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ORIGINAL ARTICLE

Evaluation of the Antioxidant and Gastric Antiulcerogenic Activities of the Hydroalcoholic Extract and Leaf Fractions of *Solanum stipulaceum* Roem. and Schult.

Cirlane Alves Araujo de Lima, Daniela Feitosa de Oliveira, Andressa da Silva, Robson Silva de Lima, Jymmys Lopes dos Santos, Antônio Santos Dias, André Luiz Lima Menezes dos Santos, Jamylle Nunes de Souza Ferro[1], Andrea Yu Kwan Villar Shan, Brancilene Santos de Araujo, Emiliano de Oliveira Barreto[1], Maria Silene da Silva[2], Josemar Sena Batista, Sara Maria Thomazzi, Sonia Piacente[3], Cosimo Pizza[3], Charles dos Santos Estevam

Department of Physiology, Federal University of Sergipe, São Cristóvão, [1]Graduate Center, Federal Institute of Sergipe, Aracaju, SE, [2]Institute of Biological and Health Sciences, Federal University of Alagoas, Maceió, AL, Brazil, [3]Department of Pharmacy, University of Salerno, Fisciano (Salerno), Italy

ABSTRACT

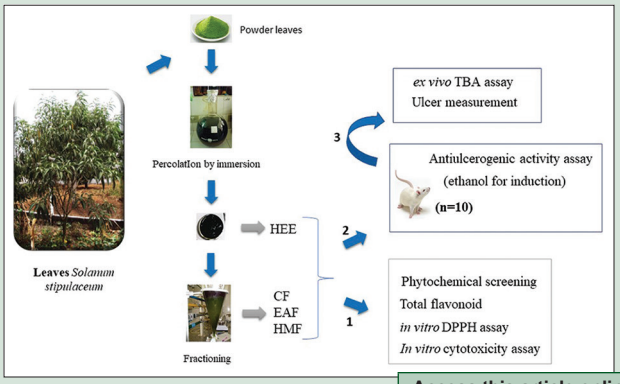
Background: An earlier ethnobotanical study showed that the species *Solanum stipulaceum* was indicated first in many orders for the treatment of gastric ulcers. **Aims:** The aim of this study was to evaluate phytochemical profile and the antioxidant, antiulcerogenic, protective redox, and cytotoxicity activities of the hydroethanol extract (HEE) and leaf fractions of this plant. **Materials and Methods:** The HEE was obtained by maceration and fractionated by liquid–liquid extraction in fractions: hexane fraction, chloroform fraction (CF), ethyl acetate fraction (EAF), and hydromethanol fraction (HMF). Phytochemical profile was evaluated by colorimetric methods. Antioxidant activity was measured by the 2,2-diphenyl-1-picrylhydrazyl (DPPH) assay. The cellular toxicity was evaluated by the 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide assay. *In vivo* antiulcerogenic activity was investigated using the ethanol-induced acute ulcer model. Afterward, protective redox activity was assessed with the plasma and liver of these animals using the thiobarbituric acid reactive substances assay. **Results:** Tannins, free steroids, triterpenes, alkaloids, and flavonoids were found in HEE, and total flavonoid measured was 5.26 ± 0.23 μg/mg of the extract. The DPPH activity was higher than 70% for HEE, EAF, and CF. Treatment with HEE (200 and 400 mg/kg) and CF, EAF, and HMF (200 mg/kg) inhibited the ulcer. CF reduces gastric lesions by 88.29% (50 mg/kg) and 85.65% (100 mg/kg). The HEE (400 mg/kg), CF, and EAF (200 mg/kg) reduced oxidative stress in the plasma and in the liver. HMF (200 mg/kg) reduced oxidative stress only in the liver. The HEE and fractions 10–50 μg/mL ($P < 0.05$) were nontoxic, maintaining cell viability at 65%. **Conclusion:** The extract and fractions have antioxidant activity *in vitro* and *ex vivo*, antiulcerogenic effect *in vivo*, and low cytotoxicity up to 50 μg/mL.

Key words: Antioxidant activity, antiulcerogenic activity, cytotoxicity, phytochemical screening, *Solanum stipulaceum*, total flavonoid

SUMMARY

- The extract of the leaves of *Solanum stipulaceum* and its fractions showed a gastroprotective effect and reduced oxidative stress in the liver and plasma, except for the HMF, which is active only in plasma and the chloroform fraction only in the liver
- The leaves of *Solanum stipulaceum* have phenolic compounds, terpenes, and alkaloids. The total amount of flavonoids in the extract and in the leaf fractions is significantly equal
- The extract and fractions were not cytotoxic in concentrations ≤50 μg/mL.

Abbreviations Used: *S. stipulaceum*: *Solanum stipulaceum*, HEE: Hydroethanol extract, HF: Hexane fraction, CF: Chloroform fraction, EAF: Ethyl acetate fraction, HMF: Hydromethanol fraction, DPPH: 2,2-diphenyl-1-picrylhydrazyl; $DPPH_{REM}$%: 2,2-diphenyl-1-picrylhydrazyl percentage of remaining; MDA: Malondialdehyde; TBA: Thiobarbituric acid; TBARS: Thiobarbituric acid reactive substances; TCA: Trichloroacetic acid; GA: Gallic acid; PBS: Phosphate-buffered saline; EQ: Equivalent of quercetin; MTT: 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide; RPMI: Roswell Park Memorial Institute; TNF: Tumor necrosis factor; ROS: Reactive oxygen species; PI: Percentage of inhibition; EC: Efficient concentration; AAI: Antioxidant activity index; ANOVA: Analysis of variance; HUFS: Herbarium of the Federal University of Sergipe; CAPES: Coordination for the Improvement of Higher Education Personnel; SISGEN: National Genetic Resource and the Associated Traditional Knowledge.



