
Antidiarrheal and antinociceptive activities of ethanol extract and its chloroform and pet ether fraction of *Phrynium imbricatum* (Roxb.) leaves in mice

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Abstract

Background: The objective of the study was to evaluate the antidiarrheal and antinociceptive activities of ethanol extract and its chloroform and pet ether fraction of *Phrynium imbricatum* (Roxb.) leaves in mice.

Methods: In the present study, the dried leaves of *P. imbricatum* were subjected to extraction with ethanol, and then it was fractioned by chloroform and pet ether solvent. Antidiarrheal effects were tested by using castor oil-induced diarrhea, castor oil-induced enteropooling, and gastrointestinal transit test. Antinociceptive activity was evaluated by using the acetic acid-induced writhing test and formalin-induced paw licking test.

Results: The standard drug loperamide (5 mg/kg) showed significant (p<0.001) inhibitory activity against castor oil-induced diarrhea, in which all the examined treatments decreased the frequency of defecation and were found to possess an anti-castor oil-induced enteropooling effect in mice by reducing both weight and volume of intestinal content significantly, and reducing the propulsive movement in castor oil-induced gastrointestinal transit using charcoal meal in mice. The results showed that the ethanol extract of *P. imbricatum* leaves has significant dose-dependent antinociceptive activity, and among its two different fractions, the pet ether fraction significantly inhibited the abdominal writhing induced by acetic acid and the licking times in formalin test at both phases.

Conclusions: These findings suggest that the plant may be a potential source for the development of a new antinociceptive drug and slightly suitable for diarrhea, as it exhibited lower activity. Our observations resemble previously published data on *P. imbricatum* leaves.

Keywords: acetic acid; antidiarrheal; antinociceptive; castor oil; intestinal transit; *Phrynium imbricatum*.

Introduction

From the past decades, plants and their products have been utilized by humans for the treatment of various diseases [1]. In present days, use of medicinal plants is increasing over the past decade as an alternative way to improve the quality of life and maintain good health. Also, huge numbers of present pharmaceutical products are discovered from medicinal plants. Thus, it appears that plants are potential sources for new drug discovery and drug development. Bangladesh has abundant medicinal plants, and proper scientific assessments are required to explore these plants for treating various diseases [2].

Diarrhea-related syndrome is a foremost cause of mortality, especially among children in developing countries, resulting in a major health-care problem. As per World Health Organization (WHO) estimation for the year 1998, there were
about 7.1 million deaths due to diarrhea [3]. In Bangladesh, 33% of the total child deaths are caused by diarrhea [4]. The WHO launched a Diarrhea Control Program that emphasized utilization of traditional medicines to combat episodes of diarrhea [5]. Diarrhea is shown by several mechanisms such as increased gut motility along with increased secretion of ions and diminished retention of liquid, and subsequently a loss of electrolytes, particularly Na⁺ and water [6]. Diarrhea is most common in crowded living conditions coupled with poor hygiene and malnutrition. Thus, to combat diarrhea, people should be clean and eat a healthy diet. Many medications and alternative medicines are available throughout the world. Conventional treatments are being neglected because antidiarrheal treatments are mostly focused on corrective fluid balance theory. Corrective fluid balance and oral rehydration solution mostly satisfy their objectives, due to their non-toxic and friendly effects. Thus, it becomes important to search for other alternatives such as plants. For these outcomes, natural medicine has made a rebound, enhancing the satisfaction of our present and future health needs [7]. Nevertheless, new agents are still required for better treatment of diarrhea [8, 9].

Pain is a manifestation of an uncomfortable feeling due to distinctive diseases and injuries. Pain is also an unpleasant sensory and emotional experience associated with actual or potential tissue damage. By acting on the central or peripheral pain mechanism, analgesic compounds selectively relieve pain without significant alteration of consciousness. Analgesics are applied to block central or peripheral pain stimuli. Several types of drugs are available, like non-steroidal anti-inflammatory drugs (NSAIDs), opioids, etc., for pain management. These synthetic and semi-synthetic pharmaceutical products are not completely free from side effects [10, 11]. In pursuit of safer and effective analgesic drugs to come into being from medicinal plants, more research and development should be carried out because many species of plants are used worldwide for relieving pain [12].

