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Original Article

Chronic hypoglycemic effect and phytochemical composition of *Smilax moranensis* roots

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ABSTRACT

Smilax moranensis M.Martens & Galeotti, Smilacaceae, root is a medicinal plant used among the Chatinos in Oaxaca, Mexico, to control type 2 diabetes. The objectives of the study were to isolate the bioactive compounds from the roots of *Smilax* and evaluate the chronic hypoglycemic effect of the ethanol–water extract. The main compounds were isolated from the methanolic extract *via* conventional phytochemical methods. The dried roots of *S. moranensis* were extracted with methanol and chromatographed on Sephadex LH 20. Fractions were chromatographed and purified on a silica gel chromatography column. The ethanol–water extract was orally administered to hyperglycemic rats for a period of 42 days, and glucose, glycated hemoglobin (*HbA1c*), and triacylglycerides were measured. Moreover, very-low-density lipoprotein was calculated. During the chemical investigation, three compounds were isolated and characterized, namely, 3-*O*-caffeoyl-quinic acid, 5-*O*-caffeoyl-quinic acid and *trans*-resveratrol, using various spectroscopic techniques. Animal experiments confirmed that the plant extract could control both the glucose and *HbA1c* levels. In conclusion, this study confirms that the roots of *S. moranensis* have hypoglycemic properties and suggests that the isolated compounds are potentially involved in this effect.

Introduction

Type 2 diabetes affects people worldwide, and millions of people are suffering from the current pandemic; at present, nearly half a billion people live with diabetes, and low- and middle-income countries carry almost 80% of the burden (IDF, 2017). Deficient β-cell insulin secretion, frequently in the presence of insulin resistance, appears to be the common denominator of the disease (ADA, 2017).

The genus *Smilax*, commonly called sarsaparilla, comprises approximately 370 species that are widely distributed in tropical and temperate zones throughout the world, especially East Asia and North America (Wu et al., 2010; Zhou et al., 2017). Several reports about the *Smilax* genus detail its pharmacological activities as a diuretic as well as its ability to treat rheumatic arthritis, gout, cancer, diabetes and inflammatory diseases (Wu et al., 2010; Andrade-Cetto, 2011; Khaligh et al., 2016). Previous phytochemical studies of *Smilax* species have identified several secondary metabolites, including steroidal saponins, flavonoids, lignans, chlorogenic acids and stilbenes, which some have been found to have cytotoxic, anti-inflammatory, antioxidant, antihemolytic and antibacterial activities (Xu et al., 2005; Wu et al., 2010; Chen et al., 2014; Khaligh et al., 2016; Shu et al., 2017a,b; Zubair et al., 2017).

Smilax moranensis M.Martens & Galeotti, commonly named "cocolmecatl", belongs to the Smilaceae family. It is a Mexican woody vine with stems covered in spikes and is widely distributed in warm and temperate climates between 600 and 1900 m.a.s.l. In Mexico, the root infusions of *S. moranensis* have been used in traditional medicine as a diuretic and for the treatment of diabetes (Alambrillo et al., 2009; Andrade-Cetto, 2011). Previously, we documented that in the community of Santos-Reyes, Nopala, Oaxaca, Mexico, the Chatino population uses *S. moranensis* roots for the treatment of type 2 diabetes. Furthermore, we found that this plant has an acute hypoglycemic effect 60 min after administration in streptozotocin-induced diabetic rats (Andrade-Cetto, 2011). The present study is focused on evaluation of the chronic hypoglycemic activity of the ethanol–water extract of *S. moranensis* in streptozotocin-induced diabetic rats and isolation of bioactive compounds from the roots.

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Materials and methods

Plant material and extracts

The roots of *Smilax moranensis* M.Martens & Galeotti, Smilacaceae, were originally collected in Santos-Reyes Nopala, Oaxaca, México, in 2011 and 2013. A voucher specimen (IMSS 15815) has been deposited at the Medicinal Plant Herbarium IMSS. New and fresh plant material was collected as needed.

