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Research Paper

# *Hancornia speciosa* Gomes (Apocynaceae) as a potential anti-diabetic drug

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ABSTRACT

*Ethnopharmacological relevance:* The leaves of *Hancornia speciosa* Gomes are traditionally used to treat diabetes in Brazil. The aim of the study is to evaluate the potential anti-diabetic effect of *Hancornia speciosa* extract and derived fractions.
*Materials and Methods:* The ethanolic extract from *Hancornia speciosa* leaves and chromatographic fractions thereof were evaluated on α-glucosidase assay, on hyperglycemic effect and glucose uptake. The chemical composition of the extract and its most active fraction was investigated by ESI–LC–MS.
*Results:* The ethanolic extract and derived fractions inhibited α-glucosidase in vitro. However, only the crude extract and the dichloromethane fraction inhibited the hyperglycemic effect induced by starch or glucose. Both the extract and dichloromethane fraction were also able to increase glucose uptake in adipocytes. Bornesitol, quinic acid, and chorogenic acid were identified in the extract, along with flavonoid glycosides, whereas the dichloromethane fraction is majorly composed by esters of lupeol and/or α/β-amirin.
*Conclusions: Hancornia speciosa* has a potential anti-diabetic effect through a mechanism dependent on inhibition of α-glucosidase and increase on glucose uptake. These results give support to the use on traditional medicine of this medicinal plant.



## 1. Introduction

Diabetes is defined as a metabolic disorder characterized by chronic hyperglycemia with disturbances of protein and fat metabolism resulting from defects in insulin secretion, insulin action, or both (WHO, 2013). Diabetes type 2 is the most frequent and has been evolved into a global epidemic (WHO, 2013).

Despite the great number of hypoglycemic drugs available, most of them exhibit undesirable side effects, high cost and ultimately cannot efficiently control alone the glycemia (Hung et al., 2012), unless if associated with the adoption of lifestyle measures, such as physical activity, control of a healthy body weight, avoid smoking and maintenance of a healthy diet (WHO, 2013).

Several plant species are traditionally used as anti-diabetic around the world and some of them have had their efficacy corroborated by pre-clinical and clinical assays (Hung et al., 2012; Chang et al., 2013; Mata et al., 2013). *Hancornia speciosa* Gomes (Apocynaceae), popularly known as mangabeira or mangaba, is a tree widely distributed in the *Cerrado* biome from Brazil. It is traditionally used as anti-diabetic, as anti-hypertensive and to treat obesity (Hirschmann and de Arias, 1990; Rodrigues and Carvalho, 2001; Macedo and Ferreira, 2004). The potential anti-hypertensive, chemopreventive, antioxidant and anti-inflammatory effects of *Hancornia speciosa* have been consistently demonstrated by our research group (Ferreira et al., 2007a; Ferreira et al., 2007b; Endringer et al., 2009; Endringer et al., 2010; Silva et al., 2011).

The lack of a scientific report supporting the large use of this plant for the treatment of diabetes led us to investigate the potential anti-diabetic effect of *Hancornia speciosa* using in vitro and in vivo methods.

## 2. Material and methods

### 2.1. Preparation of the extract and fractions

The leaves of *Hancornia speciosa* were furnished by Empresa Estadual de Pesquisa Agropecuária da Paraíba (EMEPA), João Pessoa,

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Brazil. The vegetal material was collected in April 2009 from the Active Germplasm Bank of Mangaba (BAG-Mangaba) in João Pessoa (geographic coordinates: 6° 33' 13" S and 34° 48' 31" W; altitude of 30 m). After drying at 40 °C for 72 h, the plant material was powdered (100 g) and percolated with 96% ethanol to afford a green dark residue (EEH, 23.45 g) after solvent elimination under reduced pressure. A portion of EEH (20 g) was submitted to chromatography on a silica gel 60 (0.2–5 mm) column (330 × 60 $mm^2$ i.d.) eluted with solvents of increasing polarity. After solvent elimination in a rotatory evaporator, the n-hexane (0.031 g), DCM (1.198 g), DCM/EtOAc; (1:1) (1.252 g), EtOAc (0.108 g), EtOAc/MeOH; (1:1) (16.0 g) and MeOH (0.827 g) fractions were obtained.

### 2.2. α-glucosidase inhibition assay in vitro

The inhibitory activity against α-glucosidase was carried out spectrophotometrically in a 96-well microplate reader as previously described (Pereira et al., 2012a). A reaction mixture containing 0.04 U/mL of enzyme, sample (0.3–1000 μg/mL) and 0.1 mM of reduced glutathione was preincubated for 15 min. The reaction was started by the addition of 0.85 mM p-nitrophenyl-α-D-glucopyranoside (*p*NPG) as substrate. After 25 min incubation at 37 °C, sodium carbonate was added to stop the reaction. Absorbance readings were recorded at 405 nm (Thermoplate Reader, USA). Samples were dissolved in DMSO and other reagents in 0.1 mM phosphate buffer pH 6.8. Acarbose (0.1–100 ng/mL) was used as a reference drug. The inhibitory effect was expressed as a percentage of inhibition related to the control.

