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Original Research Article

Chemical Study, Antioxidant Capacity, and Hypoglycemic Activity of *Malva pseudolavatera* Webb & Berthel and *Malva sylvestris* L. (*Malvaceae*), Grown in Ecuador

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ABSTRACT

Malva pseudolavatera and *M. sylvestris* (*Malvaceae*) are two species in great demand in Ecuador for their medicinal virtues. However, the chemical and biological information on *M. psudolavatera* is scarce. The present study aims to carry out a comparative chemical study of the antioxidant and hypoglycemic activities of the leaves of the species *M. pseudolavatera* and *M. sylvestris* to justify their traditional use. Extracts were made with 80% hydroalcoholic mixture from the leaves of *M. sylvestris* and *M. pseudolavatera*. The chemical composition was analyzed by Liquid chromatography coupled to mass spectrometry. The antioxidant capacity was performed by the Ferric Reducing Antioxidant Power, 2,2-diphenyl-1-picrylhydrazyl and 2,2'-azino-bis(3-ethylbenzothiazoline-6-sulfonic acid techniques and the hypoglycemic activity in Wistar rats at the doses of 250, 500 and 1000 mg/kg, of the extracts, were evaluated.
Liquid chromatography coupled to mass spectrometry analysis allowed the detection of similarities and differences in the chemical composition of both leaf extracts, the flavonoid gosipetin 3-*O*-*β*-D-glucopyranosyl-8-*O*-*β*-D-glucuronopyranoside, was found in both species and for *M. pseudolavatera* is reported for the first time. The extract of *M. sylvestris* showed antioxidant capacity and hypoglycemic activity, superior to the extract of *M. pseudolavatera*.
The results of this research report for the first time the phenolic and flavonoid composition, as well as the antioxidant and hypoglycemic activity of the extracts of the leaves of *M. pseudolavatera* and its comparison with that of *M. sylvestris* for which there were previous reports.

Keywords: Antioxidant activity, Hypoglycemic activity, Liquid chromatography, *Malva species*.

Introduction

Malvaceae is a family belonging to the *Malvales* order that includes herbaceous and woody plants or shrubs. It is distributed in temperate and warm regions of both hemispheres, with 243 genera and 4225 species.[1] Within this family, the *Malva* genus presents some 30 accepted species of herbaceous plants located in the temperate, subtropical, and tropical zones of Africa, Asia, and Europe, although it is found in other parts of the planet since it adapts well if the environmental condition is favourable.[2]
Many species of the genus *Malva* are traditionally used, of which *Malva sylvestris* is used as medicine and food.[3-8] It is traditionally used in skin conditions, in injuries and internal or external inflammation, as antimicrobial agents for burns.[9-12] It has als0 been shown to have mucolytic, hepatoprotective and hypoglycemic properties.[13-15]

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In the Mediterranean region, it is used ss food and in the form of infusions or herbal teas.[16-18]
In Ecuador, different species of this genus grow and/or are cultivated, of which one of the most used is *M. pseudolavatera*, however, there are no reports in the literature on its composition and properties. Another species consumed in the country is *M. sylvestris*, for which there is abundant information on its chemical composition, medicinal properties, and biological activity.
Chemical components of the species include phenolic acids such as coumarinic, chlorogenic and caffeic acid; flavonoids, tannins, anthraquinone derivatives, oleic, palmitic, and stearic acids.[19, 20] The flowers and leaves contain mucilage which on hydrolysis yield arabinose, glucose, rhamnose, galacturonic acid, small amounts of tannins, anthocyanosides, coumarins, flavonoids, polyphenols, niacin, folic acid, vitamin A, vitamin C, and vitamin E. Besides, the presence of sesquiterpenes, diterpenes, and monoterpenes has been reported.[21-24]
Taking into account that the species mostly in demand in Ecuador is *M. pseudolavatera* and that for it there is no scientific information on its composition and properties, the objective of this work was to study the chemical composition, antioxidant capacity and hypoglycemic activity of this species and compare it with *M. sylvestris*, to validate the actions attributed in traditional medicine.

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Materials and Methods

Collection and drying of plant material

The leaves of *M. sylvestris* and *M. pseudolavatera* were collected in November 2019 in the city of Riobamba, province of Chimborazo, in the Andes at 2750 meters above sea level (masl), with the coordinates: 1°40'15.5"S 78°38'49.6"W. A sample of each species was deposited in the GUAY herbarium of the Faculty of Natural Sciences of the University of Guayaquil, Ecuador, where the herbarium numbers were assigned, 13118 for *M. sylvestris* and 13119 for *M. pseudolavatera*. The species were genetically characterized by Sarmiento-Tomalá *et al*.[25] The leaves were washed with potable water and dried in a Mettler Toledo oven at 40°C to a constant weight, they were crushed in a Pulvex mill with blades to a particle size of 2 mm.

