



Effect of *Byrsonima sericea* DC. leaf extracts on mice gastrointestinal tract

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ABSTRACT

Byrsonima sericea DC. (Malpighiaceae) leaves are popularly folk medicine in Brazil used to treat gastro-intestinal disorders including diarrhea and gastric diseases. Ethanol extract (BSEE), ethyl acetate extract (BSEAE) and hexane extract (BSHE) of the leaf part of *Byrsonima sericea* DC were characterized for their total phenolics, proanthocyanidins and flavonoids content. The total antioxidant capacity of extracts was determined. The ethnopharmacological use of *B. sericea* leaves was evaluated by assaying BSEE for gastroprotective activity in stomach ulcer induced by indomethacin, intestinal motility and toxicity. Abundance of phenols mainly tannins was found in BSEE. Total phenolics, flavonoids and proanthocyanidins content in BSEE were found to be 0.371, 0.172 and 1.3×10^{-4} (mg/g) respectively. BSEE showed concentration dependent significant scavenging of DPPH values 90.0 (%) respectively. Moreover, oral doses of 500 and 1000 mg/kg did not cause mortality, and there was no difference in animals weight, organs relative weight and alanine transaminase (ALT) and aspartate transaminase (AST), as compared to the control group. Doses of 250, 500 and 1000 mg/kg inhibited the gastric lesions induced by indomethacin in 52, 60 and 62 % respectively. The dose of 1000 mg/kg decreased intestinal motility in animals. The presence of phenolic compounds, including tannins could be associated with the anti-diarrheal action and the antioxidant properties could collaborate to the gastroprotective and anti-diarrheal activities, confirming its popular use of the plant.

1. Introduction

Currently, the use of medicinal plants has become a widely accepted alternative resource for the growing population and the medical community. Plants that are used for therapeutic purposes include those which have antioxidant activity since the oxidation of biomolecules can be involved in the onset of various degenerative diseases [1]. Plants produce a variety of substances used in the control of oxidative stress, caused by the action of sunlight and oxygen, such substances may represent a source of new compounds with antioxidant activity [2]. Among the diseases generated by the action of oxidizing species are peptic ulcers which affect a considerable number of people in the world. Gastric lesions occur when there is an imbalance between aggressive factors and mucosal protective factors [3]. The gastric mucosa defense mechanisms involve multiple factors, especially NO nitric oxide

and Prostaglandin production, which regulates blood flow, gastric acid secretion, bicarbonate and mucous formation [4]. Natural antioxidants such as polyphenols and carotenoids are being extensively researched to establish their efficiencies in the gastrointestinal tract absorption, bioavailability, mechanisms of action and instructions for safe use in humans [5]. Phenolic compounds mainly tannins are known for their anti-diarrheal properties. Tannins may be employed medicinally in anti-diarrheal, hemostatic, and anti hemorrhoidal compounds. The anti-inflammatory effects of tannins help control all indications of gastritis, esophagitis, enteritis, and irritating bowel disorders [6].

Byrsonima sericea DC. (Malpighiaceae), popularly known as beach murici, and some other species of the genus are commonly used to treat gastric disorders, skin infections, snake bites and also an anti-diarrheal [7–9]. This study aimed to determine on mice the anti-diarrheal action by measuring the intestinal motility, the acute toxicity,

Abbreviations: BSEE, *Byrsonima sericea* ethanol extract; BSEAE, *Byrsonima sericea* ethyl acetate extract; BSHE, *Byrsonima sericea* hexane extract; ALT, alanine transaminase; AST, aspartate transaminase; PI, inhibition potential; DMSO, dimethyl sulfoxide; ANOVA, one-way analysis of variance; SD, standard deviation; DPPH, 2,2-diphenyl-1-picrylhydrazyl; PGE₂, prostaglandin; HPLC-DAD, high performance liquid chromatography-diode array detector; im, intra-muscular; MS, mass spectrometry; NSAIDs, nonsteroidal anti-inflammatory drugs

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gastroprotection, and *in vitro* antioxidant activity of extracts from the leaves of *B. sericea*.

