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

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

METHODS
A 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 uncover antimotility mechanisms of action and castor oil-induced entero-pooling to elucidate antisecretary 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 for all tested doses. That last effect seems to be modulated by nitric oxide, K+ 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 as andiarheics, due to their ethnopharmacological support and promising results from research.

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 hydrochloride (2 mg; Janssen Cilag Farmacêutica Ltda®, Brazil); activated charcoal meal (Proquímios®, Brazil); and glibenclamide, L-N<sub>N</sub>itroarginine methyl ester (L-NAME), propranolol and yohimbine (all from Sigma-Aldrich®, United States).

**Plant materials**
Plant samples used in the antidiarrheal activity evaluation in mice were obtained from the leaves of *M. erythroxylon* Reissek. Plants were collected in the city of Mamanguape, Paraiba state, Brazil and identified by Dr Zélma Glebya Maciel Quirino, botanist from Centro de Ciências Aplicadas e Educação/Federal University of Paraiba (UFPB; Paraíba, Brazil). A voucher number 6051 (JPB) was deposited in the Herbarium Lauro Pires Xavier of the Department of Botany of UFPB.

Plants were dried at 40 °C for 4 d, powdered and macerated with 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 for all tested doses. That last effect seems to be modulated by nitric oxide, K+ channels and tissue adrenergic receptors. Besides, the extract also presented antisecretory effect due to a decrease of intestinal fluid accumulation.

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96% ethanol for 3 d. The solution was filtered and evaporated to dryness under reduced pressure at 40 °C. 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 the Central Animal House of Instituto de Pesquisa em Fármacos e Medicamentos (IPeFarM) of the UFPB. They were kept at temperatures between 23-25 °C, with a 12-h light/dark cycle in the animal house, fed with Purina® chow and water *ad libitum* for 2 wk 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 a yellow color appeared upon AlCl₃ reagent addition and tannins when a green or black color was produced with FeCl₃. For the detection of steroids 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, following treatment of the 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 \(^1\text{H}-\text{nuclear magnetic resonance (NMR)}\) and \(^{13}\text{C}-\text{NMR} \) spectroscopy. The \(^1\text{H}-\text{NMR} \) and \(^{13}\text{C}-\text{NMR} \) spectra were obtained by Varian Mercury NMR spectrometer (UNICAL) operating at 200 MHz (\(^1\text{H}\)) and 50 MHz (\(^{13}\text{C}\)). The sample was prepared for analysis by dissolving an amount of EtOHE-Me in deuterated chloroform (CDCl₃; Cambridge Isotope Laboratories, United States). Chemical shifts (δ) were expressed in parts per million (ppm), and for \(^1\text{H}-\text{NMR} \) they were referenced to the characteristic peaks of protons belonging to non-deuterated fractions of the solvent (δH 7.24). For \(^{13}\text{C}-\text{NMR} \), the same parameters were utilized (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 LD\(_{50}\), 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 water and food consumption and 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, 10 mL/kg of castor oil was administered orally to each animal in order to induce diarrhea. Feces were counted for 4 h and classified according to their consistency in solids, semisolids or liquids. Then, the Evacuation Index (EI), Percentual of Wet feces (%) and Diarrheal Inhibition (%) were calculated.

\[
\text{EI} = \sum (\text{solid stools} \times 1) + (\text{semisolid stools} \times 2) + (\text{liquid} \times 3)
\]

\[
\% \text{ DI} = (\text{Mean of saline group} - \text{mean of treated group})/\text{Mean of saline group} \times 100
\]

**Effects of ETOHE-Me on gastric emptying:** Alterations in gastric emptying were assessed according to the model described by Scarpignato et al\(^{[28]}\). After 1 h of pretreatment as described above, 0.4 mL of semisolid colored marker (phenol red 0.05% in 1.5% carboxymethylcellulose) was administered to the non-treated control group (the zero-time control group) and the mice were euthanized immediately. The treated groups received this marker and euthanized 30 min after administration. The abdominal cavity was opened for stomach removal, with necessity of ligature of the pyloric and lower esophageal sphincters to avoid 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 into duplicate microplates and the spectrophotometric reading was made for wavelength equal to 570 nm. The results were expressed as percentage of...
gastric emptying in relation to the control (zero-time group).