Phrynium imbricatum (Family: Marantaceae) is a rigid herb (leaves large, oblong; spikes oblong; bracts oblong with obuse, minutely toothed tips; fruits usually three-seeded) that occurs in the forests of Chittagong, Chittagong Hill Tracts, Cox’s Bazar, and Sylhet. A paste prepared from leaves of P. imbricatum, Blumea clarkei, and an unidentified species (locally called Khedom gas) is applied to affected areas and bandaged for the treatment of fractures (Chakma) [13]. Leaves of P. imbricatum have antiarthritic, membrane-stabilizing, and anthelmintic activities [14, 15]. Considering the significance of plants as a vital source of medicine even today, in the present study, we subjected a plant, P. imbricatum (Roxb.), which may be a potential source.

This study intends to explore the ethanol extract and its chloroform and pet ether fractions of P. imbricatum leaves for their antidiarrheal effect by using three standard methods of bioassay: castor oil-induced diarrhea, castor oil-induced enteropooling, and gastrointestinal transit test. Meanwhile, antinociceptive activity was also evaluated by using the acetic acid-induced writhing test and formalin-induced paw licking test.

Materials and methods

Plant collection and identification

Leaves of P. imbricatum (accession no. 1315 CTGUH) were collected from Alutila, Khagrachari, Chittagong, Bangladesh, in the month of September 2014 at the last time of its flowering. It was authenticated by Dr. Shaikh Bokhtear Uddin, Professor, Department of Botany, University of Chittagong, Chittagong, Bangladesh.

Extraction and fractionations

Leaves were cleaned with fresh water and dried for a period of 10 days under shade and then powdered with a mechanical grinder, passing through sieve no. 40, and stored in a tight container. The powdered whole plant (850 g) of P. imbricatum was soaked in 1.5 L ethanol for 7 days with occasional shaking and stirring, and filtered through a cotton plug followed by Whatman filter paper no. 1. The extract was then concentrated by using a rotary evaporator at reduced temperature and pressure. A portion (55 g) of the concentrated ethanol extract (EEPI) was fractioned by using the modified Kupchan partitioning method [16, 17] into chloroform (CHFPI, 8 g) and pet ether (PEFPI, 14 g) fractions.

Chemicals and reagents

All chemicals and reagents were analytical grade. Ethanol, chloroform, and pet ether were purchased from Merck (Darmstadt, Germany). Normal saline solution was purchased from Beximco Infusion Ltd. Loperamide (Square Pharmaceuticals Ltd., Pubna, Bangladesh), castor oil (KEL’s Health Care, Madrid, Spain), charcoal meal (10% activated charcoal in 5% gum acacia) were used. Diclofenac sodium (Beximco Pharmaceuticals Ltd., Dhaka, Bangladesh), formaldehyde (MERCK, Mumbai, India), acetic acid from Merck (Darmstadt, Germany), normal saline solution (0.9% NaCl), and Tween-80 (BDH Chemicals, Hunter Boulevard, Magna Park, Lutterworth, Leicestershire, UK) were used.

Animals and ethical approval for using animals

Swiss albino mice, weighing about 25–35 g, were collected from Jahanagar Nagar University, Savar, Bangladesh. The animals were furnished...
with standard laboratory nourishment and refined water ad libitum and maintained at natural regular day-night cycle with legitimate ventilation in the room. All experiments were conducted in an isolated and noiseless condition. The experiments on animals were carried out according to the guidelines of National Institutes of Health and International Council for Laboratory Animal Science, which are internationally acceptable for proper use of laboratory animals. According to the aforementioned guidelines, the current study protocol was reviewed and approved by the ethical review committee and also by the Planning and Development Committee of the Department of Pharmacy, International Islamic University Chittagong, Bangladesh, under the name Pharm 06/10-'15-636. The animals were acclimatized to laboratory conditions for 7 days prior to experimentation.