2. Material and methods

2.1. Reagents

All the compounds and organic solvents were purchased from Merck unless otherwise stated. 2,2-Diphenyl-1-picrylhydrazyl (DPPH), Silica gel 60 (70–230 mesh ASTM) and Quercetin were procured from Sigma Chemical Co. (St. Louis, MO, USA). prostaglandin analog 16,16-dimethyl PGE2 (Misoprostol) from Continental Pharma (Cytotec®, Italy) and indomethacin purchased from Sigma–Aldrich (St. Louis, MO, USA). Double distilled water was used throughout the experiment.

2.2. Plant collection

Fresh leaves of *B. sericea* were directly collected from the plantation farms from Itaperi Campus of the State University of Ceará, Fortaleza region of Brazil in April 2008. The leaves were scientifically defined by Dr. Afrânio G. Fernandes, botanist in herbarium of Biological Sciences Department, Faculty of Sciences, State University of Ceará, Fortaleza, Brazil. A voucher specimen of the plant was prepared and deposited in the Herbarium Prisco Bezerra, under number 39.451.

2.3. Extraction of *B. sericea* leaves

The fresh leaves were immersed in ethanol (70 %) for one week at room temperature for the extraction of active ingredients. The second stage is fractionation of the obtained solid, which was carried out in three stages using a Buchner funnel with silica gel 60 on a filter paper. The resulting material was washed with hexane (1 L), ethyl acetate (1 L) and ethanol (1 L). The solvents used to elute the solid crude extract were hexane, ethyl acetate, and ethanol (1:1 v/v), to obtain fractions referred to as hexane extracts (BSHE) in ethyl acetate (BSEAE) and ethanol (BSEE). For further testing, the resulting ethanol fraction, BSEE was separated and evaporated to dryness and their yields were calculated.

Dried leaves (1240 g) of *B. sericeae* were macerated with 70 % ethanol obtaining 153 g of crude ethanolic extract which was subjected to a silica gel chromatographic column. After elution with organic solvents hexane, ethyl acetate and ethanol, followed by evaporation of these solvents three extracts were obtained: hexane extract - BSHE (1.1 g, 0.7 %); ethyl acetate extract - BSEAE (5.6 g, 3.7 %) and ethanol extract - BSEE (126.2 g, 82.3 %) [10].

2.4. Phytochemical analysis

The extracts were subjected to qualitative phytochemical tests to evaluate classes of compounds, following the methodology de Matos [11]. These tests are based on the addition of specific reagents in aliquots of the extract and observation on color changes or precipitates.

2.5. Evaluation of DPPH radical scavenging activity

According to the method developed by [12], 0.1 ml of sample methanol solution (100 ppm, 1 mg / 10 mL) was added to a test tube containing 3.9 ml of 6.5×10^{-5} M DPPH (1,1-diphenyl-2-picrylhydrazyl) in percentage inhibition ethanolic solution. The absorbance is read at 515 nm. To calculate the sample inhibition potential of DPPH in terms of percentage inhibition (PI %), the equation was: $PI = \frac{A_{DPPH} - A_{sample}}{A_{DPPH}} \times 100$. The test was performed in triplicate, and the results were considered positive if the absorbance decreased with time.

2.6. Determination of total phenolic content (TPC)

Total phenolic content of each extract was determined by the Folin–Ciocalteu method described by [13]. The extract's solution at 10 mg/mL was added to 5 ml of Folin–Ciocalteu reagent (diluted in 10 parts) and 4 ml of sodium carbonate solution (8 %). The reading is taken after 30 min in a UV/VIS spectrophotometer set at 765 nm, against the blank containing deionized water, instead of sample extract. All determinations were done in triplicate and compared with quercetin (control) regression curve to find total phenol content in mg/mL. The total phenol content, in mg/g extract, was determined using the equation $C = c \cdot V/m$, where: C = total phenolics (mg/g extract), c = concentration of total phenol (mg/mL) obtained from the standard curve plotted in Microcal Origin 6.0 software, V = Volume of extract (mL), m = weight of crude extract (g). To prepare the calibration curve, aliquots of 1 ml of ethanol solution of quercetin at concentrations of 0.024, 0.075, 0.150 and 0.3 mg/ml were analyzed by Folin–Ciocalteu procedures.

