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ORIGINAL PAPER

# Protective effect of *Chresta martii* extract against indomethacin-induced gastric lesions in mice

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**Abstract** *Chresta martii* (Asteraceae) is a plant found in the Xingó region (semi-arid area) in Northeastearn Brazil, and is recognized by the local population as a traditional herb used to treat gastric diseases. This is the first report of the chemical composition, acute toxicity, and gastroprotective effect in mice of the hydroalcoholic extract (HAE) from the aerial parts (leaves and flowers) of *Chresta martii*. Animals received HAE doses from 10 to 2000 mg/kg, i.p. or 50 to 3000 mg/kg, p.o.) and were observed over 48 h for toxicity signs and mortality; sub-chronic toxicity was evaluated through 14 days treatment with once-daily HAE doses (400 mg/kg, p.o.). The gastroprotective effect of HAE was demonstrated on the indomethacin-induced gastric ulcer model after the administration of extracts. Data comparison of ulcer index averages between saline and HAE (100 or 400 mg/kg, p.o.) groups showed significant ($P < 0.01$) inhibition (71.73 and 76.72 %, respectively) of indomethacin-induced gastric lesions. Histological analyses showed significant ($P < 0.05$) inhibition of leukocyte migration in HAE-treated groups. A fingerprint of the HAE obtained by

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HPLC/UV/MS analysis showed major peaks characteristic of sesquiterpene lactones. Compound **1** was isolated and elucidated as a new natural product. Its capacity to prevent leukocyte chemotaxis was demonstrated in vitro, corroborating the pharmacological effects observed for *C. martii* HAE.

**Keywords** *Chresta martii* · Asteraceae · Sesquiterpene lactones · Gastric ulcer

## Introduction

Despite progress in diagnosis and treatment, peptic ulcer disease remains a common reason for hospitalization and operation. Gastric ulcers may arise from several factors including infection (*Helicobacter pylori*) together with an imbalance between aggressive (acid, pepsin) and protective factors (prostaglandin, mucus and bicarbonate, gastric mucosal blood flow and motility) [1].

The current medicinal treatment of peptic ulcer is generally based on the inhibition of gastric acid secretion by $H_2$-receptor antagonists (ranitidine, cimetidine) and antimuscarinics, as well as on acid-independent therapy provided by sucralfate and bismuth. In the case of *H. pylori* infection, antibiotics are also used. Drugs providing antisecretory activity coupled with cytoprotective effects could represent a promising approach for the successful treatment of peptic gastric ulcer [2].

In recent years, there has been growing interest in alternative therapies and the use of natural products, especially those derived from plants. In traditional medicine, several plants and herbs have been used to treat gastrointestinal disorders [3].

In this regard, *Chresta martii* (DC.) H. Rob. (Asteraceae), found in the Xingó region (semi-arid area) in Northeastern Brazil, whose genus is considered a synonym for *Argyrovernonia* [4], is recognized by the local population as a traditional herb used to treat gastric diseases [5–7]. This plant has recently been studied botanically and considered as a new species [8] and this is the first report of its chemical profiling and pharmacological properties. The acute toxicity profile of hydroalcoholic extract (HAE) from *C. martii*, the evaluation of its antiulcerogenic effect, as well as its phytochemical analysis with the isolation of a new active natural product is reported here.

## Materials and methods

### Animals

Male Swiss mice (25–30 g) were housed at 22 ± 2 °C under a 12/12-h light/dark cycle, and food and water were supplied ad libitum. Animals were fasted for 18–24 h before the start of experiments. All efforts were made to minimize animal suffering and the number of animals used. All animal treatments and surgical procedures were performed in accordance with the "Guide for the Care and Use of Laboratory Animals" from the Brazilian Science Society in Laboratory Animals (SBCAL). All experiments were in accordance with the Guidelines for the Care and Use of Laboratory Animals and were approved by Committee on Animal Ethics (EAEC), Federal University of Pernambuco (process # 23076009313/2003-04).

### Plant material and extract preparation

Aerial parts (leaves and flowers) of *C. martii* were collected on 31 July in the Xingó region, Sergipe, Brazil (longitude −37.940 and latitude ranging from −9.5563 to −9.5548, altitude 130 m). After its authentication by Dr. Nádia Roque (Botany Department of Biology Institute—Federal University of Bahia, Brazil) a voucher specimen (protocol # 14602) was deposited in Vale do Acaraú State University herbarium (Sobral, Ceará, Brazil).

