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Artigo

ANTI-HYPERGLYCEMIC, ANTIOXIDANT, AND ANTI-INFLAMMATORY ACTIVITIES OF EXTRACTS AND METABOLITES FROM *Sida acuta* AND *Sida rhombifolia*

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Species of genus *Sida* are used around the world for a large amount of therapeutic treatments, including hyperglycemia. α-Glucosidase inhibitors are recognized as valuable tools for reducing postprandial hyperglycemia by retarding absorption of glucose. The effect of extracts and isolated compounds of *S. acuta* and *S. rhombifolia* on inhibition of α-glucosidase as primary screening of anti-hyperglycemic activity was tested using yeast and mammalian α-glucosidases. When yeast α-glucosidase was used the acetone extracts of *S. acuta* and *S. rhombifolia* showed IC_{50} values of 8.49 ± 0.66 and 8.10 ± 0.34 μg mL^{-1}, respectively, and the most active compound was *p*-hydroxyphenethyl *trans*-ferulate (IC_{50} 19.24 ± 1.73 μmol L^{-1}) followed by β-sitosteryl glucopyranoside (IC_{50} 32.70 ± 1.35 μmol L^{-1}). However, the activity of extracts and isolated compounds decreased significantly when mammalian α-glucosidase was used, indicating that substrates affinity is higher for type 1 enzymes. The antioxidant and anti-inflammatory activities of extracts and isolates were also tested since many diabetic complications are associated to the oxidative stress and inflammatory immune responses. Acetone extracts were the most active in all evaluations. *p*-Hydroxyphenethyl *trans*-ferulate**,** could be associated to these activities, since it was active in the three evaluations. This effect could be related to its phenolic character.

Keywords: α-glucosidase; DPPH; TPA; *Sida acuta*; *Sida rhombifolia.*

INTRODUCTION

The genus *Sida* (Malvaceae) groups around 200 species spread worldwide in tropical and warm regions, 35 of which occur in Mexico, included *S. acuta* and *S. rhombifolia*.[1] Species of this genus have a large amount of therapeutic uses; in Africa they are used to treat malaria, gastrointestinal infections, varicella, variola, and hepatitis B;[2,3] in Asia they serve as tonic, antipyretic, and to cure disorders of the nervous system, hyperglycemia, and liver and blood problems;[4,5] and in Central America they are taken as medication for fever, asthma, renal inflammation, ulcers, and worm infections.[6] Their use as antituberculosis, hypotensive and cytotoxic agents, and in urinary and cardiac diseases is also common.[7] In Mexico *S. acuta* is used to treat fever, and stomach and teeth ache and *S. rhombifolia* is employed as disinfectant and to cure diarrhea, ulcers and tumours.[8] As a result of the abundant activities of these plants there are also many pharmacological evaluations; their analgesic,[9] antidiarreic,[10] anti-oxidant, vasorelaxant,[11] and anti-hyperglycemic activities,[12] among others, have been tested, mainly from extracts of different species.[7] Previous chemical reports show the presence of ecdysteroids,[13,14] alkaloids,[11,15] and flavonoids,[11,16] as main secondary metabolites in *S. acuta* and *S. rhombifolia*.

Diabetes mellitus (DM), a chronic metabolic disease characterized by high level of glycemia, is becoming a serious problem around the world. There are two major types of DM, type-1 is insulin dependent and arises from defects in the insulin gene causing an inefficient or no insulin production by the pancreas, and type-2 is a noninsulin dependent disease characterized by the reduction of insulin secretion and resistance to the metabolic effects of insulin play role. Worldwide, in 2013 there were about 380 million and for 2035 is expected to be 592 million diabetic patients. Among them, 90% are DM type-2 cases.[17] α-Glucosidase inhibitors are one of the six groups of drugs in the prescription of DM. They act by decreasing the postprandial hyperglycemia through the inhibition of the carbohydrate-hydrolysing enzymes, delaying the release of glucose from the oligosaccharides.[18] Additionally, many diabetic complications are associated to oxidative stress and inflammatory immune responses. Free radicals and elevated circulating inflammatory markers can predict the development of diabetes mellitus. Thus, several drugs with anti-inflammatory properties lower glycemia and possibly decrease the risk of developing type 2 diabetes, and in the same way, the presence of antioxidant sources have potential benefits in obesity related diseases as DM.[19] On the bases of the utilization in popular medicine of the plants of the genus *Sida* related above, and since there are no chemical reports on mexican *S. acuta* and *S. rhombifolia*, this paper describes their chemical composition, the effect as α-glucosidase inhibitors, and the antioxidant and anti-inflammatory activities of extracts and isolates of these *Sida* species.

