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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 α-amylase, α-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 α-amylase and α-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 (α-amylase, α-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 α-amylase and α-glucosidase were estimated using soluble starch and ρ-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^{2+}-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 α-amylase, α-glucosidase and aldose reductase activities in a dose-dependent manner, having inhibitory preference for these enzymes in the order of aldose reductase>α-glucosidase>α-amylase. With lower half-maximal inhibitory concentrations (IC_{50}) against α-amylase, α-glucosidase, and aldose reductase, *M. indica* had stronger inhibitory potency against these enzymes than *M. urens*. Extracts of both seeds also inhibited Fe^{2+}-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)[2], DM is nearing a pandemic. About 90% to 95% of diabetic cases are diagnosed with type 2 diabetes (T2D)[3].

T2D is characterized by chronic hyperglycemia due to insulin resistance and loss of pancreas β-cell function[4]. 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[5]. 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[6].

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[7]. Phenolic compounds are known to modulate glucose metabolism by several mechanisms including inhibition of carbohydrate digesting enzymes and aldose reductase[8,9].

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

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[11,12] to ethnomedicinal applications[13,14]. The phytochemical composition and antioxidant activities of seeds extracts of these two plants had earlier been reported[15]. 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 3 000 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 ×150 mm) packed with 5 μm 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%,