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ORIGINAL ARTICLE

Protective Effect of *Struthanthus marginatus* on Ethanol-induced Gastric Damage in Mice

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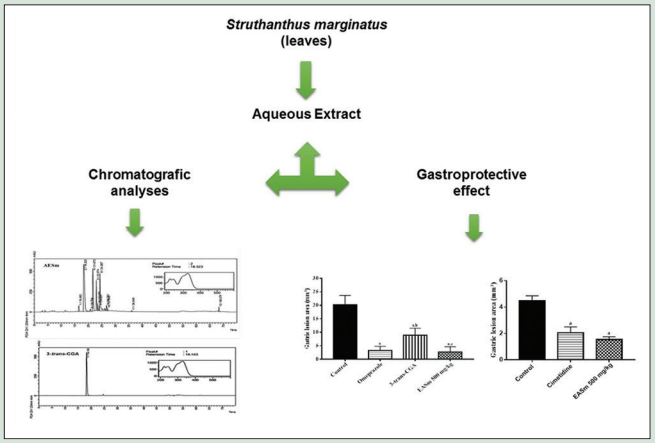
ABSTRACT

Background: Our research group previously characterized the antioxidant and gastroprotective effects of *Struthanthus marginatus* (Loranthaceae), a medicinal herb used in Brazil as a healing agent. **Objective**: The aim of this study is to evaluate the chemical composition of aqueous extract of *S. marginatus* (AESm), as well as the mechanisms underlying its gastroprotective and ulcer healing properties using different protocols in mice. **Materials and Methods:** Gas chromatography-mass spectrometry and liquid chromatography/electrospray ionization-mass spectrometry-mass spectrometry/diode array detection analyses to evaluate the chemical composition of AESm were conducted. The antisecretory activity (basal or stimulated) was determined using the pyloric ligature method. The gastroprotective action of nitric oxide and sulfydryl groups (–SH groups) were evaluated using ethanol-induced gastric ulcer model. The healing ability was evaluated using an acetic acid-induced chronic ulcer. **Results:** Chromatographic analyses of AESm permitted to identify several compounds, including 3-*trans*-caffeoylquinic acid (3-*trans*-CGA), quercetin, and kaempferol as the major constituents. Oral treatment of animals with AESm (500 mg/kg/day) reduced the severity of ethanol-induced gastric damage similar to omeprazole and in a more pronounced manner than 3-*trans*-CGA. Such effect was significantly reduced in animals pretreated with N_{ω}-nitro-L-arginine methyl ester. In addition, AESm inhibited gastric acid secretion in pylorus-ligated mice stimulated with histamine or pilocarpine similar to atropine or cimetidine, respectively. A decrease in acetic acid-induced gastric ulcers similar to that promoted by cimetidine was also observed. **Conclusion:** The results show that *S. marginatus* is rich in flavonoids and that these compounds contribute directly to the gastroprotective and ulcer healing effects of this herb. The inhibition of gastric secretion is the possible gastroprotective mechanism.

Key words: Antiulcer activity, gastric ulcers, herbal medicine, *Struthanthus marginatus*

SUMMARY

- *Struthanthus marginatus* aqueous extract (AESm) is rich in flavonoids, especially 3-trans CGA, quercetin, and kaempferol
- Gastroprotection of AESm may be related to a mechanism of reduction of gastric secretion and partially to the participation of nitric oxide
- This species has healing properties in an experimental model of chronic ulcers in mice.



Abbreviations Used: AESm: Aqueous Extract of *S. marginatus*; 3-trans-CGA= 3-trans-caffeoylquinic acid.

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INTRODUCTION

Peptic ulcer is one of the most common gastrointestinal disorders that affect a considerable number of people worldwide and whose incidence and prevalence are increasing worldwide.[1] The pathophysiology of gastric ulcers is associated with an imbalance between aggressive and protective factors in the stomach.[2] Noxious factors include alcohol ingestion, acid and pepsin secretion, poor diet, stress, reactive oxygen species, the use of nonsteroidal anti-inflammatory drugs, and infection with *Helicobacter pylori*.[3]

The current medical treatment of peptic ulcers consists of the inhibition of acid secretion by proton-pump inhibitors, H_2 receptor antagonists and antimuscarinic agents, as well as acid-independent treatment with agents such as sucralfate or bismuth. In addition, antibiotics are used for the treatment of infection with *H. pylori*.[4] Despite advances in peptic ulcer treatment which considerably reduced morbidity and mortality, the available treatment options are not effective and are associated with many adverse effects such as hypersensitivity, impotence, arrhythmia, hematopoietic disorders, gynecomastia, and long-term antibiotic

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resistance.[5,6] Moreover, clinical evaluation of these drugs revealed the development of tolerance and recurrence.[7]

Within this context, alternative therapies for peptic ulcers have gained interest in the scientific community, especially the validation of plant extracts in experimental peptic ulcer models, which could serve as a source of new antiulcer agents.[8,9] The main effects related to the gastroprotective activity of these extracts are their antioxidant, antisecretory, and cytoprotective properties.[10]