### 2.3. Glucose uptake assay

Adipocytes were isolated from epididymal fat pads by the method of Rodbell (1964). After isolation, adipocytes were incubated with EEH and DCM (100 μg/mL) and insulin (50 ng/mL) for 45 min. The uptake of 2-deoxy-[$^3$H]glucose was used to determine the rate of glucose transport as previously described (Green, 1987). Briefly, glucose uptake was initiated by the addition of 2-deoxy-[$^3$H]glucose (0.2 μCi/tube) for 3 min. Thereafter, cells were separated by centrifugation through silicone oil and cell-associated radioactivity was determined by scintillation counting.

### 2.4. Animals

Animal experimental protocols conformed international guidelines and were approved by the local ethics committee [protocol 163/2010, UFMG]. Male Swiss mice (10–12 week old) were maintained with free access to standard diet (Nuvilab, Brazil) and tap water was supplied ad libitum, at a constant temperature (23 ± 2 °C), with a 12:12 h dark/light cycle. All experiments were carried out using at least five animals per group.

### 2.5. Acute effect of EEH and its fractions on blood glucose in mice

The indirect evaluation of α-glucosidase inhibition in animals was performed after cornstarch overload. Swiss mice were deprived of food for 6 h before the test but allowed free access of tap water throughout the experiment. Blood samples were collected from the tail tip before EEH and fractions administration (time 0). EEH, fractions, and cornstarch were administrated orally. EEH and fractions (300 mg/kg) were dissolved in 50% labrasol. After 20 min, cornstarch (2 g/kg) was administrated, and blood samples were collected at 15, 30, 60 and 120 min after starch overload. Acarbose (10 mg/kg) was used as a reference drug.

In order to evaluate the mechanism of action of the samples, the glucose tolerance test was performed as described above, except that glucose replaced cornstarch and glibenclamide (5 mg/kg) replaced acarbose as reference drug.

### 2.6. Drugs

Unless otherwise mentioned, all reagents were purchased from Sigma-Aldrich. Blood glucose concentration was determined with an Accu-Chek active glucose meter (Roche Diagnostics, Brazil). Labrasol was purchased from Gatefossé SAS (France).

### 2.7. Statistical analysis

The percentage of α-glucosidase inhibition was evaluated using the $pIC_{50}$ ($-\log$ of the sample concentration that inhibits activity of the enzyme by 50%), which was calculated using non-linear regression curve and expressed as mean ± S.E.M.. Two-way or one-way ANOVA analysis followed by the Bonferroni post-test were employed, and significance was accepted at $P < 0.05$.

### 2.8. Chemical characterization of EEH and DCM by ESI–LC–MS

The chemical composition of EEH and DCM was analyzed using an Acquity UPLC system (Waters, Milford, USA) with a Photodiode Array (PDA) detector and interfaced to a triple quadrupole mass spectrometer (TQD) (Waters Micromass, Manchester, UK). The analyses were carried out on a reverse phase column (Acquity UPLC BEH C18, Waters, Ireland; 50 × 2.1 $mm^2$ i.d., 1.7 μm) in combination with a guard column (Acquity UPLC BEH C18 VanGuard pre-column, Waters, Ireland; 2.1 × 5 $mm^2$ i.d., 1.7 μm), using a gradient elution of water (A) and acetonitrile (B), both containing 0.1% v/v formic acid (5–95% B in 10 min; 95–5% B in 1 min, followed by 2 min of isocratic elution), at a flow rate of 0.3 mL/min, and temperature of 40 °C. Sample volume injected was 3 μL for EEH (2 mg/mL) and 5 μL for DCM (5 mg/mL), both prepared as MeOH solutions. For the ESI (negative and positive mode) source, the following inlet conditions were applied: capillary voltage 3.54 kV; cone voltage 17 V; source temperature 120 °C; desolvation temperature 300 °C. The mass range was set at *m*/*z* 100–2000.

## 3. Results and discussion

Inhibition of α-glucosidase has been proven to be a valid therapeutic option for the prevention of type 2 diabetes (Chiasson et al., 2002). In the present study, the effect of EEH and its derived chromatographic fractions on α-glucosidase were evaluated in vitro (Fig. 1). EEH, DCM, DCM:EtOAc, EtOAc, EtOAc:MeOH and MeOH fractions promoted a concentration-dependent inhibition of α-glucosidase activity (Fig. 1A) with similar potency, excepting EtOAc, which showed a lower $pIC_{50}$ value (Fig. 1B). The small yielding of the hexane fraction did not allow us to perform the test with α-glucosidase. Considering the difference of polarity among the fractions and the crude extract, the obtained results suggest that the different classes of compounds may account for the α-glucosidase inhibition. The idea of a broad class of natural products as inhibitors of α-glucosidase is compatible with reports from the literature (Reddy et al., 2009; Bräunlich et al., 2013). L-bornesitol, quinic acid, and rutin were previously reported in an ethanol extract from *Hancornia speciosa* leaves (Endringer et al., 2009). In our experimental conditions, L-bornesitol did not inhibit α-glucosidase (data not shown). Quinic acid and rutin derivatives are well-known inhibitors of α-glucosidase (Iwai et al., 2006; Pereira et al., 2011), and probably contribute to the observed effect. Moreover, chemical studies with *Hancornia speciosa* leaves have also indicated the presence of terpenoids, steroids and tannins (Honda et al., 1990; Brandão et al., 2011), which could also play a role in the inhibitory effect of this plant on α-glucosidase.

The starch tolerance test demonstrated that EEH, DCM, and MeOH fractions were able to decrease the plasma concentration of