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***Basic Study***

***Maytenus erythroxylon* Reissek (Celastraceae) ethanol extract presents antidiarrheal activity *via* antimotility and antisecretory mechanisms**

Formiga RO *et al*. *Maytenus erythroxylon*

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**Abstract**

***AIM***

To investigate the acute toxicity, the phytochemical profile, the antidiarrheal activity and the mechanisms of action of *Maytenus erythroxylon* (*M. erythroxylon*) ethanol extract.

***METHODS***

Castor oil-induced diarrhea model was used to evaluate antidiarrheal activity. Intestinal transit and gastric emptying protocols were used to evaluate a possible antimotility effect. KATP channels, nitric oxide, presynaptic α2-adrenergic and tissue adrenergic receptors were investigated to underline antimotility mechanisms of action and castor oil-induced enteropooling to elucidate antisecretory mechanisms.

***RESULTS***

All tested doses of the extract (62.5; 125, 250 and 500 mg/kg) possessed antidiarrheal activity, with a significant decrease of the evacuation index. This activity is possibly related to a reduced gastric emptying (125, 250 and 500 mg/kg) and to a decreased percentage of intestinal transit in all tested doses. That last effect seems to be modulated by nitric oxide, KATP channels and tissue adrenergic receptors. Besides, the extract also presented antisecretory effect due to a decrease of intestinal fluid accumulation.

***CONCLUSION***

The antidiarrheal effect of *M.* *erythroxylon* found in this study involves antimotility and antisecretory mechanisms that may be attributed to the chemical compounds found in this species: saponins, flavonoids, tannins, triterpenes and steroids

**Key words:** Medicinal plants; Celastraceae; *Maytenus erythroxylon*; Diarrhea; Antidiarrheal activity

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**Core tip:** *Maytenus erythroxylon* Reissek, known as “casca grossa” and “bom-nome” in Brazil, is a species with indication to treat gastrointestinal disorders, like ulcers and diarrhea. Diarrhea is a pathological condition characterized by an increase in three or more defecations in 24 h, being of multiple origin, whether infectious or not. There is a search for new therapeutic alternatives for the treatment of diarrhea, since the current drugs on the market present serious undesirable effects. Species of *Maytenus* genus appear in this scenario due to their ethnopharmacological support and researches that point promising results, as antidiarrheics.

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**INTRODUCTION**

*Maytenus erythroxylon* Reissek (Celastraceae), popularly known as "bom nome"[1] and "casca-grossa", is a small shrubby tree, measuring about 3.8 m high[2] and used traditionally to treat diseases of the gastrointestinal tract.

Studies with *Maytenus* genus have presented promising results for treatment of gastrointestinal disorders, like peptic ulcers[3-7] and diarrhea[6,8]. Besides, a lot of *Maytenus* species possess popular indication for treatment of diarrhea, such as *M. rigida* Mart[6]. and *M. senegalensis* Lam. Exell[9], being most of their biological activities attributed to the presence of phenolic compounds, particularly flavonoids, tannins, glycosides, terpenes, steroids and alkaloids[10], which have already been referenced in pharmacologic studies as antidiarrheal agents[11-14].

Diarrhea is a debilitating gastrointestinal condition[15] that involves an increase of unformed stools and also of the defecation frequency (three times or more in a day)[16]. The etiology of diarrehal disorders is multifactorial, attributed to factors such as infectious agents, microorganisms and their toxins, increased fluid secretion, malabsorption of biliary salts[17], food allergies[18] and some medications, like antibiotics[19]. It is responsible for up to 5 million deaths each year[20], especially of children of less than 5 years, corresponding 500000 deaths annually in developing countries[21], associated with factors such as poor home environments, undernutrition and lack of access to essential services[22].

Available drugs used in diarrhea pharmacotherapy are related to contraindications and undesirable effects, like bronchospasm, vomiting and fever[16]. In this context, World Health Organization (WHO) created a Diarrheal Disease Control Program that stimulates studies with natural products, especially traditional medicinal plants, to the management of diarrhea worldwide[23].