Struthanthus marginatus (Desr.) Blume, popularly known as "erva-de-passarinho," is a hemiparasitic plant of the family Loranthaceae that comprises approximately 1400 species.[11,12] Brandao *et al*.[13] indicated its use for the treatment of bronchitis and leucorrhoea. Its use as a healing agent has also been reported.[14]

A previous pharmacological study performed in our laboratory investigated the gastroprotective effect of different extracts prepared from the leaves of *S. marginatus* in rat models of acute gastric ulcers. The results showed a significant reduction in gastric secretion, stimulation of mucus production, and more pronounced antioxidant activity for the aqueous extract.[15] In addition, in the same study, evaluation of acute toxicology revealed that the aqueous extract of *S. marginatus* (AESm) did not induce any signs of toxicity.

There are few chemical and pharmacological studies of this plant species. Therefore, the objective of this study was to evaluate the chemical composition of *S. marginatus* aqueous extract (AESm), as well as the mechanisms underlying its gastroprotective and ulcer healing properties.

MATERIALS AND METHODS

Reagents and chemicals

3--Trans-caffeoylquinic acid (3-*trans*-CGA), N, O-bis (trimethylsilyl) trifluoroacetamide, 1% trimethylchlorosilane (BSTFA/TMCS), carbenoxolone, histamine, NG-nitro-L-arginine methyl ester (L-NAME), N-ethylmaleimide (NEM), cimetidine, and omeprazole were purchased from Sigma-Aldrich (USA). Acetonitrile, atropine, and pilocarpine were purchased from Merck (Brazil). All other chemicals and reagents used in the study were of analytical grade.

Plant material

The leaves of S. *marginatus* were collected in São José de Ribamar, Maranhão State, Brazil (W:-44.131876; S:-2.555392). The plant material was identified by Dr. Marie Sugiyama from the Institute of Botany (São Paulo, SP, Brazil), and a voucher specimen was deposited in the Maria Eneyda P. Kauffman Fidalgo Herbarium under voucher number SP397.724.

Extraction of *Struthanthus marginatus* leaves

The aqueous extract of the air-dried and powdered leaves (20 g) of *S. marginatus* was prepared as described by Freire *et al*.[15] The aqueous extract (AESm) was obtained by infusion of the leaves at 72°C for 30 min. The infusion was filtered, concentrated under vacuum at 55°C (Heidolph Laborota 4000) and freeze-dried (Terroni Fauvel– LB 3000TT), in a yield 26%. The phytochemical screening determined the presence of tannins hydrolysable, flavanonols, and flavanones.

Identification of the constituents of *Struthanthus marginatus* leaves

Gas chromatography coupled to mass spectrometry

After extraction and derivatization as described by Roessner *et al*.,[16] analysis of the AESm was carried out using an Agilent GC 6890 and MSD 5973N gas chromatograph (Agilent, USA) operated in the electronic impact mode (70 eV). The temperatures of the injector and detector ports were maintained at 230°C and 250°C, respectively, and the ion source temperature was maintained at 200°C. Helium was used as the carrier gas at a flow rate of 1 mL/min. The following temperature program was used throughout the analysis: constant isothermal heating at 70°C for 5 min, followed by increments from 70°C to 310°C at a rate of 5°C/min and maintained at 310°C before a final minute of heating. The temperature was then equilibrated before automatic injection of the next sample. The mass spectra were recorded at 2 scans/s with a scanning range of m/z 50-650 (*m*/z = mass-to-charge ratio) in Dalton units. The total ion current (TIC) and mass spectral data were processed using the ChemStation program (Agilent Technologies, USA). The mass spectra were compared with the database and library of the NIST software (National Institute of Standards and Technology Mass Spectral Library 08 MS Search Program v. 2.0).

High-performance liquid chromatography coupled to ultraviolet/visible detection

The AESm and 3-*trans*-CGA (authentic standard) were analyzed on an analytical column using a Varian system (Pro Star model 310, Varian Star Workstation, 6.0, Agilent, USA). The mobile phase A consisted of 0.1% acetic acid in Milli-Q water (Merck) and acetonitrile. The following gradient program was used: 95% A and 5% B (0–5 min); 95%–90% A (5–10 min); 90%–85% A (10–12 min); 85%–82% A (12–18 min); 82%–75% A (18–20 min); 75%–72% A (20–25 min), and 72%–70% A (25–50 min). The total run time was 50 min. The analytical column was a C18 Pursuit column (5 μm particle size, 250 mm × 4.60 mm i. d.; Varian) protected by a 2-mm C-18 Pursuit pre-column (5 μm). The samples were diluted in Milli-Q water and filtered through a Millipore filter (Millex, PVDF 0.45 mm). The injected sample volume was 20 μL, and the compounds were detected at a wavelength (λ) of 254 nm. The flow rate of the mobile phase was 0.6 mL/min. The results are expressed as retention time (R_t) in minutes. The concentrations were determined based on the ratio of the integrated peak area and are expressed as a percentage (%). All analyses were performed under the same conditions.

