



Original Article

Phytochemical composition and chronic hypoglycemic effect of *Rhizophora mangle* cortex on STZ-NA-induced diabetic rats



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ABSTRACT

Type 2 diabetes is a major health problem in Mexico, as it is in other countries, is a chronic condition that develops when the body cannot produce enough insulin or cannot use it appropriately. Both insulin deficiency and insulin resistance lead to high blood glucose levels. In Mexico, people with diabetes are known to use the decoction of red mangrove (*Rhizophora mangle* L., Rhizophoraceae) bark to control blood glucose levels. Therefore, in this study, we sought to investigate the chronic hypoglycemic and hypolipidemic effects of *R. mangle*; we also elucidate some of the major phytochemical compounds of *R. mangle*. To analyze the hypoglycemic and hypolipidemic effects, we used rats with streptozotocin–nicotinamide-induced hyperglycemia; the rats were classified into four groups (six rats each), based on the treatment given, as follows: group 1, non-hyperglycemic control; group 2, hyperglycemic control; group 3, glibenclamide (5 mg/kg body weight); and group 4, *Rhizophora* ethanol–water extract (90 mg/kg). The extract or glibenclamide was orally administered, dissolved in 1.5 ml of physiological NaCl-solution, twice a day (in the morning and in the evening) over a period of 42 days. The methanolic extract was used to elucidate the main compounds present in *R. mangle* via conventional phytochemical methods, such as TLC, HPLC, UPLC–DAD–MS, and NMR. The following compounds were detected: cinchonins Ia and Ib, catechin-3-O-rhamnopyranoside, epicatechin, lyoniside, and nudiposide. The daily administration of *Rhizophora* ethanol–water extract, similar to the traditional usage to control type 2 diabetes, was shown to exert chronic hypoglycemic and hypolipidemic effects. This effect may be associated with the constituents in the extract. These findings suggest that *R. mangle* and its constituents could be potentially used to treat type 2 diabetes.

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Introduction

Diabetes is a chronic condition that occurs when the body cannot produce enough insulin or cannot use it appropriately. In type 2 diabetes (T2D), the body can produce insulin but becomes resistant to it, causing ineffectiveness of insulin. Consequently, insulin levels may become insufficient, and thus insulin resistance and insulin deficiency result in high blood glucose levels (IDF, 2015) and (ADA, 2015). Individuals with T2D suffer from insulin resistance and usually relative, rather than absolute, insulin deficiency. However, at least initially, and often throughout their lifetime, these individuals may not require insulin treatment to survive.

In 2015, the IDF estimated that 415 million people are living with diabetes worldwide; Mexico stands sixth among the top ten countries, with 11.5 million people (IDF, 2015). Diabetes-associated complications, such as cardiovascular disease, blindness, kidney failure, and lower-limb amputation, are a major cause of disability, low quality of life, and premature death.

Among the World Health Organization list of essential drugs used for the treatment of diabetes, metformin (a biguanide) and gliclazide (a sulfonylurea) are well-established medications and they should be available and easily accessible (according to need), to all patients with T2D (IDF, 2015). It is of significance that metformin was originally isolated from the French lilac (*Galega officinalis*) (Witters, 2001).

Plants have been used for medicinal purposes in Mexico since pre-Hispanic times. The high prevalence of T2D among Mexicans, associated to vulnerable economic stability, and the fact that people trust the effectiveness of medicinal plants have led to the increased use of plants to treat T2D. These factors have made it essential to

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study the pharmacological and phytochemical properties of plants with hypoglycemic properties in Mexico.

Rhizophora mangle L., Rhizophoraceae (Andrade-Cetto and Heinrich, 2005), traditionally known as “mangrove” or “red mangrove,” is widely used for the treatment of diabetes in Mexico. It is a 25-m-tall tree that grows in mangroves and distributed along the Pacific and Gulf Coasts of Mexico. It has a tall, straight trunk with abundant roots, a round treetop with sympodial branching, bitter-red wood, and cortex (Pennington and Sarukhán, 1998).

The anti-hyperglycemic effect of the plant was previously reported by Alarcon-Aguilara et al. (1998), they test the effect of 28 plants in rabbits under a glucose tolerance test, the results obtained from the variance analysis showed that *R. mangle* significantly decreased the hyperglycemic peak by 16.1%.

In a previous study performed by our group, the ethnobotanical relevance and the acute hypoglycemic effect of *R. mangle* were reported (Andrade-Cetto and Mares, 2012), in that study we confirm that the dose of 90 mg/kg has the better hypoglycemic effect, this dose is the traditional used dose multiplied by 10. In the present study, we aimed to examine the chronic hypoglycemic effect of the ethanolic extract of the bark of *R. mangle* in streptozotocin–nicotinamide (STZ-NA)-induced diabetic rats; we also evaluated the lipid profile and glycated hemoglobin after chronic administration. In addition, we sought to characterize the major phytochemical compounds present in the plant cortex.