2.7. Determination of flavonoid content

An aliquot of 1 ml of the ethanol extract (10 mg/mL) was mixed with 1 ml of aluminum chloride in ethanol (2 g/mL) and the volume completed with ethanol to 25.0 mL. The absorbance reading is taken after 40 min at 20 °C at a wavelength of 425 nm. The blank was prepared with 1 ml of the extract solution and a drop of acetic acid and then diluted to 25 mL. Quercetin was used as a reference in the measurement of flavonoids. The absorbances of the standard solution of quercetin was measured using the same sample procedures, in triplicate. The amount of flavonoids in the plants extract was compared to quercetin and calculated by the formula: $X = (A \times m_o \times 10) / (A_o \times m)$, where: A = absorbance of the extract solution, X = amount of flavonoid (mg/g extract), A_o = Absorbance of standard solution of quercetin, m = weight of the extract (g), m_o = weight of quercetin in solution (g) [14].

2.8. Determination of proanthocyanidins - vanillin methodology

1.0 ml of extract in ethanol (1 g/100 ml) was placed in a test tube, than 2.0 ml of a solution of 2 % vanillin in 70 % sulfuric acid (diluted 2 g of vanillin in 100 ml of 70 % sulfuric acid) was added and the mixture was kept at 20 °C during 15 min. The absorbance reading was taken at a wavelength of 500 nm. The same procedure was made for the calibration curve which was used a standard tannic acid, in the concentration range from 2.4–40 µg/mL [15].

2.9. Experimental animals

Swiss mice were used, females, weighing between 20–25 g were obtained from the Central Animal Facility of the Federal University of Ceará. The animals were maintained under appropriate conditions of light and temperature, receiving food and water ad libitum. The Institutional Ethics Committee on the Care and Use of Animals for experimentation approved the experimental protocols (Nº 07520831-8), and all experiments were performed in accordance with the guidelines of the National Institute of Health, Bethesda, USA.

2.10. Evaluation of the toxicity of BSEE in mice

The animals were divided into experimental groups (n = 8): wherein BSEE two groups received doses of 500 or 1000 mg/kg po, and a control group received vehicle (0.9 % saline, 5 % DMSO) once a day for a period of 5 consecutive days. The animals were weighed on day 1 and day 5 for monitoring the weight. The blood was collected by puncture through the retro-orbital plexus, centrifuged at 3000g for 7 min and serum was separated for the analysis of AST and ALT levels

on day zero before the first dose of BSEE 5 and day, the last day of treatment. Sera were analyzed at the Laboratory of Hematology at the General Hospital of Fortaleza (HGF) in equipment Konelab by UV kinetic method recommended by the International Federation of Clinical Chemistry (IFCC). To determine the relative weight of the organs (liver, spleen, and kidney), all the animals were sacrificed by cervical dislocation at the fifth day of treatment and the organs were removed and weighted [16].

2.11. Indomethacin induced gastric lesion

Animals in a fasting period of 18 h (n = 8) were treated with BSEE (125, 250, 500 and 1000 mg/kg, po) or misoprostol (50 µg/kg, po) or vehicle (0.9 % saline in 0.5 % DMSO, 10 ml/Kg). After one hour the animals were treated orally with indomethacin (50 mg/kg po). Eight hours after indomethacin administration, the animals were sacrificed by cervical dislocation, their stomachs removed and instilled with 5 % formalin for 15 min [17]. Then opened along the greater curvature, washed with saline and inspected for assignments scores according to Szabo et al. [18], the following scale: 0 = no petechiae; 2 = up to 5 petechiae and bleeding or erosion up to 1 mm deep; 3 = up to 5 to petechiae with bleeding or erosion above 1 mm deep. The average scores attributed ulcers for each animal was calculated and compared between groups. All animals were fasted 24 h before indomethacin oral treatment; during this period, the mice were kept in wide wire mesh-bottom cages to avoid coprophagia in addition, water access was prevented for 2 h before the indomethacin dosing.

2.12. Determination of gastric wall mucus

Gastric wall mucus was determined in Indomethacin induced gastric lesion models were carried out according to the adaptation of the method suggested by Corne et al. [19]. The glandular segments from stomachs were collected and weighed. Each segment was transferred to 1 % Alcian blue solution (0.16 M sucrose in 0.05 M sodium acetate, pH 5.8). Excess dye was removed by washing the segments with 0.25 M sucrose solution. The mucus dye complex was extracted by placing the segments in 0.5 M magnesium chloride for 2 h. The dye extract was mixed with diethyl ether, centrifuged at 1400 × g for 10 min, and the absorbance of the supernatant was measured at 598 nm. The quantity of Alcian blue extracted (µg/g of glandular tissue) was then calculated using a standard curve of Alcian blue.