EXPERIMENTAL

General

Melting points were determined using a Fisher Jones melting point apparatus and are uncorrected. IR spectra were recorded on a Nicolet Magna-IR 750 spectrometer. 1D and 2D NMR spectra were obtained on a Bruker Avance III 400 MHz or on a Varian-Unity Inova 500 MHz spectrometer with tetramethylsilane (TMS) as internal standard. For Direct Analysis in Real Time Mass Spectroscopy (DARTMS) a JEOL AccuTOF JMS-T100LC DART mass spectrometer was used. ESIMS were performed on an ESI ion trap Bruker Esquire 6000 mass spectrometer. FABMS were obtained on a JEOL JMS-SX102A mass spectrometer operated with an acceleration voltage of 10 kV, and samples were desorbed from a nitrobenzyl alcohol matrix using 6 kV xenon atoms. All MS analyses were performed at low resolution. Vacuum column chromatography (VCC) was performed using Silica gel 60 G (Merck, Darmstadt, Germany). Flash column chromatography (FCC) was run using Silica gel 60

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(230-400 Macherey-Nagel). TLC was carried out on Silica gel 60 GF_{254} and preparative TLC on Silica gel GF_{254} (Macherey-Nagel), layer thickness 2.0 mm.

Plant Material

S. acuta Bum. f. and *S. rhombifolia* L. were collected at the archeological zone La Joya, Medellin de Bravo district, Veracruz, México, in March 2012. Voucher specimens were deposited at the Herbarium del Instituto de Biología, UNAM, México (MEXU 1319742 for *S. acuta* and MEXU 1241720 for *S. rhombifolia*).