Materials and methods

Plant extracts

Based on the results of the previous study in which the water and ethanol–water extracts (EW) were tested (Andrade-Cetto and Mares, 2012), we selected the ethanol–water extract which is similar to the traditional used infusion and presented better activity (Fig. 1). New botanical samples of *Rhizophora mangle* L., Rhizophoraceae, were collected with the help of informants in Manialtepec, Oaxaca Mexico, the original plant was deposited at the IMSS, Herbarium in Mexico City with the voucher number IMMSM15816. The extract to be used in pharmacological tests was prepared as previously described; in brief, a 50 g sample of the plant material was added to 500 ml of an ethanol–water mixture (1:1), it was then heated at 40 °C for 4 h before being filtered for three times. This was followed by elimination of the solvent under reduced pressure in a Büchi rotary evaporator. The yield of the extract ethanol–water (1:1) was 14.75 g.

For the phytochemical identification of the main compounds of the cortex, the methanolic extract (ME) was prepared using 200 g of plant material through Soxhlet extraction. Defatting with hexane (24 h) followed by methanol (MeOH) extraction (48 h), and the resulting extract evaporated under reduced pressure until it reached dryness producing 15 g of ME.

Compounds isolation

HPLC–DAD–MS analysis was performed to confirm that the ethanol–water extract used in the pharmacological testing has a similar phytochemical profile than the water and methanolic extracts (Fig. 1), but the last one was more accessible for the isolation process.

A sample of ME (3 g) was dissolved in MeOH and partitioned with hexane to yield a hexane soluble fraction (HSF; 50 mg), a MeOH-soluble fraction (MSF, 2.90 g), and a red precipitate (RP; 28 mg).

The MSF was subjected to column chromatography (CC) on 360 g of silica gel (70–230 mesh, Merck) starting with hexane 100% (400 ml), increasing the polarity with EtOAc using a mixture of hexane/EtOAc as eluent, until 100% (500 ml), and subsequently with MeOH until 100% (500 ml). This process led to fourteen primary fractions (MSF1–MSF14). Fraction MSF8 (400 mg) was subjected to silica gel CC eluted with EtOAc/MeOH (10:0–0:10), this process led to five subfractions (MSF 8.1–MSF8.5). Preparative thin layer chromatography (TLC) (Macherey & Nagel, 0.25 mm) of fraction MSF8.2 (20 mg) using EtOAc/MeOH/H₂O, 7:2:1, as eluent resulted in the isolation of a mixture of **1** and **2** (10 mg). Preparative TLC (EtOAc/MeOH/H₂O, 7:2:1) of fraction MSF8.3 (80 mg) resulted in the isolation of **3** (23.7 mg) and **4** (13 mg). FSM8.4 was resolved by HPLC (Nucleosil 250 × 10 mm i.d., 5 μm, C18, Macherey & Nagel); using a gradient of MeCN/H₂O starting with 20/80 to 70/30 during 17 min (3 ml/min; 250 and 280 nm UV-det.) to obtain 10 mg of a mixture of **5** and **6** with an Rt 10.5 min.

An efficient method based on HPLC–DAD–MS technique was used for identifying the isolated compounds from the methanolic extract corresponding to (**1**–**6**) in the water and ethanol–water extract. The components were separated on a Kinetex HPLC/UPLC XB-C18 column (50 × 2.1 mm i.d., 2.6 μm) at 25 °C. The mobile phase consisted of a water gradient (containing 0.1% FA) (A) and acetonitrile (B). The following gradient elution program was used: 1% B during 0.5 min, 1–35% B 0.5–15 min, 35–100% B 15–18 min, 100–1% B 18–20 min at a flow rate of 0.2 ml min⁻¹, the injection volumen was 3 μl. Majority compounds of the traditional decoction and the ethanol–water extract were identified and are shown in Fig. 1.

General experimental procedures

NMR spectra including HSQC, HMBC, COSY, and TOCSY were recorded in a Varian Inova spectrometer at 500 (¹H) and 125 MHz (¹³C) or a JEOL-ECA at 300 (¹H) and 75 MHz (¹³C); chemical shifts were recorded as δ values. HRESIMS were recorded on a Thermo Scientific LTQ Orbitrap XL hybrid FTMS (Fourier transform mass spectrometer). Data were collected in both positive and negative ionization modes via a liquid chromatographic/autosampler system that consisted of an Acquity UPLC system. Profile HPLC–DAD–MS were performed using an Agilent 1200 Infinity system equipped with a G1312–95006 Binary pump, G1329–90012 Autosampler, controlled by Agilent ChemStation software, coupled to a Waters diode array detector (DAD) and a Squire 6000 Bruker ESI-MS in negative mode ion polarity.

Analytical and preparative HPLC analyses were performed in an Agilent 1260 Infinity system equipped with a G1311B Quaternary pump, G1367E Autosampler, G1315C DAD VL+ and controlled by Agilent ChemStation software. For analytical and semipreparative HPLC, Macherey–Nagel (Nucleosil C₁₈, 250 × 4.6 mm i.d., 5 μm), Macherey–Nagel (Nucleosil C₁₈, 250 × 10 mm i.d., 5 μm) columns, respectively, were used. Column chromatography (CC) was carried out on silica gel (70–230 mesh, Merck). Thin-layer chromatography analysis was carried out on silica gel 60 F₂₅₄ plates (Macherey & Nagel) using ceric sulfate (10%) solution in H₂SO₄ as color reagent.