2.13. Test of intestinal motility in mice

Mice (n = 8) in a fasting period of 18 h, were pretreated with BSEE (1000 mg / kg, po) or atropine (1 mg / kg, im) or vehicle (0.9 % saline in 0.5 % DMSO, 10 ml/Kg). After 1 h, receiving the treatments, the animals received 0.5 ml / animal charcoal suspension in 10 % gum arabic solution 5 % vo After 45 min. the mice were sacrificed by cervical dislocation, and performed the immediate removal of the intestine from the pylorus to the beginning of the cecum. The distance traveled by the charcoal suspension was measured from the complete length of the small intestine. The result was expressed as a percentage of the total length of the small intestine [20].

2.14. Statistical analysis

Statistical analysis was performed using the program Graph Pad Prism 3.0 (USA). The results were expressed as the mean ± standard deviation (SD). Comparison of the means was performed using analysis of variance (ANOVA) followed by Student-Newman-Keul and Dunnett's test for parametric data and Kruskal-Wallis test followed by Dunn's test for nonparametric data A value of p < 0.05 was considered significant.

Table 1

Determination of main phenolic constituents of *Byrsonima sericea* extracts expressed in mg/g extract and antioxidant activity by DPPH inhibition percentage.

| Sample | Total phenols (mgEqGA/g extract) | Flavonoids (mgEqQ/g extract) | Proanthocyanidins (mgEqTA/g extract) | % DPPH Inhibition |
|-----------|----------------------------------|------------------------------|--------------------------------------|-------------------|
| BSEE | 0.371 | 0.172 | 1.3×10^{-4} | 90.0 |
| BSEAE | 0.223 | 0.125 | 2.1×10^{-6} | 87.9 |
| BSHE | 0.070 | 0.040 | 0.3×10^{-7} | 21.0 |
| Quercetin | – | – | – | 94.6 |

BSEE = Ethanol extract; BSEAE = ethyl acetate extract; BSHE = Hexane extract.

(–) = not found; mgEqGA/g extract = milligrams equivalent gallic acid/gram of extract; mgEqQ/g extract = milligrams equivalent quercetin/gram of extract; mgEqTA/g extract = milligrams equivalent tannic acid/gram of extract.

3. Results

The phytochemical qualitative analysis of the extracts demonstrated in BSEE and BSEAE the presence phenols, tannins, leucoanthocyanidins, flavonoids, steroids, triterpenes and alkaloids. The presence of fatty acids and steroids and the absence of phenolic compounds were observed in BSHE.

The total phenolic content of BSEE extract was 0.371 mgEqGA/g extract. The BSEE inhibit the free radical DPPH in terms of 90 % close to the standard quercetin 94 %. The flavonoid content in BSEE was quantitatively estimated as 0.172 mgEqQ/g extract as shown in Table 1. The BSEE exhibited proanthocyanidins (condensed tannins) content of 1.3×10^{-4} mgEqTA/g extract (milligrams equivalent tannic acid/gram of extract).

The administration of BSEE doses did not modify the serum levels of AST and ALT in animals. The bodyweight of the animals showed no significant difference when compared to the control group. The relative weight of the analyzed organs (kidney, spleen and liver) also did not change. BSEE in doses of 250, 500 and 1000 mg/kg significantly reduced the indomethacin-induced gastric lesions, inhibiting 52, 60 and 62 % respectively (Fig. 1).