Extraction and isolation

Dried and ground aerial parts (970 g) of *S. acuta* were successively extracted with hexane, acetone and methanol, 5 L of each. Solvents were removed at reduced pressure to obtain the corresponding extracts. The hexane extract (5.6 g) was subjected to VCC eluting with hexane-EtOAc mixtures of increasing polarity to obtain 60 fractions of 75 mL. Fractions eluted with hexane-EtOAc 19:1 afforded 180 mg of a mixture β-sitosterol (**1**)-stigmasterol (**2**). The acetone extract (10 g) was purified by VCC eluted with hexane-acetone gradient mixtures to collect 150 fractions of 150 mL. Fractions eluted with hexane-acetone 19:1 produced the mixture **1**-**2** (28 mg). Purification of its mother liquors (620 mg) by FCC eluted with hexane-acetone 9:1 (30 fractions of 15 mL) led to the isolation of 13^2-hydroxyphaeophytin a (**3**, 17 mg). Fractions obtained with hexane-acetone 4:1 produced β-sitosteryl glucopyranoside (**4**, 80 mg). Fraction eluted with hexane-acetone 7:3 were treated with charcoal/acetone and the resulting mixture (220 mg) was purified by FCC eluted with hexane-acetone 1:1 (45 fractions of 10 mL) to obtain 35 mg of a mixture which by preparative TLC (hexane-acetone 1:1) afforded *p*-hydroxyphenethyl *trans*-ferulate (**5**, 6 mg). The methanol extract (74 g) was fractionated through a VCC using EtOAc-MeOH gradient system to obtain 95 fractions of 500 mL which by TLC analysis were grouped in: fraction A obtained with EtOAc, fraction B obtained with EtOAc-MeOH 9:1, and fraction C obtained with EtOAc-MeOH 4:1. Fraction A (3.5 g) was treated with charcoal/acetone and purified by VCC using EtOAc-MeOH gradient system as eluent to collect 50 fractions of 50 mL which were grouped by TLC analysis in fractions A1 and A2 eluted with EtOAc and EtOAc-MeOH 49:1, respectively. Fraction A1 (850 mg) was submitted to a FCC eluted with EtOAc-MeOH 19:1 (30 fractions of 20 mL) to afford 20-hydroxyecdisone (**6**, 46 mg) and a mixture (340 mg) which was purified by FCC eluted with hexane-acetone 1:1 (20 fractions of 10 mL) followed by preparative TLC (hexane-acetone 1:1) to obtain 5 mg of **5**. Fraction A2 (450 mg) purified by FCC eluted with EtOAc-MeOH 9:1 (30 fractions of 15 mL) yielded a mixture (45 mg) which by preparative RPTLC (H_2O-MeOH 1:1) led to the isolation of 20-hydroxy-24-hydroxymethylecdysone (**7**, 10 mg). From fraction B only β-sitosteryl glucopyranoside (**4**, 110 mg) was isolated. Fraction C (4.8 g) was subjected to VCC (CH_2Cl_2-MeOH gradient system) to obtain fraction C1, from fractions eluted with CH_2Cl_2-MeOH 4:1. Fraction C1 (859 mg) was purified by Sephadex LH 20 column eluted with MeOH (25 fractions of 30 mL) to obtain uridine (**10,** 42 mg). Dried and ground aerial parts (1.8 Kg) of *S. rhombifolia* were worked out as described for *S. acuta*. The hexane extract (10 g) produced only a mixture of β-sitosterol-stigmasterol (**1**-**2**, 280 mg). The acetone extract (13 g) was purified by VCC eluted with hexane-acetone gradient mixtures to obtain 125 fractions of 150 mL. Fractions eluted with hexane-acetone 9:1 produced **1** and **2** as a mixture (48 mg). Fractions eluted with hexane-acetone 1:1 were treated with charcoal/acetone to obtain a mixture (440 mg) which by FCC eluted with hexane-acetone 2:3 (30 fractions of 15 mL) produced 20-hydroxyecdysone 20,22-monoacetonide (**9**, 40 mg) and 5 mg of **5**. The methanol extract (105 g) was fractionated thought VCC using EtOAc-MeOH gradient system as eluent to collect 95 fractions of 500 mL. Fractions eluted with EtOAc were treated with charcoal/acetone to obtain a mixture (3.4 g) which was purified by VCC eluted with hexane-acetone gradient system (60 fractions of 50 mL) to obtain β-sitosteryl glucopyranoside (150 mg) and fraction D. Fraction D (1.1 g) was purified by FCC eluted with hexane-acetone 1:1 (50 fractions of 50 mL) to afford a mixture (200 mg) which was rechromatographed on a FCC eluted with CH_2Cl_2-acetone 4:1 (20 fractions of 100 mL) to obtain **5** (8 mg). Fractions eluted with EtOAc-MeOH 19:1 (10 g) were purified by VCC (hexane-acetone gradient system) to obtain 84 fractions of 200 ml which were analyzed by TLC and grouped in: fraction E eluted with hexane-acetone 3:2, fraction F with hexane-acetone 1:1, fraction G with hexane-acetone 2:3, and fraction H with acetone. Fraction E produced 200 mg of β-sitosteryl glucopyranoside, and from fraction F 240 mg of **6** were isolated. Fraction G (649 mg) was purified by FCC (CH_2Cl_2-MeOH 9:1) to obtain 35 fractions of 25 mL which were grouped by TLC analysis in fractions G1 and G2. Fraction G1 (95 mg) was purified by FCC (CH_2Cl_2-MeOH 85:15) to obtain 25 fractions of 5 mL and led to the isolation of **6** (55 mg). Fraction G2 (75 mg) was purified by preparative RPTLC (H_2O-MeOH 1:1) to obtain **7** (10 mg). Fraction H (2.5 g) was purified by VCC (CH_2Cl_2-MeOH gradient system) to obtain 70 fractions of 50 mL which were grouped by TLC analysis in: fraction H1 eluted with CH_2Cl_2-MeOH 9:1, fraction H2 obtained with CH_2Cl_2-MeOH 4:1 and fraction H3 collected with CH_2Cl_2-MeOH 7:3. Fraction H1 (80 mg) was purified by FCC elued with AcOEt-MeOH 85:15 (25 fractions of 5 mL) to obtain 28 mg of inosine (**8**). Fraction H2 (215 mg) was purified by a preparative TLC (CH_2Cl_2-MeOH 7:3) followed of a preparative RPTLC (H_2O-MeOH 3:2) to obtain 25-acetoxy-20-hydroxyecdysone 3-*O*-β-D-glucopyranoside (**11,** 20 mg). Fraction H3 (503 mg) were submitted to a Sephadex LH 20 column eluted with H_2O-MeOH 9:1 (20 fractions of 20 mL) to obtain glycerol (30 mg).

p-Hydroxyphenethyl trans-ferulate (**5**): colorless needles; mp 164-167 ºC; IR (KBr) ν_{max} / cm^{-1}: 3475, 1676; ^1H NMR (CD_3OD, 500 MHz) and ^{13}C NMR (CD_3OD, 125 MHz) see supporting material; DARTMS *m/z* 314 $[M]^+$.

20-Hydroxyecdysone (**6**): White amorphous powder; IR (KBr) ν_{max} / cm^{-1}: 3347, 1645; ^1H NMR (CD_3OD, 400 MHz) and ^{13}C NMR (CD_3OD, 100 MHz) see supporting material. DARTMS *m/z* 481 $[M+H]^+$.

20-Hydroxy-24-hydroxymethylecdysone (**7**): White amorphous powder; IR (KBr) ν_{max} / cm^{-1}: 3390, 1659; ^1H NMR (CD_3OD, 500 MHz) and ^{13}C NMR (CD_3OD, 125 MHz) see supporting material; FABMS *m/z* 511 $[M+H]^+$.