Correspondence:
Dr. Andrea Yu Kwan Villar Shan,
Department of Physiology, Federal University of Sergipe, University city Prof.José Aloisio de Campos, Av. Marechal Rondon s/n, Jd Rosa Elze, São Cristóvão, SE, CP 49000-000, Brazil.
E-mail: andreashan@ymail.com
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INTRODUCTION

People identified the first aid properties of medicinal plants since the beginning of humanity, which were successively transferred to their descendants and produced scientific discoveries through studies based on their popular use.[1] The approach used to select plants based on popular indication is denominated ethnodirected research.[2]

According to Di Stasi and Hiruma-Lima,[3] it strongly contributes to plant bioprospection because the popular knowledge comes from a dynamic culture and is daily enriched so that new plant species become potentially useful to pharmacological and chemical researches aimed to produce medications. However, the frequent use of these plants by the population does not guarantee full benefits due to the toxicity risk.[4] Therefore, at least preclinical tests should be performed before a plant can be considered safe for use. In this context, several research areas, such as ethnobotany, chemistry, and pharmacology, have been contributing to produce phytotherapics effective against several illnesses, which were associated with less toxicity.[5]

Plants contain several secondary metabolites classified in three main groups: terpenes, phenolic, and nitrogenated compounds, with varied degrees of toxicity as well as other biological properties, such as antioxidant and anti-inflammatory effects.[6] Flavonoids, classified as phenolic compounds, are responsible for the great part of the antioxidant activity in plants, constituting a strong ally of the human diet against pathologies associated with oxidative stress.[7] The expression "oxidative stress" refers to the production of substances toxic to tissues or the damage caused by the excess of free radicals produced due to biological dysfunctions in aerobic respiration and general metabolism.[8] Among these dysfunctions, gastric ulcers can be cited. According to Mei *et al.*,[9] oxygen-free radicals are involved in the mechanism of chronic ulceration in the gastric mucosa, while the elimination of free radical is implied in ulcer cicatrization.

The literature has shown plants with antiulcerogenic activity such as *Abarema cochliocarpos* (Gomes) Barneby and Grimes,[10] *Trichosanthes dioica* Roxb.,[11] and *Campomanesia xanthocarpa* O. Berg.[12] Several other medicinal plants nominated by traditional populations present antiulcerogenic activity.

In a previous ethnobotanical study, carried out by some members of this work, it was observed that the species *Solanum stipulaceum* Willd. Ex Roem. and Schult. (Sacatinga) was the most salient among 16 cited species indicated for the treatment of gastric ulcer by specialists from Vila Capim, in the city of Arapiraca, State of Alagoas, Brazil.[13] The aqueous and ethanolic extracts of the leaves of this plant tested in this study showed gastroprotective effect. Considering that *S. stipulaceum* leaf extract is popularly used and has pharmacological activities, the present study aimed to complement the ethnobotanical knowledge of this plant, investigating its phytochemical profile and mainly evaluating its gastric antiulcer effect *in vivo*, as well as antioxidant effects *in vitro* and *ex vivo* hydroethanol extract (HEE) and its hexane fraction (HF), chloroform fraction (CF), ethyl acetate fraction (EAF), and hydromethanol fractions (HMF). It is noteworthy that this species is native and endemic to the Cerrado, Caatinga, and Atlantic Forest biomes in Brazil, and there is little knowledge of its antioxidant and antiulcerogenic activities.

MATERIALS AND METHODS

Plant material harvesting and identification

Leaves of *S. stipulaceum* Willd. Ex Roem. and Schult. were collected on September 22, 2013, in the town Birth, city of São Francisco, State of Sergipe, at latitude 10° 23′13.0″ and longitude 36° 54′14.1″. A floral branch was collected and deposited in the Herbarium of the Federal University of Sergipe (HUFS) under record number ASE 30169 (consult in splink.cria.org.br) The species was identified in the HUFS as *S. stipulaceum*, family Solanaceae, with its name verified in www.theplantlist.org (record 29608065), after identification was registered in the National Genetic Resource and the Associated Traditional knowledge (SISGEN) under the number AD57577.

Extract preparation and fractioning

The collected leaves of *S. stipulaceum* were dried in a stove at 40°C with forced air circulation. Then, they were triturated and reduced to 900 g of powder. Afterward, 900 g of the leaf powder was percolated by immersion in ethanol 90% for 7 days. Then, the solution was concentrated on rotary evaporator at 45°C under reduced pressure to give 129.39 g (yield of 14.38%) of HEE, from which 105 g was dissolved in methanol: water (2:3) and fractioned using hexane, chloroform, and ethyl acetate to give HF (30.55 g; yield of 29.09%), CF (6.95 g; yield of 6.62%), and EAF (3.33 g; yield of 3.17%), respectively, with the remaining residue being called HMF (39.19 g; yield of 37.32%).

Phytochemical screening

To identify the classes of secondary metabolite present in them, HEE and HEE fractions were treated with classical reagents to give color development and/or characteristic precipitations.[14,15] All analyses were done with solutions at 1 mg/mL of each extract or fraction in ethanol using an ultrasound bath for solubilization.