Air-dried and powdered aerial parts of the plant (100 g) were extracted using 50 % ethanol at room temperature (28 ± 3 °C) for 48 h. After filtration, the dark green solution was concentrated at 50 °C under reduced pressure to dryness and kept in a freezer. Fresh dilution of dried extract in saline solution (0.9 % NaCl) was prepared on the day of experiments, and administered orally or intraperitoneally in different doses.

### Acute toxicity study

The intraperitoneal (i.p.) or enteral (p.o.) acute toxicity $LD_{50}$ of *C. martii* HAE was evaluated in mice ($n = 10$/group) as described previously [9]. Ranging doses (10–2000 mg/kg, i.p. or 50–3000 mg/kg, p.o.) or saline solution (5 mL/kg p.o.) were administered to the animals as a single dose. Animals were observed over a 48-h period for toxicity signs and mortality.

### Sub-chronic toxicity study

Body mass loss, liver weight alteration, blood cell count alterations and the biochemical parameters aspartate aminotransferase (AST), alanine aminotransferase (ALT), amylase and lipase were evaluated after once-daily subchronic treatment of HAE (400 mg/kg, p.o.) or saline solution (5 mL/kg p.o.) for fourteen consecutive days. On the 15th day, all the animals were anesthetized with tribromoethanol (200 mg/kg, i.p.), and blood samples were collected from the retro-orbital plexus. Hematology analysis was performed using an automated hematology

analyzer (Pentra 80, Horiba ABX, Montpellier, France). Blood samples were analyzed to measure the following parameters: erythrocyte count, hemoglobin concentration, hematocrit, platelet count, leukocyte count, and differential cell count (neutrophils, monocytes and lymphocytes). For serum biochemistry analysis, the blood was centrifuged at 3000*g* for 15 min after collection. The serum samples were stored at −80 °C prior to analysis. The following serum biochemistry parameters were determined by enzymatic and colorimetric tests from Labtest Diagnóstica (Lagoa Santa/MG, Brazil): AST, ALT, amylase and lipase. After killing, the liver was removed and weighed. Spleen, kidney and heart were macroscopically analyzed for possible ulcerative lesions or hemorrhaging.

### Indomethacin-induced gastric lesions

Gastric ulceration was induced in 24-h fasted mice by the administration of indomethacin [40 mg/kg, subcutaneously (s.c.)]. Mice (6 per group) were pre-treated with HAE (100 or 400 mg/kg, p.o.), omeprazole (30 mg/kg, p.o.), used as a positive control, or saline (5 mL/kg; p.o.) 1 h before the challenge. A non-treated group (no indomethacin) was used as control for histological analyses. The animals were killed in a $CO_2$ chamber 6 h after the ulcerogenic procedure. The stomach was removed and opened along its greater curvature. The ulcer index was evaluated using the quantitative method for assessing the extent of experimental gastric erosions and ulcers as described by Szabo et al. [10]. The percentage inhibition was calculated in relation to the saline group according to the following formula: %inhibition = UIt/UIs × 100, where UIt and UIs correspond to ulcer index of treated and ulcer index of saline groups, respectively.

### Histopathological analysis

The H&E-stained gastric samples were semi-quantitatively evaluated for inflammatory infiltration to provide a (0–3) score grade: 0, no infiltration; 1, very mild infiltration; 2, mild infiltration; 3, moderate infiltration; and 4, marked infiltration. These observations were made by an experienced pathologist who was blinded to the treatment protocol.

### Statistical analysis

All values are expressed as mean ± SEM. For macroscopic (ulcer index) and histological assessment, the Kruskal–Wallis nonparametric test was used, followed by Dunn's test for multiple comparisons. Student's *t*-test was used to evaluate sub-chronic toxicity data. $P < 0.05$ was considered statistically significant.