**Acute toxicity study**

For acute toxicity study, 40 Swiss albino female mice were used. According to the method of Walum, the mice were divided into four groups of five animals each [18]. Different doses (1000, 2000, 3000, and 4000 mg/kg) of ethanol extract and its chloroform and pet ether fractions of *P. imbricatum* leaves were administered via a stomach tube. Then, the animals were observed for general toxicity signs.

**In vivo antidiarrheal activity**

**Castor oil-induced diarrhea:** The experiment employed the method described by Awouters et al. [19]. Mice were fasted for 18 h before the test with free access to water and divided into five groups of five animals each. Group I was treated as control [saline 2 mL/kg body weight (b.wt.) p.o.]; group II received standard drug (loperamide 5 mg/kg b.wt. p.o.); group III–IV received ethanol extract of *P. imbricatum* (200 and 400 mg/kg b.wt. p.o.); group V–VI received chloroform fraction of ethanol extract (200 and 400 mg/kg b.wt. p.o.); and group VII–VIII received pet ether fraction of ethanol extract (200 and 400 mg/kg b.wt. p.o.). Then, 1 h later, castor oil was administered orally to these animals to induce diarrhea. The mice were then housed singly in cages lined with white blotting paper. The papers were changed every hour. The total number of both dry and wet feces excreted were counted every hour for a period of 4 h and compared with the control group. The total number of diarrheal feces of the control group was considered 100%.

**Castor oil-induced enteropooling:** Intraluminal fluid accumulation was determined by the method of Robert et al. [20]. Mice fasted for 18 h were divided into five groups of five animals each. Group I served as control (saline 2 mL/kg b.wt. intraperitoneally); group II received standard drug (loperamide 5 mg/kg b.wt.); group III–IV received ethanol extract of *P. imbricatum* (200 and 400 mg/kg b.wt. p.o.); group V–VI received chloroform fraction of ethanol extract (200 and 400 mg/kg b.wt. p.o.); and group VII–VIII received pet ether fraction of ethanol extract (200 and 400 mg/kg b.wt. p.o.). Then, 1 h later, castor oil was administered orally to these animals to induce diarrhea. Two hours later, the mice were sacrificed by an overdose of chloroform anesthesia, and the distance traveled by the charcoal meal from the pylorus to the cecum was measured and expressed as a percentage of the total distance of the intestine.

**Antinociceptive activity**

**Acetic acid-induced writhing test:** Mice were divided into eight groups of five animals in each. For the writhing test, 0.6% (v/v) acetic acid solution (10 mL/kg b.wt.) was injected intraperitoneally to each mouse, and the number of writhing and stretching was counted over 20 min [22, 23]. Group I served as control (received normal saline 10 mL/kg); group II received diclofenac sodium 10 mg/kg as a standard; and groups III–VIII were treated with ethanol extract and its chloroform and pet ether fractions (200 and 400 mg/kg) orally 30 min before acetic acid injection. Analgesic activity was expressed as writhing inhibition (%) and was calculated for each animal using the following formula:

\[
W_{s} - W_{c} \times 100
\]

where \(W_{s}\) is the mean number of writhings of the control and \(W_{c}\) is the mean number of writhings of the test sample.

**Formalin test:** A total of 20 \(\mu\)L of 2.5% formalin in saline was injected subcutaneously to a hind paw of the mice after 30 min administration of diclofenac sodium 10 mg/kg, ethanol extract and its chloroform, and pet ether fractions at 200 and 400 mg/kg p.o. dose to groups II–VIII, respectively. Group I as control received only formalin (20 \(\mu\)L of 2.5%) during the experiment. The time spent licking or the biting responses to the injected paw was taken as an indicator of pain response, and the data were expressed as total licking time in the early phase (0–5 min) and late phase (15–30 min) after formalin injection [24]. The total time spent licking or biting the wounded paw (pain response) was determined with the aid of a stopwatch. The percentage of inhibition of pain was determined as the previous equation for early- and late-phase pain.