Total flavonoid quantification

Aliquots of the extract and fractions at 1 mg/mL in methanol were mixed with 2% aluminum chloride in ethanol to give the flavonoid–aluminum complex. Afterward, the absorbance of these mixtures was spectrophotometrically measured at 420 nm. The analyses were performed in three repetitions, with three replicates each, and the total flavonoid content was expressed as μg equivalent of quercetin per mg of the extract or fractions (EQ μg/mg).[16]

In vitro antioxidant activity by 2,2-diphenyl-1-picrylhydrazyl assay

A solution of 2,2-diphenyl-1-picryl-hydrazil (DPPH) in methanol at 40 μg / mL was mixed with aliquots of the extract and fractions to obtain different final concentrations in a final reaction volume of 3 mL. The negative control and positive control were prepared using the same procedure, however, using methanol and DPPH and gallic acid (GA) and DPPH, respectively. The absorbance of each mixture was measured at 515 nm after 1, 5, 10, 20, 30, 40, 50 and 60 min of reaction in three repetitions in triplicates. The percentage of remaining DPPH ($DPPH_{REM}$%) was calculated according to Brand-Williams *et al.*[17] using the equation: $DPPH_{REM} = [DPPH]_T/[DPPH]_{To} \times 100$, where $[DPPH]_T$ is the final concentration of DPPH after 60 min of reaction with the antioxidant and $[DPPH]_{To}$ is the initial concentration of DPPH. Then, $DPPH_{REM}$ was used to calculate the percentage of inhibition (PI), using the equation: $PI = 100 - DPPH_{REM}$.

The quantity of antioxidant needed to reduce the $[DPPH]_{To}$ by 50% (EC_{50}) was found by plotting $DPPH_{REM}$% after 60 min of reaction against the extract or fraction concentrations. The antioxidant effect was also expressed as the antioxidant activity index (AAI), which was calculated in accordance with Scherer and Godoy[18] using the equation: $AAI = [DPPH]_T/EC_{50}$ (both in μg/mL). The antioxidant activity is considered poor when the value of AAI is < 0.5, moderate when the AAI is between 0.5 and 1.0, strong when the AAI is between 1.0 and 2.0, and very strong when the value of AAI is higher than 2.0.

Antiulcerogenic activity assay

Three-month-old Wistar rats (100 animals) were maintained in cages in groups of 10 males and 10 females/cage (200–220 g). They were kept under controlled temperature (25°C) with light/dark cycles of 12 h and free access to water and food (NUVILAB CR-1 brand). The experimental protocols were approved by the Committee of Ethics in Animal Research of the Federal University of Sergipe under protocol number 05/15.

The antiulcerogenic activity was evaluated using the method described by Morimoto *et al.*[19] with modifications. Wistar rats were fasted for 20 h. The experimental groups (n = 7, 4 males and 3 females) received HEE at 100, 200, and 400 mg/kg and CF, EAF, and HMF at 200 mg/kg. The fraction that showed the highest ulcer inhibition was further evaluated at 50 and 100 mg/kg as well. The positive control group was treated with ranitidine at 50 mg/kg (0.33 mL/100 g), while 5% Tween 80 (1 mL/100 g) was used to treat rats in the negative control group. All treatments were administered orally. After 1 h of receiving these pretreatments, animals were orally treated with absolute ethanol (0.4 mL/100 g) for induction of gastric ulcer. Thirty minutes after ethanol administration, animals were anesthetized with ketamine at 130 mg/kg and xylazine at 17.6 mg/kg and euthanized by decapitation with a guillotine. Afterward, animal stomachs were removed, opened by the great curvature, and washed with distilled water for evaluation of the gastric ulcer. Stomachs were photographed and lesion areas were quantified with the help of the Software Avsoft Bioview 4.0.2, (Avsoft Softwares Laboratoriais, Campinas, São Paulo, Brazil).[20]

Evaluation of the *ex vivo* antioxidant activity

The same animals used to evaluate the antiulcerogenic activity through the ethanol inducer were used to collect blood and to remove the liver, soon after euthanasia in order to evaluate the antioxidant activity *ex vivo* against the same inducer. Lipoperoxidation in the collected tissues was evaluated using the thiobarbituric acid reactive substances (TBARS) assay.[21-23] After euthanasia and stomach removal, 4 mL of blood was collected from each animal and immediately centrifuged at 1310 g/15 min. The supernatant was stored at −70°C. Livers were also removed, weighed, homogenized in 5 mL of phosphate-buffered saline (PBS), and centrifuged at 1310 g/15 min, with the supernatant being collected and stored at −70°C. Malondialdehyde (MDA) marker was measured in all samples to evaluate the oxidative stress.

Aliquots of 200 µL of the blood and liver samples were added to a mixture consisting of equal parts of 15% trichloroacetic acid, 0.25N HCL, and 0.375% thiobarbituric acid, plus 2.5 mM butylated hydroxytoluene and 40 µL of 8.1% sodium dodecyl sulfate. These mixtures were heated at 95°C in a stove for 30 min, after which the pH was adjusted to 0.9 with HCL. After cooling to room temperature and addition of 4 mL of n-butanol, the material was centrifuged at 1310 g for 15 min and the absorbance of the supernatant was measured at 532 nm. MDA concentration was determined using the coefficient of molar extinction 15400 L/mol. cm. TBARS were expressed as nmol Eq MDA/mL of blood and nmol Eq MDA/mg for liver samples.