Table 2 shows the effects of BSEE on body weight changes and relative organ weight. Groups of animals administered 500 and 1000 mg/kg did not differ significantly in body weight and relative organ weight when compared with the control. ALT and AST activities were did not

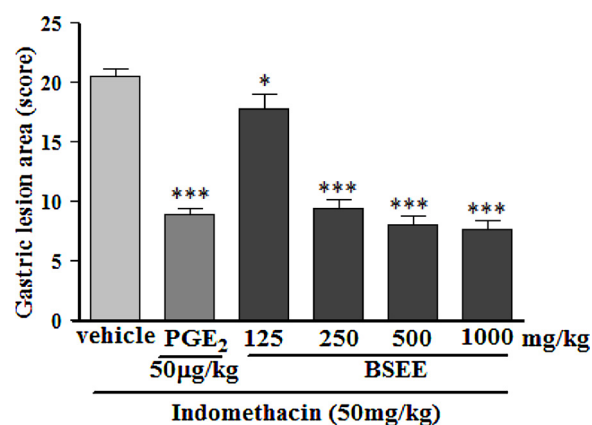


Fig. 1. Effect of indomethacin on the *Byrsonima sericea* leaf ethanolic extracts (BSEE) gastroprotection in mice. Values represent the mean ± standard error of mean (S.E.M) for 8 animals/group. The animals were treated with BSEE (125, 250, 500 and 1000 mg / kg, po) or misoprostol (50 µg/kg, po). Indomethacin (50 mg / kg, po) was administered 1 h after treatment with BSEE and misoprostol. * p < 0.05 *** p < 0.001 vs. vehicle (ANOVA and Kruskal-Wallis test followed by Dunn's test).

Table 2

Serum levels of AST and ALT U/L, animal weight (g) and relative organ weight (mg/g) of treated mice at the dose of *Byrsonima sericea* leaf ethanolic extracts (BSEE) 500 and 1000 mg/kg.

| Groups | AST (U/L) | | ALT (U/L) | | Body weight (g) | | Organs (mg/g) | | |
|---------|-----------|----------|-----------|-----------|-----------------|----------|---------------|-------------|------------|
| | Day 0 | Day 5 | Day 0 | Day 5 | Day 0 | Day 5 | Liver | Kidney | Spleen |
| Vehicle | 145 ± 55 | 118 ± 22 | 53 ± 10 | 70 ± 26.8 | 25 ± 2 | 25 ± 2 | 45 ± 0.8 | 0.79 ± 0.1 | 0.46 ± 0.1 |
| 500 | 104 ± 11 | 118 ± 21 | 49 ± 1 | 48 ± 14.7 | 26 ± 2 | 25 ± 2 | 39 ± 0.4 | 0.58 ± 0.06 | 0.5 ± 0.1 |
| 1000 | 111 ± 1 | 158 ± 52 | 43 ± 7 | 64 ± 18.2 | 25 ± 05 | 21 ± 0.3 | 45 ± 0.9 | 0.66 ± 0.1 | 0.3 ± 0.1 |

Results were expressed as Mean ± SEM. n = 8. p < 0.05 vs. Vehicle. Data were analyzed by ANOVA followed by Newman-Keuls test.

Table 3

Percentage of the distance traveled by the charcoal in the mice intestine after *Byrsonimasericea* leaf ethanolic extracts (BSEE) administration.

| Treatment | Dose (mg/kg) | Distance travelled by the charcoal (%) ± DP | % inhibition |
|-----------------|--------------|---|--------------------|
| Atropine | 1 | 47.17 ± 13.31 | 52.83 ^a |
| BSEE | 1000 | 49.41 ± 13.77 | 50.59 ^a |
| Vehicle (mL/kg) | 10 | 74.23 ± 12.25 | 25.77 |

Results were expressed as Mean ± SEM. n = 8.

^a p < 0.05 vs. Vehicle. Data were analyzed by ANOVA followed by Dunnett test.

significantly increased at the, 500 and 1000 mg/kg dose levels.

The doses of BSEE and atropine showed the decrease in the propulsion of the charcoal suspension as compared to the control group. The distance traveled by the charcoal in the BSEE treated group was found to be 50.59 % at the dose of 1000 mg/kg; whereas the standard showed 52.83 % compared to the control group. The activity of BSEE at a dose of 1000 mg/kg on the charcoal test was found to be closer resemblance when compared to atropine as shown in Table 3.

The results of indomethacin lesion tests showed the gastroprotective activity of BSEE, revealing the dose of 250 mg/kg as the most efficient, reducing the injured area, which was statistically similar to the reduction obtained with misoprostol, the reference drug used in this experiment as seen in Fig. 1. Based on these results, we hypothesized that BSEE can protect gastric mucosa from injury by increasing mucous production, however, there was no significant difference between any of the groups tested, as observed in Fig. 2.