From this perspective, the present study aimed to present the phytochemical profile, the acute toxicity, the antidiarrheal activity and the mechanisms of action of the ethanol extract obtained from the aerial parts from *Maytenus erythroxylon* (EtOHE-*Me*).

**MATERIALS AND METHODS**

***Reagents***

The drugs and reagents were prepared immediately before use. The following drugs were used: Carboxymethylcellulose (Formula Brasil®, Brazil); castor oil (Tayuyna Lab Ltda®, Brazil); loperamide hydrocloride 2 mg (Janssen Cilag Farmacêutica Ltda®, Brazil); activated charcoal meal (Proquímios®, Brazil). Glibenclamide, L-NG-Nitroarginine methyl ester (L-NAME), propranolol and yohimbine (Sigma-Aldrich®, USA).

***Plant material***

Plant samples used in the antidiarrheal activity evaluation in mice were obtained from the leaves of *M. erythroxylon* Reissek. It was collected in the city of Mamanguape, Paraíba state, Brazil and identified by Dr. Zelma Glebya Maciel Quirino, botanist from CCAE/UFPB. A voucher number 6051 (JPB) was deposited in the Herbarium Lauro Pires Xavier of the Departament of Botany of UFPB. The aerial parts (665 g) of *M. erythroxylon* were air-dried at 40 ºC for 4 d, powdered and macerated with 96% ethanol for 3 days. The solution was filtered and evaporated to dryness under reduced pressure at 40 ºC. The yield (w/w) of the crude ethanol extract of *Maytenus erythroxylon* (EtOHE-*Me*) was 55.5 g (8%).

***Animals***

Swiss adult male and female mice (*Mus musculus*) weighing between 25-35 g were obtained from Central Animal House of Instituto de Pesquisas Farmacêuticas (IPeFarm) of the Universidade Federal da Paraíba (UFPB), Paraíba, Brazil. They were kept at temperatures between 23-25 °C, with a 12-h light/dark cycle in the animal house, fed with Purina® and water *ad libitum* for two weeks prior to experimentation. Intragastric gavage administration was carried out with conscious animals, using straight gavage needles appropriate for the animal size. All animals were euthanized by barbiturate overdose (intravenous injection, 150 mg/kg pentobarbital sodium) for tissue collection.

***Phytochemical screening of EtOHE-Me***

EtOHE-*Me* was subjected to preliminary phytochemical screening[24] for the detection of the presence of various phytoconstituents (alkaloids, saponins, steroids, triterpenoids, flavonoids and tannins). Alkaloids were detected using the Dragendorff's reagent, resulting in the appearance of a precipitate at the bottom of the test tube. Flavonoids were considered present when it appeared a yellow color with AlCl3 reagent addition and for tannins when a green or black color was produced with FeCl3. For the detection of sterols and triterpenes, petroleum ether was used and extracted with CHCl3. Sterols were detected when a green to pink color appeared and pink to purple color for terpenes, after treatment of CHCl3 layer with acetic anhydride and concentrated HCl. Saponins were detected when persistent froth appeared after vigorous shaking of diluted samples.

The metabolic fingerprinting assessment of EtOHE-Me was also performed by 1H-NMR and 13C-NMR (Nuclear Magnetic Resonance) spectroscopy. The 1H-NMR and 13C-NMR spectra were obtained by Varian Mercury NMR spectrometer (UNICAL) operating at 200 MHz (1H) and 50 MHz (13C). The sample for analysis was prepared by dissolving an amount of EtOHE-Me in deuterated chloroform (Cambridge Isotope Laboratories - CDCl3). Chemical shifts (δ) were expressed in parts per million (ppm) and for 1H-NMR it was referenced the characteristic peaks of protons belonging to not deuterated fractions of the solvent (δH 7.24). For 13C-NMR it was utilized the same parameters (δC 77.0).