In vitro cytotoxicity

Cytotoxic effect of *S. stipulaceum* leaf extract and fractions was measured in macrophages using the 3-(4,5-dimethylthiazol-2-yl)-2, 5-diphenyltetrazolium bromide (MTT) method.[24] J774 macrophages were distributed in 96-well microplates (1.5×10^5 cells/well) and incubated for 2 h at 37°C. Afterward, the adherent cells were pretreated with HEE, HF, CF, EAF, and HMF at 10, 50, and 100 µg/mL prepared in RPMI-1640 medium supplemented with 10% fetal bovine serum, 1% L-glutamine, and 1% gentamicin. The plates were further incubated for 24 h at 37°C. Following this, the medium was changed for fresh RPMI-1640 containing 5 mg/mL of MTT, and the plates were again incubated at 37°C for 4 h. Then, the supernatant was discarded, and dimethyl sulfoxide (150 µl/well) was added to the wells in each culture plate. After an additional 15 min of incubation at room temperature, the absorbance of the soluble product, formazan, was measured at 540 nm using a microplate reader (Thermo Plate, RS MODEL 232). Four individual cavities were evaluated by treatment. Cytotoxicities were determined using the following formula: (absorbance of treated cells/absorbance of untreated cells) × 100.

Statistical analysis

Chemical and *in vitro* antioxidant assays were performed in three repetitions with three replicates each. Pharmacological experiments were done using groups of 7 animals. Data were expressed as mean ± standard error. The statistical differences among means were determined by one-way analysis of variance, followed by Tukey's *post hoc* test at $P < 0.05$ for flavonoid content analysis and DPPH antioxidant results, while the *post hoc* test of Bonferroni at $P < 0.05$ was used for the antiulcerogenic and *ex vivo* antioxidant activities as well as for cytotoxicity. Statistical analyses were performed using the software GraphPad Prism v. 5.0 (Dr. Harvey Motulsky, GraphPad Software, San Diego, California, United States of America).

RESULTS

Phytochemical screening

Different groups of secondary metabolites were found in the extract and fractions of *S. stipulaceum* leaves, as it is shown in Table 1. Phenolic compounds such as catechinic tannins, flavonols, flavonoids, or xanthones were detected in HEE, CF, EAF, and HMF but not in HF.

Another important group of compounds found in the leaves of *S. stipulaceum* was the terpenes, being detected three subgroups: pentacyclic triterpenes, saponins, and free steroids. Alkaloids, which are nitrogenated compounds, were also detected [Table 1].

Total flavonoid content

Table 2 shows there were no significant differences ($P < 0.05$) regarding the amount of flavonoids in the extract and fractions obtained from the leaves of *S. stipulaceum*. The HF was not evaluated by the absence of phenolic compounds.

Table 1: Chemical composition of the extract and fractions obtained from leaves of *Solanum stipulaceum* Willd. Ex Roem. and Schult.

Secondary metabolite groups	HEE fractions				
	HEE	HF	CF	EAF	HMF
Catechinic tannins	−	−	+	+	+
Free steroids	+	+	−	−	−
Pentacyclic triterpenes	+	−	+	−	−
Alkaloids	+	−	−	+	−
Saponins	−	−	−	−	+
Flavonols	+	−	−	−	−
Flavononols	+	−	−	−	−
Xanthones	+	−	−	−	−

HEE: Hydroethanol extract; HF: Hexane fraction; CF: Chloroform fraction; EAF: Ethyl acetate fraction; HMF: Hydromethanol fraction

Table 2: Total flavonoid content of the extract and fractions obtained from the leaves of *Solanum stipulaceum* Willd. Ex Roem. and Schult.

Sample	Flavonoid total content (EQ µg/mg of extract or fraction±SE)
HEE	5.26±0.23[a]
CF	4.50±0.10[a]
EAF	4.70±0.33[a]
HMF	4.05±0.50[a]

Means±SE with similar lower case letters indicate they are not significantly different at $P<0.05$ (ANOVA, followed by Tukey's *post hoc* test).
HEE: Hydroethanol extract; CF: Chloroform fraction; EAF: Ethyl acetate fraction; HMF: Hydromethanol fraction; SE: Standard error; EQ: Equivalent of quercetin; ANOVA: Analysis of variance

2,2-diphenyl-1-picrylhydrazyl antioxidant activity

In the antioxidant assay, the EAF presented a higher free radical sequestration capacity with a lower EC_{50} value, which did not differ significantly ($P < 0.05$) from the positive control (GA) and CF and HEE. However, CF and HEE differed significantly ($P < 0.05$) from GA. The highest PI values were obtained for EAF, HEE, and CF. HF showed the highest value of EC_{50} and lower PI value, showing that this fraction has low antioxidant activity.

The antioxidant potential of EAF, HEE, and CF against DPPH was further demonstrated by the AAI analysis [Table 3], which showed that their antioxidant activities were moderate, with AAI values between 0.5 and 1.0. The activities of the other fractions were poor, with AAI below 0.5.

Antiulcerogenic activity

When the antiulcerative effect of *S. stipulaceum* extract and fractions was investigated, it was shown that HEE has a significant ($P < 0.05$) gastroprotective activity at 200 and 400 mg/kg, with inhibitions of 61.00% and 88.32%, respectively [Figure 1a]. The antiulcerogenic effect produced at 400 mg/kg was comparable to ranitidine at 50 mg/kg, with an inhibition of 96.28%. HEE showed a dose–response behavior.