Two types of extracts were prepared: the methanolic extract (ME) was prepared for compound isolation, and the ethanol–water extract (EW) was prepared for animal testing. In a previous study, we demonstrated that the aqueous extract, analogous to the traditionally used tea, has a similar composition to the EW extract and that the latter possesses a better hypoglycemic effect than the former. In the present study, we selected the dose of 80 mg/kg EW previously reported (Andrade-Cetto, 2011).

For the preparation of the EW, 20 g of air-dried and powdered roots of *S. moranensis* were extracted in a mixture of 250 ml water: 250 ml ethanol for 15 min. Subsequently, the extract was filtered under vacuum over a diatomaceous earth layer. The filtrate was frozen for 24 h and then lyophilized. The dried extract (1.4 g) was stored at −40 °C in a Revco® freezer for further investigation.

For phytochemical identification of the main compounds in the roots, the ME was prepared using 120 g of powdered *S. moranensis* roots through Soxhlet extraction. The resulting mixture was defatted with hexane (48 h) and then extracted (48 h) with methanol (MeOH). The resulting extract was evaporated under reduced pressure until dry, producing 24 g of the ME, which was refrigerated for further studies.

A portion of the ME (8 g) was resuspended in EtOH:H_2O (1:1, 200 ml) and partitioned with CH_2Cl_2 (400 ml) to obtain the dichloromethane subfraction (220 mg).

General experimental procedures

Compounds were monitored by thin-layer chromatography (TLC) on 0.25-mm silica gel plates visualized under UV light or stained with ceric ammonium sulfate. Column chromatography was performed using Sephadex LH-20 (bead size 25–100 μm, Sigma–Aldrich) and silica gel 60 (particle size 230–400 mesh, Sigma–Aldrich). The solvent mixtures employed in TLC analysis and flash column chromatography purification are reported as volume by volume. Proton nuclear magnetic resonance (^{1}H NMR) spectra were recorded on a Bruker Avance III (400 MHz) instrument using acetone-d_6 (CD_3COCD_3), methanol-d_4 (CD_3OD) and water-d_2 (D_2O) as the solvents. Carbon nuclear magnetic resonance (^{13}C NMR) spectra were recorded using an NMR spectrometer at 100 MHz. 2D (HSQC, 1H–1H COSY, HMBC, and NOESY) NMR spectra were measured on a Bruker Avance III (400 MHz) spectrometer at 25 °C. Low-resolution mass spectra (LRMS) were recorded on a JEOL JMS-AX505HA mass spectrometer. Analytical HPLC analyses were performed on an Agilent 1260 Infinity system equipped with a G1311B quaternary pump, a G1367E autosampler, and a G1315C DAD. The separation was carried out using a Phenomenex® Luna Polar C_{18} (50 × 2.1 mm id., 1.6 μm) reversed-phase column, and the control of the equipment, data acquisition, and processing and management of the chromatographic information were performed by OpenLab CDS ChemStation Edition (2001–2013) software. All solvents were purchased from JT Baker as HPLC grade.

Chromatographic profiles

Chromatographic conditions for EW and ME were developed as follows: flow rate of 0.30 ml/min with water containing 0.1% formic acid as solvent A and acetonitrile (MeCN) as solvent B using a gradient elution of 94:06 (A:B) at 0–15 min, 88:12 (A:B) at 15–30 min, 78:22 (A:B) at 30–31 min, 05:95 (A:B) at 32–33 min and 94:06 (A:B) at 35 min. The column temperature was kept at 35 °C. Working solutions for the EW and ME of *S. moranensis* were prepared by dissolving 20.6 and 20.7 mg of the extract in 1 ml of water and methanol, respectively. The samples were injected (2 μl) using an autosampler.

Compound isolation

HPLC analysis was performed to confirm that the EW used in the pharmacological testing had a similar phytochemical profile to the ME (Fig. 1), as the latter was more accessible for the isolation process.