***Toxicological evaluation***

**Investigation of the acute toxicity of EtOHE-Me in mice:** The toxicological research was conducted in order to assess behavioral parameters and to determine LD50, according to the model described by Almeida *et al*[25] and Anvisa[26]. Male and female mice (*n* = 7) were fasted for 12 h and treated with EtOHE-*Me* orally in a single dose (2000 mg/kg - solubilized in saline solution 0.9%) for two groups (male and female mice). Simultaneously, two other groups (male and female) were treated with NaCl 0.9% (10 mL/kg). Then, a behavioral screening was carried out and signs and symptoms of acute toxicity were observed and noted for 72 h. For 14 d the animals were evaluated with respect to the consumption of water and food, body weight gain and to observe if there were deaths. At the end of the experiment, the animals were euthanized for macroscopic analysis of organs (heart, spleen, liver and kidneys).

***Pharmacological assays***

**Effect of EtOHE-Me on castor oil-induced diarrhea in mice:** The antidiarrheal activity was evaluated according to the model described by Awouters *et al*[27]. Male mice were divided into six groups (*n* = 7) and pretreated orally with NaCl 0.9% (10 mL/kg), loperamide 5 mg/kg and EtOHE-*Me* (62.5, 125, 250 and 500 mg/kg). After 1 h, it was administered 10 mL/kg of castor oil orally to each animal to induce diarrhea. The counting of feaces were assessed for 4 h and classified according to their consistency in solids, semisolids or liquids. Then, it was calculated the Evacuation Index (EI), Percentual of Wet feaces (%) and Diarrheal Inhibition (%).

EI = ∑ (solid stools × 1) + (semisolid stools × 2) + (liquid 3 × 3)

% DI = (Mean of saline group – mean of treated group) × 100

Mean of saline group

**Effects of EtOHE-Me on gastric emptying:** The alterations on gastric empyting were assessed according to the model described by Scarpignato *et al*[28]. After 1 h of pretreatment mentioned before, 0.4 mL of semisolid colored marker (phenol red 0.05% in 1.5% carboxymethylcellulose) was administered to a not-treated control group named “zero time control group” that were euthanized immediately. The treated groups received this marker and then euthanized 30 min after administration. The abdominal cavity was opened for the stomach removal, being necessary the ligation of the pyloric and lower esophageal sphincters, avoiding loss of the stomach contents. The gastric content was collected in Falcon® tubes, solubilized in 7 mL of distilled water and centrifuged at 3000 rpm for 15 min. Then, 1 mL of the supernatant was mixed with 1 mL of 0.025 N NaOH and stirred using a vortex. From this material, 150 µL were pipetted in duplicate microplate and spectrophotometric reading was held in wavelength equal to 570 nm. The results were expressed as percentage of gastric emptying in relation to the control (“zero time group”).

%Gastric emptying = 100 – mean absorbance of sample × 100

 mean absorbance of zero time control group

**Effects of EtOHE-Me on normal intestinal transit:** The alterations on normal intestinal transit were evaluated according to the model described by Stickney and Northup[29]. After 60 minutes of the pretreatment, it was administered 10 mL/kg (p.o.) the red marker (phenol red 0.05% in 1.5% carboxymethylcellulose). After 30 minutes, the animals were euthanized for removal of the small intestine (pylorus to the ileocaecal junction). With a ruler, the total length of the small intestine and the distance traveled by the black marker (last portion comprising at least one continuous score) were measured to calculate the percentage of the charcoal meal rout depending on the total length of the intestine.

%Intestinal transit = Length traveled by charcoal meal × 100

 Total intestinal length

***Antimotility mechanisms of action of EtOHE-Me***

The antimotility mechanisms of action were evaluated according to the model described by Santos and Rao[30]. Male mice were fasted for 24 hours and subsequently treated orally with NaCl 0.9% (10 mL/kg), EtOHE-*Me* in its best dose (500 mg/kg), and to obtain information about the mechanism of action, it was experienced the administration of different drugs acting by a well-known mechanism alone and in association with EtOHE-*Me,* such as glibenclamide (1 mg/kg i.p.), a blocker of KATP channels, L-NAME (1 mg/kg i.p.), an inhibitor of nitric oxide synthase (NOs), propranolol (1 mg/kg i.p.), a non-selective adrenergic antagonist and yohimbine (1 mg/kg i.p.), a presynaptic α2-adrenergic antagonist. These drugs were dissolved in NaCl 0.9% and given 30 min before extract administration. After 60 min it was administered 10 mL/kg (p.o.) of the black marker (5% charcoal suspension in 5% Arabic gum) and 30 minutes later, the animals were euthanized for removal of the small intestine to calculate the percentage of intestinal transit.