A preliminary test was performed with at fractions to evaluate its probable antiulcerogenic activity. We found that HF did not inhibit gastric ulcer induced by ethanol, since the treated animals presented intense pain and their stomachs were severely ulcerated. In view of the above, we decided not to continue the experiments with this fraction. The protective effect of CF, EAF and HMF at 200 mg/kg evaluated significantly inhibited ($P < 0.05$) gastric ulcer by 96.80%, 62.47%, and 58.09%, respectively [Figure 1b]. As CF at 200 mg/kg inhibited gastric ulcer at the same level as ranitidine at 50 mg/kg (94.44%), it was further investigated at 50 and 100 mg/kg. CF significantly ($P < 0.05$) reduced the gastric lesions by 88.29% and 85.65%, respectively [Figure 2].

Table 3: Antioxidant activity of the extract and fractions obtained from the leaves of *Solanum stipulaceum* against the free radical 2,2-diphenyl-1-picrylhydrazyl

Samples	IP (%)	AAI	EC_{50} (μg/mL±SE)
GA	84.45	2.48	14.72±0.49[a]
HEE	77.30	0.68	73.46±8.32[b]
HF	19.86	0.08	466.29±20.68[d]
CF	73.35	0.56	72.38±1.88[b]
EAF	77.89	0.74	53.30±2.20[ab]
HMF	69.38	0.32	127.53±8.72[c]

IP was calculated using the 60 min $DPPH_{REM}$ data for the concentration 40 μg/mL. AAI was calculated by the formula $(DPPH)_T/EC_{50}$ (both in μg/mL). EC_{50} for DPPH was calculated by plotting 60 min $DPPH_{REM}$ against extract or fraction concentration and expressed as mean±SE. Means with similar lower case letters indicate they are not significantly different at least at $P<0.05$ (ANOVA, followed by Tukey's *post hoc* test). The IP was obtained using the percentage of remaining data of the highest concentrations of each extract or fraction. Subtracting from 100. PI: Percentage of inhibition; AAI: Antioxidant activity index; GA: Gallic acid; DPPH: 2,2-diphenyl-1-picrylhydrazyl percentage of remaining; SE: Standard error; HEE: Hydroethanol extract; CF: Chloroform fraction; EAF: Ethyl acetate fraction; HMF: Hydromethanol fraction; HF: Hexane fraction; ANOVA: Analysis of variance

Reduction of the oxidative stress induced by extract and fractions

HEE at 400 mg/kg significantly ($P < 0.05$) reduced the oxidative stress in plasma [Figure 3a] and liver [Figure 3b] by 59.09% and 44.14%, respectively, compared with positive control (ranitidine).

Regarding fractions, CF and EAF at 200 mg/kg significantly ($P < 0.05$) reduced the oxidative stress damage in plasma [Figure 4a] by 44.44% and 36.86%, respectively. CF, EAF, and HMF at 200 mg/kg were also able to reduce the oxidative stress damage in the liver [Figure 4b] by 51.57%, 35.53%, and 47.79%, respectively.

Considering that CF inhibited ulcer formation at 50 and 100 mg/kg, the protective effect against the oxidative stress was investigated at these concentrations. CF significantly ($P < 0.05$) reduced liver oxidative stress by 55.66% at 50 mg/kg, showing no effect in the plasma [Figure 5].

Cytotoxic effect of *Solanum stipulaceum* extracts and fractions

Cell toxicity of HEE, HF, CF, EAF, and HMF was assessed using J774 macrophages at 10, 50, and 100 μg/mL by the MTT assay. At concentrations of 10 and 50 μg/mL, extracts and fractions did not differ significantly ($P < 0.05$) from the positive control RPMI, that is nontoxic, maintaining cell viability at 65% [Figure 6a and b]. HEE differed statistically from CF and EAF, and the concentration of 50 μg/mL in CF and EAF differed statistically from HEE. However, the same performance was not observed at a concentration of 100 μg/mL, since all samples of *S.*

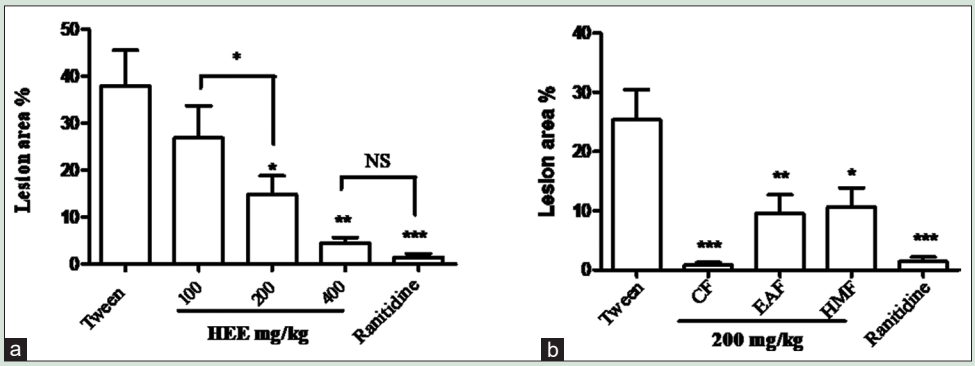

Figure 1: (a) Effect of the hydroethanol extract obtained from leaves of *Solanum stipulaceum* (100, 200, and 400 mg/kg) and ranitidine (50 mg/kg) against gastric ulcers induced by ethanol administration and (b) effect of chloroform, ethyl acetate, and hydromethanol fractions obtained from the hydroethanol extract of *Solanum stipulaceum* leaves at 200 mg/kg and ranitidine (50 mg/kg) against gastric ulcers induced by ethanol administration. Bars represent means ± standard error of the gastric lesion area (%) obtained from the experimental animals. Means were compared using one-way analysis of variance, followed by Bonferroni's *post hoc* test. *$P < 0.05$, **$P < 0.01$, and ***$P < 0.001$ against control (Tween)

stipulaceum differed statistically from the positive control RPMI ($P < 0.05$), that is, they presented toxicity, as it decreased cell viability. However, there was no statistical difference between CF, EAF, and HMF [Figure 6c].

