urens* seed extracts against  $\alpha$ -glucosidase activity.

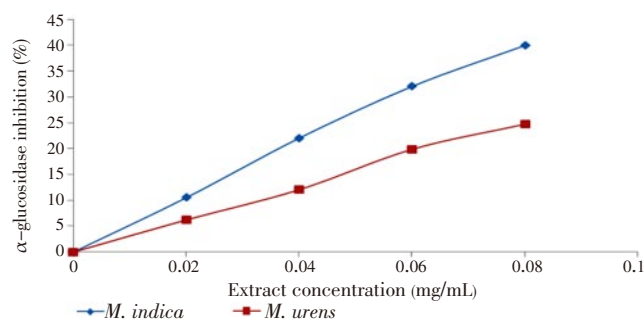
#### 4. Discussion

There has been increasing interest in the use of plant-derived hypoglycemic agents for the management of T2D and its complications in the recent time due to the side effects associated with the use of synthetic hypoglycemic drugs. In addition to the side effects, synthetic hypoglycemic drugs have other limitations such as development of resistance, irresponsiveness in some patient population, and inability to effectively control the hyperlipidemia that usually presents with T2D[22].

Phenolic compounds possess pharmacological activities including antidiabetic effects. In this study, two classes of pharmacologically important phenolic compounds were quantified, namely, flavonoids and phenolic acids. Flavonoids are synthesized from phenylalanine[23], and are widely distributed in the plant kingdom. Prominently, flavonoids have continued to receive increasing attention because of their health benefits as antioxidants in the prevention of human diseases[24]. In addition to their antioxidant properties, as natural drugs, flavonoids are known to possess antihyperglycemic activity[25], protect against the development of diabetes[26,27], and ameliorate the complications of diabetes[28,29]. Other reported pharmacological activities of flavonoids include antiallergic, antiinflammatory, hepato- and gastro-protective, antiviral, and antineoplastic activities[30].



**Figure 4.** Concentration–inhibition curve of *M. indica* and *M. urens* seed extracts against aldose reductase activity.



**Figure 5.** Concentration–inhibition curve of *M. indica* and *M. urens* seed extracts against  $\text{Fe}^{2+}$ -induced lipid peroxidation in rat pancreas homogenate.

The flavonoids quantified in the extracts were catechin, rutin, quercitrin, quercetin and kaempferol. Of these flavonoids, quercetin and rutin are consumed more abundantly in foods[31]. Rutin is known to possess various pharmacological activities including antihyperglycemic[25]. Similarly, quercetin has been reported to exhibit antidiabetic activity[32], and alleviate diabetic nephropathy in streptozotocin–diabetic rats[33]. Interestingly, the levels of quercetin quantified in both *M. indica* and *M. urens* seeds (1180 mg/kg and 10170 mg/kg respectively) in this study are over 1000 times larger than the level (generally below 10 mg/kg) in the edible parts of vegetables[34]. The phenolic acids of pharmacological importance quantified were gallic acid, caffeic, chlorogenic and ellagic acid. Caffeic acid is regarded as the most common phenolic compounds distributed in the plant flora followed by chlorogenic acid[35]. Research has shown that plants containing these phenolic acids exhibit antidiabetic activity[36,37]. Catechins, for example, have been reported to possess inhibitory properties against  $\alpha$ -glycosidase and  $\alpha$ -amylase *in vitro*[38].

Postprandial blood glucose level depends partly on the activities of carbohydrate hydrolyzing enzymes, mainly,  $\alpha$ -amylase and  $\alpha$ -glucosidase, that break down dietary starch and sugars into glucose[39].  $\alpha$ -amylase is present in the saliva and pancreatic juice, and is responsible for cleaving the  $\alpha$ -1,4 bonds of starch releasing dextrin, maltose, and maltotriose[40]. The glycosidic linkages of

these oligosaccharides are subsequently hydrolyzed by  $\alpha$ -glucosidase present in the ciliary membrane of small intestine to produce glucose molecules which are then absorbed<sup>[41]</sup>. Consequently, the inhibition of  $\alpha$ -amylases and  $\alpha$ -glucosidases activities is one of the most effective approaches to control hyperglycemia in type 2 diabetic patients<sup>[18]</sup>.