Preparation of extracts

Ethanol extracts of the plant material (20 g of plant sample/100 mL of 80% hydroalcoholic mixture) was prepared by the maceration method with sporadic stirring, for seven days at room temperature (30 ± 2°C).[26]

For the hypoglycemic activity test, the extract was made by maceration of 1 kg of dried and pulverized leaves in 1 L of 80% hydroalcoholic mixture, which was concentrated to dryness in a Heidolph rotary evaporator (model 4001) at a reduced pressure, 50 rpm, and temperature of 60°C. The dried extract was used to prepare the different aqueous solutions to be administered to the experimental animals.

Chemical composition analysis by Liquid Chromatography-Mass Spectrometry (LC-MS)

The hydroalcoholic extracts were dried, dissolved in methanol, and passed through an RP-18 column. In a 5 g column (RP-18) 100 mg of each sample was added and eluted with MeOH: H_2O (8:2 v/v). The eluate was concentrated to dryness in a rotary evaporator at 40°C. Of the solid residues, 10 mg was dissolved in 1 mL of HPLC grade methanol in a 2 mL Eppendorf vial. The vial was placed in an ultrasonic bath (1-3 min) and filtered using a sterile syringe (3 mL) with a 0.20 µm Titan 3 filter. The filtrate was directly transferred to a glass HPLC vials (2 mL) and injected into the liquid chromatograph-diode array detector-mass spectrometry / electrospray ionization (LC-DAD-MS / ESI).

Analyses were performed on a Ultra high pressure liquid chromatography (UPLC) system (Thermo Scientific) with a Dionex ultimate 3000 RS LC quaternary pump, Dionex ultimate 3000 RS autosampler, Dionex Ultimate 3000 RS diode array detector, and LTQ ion linear trap mass spectrometer. XL, equipped with Xcalibur 3.1 software. The separations were performed on an Accucore RP-MS column (100 mm x 2.1 mm, 2.6 µm, Thermo scientific) protected by a guard column (4 mm x 2 mm). Elution was carried out at a flow rate of 0.4 mL/min and the injection volume was 2 µL. The mobile phase consisted of acetonitrile (Merck, solvent A) and formic acid (Merck, 0.1%, solvent B). The gradient used was 0-3 min, 85-15% B; 3-18 min, 70-30% B; 18-22 min, 57-43% B; 22-25 min, 45-55% B; 25-27 min, 32-68% B; 27-28 min. 22-78% B, 28-31 min,100% B.

Mass analysis was performed with a negative mode "ESI" interface. Data were acquired in the "Full Scan" mode (m/z range 100 to 1800). The instrumental parameters were capillary temperature 225°C; capillary voltage -50 V; spray voltage 5 kV; main gas flow 34 (nitrogen gas, arbitrary units); makeup gas flow 5 and sweep gas flow 3. The tuning file was optimized with a direct infusion of a quercetin solution with a concentration of 10 µg/mL.

In this study, the mass spectra of each compound identified in the extract was analysed and compared with compounds identified in the literature.

Antioxidant capacity

The antioxidant *capacity* was evaluated by three methods. The hydroalcoholic extracts of the leaves of the two species were analyzed.

Determination of Total Reduction Potential (FRAP)

Each determination was carried out in triplicate, in a UV-visible spectrophotometer (Rayleigh UV-1601, China). The method used was that described by Benzie and Strain.[27]

The results were expressed as µmol equivalents of ascorbic acid (EAA) and µmol equivalents of $FeSO_4$, interpolating the optical density (OD) of the samples in the calibration curves of both reference substances at the concentrations of 100, 200, 400, 800, and 1000 µM.

Determination of the scavenging capacity of DPPH radical

The results were expressed as a percentage of inhibition of the DPPH radical, according to the procedure described by Brand-Williams *et al*.[28] and Kedare and Singh.[29] The leaves extracts and reference substances; Vitamin C (99 % purity, Sigma Aldrich) and Trolox (Sigma Aldrich) were tested at concentrations of 2, 6, 10, 15, and 20 µg/mL. The percentage inhibition was calculated using the formula:

$$\% \text{ inhibition DPPH radical} = \frac{Ab - Am}{Ab} \times 100$$

Where; Ab: absorbance of blank, Am: absorbance of the sample.

The 50% inhibitory concentration (IC_{50}) was determined with the aid of the Graphprism 5.0 statistical program.

Determination of the abduction capacity of $ABTS^{\bullet+}$ radical

The methodology proposed by Re *et al*.[30] and Arnao *et al*.[31] was used. The extracts and reference substances Vitamin C and Trolox were tested at concentrations of 100, 200, 300, 500 and 600 µg/mL. The results were expressed as a percentage of inhibition of the $ABTS^{\bullet+}$ radical, according to the formula:

$$\% \text{ inhibition} = \frac{A_{734}(ABTS) - A_{734}\ (Antioxidant)}{A_{734}(ABTS)} \times 100$$

The mean inhibitory concentration (IC_{50}) was determined with the aid of the Graphprism 5.0 statistical program.

Hypoglycemic activity

The method described by Pinzón *et al*.[32] was followed for this trial.

Animals

Female albino rats (Wistar) raised in the animal house of the Faculty of Chemical Sciences of the University of Guayaquil-Ecuador, whose weights were between 180-210 g, were used.