\[
\text{% gastric emptying} = \frac{(100 - \text{mean absorbance of sample})}{\text{Mean absorbance of zero-time control group}} \times 100
\]

**Effects of EtOHE-Me on normal intestinal transit:**
Alterations in normal intestinal transit were evaluated according to the model described by Stickney and Northup[29]. After 60 min of the pretreatment, 10 mL/kg (p.o.) Black marker (5% charcoal suspension in 5% Arabic gum) was administered. After 30 min, the animals were euthanized for removal of the small intestine (pylorus to the ileocecal junction). Using 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 route depending on the total length of the intestine.

\[
\text{% intestinal transit} = \frac{\text{length traveled by charcoal meal}}{\text{Total intestinal length}} \times 100
\]

**Antimotility mechanisms of action of EtOHE-Me**
The antimotility mechanisms of action were evaluated according to Santos and Rao[30]. Male mice were fasted for 24 h and subsequently treated orally with NaCl 0.9% (10 mL/kg), loperamide 5 mg/kg and EtOHE-Me at its best dose (500 mg/kg). To obtain information about the mechanism of action, different drugs acting via a well-known mechanism were administered either alone and in association with EtOHE-Me, such as glibenclamide (1 mg/kg i.p.), a blocker of K<sub>ATP</sub> 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 α-adrenergic antagonist. These drugs were dissolved in NaCl 0.9% and given 30 min before extract administration. After 60 min, 10 mL/kg (p.o.) of the black marker (5% charcoal suspension in 5% Arabic gum) was administered and 30 min 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 antisecretory 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 at its best dose (500 mg/kg). After 1 h, 10 mL/kg of castor oil was administered to animals orally. Then, 1 h later, the animals were euthanized for removal of the small intestine, after which the intestinal content was measured with the aid of a graduated cylinder.

**Ethical consideration**
All protocols performed in the present study were 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 (No. 0105/14).

**Statistical analysis**
Parametric data were expressed as mean ± SD and non-parametric data 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 (United States) 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 EtOHE-Me, such as glibenclamide (1 mg/kg i.p.), a blocker of K<sub>ATP</sub> 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 α-adrenergic antagonist. These drugs were dissolved in NaCl 0.9% and given 30 min before extract administration. After 60 min, 10 mL/kg (p.o.) of the black marker (5% charcoal suspension in 5% Arabic gum) was administered and 30 min later, the animals were euthanized for removal of the small intestine to calculate the percentage of intestinal transit.

The NMR spectrum 1 of EtOHE-Me showed the presence of signals relating to quaternary, metinic, methylene and methyl carbons, suggesting the presence of terpenes. It was observed in the regions 8C 124.15 and 8C 145.06 of the spectrum signals that suggest the presence of olefinic carbons referring to pentacyclic triterpenes. There was also present a signal in 8C 80.69 referring to carboxilin carbon. The signal 8C 173.86 suggested the presence of carboxyl of an acid or esters of triterpene. The chemical shifts at the 6.68 8C region are characteristic of methyl carbons of friedelan pentacyclic triterpenes, indicating the presence of ketone compounds at C-3.

The NMR spectrum 2 of EtOHE-Me 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 8H 5.28 and 8H 5.03 are characteristic of olefinic hydrogens. The spectra showed no signals in the aromatic region (8H 6.5 and 8H 8.0).

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

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

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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, evidenced by lack of death during 14 d of the experiment and no apparent behavioral changes. Furthermore, there were no changes in body weight (Table 2) or organ weights of treated animals (Table 3), and no 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 the control group treated only with vehicle (NaCl 0.9%) showed intense signs of diarrhea, with respective evacuation index of 21 (19–25) and 47% wet feces. Pretreatment with EtOHE-Me at all doses (62.5, 125, 250 and 500 mg/kg) decreased the evacuation index of 8 (5–11) with 62% showing diarrhea inhibition (P < 0.05), 7 (6–8) showing 66% (P < 0.05), 6.5 (3–7) showing 69% (P < 0.05) and 4 (3–5) showing 80% (P < 0.001) respectively, when compared with the NaCl 0.9% control group. The standard anti diarrheal drug loperamide (5 mg/kg) produced a significant inhibition of all parameters evaluated (Table 5).