**Intermodel relationship:** Generally, different models are used to ensure an activity. By the intermodel relationship, the way an extract with a definite dose shows activity in a different model is estimated. Thus, if this extract shows the same ratio value in all models, it
proves that it has same activity through all the models. If the value of the ratio is near 1, then it has good activity. The ratio is calculated as follows:

$$\frac{\% \text{ inhibition by the standard}}{\% \text{ inhibition by the extract at definite dose}}$$

where ratio value <1 = very good activity; 1 < ratio value <2 = good activity; 2 < ratio value <3 = moderate activity; 3 < ratio value <5 = low activity; and 5 < ratio value = very low activity.

**Statistical analysis**

The results were expressed as the mean ± SEM. The results were statistically analyzed using repeated measures analysis of variance with Dunnett’s and Bonferroni multiple comparisons when compared against control in all models of antidiarrheal and antinociceptive activity. Values of p <0.05, p <0.01, and p <0.001 were considered statistically significant. The statistical program used was SPSS (Statistical Package for Social Science, version 16.0; IBM Corporation, NY, USA).

**Results**

**Acute toxicity study**

No behavioral, neurological, or physical changes characterized by symptoms, such as reduced motor activity, restlessness, convulsions, coma, diarrhea, and lacrimation, at the limit dose of 4000 mg/kg of ethanol extract and its different fractions of *P. imbricatum* were seen among test animals during the observation period. In addition, no mortality was observed at the test dose. Thus, the median lethal dose (LD50) of the plant extract was found to be >4000 mg/kg.

**In vivo antidiarrheal activity**

**Castor oil-induced diarrhea**

The ethanol extracts of *P. imbricatum* leaves and its fractions were found to be effective in a dose-dependent manner against castor oil-induced diarrhea in experimental mice. At the dose of 400 mg/kg, every extract produced a significant decrease in the severity of diarrhea in terms of reduction in the frequency of defecation and consistency of feces in albino mice. At the same dose, the extracts showed significant antidiarrheal activity (p<0.001), showing 41.38%, 37.93%, and 44.83% reduction in the frequency of defecation in EEPI, CHFPI, and PEFPI, respectively, comparable to that of the standard drug loperamide that showed 59.70% ± 2.99% reduction in diarrhea (Table 1).

**Castor oil-induced enteropooling**

As presented in Table 2, all test doses of the extract and fractions significantly reduced the intestinal weight and volume in a dose-dependent manner. Castor oil caused accumulation of water and electrolytes in the intestinal loop. Treatment with the *P. imbricatum* leaf extract and its fractions (200 and 400 mg/kg) produced a significant (p<0.05, p<0.01) 31.64%, 28.36%, and 33.44% inhibition of intestinal content in EEPI, CHFPI, and PEFPI, respectively, comparable to that of the standard drug loperamide that showed 59.70% ± 2.99% reduction in diarrhea (Table 1).