Preparation of the doses to be administered

From the dry extracts, the amounts to be redissolved in 6 mL of water were calculated to obtain concentrations of 250 mg/kg, 500 mg/kg and 1000 mg/kg, of the extracts. 1 mL was administered to each treatment group, orally for seven days with the use of an intragastric cannula.[33]

Procedure

Nine treatment groups of six rats each were made (Table 1). Before blood glucose determination, food was removed a few hours before to avoid alterations in glucose levels.

Blood samples were taken by an orbital puncture. The glucose measurement was verified with an Active Accu-chek glucometer. After taking the glucose values of each group, a redistribution of the groups of bio models was carried out, where all the averages of the groups were similar. Subsequently, water and food were administered *ad libitum* and each group received its treatment except for group 3 (positive control that received physiological saline solutions). For group 3, on the fifth day 30 min before alloxan induction (100 mg/kg), was administered intraperitoneally, 5 mg/kg of metformin dissolved in physiological saline, continuing the administration on the sixth and seventh day. Groups 3-9 on the fifth day of treatment were administered 100 mg/kg of alloxan dissolved in physiological saline solutions intraperitoneally,[34] continuing until the seventh day with their respective treatments. On the seventh day, 30 mins after the administration of the treatments, the final glycemia was determined.

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Table 1: Test groups for the normoglycemic evaluation

Groups	Identification	Drug	Dose volume to administer/(mg/kg)
1	Normal control	ClNa 0.9 %	1 mL/kg
2	Negative control	ClNa 0.9 %	1 mL/kg
3	Positive control	Metformin	100 mg/kg
4	Extract	*M. sylvestris*	1 mL solution. 250 mg/kg
5	Extract	*M. sylvestris*	1 mL solution. 500 mg/kg
6	Extract	*M. sylvestris*	1 mL solution. 1000 mg/kg
7	Extract	*M. pseudolvatera*	1 mL solution 250 mg/kg
8	Extract	*M. pseudolvatera*	1 mL solution. 500 mg/kg
9	Extract	*M. pseudolvatera*	1 mL solution. 1000 mg/kg

Ethical consideration

The trial was approved by the quality committee of the Faculty (BIO-IP-012-2019), the experiment complied with all the procedures established for the treatment of experimental animals. On completion of the experiment, animals were euthanized with ketamine overdose, in accordance with the refinement procedures to avoid pain and suffering of the animals. The Bioethics and Biosafety Standards established by The World Medical Association[35] were also considered.

Statistical analysis:

Data from the pharmacological trials were analyzed by one-way ANOVA, followed by Tukey's test of multiple comparisons of means with significant level at $p \leq 0.05$. For the statistical processing and analysis of the results, the statistical program SPSS for Windows version 8.0 was used. The experimental values were expressed as the mean ± standard deviation (SD).

Results and Discussion

Analysis of the alcoholic extracts of the leaves by LC-MS

In Figure 1, the total ion current chromatogram TIC (-) is presented in the retention time range of 1-30 min. Some differences were evident in the alcoholic extracts of the leaves of *M. sylvestris* and *M. pseudolavatera*. The characterization of each chromatographic peak was carried out by the analysis of their mass fragmentation and the comparison of these with those reported in the literature for compounds isolated from the leaves of *M. sylvestris* and other species of the genus *Malva.*

The filtered chromatogram (extracted ion chromatogram) with m/z 655 showed the presence of a single chromatographic peak at 7.01 min (Figure 2). The signal originating at m/z 655 (TIC negative ion mode) was deduced as the pseudo molecular ion $[MH]^-$, taking into account that the fragment ion m/z 479 $[MH\text{-}176]^-$, present in the MS2 spectrum, is typical of the loss of a glucuronic acid residue attached to an aglycone through an O-C bond. The inferred nominal molecular mass for this compound (656 Da) coincides with gossypetin 3-*O*-*β*-D-glucopyranosyl-8-*O*-*β*-D-glucuronopyranoside (Figure 3), a compound isolated from the leaves of *Malva sylvestris* L.[36] The fragment ion m/z 479 matches the expected mass for a fragment ion consisting of a hexose residue (most likely glucose) and gossypetin. The previous report of the glycoside in *M. sylvestris*, suggests that this is the compound identified in the current study.

The MS/MS spectrum indicates that the loss of the glucuronic acid residue from C-8 is favoured over the loss of glucose from C-3. The difference in masses of these two residues allows us to infer the priority fragmentation without a doubt since the first causes the loss of 176 Da and the hexoses 162 Da.

Similarly, the analysis was carried out for other chromatographic peaks and mass spectra. For the ion m/z 639, two chromatographic peaks were observed at 7.83 and 15.90 min, respectively. The first chromatographic signal (compound 2) resulted in an MS2 spectrum with a base peak at m/z 463, which indicated also the loss of 176 Da and therefore the presence of a glucuronic acid residue in a similar way to the aforementioned gossypetin derivative, for this compound was 640 Da and differs by 16 Da (one oxygen atom) from compound 1. Therefore, it is plausible to consider that the structure of this compound also corresponds to a flavonoid containing a glucuronic acid residue, a hexose (most likely glucose) and a flavonoid-type aglycone with one hydroxyl group less in its backbone. Compound 2 could be a new report for the species and its aglycone could be a flavonol or a flavone. The compound that eluted at 15.90 min could not be characterized and its spectra did not appear to be related to the structure of a flavonoid.