20-Hydroxyecdysone 20,22-monoacetonide (**9**): White amorphous powder; IR (KBr) ν_{max} / cm^{-1}: 3324, 1661; ^1H NMR (CD_3OD, 400 MHz) and ^{13}C NMR (CD_3OD, 100 MHz) see supporting material. DARTMS *m/z* 521 $[M+H]^+$.

*25-Acetoxy-20-hydroxyecdysone-3-O-*β-D-*glucopyranoside* (**11**): White amorphous powder; IR (KBr) ν_{max} / cm^{-1}: 3371, 1706, 1646; ^1H NMR (CD_3OD, 400 MHz) and ^{13}C NMR (CD_3OD, 100 MHz) see supporting material. ESIMS 707 $[M+Na]^+$.

Evaluation of yeast α-glucosidase activity

α-Glucosidase activity was evaluated using an adapted method of S. Xiao-Ping *et al.*[20] A solution (25 μL) of samples in DMSO-H_2O 1:1

was added to 150 μL of phosphate buffer solution (PBS, 67 mmol L^{-1}, pH 6.8) and incubated at 37 °C for 10 min with 25 μL of glutation (3 mmol L^{-1} in PBS) and 25 μL (0.2 U mL^{-1}) of α-glucosidase type I (Sigma cat. G5003-100UN). The substrate solution (25 μL, 23.2 mmol L^{-1} *p*-nitrophenyl-α-D-glucopyranoside, Sigma N1377, in PBS) was then added and incubated with agitation for 15 min at 37 °C. Reaction mixture was stopped with 50 μL of Na_2CO_3 (1 mol L^{-1}) and after 5 min of agitation the optical density was determined at 405 nm. Acarbose and quercetin were used as positive controls. The inhibition percentage was calculated by the equation: Inhibition (%) = $[OD_{control} - (OD_{sample} - OD_{background}) / OD_{control}] \times 100$.

Where $OD_{control}$, OD_{sample} and $OD_{background}$ are defined as the absorbance of 100% enzyme activity, test sample with enzyme, and test sample without enzyme, respectively. The concentration of an inhibitor required for inhibit the 50 % of enzyme activity under the mentioned assay conditions is defined as IC_{50} value. All samples were tested in triplicated.

Mammalian α-glucosidase inhibition assay

Mammalian α-glucosidase was prepared following the modified method of Jo.[21] Rat-intestinal acetone powder (100 mg) was rehydrated with 4 mL of 67 mmol L^{-1} ice cold phosphate buffer (pH 6.8). After homogenization for 3 minutes at 4 °C, the suspension was centrifuged (13,400 rcf, 4 °C, 30 min) and the resulting supernatant was used for the assay. A reaction mixture containing 150 μL phosphate buffer (67 mM, pH 6.8), 25 μL of α-glucosidase and 25 μL of sample (in DMSO 50%) at different concentrations was pre-incubated for 10 min at 37 °C, and 25 μL of 23.2 mmol L^{-1} p-nitrophenyl-α-D-glucopyranoside were added. After 15 min incubation at 37 °C, the reaction was stopped by adding 50 μL of Na_2CO_3 (1 mol L^{-1}). Acarbose, and quercetin were used as a positive controls and DMSO 5% as negative control. Enzyme activity was quantified by measuring the absorbance at 405 nm in a BioTek microplate reader Synergy HT. Experiments were done in triplicates. The percentage of enzyme inhibition by the sample was calculated by the following formula: Inhibition (%) = $[(AC - AS) / AC)] \times 100$, where AC is the absorbance of the negative control and AS is the absorbance of the tested sample.

Scavenging activity on free radical 2,2-diphenyl-1-picrylhydrazyl (DPPH)

Free radical scavenging activity was measured using an adapted method of Mellors and Tappel, as previously reported.[22]

Evaluation of the anti-inflammatory activity

Animals: Male NIH mice weighing 25-30 g were maintained under standard laboratory, conditions in the animal house (temperature 22 ± 4 °C) with a 12/12 h light-dark cycle, according with the Mexican official norm MON-062-Z00-1999. They were fed laboratory diet and water *ad libitum*.

TPA-induced edema model. The TPA-induced ear edema assay in mice was performed as previously reported.[23] (Table 3).

Statistical analysis

All data were represented as percentage mean ± standard error of mean (SEM). The statistical analysis was done by means of Student's t-test, whereas analysis of variance ANOVA followed by Dunnett test were used to compare several groups with a control. P values p≤0.05 and p≤0.01 were considered to be significant.