Liquid chromatography/electrospray ionization-mass spectrometry-mass spectrometry/diode array detection

Liquid chromatography (LC) was carried out on a Shimadzu HPLC-10 Avp PDA, SPD-M10A VP diode array detection (DAD) (Japan) coupled to a mass spectrometer (Esquire 3000 Plus Ion-Trap, Brüker Daltonik, Bremen, Germany) with electrospray ionization (ESI). The conditions for LC-DAD were a C-18 column (250 mm × 4.6 mm) maintained at 20°C and transitioning of the linear gradient mobile phase from 95% water (Milli-Q) with 0.1% glacial acetic acid (pump A) and 5% acetonitrile (Merck, pump B) to 100% acetonitrile for 50 min at a flow rate of 0.9 mL/min. The injection volume was 20 μL. The chromatograms were recorded at 280 nm, and the R_t was determined in minutes. The conditions for LC/ESI-mass spectrometry (LC/ESI-MS-MS) were as follows: ion source electrospray voltage of 40 V, capillary voltage of 4000 V, and capillary temperature of 320°C. The collision gas was ultra high purity helium, and the nebulizing gas was high-purity nitrogen. Nebulization was aided with a coaxial nitrogen sheath gas provided at a pressure of 27 psi. Desolvation was facilitated using a countercurrent nitrogen flow set at 7.0 L/min. The analysis was performed using full-scan mass spectra in the positive ionization mode and data-dependent MS-MS scans from m/z 100–3000 Da. Compounds were identified based on the wavelength (λ) of the ultraviolet spectrum and R_t data of the chromatographic peaks observed on the chromatogram (LC-DAD). R_t of the peaks which were located in the extracted TIC by LC/ESI-MS-MS^2 as they produced the parent ion or protonated molecules [M + H] + which [M + H] + = m/z (mass-to-charge ratio) in Dalton units and fragmentation ions observed in the mass spectrum from selected parent ion [M + H] +. Data obtained for 3-*trans*-CGA (authentic standard) and literature data were used to confirm the identification.

Animals

Swiss mice of either sex weighing 30 ± 5 g were provided by the animal house of the Federal University of Maranhão (São Luís, Maranhão, Brazil). These were kept under standard environmental conditions (12 h dark/light cycle) and temperature (22°C ± 2°C). Water and food were made available *ad libitum*. All the experimental protocols were submitted to and approved by the Animal Experimentation Ethics Committee of the State University of Maranhão (UEMA), under license no. 879, in accordance with the COBEA (Brazilian College of Animal Experimentation). At the end of the experiments, the animals were euthanized with an overdose of the anesthetic (xylazine and ketamine).

Evaluation of the anti-ulcerogenic activity and healing properties of aqueous extract of *Struthanthus marginatus*

Evaluation of mucosal protective factors

Each experimental model included the following groups depending on the specificity of each model: positive control (omeprazole, a proton pump inhibitor; carbenoxolone, a cytoprotective agent; cimetidine, a H_2-receptor antagonist; atropine, a muscarinic antagonist, and 3-*trans*-CGA, the standard antioxidant drug), negative control (0.9% saline), or AESm. Previous pharmacological studies from our group using the aqueous extract of dried leaves of *S. marginatus* showed antiulcerogenic activity at doses of 125, 250, and 500 mg/kg in acute gastric ulcer models.[15] The dose of 500 mg/kg was chosen to shed light on the mechanisms underlying its gastroprotective effect since this dose was found to be the most effective.

Ethanol-induced gastric ulcer

After fasting for 16 h, the animals (n = 6/group) were orally treated with 10 mL/kg water (control), omeprazole (30 mg/kg), 3-*trans*-CGA (100 mg/kg), or AESm (500 mg/kg) 1 h before the oral administration of 75% ethanol (10 mL/kg), as previously described by Robert *et al.*[17] with some modifications. The animals were euthanized 1 h after ethanol treatment and their stomachs were excised, opened along the greater curvature, washed with saline (0.9%), and fixed between two glass plates. The mucosa was examined for the demarcation of gastric ulcers and the ulcer area was measured using computerized planimetry (mm^2) using the Image J® software (NIH, USA).

Determination of the role of prostaglandins, nitric oxide, and sulfhydryl groups (–SH) in gastroprotection

The method described by Matsuda *et al.*[18] was used, with some modifications. Fasted Swiss mice (n = 6) were divided into 12 groups; of these, three groups were pretreated with saline (10 mL/kg), three with the cyclooxygenase inhibitor indomethacin (10 mg/kg, s. c.), three with the nitric oxide (NO) synthase inhibitor N_ω-nitro-L-arginine methyl ester (L-NAME, 70 mg/kg), and three with the sulfhydryl compound blocker NEM (10 mg/kg) administered intraperitoneally (i. p.). After 30 min, all groups received the respective oral treatment: water (10 mL/kg), AESm (500 mg/kg), or carbenoxolone (100 mg/kg). After 60 min, all groups received 75% ethanol (10 mL/kg) orally for gastric ulcer induction. The animals were euthanized 60 min after ethanol administration and their stomachs were excised. Gastric injury was determined as described above.