### HPLC/UV/APCI-MS analysis

The HPLC/UV/APCI-MS analysis of *C. martii* HAE was performed on an HP-1100 liquid chromatography system (Hewlett-Packard, Palo Alto, CA, USA) equipped with a binary pump, a DAD and an autosampler. MS analyses were made on a Finnigan MAT (San Jose, CA, USA) LCQ ion trap mass with an atmospheric pressure chemical ionization (APCI) interface, with the following conditions: capillary temperature, 150 °C; vaporizer temperature, 370 °C; positive mode; sheath gas flow, 60 psi (414 kPa); corona needle current, 5 μA; collision energy, 15 eV. The separation was achieved on a Waters Nova-Pak $C_{18}$ column (250 × 4.6 mm i.d.; 5 μm) eluted with a linear gradient of methanol–water containing 0.1 % formic acid from 10:90 to 100:0 in 40 min. The flow rate was 1 mL/min; UV spectra were recorded at 210, 254 and 366 nm.

### Centrifugal partition chromatography

Fractionation was conducted on a counter-current chromatograph CCC-IOOO (Pharma-Tech Research Corporation, Baltimore, MD, USA) equipped with dynamic coils of 650 mL total volume. The rotation speed was set at 1000 rpm. Two LC-300 pumps (Scientific Systems Inc., State College, PA, USA) were used to pump either upper or lower phase into the coils at a flow rate of 3.0 mL/min for each phase. The HAE was diluted in 30 mL of a mixture of upper and lower phases (1:1) and introduced through the injection loop. Crude extract (9 g) was chromatographed using the solvent system chloroform–methanol–water (45:11:44) with the upper phase as the mobile phase, followed by phase inversion after 6 h. By this method, the extract was first fractionated into seven major fractions (AI–AVII).

### Column liquid chromatography

Fraction AII (2 g) was chromatographed on a silica gel 60 (63–200 μm, Merck, Germany) column (750 × 20 mm). The mobile phase consisted of hexane, ethyl acetate and methanol in increasing polarity proportions. From the 24 fractions obtained, compound **1** (50 mg) could be isolated (Fig. 3).

### NMR analyses

$^{1}H$ and $^{13}C$ NMR spectra were recorded on a Unity Inova 500 spectrometer at 500 and 125 MHz, respectively, with the compound dissolved in $CD_2Cl_2$. Chemical shifts were recorded in ppm with $\delta$ relative to tetramethylsilane (TMS) as internal standard.

Compound **1**

$^{1}$H-NMR (500 MHz), $CD_2Cl_2$: $\delta$ 1.36 (3 H, s, H-15), 1.38 (3 H, s, H-14), 2.04 (1H, ddd, H-1), 2.51 (1H, m, H-1), 2.54 (H, d, H-2), 2.62 (H, dd, H-2), 2.73 (1H, ddd, H-5), 2.83 (1H, dd, H-5), 4.51 (2H, d, H-13), 5.43 (1H, d, H-6), 5.83 (1H, s, H-9).

$^{13}$C-NMR (125 MHz), $CD_2Cl_2$: $\delta$ 26.7 (C-14), 27.2 (C-15), 33.9 (C-2), 38.7 (C-1), 47.5 (C-5), 56.0 (C-13), 67.2 (C-6), 74.7 (C-10), 76.2 (C-4), 113.6 (C-9), 121.6 (C-11), 145.1 (C-8), 146.7 (C-7), 168.5 (C-12), 213.1 (C-3).

HRESI/MS 295.1182 $[M+H]^+$, calculated for $C_{15}H_{19}O_6$.

In-vitro inhibition of neutrophil chemotaxis

*C. martii* HAE and compound **1** were investigated for their ability to inhibit neutrophil chemotaxis in vitro. This evaluation was performed using Boyden's method [11] modified by Zigmond and Hirsch [12].

The collection of chemotactic factor, the collection and preparation of neutrophils and the determination of their migration through the filters were conducted as previously described [13].

Readings were taken for 10 fields of two filters for each sample, and the result was expressed as mean ± SD. The chemotaxis experiment was analyzed using Student's *t* test. $P < 0.05$ was considered statistically significant, and $P < 0.001$ was considered highly significant.

## Results

Acute toxicity assay

HAE (50, 100, 2000 or 3000 mg/kg) administered orally (p.o.) did not result in mortality or altered pathognomonic behavior of the central nervous system. Similarly, low doses of HAE (10 or 100 mg/kg) injected intraperitoneally (i.p.) did not change animals' behavior. However, HAE (1000 mg/kg, i.p.) caused 2 deaths up to 6 h after the treatment. Furthermore, HAE (2000 mg/kg; i.p.) caused more than 50 % of mortality up to 6 h after the treatment, followed by 2 and 1 deaths over 24 and 36 h, respectively, from the beginning of the assay. All deaths were preceded by reduction of locomotion and eyelid ptosis.