Experimental animals

Eight-week-old Wistar rats weighing 200–250 g were obtained from the Bioterium of the Science School, UNAM, and were acclimated with free access to food and water for at least one week in an air-conditioned room (25 °C with 55% humidity) on a 12 h light–dark cycle prior to performing the experiments. The animals were handled according to the National Institute of Health, USA (Committee for the Update of the Guide for the Care and Use of Laboratory Animals, 2011). Experimental diabetes was induced

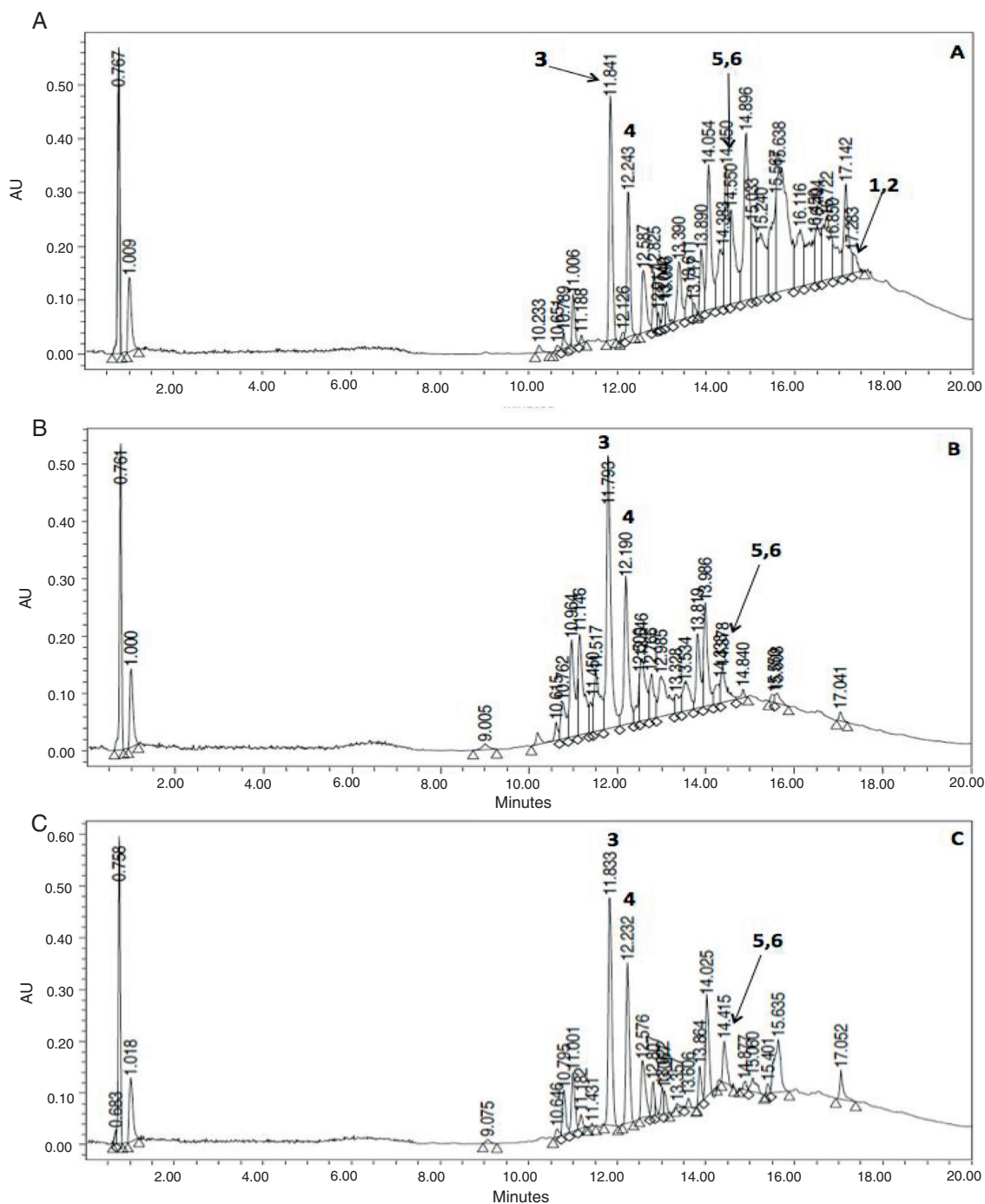


Fig. 1. HPLC–DAD profile comparison of the methanolic, ethanol–water and water extracts. (A) Methanolic extract, (B) water extract, (C) ethanol–water extract.

as described by Masiello et al. (1998). In brief, the rats were fasted overnight and injected intraperitoneally with 150 mg/kg nicotinamide (NA) (Sigma, N3376) 15 min before an intravenous injection of 65 mg/kg streptozotocin (STZ) in citrate buffer (Sigma, S0130). Diabetes was identified by polydipsia, polyuria and by measuring non-fasting plasma glucose levels 48 h after injection. Animals which did not develop more than 250 mg/dl glucose levels were rejected.