Determination of antisecretory activity

The assay was performed using the method described by Shay *et al.*[19] and Rozza *et al.*,[20] with some modifications. The animals were divided into groups (n = 6) according to the treatment used. After fasting for 18 h, the animals were anesthetized with ketamine (25 mg/kg) and xylazine (10 mg/kg, i. p.). The groups receiving water (vehicle, 10 mL/kg) or AESm (500 mg/kg), which was injected intraduodenally (i. d.), were used for the evaluation of secretion under basal conditions. Furthermore, we tested the effects of AESm, and the specific antagonist atropine (1 mg/kg) injected s. c. and of cimetidine (60 mg/kg, i. d.) on gastric acid secretion induced in mice by 4-h pylorus ligation with pilocarpine (1 mg/kg) or histamine (20 mg/kg) injected s. c. 1 h after surgery. The animals were euthanized 4 h after pylorus ligation. The gastric secretion was collected and centrifuged for 30 min at 176×g. The volume (mL), pH, and total acidity (mEquiv.[H+]/mL/4 h) of gastric secretion were determined.

Acetic acid-induced gastric ulcer

The method described by Takagi *et al.*[21] was used, with some modifications. For this experiment, fasted animals were divided into three groups (n = 6). Under anesthesia (25 mg/kg ketamine and 10 mg/kg xylazine, i. p.), the animals were subjected to laparotomy through a midline epigastric incision. The abdomen was exposed, and 50 µL of a 30% acetic acid solution was injected into the subserosal layer at the fundo-antral junction. The stomach was washed with saline, and the abdomen was closed. After recovery from anesthesia, the animals were treated orally with vehicle (water, 10 mL/kg), AESm (125 and 500 mg/kg), or cimetidine (100 mg/kg) once a day for 14 days, starting 1 day after surgery. The animals were euthanized on the day after the last administration (day 15). The stomachs were excised, and the gastric injury was determined as described above.

Statistical analysis

Values were expressed as a mean ± standard error of the mean. The differences between groups were determined using analysis of variance followed by Tukey's test. Statistical analysis was performed using GraphPad Prism 6. Results were considered statistically significant when the value of $P < 0.05$.

RESULTS

Chemical analysis of aqueous extract of *Struthanthus marginatus*

The compounds identified in the AESm by gas chromatography–mass spectrometry (GC-MS) are shown in Table 1, which list the R_t and the proposed compound detected. The main compounds detected in AESm were 3-*trans*-caffeoylquinic acid (3-*trans*-CGA), detected at an R_t of 49.40 min, the isomers 4-*trans*-CGA and 5-*trans*-CGA, detected at R_t of 50.18 min and 50.37 min, respectively, and the flavonoid quercetin, detected at an R_t of 49.7 min.

The exploratory analysis (by high-performance LC coupled to ultraviolet) of the AESm recorded at 254 nm revealed the following R_t of the main peaks and the respective areas are reported in parentheses as percentage: 28.0 min (27.7%), 29.5 min (16.0%), 30.4 min (9.8%), and 32.1 min (6.5%). The areas of the other peaks were <3.8%. The chromatographic profile of the compound isolated from the AESm by preparative HPLC (LaPrep System, Merck; data not shown) had a peak at 28.0 min (98%), similar to that observed for 3-*trans*-CGA (authentic standard).

The chromatographic profiles of AESm obtained by LC-DAD are shown in Figure 1a, where the peak at 17.959 min showed molecular absorption in the ultraviolet spectra similar to the authentic standard, 3-trans-CGA [Figure 1b], at 18.163 min.

The analysis of the AESm by LC/ESI-MS-MS in Table 2 provides the peaks, R_t of the peaks that were located in the extracted ion chromatogram (TIC) and that produced the parent ion [M + H] +

or protonated molecules (m/z: mass-to-charge ratio in Dalton) and compounds.

Antiulcerogenic activity

The oral administration of 75% ethanol (10 mL/kg) induced gastric mucosal damage (19.9 ± 5.6 mm^2). Pretreatment of mice with AESm (500 mg/kg) decreased the ethanol-induced gastric ulcer area by 50.1% compared to the vehicle group ($P < 0.05$). Omeprazole (30 mg/kg) and 3-*trans*-CGA (100 mg/kg) used as positive controls also significantly inhibited ethanol-induced gastric mucosal damage in mice by 80.98% and 48.7%, respectively. In addition, AESm (500 mg/kg) exhibited better antiulcerogenic activity than 3-*trans*-CGA ($P < 0.001$) and similar activity compared to omeprazole [Figure 2].