***Antisecretory mechanisms of action of EtOHE-Me***

The anti-secretory mechanism of action was evaluated according to Ezeja and Anaga[31] using the castor oil-induced enteropooling model. The animals were fasted for 24 h and treated orally with NaCl 0.9% (10 mL/kg), loperamide 5 mg/kg and EtOHE-*Me* in its best dose (500 mg/kg). After 1 h, 10 mL/kg of castor oil was administered to animals orally. Then, 1h later the animals were euthanized for the removal of the small intestine and, after that, the intestinal content was measured with the aid of a graduted cylinder.

***Ethical consideration***

All protocols performed in the present study are in accordance with international principles for research with laboratory animals[32].

***Animal care and use statement***

All experimental procedures were approved by the Institutional Committee for Ethics in Animal Use from UFPB with number 0105/14.

***Statistical analysis***

Parametric data were expressed as mean ± standard deviation (SD) and non-parametric expressed as median (minimum–maximum values). The data were subjected to *t*-test to compare two groups (control and treated group) and variance analysis (one way ANOVA) to compare more than two groups, followed by a Dunnett and Tukey test (parametric) or Kruskal-Wallis followed by Dunn test (non-parametric). *P <* 0.05 was considered as statistically significant. GraphPad Software© 5.0 Inc., San Diego, CA, USA software was used for data processing.

**RESULTS**

***Phytochemical screening of EtOHE-Me***

In the present study, the results demonstrated the presence of saponins, flavonoids, tannins, steroids and triterpenes in EtOHE-*Me* (Table 1).

The NMR spectrum 1 of 13C (50 MHz, CDCl3) showed the presence of signals relating to quaternary, metinic, methylene and methyl carbons suggesting the presence of terpenes. It was observed in the regions δC 124.15 and δC 145.06 of the spectrum signals that suggest the presence of olefinic carbons referring to pentacyclic triterpenes.

It was also presented a signal in δC 80.69 referring to carbinolic carbon. The presence of a signal δC 173.86 suggested the presence of carbonyl of an acid or esters of triterpene. The chemical shifts at 6.68 δC region are characteristic of methyl carbons of friedelan pentacyclic triterpenes, which indicates the presence of ketone compounds at C-3.

The NMR spectrum 2 of 1H (200 MHz, CDCl3) showed an envelope of signals in the region between 2.22 to 0.78 ppm, characteristic of protons from terpenes. The chemical shifts in the region of δH 5.28 and δH 5.03 are characteristic of olefinic hydrogens. The spectra showed no signals in the aromatic region (δH 6.5 a δH 8.0).

***Investigation of the acute toxicity of EtOHE-Me in mice***

The results showed low toxicity after the single dose administration (2000 mg/kg) of EtOHE-Me due to lack of death during 14 d of experiment and no apparent behavioral changes. Furthermore, there were no changes in body (Table 2) and organs weight of treated animals (Table 3) or changes in the consumption of water and food when compared to the group treated only with NaCl 0.9% (Table 4).

***Effect of EtOHE-Me on castor oil-induced diarrhea in mice***

In the present study, mice in control group treated only with vehicle (NaCl 0.9%) showed intense signs of diarrhea, with respective evacuation index 21 (19-25) and 47% of wet faeces. Pretreatment with EtOHE-Me in all doses (62.5, 125, 250 and 500 mg/kg) decreased the evacuation index in 8 (5-11) and 62% of diarrhea inhibition (*P <* 0,05), 7 (6-8), 66% (*P <* 0,05), 6.5 (3-7), 69% (*P <* 0.05) and 4 (3-5), 80% (*P <* 0.001), respectively when compared with NaCl 0.9% control group. The standard antidiarrheal drug loperamide (5 mg/kg) produced a significant inhibition of all parameters evaluated (Table 5).