**Table 1**: Effect of *P. imbricatum* extracts on feces count in castor oil-induced diarrhea in mice.

<table>
<thead>
<tr>
<th>Treatment</th>
<th>Total number of feces</th>
<th>% Inhibition of defecation</th>
<th>Total number of diarrheal feces</th>
<th>% Inhibition of diarrhea</th>
</tr>
</thead>
<tbody>
<tr>
<td>Saline (2 mL/kg p.o.)</td>
<td>13.4 ± 0.245</td>
<td>–</td>
<td>5.8 ± 0.2</td>
<td>–</td>
</tr>
<tr>
<td>Loperamide (5 mg/kg p.o.)</td>
<td>5.4 ± 0.400</td>
<td>59.7</td>
<td>2.2 ± 0.374a</td>
<td>62.07</td>
</tr>
<tr>
<td>EEPI (200 mg/mL p.o.)</td>
<td>9.8 ± 0.200</td>
<td>26.87</td>
<td>4.4 ± 0.245a</td>
<td>24.14</td>
</tr>
<tr>
<td>EEPI (400 mg/mL p.o.)</td>
<td>7.6 ± 0.678</td>
<td>43.28</td>
<td>3.4 ± 0.400b</td>
<td>41.38</td>
</tr>
<tr>
<td>CHFPI (200 mg/mL p.o.)</td>
<td>10.4 ± 0.510b</td>
<td>22.39</td>
<td>4.6 ± 0.245b</td>
<td>22.69</td>
</tr>
<tr>
<td>CHFPI (400 mg/mL p.o.)</td>
<td>7.8 ± 0.583b</td>
<td>41.79</td>
<td>3.6 ± 0.510b</td>
<td>37.93</td>
</tr>
<tr>
<td>PEFPI (200 mg/mL p.o.)</td>
<td>9.6 ± 0.510a</td>
<td>28.36</td>
<td>4.2 ± 0.374a</td>
<td>27.59</td>
</tr>
<tr>
<td>PEFPI (400 mg/mL p.o.)</td>
<td>7.4 ± 0.400</td>
<td>44.78</td>
<td>3.2 ± 0.583c</td>
<td>44.83</td>
</tr>
</tbody>
</table>

Values are mean ± SEM (n = 5). EEPI, ethanol extract of *P. imbricatum*; CHFPI, chloroform fraction of the ethanol fraction; and PEFPI, pet ether fraction of the ethanol extract. Repeated measures ANOVA with Dunnett’s and Bonferroni multiple comparisons as compared to negative control (saline) were performed for this data set. Statistical representation of the total number of feces and total number of diarrheal feces by ethanol extract and its chloroform and pet ether fraction of *P. imbricatum* leaves, positive antidiarrheal control (loperamide, 5 mg/kg p.o.), was processed by Dunnett’s and Bonferroni tests using SPSS for Windows, version 16.0. *p <0.05, †p <0.01, and ‡p <0.001 for Dunnett’s test and *p <0.05, †p <0.01, and ‡p <0.001 for Bonferroni test.
respectively, at 400 mg/kg b.wt., comparable to that of the standard drug loperamide that showed 48.2% inhibition of intestinal content (Table 2).

Gastrointestinal motility test

The effect of *P. imbricatum* extract and its fractions on the intestinal transit is depicted in Table 3. All doses of the extracts were successful in producing a significant alteration in the percentage inhibition of intestinal motility compared to the negative control. The negative control (saline) resulted in 84.85%±2.88% intestinal motility by the marker – charcoal meal. The 200 and 400 mg/kg oral doses of the ethanol extract of *P. imbricatum* and their fractions exhibited 18.87%–26.41%, 18.27%–25.94%, and 19.45%–26.48% inhibition in EEPI, CHFPI, and PEFPI intestinal motility, respectively (Table 3). However, the standard drug loperamide (5 mg/kg) demonstrated a significant inhibition (43.36%) in intestinal motility.

### Intermodel relationship of sample doses and standard for antidiarrheal activity

The calculated ratio values of intermodel relationships are presented in Table 4. From the chart, it was proved that in all the used models, the extract and its all fractions with their 200 and 400 mg/kg doses showed good to moderate activity. The ratio values of a dose of tested treatments are almost the same in all models. Thus, the tests were appropriate because that means in all the models, the extract and its all fractions exhibited the same results. At 400 mg/kg dose, all tested treatments exhibited good activity; at 200 mg/kg dose, they showed moderate activity.

### Antinociceptive activity

#### Acetic acid test

The effects of *P. imbricatum* extract and its fractions on acetic acid-induced writhing in mice are depicted in Table 5. Oral administration of the extract moderately...
(p<0.01) inhibited the writhing response induced by acetic acid, which was compared to the reference drug.