A small portion of the ME (4 g) was chromatographed on an 80 g Sephadex LH-20 column (20 cm length × 3.5 cm diameter) and eluted with MeOH. A total of 51 fractions (30 ml each) were collected and monitored by TLC. These fractions were pooled into nine collective fractions (Fr. 1–9) based on similar TLC profiles. Fr. 5 was chromatographed on Sephadex LH-20 *via* isocratic elution with 50% EtOAc/MeOH to afford eight fractions (F5.1–F5.8), isolated compound **1** (35 mg, MS (DART+): *m/z* 355 $[M+H]^+$) and a mixture of compound **1** and compound **2** (19 mg, MS (DART+): *m/z* 355 $[M+H]^+$).

On the other hand, the CH_2Cl_2 subfraction (220 mg) was subjected to fractionation on 4 g of silica gel and eluted with a gradient of hexane:EtOAc (from 8:2 to 0:1) and EtOAc:MeOH (from 95:05 to 0:100) to obtain 52 fractions (10 ml each). These fractions were pooled into twelve fractions (Fr. 1–12) on the basis of similar TLC profiles. Fr. 3 (19.2 mg) was subjected to silica gel column chromatography (1.5 g) and eluted with a gradient of hexane:EtOAc (from 8:2 to 0:1) to isolate compound **3** (6 mg, MS (DART+): *m/z* 229 $[M+H]^+$).

The HPLC-DAD technique was used to identify the compounds isolated from the ME that corresponded to the compounds in the EW. The compounds (**1**–**3**) were identified by comparing them to identified compounds using the retention time, by analyzing their absorbance spectrum profiles and by running the samples after the addition of pure compound.

Experimental animals

The performed methods were previously reported (Andrade-Cetto et al., 2017); in brief, Wistar rats weighing 200–250 g were obtained from the Bioterium of the Science School, UNAM, and acclimated with free access to water and food for at least seven days in an air-conditioned room (25 °C with 55% humidity) under a 12 h light–dark cycle. Experimental diabetes was induced as described by Masiello et al. (1998): the rats were fasted overnight, and 15 min before an intravenous injection of 65 mg/kg streptozotocin (STZ) in citrate buffer (Sigma, S0130), they were injected intraperitoneally with 150 mg/kg nicotinamide (NA) (Sigma, N3376). Diabetes was identified by polydipsia and polyuria and by measurement of the nonfasting plasma glucose levels 48 h after injection. Animals that did not develop glucose levels greater than 250 mg/dl were rejected.

The hyperglycemic animals were classified into four groups, with each group consisting of six rats: the “normal control” group received 1.5 ml of physiological NaCl solution (vehicle); the “hyperglycemic control” group also received 1.5 ml of physiological NaCl solution; the “gliben” group received a standard oral hypoglycemic agent, glibenclamide (5 mg/kg bodyweight (bw)), in the same vehicle; and the “Smilax” group received Sm-EW (80 mg/kg bw) dissolved in 1.5 ml of physiological NaCl solution. The hypoglycemic agent and the extract were orally administered