***Effects of EtOHE-Me on gastric emptying of mice***

The results showed that the animals treated with NaCl 0.9% showed 79% of gastric empyting and the treatment with EtOHE-Me (125, 250, 500 mg/kg) and loperamide significantly reduced gastric emptying in 66% (*P <* 0.05), 45% (*P <* 0.001), 47% (*P <* 0.001) and 53% (*P <* 0.001) respectively, when compared to the NaCl 0.9% control group (Figure 1).

***Effects of EtOHE-Me on intestinal transit of mice***

The distance travelled by charcoal in terms of percent of the total length of intestine was 76% in the NaCl 0.9% control group. The treatment with loperamide and EtOHE-Me in all doses produced significant (*P <* 0.001) reduction in the percentage of intestinal transit in 25, 57, 49, 41 and 35%, respectively, when compared to the control group (Figure 2).

***Antimotility mechanisms of action of EtOHE-Me***

The results in this protocol showed that the distance travelled by charcoal meal was 78% in the NaCl 0.9% control group. The treatment with EtOHE-Me in its best dose (500 mg/kg) produced significant (*P <* 0.001) reduction in the percentage of intestinal transit (36%), when compared to NaCl 0.9% group. Although, when EtOHE-Me was associated with standard drugs L-NAME, glibenclamide and propranolol it was observed an increase of the intestinal transit to 78%, 70% and 74%, respectively. The same effect was not reproduced when EtOHE-*Me* was administrated along with yohimbine (36% of intestinal transit) (Figure 3).

***Antisecretory mechanisms of action of EtOHE-Me***

EtOHE-Me in its best dose (500 mg/kg) reduced intestinal fluid in 0.6429 ± 0.1272, with 51% of fluid inhibition (*P <* 0.001), when compared to the NaCl 0.9% control group (1.325 ± 0.2053) (Figure 4).

**DISCUSSION**

The phytochemical screening showed the presence of saponins, flavonoids, tannins, triterpenes and steroids in EtOHE-*Me*. Therefore, the absence of signals in the aromatic region along with the previous isolated fridelane terpene from *Maytenus erythroxylon,* 3β-friedelinol[33], corroborate that the signals presented in the 1H and 13C NMR spectra of the extract sample evaluated are from terpenes. The compounds found in the extract are mostly liked to increased water and electrolyte absorption in the colon and decreased intestinal irritability, reduced intestinal propulsion and spasmolitic effect[11-14]. Considering those findings*,* they might be responsible for the biological activities evidenced in the present study.

The studies of acute toxicity are important to determine the LD50 and set doses to be used in later experimental models[26]. The single dose administration of EtOHE-*Me* did not alter any parameter evaluated and showed no deaths, being LD50 considered over 2000 mg/kg (p.o.) and the extract safe for pharmacological studies.

Then, it was investigated if *Maytenus erythroxylon* ethanol extract possessed antidiarrheal effect. For that, it was used the castor oil-induced diarrhea model in mice. Castor oil is a potent laxative agent and induce diarrhea through its active compound, the ricinoleic acid[34], which acts in the upper small intestine where castor oil is hydrolyzed. It produces cytotoxicity to epithelial cells[35]**,** decreases absorption[36], increases water flux[37], increases fluid and electrolyte accumulation[38], enhances intestinal motility and alters the gastric contractions[39], being those effects similar to physiopathologic conditions that cause diarrhea in humans. Castor oil produces its laxative effect in association with the release of platelet activating factor (PAF), nitric oxide (NO), tachykinins (TK), cAMP[26,40] and prostaglandins *via* EP3 and EP4 receptors biding[41].