RESULTS AND DISCUSSION

The chemical study of the aerial parts of *S. acuta* afforded (Figure 1) a mixture of β-sitosterol (**1**)[24] and stigmasterol (**2**),[25] 13^2-hydroxyphaeophytin a (**3**),[26] β-sitosteryl glucopyranoside (**4**),[27] *p*-hydroxyphenethyl *trans*-ferulate (**5**),[28] 20-hydroxyecdisone (**6**),[28] 20-hydroxy-24-hydroxymethylecdysone (**7**),[28] and uridine (**10**).[29] *S. rhombifolia* yielded **1** and **2** as a mixture, **5** - **7**, inosine (**8**),[30] 20-hydroxyecdysone 20,22-monoacetonide (**9**),[31] 25-acetoxy-20--hydroxyecdysone 3-*O*-β-D-glucopyranoside (**11**),[13] and glycerol.[32] Structures of the isolated products were determined by comparison of their physical constants and spectroscopic data with those reported in the literature. 13^2-Hydroxyphaeophytin a (**3**), a degradation product of chlorophyll, and 20-hydroxyecdysone 20,22-monoacetonide (**9**) could be artefacts produced in the extraction of the plant or purification of the extracts,[33] or generated by the plant metabolism. Thus, there is evidence of the presence of **3** at different stages of plant grown,[34] and compound **9** has been isolated from plants with no acetone involved in their extraction and purification process.[28, 31]

Compound **6** had a molecular ion at 481 *m/z* $[M+H]^+$ in DARTMS, and in the IR spectrum showed absorption bands for hydroxy (3347 cm^{-1}) and conjugated ketone groups (1645 cm^{-1}). Its ^{13}C NMR spectrum exhibited signals of twenty seven carbons: a carbonyl, two vynilic, six oxygenated, two quaternary, three methines, eight methylenes, and five methyls, and in the ^1H NMR spectrum the resonance of a vynilic proton at δ_H 5.80 which correlated with a carbonyl carbon (δ_C 206.4) in the HMBC spectrum, suggested an ecdysone skeleton. The presence of the singlet resonances of five methyl groups together with those of the oxymethines H-2 (δ_H 3.82, ddd, *J* = 12.0, 4.0, 2.8 Hz), H-3 (δ_H 3.93, brq, *J* = 2.8 Hz), and H-22 (δ_H 3.32, dd, *J* = 10.4, 1.6 Hz) allowed to identify compound **6** as 20-hydroxyecdysone. This compound has been isolated from several species of the genus *Vitex*,[31] and of *S. rhombifolia*,[13] and *S. spinosa.*[28]

Compound **7** showed a molecular ion at 511 *m/z* $[M+H]^+$ in FABMS. Its ^1H NMR and ^{13}C NMR data were similar to those of compound **6**, except for the presence of the resonances of a hydroxymethylene group (δ_H 3.57, dd, *J* = 11.0, 6.0 Hz and 3.50, dd, *J* = 11.0, 5.0 Hz; δ_C 64.4), which was located at C-24 by the correlations of H-28 with C-24, and of H-24 with C-23 and C-25 observed in the HMBC spectrum. Compound **7,** identified as 20-hydroxy-24--hydroxymethylecdysone, has been isolated from *S. spinosa*.[28]

Compound **9**, obtained as white amorphous powder, exhibited in the IR spectrum bands of hydroxy and conjugated ketone groups at 3424 and 1661 cm^{-1}, respectively, and a molecular ion at 521 *m/z* $[M+H]^+$ in DARTMS. The NMR spectra of **9**, as those of compounds **6** and **7**, showed an ecdysteroid structure pattern, with the additional resonances of a ketal group: two methyl groups (δ_H 1.41 and 1.33, δ_C 26.9, and 28.9) and a ketalic carbon (δ_C 106.9). Compound **9** was identified as 20-hydroxyecdysone 20,22-monoacetonide, isolated previously of *Vitex strickeri*[31] and *S. spinosa*.[28]

Compound **11** exhibited in the IR spectrum absorptions at 3371, 1706, and 1646 cm^{-1} indicative of hydroxy, carbonyl, and conjugated ketone groups, and showed a *quasi*-molecular ion at 707 *m/z* $[M+Na]^+$ in ESIMS. The NMR spectra of **11** showed the characteristic features of an ecdysone with a sugar moiety whose anomeric proton resonated at δ_H 4.34 (d, *J* = 8.0 Hz). The HMBC correlation between this proton and C-3 (δ_C 76.8) determined the localization of the sugar portion at this carbon. Additionally, the presence of the singlet signal of a methyl group at δ_H 1.95, and the resonances of a carbonyl carbon at δ_C 172.7 and a methyl at δ_C 22.4 indicated an acetyl group which was located at C-25 (δ_C 83.9) by comparing its chemical shift with those reported in the literature.[15] Compound **11** spectroscopic features were in agreement with 25-acetoxy-20-hydroxyecdysone