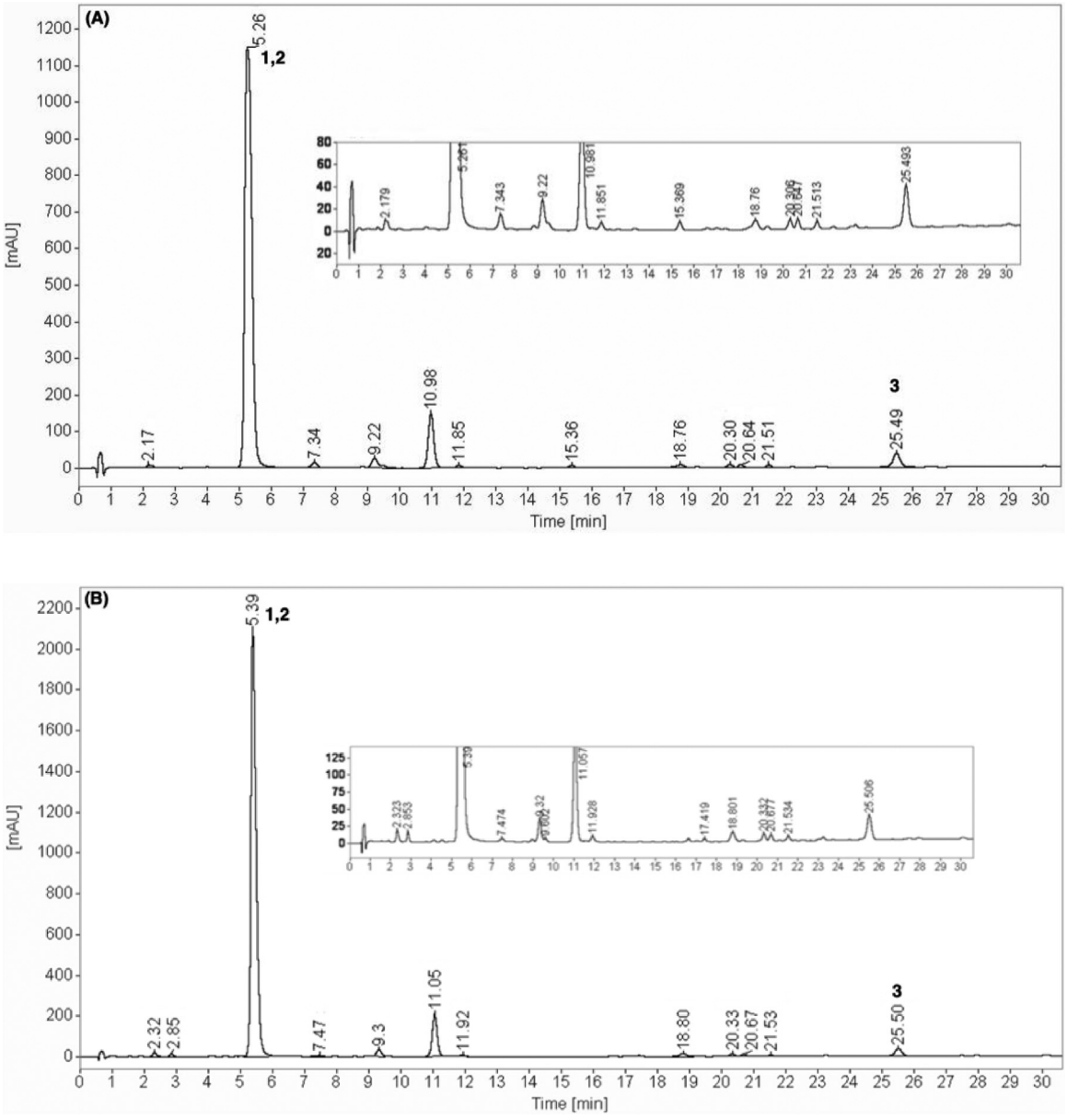


Fig. 1. HPLC-DAD profile comparison of the ethanol–water extract (A) and Methanolic extract (B).

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. All methods used in this study were approved by the Internal Council of the "Facultad de Ciencias" of the Universidad Nacional Autónoma de México. 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).

Glucose monitoring was performed weekly with glucose test strips and an Accutrend® plus glucometer in duplicate. Glycated hemoglobin (*HbA1c*) was analyzed using DCA Vantage® Siemens equipment. The lipid profiles (HDL, triacylglycerides (TG) and cholesterol) were measured with Cardio Check® and Panels® PTS strips. VLDL was calculated using the following equation: VLDL = 0.2 × TG. *HbA1c* and lipid profiles were measured on days 0, 14, 28 and 42 after the initiation of administration of treatments.

Statistical methods

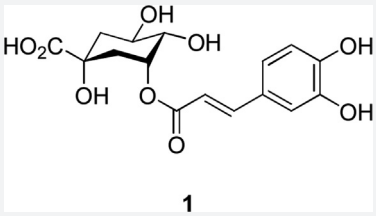
The data were statistically analyzed by an unpaired t-test with the help of GraphPad Prism software. The plasma glucose levels were expressed as the mean (S.E.M.).