EtOHE-*Me* presented antidiarrheal activity, decreasing the evacuation index in all doses with crescent percentuals of diarrhea inhibition, as well as, the standard drug loperamide. These results corroborate a study by Santos *et al*[6] with *Maytenus rigida* Mart. ethanolic extract, which was able to reduce the total number of faecal output and the diarrhoeic faeces in all tested doses.

In order to evaluate if EtOHE-*Me* presented effects in gastrointestinal motility, it was performed the gastric empyting and intestinal transit protocols. The findings suggested an anti-motility activity mediated by EtOHE-*Me,* since it was efficient in decreasing gastric emptying and intestinal transit. Similar results were found for a flavonoid rich fraction of *Maytenus ilicifolia* Reissek, which was able to inhibit the intestinal transit in a more potent way than the gastric emptying[8]. Those results suggest the presence of different mechanisms of action in the different segments of the gastrointestinal system, being probably not liked to gastric disfunction, since *Maytenus* species are well known for enhancing the protective effects of the stomach preserving its normal physiology[3-7].

The control of gastrointestinal motility is very complex and involves multiple signaling pathways, such as nitric oxide, gastrin, opioids, 5-hydroxytryptamine (5-HT), dopamine, catecholamines and acetylcholine[42]. Thus, it was assessed the mechanistic studies targeting nitrergic and adrenergic pathways, as well as, the participation of KATP channels involved in the antimotility effect previously evaluated. For that matter, it was used for blocking these pathways drugs with well-known mechanisms, glibenclamide, a KATP channels blocker, L-NAME, an inhibitor of nitric oxide synthase (NOs), propranolol, a non-selective adrenergic antagonist and yohimbine, a presynaptic α2-adrenergic antagonist.

The results found in this experiment suggested the participation of nitric oxide and KATP channels, that might involve the NO-cGMP-KATPpathway, as well as, the participation of tissue adrenergic receptors in the antimotility activity, due to the effect reversal when EtOHE-*Me* was administered along the respective blockers. It is also possible to suggest that this effect does not involve presynaptic α2-adrenergic receptors, since EtOHE-*Me* kept decreasing intestinal transit in the presence of yohimbine, a blocker of this pathway.

In order to determine if antidiarrheal activity of EtOHE-*Me* was also associated with a reduction of fluid accumulation, it was performed the castor oil induced-enteropooling model. It is possible to suggest by the present results that EtOHE-*Me* acts reducing diarrhea also due to antisecretory effect and this mechanism of action might be related to inhibition of secretion, reducing intraluminal fluid accumulation and/or enhancing water and ion absorption.

Species such as *Psidium guajava* and *Anacardium occidentale*, largely used in traditional medicine as antidiarrheic[24], have already demonstrated a decrease of fluid accumulation underlining their antidiarrheal property[43,44].

Thus, this work showed for the very first time that the ethanol extract of *Maytenus erythroxylon* potently reduced the diarrheal episodes, due to inhibition of gastrointestinal motility, *via* nitrergic, KATP channels and tissue adrenergic receptors modulation, as well as, by its antisecretory activity. Those results must be closely related to the secondary metabolites found in the extract: saponins, flavonoids, tannins, triterpenes and steroids. These effects accompanied by the safety of itsadministration, evaluated in this study, validate the popular utilization of *Maytenus erythroxylon*.

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**COMMENTS**

***Background***

A variety of herbal medicines from *Maytenus* genus have been shown to produce results in the treatment of diarrhea in folk medicine, such as *M. rigida* and *M. ilicifolia,* being this activity already validated by pharmacological studies. *M. erythroxylon*, the species selected for this study, popularly known as “bom-nome” and “casca grossa”, in folk medicines is used to treat gastrointestinal disorders. Given the need for new antidiarrheal therapies, this study aimed to evaluate for the first time the antidiarrheal activity of this species, as well as, its mechanisms of action, the acute toxicity and phytochemical profile, validating its popular use and contributing to the search for new therapies for diarrhea.