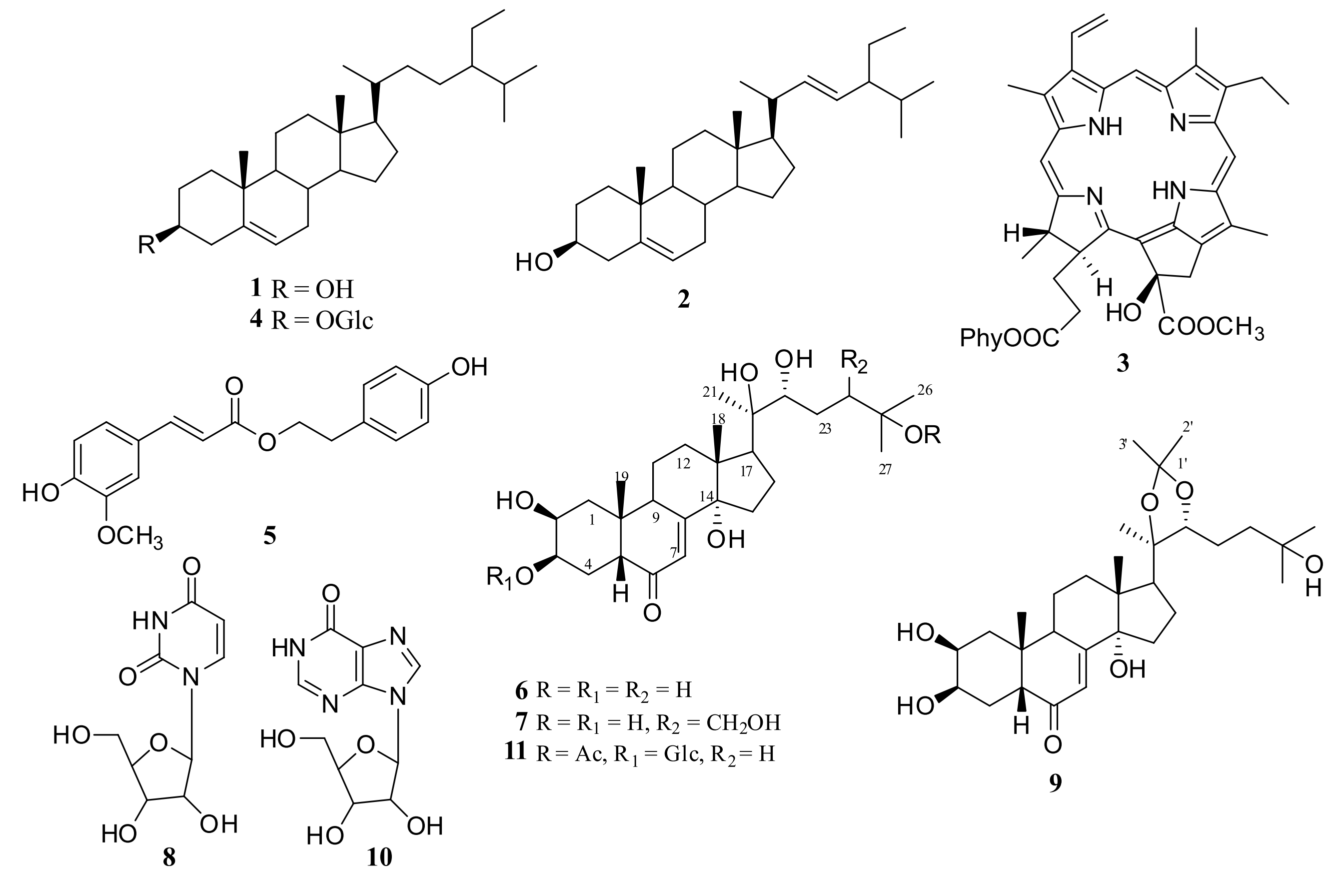

***Figure 1.** Compounds isolated from Sida acuta and Sida rhombifolia*

3-*O*-β-D-glucopyranoside, previously isolated from *S. rhombifolia*.[13]

In previous reports, a slight reduction on blood glucose level in normal glycemic rats produced by the methanolic extract of leaves of *S. acuta* has been stablished,[5] and a dose-dependent anti-hyperglicemic effect induced on diabetic rats by the methanolic and aqueous extracts of *S. rhombifolia* related to their antioxidant properties, was pointed out.[12] Additionaly, the antioxidant effect of *S. acuta* and *S. rhombifolia* (L.) *ssp. retusa* extracts has been reported.[2,35] However, there is no information of the effect of *S. acuta* and *S. rhombifolia* on α-glucosidase, neither on the possible anti-hyperglicemic or antioxidant active compounds, therefore, the α-glucosidase and antioxidant activities of extracts and isolates were determined. Moreover the anti-inflammatory effect of extracts and isolated compounds was also determined.