Results and discussion

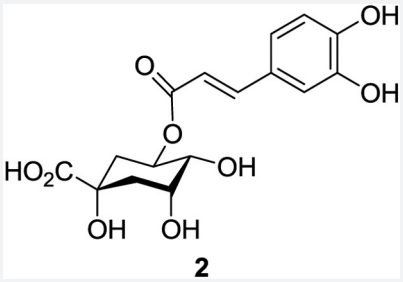
Yield of organic extracts

The ME of the roots of *S. moranensis* was phytochemically investigated for the first time in this study. The percent yield of

Table 1
Spectral data of compounds 1–2 (D_2O, 400–100 MHz).



1



2

Position	δ_H, multiplicity, (*J* in Hz)	δ_C	δ_H, multiplicity, (*J* in Hz)	δ_C
1′		127.0		126.9
2′	7.10, d, (2.1)	115.0	7.10, d, (2.1)	114.6
3′		144.2		144.1
4′		147.0		147.0
5′	6.87, d, (8.2)	116.2	6.87, d, (8.2)	116.1
6′	7.00, dd, (8.3, 2.1)	122.6	7.00, dd, (8.3, 2.1)	122.6
7′	7.55, d, (16.0)	146.0	7.55, d, (16.0)	146.0
8′	6.29, d, (15.9)	115.1	6.29, d, (15.9)	115.1
9′		169.1		169.0
1		76.84		76.9
2a	2.12, ddd (13.3, 4.8, 2.7)	37.3	1.80, dd, (13.5, 10.8)	37.2
2e	1.99–1.90, m		1.90–1.85, m	
3	5.32, ddd (11.1, 9.7, 4.7)	71.1	3.56, dd, (9.3, 3.3)	75.2
4	4.24, q, (3.4)	72.8	4.15, q, (3.5)	70.4
5	3.84, dd, (9.8, 3.3)	70.6	4.04, ddd (10.6, 9.2, 4.7)	67.0
6a	2.06, dd, (15.0, 3.2)	38.4	2.12–2.06, m	40.6
6e	1.99–1.90 (m)		1.90–1.85, m	
7		180.8		181.2

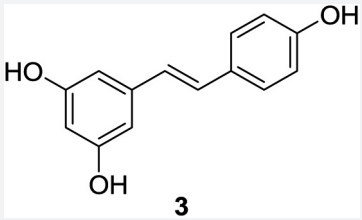
methanolic extraction was 20% (w/w). After extraction by partition with CH_2Cl_2, 5.12% of the yield was obtained from the dichloromethane subfraction, and 7% (w/w) of the yield was obtained from the water extract.

Structure elucidation of isolated compounds

Chromatographic fractionation of the ME from the roots of *S. moranensis* led to the isolation of three known compounds: 3-*O*-caffeoyl-quinic acid (**1**), 5-*O*-caffeoyl-quinic acid (**2**) and *trans*-resveratrol (**3**); their spectroscopic data are presented in Tables 1 and 2, and the values were compared to reported literature values (Huang et al., 2000; Nakatani et al., 2000; Tolonen et al., 2002; Villarino et al., 2011). HPLC analysis was carried out on the ME and EW. As a result, **1**, **2** and **3** were identified in the chromatographic profile of the extracts. Compounds **1**–**3** were isolated for the first time from the plant; however, some of the compounds were previously isolated from other species of *Smilax* (Khaligh et al., 2016).

The chromatographic profile of the extracts (Fig. 1) indicates that **1**–**3** are some of the main compounds found in *S. moranensis*; however, it was necessary to continue the chemical analysis to better understand the plant's chemical profile.

Table 2
Spectral data of compound **3** (CD_3COCD_3, 400–100 MHz).