The hyperglycemic animals were classified into four groups (1–4) each of them with six rats. Group 1 as normal control received 1.5 ml of physiological NaCl-solution (vehicle), group 2

as hyperglycemic control received also 1.5 ml of physiological NaCl-solution, group 3 was given a standard oral hypoglycemic agent, glibenclamide (5 mg/kg bodyweight (bw)), in the same vehicle, while group 4 received Rm-EW (90 mg/kg bw) dissolved in 1.5 ml of physiological NaCl-solution. The extract or the hypoglycemic agent was orally administered twice a day (in the morning and in the evening) over a period of 42 days. All groups were fed Purina Rodent Laboratory Chow 5001.

Blood samples were obtained from the tail vein, the animals were handled according to procedures outlined by the Committee

for the Update of the Guide for the [Care and Use of Laboratory Animals \(2015\)](#). All methods used in this study were approved by the Internal Council of the “Facultad de Ciencias” of the Universidad Nacional Autónoma de México. Glucose monitoring was performed weekly and analyzed with glucose test strips and a glucometer Accutrend® plus. Glycated hemoglobin (HbA1c) was analyzed in a DCA Vantage® Siemens, equipment. The lipid profile (HDL TG and cholesterol) were measured with Cardio Check® and strips Panels® PTS. VLDL was calculated using the following $VLDL = 0.2 \times TG$. Both HbA1c and lipid profiles were measured on days 0, 14, 28 and 42 after the initiation of administration of treatments.

Statistical methods

The data were statistically analyzed by unpaired *t*-test with the help of the software GraphPad Prism. The plasma glucose levels were expressed as the mean (S.E.M.).

Results and discussion

Ethnobotany

Traditional use of *R. mangle* cortex for the treatment of type 2 diabetes was confirmed by the traditional healers who assisted during the plant collection in Manialtepec, Oaxaca, Mexico. For this purpose, a rough approximation of 20 g of plant cortex are boiled in 500 ml of water for 15 min, once the decoction is cold, it is consumed throughout the day as the so called “agua de uso”.

Compound identification

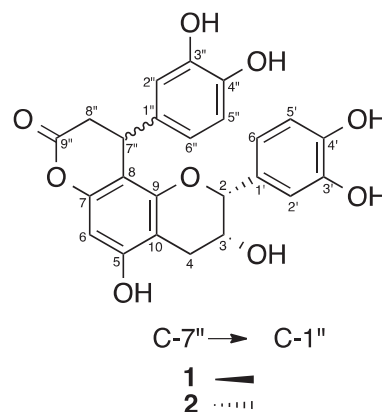
Cinchonains 1a and 1b (1 and 2)

Compounds **1** and **2** were identified by their ¹H and ¹³C NMR spectral data, included 2D experiments (COSY, HSQC, HMBC, NOESY), and mass spectral data, which allowed the identification of compounds **1** and **2** as a mixture.

The mass spectrum (ESI-MS negative ion mode), showed only one *pseudo* molecular ion at *m/z* 451.66 [M–H][–] indicating a molecular formula C₂₄H₂₀O₉ for both compounds **1** and **2**, while the ¹H and ¹³C NMR spectra showed duplicated signals. The ¹H NMR spectrum (CD₃OD, 300 MHz) showed pairs of signals at $\delta_{H(1/2)}$ 4.81/4.87 (br s, H-2), $\delta_{H(1/2)}$ 4.25/4.19 (m, H-3) and $\delta_{H(1/2)}$ 2.90/2.86 (m, H-4) suggesting the presence of a flavan-3-ol moiety in the molecule. The typical ABX spin system due to the aromatic protons of the B-ring, were also present as pairs of signals, at $\delta_{H(1/2)}$ 6.97/6.84 (d, *J* = 1.7/1.9 Hz, H-2'), $\delta_{H(1/2)}$ 6.75/6.69 (d, *J* = 8.2/8.1 Hz, H-5') and $\delta_{H(1/2)}$ 6.80 (dd, *J*₍₁₎ = 1.7, 7.9)/6.62 (dd, *J*₍₂₎ = 2.1, 8.1 Hz) (H-6'). Two singlet signals at $\delta_{H(1/2)}$ 6.20/6.21 suggested a tri-substituted A-ring. The presence of a phenylpropanoid moiety related with caffeic acid was indicated by an extra aromatic ABX system with signals at δ_H 6.64 (d, *J* = 7.6 Hz, 1H, H-5''), 6.44 (dd, *J* = 8.0, 2.2 Hz, 1H, H-6''), 6.55 (d, *J* = 2.2 Hz, 1H, H-2''), in addition to an aliphatic ABX system due to the methine CH-7'' and the methylene CH₂-8'', at δ_H 4.55 (dd, *J* = 6.8, 1.3 Hz, 1H, H-7''), and 3.0 (m, 2H, H-8'').