Effect of aqueous extract of *Struthanthus marginatus* on stimulated gastric acid secretion

As shown in Table 3, administration of AESm (500 mg/kg, i. d.) reduced the volume and total acidity of gastric acid secretion and increased gastric juice pH compared to control animals after 4 h of pylorus ligation. Pilocarpine increased the volume and total acidity of gastric secretion and significantly reduced gastric juice pH by 1.06 units. Pretreatment of the animals with AESm reduced pilocarpine-stimulated gastric secretion and total acidity and increased the pH by 2.57 units.

Similarly, histamine increased the volume and total acidity of gastric secretion in pylorus-ligated mice and reduced the pH by 1.1. Pretreatment of the animals with AESm reduced histamine-stimulated gastric secretion and total acidity and increased the pH by 2.35 units [Table 3].

Table 1: Chemical constituents of aqueous extract of *Struthanthus marginatus* leaves by Gas chromatography-mass spectrometry

Compound	Retention time (min)
Glycolic acid	8.97
Succinic acid	16.19
Glyceric acid	16.88
Malic acid	20.99
Erythritol/threitol	21.65
Benzoic acid	22.52
Threonic acid	22.95
Shikimic acid	28.34
Fructose	28.39
Quinic acid	29.52
Benzoic acid	31.17
Hexadecanoic acid	32.41
Nnmyo inositol	33.83
Octadecanoic acid	35.91
Sucrose	43.13
3-*trans*-p-coumaroyl quinic acid	47.46
3-*trans*-CGA	49.40
Quercetin	49.72
4-*trans*-CGA	50.18
5-*trans*-CGA	50.37

CGA: Caffeoylquinic acid

Involvement of prostaglandins, sulfhydryl compounds (–SH groups) and nitric oxide in gastroprotection

The cyclooxygenase inhibitor indomethacin, the sulfhydryl compound blocker NEM, and the NO synthase inhibitor L-NAME increased gastric damage in all groups compared to the groups pretreated with saline [Table 4]. In animals pretreated with saline, treatment with AESm (500 mg/kg) had a gastroprotective effect as expected, since the extract inhibited the formation of gastric ulcers induced by ethanol.

This gastroprotective effect of AESm (500 mg/kg) was maintained even after the depletion of sulfhydryl groups by pretreatment with NEM or after reducing the production of prostaglandins by pretreatment with indomethacin. However, the gastroprotective effect of AESm was significantly reduced in rats pretreated with L-NAME ($P = 0.1189$) [Table 4].

Acetic acid-induced gastric ulcer

In the acetic acid model, oral administration of AESm (500 mg/kg) for 14 consecutive days decreased the chronic ulcer area by 65% (1.53 ± 0.22 mm2) [Figure 3]. Cimetidine (100 mg/kg) accelerated gastric ulcer healing, significantly reducing the ulcer area to 2.06 ± 0.44 mm^2 (54%) when compared to the control group (4.46 ± 0.4 mm^2) [Figure 3].

DISCUSSION

The results of the present study show that the AESm promotes gastroprotection and the healing of acute and chronic gastric ulcers mediated by the partial contribution of NO and a reduction in gastric secretion. This is the first study to identify the components of *S. marginatus* and to establish the mechanisms of action involved in the gastroprotective and ulcer healing properties of this plant. The results showed that this species is rich in flavonoids including 3-*trans*-CGA, quercetin, and kaempferol, which are related to the antioxidant activity of AESm previously described by our research group.[15]

The preliminary identification of compounds in AESm was based on GC-MS data, which identified several organic acids, including 3-*trans*-CGA, quercetin, and sugars. Analysis by LC/ESI-MS-MS/DAD

Table 2: Phenolic compounds identified in the aqueous extract of *Struthanthus marginatus* leaves by LC/ESI- MS-MS analysis

Peak number	R_t (min)	$(M+H)^+$ *m/z**	Ions detected (MS^2) *m/z***	Compounds
1	16.46	355	355, 163 (100%), 145	4-*trans* CGA[a]
2	18.32	355	355, 163 (100%), 145	3-*trans* CGA[a,b]
3	20.76	355	355, 163 (100%), 145	5-*trans* CGA[a]
4	21.67	337	337, 163 (100%) 145	Caffeoylshikimic acid[c]
5	22.97	597	597, 465, 303 (100%), 228	Quercetin-3-O-hexosyl O-pentosyl
6	23.47	339	-	*p*-Coumaroylquinic acid
7	24.03	581	581, 449, 287 (100%)	Kampferol-3-O-hexosyl-O-pentosyl
8	26.72	453	453, 435 (453 - H_2O), 343 (100%), 301, 191, 163	Shikimoyl-*p*-coumaroytartaric acid[c]
9	27.04	471	471, 163 (100%), 153, 145	Quinoyl-*p*-coumaroytartaric acid[c]

*R_t (Retention time) of the peaks that produced the parent ion $(M+H)^+$ or protonated molecules. m/z: mass-to-charge ratio in Dalton, **Fragment ions observed in the mass spectra (MS^2) and the base peak (100%), [a]Isomeric compounds identified based on retention time, [b]Data for 3-trans-CGA (Sigma-Aldrich): R_t=18.2 min and *m/z*=355 $(M+H)^+$, 163 (100%), 145 analyzed under the same conditions, [c]O-pentosyl position not determined and hexosyl and pentosyl structures not identified. CGA: Caffeoylquinic acid; LC/ESI- MS-MS: Liquid chromatography/electrospray ionization-mass spectrometry-mass spectrometry