Sub-chronic toxicity essay

HAE (400 mg/kg, p.o.) treatment for 14 days did not alter blood cell counts, hemoglobin, hematocrit, or liver mass. No macroscopic alterations were observed in internal organs. Indicators of pancreatic function (amylase and lipase) were not altered. ALT and AST values were significantly different from the saline group (Table 1). The body mass curve of the HAE-treated group was not significantly different from that of the saline-treated group (Fig. 1).

Effect of HAE on ulcer index

Indomethacin administration (40 mg/kg, s.c.) produced acute hemorrhagic damage. HAE (100 or 400 mg/kg, p.o.) significantly reduced ($P < 0.001$) the gastric lesion score compared to the saline group. Gastric protection by HAE was similar to the effects observed with omeprazole (30 mg/kg, p.o.) (Table 2).

Histopathological analysis

When compared to the non-treated group (no indomethacin) (Fig. 2a), the gastric mucosa of the saline group (saline + indomethacin) showed marked inflammatory cell accumulation together with disruption of the superficial layers (Fig. 2b). HAE (100 mg/kg) attenuated mucosal erosion as shown in Figure 2c. Histological analysis of omeprazole (30 mg/kg, p.o.) and HAE (100 and 400 mg/kg) groups revealed a significant ($P < 0.05$) decrease in inflammatory cell infiltrate [1 (0–1), 1 (0–1) and 1 (0–1)], respectively (Table 3).

Phytochemical analysis

A fingerprint of the HAE obtained by HPLC/UV/MS analysis is presented in Figure 3. Major peaks were detected in the extract and the presence of flavonoids was excluded. Compounds presented similar UV spectra with a maximum at c. 280 nm and a slight shoulder at 220 nm, which is characteristic of sesquiterpene lactones [14]. The mass spectra of some compounds, such as **1,** showed a low base peak at 295.1, corroborating the range of mass for sesquiterpene lactones.

Compound **1**

Compound **1** could be purified and elucidated as a new sesquiterpene lactone based on the analyses of its spectroscopic data. The $^{1}$H and $^{13}$C NMR data, together with HMBC correlations, are presented in Table 4.

In-vitro inhibition of neutrophil chemotaxis

When investigated for the ability to reduce leukocyte migration in vitro, HAE significantly reduced the migration distance ($P < 0.05$) at 100 μg/mL. At the same concentration, the isolated sesquiterpene lactone (compound **1**) was able to inhibit leukocyte migration with high significance ($P < 0.001$), which was comparable to the positive control indomethacin (Fig. 4).

**Table 1** Effects of HAE on blood parameters (AST, ALT, amylase, lipase and cell count) and liver mass after once-daily sub-chronic treatment of HAE or saline solution for fourteen consecutive days

| Blood parameter/treatment | Saline (5 mL/kg p.o.) | HAE (400 mg/kg p.o.) |
|---|---|---|
| AST (U/L) | 80.69 ± 4.33 | 110.3 ± 7.24# |
| ALT (U/L) | 51.36 ± 5.54 | 91.79 ± 8.43# |
| Amylase (U/dL) | 733.2 ± 11.48 | 683.5 ± 17.92 |
| Lipase (U/dL) | 276.0 ± 5.86 | 268.5 ± 13.73 |
| Erythrocyte ($10^6$/mm$^3$) | 7.676 ± 0.17 | 7.793 ± 0.17 |
| Hemoglobin (g/dL) | 12.84 ± 0.34 | 13.14 ± 0.30 |
| Hematocrit (%) | 38.67 ± 1.11 | 40.26 ± 1.05 |
| Total leukocytes (cells/mm$^3$) | 2,080 ± 196.0 | 2,471 ± 259.8 |
| Neutrophils (cells/mm$^3$) | 211.9 ± 45.35 | 181.3 ± 66.89 |
| Lymphocytes (cells/mm$^3$) | 1,431 ± 86.20 | 1,974 ± 212.1 |
| Monocytes (cells/mm$^3$) | 437.3 ± 95.71 | 316.4 ± 72.83 |
| Platelets (mm$^3$) | 601,300 ± 43,376 | 514,857 ± 56,340 |
| Liver mass (g) | 2.120 ± 0.067 | 1.925 ± 0.069 |