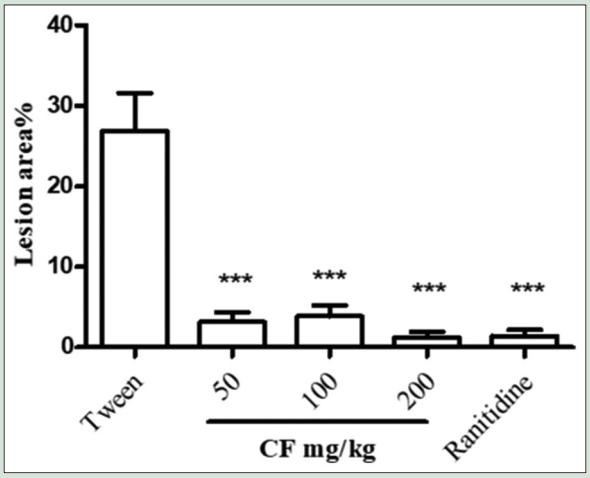

Figure 2: Effect of the chloroform fraction obtained from the hydroethanol extract from the leaves of *Solanum stipulaceum* (50, 100, and 200 mg/kg) and ranitidine (50 mg/kg) on the gastric lesions induced by ethanol. Bars represent means ± standard error of the gastric lesion area (%) obtained from the experimental animals. Means were compared using one-way analysis of variance, followed by Bonferroni's *post hoc* test. ***$P < 0.001$ against control (Tween)

DISCUSSION

Substances belonging to the classes of catechinic tannins, flavonols, flavononols, or xanthones, which were found in the extract and fractions of *S. stipulaceum* leaves, showed biological activities in other studies on inflammation and gastric ulcers[25] and as antioxidant agents.[26] Flavonols and flavononols presented antioxidant and antitumor activities and were shown to protect renal, cardiovascular, and hepatic systems,[27] while xanthones had hepatoprotective, antitumor, anti-inflammatory, antioxidant, antiulcer, and immunomodulatory activities.[28] Pentacyclic triterpenes, such as squalene, are known for their antiulcerogenic,[29,30] anti-inflammatory,[31] antifungal,[32] hepatoprotective,[33] antitumor, and anti-HIV[34] activities. Saponins, which are steroid glycosides or polycyclic terpenes,[35] act as immunoadjuvants and hemolytic agents[36] and have shown anti-inflammatory activity.[37] Free steroids, which are direct derivatives of isoprene, possess antitumor,[38] anti-inflammatory, and gastrointestinal[39] activities. Alkaloids have shown antitumor,[40] anticholinergic,[41] and hepatoprotective[42] activities.

The total flavonoid contents found in extract and fractions of *S. stipulaceum* leaves were higher than observed for leaves of other plants in the same genus, such as *Solanum nigrum*, whose methanol and aqueous leaf extract showed 0.73 ± 0.06 and 0.91 ± 0.04 EQ mg/g extract, respectively,[43] and *Solanum fastigiatum*, which had a total flavonoid content of 0.173 ± 0.03 EQ mg/g extract.[44] Flavonoids are usually found in the aerial parts of plants and are considered stable compounds because they resist oxidation and pH variations.[45] They can prevent the formation of free radicals and inhibit their actions in the geneses of several illnesses.[46] Differences in total flavonoid contents depend on genetic variations, environmental factors such as radiation

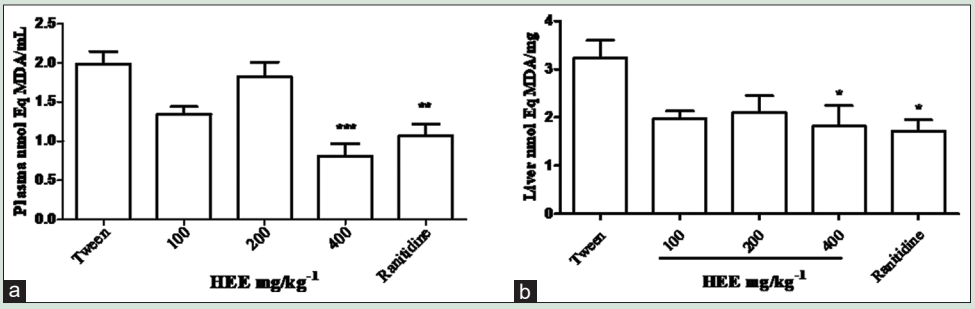

Figure 3: Effect of the hydroethanol extract of *Solanum stipulaceum* (100, 200, and 400 mg/kg) and ranitidine (50 mg/kg) in the reduction of the *ex vivo* oxidative stress induced by ethanol orally administrated in (a) blood and (b) liver. Bars represent means ± standard error of the nmol Eq MDA/mL of blood and nmol Eq MDA/mg for liver samples. Means were compared using one-way analysis of variance, followed by Bonferroni's *post hoc* test. *$P < 0.05$, ** $P < 0.01$, and ***$P < 0.001$ against control (ranitidine)