Plant-derived phenolic compounds have the potential to inhibit both  $\alpha$ -amylase and  $\alpha$ -glucosidase, making them natural pharmacological agents for the management of postprandial hyperglycemia and its associated complications<sup>[42]</sup>. The results obtained in this study revealed that extracts of *M. indica* and *M. urens* seeds potentially inhibited the activities of  $\alpha$ -amylase,  $\alpha$ -glucosidase and aldose reductase. Of interest is their inhibitory preference for these three enzymes which was in the order of aldose reductase >  $\alpha$ -glucosidase >  $\alpha$ -amylase. The stronger inhibition of  $\alpha$ -glucosidase over  $\alpha$ -amylase by both extracts observed in this study could confer them with the pharmacological advantage of lesser clinical side effects than acarbose and other synthetic oral hypoglycemic drugs currently used to manage hyperglycemia in diabetic patients. In contrast to this observed inhibitory preference, acarbose has been reported to inhibit both  $\alpha$ -amylase and  $\alpha$ -glucosidase<sup>[43]</sup>, but with preference for pancreatic  $\alpha$ -amylase<sup>[44]</sup>. This excessive inhibition of  $\alpha$ -amylase underlies the clinical side effects of acarbose including diarrhea, flatulence and abdominal discomfort<sup>[17,44]</sup>. This finding is in agreement with the report of Kwon *et al.*<sup>[17]</sup> that unlike acarbose, plant-derived  $\alpha$ -amylase and  $\alpha$ -glucosidase inhibitors have a stronger inhibitory effect against  $\alpha$ -glucosidase activity than  $\alpha$ -amylase activity. Diarrhea in diabetic patients could exacerbate their nutritional status through loss of both essential and nonessential nutrients, and body fluids.

The inhibitory effect of plant extracts against aldose reductase activity is a possible therapeutic approach to ameliorate diabetic complications<sup>[45,46]</sup>. Aldose reductase is the first and rate-limiting enzyme of the polyol pathway that catalyzes the reduction of glucose to sorbitol using NADPH as a cofactor<sup>[47]</sup>. This aldose reductase-catalyzed reaction is favoured under the hyperglycemic condition that characterizes T2D, and is believed to be the metabolic bases for diabetic complications in complication-prone tissues with high aldose reductase activity such as the eye, kidney, heart and nerves<sup>[48]</sup>. Previous studies have provided evidence for the involvement of aldose reductase in diabetic complications such as neuropathy, retinopathy, nephropathy and cataract<sup>[47,49]</sup>. In this study, both *M. indica* and *M. urens* seeds extracts displayed a dose-dependent inhibition of aldose reductase activity, suggesting that both plants have the potential to mitigate the complications of T2D. However, based on their  $IC_{50}$  values (0.71 mg/mL and 1.13 mg/mL for *M. indica* and *M. urens*, respectively), *M. indica* was more potent in inhibiting aldose reductase

activity than *M. urens*. This aldose reductase inhibitory effect of the extracts could be attributed to their flavonoids; the reason being that flavonoids have been reported to function as anti-diabetic complications agents due to their antioxidant and free radical scavenging activities<sup>[9]</sup>.

Lipid peroxide-mediated tissue damages have been reported in T2D<sup>[50]</sup>. The peroxidation of lipids disrupts the fluidity and permeability of the membrane leading to the oxidative damage of unsaturated fatty acids components of biological membranes. In this study, lipid peroxidation in rat pancreas homogenate was induced with  $Fe^{2+}$ . The accumulation of  $Fe^{2+}$  in the acinar cells and in the islets of Langerhan, results in the oxidative destruction of the  $\beta$ -cells of the pancreas associated with DM<sup>[51]</sup>. Therefore the ability of *M. indica* and *M. urens* extracts to inhibit  $Fe^{2+}$ -induced lipid peroxidation in rat pancreas homogenate is an indication that both extracts could mitigate the oxidative damage of the pancreas cells in T2D. Comparatively, *M. indica* with a lower  $IC_{50}$  had a stronger inhibitory potential against rat pancreas lipid peroxidation potential than *M. urens*.