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

| Sex       | Weight gain1 (NaCl 0.9%) | Vehicle (2000 mg/kg) | EtOHE-Me (2000 mg/kg) |
|-----------|--------------------------|---------------------|----------------------|
| Female    | Initial 30.78 ± 2.30      | 28.09 ± 2.53NS       |                      |
|           | Final 35.51 ± 2.00        | 34.65 ± 3.37NS       |                      |
| Male      | Initial 31.41 ± 2.00      | 30.71 ± 2.26NS       |                      |
|           | Final 39.06 ± 1.32        | 38.93 ± 1.83NS       |                      |

1Data are presented in g and expressed as mean ± SD. NSNo significant differences (P > 0.05) between treated (EtOHE-Me) vs non-treated (NaCl 0.9%) mice. EtOHE-Me: Ethanol extract of *Maytenus erythroxylon*.

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

| Sex    | Organ index1 | 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   |                      |
|        | Spleen 5.53 ± 0.94 | 4.78 ± 0.37NS       |                      |

1Data are presented in mg/g and expressed as mean ± SD. NSNo significant differences (P > 0.05) between treated (EtOHE-Me) vs non-treated (NaCl 0.9%) mice. EtOHE-Me: Ethanol extract of *Maytenus erythroxylon*.

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

| Intake | Vehicle (NaCl 0.9%) | EtOHE-Me (2000 mg/kg) |
|--------|---------------------|----------------------|
| Water consumption (mL) | |                       |
| Female | Initial 30.78 ± 2.30 | 28.09 ± 2.53NS       |                      |
|        | Final 35.51 ± 2.00   | 34.65 ± 3.37NS       |                      |
| Food consumption (g) | |                       |
| Female | Initial 31.41 ± 2.00 | 30.71 ± 2.26NS       |                      |
|        | Final 39.06 ± 1.32   | 38.93 ± 1.83NS       |                      |

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

| Treatment, as p.o. | Dose in mg/kg | Evacuation index | Wet feces | Inhibition of diarrhea |
|--------------------|---------------|------------------|-----------|------------------------|
| NaCl 0.9%          | -             | 21 (19-25)       | 47%       | -                      |
| Loperamide         | 5             | 0 (0-4)          | 0%        | 100%                   |
| EtOHE-Me           | 62.5          | 8 (5-11)         | 4%        | 62%                    |
|                    | 125           | 7 (6-8)          | 2%        | 66%                    |
|                    | 250           | 6.5 (3-7)        | 0%        | 69%                    |
|                    | 500           | 4 (3-5)          | 0%        | 81%                    |

1Significant differences between treated groups vs NaCl 0.9% control group (P < 0.05); Significant differences between loperamide group vs NaCl 0.9% control group (P < 0.001); 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*.

**Effects of EtOHE-Me on gastric emptying of mice**
The animals treated with NaCl 0.9% showed 79% of gastric emptying and and the treatment with EtOHE-Me (125, 250 or 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 distance travelled by charcoal meal was 78% in the NaCl 0.9% control group. The treatment with EtOHE-Me at its best dose (500 mg/kg) produced significant (P < 0.001) reduction in the percentage of intestinal transit (36%), when compared to the NaCl
Me, 66% signals in the aromatic region along with the previous steroids in EtOHE-Me.

**DISCUSSION**

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 fidelane terpene from Maytenus erythroxylon, 3β-friedelanol [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 likely to increase water and electrolyte absorption in the colon, decrease intestinal irritability, and reduce 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 [20]. The single-dose administration of EtOHE-Me did not alter any parameter evaluated and showed no deaths, with LD50 considered over 2000 mg/kg (p.o.) and the extract considered safe for pharmacological studies.

Then, it was investigated whether Maytenus erythroxylon ethanol extract possessed antidiarrheal effect. For that, the castor oil-induced diarrhea model in mice was used. Castor oil is a potent laxative agent and induces diarrhea through its active compound, the ricinoleic acid [34], which acts in the upper small intestine where castor oil is hydrolyzed. It produces cytotoxicity of 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], representing 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, nitric oxide (NO), tachykinins (TKs), cAMP [35,36] and prostaglandins via EP1 and EP3 receptors' binding [41].