**Formalin test**

The ethanol extract of *P. imbricatum* and its fractions at doses of 200 and 400 mg/kg significantly inhibited the two phases of the formalin test. For the early phase, the ethanol extract and its fractions showed different values in different concentrations; for the 400 mg/kg dose, they showed 47.33%, 45.23%, and 53.32% inhibition for EEPI, CHFPI, and PEFPI, respectively. For the late phase, the ethanol extract and its fractions showed different significant values in different doses, and for the 400 mg/kg dose, they showed 53.04%, 49.23%, and 55.44%, for EEPI, CHFPI, and PEFPI, respectively. The summary of the results is shown in Table 6.

### Discussion

Many models are available for the evaluation of antidiarrheal activity. Among them, stimulating water adsorption, reducing the intraluminal fluid accumulation, delaying intestinal transit, and suppressing gut motility are the most popular [25]. Ethanol extract and its fractions of *P. imbricatum* (Roxb.) exhibited antidiarrheal activity in a dose-dependent manner. Antidiarrheal activity was observed at doses of 200 and 400 mg/kg in all methods, and was significant when compared to the standard loperamide.

Four notable pathophysiologies are responsible for frequent bowel movement. These are expanded luminal osmolarity (osmotic diarrhea), increased electrolyte secretion (secretory diarrhea), reduced electrolyte absorption, and disturbed intestinal motility [26]. Disturbed intestinal motility is mostly linked with reduced gastrointestinal motility and the secretions.
Purgatives stimulate evacuation of the bowel by loosening bowel content and promoting bowel movement. For example, castor oil, used as an inducer of diarrhea in the study, is known for its purgative and laxative effects because of the active component, retinoic acid. The dynamics of castor oil is known to change the electrolyte permeability of the intestinal membrane by increasing prostaglandin biosynthesis and discharges. It causes pseudo-diarrhea almost similar to pathophysiologic conditions that cause diarrhea [27, 28]. In light of this, castor oil was utilized to promote diarrhea in the experimental animals in this study.

Different researchers around the world have shown that castor oil causes diarrhea 1–2 h just after administration of 0.1–0.3 mL in mice [28]. In our experiment, frequent bowel movement (diarrhea) was observed within 1 h in most of the experimental animals because of the high dose of castor oil (0.5 mL/mice). Only those mice that demonstrated this diarrheal reaction were chosen for this trial.

The ethanol extract of *P. imbricatum* significantly (p<0.05–0.001) reduced the fecal output produced by castor oil. At doses of 200 and 400 mg/kg (p.o.), all the fractions significantly (p<0.05–0.001) and dose dependently delayed the onset of diarrhea induced by castor oil when compared with the untreated controls. PEFPI showed the maximum effect in this model. PEFPI (200 mg/kg, p.o.) reduced the number of fecal episodes by 27.59%, while the dose of 400 mg/kg (p.o.) significantly (p<0.001) reduced the number of animals suffering from diarrhea by reducing defecation by 44.83%. Loperamide (5 mg/kg, p.o.) profoundly (p<0.001) reduced the fecal output produced by castor oil, which reduced the number of fecal episodes by 62.07%. Antidiarrheal activity was best observed after 4 h at 400 mg/kg dose of *P. imbricatum* extract, which can be best comparable with the standard drug loperamide (Table 1).

The extract and its fractions showed a noticeable effect in the castor oil-induced enteropooling test in the mice (Table 2). The intestinal volume was decreased by 31.64% for ethanol extract, 28.36% for CHFPI, and 33.44% for PEFPI at the dose of 400 mg/kg, which were statistically significant (p<0.05–0.001). The standard drug, loperamide (5 mg/kg), also significantly inhibited intestinal fluid accumulation (48.2%) (p<0.01 in Dunnett’s test).
test), and the effects of the extract and fractions were less potent in comparison to the standard drug.

The gastrointestinal distance traveled by the charcoal meal in the mice was significantly (p < 0.01) lessened by all the extracts compared with the control group. Loperamide (5 mg/kg) produced a marked decrease (43.36%) in the propulsion of charcoal meal through the gastrointestinal tract, and the extract and fractions showed the same results as previously mentioned (Table 3).