The predominance of pharmacologically important flavonoids and phenolic acids in the extracts of *M. indica* and *M. urens* seeds could be responsible for the inhibitory effect of the extracts against the activities of  $\alpha$ -amylase,  $\alpha$ -glucosidase and aldose reductase, as well as lipid peroxidation observed in this study. Thus, the inhibition of the activities of these carbohydrate-metabolizing enzymes by the extracts may be a possible mechanism of action supporting their use for the management of hyperglycemia and its associated complications in T2D. Comparatively, *M. indica* may be more pharmacologically potent in inhibiting the activities these enzymes than *M. urens*.

### Conflict of interest statement

We declare that we have no conflict of interest.

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### Comments

#### Background

Diabetes-specific microvascular disease is a leading cause of blindness, renal failure and nerve damage. Four main molecular mechanisms have been implicated in glucose-mediated vascular damage. All seems to reflect a single hyperglycaemia-induced process of overproduction of superoxide by the mitochondrial electron-transport chain. This integrating paradigm provides a new conceptual framework for future research and drug discovery. Therefore, there is need of identifying novel and potent sources of phenolic compounds with inhibitory activity against key enzymes involved in complication of T2D.

### Research frontiers

The present study reveals the presence of major flavonoids and phenolic acids with pharmacological importance, such as gallic acid, chlorogenic acid, caffeic acid, ellagic acid, catechin, rutin, quercitrin, quercetin and kaempferol and potential inhibitory activity (*in vitro*) of extracts of both the seeds against  $\alpha$ -amylase,  $\alpha$ -glucosidase and aldose reductase activities in a dose-dependent manner.

### Related reports

Dietary phytochemicals, of which polyphenols form a considerable part, may affect the risk of obesity-associated chronic diseases such as T2D. Fruits, vegetables, berries, beverages and herbal medicines have been reported to be widely used against various ailments ranging from obesity to diabetes in the traditional medicine. Besides, there is available evidence in the literature for phenolic compounds to be associated with antioxidant activity and may modify imbalanced lipid and glucose homeostasis thereby reducing the risk of the metabolic syndrome and T2D complications.

### Innovations and breakthroughs

Seeds of *M. indica* and *M. urens* are currently underutilized as compared to their potential health beneficial effects. In the present study, authors have demonstrated the potential role of underutilized seeds in managing hyperglycemia and preventing the complications associated with it in T2D through the inhibitory activity of key enzymes, which may be due to their shown corresponding polyphenol content.

### Applications

From the literature survey it has been found that the studied seeds are safe to humans. This scientific study supports and suggests the use of these seeds as an excellent source of pharmacologically important flavonoids and phenolic acids in nutraceutical, food and medicinal industries.

### Peer review

This is a valuable research work in which authors have demonstrated the potential inhibitor against the activities of  $\alpha$ -amylase,  $\alpha$ -glucosidase and aldose reductase, as well as lipid peroxidation. Reverse phase chromatographic quantification of the major flavonoids and phenolic acids in the seeds extracts was carried out using HPLC-DAD. The inhibitory activities of the extracts against  $\alpha$ -amylase and  $\alpha$ -glucosidase were estimated using soluble starch and PNPG as their respective substrates. Inhibition of aldose reductase activity was assayed using partially purified lens homogenate of normal male rat as source of enzyme; inhibition of  $\text{Fe}^{2+}$ -induced lipid peroxidation by extracts was tested in rat pancreas homogenate. In addition, *M. indica* may be more pharmacologically potent in inhibiting the activities these enzymes than *M. urens*.

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