Because the substrate specificity of α-glucosidase differs greatly depending on the source, and is well established that α-glucosidase inhibitors show variable activities upon the origin of the enzyme,[36] yeast (type 1) and mammalian (type 2) α-glucosidases were used to determine the inhibitory activity of *S. acuta* and *S. rhombifolia* extracts and isolated metabolites (Table 1). Acarbose and quercetin were used as reference compounds since acarbose would be more active on mammalian α-glucosidase[37] while quercetin specificity would be higher for yeast α-glucosidase.[38]

Results (Table 1) show that, in primary screening, using yeast α-glucosidase the acetone extracts exhibited the highest inhibition of enzyme (82.45 and 88.52%), while the methanol extracts were barely active and no activity was detected in hexane extracts. Among the isolated compounds β-sitosteryl glucopyranoside (**4**) and *p*-hydroxyphenethyl *trans*-ferulate (**5**) with 86.33 and 82.93% of enzyme inhibition, respectively, were the more actives. However, the activity of extracts and isolates decreased significantly in the mammalian α-glucosidase test, indicating that the substrates affinity is higher for type 1 enzymes. In the concentration-response evaluation the acetone extracts showed concentration-dependent activities (Table 1S) with IC_{50} values of 8.49 ± 0.66 and 8.10 ± 0.34 μg mL^{-1} for *S. acuta* and *S. rhombifolia*, respectively. *p*-Hydroxyphenethyl *trans*-ferulate (**5**) was the most active compound (IC_{50} 19.24 ± 1.73 μmol L^{-1}), near to the reference compound quercetin (IC_{50} 15.61 ± 1.68 μmol L^{-1}), followed by β-sitosteryl glucopyranoside (**4**, IC_{50} 32.70 ± 1.35 μmol L^{-1}).

In primary screening of DPPH free radical scavenging activity only the acetone and methanol extracts of *S. acuta* and *S. rhombifolia* and compound **5** showed antioxidant properties (Table 2). In concentration-response evaluation (Table 2S) extracts showed rather moderated activities with IC_{50} of 220.54 ± 6.47 and 221.50 ± 5.55 μg mL^{-1} for the acetone extracts of *S. acuta* and *S. rhombifolia*, respectively, which were the most actives. Compound **5**, showed the highest activity with IC_{50} of 46.18 ± 0.83 μmol L^{-1}, but was less active than α-tocopherol (IC_{50} 31.74 ± 1.04 μmol L^{-1}), the reference compound.

The anti-inflammatory activity of extracts and compounds **4**, **5**-**7**, **9**, and **11** was tested on the TPA model of induced acute inflammation.[39] The effect on the edema (Table 3) of the acetone extracts *S. acuta* and *S. rhombifolia* was mild (46.36% and 42.23%, respectively) while the hexane and methanol extracts were not active. Among the tested compounds only compound **5** showed 48.49% of edema inhibition which was moderated compared with the reference compound indomethacin (83.73%).

CONCLUSIONS

The chemical composition of *S. acuta* and *S. rhombifolia* collected in Mexico is in agreement with that reported for the genus *Sida* so far. On biological screening the acetone extracts of *S.acuta*

Table 1. Effect of extracts and isolated compounds from *S. acuta* and *S. rhombifolia* on yeast and mammalian α-glucosidase inhibition

Sample	Mammalian α-glucosidase[a]	Yeast α-glucosidase	
	Inhibition (%)[b,c]	Inhibition (%)[b,c]	IC_{50} (μmol L^{-1})
Hexane extract of *S. acuta* (SAH)	0.37	-21.57	nd
Hexane extract of *S. rhombifolia* (SRH)	0.83	-0.91	nd
Acetone extract of *S. acuta* (SAA)	18.21	82.45	8.49 ± 0.66[d]
Acetone extract of *S. rhombifolia* (SRA)	11.88	88.52	8.10 ± 0.34[d]
Methanol extract of *S. acuta* (SAM)	8.28	24.02	nd
Methanol extract of *S. rhombifolia* (SRM)	9.63	39.30	nd
Compound **1**-**2**	-0.69	32.00	nd
Compound **4**	-0.49	86.33	32.70 ± 1.35
Compound **5**	-0.36	82.93	19.24 ± 1.73
Compound **6**	-3.32	-1.66	nd
Compound **7**	2.14	-4.09	nd
Compound **8**	7.31	-2.46	nd
Compound **9**	2.40	-1.33	nd
Compound **10**	4.17	-0.64	nd
Compound **11**	10.32	0.95	nd
Acarbose	55.94	10.6	7.26 ± 0.34[e]
Quercetin	16.11	92.16	15.61 ± 1.68

[a]IC_{50} of extracts and tested metabolites on mammalian α-glucosidase was not determined. [b]Concentrations of 100 μg mL^{-1} for extracts. [C]Concentrations of 100 μmol L^{-1} for pure compounds. [d]μg mL^{-1}. [e]mmol L^{-1}. The IC_{50} values were calculated from the dose response curve of seven concentrations of each tested sample in triplicated. All values are mean ± SD (n = 3).