EtOHE-Me presented antidiarrheal activity, decreasing the evacuation index at all doses, with crescent percentiles of diarrhea inhibition, along with the standard drug loperamide. These results corroborate a study by Santos et al. [6] with Maytenus rigid Mart. ethanolic extract, which was shown to be able to reduce the total number of fecal output and the diarrheic feces for all tested doses.

In order to evaluate if EtOHE-Me affected gastrointestinal motility, gastric emptying and intestinal transit protocols were performed. The findings suggested an antimotility 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 suggested the presence of different mechanisms of action in the different segments of the gastrointestinal system, and likely not liked to gastric dysfunction, since Maytenus species are well known for enhancing the protective effects of the stomach preserving its normal physiology [26-40].

The control of gastrointestinal motility is very complex and involves multiple signaling pathways, such as NO, gastrin, opioids, 5-hydroxytryptamine,
Thus, the mechanistic studies targeting nitrergic and adrenergic pathways were assessed, as well as, the participation of K\(_{ATP}\) channels involved in the antimotility effect previously evaluated. For that matter, we used drugs with well-known mechanisms for blocking these pathways, including glibenclamide, a K\(_{ATP}\) channels blocker, L-NAME, an inhibitor of NOs, propranolol, a non-selective adrenergic antagonist, and yohimbine, a presynaptic \(\alpha_2\)-adrenergic antagonist.

The results from this experiment suggested the participation of NO and K\(_{ATP}\) channels, that might involve the NO-cGMP-K\(_{ATP}\) pathway, as well as of tissue adrenergic receptors in the antimotility activity, due to the effect reversal when ETOHE-Me was administered along with the respective blockers. It is also possible to suggest that this effect does not involve presynaptic \(\alpha_2\)-adrenergic receptors, since EtOHE-Me still decreased 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 in fluid accumulation, the castor oil induced-enteropooling model was used. It is possible to suggest through the present results that EtOHE-Me ability to reduce diarrhea may also be due to its antisecretory effect and that 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 antidiarrheics\[24\], have already demonstrated a decrease of fluid accumulation, underlining their antidiarrheal properties\[43,44\].

Thus, this work showed, for the very first time, dopamine, catecholamines and acetylcholine\[42\]. This, thus, the mechanistic studies targeting nitrergic and adrenergic pathways were assessed, as well as, the participation of K\(_{ATP}\) channels involved in the antimotility effect previously evaluated. For that matter, we used drugs with well-known mechanisms for blocking these pathways, including glibenclamide, a K\(_{ATP}\) channels blocker, L-NAME, an inhibitor of NOs, propranolol, a non-selective adrenergic antagonist, and yohimbine, a presynaptic \(\alpha_2\)-adrenergic antagonist.

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Thus, this work showed, for the very first time,
that the ethanol extract of Maytenus erythroxylon potently reduced diarrheal episodes, due to inhibition of gastrointestinal motility via nitricergic pathways and KATP channels, through tissue adrenergic receptors modulation, and 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 its administration, validate the popular utilization of Maytenus erythroxylon.

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COMMENTS

Background

A variety of herbal medicines from the Maytenus genus, such as M. rigida and M. ilicifolia, have been shown to produce results in the treatment of diarrhea in folk medicine, and this activity has already been validated by pharmacological studies. M. erythroxylon, the species selected for this study, popularly known as “bom-rome” and “casca grossa”, in folk medicine 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-inflammatory and antidiarrheic effects. Maytenus erythroxylon is a species with folk use to treat ulcers and diarrhea, but 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 Reissek and contributes to the search for new therapies for diarrhea.

Terminology

The antidiarrheal activity of ethanol extract (EtOHE) obtained from the leaves of M. erythroxylon (EIOHE-Me) was assessed in the present study. In addition, the lethal dose 50% (LD50) was evaluated, along with behavioral alterations and the phytochemical profile of this extract by means of colorimetric reactions and nuclear magnetic resonance spectroscopy.

Peer-review

The authors demonstrated that EIOHE-Me displayed an antidiarrheal effect in the castor oil-induced diarrhea mouse model and showed that this activity is related to a decrease in gastric emptying and intestinal transit, with this last result being 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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June 28, 2017 | Volume 23 | Issue 24 | 4389

Formiga RO et al. Maytenus erythroxylon

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