At retention time 7.73 min and 11.20 min, two compounds (compounds 3 and 4) were detected, with m/z 785, indicating the presence of two isobars. Both showed the loss of a glucuronic acid residue (-176 Da) by originating the same base peak (m/z 609) in their respective MS2 spectra. In turn, both differ by 146 Da from the compound characterized by m/z 639 (compound 2); The 146 Da difference is characteristic of glycosides that contain a rhamnose residue. Compound 3 (nominal mass 786 Da) could contain a residue of glucuronic acid, a hexose (glucose) and rhamnose, (rhamnosylglucosides), quite common in flavonoids and can occur in various isomeric forms including 1→2, 1→4 linkages and 1→6, where glucose binds aglycone and rhamnose is the external sugar. The other isobars (compound 4; 11.20 min) could be an isomer of compound 3 and could differ both in the type of rhamnosylglucose residue and in the position of it substitutes on the aglycone nucleus (supposedly a flavonoid). The differentiation of these structures could be carried out by NMR, although there are studies that show that it is also possible through MS^n experiments.[37]

When obtaining the filtered chromatogram with ion m/z 593, two chromatographic peaks were observed at 15.92 min (compound 5) and 16.57 min (compound 6). Both isobars evidenced the loss of 308 Da in their corresponding MS2 spectra, to yield the base peak m/z 285 $[M\text{-}H\text{-}308]^-$. The 308 Da fragment is seen in glycosides that release rhamnosylglycoside residues during fragmentation and is common in flavonoids as well. In turn, the fragment ion m/z 285 coincides with the value of the pseudo molecular ion of several isomeric flavonoids, among which are luteolin, fisetin, kaempferol, and isoscutellarein.[37] With the available mass spectra, it was not possible to differentiate between them. The presence of two rhamnosylglucosides with a flavonoid-like aglycone appears to be evident. The signal at 15.92 min is one of the strongest in the TIC (-) chromatogram. These compounds were not found as previous reports of *M. sylvestris* and could constitute a difference concerning the species that grow in other regions. If the above approach is confirmed, the isolation and NMR characterization of such compounds could be useful to unequivocally establish their structures. Confirmation that these compounds were flavonoids was obtained by analysis of their UV spectra.

Table 2 shows the compounds that could be identified for both species by comparison with data from the literature or by analyzing their mass

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spectra, as well as the structural proposals for compounds 2 to 6. Organic acids and glycosides of flavonoids, mainly 4-hydroxydihydrocinnamic acid and the two rhamnosylglucosyl flavonoids (compounds 5 and 6) were the compounds identified in the two extracts studied and therefore could be chemical makers of both species. Also, several isomers of hydroxybenzoic acid were detected. Compounds 2-6 seem not to have been reported in some previously studied *Malva* species and could contribute to the chemical novelty of the analyzed extracts.

Some studies have shown that some species of the *Malva* genus and in particular *M. sylvestris*, present flavonoids in various plant organs, they are rich in flavones and phenols among other compounds.[38, 39] However, for the species *M. pseudolavatera* there is no referenced information.

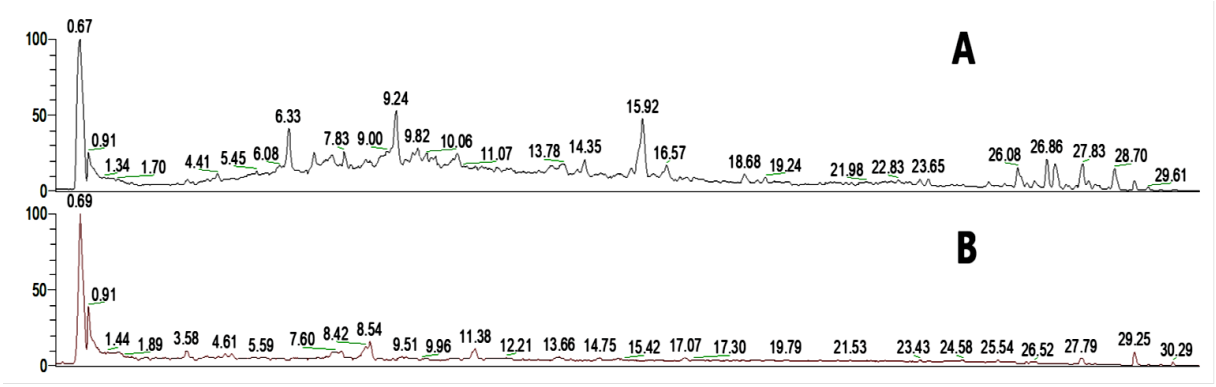

Figure 1: TIC (-) chromatograms of *Malva hydroalcoholic* extracts
Legend: **A** = *M. sylvestris*; **B** = *M. pseudolavatera*