Data are presented as mean ± SEM. Student's *t* test

*HAE Chresta martii* hydroalcoholic extract

# $P < 0.05$ versus saline group

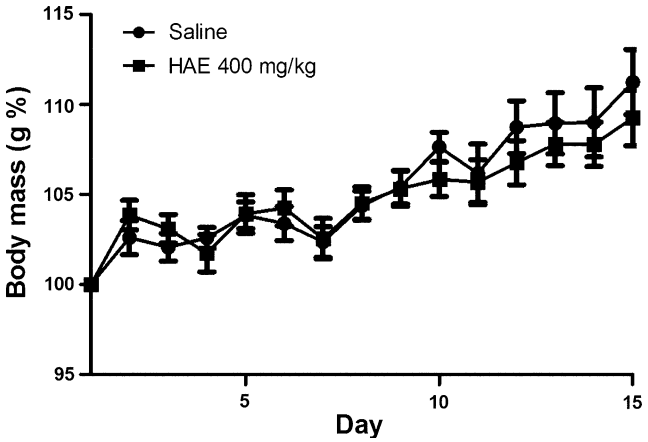


**Fig. 1** Body mass evolution of mice treated with HAE (400 mg/kg) for 14 days. Results are expressed as percentage of initial mass mean. There were no significant differences between saline and HAE-treated groups during the experiment

**Table 2** Effects of HAE on the ulcer index (macroscopic analysis) inhibition after indomethacin challenge

| Treatments | Ulcer index | % Inhibition |
|---|---|---|
| Saline | 22.25 ± 1.8 | – |
| Omeprazole 30 mg/kg, p.o. | 9.31 ± 2.0 | 58.15* |
| HAE 100 mg/kg, p.o. | 10.09 ± 1.6 | 54.66* |
| HAE 400 mg/kg, p.o. | 4.16 ± 1.2 | 81.30* |

Kruskal–Wallis nonparametric test followed by Dunn's test was used for multiple comparisons of macroscopic assessment

*HAE Chresta martii* hydroalcoholic extract

* $P < 0.01$ versus saline group

## Discussion

In the acute toxicity study, all the animals treated orally remained alive and did not manifest any significant visible signs of toxicity at the doses evaluated. There were no abnormal signs, behavioral changes, or macroscopic alterations at any time during the observation period. However, HAE at the highest doses (1000 and 2000 mg/kg) given intraperitoneally caused death preceded by observable adverse symptoms. These doses are, respectively, 10 and 20 times the minimum dose which gave gastric protection (100 mg/kg p.o.) in the experimental ulcer model used. This suggests that the extract has a wide safety window. The absence of any deleterious symptoms with oral use denotes low toxicity for this preparation when administrated in single doses.

Serum ALT and AST activities are used as indicators of chemically induced liver damage [15]. The ALT and AST values of our control (saline) group were similar to other reports [16, 17]. Results of the sub-chronic study revealed that there were significant ($P < 0.05$) increases in the means of serum ALT and AST levels in the HAE group when compared to the saline group. The literature shows that 2- to 3-fold increases in ALT levels above the upper limit of normal are needed to be considered indicative of hepatocellular injury [18]. Our study results did not reach these values (1.78 and 1.36 times the saline values of ALT and AST, respectively), and we compared it to mean, not to upper limits. Moreover, these parameters cannot be evaluated in isolation. The determination of this kind of drug-induced toxic effect must be based on the magnitude of the changes in all concurrently evaluated parameters [18]. Other parameters evaluated in our sub-chronic essay (macroscopic organ evaluation, serum lipase and amylase, body mass changes in time, blood parameters and liver mass) did not shown any alterations. Furthermore, the dose used in sub-chronic toxicity was 4 times the therapeutic dose used in this report (400 vs. 100 mg/kg). Pancreatic function indicators, amylase and lipase, did not differ between the HAE and saline groups. These considerations indicate a suitable safety margin for the oral administration of HAE.