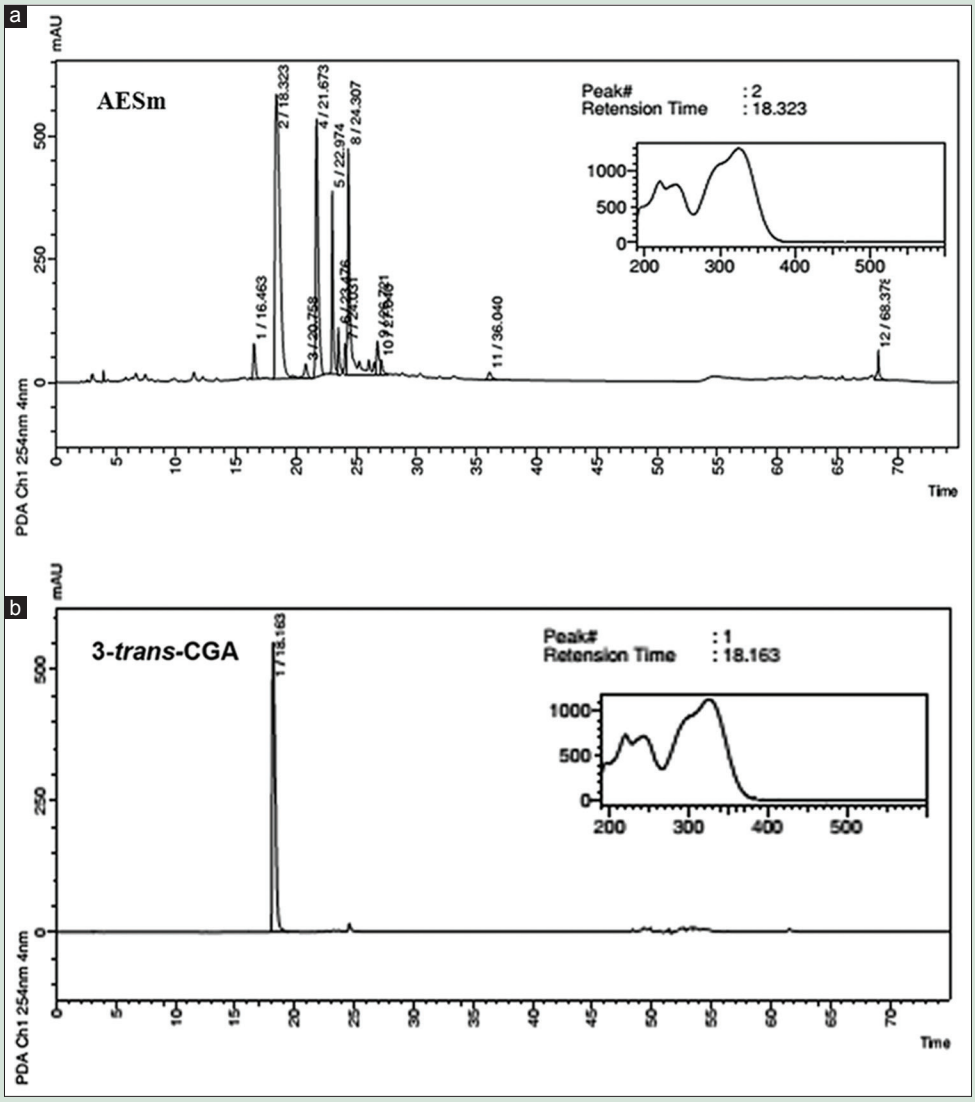

Figure 1: Chromatographic profiles of the aqueous extract of *Struthanthus marginatus* (a) and 3-*trans*-caffeoylquinic acid, authentic standard, (b) obtained by liquid chromatography-diode array detection at 280 nm. The inserted panel on the right shows the detailed ultraviolet spectrum of 3-*trans*-caffeoylquinic acid

Table 3: Volume (mL), total acidity (mEq [H+]/mL) and pH of gastric secretion in mice treated with *Struthanthus marginatus*, atropine or cimetidine

Treatment	Gastric volume (mL)	Total acidity (mEq[H⁺]/mL/4 h)	pH
Control	0.23±0.02	0.11±0.01	4.55±0.21
AESm	0.13±0.02[a]	0.04±0.002[a]	6.50±0.14[a]
Pilocarpine	0.34±0.08[a]	0.18±0.20[a]	3.50±0.01[a]
Pilocarpine + atropine	0.11±0.03[b]	0.05±0.01[b]	5.90±0.07[b]
Pilocarpine + AESm	0.18±0.07[b]	0.05±0.01[b]	6.10±0.44[b]
Histamine	0.52±0.08[a]	0.20±0.03[a]	3.40±0.24[a]
Histamine + cimetidine	0.18±0.03[c]	0.05±0.01[c]	5.90±0.10[c]
Histamine + AESm	0.25±0.07[c]	0.07±0.01[c]	5.72±0.44[c]