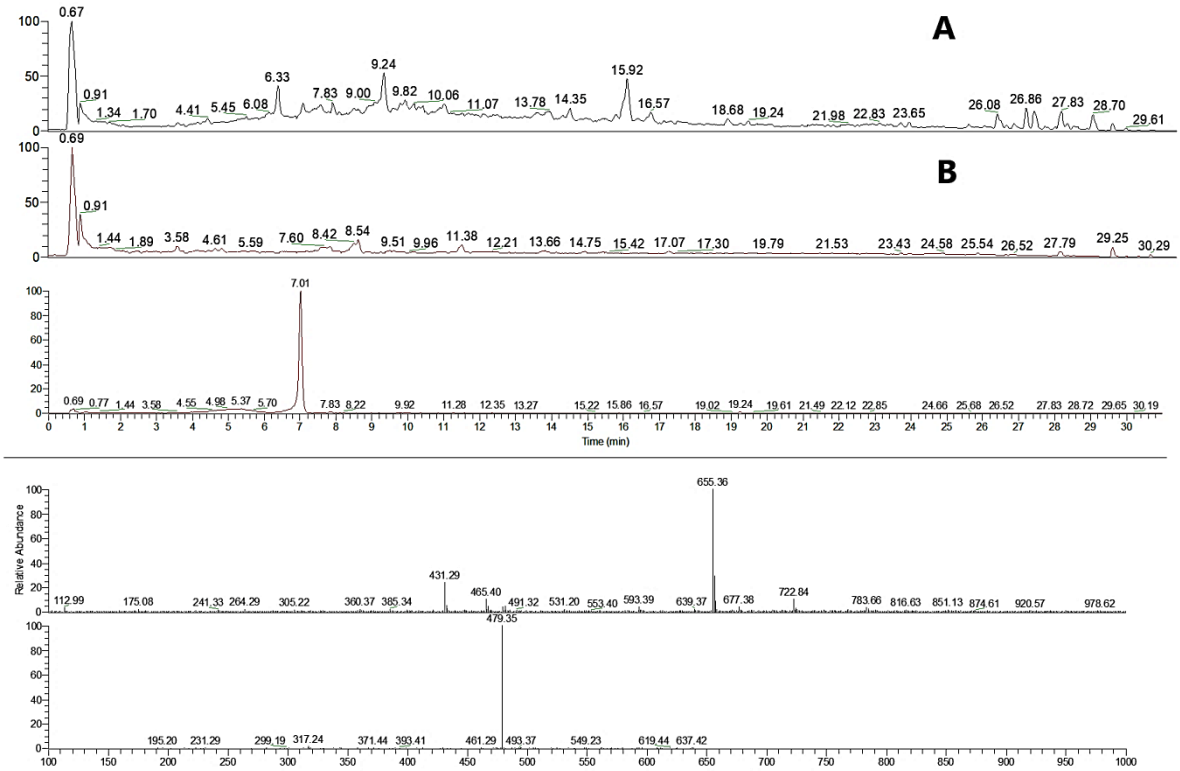

Figure 2: TIC (-) chromatogram, filtered chromatogram with m/z 655, mass spectrum and MS2 of compound eluting at 7.01 min.
Legend: **A** = *M. sylvestris*; **B** = *M. pseudolavatera*

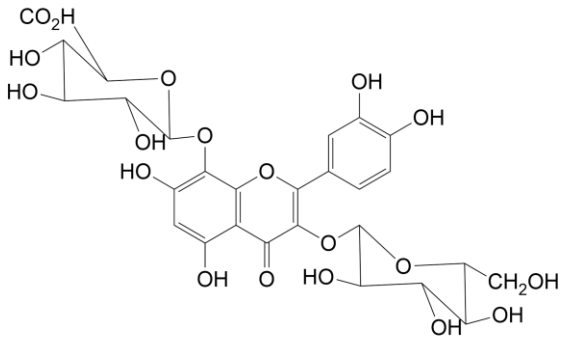

Figure 3: Structure of gossypetin 3- O-*β*-D-glucopyranosyl-8-O-*β*-D-glucuronopyranoside

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Table 2: Compounds identified by HPLC-MS/ESI

Tr (min)	$[M-H]^-$ m/z	MS^2 m/z (%)	*M. sylvestris*	*M. pseudolavatera*	References
0.7	133	115, 71	Malic acid	Malic acid	[28]
3.74	137	137, 93	Hydroxybenzoic acid	Hydroxybenzoic acid	[29]
4.08	137	137, 93	Hydroxybenzoic acid	-	[29]
5.19	153	135, 109	-	Protocatechic acid	[28]
6.7	151	151, 107	4-methoxybenzoic acid	4-methoxybenzoic acid	[29]
7.73	165	147	4-hydroxydihydrocinnamic acid	4-hydroxydihydrocinnamic acid	[29]
10.06	137	137, 93	-	Hydroxybenzoic acid	[29]
7.01	655	479 (100), 461, 393, 371, 317	gossypetin 3-*O*-*β*-D-glucopyranosyl-8-*O*-*β*-D-glucuronopyranoside (**compound 1)**	gossypetin 3-*O*-*β*-D-glucopyranosyl-8-*O*-*β*-D-glucuronopyranoside (**compound 1)**	[29]
7.83	639	477, 463 (100), 301	Glucosylglucuronosylflavonoid (**compound 2)**	-	
7.73	785	609 (100)	Rhamnosylglucosylglucuronosyl flavonoid **(compound 3)**	-	
11.20	785	609 (100)	Rhamnosylglucosylglucuronosyl flavonoid **(compound 4)**	-	
15.92	593	447, 285 (100)	Rhamnosylglucosylflavonoide **(compound 5)**	Rhamnosylglucosylflavonoid **(compound 5)**	
16.57	593	447, 285 (100)	Ramnosylglucosylflavonoide **compound 6**	Rhamnosylglucosylflavonoid compound 6	
17.85	285	257, 241, 151, 133	kaempferol	-	Confirmed with standard