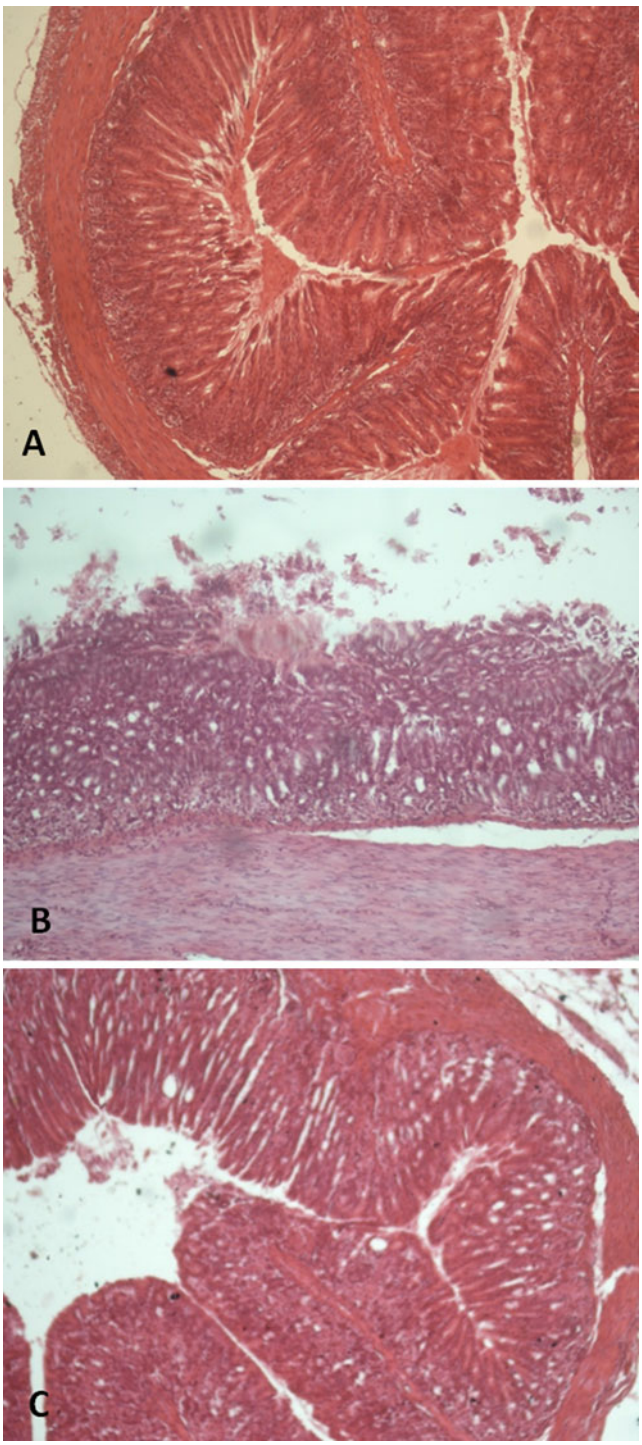


**Fig. 2** Photomicrographs of gastric mucosa: **a** non-treated group with normal conformation of mucosa; **b** saline group (animals that received indomethacin + saline) showing loss of papillae conformation, with epithelial disruption and gastric lumen enhancement; **c** animals treated with indomethacin + HAE 100 mg/kg, showing almost totally anatomical conformation, preservation of papillae and gastric luminal space (H&E ×10)

**Table 3** Effects of HAE on gastric histological assessment after indomethacin challenge

| Treatments | Inflammatory cells score (0–4) |
|---|---|
| Not treated[a] | 0 |
| Saline | 2 (1–3) |
| Omeprazole 30 mg/kg | 1 (0–1)** |
| HAE 100 mg/kg | 1 (0–1)** |
| HAE 400 mg/kg | 1 (0–1)** |

Data shown are medians with minimum and maximum scores shown in brackets. Kruskal–Wallis nonparametric test followed by Dunn's test was used for multiple comparisons of histological assessment