The above data agree with those published for the mixture of cinchonains 1a (**1**) and 1b (**2**), isolated from *Trichilia catigua*, Meliaceae (Pizzolatti et al., 2002). The ¹³C NMR data and 2D NMR experiments (COSY, HSQC, HMBC, NOESY; 300 MHz, acetone-D₆) confirmed the above assignments. HMBC correlation of H-8'' and H-7'' with the tertiary carbon C-8 ($\delta_{C(1/2)}$ 105.7/105.9), indicating that the phenylpropanoid unit was attached to the C-8 position of the epicatechin moiety. The relative configuration at the C-7'' stereogenic center of compounds **1** and **2** was established based on the correlations observed in the NOESY experiment. Thus, for compound **1** a correlation between H-2 and H-2''/6'' was observed;

while for compound **2** NOESY correlation between H-7'' and H-2'/6'' was observed. The same NOESY interactions were previously reported by Resende et al. (2011) for cinchonains 1a and 1b.

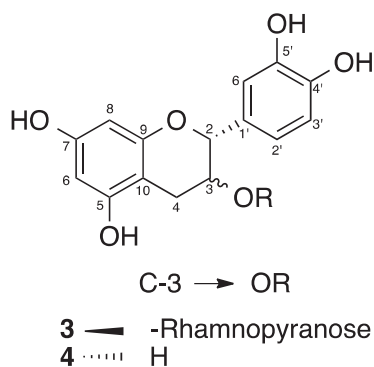


Catechin-3-O-rhamnopyranoside (3)

Compound **3** was obtained as a yellowish amorphous solid, the molecular formula C₂₁H₂₄O₁₀ was deduced from the ESI-MS [M+H]⁺ ion at *m/z* 437.314 and 435.73 [M–H][–]. The ¹H and ¹³C NMR spectra and 2D NMR experiments (COSY, HSQC, HMBC, NOESY, TOCSY; 500 MHz, CD₃OD) indicating the presence of catechin and rhamnose moieties in the molecule. The ¹H NMR spectrum showed the ABX aromatic system with signals at $\delta_{H/C}$ 6.84/115.1 (d, *J* = 2.0 Hz, 1H, H-2'/C-2'), 6.76/116.1 (d, *J* = 8.0 Hz, 1H, H-5'/C-5'), 6.71/119.8 (dd, *J* = 8.0, 1.8 Hz, 1H, H-6'/C-6') due to the B-ring protons. An AX system associated with two-meta coupled aromatic protons with signals at $\delta_{H/C}$ 5.94/96.4 (d, *J* = 2.5 Hz, 1H, H-6/C-6) and 5.86/95.5 (d, *J* = 2.5 Hz, 1H, H-8/C-8) and an AMX₂ spin system with resonances at $\delta_{H/C}$ 4.62/81.1 (d, *J* = 8.0 Hz, 1H, H-2/C-2), 3.93/75.9 (m, 1H, H-3/C-3), and at 2.64/27.9 (dd, *J* = 16.5, 8.5 Hz, 1H, H-4 α /C-4), and 2.88/27.9 (dd, *J* = 16.5, 5.5 Hz, 1H, H-4 β /C-4), indicated the presence of a flavan 3-ol skeleton. The *J*_{2,3} coupling of 8.0 Hz together with the chemical shift of C-2 at $\delta_{H/C}$ 4.62/81.1 indicate a 2,3-*trans* stereochemistry of the catechin. The HMBC spectrum shows correlation of H-3 (δ_H 3.93) with the anomeric carbon signal at δ_C 102.2 (C-1'') indicating a 3-O-linkage of the rhamnose to the flavan. All the above data agreed with those for catechin-3-O-rhamnopyranoside (Ishimaru et al., 1987). ESI-MS (positive ion mode): [M+H]⁺ *m/z* 437.314.

Epicatechin (4)

Compound **4** was obtained as a red amorphous powder. ESI-MS positive ion mode: [M+H]⁺ 291.152 and 289.58 [M–H][–]. The ¹H NMR revealed an ABX-type aromatic spin system with proton signals at $\delta_{H/C}$ 7.01/115.3 (d, *J* = 2.0 Hz, 1H, H-2'/C-2'), 6.78/115.9 (d, *J* = 8.0 Hz, 1H, H-5'/C-5'), 6.83/119.4 (dd, *J* = 8.0, 1.8 Hz, 1H, H-6'/C-6'), and an AB meta coupled aromatic system with signals at $\delta_{H/C}$ 5.94/96.4 (d, *J* = 2.5 Hz, 1H, H-6/C-6) and 5.91/95.8 (d, *J* = 2.5 Hz, 1H, H-8/C-8). The presence of an AMX₂ system with resonances at lower frequencies $\delta_{H/C}$ 4.90/79.8 (d, *J* = 3.5 Hz, 1H, H-2/C-2), 4.18/67.5 (ddd, *J* = 1.54, 3.10, 4.64, 1H, H-3/C-3), and 2.74/29.2 (dd, *J* = 16.8, 2.8 Hz, 1H, H-4 α) and 2.88/29.2 (dd, *J* = 16.6, 4.3 Hz, 1H, H-4 β), indicate the presence of a flavan 3-ol skeleton. The *J*_{2,3} coupling of 3.5 Hz together with the chemical shift of the methine C-2 at $\delta_{H/C}$ 4.90/79.8, indicating a 2,3-*cis* stereochemistry. Thus, compound **4** was identified as epicatechin. Comparison with published data confirmed the above assumption, (Schroeter et al., 2006).