Antioxidant activity

The antioxidant activity of the extracts was evaluated by three methods, since it is known that antioxidants can act by multiple mechanisms, depending on the reaction system or the radical or oxidant source.

In the FRAP test (Figure 4), antioxidant activity was evidenced in a concentration-dependent manner. There was a tendency to increase the antioxidant capacity by FRAP as the concentration of the extract increased. The results made it possible to suggest that the hydroalcoholic extract of *M. sylvestris* has greater ferro-reducing activity than the extract of *M. pseudolavatera*, which translates into high values of μM equivalents expressed as a function of the reference substances tested.

In the DPPH test (Figure 5) it was observed that as the concentration of the extract increased, the inhibition of the radical increased.

An important aspect to consider is the determination of the IC_{50}. In this sense, the extracts showed good anti-radical activity with a similar IC_{50}, although the highest activity was for vitamin C (lower IC_{50} value). Table 3 shows the results obtained for the IC_{50} of the extracts in the DPPH test, where it was observed that there were no significant differences between the extracts studied.

For the ABTS test (Figure 6), there was a tendency to increase the inhibition capacity of the radical as the concentration increases. For the percentages of inhibition of the radical ABTS, significant differences were observed between the samples tested. The extracts showed at the minimum concentration (100 μg/mL) a sequestration capacity greater than 50%, even greater, at the same concentration evaluated for the two reference substances. At concentrations of 200 and 300 μg/mL, the extracts exhibited a higher percentage of inhibition than Trolox, which suggests a high antioxidant activity. Of the evaluated samples, the one that presented the lowest IC_{50} and therefore the highest antioxidant activity was vitamin C. However, the two extracts were able to inhibit 50% of the radical with a similar IC_{50}, also showing anti-radical activity (Table 4).

The leaves, flowers, floral stems, and immature fruits of the species *M. sylvestris* have shown antioxidant capacities. The extracts from the leaves have very strong antioxidant properties, including the scavenging of radicals, attributable to the presence of flavonoids, tocopherols, phenols, carotenoids, and antioxidant elements present in them.[40-43]

The evaluation of the antioxidant activity of the leaves of *M. sylvestris*, using the DPPH test, compared with the antioxidant activity of Trolox, which demonstrated that the leaves of *M. sylvestris* presented a higher antioxidant activity compared to the other species.[44, 45]

Hypoglycemic activity

Figures 7 and 8 show the initial glucose concentration for the study groups and the glucose values at the end of the experiment, after the application of the treatments.

The positive control group treated with the hypoglycemic drug metformin before and after induction with alloxan, presented a blood glucose concentration of 119.67 ± 43.50 mg/dL, because this drug increases the basal rate of the transport of glucose. The negative control group presented a significant difference with the rest of the groups, with an average glucose value of 455 ± 113.86 mg/dL.

For the groups treated with the extracts of *M. pseudolavatera*, the glucose values were 124.33 ± 7.50 mg/dL at dose of 1000 mg/kg, 125.40 ± 21.73 mg/dL at dose of 500 mg/kg and 129.40 ± 32.84 mg/dL at dose of 250 mg/kg, there was no significant difference between them. The glucose values of the group treated with *M. sylvestris* extract were 146.50 ± 11.71 mg/dL, 138.67 ± 21.29 mg/dL and 141.83 ± 6.24 mg/dL at doses of 1000, 500 and 250 mg/kg (*p.o*), respectively. Although, there were no significant differences between the doses, but for similar doses of the *M. pseudolavatera* extract, there were significant differences ($p < 0.05$). This study demonstrated that the extract of the species *M. pseudolavatera* has a greater normoglycemic effect than that of *M. sylvestris*.