*HAE*, *Chresta martii* hydroalcoholic extract

**$P < 0.05$ versus saline group

[a] Non-treated group received no indomethacin

Our results clearly demonstrated that HAE protects against indomethacin-induced gastric mucosal lesions in mice, as shown by a significant decrease in the mean scores in comparison to the saline group. The integrity of gastric mucosal defense depends on continuous generation of prostaglandin $E_2$ ($PGE_2$) and prostacyclin ($PGI_2$), mediated by cyclooxygenases 1 (COX1) and 2 (COX2), which catalyze the rate-limiting step in the conversion of arachidonic acid to prostaglandins (PGs). $PGE_2$ and $PGI_2$ are both potent vasodilators and control many aspects of gastric mucosal defense and healing [19, 20]. These prostaglandins play an important role in maintenance of gastric mucosa blood flow, stimulation of mucus secretion, inhibition of neutrophil adherence and activation, and in the ability to protect the stomach against ulcerogenic agents [21–24]. Leukotrienes, having the same precursor as PGs (arachidonic acid), play an important role in gastric mucosa protective functions. Leukotriene production is related to vasoconstriction and neutrophil chemoattraction [25]. Inhibition of COX causes an imbalance in mucosal levels of leukotrienes and prostaglandins, favoring the production of the former [14].

According to Souza et al. [26], indomethacin-induced gastric lesions are dependent on neutrophil infiltration and nitric oxide (NO) generation through the inducible nitric oxide pathway. Our results of the histological assessment reveal that HAE (100 or 400 mg/kg, p.o.) reduced inflammatory cell migration to the gastric mucosa (Table 3). This reduction in neutrophils may, perhaps, contribute to the gastroprotection exhibited by the HAE.

Previous studies [27] showed that reactive oxygen species (ROS) play a vital role in indomethacin-induced gastric damage via oxidation of important cellular biomolecules such as lipids, proteins and DNA. The ROS-mediated degradation of the cell membrane results in the formation of lipid peroxides and initiates a variety of

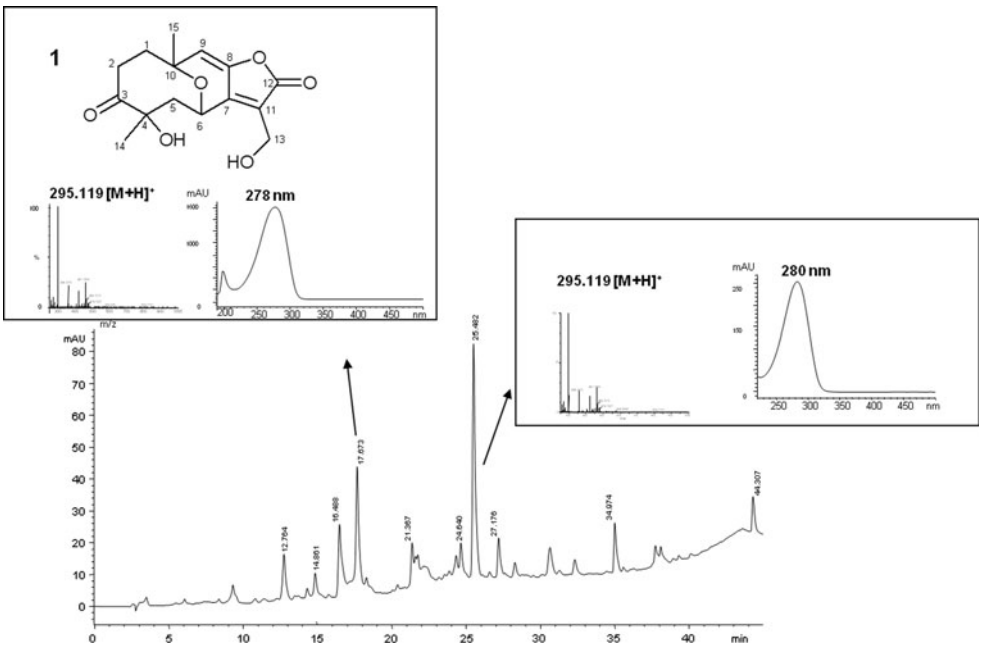


**Fig. 3** HPLC/UV/APCI-MS chromatogram of *C. martii* hydroalcoholic extract (C-18 column, UV 254 nm, APCI positive mode, mobile phase: MeOH−$H_2O$ + formic acid 0.2 % in gradient mode) and elucidated structure for compound **1**