3

Position	δ_H, multiplicity, (*J* in Hz)	δ_C
1		141.0
2	6.50, d, (2.2)	105.8
3		159.7
4	6.50, t, (2.2)	102.8
5		159.7
6	6.50, d, (2.2)	105.8
1′		130.1
2′	6.80, d, (8.6)	128.8
3′	7.38, d, (8.3)	116.5
4′		158.3
5′	7.38, d, (8.3)	116.5
6′	6.80, d, (8.6)	128.8
α	6.84, d, (16.3)	127.0
β	6.98, d, (16.4)	129.2

Chronic hypoglycemic effect

The results presented in Table 3 show that the non-hyperglycemic control group as well as the hyperglycemic group presented stable values over the 42-day period; however, the hyperglycemic group presented higher values than the normal control group. These observations are supported by the statistical significance. The control drug glibenclamide was able to induce hypoglycemia starting at day 7 and ongoing until day 42. It is important to note that in this model, some β-cells are still functional and decrease over time (Masiello et al., 1998). The extract of the *S. moranensis* root was also able to induce hypoglycemia starting at day 7 until day 42. The root extract exhibited a better effect than the control drug, which was reinforced by the *HbA1c* levels (Table 4): compared to the hyperglycemic group, the control drug and extract groups present a statistically significant effect from days 28 and 14, respectively. The control of the *HbA1c* levels begins earlier with the

Table 3
Chronic hypoglycemic effect of *Smilax moranensis* root on STZ-NA induced hyperglycemic rats.

Glucose groups	T0 (mg/dl)	T7 (mg/dl)	T14 (mg/dl)	T21 (mg/dl)	T28 (mg/dl)	T35 (mg/dl)	T42 (mg/dl)
1 Norm.	124 ± 2	129 ± 2	125 ± 2	127 ± 1	126 ± 4	118 ± 4	129 ± 2
2 Hyperg.	174 ± 5[1]	171 ± 2[1]	172 ± 5[1]	173 ± 8[1]	168 ± 4[1]	162 ± 3[1]	168 ± 4[1]
3 Glib. 5 mg/kg	179 ± 2	128 ± 5[a,1]	147 ± 9[a]	132 ± 4[a,1]	149 ± 5[a]	133 ± 5[a,1]	152 ± 5[a]
4 Sm-EW 80 mg/kg	190 ± 4	146 ± 5[a,1]	136 ± 3[a,1]	140 ± 8[a,1]	123 ± 6[a,1]	135 ± 2[a,1]	146 ± 3[a,1]

The values represent the mean ± SEM. Numbers in the same column indicate statistically significant differences compared with the respective control group. a,1 ($p < 0.05$). Letters in the same row indicate statistically significant differences compared with time 0.

extract than with the control drug. The TG and VLDL levels are not modified by the control drug or the extract.

Diabetes is an important public health problem. Most people with diabetes are affected by type 2 diabetes. With concerns about the increase in the number of people suffering from diabetes, the search for alternatives to control this disease is of great importance. One method is the use of medicinal plants, which have been the source of new molecules to help cure or treat diseases.

In this study, we evaluated the phytochemical composition of the ME and the hypoglycemic activity of the EW from the roots of *S. moranensis*, a plant commonly used in Santos-Reyes in the Oaxaca province of Mexico for the management of type 2 diabetes mellitus. We confirmed that the use of the plant for 42 days could control glucose and *HbA1c* levels.