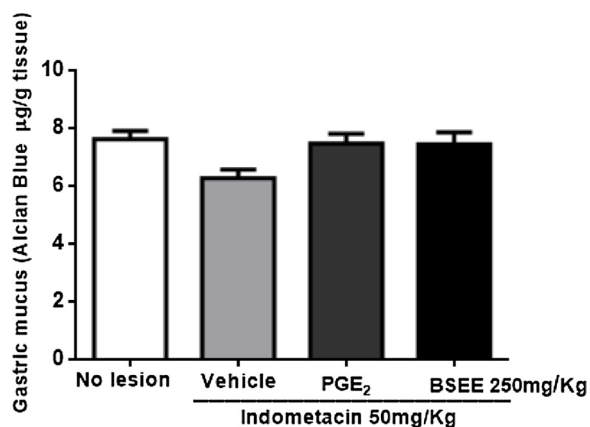


Fig. 2. Effect of indomethacin on the *Byrsonima sericea* leaf ethanolic extracts (BSEE) on Gastric mucus in mice. Values represent the mean ± standard error of mean (S.E.M) for 8 animals/group. The animals were treated with E.E. (250 mg/kg, po) or misoprostol (50 mg/kg, po). Indomethacin (50 mg / kg, po) was administered 1 h after treatment with BSEE and misoprostol. (ANOVA).

4. Discussion

The present study was carried on BSHE, BSEAE and BSEE extracts to investigate the presence of medicinally important phytochemicals in the leaves of different varieties. Both the extracts (BSEE and BSEAE) revealed the presence of various phytochemicals such as phenols, tannins, leucoanthocyanidins, flavonoids, steroids, triterpenes and alkaloids [21]. The presence of fatty acids and steroids and the absence of phenolic compounds were observed in BSHE. The chemical composition of this plant extract was further confirmed from other previous studies demonstrated by HPLC-DAD and MS techniques which revealed the presence of different antioxidant phenolic compounds such as rutin, isoquercitrin, kaempferol 3-O-rutinoside, and quercetin.

The presence of high amounts of phenolic compounds, such as flavonoids, found in plants possess many biological activities includes antioxidant power, contributing to the body's defenses. The bioactivity of phenolics can be attributed to their ability to chelate metals, inhibit lipoxygenase and sequester free radicals [22] which may be attributable for the antioxidant capacity [10,20]. Regarding the antioxidant activity (DPPH), the high antioxidant potential increased in BSEE and BSAE extracts. The BSEE and BSEAE inhibit the free radical DPPH in terms of 90 % and 87.9 % respectively. Both percentages were close to the standard quercetin 94 %. However, BSHE was less active obtained a percentage inhibition of 21 %, which is coherent due to the absence of phenolic compounds. The same antioxidant activity of *B. crassifolia* leaves was previously described by Boscolo et al. [23]. Therefore the highest amount of total phenolics found in the BSEE may justify the highest free radical scavenger since phenolic compounds are responsible for the inhibition of the radical species [22] [24]. The high content of chemical compounds with antioxidant characteristics such as polyphenols, tannins, and flavonoids were found in the ethanol and ethyl acetate extracts. Other *Byrsonima* species as *B. crassifolia* was reported as presenting compounds such as quercetin, quercetin 3-O-β-D-glucopyranoside, epicatechin and catechin [25] and *B. verbascifolia* showed the presence of quercetin, isoquercetin, 3-arabinosyl-quercetin, gallic tannins and saponins, compounds with known antioxidant activities [26]. The flavonoids and proanthocyanidins positives results agreed with the data found by Guilhon-Simplicio and Pereira et al. [27], which pointed to the presence of flavonoids and terpenes, especially triterpenes for species of the *Byrsonima* genre. Indomethacin has higher ulcerogenic potential than other NSAIDs, thus it was considered as the drug of choice for gastric ulcer experimental induction [28,29]. There is a previous study showing the gastroprotective effect of *B. sericea* leaf extract [10], where some known antioxidant phenolic compounds were characterized in the leaf extract.