**Table 4** NMR data of compound **1** in $CD_2Cl_2$

| Position | $^{13}$C NMR | $^{1}$H NMR | HMBC ($^{1}$H → $^{13}$C) |
|---|---|---|---|
| 1 | 38.7 | 2.04–2.51 | C-2, C-3, C-10, C-15 |
| 2 | 33.9 | 2.54–2.62 | C-3, C-4, C-10 |
| 3 | 213.1 | | |
| 4 | 76.2 | | |
| 5 | 47.5 | 2.73–2.83 | C-3, C-4 |
| 6 | 67.2 | 5.43 | C-5, C-7, C-8, C-10 |
| 7 | 146.7 | | |
| 8 | 145.1 | | |
| 9 | 113.6 | 5.83 | C-7, C-8, C-10 |
| 10 | 74.7 | | |
| 11 | 121.6 | | |
| 12 | 168.5 | | |
| 13 | 56.0 | 4.51 | C-7, C-11, C-12 |
| 14 | 26.7 | 1.38 | C-3, C-4, C-5 |
| 15 | 27.2 | 1.36 | C-1, C-9, C-10 |

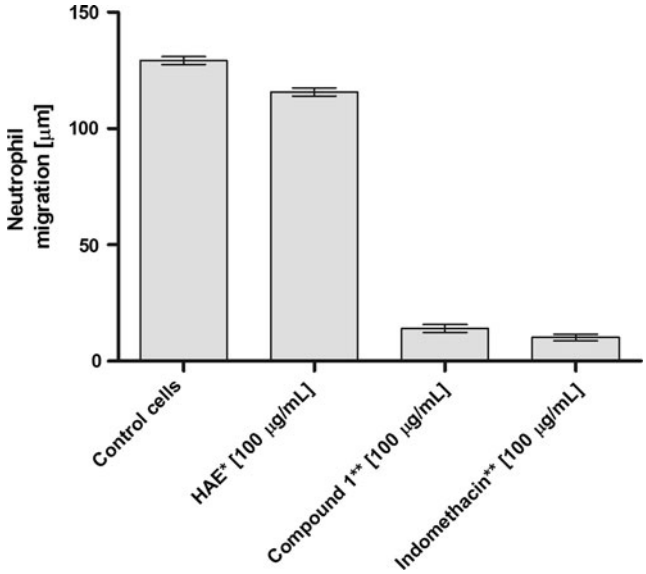


**Fig. 4** Effect of HAE and compound **1** on in-vitro neutrophil migration towards a chemotactic factor (lipopolysaccharide) gradient in Boyden chamber chemotaxis assay. Positive control: indomethacin; $*P < 0.05$; $**P < 0.001$

deleterious events, including mucosal lesions, increased vascular permeability and depletion of the mucus layer [28]. Nonprotein sulfhydryl groups, such as glutathione, promote cytoprotection by preventing free-radical oxidative damage in various tissues, including the gastric mucosa [29]. Indomethacin causes gastric erosions with increased lipid peroxidation and decreased glutathione peroxidase activity [27].

Some species from Asteraceae genera (*Eremanthus erythropappus, Baccharis illinita* and *Baccharis trimera, Senecio brasiliensis*) have demonstrated anti-ulcerogenic activity without deleterious side effects [3, 30–32]. Active compounds of various plants, such as flavonoids, triterpenes and tannins, may be regarded as possible active substances against gastric lesions [33]. In our study, we demonstrated the presence of sesquiterpene lactones in *C. martii* HAE. Some authors have demonstrated that sesquiterpene lactones have gastroprotective effects in experimental models of ulcer [34–39]. In fact, sesquiterpene lactones are related to preservation of endogenous non-protein sulfhydryl groups such as glutathione [40]. These compounds can be related to the gastroprotective

effect observed in our study. The isolated sesquiterpene lactone **1** was able to significantly inhibit neutrophil migration in a Boyden chamber in-vitro assay. This result corroborates the hypothesis that sesquiterpene lactones of this kind are responsible for the observed gastroprotection of HAE.

In conclusion, the low toxicity and the gastroprotective effect of *C. martii* HAE were demonstrated in mice, supporting the traditional use of this plant to treat gastric disorders. This plant provided a new sesquiterpene lactone with neutrophil antichemotactic activity. According to the HPLC/UV/MS fingerprint of *C. martii* HAE, this new plant species seems to be promising in providing new sesquiterpene lactones to be investigated individually for their complete structure elucidation and mechanisms of action in gastric protection.

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