Other species of the *Smilax* genus have been studied for their hypoglycemic effects: the ME of the aerial parts of *Smilax zeylanica*, a plant used in traditional Chinese and Indian medicine for the treatment of venereal diseases, at doses of 200 mg/kg and 400 mg/kg decreased blood glucose levels in streptozotocin-induced diabetic rats after 15 and 28 days, respectively. In addition, the authors of the study observed that the methanolic extract is safe at doses up to 2000 mg/kg (Rajesh et al., 2009; Rajesh and Perumal, 2014). In another study, (Syiem and Warjri, 2013) reported that the aqueous extract from the leaves of *Smilax perfoliata* decreased blood glucose levels in alloxan-induced diabetic mice at a concentration of 250 mg/kg after 2 h. In a study conducted by Fukunaga et al. (1997), the authors observed that the ME of *Smilax glabra* roots had a hypoglycemic effect in KK-Ay mice and epinephrine-induced hyperglycemic mice; however, the extract did not induce a decrease in the blood glucose levels in streptozotocin-induced diabetic mice. In 2012, Solomon Raju et al. (2012) studied the aqueous, alcoholic and petroleum ether extracts of *Smilax china* in a rat model of alloxan-induced diabetes and found that oral administration of the alcoholic and aqueous extracts (200 mg/kg) for 7 days produced a significant decrease in the blood glucose levels. The aqueous extract of the roots of *Smilax officinalis*, a South American species that is used as a diuretic and as a treatment for syphilis and some infections of the skin, was observed to induce almost 100% inhibition of alpha-glucosidases at a concentration of 2.5 mg in an *in vitro* assay (Ranilla et al., 2010). In 2018, Pérez-Najera and colleagues reported that the EW from the roots of *Smilax aristolochiifolia*, a Mexican species with hematopoietic, hypoglycemic and hypotensive effects, acts as a noncompetitive inhibitor of alpha-glucosidases by binding to two sites that are different from the active site. However, the hypoglycemic effect in an *in vivo* model has not been demonstrated (Pérez-Nájera et al., 2018). Although several reports on the global use of plants of the genus *Smilax* as hypoglycemics exist, until now, no mechanism of action has been proposed. Nevertheless, our results agree with those from previous reports: the tested extract presented a chronic hypoglycemic effect.

We compared the content of the isolated phytochemicals with the components of the ME and found that only *trans*-resveratrol (**3**) and chlorogenic acids (CQA) **1** and **2** are present in the extract. CQA, especially those derived from caffeic acid, are found mostly in plants, where they are produced by the esterification of a C6-C3 *trans*-hydroxycinammic acid (caffeic acid) with quinic acid. 5-*O*-Caffeoyl-quinic acid is the dominant isomer in most plants and the biosynthesis pathway is described in Scheme 1 (Dewick, 2009; Clifford et al., 2017); however, 3-*O*-caffeoyl-quinic acid and 4-*O*-caffeoyl-quinic acid have also been isolated in large quantities. Although it has been established that 5-*O*-caffeoyl-quinic acid can be transformed into 4-*O*-caffeoyl-quinic acid and 3-*O*-caffeoyl-quinic acid by the migration of cinnamoyl moieties from one quinic acid alcohol group to another (transesterification reactions) depending on their temperature and pH conditions of the medium; it has not been possible to clearly demonstrate the proposal that these migrations can occur in the plant from an enzymatic perspective (Deshpande et al., 2014; Clifford et al., 2017).

Several reports suggest that CQA have antioxidant, anti-inflammatory, cardioprotective, anticancer and antidiabetic activity (Naveed et al., 2018). Moreover, some studies show that CQA has the ability to lower blood glucose levels in streptozotocin–nicotinamide-induced diabetic rats alone or in combination with other phenolic compounds (Karthikesan et al., 2010). The hypoglycemic properties of CQA may be due to the inhibition of hepatic glucose-6-phosphatase (involved in hepatic glucose release) and the inhibition of glucose absorption in the small intestinal tract (inhibition of α-glucosidases) (Naveed et al., 2018).

On the other hand, *trans*-resveratrol is a stilbene with anti-inflammatory, anticancer and antioxidant activity. Resveratrol helps prevent human diseases such as arteriosclerosis, cancer and diabetes owing to its antioxidant, anti-inflammatory, and antiestrogenic properties and its modulatory activity on lipid metabolism. Animal studies suggest that resveratrol may blunt metabolic complications induced by a high-fat diet because it is a potent activator of SIRT1, a deacetylase enzyme known to have beneficial effects on glucose homeostasis and insulin sensitivity in animal models of insulin resistance (Commodari et al., 2005; Kitada and Koya, 2013; Timmers et al., 2016).