Lyoniside and nudiposide (5 and 6)

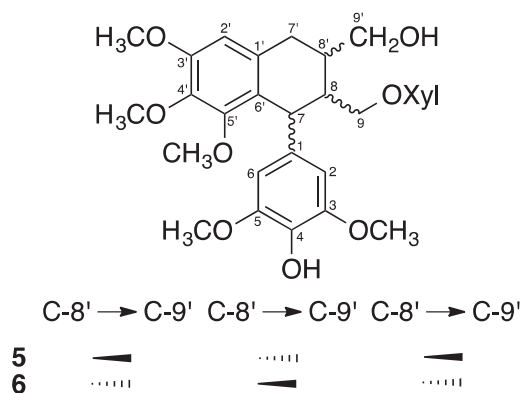
Compounds **5** and **6** were isolated as a brown amorphous solid. The UV spectrum showed absorptions at 236, and 276 nm (MeOH). The molecular formula for **5** and **6** $C_{27}H_{36}O_{12}$ was deduced from the pseudo-molecular ion peaks at m/z 575.446 $[M+Na]^+$, and 551.65 $[M-H]^-$, obtained by ESI-MS in positive and negative modes, respectively. The identification of **5** and **6** was possible by careful analysis of 1H and ^{13}C NMR spectra, included 1D and 2D experiments (COSY, HSQC, HMBC, TOCSY); at 500 MHz, using D_2O as solvent. The ^{13}C NMR spectrum of the mixture showed some dual signals, while the 1H NMR spectrum showed differences only in the chemical shifts of the anomeric proton H-1'', and some alicyclic protons of the xylose moiety. The 1H and ^{13}C NMR spectra displayed the typical signals of a lignan skeleton, including an aromatic proton singlet at $\delta_{H/C}$ 6.81/108.8 (s, 1H, H-2'/C-2'), and two equivalent aromatic protons singlet at $\delta_{H/C}$ 6.53/106.9 (s, 2H, H-2, H-6/C-2, C-6) for **5**; and at $\delta_{H/C}$ 6.81/108.7 (s, 1H, H-2'/C-2'), and 6.55/106.9 (s, 2H, H-2, H-6/C-2, C-6) for **6**. The 1H and ^{13}C NMR spectra also showed four singlet signals due to five methoxyl groups, two of them symmetrically equivalents [$\delta_{H/C}$ 3.91/57.1 (s, 3H, OMe-3'), 3.81/57.3 (s, 6H, OMe-3, OMe-5), 3.43/60.7 (s, 3H, OMe-5'), 3.91/57.1 (s, 3H, OMe-3'), 3.82/57.3 (s, 6H, OMe-3, OMe-5), 3.42/60.7 (s, 3H, OMe-5')] for **6**. The NMR spectra of **5** and **6** revealed other signals, consistent with the presence of a xylose moiety [$\delta_{H/C}$ 4.37/104.7 (d, $J=7.8$ Hz, H-1'', anomeric proton), 3.34/73.9 (dd, $J=8.5$ Hz, 16.5 Hz, 1H, H-2''/C-2''), 3.47/76.5 (t, $J=9.2$ Hz, 1H, H-3''/C-3''), 3.63/70.2 (m, 1H, H-4''/C-4''), 3.59/66.0 (dd, $J=11.3$ Hz, 4.3 Hz, 1H, H-5 α ''/C-5''), 3.95/66.0 (m, 1H, H-5 β ''/C-5'') for **5**; and 4.07/103.4 (d, $J=7.5$ Hz, H-1'', anomeric proton), 3.28/73.7 (m, 1H, H-2''/C-2''), 3.63/70.1 (m, 1H, H-3''/C-3''), 3.09/76.6 (t, $J=11.1$ Hz, 1H, H-4''/C-4''), 3.59/66.1 (dd, $J=11.3$ Hz, 4.3 Hz, 1H, H-5 α ''/C-5''), 3.95/66.1 (m, 1H, H-5 β ''/C-5'') for **6**. The value of the coupling constant ($J=7.5$ Hz) between the anomeric proton and C-2'' position, suggested the β -orientation of the glycosidic linkage in **5** and **6**. The HMBC correlation from H-1'' (δ_H 4.37 for **5**; 4.07 for **6**) to C-9 (δ_C 71.6 for **5** and **6**) indicated that the xylose unit was linked to the oxygen at C-9. The above data

Table 1

Chronic hypoglycemic effect of *Rhizophora mangle* cortex on STZ-NA induced diabetic rats. The values represent the mean \pm SEM. Superscripted letters in the same row indicate statistically significant differences compared with time 0. Superscripted numbers in the same column indicate statistically significant differences compared with the respective control group (a,1) ($p < 0.05$).