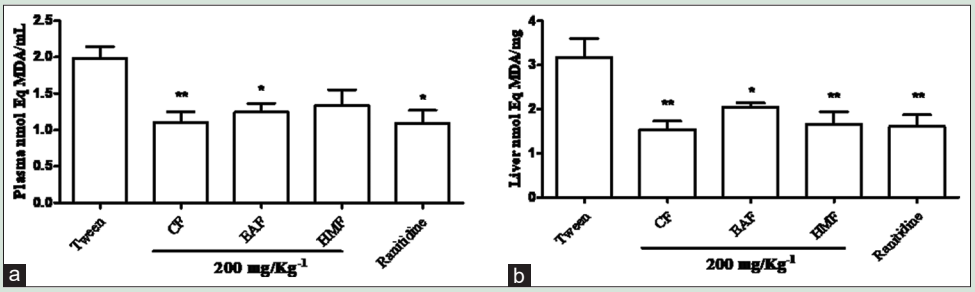

Figure 4: Effect of chloroform, ethyl acetate, and hydromethanol fractions obtained from the hydroethanol extract of *Solanum stipulaceum* leaves (200 mg/kg) and ranitidine (50 mg/kg) in the reduction of the *ex vivo* oxidative stress induced by ethanol orally administrated in (a) blood and (b) liver. Bars represent means ± standard error of nmol Eq MDA/mL of blood and nmol Eq MDA/mg for liver samples. Means were compared using one-way analysis of variance, followed by Bonferroni's *post hoc* test. *$P < 0.05$ and **$P < 0.01$ against control (ranitidine)

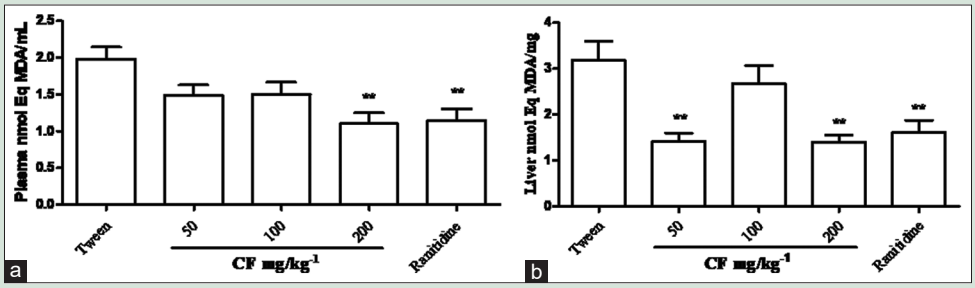

Figure 5: Effect of chloroform fraction obtained from the hydroethanol extract of *Solanum stipulaceum* leaves (50, 100, and 200 mg/kg) and ranitidine (50 mg/kg) in the reduction of the *ex vivo* oxidative stress induced by ethanol orally administrated in (a) blood and (b) liver. Bars represent means ± standard error of nmol Eq MDA/mL of blood and nmol Eq MDA/mg for liver samples. Means were compared using one-way analysis of variance, followed by Bonferroni's *post hoc* test. $^{**}P < 0.01$ against control (ranitidine)