The results demonstrated that BSEE has no adverse effects on the oral acute toxicity study. The animals that received BSEE doses of 500 and 1000 mg/kg did not differ in body weight when compared to animals receiving vehicle alone. According to Jahn & Gunzel [30] monitoring the bodyweight of the animal is an important indicator for assessing the toxicity of a substance. There were also no changes in the relative weight of the organs examined (kidney, spleen, and liver). Existing studies on some species of the genus as *B. fagifolia* and *B. basiloba* [31] showed low toxicity at a dose of 1000 mg/kg and the species

B. crassa and *B. intermedia* showed toxicity at a dose of 500 mg/kg. Gastric lesions in gastric diseases occur either by an imbalance generated by the increase in aggressive agents (acid and pepsin) or by reduction of protective agents (mucus, bicarbonate and blood flow). Acid secretion is regulated by hormonal, paracrine and neural factors represented by gastrin, histamine, and acetylcholine respectively [32]. In the model of indomethacin-induced gastric lesions, BSEE (250 mg/kg) was effective in preventing damage to the mucosa. The production and release of mucus is directly influenced by prostaglandins [33], especially PGE₂, and indirectly by NO by increasing the gastric microcirculation and sulfhydryl compounds [34]. The misoprostol used in this study as a negative control is a synthetic prostaglandin analogue, providing gastroprotection by increasing the secretion of mucus and bicarbonate, resulting in decreased acid secretion. The BSEE at doses of 250, 500 and 1000 mg/kg seems to have influence on one or more factors involved in the pathology of indomethacin ulcer. The treatment with BSEE was able to reduce the gastric lesions caused by indomethacin, indicating the probable involvement of prostaglandins in the protection mechanism. Non-steroidal anti-inflammatory drugs such as aspirin and indomethacin are known to induce ulcers during the course of therapy with anti-inflammatory by inhibiting prostaglandin synthesis via the cyclooxygenase [35–37]. The major effects of prostaglandins in the stomach related to the defense of the gastric mucosa, the stimulation of the secretion of mucus and bicarbonate and maintenance of blood flow [38].

The intestinal transit test in mice is essential to understand the influence of natural products on digestive motility. The reduction caused by BSEE indicates a probable anticholinergic effect by blocking of M₃ receptors. These intestinal movements were reduced by the action of atropine the muscarinic antagonist of M₃ receptors. The assay of intestinal transit in mice showed significantly reduction on the motility of the animals after administration of BSEE (250 mg/kg) as compared with the control. Several studies have reported that tannins and flavonoids show anti-diarrheal activity by decreasing intestinal tract motility [39]. According to Parmar & Ghosh, [40], many flavonoids isolated from medicinal plants have gastroprotective activity being catechins and flavonoids best known as presenting antiulcer activity and act by inhibiting the enzyme histidine decarboxylase, which could explain the reduction of bowel motility. Additional studies have reported that the *B. crassifolia* volatiles were obtained from fruits, glycolipids, triterpenes, triterpene acids, catechins, flavonoids from leaves [7,25] and the log tannins and proanthocyanidins [22]. According to scientific reports some flavonoids are known for their anti-diarrheal action among them are quercetin, and ternatin and this activity has been observed through experiments in rats with chronic diarrhea and bowel motility in mice [41,42]. The results of the present study agree with Figueiredo et al. [43] which evaluated the anti-diarrhea activity of methanolic and hydromethanolic extracts of leaves *B. cinera* [31] in mice at doses of 1000 mg/kg, with a significant reduction in intestinal motility. The high content of chemical compounds with antioxidant characteristics such as polyphenols, tannins, and flavonoids were found in the ethanol and ethyl acetate extracts. Other *Byrsonima* species as *B. crassifolia* was reported as presenting compounds such as quercetin, quercetin 3-O-β-D-glucopyranoside, epicatechin and catechin [25] and *B. verbascifolia* showed the presence of well-known antioxidant compounds [26].

In conclusion, the non-toxicity of *B. sericea* combined with antioxidant constituents that protect the gastrointestinal system corroborate their ethnopharmacological use without reported side effects. The presence of phenolic compounds, including tannins could be associated with the anti-diarrheal action and the antioxidant properties could collaborate to the gastroprotection of the plant. However, there is a need for further studies related to the species in order to isolate and assess the activity of their compounds.

Declaration of Competing Interest

The authors declare no conflict of interest.

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