Results are the mean±SEM of six rats. [a]$P<0.05$ versus control, [b]$P<0.05$ versus pilocarpine, [c]$P<0.05$ versus histamine (ANOVA followed by Tukey's multiple comparison test). Control (water, 10 mL/kg), AESm (500 mg/kg), cimetidine (60 mg/kg, i.d.), histamine (20 mg/kg), pilocarpine (1.0 mg/kg), Atropine (1 mg/kg) (s.c.). AESm: Aqueous extract of *Struthanthus marginatus*; SEM: Standard error of the mean

confirmed the presence of CGA and identified the flavonoid glycosides quercetin and kaempferol.

Studies have shown that the antiulcer or gastroprotective properties of several plants can be attributed to the antioxidant activity of their constituents.[7] In this respect, 3-*trans*-CGA as the major compound, including its isomers 4-*trans*-CGA and 5-*trans*-CGA and other derivatives, flavonoids and antioxidants in AESm may contribute to the activity observed.[22,23] Quercetin and kaempferol have also been associated with the antiulcer activity.[24-28]

For evaluation of the gastroprotective effect of AESm in an acute gastric ulcer model induced by ethanol in mice, in addition to omeprazole as the positive control, we included a group treated with 3-*trans*-CGA (authentic standard) to investigate the association of this activity with the major compound of the extract. The results showed that treatment with AESm reduced the gastric ulcer area similar to omeprazole. A significant reduction was also found for its major compound, but this effect was lower than that observed for omeprazole and AESm. These

Table 4: Effect of oral administration of *Struthanthus marginatus* on gastric damage induced by ethanol in Swiss mice pretreated with NEM, indomethacin or L-NAME

Pretreatment (i.p.)	Treatment (oral)	Dose (mg/kg)	Ulcer area (mm²)	Inhibition (%)
Saline	Control		19.70±3.44	-
	Carbenoxolone	100	3.76±1.45[a]	80.91
	AESm	500	2.80±0.73[a]	85.80
NEM	Control		32.07±4.10[a]	-
	Carbenoxolone	100	12.60±2.90[b]	60.71
	AESm	500	4.31±0.60[b]	86.60
Indomethacin	Control		28.17±2.62[a]	-
	Carbenoxolone	100	8.04±1.74[c]	71.45
	AESm	500	9.54±2.62[c]	66.05
L-NAME	Control		43.62±6.52[a]	-
	Carbenoxolone	100	19.57±3.46[d]	55.13
	AESm	500	26.05±2.92	40.30

Results are the mean±SEM of six rats. [a]P<0.05 versus saline, [b]P<0.05 versus NEM, [c]P<0.05 versus indomethacin, [d]P<0.05 versus L-NAME (ANOVA followed by Tukey's multiple comparison test). L-NAME: N_{ω}-nitro-L-arginine methyl ester; NEM: N-ethylmaleimide; SEM: Standard error of the mean; AESm: Aqueous extract of *Struthanthus marginatus*

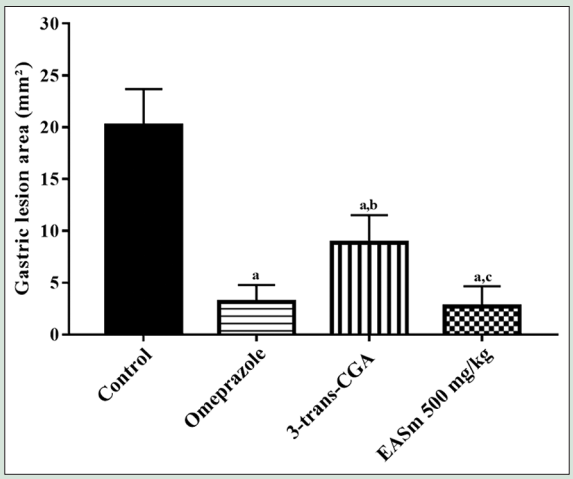

Figure 2: Effects of the aqueous extract of *Struthanthus marginatus*, omeprazole and 3-*trans*-caffeoylquinic acid on gastric ulcers induced by 75% ethanol in mice. The results are expressed as the mean ± standard error of the mean (n = 6). [a]P < 0.001 *vs* control; [b]P < 0.001 versus omeprazole; [c]P < 0.001 versus 3-*trans*-caffeoylquinic acid (analysis of variance followed by Tukey's multiple comparison test)