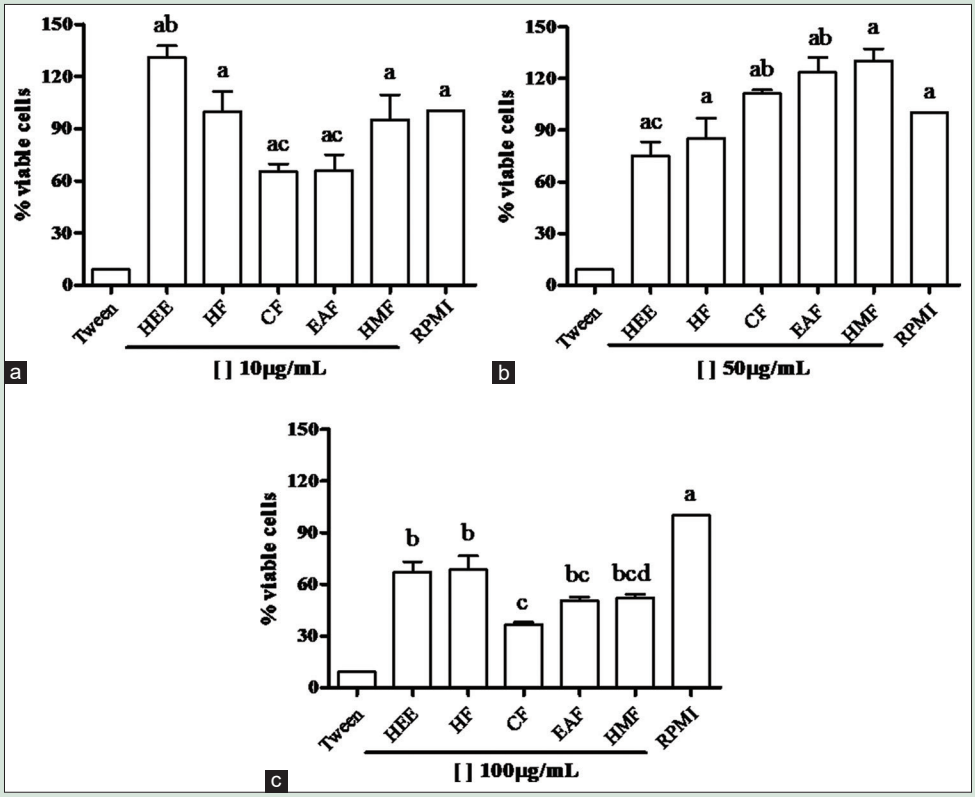

Figure 6: Effect of the hydroethanol extract, hexane, chloroform, ethyl acetate, and hydromethanol fractions obtained from leaves of *Solanum stipulaceum* on cell viability of J774 macrophages in the 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide assay. Cells were treated with the extract and fractions at (a) 10 µg/mL, (b) 50 µg/mL, and (c) 100 µg/mL during 24 h. Bars represent the percentage of viable cells compared with negative control as means ± standard error. Means were compared using one-way analysis of variance, followed by Bonferroni's *post hoc* test. Bars with the same letters indicate they are not significantly different at $P < 0.05$

and temperature, and edaphoclimatic conditions such as infections as well as silicon and nitrogen concentrations in the soil.[47-49] In the other hand, independent of their content, the bioavailability, metabolism, and biological activities of flavonoids are subjected to the configuration and quantity of hydroxyl groups and the substitution of functional groups in the nuclear structure[50] because a specific flavonoid or a set of these substances are needed to treat a certain illness.

Secondary metabolites of plants have been associated with gastric protection. According to Murdiati *et al.*,[51] tannins can form stable complexes with pepsin to precipitate this protein. It is also reported that sesquiterpenes can reduce gastric acid secretion by directly blocking muscarinic receptors or interacting through a second intracellular messenger.[52] Catechinic tannins were found in CF, EAF, and HMF, while pentacyclic terpenes were found in HEE and CF. Both groups of secondary metabolites were found in the CF, which was more potent in inhibiting the gastric lesions. However, the influence of other metabolites in these extract and fractions as gastric protectors cannot be discarded. This is the case for flavonoids, which are known for their antiulcerogenic and antioxidant.[53]

Reduction of oxidative stress in the plasma and liver by HEE (400 mg/kg) is consistent with results for antiulcerogenic activity, since HEE was the most effective in inhibiting gastric lesions (88.32%) and

reducing oxidative stress (59.09% for plasma and 44.14% for liver). In addition, CF (200 mg/kg) gave the best results for antiulcerogenic activity (96.80%) and oxidative stress inhibition (44.44% and 51.57% for plasma and liver, respectively). In this study, there was no significant difference in flavonoid content of extracts and fractions. Therefore, the antioxidant effect of *S. stipulaceum* in the plasma and liver is probably related to the type of phenolic compounds present in each extract or fraction and their potential to eliminated reactive oxygen species through hydroxyl groups present in their structures.[54,55] In addition, it is known that ingested polyphenols are metabolized in the liver before their release in bloodstream.[54] Therefore, this organ may have a higher content of polyphenols, serving as a deposit for these compounds, which will minimize free radicals faster and in a more efficient way than in the blood. It can also be suggested that the polar and nonpolar nature of HEE and CF, respectively, may have influenced the results because polyphenols present in CF may be nonpolar, so they are better absorbed in tissues such as the liver to reduce the oxidative stress.[54]

According to Marroni,[23] quercetin and other flavonoids may inhibit excessive production of nitric oxide and tumor necrosis factor which may be involved in gastrointestinal mucosa injury, which explain their protective effect against an ulcerative process. Given that both reduction and overproduction can compromise the integrity of the gastric mucosa because endogenous nitric oxide has a cytoprotective role acting in the maintenance of the integrity of the mucosa, in the regulation of acid and alkaline secretions, mucus secretion and blood flow.[23,56-58] Flavonoids have the role of purifying active species of oxygen and chelating metals and quercetin purifies superoxide anions and hydroxyl radicals, which can minimize oxygen-reactive species, increase the hepatic reaction to them, and contribute to the reduction of the hepatic oxidative stress.[59] Flavonoids kaempferol, quercetin, and tyrosine were already identified in *S. stipulaceum*,[60] and their antioxidant activities are well registered in the literature.[61-63]

However, results for the cellular toxicity assay suggested that extracts and fractions of *S. stipulaceum* can cause cell damage at 100 μg/mL. According to Haraguchi,[64] the toxic effect of a plant does not depend uniquely of its chemical composition, but it is linked to the quantity and nature of the substances as well as the administration pathway. Despite the leaf extracts and fractions *in vitro* apparent toxicity toward J774 macrophages at 100 μg/mL, a previous study in Swiss mice with the aqueous and ethanol extracts of *S. stipulaceum* at 5000 mg/kg did not present any visible symptoms of toxicity.[13]

CONCLUSION

The findings of the present study showed that *S. stipulaceum* leaf extract and fractions, which are rich in terpenes, phenolic compounds, and alkaloids, have *in vitro* and *ex vivo* antioxidant activity, *in vivo* antiulcerogenic effect, and low cytotoxicity until 50 μg/mL against J744 macrophages. For better elucidation of their biological activities, complementary studies for the isolation and identification of secondary metabolites should be done.

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Conflicts of interest

There are no conflicts of interest.

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