***Research frontiers***

*Maytenus* genus presents a variety of species with promising results in pharmacological trials, including the ones evaluating biological activities in the gastrointestinal tract, as gastroprotective, anti-inflamatory and antidiarrheic effects. *Maytenus erythroxylon* is species with folk use to treat ulcers and diarrhea with no toxicological, pharmacological and phytochemical studies in the literature. Thus, this species was selected for the present study in order to contribute to its validation and promote new therapies for the treatment of diarrhea.

***Innovations and breakthroughs***

This study evaluated for the first time the antidiarrheal effect promoted by the species *M. erythroxylon* Reissek in animal models, as well as, its acute toxicity and phytochemical profile.

***Applications***

This study validated the popular use of *M. erythroxylon* Reissekand contributes to the search for new therapies for diarrhea.

***Terminology***

The antidiarrheal activity of ethanol extract (EtOHE) obtained from the leaves of *M. erythroxylon* (EtOHE-*Me)* was studied in the present study. Besides, it was also evaluated the lethal dose 50% (LD50), behavioral alterations and the phytochemical profile of this extract, using for that colorimetric reactions and nuclear magnetic resonance spectroscopy.

***Peer-review***

Authors demonstrated that EtOHE-*Me* displayed antidiarrheal effect in the castor oil-induced diarrhea in mice, and this activity is related to a decrease of gastric emptying and the intestinal transit, being this last result related to nitric oxide, KATP and tissue adrenergic receptors. It was also shown that the antidiarrheal activity is associated with antisecretory mechanisms.

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**Table 1 Preliminary phytochemical screening of EtOHE-*Me***

|  |  |
| --- | --- |
| Test  | Result  |
| Alkaloids | -  |
| Flavonoids  | +  |
| Tannins  | +  |
| Steroids and triterpenoids  | +  |
| Saponins  | +  |

 (+) Present, (-) Absent. EtOHE-*Me*: Ethanol extract of *Maytenus erythroxylon*.

**Table 2 Effect of the oral administration of EtOH extract obtained from the leaves of *Maytenus erythroxylon* over the weight gain of male and female mice for 14 d**

|  |  |  |  |
| --- | --- | --- | --- |
| **Weight gain (g)**  |  | **Vehicle****(NaCl 0.9%)** | **EtOHE-*Me*****(2000 mg/kg)** |
|  | Female |  |  |
| Inicial  |  | 30.78 ± 2.30 | 28.09 ± 2.53NS |
| Final  |  | 35.51 ± 2.00 | 34.65 ± 3.37NS |
|  | Male  |  |  |
| Inicial  |  | 31.41 ± 2.00 | 30.71 ± 2.26NS |
| Final  |  | 39.06 ± 1.32 | 38.93 ± 1.83NS |

NSIndicates no significant differences (*P >* 0.05) between treated (EtOHE-*Me*) *vs* non-treated (NaCl 0.9%). Data are expressed as mean ± SD. EtOHE-*Me*: Ethanol extract of *Maytenus erythroxylon*.

**Table 3 Effect of the oral administration of EtOH extract obtained from the leaves of *Maytenus erythroxylon* over the organ index of male and female mice for 14 d**

|  |  |  |  |
| --- | --- | --- | --- |
| Organ Index (mg/g) |  | Vehicle(NaCl 0.9 %) | EtOHE-*Me*(2000 mg/kg) |
|  | Female |  |  |
| Liver  |  | 52.66 ± 6.68 | 53.59 ± 41.61NS |
| Heart |  | 4.27 ± 0.74 | 3.86 ± 0.56NS |
| Kidneys |  | 11.40 ± 0.81 | 10.81 ± 2.31NS |
| Spleen  |  | 5.53 ± 0.83 | 5.16 ± 1.03NS |
|  | Male  |  |  |
| Liver  |  | 51.59 ± 2.57 | 52.33 ± 4.16NS |
| Heart  |  | 4.71 ± 0.75 | 4.24 ± 0.45NS |
| Kidneys |  | 12.18 ± 1.31 | 13.16 ± 0.97NS |
| Slpeen |  | 5.53 ± 0.94 | 4.78 ± 0.37NS |

NSIndicates no significant differences (*P >* 0.05) between treated (EtOHE-*Me*) *vs* non-treated (NaCl 0.9%). Data are expressed as mean ± SD. EtOHE-*Me*: Ethanol extract of *Maytenus erythroxylon*.