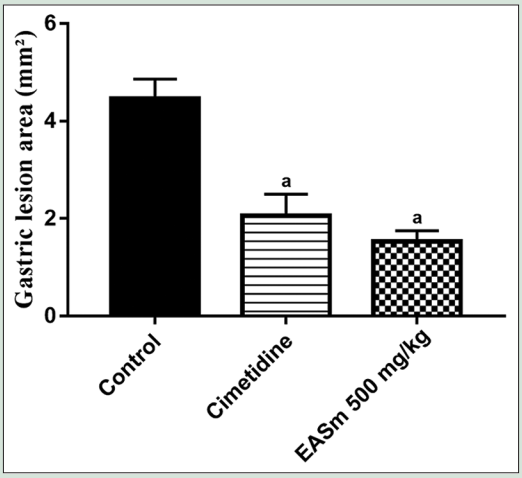

Figure 3: Effect of the aqueous extract of *Struthanthus marginatus* and cimetidine on gastric ulcers induced by 30% acetic acid in mice. Results are the mean ± standard error of the mean of six rats. [a]P < 0.001 versus control (analysis of variance followed by Tukey's multiple comparison test)

findings suggest that the protective effect of *S. marginatus* on the gastric mucosa is not only exclusively related to 3-*trans*-CGA but also to other components present in the extract and identified in this study, possibly flavonoids whose antiulcer activity has been extensively reported in the literature.[26-29]

The activity of the *S. marginatus* extract on gastric acid secretion, an important site of action of drugs used to treat peptic ulcers, was evaluated. AESm inhibited both basal secretion and histamine- or pilocarpine-stimulated gastric acid secretion. In addition, AESm reduced the volume and total acidity of gastric acid secretion and increased the pH after pylorus ligation [Table 3], findings indicating the involvement of this activity in the protection of the gastric mucosa. We, therefore, suggest the blockade or inhibition of a common target in the cascade of events that lead to gastric acid secretion, such as H^+/K^+-ATPase.[30] According to Beil *et al*.[31] and Freitas *et al*.,[32] the flavonoids quercetin and kaempferol can act as proton pump inhibitors and may be related to the effect of the extract on gastric acid secretion, considering that they were identified in the present study as major compounds of AESm [Table 2].

To investigate the mechanism of action underlying the gastroprotective effect of *S. marginatus*, this study also evaluated the involvement of endogenous NO, sulfhydryl compounds, and prostaglandins in the antiulcer activity of the extract, which are important defense mechanisms of the gastric mucosa. In mice pretreated with saline, AESm reduced the area of ethanol-induced gastric damage [Table 4], while administration of the extract to animals pretreated with L-NAME resulted in a decrease of the gastroprotective effect of *S. marginatus*, suggesting the partial involvement of NO since the effect of AESm was not completely abolished [Table 4]. Experimental studies have shown that NO released from the gastric epithelium plays an important role in the modulation of gastric defense.[23,33] NO increases the production of cyclic guanosine monophosphate through the activation of guanylate cyclase, which is involved in the synthesis and secretion of mucus. In addition to increasing local blood flow, endogenous and exogenous NO protects the gastric mucosa against damage induced by ethanol and endothelin-1.[2] NO also participates in other physiological mechanisms of gastroprotection that involve growth factors and hormones.[34]

Prostaglandins and sulfhydryl compounds are endogenous substances that play a mechanistic role in gastroprotection, since SH alkylators such as NEM counteract virtually any form of gastroprotection.[35] However,

the cyclooxygenase inhibitor indomethacin diminished but never abolished gastroprotection by other drugs.[36] Within this context, since ethanol-induced gastric damage is associated with a significant decrease in mucosal sulfhydryl and prostaglandin levels, this study evaluated the participation of these defense mechanisms in the antiulcer activity of AESm. Pretreatment of mice with an SH-blocker (NEM) did not alter the gastroprotection mediated by AESm and produced no significant increase in gastric damage compared to the group pretreated with saline, indicating the absence of involvement of SH-containing compounds. Furthermore, the gastroprotective effect of AESm was not affected by pretreatment of mice with indomethacin, suggesting that prostaglandins play no role in the antiulcer effect of the extract.

We also evaluated the effects of the extract on chronic ulcers induced by acetic acid. This model is extremely useful for pathophysiological and pharmacological studies of peptic ulcers.[37] Changes in the levels of prostaglandins, growth factors, NO, cytokines, and the amount of mucus may be involved in this type of injury.[38] The results showed that, similar to cimetidine, the treatment of animals with AESm significantly reduced the ulcer area compared to control, demonstrating the healing property of the extract. The observation of this activity in a chronic ulcer model is an interesting finding since previous studies have shown the gastroprotective effect of the plant in acute ulcer models in mice induced by different ulcerogenic agents such as ethanol, indomethacin, and stress (factors considered to be harmful to the human gastric mucosa) in which the extract seemed to increase the production of gastric mucus.[15]

The present findings suggest that the AESm protects the gastric mucosa against acute injury induced by ethanol and accelerates healing in an acetic acid-induced chronic ulcer model. These properties may be attributed in part to the antisecretory effects of the extract. Furthermore, the gastroprotective effect of the plant involves the release of endogenous NO, although the antioxidant compounds present in the extract may also effectively contribute to the activity observed. The present results support the ethnopharmacological use of the species and highlight its potential as gastroprotective herbal medicine.

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Conflicts of interest

There are no conflicts of interest.

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