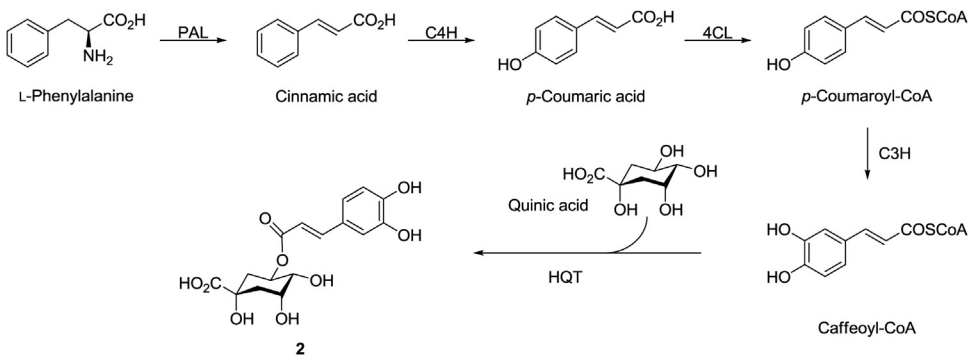
Therefore, the presence of these three compounds could explain the hypoglycemic effect of the EW extract from the roots of *S. moranensis* observed in this study.

In conclusion, this study confirms that the EW of *S. moranensis* possesses hypoglycemic activity and can decrease blood glucose and *HbA1c* levels. This work is the first report on the presence of *trans*-resveratrol, 5-*O*-caffeoyl-quinic acid and 3-*O*-caffeoyl-quinic acid in the roots of *S. moranensis*. Due to the similarities between the plant extract and glibenclamide in their observed effects, it is possible that the extract affects plasma insulin concentrations. Moreover, some of the isolated compounds can block hepatic glucose output, which can also contribute to the hypoglycemic effect; however, more studies are needed to exactly understand how the plant produces this hypoglycemic effect.

Table 4
HbA1c, Tg and LDL values of the *Smilax moranensis root* on STZ-NA induced hyperglycemic rats.

	T0			T14			T28			T42		
Groups	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 ± 0.1	71 ± 4.0	14 ± 1	3.6 ± 0.1	78 ± 12	15 ± 3	3.5 ± 0.1	66 ± 3	12 ± 1	3.6 ± 0.1	68 ± 12	13 ± 2
2 Hyperg.	3.7 ± 0.1	53 ± 7[1]	10 ± 1[1]	4.1 ± 0.2[1]	77 ± 4	15 ± 3	4.2 ± 0.1[1,a]	119 ± 21[1a]	24 ± 3[1a]	4.3 ± 0.1[1a]	113 ± 4[a,1]	22 ± 5[a]
3 Glib. 5 mg/kg	3.8 ± 0.05	69 ± 4	14 ± 2	4.2 ± 0.1	90 ± 4	18 ± 3	3.9 ± 0.1[1]	100 ± 16	20 ± 3	3.9 ± 0.2[1]	115 ± 4	23 ± 4[a]
4 Sm-EW 80 mg/kg	3.4 ± 0.5	79 ± 5	16 ± 2[1]	3.7 ± 0.1[a]	76 ± 2	15 ± 3	3.6 ± 0.2[a]	62 ± 4[1]	12 ± 1[1]	3.9 ± 0.2[a]	74 ± 10[1]	15 ± 2

The values represent the mean ± SEM. Letters in the same row indicate statistically significant differences compared with time 0. Numbers in the same column indicate statistically significant differences compared with the respective control group. a,1 ($p < 0.05$).



Scheme 1. Schematic representation of chlorogenic acid biosynthetic pathway. Key to enzymes: PAL, phenylalanine ammonia lyase; C4H, cinnamate 4-hydroxylase; 4CL, 4-hydroxycinnamoyl CoA ligase; C3H, *p*-coumarate 3′-hydroxlase; HCT, hydroxycinnamoyl CoA: quinate hydroxycinnamoyl transferase.

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

AAC idealized the study, collected the plant material, supervise the pharmacological study and get the financial support; ARP performs the phytochemical experiments; SMER developed the HPLC chromatographic profiles and reviewed the phytochemical experimental data.

Conflicts of interest

The authors declare no conflicts of interest.

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