Groups	Glucose						
	T0 (mg/dl)	T7 (mg/dl)	T14 (mg/dl)	T21 (mg/dl)	T28 (mg/dl)	T35 (mg/dl)	T42 (mg/dl)
1 Norm.	123 \pm 3	129 \pm 1	124 \pm 2	127 \pm 1	125 \pm 4	118 \pm 6	131 \pm 3
2 Hyperg.	175 \pm 0 ¹	171 \pm 2 ¹	171 \pm 10 ¹	154 \pm 4 ¹	168 \pm 7 ¹	162 \pm 4 ¹	168 \pm 9 ¹
3 Glib. 5 mg/kg	179 \pm 2	129 \pm 5 ^{a1}	148 \pm 9 ^a	132 \pm 6 ^{a1}	150 \pm 9 ^a	134 \pm 11 ^{a1}	153 \pm 11 ^a
4 Rm-EW 90 mg/kg	184 \pm 2	140 \pm 7 ^{a1}	145 \pm 3 ^{a1}	141 \pm 2 ^{a1}	146 \pm 3 ^{a1}	141 \pm 3 ^{a1}	144 \pm 5 ^{a1}

were in agreement with those for lyoniside and nudiposide (**5** and **6**) (Sadhu et al., 2007).



The major metabolites isolated (**3–6**) of ME, were identified through the elaboration of an HPLC–ESI–MS chromatographic profile of Water extract of *R. mangle*.

Efficacy in diabetic rats

We confirmed that the Stz-Na model is suitable for a chronic experiment in which glucose values are evaluated. The glucose values for the group 1 (normal) remain stable around 125 mg/dl over the 42 days of experimentation, whereas the values for the group 2 (hyperglycemic) were around 170 mg/dl in the same period; the group 2 presented statistically significant higher values as compared to the group 1 (Table 1). The glycosylated hemoglobin and triacylglycerides (Table 2) are also higher in the group 2 in contrast to the group 1 and the increase in Hb1Ac and triacylglycerides levels is significant after 14 days of the injection.

The standard hypoglycemic agent glibenclamide could control the glucose levels from day 7 and the Hb1Ac after 28 days. The Rm-EW extract controls the glucose values from day 7, and the Hb1Ac after 28 days, the Hb1Ac results are statistically different from their own time 0 but not from group 2, the Rm-EW extract also controls the triacylglycerides and the VLDL levels after 28 days (Tables 1 and 2).

R. mangle traditional usage to treat type 2 diabetes is through drinking the decoction throughout the day, this way of administration is noticeable and it is not associated with meals, this means the plant is not used in the post absorptive state. This fact could be related to the action mechanism.

Some of the main components of *R. mangle* cortex were isolated from the methanol soluble fraction (MSF) by repeated column chromatography in silica gel and purified by HPLC. This process allowed the isolation and identification of two flavanones: **1** and **2** (cinchonins Ia and Ib), two flavanols: **3** (catechin-3-O- α -rhamnopyranoside) and **4** (epicatechin), and two lignan glycosides: **5** and **6** (lyoniside and nudiposide). These compounds had not been previously reported for *R. mangle*. The chromatographic profile for

Table 2

Chronic hypoglycemic effects of the *Rhizophora mangle* cortex on STZ-NA induced diabetic rats. The values represent the mean \pm SEM. Superscripted letters in the same row indicate statistically significant differences compared with time 0. Superscripted numbers in the same column indicate statistically significant differences compared with the respective control group (a,1) ($p < 0.0$).

Groups	T0			T14			T28			T42		
	HbA1c (%)	Tg (mg/dl)	vLDL (mg/dl)	HbA1c (%)	Tg (mg/dl)	vLDL (mg/dl)	HbA1c (%)	Tg (mg/dl)	vLDL (mg/dl)	HbA1c (%)	Tg (mg/dl)	vLDL (mg/dl)
1 Norm.	3.6 \pm 0.1	71 \pm 4.0	14 \pm 1	3.6 \pm 0.1	79 \pm 16	16 \pm 3	3.54 \pm 0.1	63 \pm 4	12 \pm 1	3.62 \pm 0.1	61 \pm 10	12 \pm 2
2 Hyperg.	3.7 \pm 0.1	52 \pm 1 ¹	10 \pm 1 ¹	4.1 \pm 0.2 ¹	77.6 \pm 14	15 \pm 3	4.2 \pm 0.1 ^{1a}	119 \pm 15 ^{1a}	24 \pm 3 ^{1a}	4.3 \pm 0.1 ^{1a}	113 \pm 26 ^a	23 \pm 5 ^a
3 Glib. 5 mg/kg	3.8 \pm 0.2	69 \pm 11	14 \pm 2	4.2 \pm 0.2	89 \pm 13.	18 \pm 3	3.9 \pm 0.2	100 \pm 14	20 \pm 3	3.9 \pm 0.2 ¹	115 \pm 19	23 \pm 4 ^a
4 Rm-EW 90 mg/kg	3.4 \pm 0.2	78 \pm 10	15 \pm 2 ¹	4.1 \pm 0.2 ^a	76 \pm 13	15 \pm 3	3.9 \pm 0.1	65 \pm 4 ¹	13 \pm 1 ¹	4.0 \pm 0.2	72 \pm 10	14 \pm 2