Table 2. DPPH free radical scavenging activity of extracts and isolated products from *S. acuta* and *S. rhombifolia*

Sample	Inhibition of DPPH (%)[a,b]	IC_{50} (μg mL^{-1})
SAH	6.60	nd
SRH	4.40	nd
SAA	31.00	220.54 ± 6.47
SRA	28.34	221.50 ± 5.55
SAM	20.20	295.01 ±18.19
SRM	21.28	346.28 ± 3.47
Compound **1-2**	1.23	nd
Compound **4**	1.63	nd
Compound **5**	61.02	46.18 ± 0.83[c]
Compound 6	2.30	nd
Compound **7**	8.47	nd
Compound **8**	6.16	nd
Compound **9**	-0.41	nd
Compound **11**	1.18	nd
α-tocopherol	85.79	31.74 ± 1.04[c]

[a]Concentrations of 100 μg mL^{-1} for extracts. [b]Concentrations of 100 μmol L^{-1} for pure compounds. [c]μmol L^{-1}. The IC_{50} values were calculated from the dose response curve of seven concentrations of each tested sample in triplicated. All values are mean ± SD (n = 3).

Table 3. Effect of extracts and isolated products from *S. acuta* and *S. rhombifolia* on TPA-induced mouse edema

Sample	Edema (mg)	Inhibition (%)
SAH[a]	12.33 ± 0.88	20.22*
SRH[a]	11.00 ± 0.41	28.26**
SAA[b]	7.37 ± 0.76	46.36**
SRA[b]	7.93 ± 0.38	42.23**
SAM[c]	12.80 ± 0.92	18.82
SRM[c]	13.37 ± 0.95	15.22
Compound **4**	12.73 ± 0.30	9.26
Compound **5**[c]	7.40 ± 2.16	48.49
Compound **6**[d]	14.33 ± 0.78	10.60
Compound **7**[d]	14.17 ± 0.37	11.64
Compound **9**[d]	10.80 ± 2.08	32.64**
Compound **11**[d]	13.53 ± 0.72	15.59
Indomethacin[e]	1.99 ± 0.69	83.73*

Dose: 1 mg/ear for extracts, 1 μmol/ear for pure compounds. Each value represents the mean of three animals ± standard error. Control: [a]chloroform 15.33 ± 0.08; [b]acetone 13.73 ± 0.64, [c]methanol, 15.77 ± 1.13, [d]methanol-acetone 1:1 16.03 ± 0.78, [e]acetone, 13.53 ± 1.04. Results were analysed by the *t* Student's test. *p≤0.05. **p≤0.01.

and *S. rhombifolia* behave similarly and were the most active in all evaluations. In yeast α-glucosidase test, *p*-hydroxyphenethyl *trans*-ferulate (**5**) was the most active compound, followed by β-sitosteryl glucopyranoside (**4**). They were isolated from acetone and methanol extracts of both species. However, they may not be the only active compounds since the IC_{50} of the acetone extracts are lower than those of **5** and **4**, or else there is a synergetic effect of the components of these extracts that accounts for the activity. In mammalian α-glucosidase test, the activity was considerably lower than in yeast α-glucosidase test, in both, and the increasing order of extracts activity was hexane, methanol, and acetone. This same order of activity was observed in DPPH and TPA tests, indicating a possible relation between the α-glucosidase inhibition and antioxidant and anti-inflammatory actions. Compound **5** was active in the three evaluations. This effect could be related to its phenolic character, since antioxidant phenolic compounds have been related to reduction of chronic inflammation,[40] and their ability to inhibit digestive enzymes such as α-glucosidase, α-amylase, lipase and tripsine has been reported.[41]

SUPPLEMENTARY MATERIAL

^{1}H and ^{13}C NMR data of compounds **4**-**7**, **9** and **11**; ^{1}H, ^{13}C NMR, 2D NMR spectra of compounds **4**-**7**, **9** and **11**; DARTMS of compounds **5**, **6** and **9**; concentration-response evaluation on yeast α-glucosidase for acetone extracts and compounds **4** and **5**; and concentration-response of DPPH free radical scavenging activity of acetone and methanol extracts and compound **5** are freely available at http://quimicanova.sbq.org.br in PDF.

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