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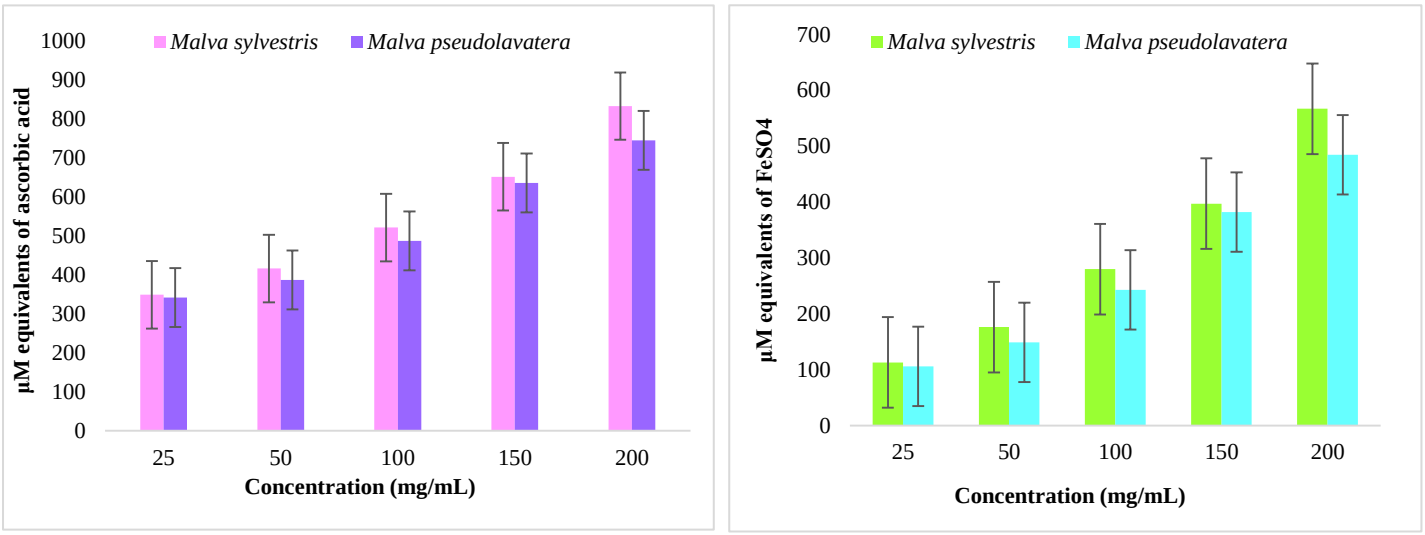

Figure 4: Micro molar (µM) equivalents of ascorbic acid and $FeSO_4$ at different concentrations of the hydroalcoholic extracts of *M. sylvestris* and *M. pseudolavatera*

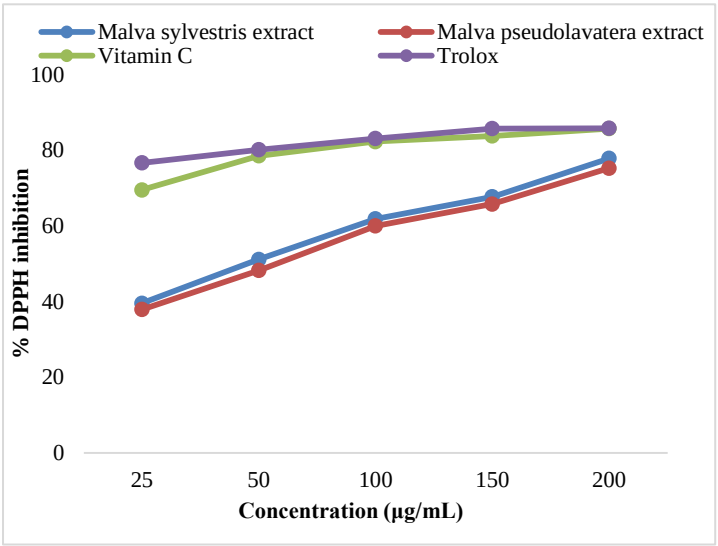

Figure 5: Behavior of the DPPH radical sequestration capacity of the hydroalcoholic extracts of *M. sylvestris* and *M. pseudolavatera* and the reference substances

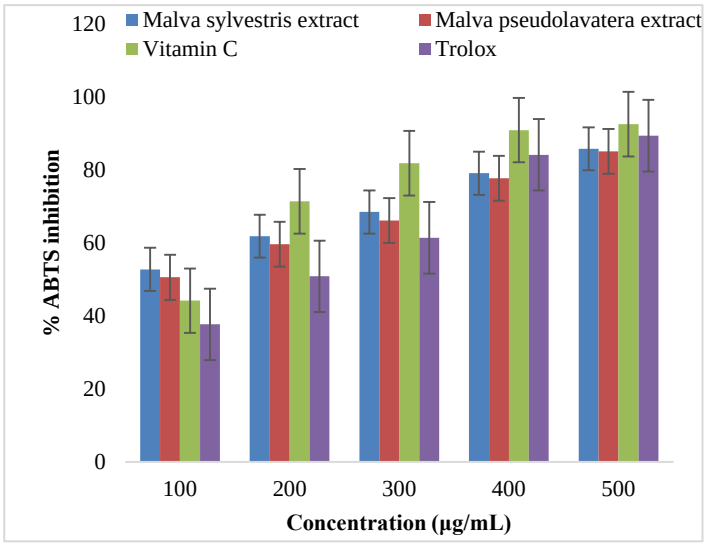

Figure 6: Behavior of the ABTS radical sequestering capacity of the hydroalcoholic extracts of *M. sylvestris*, *M. pseudolavatera* and the reference substances