It was observed from the study that in both analgesic activity assay models, the plant extract demonstrated analgesic effects. The acetic acid-induced abdominal constriction test has long been broadly utilized as an exploratory and screening tool for relieving pain or mitigating the properties of new agents [29, 30], and conventionally utilized as a model to consider the peripheral antinociceptive effect of plant extracts. This model of nociception is believed to provoke a peripheral mechanism as organization of phlogogen leads to elevated levels of cyclooxygenase (COX1 and COX2) and lipoxygenase (LOX) [31], and in an indirect way prompts the arrival of endogenous nociceptive mediators as well as other LOX products in peritoneal fluids that can instigate different peripheral nociceptive neurons sensitive to NSAIDs within the inside of the peritoneal cavity [32, 33]. Prolonged irritation of the peritoneal cavity has been associated with an increase in the PGE levels in the peritoneal fluid, which enhances capillary permeability [34] and the release of glutamate and substance P from peripheral afferent fiber terminals [35].

In the acetic acid-induced writhing test, the ethanol extract and its fractions significantly reduced the number of writhing movements induced by the intraperitoneal administration of acetic acid solution. The dose-dependent inhibition of abdominal constrictions by the ethanol extract and its fractions indicate the antinociceptive potential of the plant, which is clearly supported by our results (Table 5). Our result clearly indicates that the extract and all the fractions reduced the number of writhing. Among all the tested treatments, PEFPI (57.19% inhibition) showed maximum effect, which is well comparable with the standard drug (70.61% inhibition).

Another model of nociception that has recently been used to support the antinociceptive impact is formalin-induced paw licking test or formalin test [36, 37]. This test, which symbolizes a model of persistent pain, can also be used to explore the ability of new compounds to affect peripheral or central nociceptive pathways due to their biphasic nociceptive characteristics, known as the early phase (first 5 min) and late phase (last 15 min), resulting from the formalin administration [38]. The early phase, known as neurogenic pain, is an acute response observed immediately after the administration of formalin and persists for 5 min (0–5 min) as a result of a direct action of injected formalin on nociceptors. The late phase, classified as inflammatory pain, is a tonic reply resulting from the inflammatory processes generated by the release of inflammatory mediators such as histamine, serotonin, and bradykinin [39–41]. The extract and fractions of *P. imbricatum* (Roxb.) when administered orally at 200 and 400 mg/kg exhibited significant analgesic activity in the formalin test method, as supported by the increase in latency time when compared to control. The decrease in paw licking period was found to be dose dependent. However, maximum effect was seen at the dose of 400 mg/kg of PEFPI (at early phase, 53.32% inhibition; at late phase, 55.44% inhibition) and was comparable with the standard drug (at early phase, 73.91% inhibition; at late phase, 80.06% inhibition).

**Conclusions**

According to the results of the present investigation, it can be concluded that the ethanol extract of *P. imbricatum* leaves and its fractions have significant antidiarrheal and antinociceptive effects. The extract and fractions showed dose-dependent activity in all the examined models of antidiarrheal and antinociceptive effects. The intermodel relationship also suggested that the plant extract and its fractions showed almost similar activity in the grouped model for the activities. Thus, from the results, it can be understood that this plant has more antinociceptive potential and less antidiarrheal activity compared with the standard. This study also suggests the need for further detailed investigations of the mechanisms of action of the pharmacological effects, and also the need to isolate the active compounds responsible for those properties.

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**Author contributions:** MMH and MSHK collected the plant leaves and prepared the extract and fractions. MMH and
MSHK also carried out the study design, performed the experiments, collected and interpreted the data, prepared the manuscript, and performed statistical analysis. MSHK wrote the first draft of the manuscript. MAMD, MSIA, SMZH, RD, and MMNU helped in the experiments, data collection, and literature search. The intermodel relationship idea was uniquely given by MSHK. MMR supervised the study design and data interpretation. All the authors have accepted responsibility for the entire content of the submitted manuscript and approved submission.

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