**Table 4 Effect of the oral administration of EtOH extract obtained from the leaves of *Maytenus erythroxylon* over the consumption of water and food of male and female mice for 14 d**

|  |  |  |  |
| --- | --- | --- | --- |
| Water consumption (mL)  |  | Vehicle(NaCl 0.9 %) | EtOHE-*Me*(2000 mg/kg) |
|  | Female  | 53.93 ± 5.94 | 54.64 ± 4.58NS |
|  | Male  | 76.43 ± 4.57 | 76.07 ±4.01NS |
|  |  |  |  |
| Food comsumption (g) |
|  | Female  | 45.54 ± 4.20 | 46.33 ± 4.71NS |
|  | Male  | 59.65 ± 6.75 | 59.49 ± 2.28NS |

NSIndicates no significant differences (*P >* 0.05) between treated (EtOHE-*Me*) *vs* non-treated (NaCl 0.9%). Data are expressed as mean ± SD. EtOHE-*Me*: Ethanol extract of *Maytenus erythroxylon*.

**Table 5 Effect of oral administration of EtOHE-*Me* and loperamide in castor oil induced-diarrhea in mice**

|  |  |  |  |  |
| --- | --- | --- | --- | --- |
| Treatment(p.o.) | Dose (mg/kg) | Evacuação Index (EI) | % Wet feaces | % Inibition of Diarrhea |
| NaCl 0.9% | - | 21 (19-25) | 47 | - |
| Loperamide | 5 | 0 (0-4)2 | 0 | 100 |
| EtOHE-*Me* | 62.5 | 8 (5-11)1 | 4 | 62 |
| EtOHE-*Me* | 125 | 7 (6-8)1 | 2 | 66 |
| EtOHE-*Me* | 250 | 6,5 (3-7)1 | 0 | 69 |
| EtOHE-*Me* | 500 | 4 (3-5)1,3 | 0 | 81 |

1Indicates significant differences between treated groups *vs* NaCl 0.9% control group (*P <* 0.05); 2Indicates significant differences between loperamide group *vs* NaCl 0.9% control group (*P <* 0.001); 3Indicates significant differences between EtOHE-*Me* 250 mg/kg *vs* EtOHE-*Me* 500 mg/kg (*P <* 0.05). Data are expressed as median (minimum-maximum). EtOHE-*Me*: Ethanol extract of *Maytenus erythroxylon*.



**Figure 1 Effect of oral administration of EtOHE-*Me* and loperamide in gastric emptying of mice.** Data are presented as mean ± standard desviation. a*P <* 0.05, b*P <* 0.001, *vs* NaCl 0.9% group. EtOHE-*Me*: Ethanol extract of *Maytenus erythroxylon*.



**Figure 2** **Effect of oral administration of EtOHE-*Me* and loperamide in intestinal transit of mice.** Data are presented as mean ± standard desviation. a*P <* 0.05 *vs* NaCl 0.9% group. EtOHE-*Me*: Ethanol extract of *Maytenus erythroxylon*.







**Figure 3 Effect of oral administration of EtOHE-*Me* Glibenclamide, L-NAME, propranolol and yohimbine upon the intestinal transit of mice.** Data are presented as mean ± standard desviation. a*P <* 0.05 *vs* NaCl 0.9% group. EtOHE-*Me*: Ethanol extract of *Maytenus erythroxylon*; L-NAME: L-NG-Nitroarginine methyl ester.



**Figure 4 Effect of oral administration of EtOHE-*Me* and loperamide in cator oil induced enteropooling in mice.** Data are presented as mean ± standard desviation. a*P <* 0.05 *vs* NaCl 0.9% group.EtOHE-*Me*: Ethanol extract of *Maytenus erythroxylon*.