Table 3: Mean inhibitory concentration (IC_{50}) of the extracts of *M. sylvestris*, *M. pseudolavatera* and the reference substances in the DPPH assay

Sample	*M. sylvestris*	*M. pseudolavatera*	Vitamin C	Trolox
X/SD*	81.81/8.08[a]	82.53/7.18[a]	52.73/9.15[b]	67.80/9.05[c]

Legend: The mean X µg/mL (n = 3) / standard deviation (SD) is indicated. Different letters indicate significant differences ($p \leq 0.05$), according to Tukey's multiple comparison test

Table 4: Mean inhibitory concentration (IC_{50}) of the extracts of *M. sylvestris*, *M. pseudolavatera* and the reference substances in the ABTS assay

Sample	*M. sylvestris*	*M. pseudolavatera*	Vitamin C	Trolox
X/SD*	283,00/7.11[a]	290,00/7.49[a]	196.20/5.26[b]	283.7 0/8.18[c]

Legend: The mean X µg/mL (n = 3) / standard deviation (SD) is indicated. Different letters indicate significant differences ($p \leq 0.05$), according to Tukey's multiple comparison test

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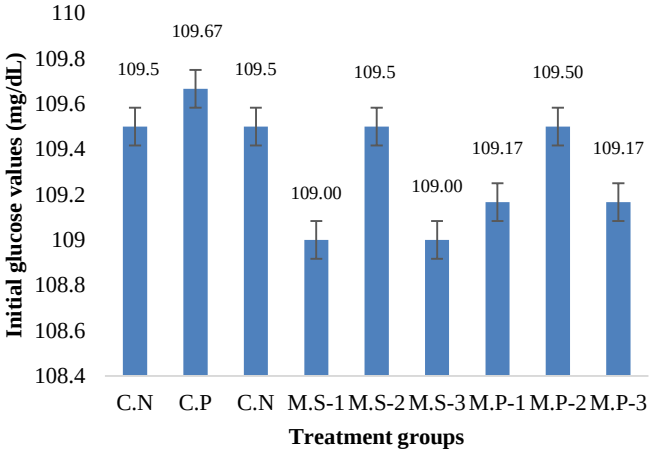

Figure 7: Initial glucose concentration for the study groups.
Legend: CN = normal control; CP = positive control; CN = negative control; MS-1 = *M. sylvestris* dose 250 mg/kg;
MS-2 = *M. sylvestris* dose 500 mg/kg; MS-3 = *M. sylvestris* dose 1000 mg/kg; MP-1 = *M. pseudolavatera* dose 250 mg/kg; MP-2 = *M. pseudolavatera* dose 500 mg/kg; MP-3 = *M. pseudolavatera* dose 1000 mg/kg.

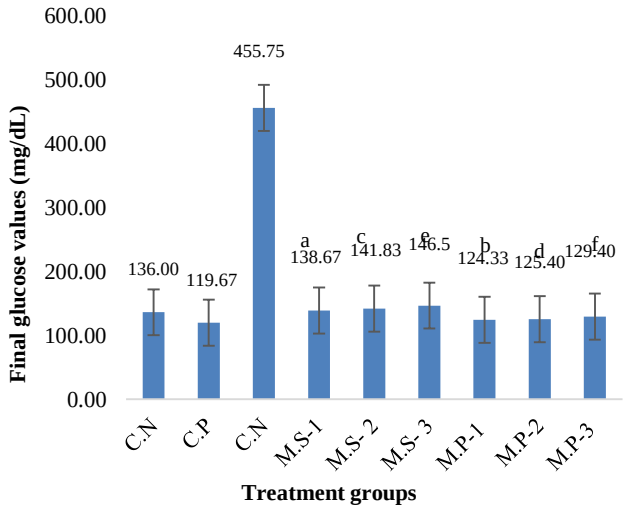

Figure 8: Final glucose concentration for the study groups.
Legend: CN = normal control; CP = positive control; CN = negative control; MS-1 = *M. sylvestris* dose 250 mg/kg;
MS-2 = *M. sylvestris* dose 500 mg/kg; MS-3 = *M. sylvestris* dose 1000 mg/kg; MP-1 = *M. pseudolavatera* dose 250 mg/kg; MP-2 = *M. pseudolavatera* dose 500 mg/kg; MP-3 = *M. pseudolavatera* dose 1000 mg/kg.
Different letters indicate significant differences between groups at the same dose for $p < .05$ n = 6.

Conclusion

The results obtained in this investigation allowed the report for the first time the phenolic and flavonoid composition, the antioxidant capacity and the normoglycemic activity of the species *M. pseudolavatera*. On the other hand, it was shown that both species had similar antioxidant capacity, but that of the *M. pseudolavatera* extract had a higher hypoglycemic activity than the *M. sylvestri* extract.

Conflict of interest

The authors declare no conflict of interest.

Authors' Declaration

The authors hereby declare that the work presented in this article is original and that any liability for claims relating to the content of this article will be borne by them.

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