all extracts (Fig. 1) indicates that 3–6 are part of the main compounds found in *R. mangle*; however, it is still necessary to continue the chemical analysis of the other subfractions for a better understanding of the chemical profile of this plant.

Among plant metabolites, phenols are found to possess a wide range of biological effects. In recent years, plant polyphenols including phenolic acids, flavonoids, stilbenes and lignans, based on *in vitro* studies, animal models and some clinical trials, have been proposed as effective supplements for diabetes management and prevention of its long-term complications (Bahadoran et al., 2013).

Based on several *in vitro*, animal models and some human studies, dietary plant polyphenols and polyphenol-rich products, modulate carbohydrate and lipid metabolism as well as attenuate hyperglycemia, dyslipidemia and insulin resistance (Bahadoran et al., 2013).

Si et al. (2011) shows that epicatechin treatment caused changes in diabetic mice. These changes are associated with a healthier and longer lifespan, including improved skeletal muscle stress output, reduced systematic inflammation markers and serum LDL cholesterol, increased hepatic antioxidant glutathione concentration and total superoxide dismutase activity, decreased circulating insulin-like growth factor-1, and improved AMP-activated protein kinase activity in the liver and skeletal muscle. Recent studies (Litterio et al., 2015) showed that epicatechin prevented hypertension in a *in vivo* model diet with 10% (w/v) fructose in the drinking water (high fructose, HF) for eight weeks on rats, decreasing superoxide anion production and elevating NOS activity, favoring an increase in NO bioavailability. Epicatechin and epicatechin-rich foods improves insulin sensitivity in high fat diet-fed in a mouse model of obesity and T2D triggered by high fat consumption in mice (Cremonini et al., 2016).

Cinchonain Ib, from *Eriobotrya japonica* (Thunb.) Lindl., Rosaceae, leaves enhanced insulin secretion from INS-1 cells (rat insulinoma cell), as well as reduced plasma insulin level in rats after 108 mg/kg oral administration, however, it did not induce any changes in blood levels (Qa'dan et al., 2009).

In diabetic rats the ethanol–water extract exerts a hypoglycemic effect after seven days of administration, the effect was sustained until day 42, the decrease in glucose levels were reflected in the Hb1Ac levels after 28 days, despite being significant not before 42 days after the beginning of the experiment. The reason for this is; Hb1Ac is formed in a non-enzymatic glycation when hemoglobin is exposed to plasma glucose, when the average amount of plasma glucose increases, the fraction of glycated hemoglobin increases. This serves as a marker for average blood glucose levels over the previous three months.

Insulin deficiency and insulin resistance cause in consequence an increase in lipolysis conducted by adipocytes. The activation of this pathway contributes to dyslipidaemia, condition present in type 2 diabetics (De Fronzo et al., 2015). In the present study, decrease of triacylglycerides and plasma glucose levels was observed, suggesting the control of insulin resistance as a possible mechanism of action. Such decrease may be due to the activation

of the AKT kinase pathway which is responsible for inhibiting the enzymes involved in lipolysis whereby the concentration of triglycerides in the blood decreases. Another important factor is that AKT also is responsible for inhibiting the activity of GSK3 increasing glycogen synthesis which decreases the release of glucose by the liver (Sunil et al., 2012), for this reason we suggest that the hypoglycemic effect of the plant may be linked to the liver glucose output.

In summary, the decoction of *R. mangle* exerts a chronic (42 days of treatment) hypoglycemic and hypolipidemic effect. This effects could be associated with the compounds present in the water extract: catechin-3-*O*-rhamnopyranoside, lyoniside, nudiposide and especially by epicatechin. However, further studies are needed to tackle the mechanism of action responsible of its effects.

Ethical disclosures

Protection of human and animal subjects. The authors declare that the procedures followed were in accordance with the regulations of the relevant clinical research ethics committee and with those of the Code of Ethics of the World Medical Association (Declaration of Helsinki).

Confidentiality of data. The authors declare that no patient data appear in this article.

Right to privacy and informed consent. The authors declare that no patient data appear in this article.

Authors' contributions

AA-C idealized the study, write the manuscript and get the financial support; GMT-V perform the pharmacological experiments; SME-R performs the phytochemical experiments; LQ reviewed the phytochemical experimental data.

Conflicts of interest

The authors declare no conflicts of interest.

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