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# Antidiarrheal Activity-Guided Fractionation of *Plumbago Zeylanica*

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**Abstract**
Diarrheal diseases pose a significant global health challenge, particularly in developing countries, emphasizing the urgent need for effective interventions. This study investigated the antidiarrheal properties of *Plumbago zeylanica* using bioactivity-guided fractionation. A dark brown ethanolic extract (11.45% w/w yield) was obtained from *P. zeylanica* roots via Soxhlet extraction. Chromatographic separation yielded 23 fractions, which were combined based on TLC analysis into four main fractions (F1-F4) with yields of 28.13%, 42.55%, 2.81%, and 22.37%, respectively. Fraction F3 exhibited an Rf value of 0.86. In a castor oil-induced diarrhea model in Wistar rats, the crude extract showed dose-dependent efficacy, with both 200 mg/kg and 400 mg/kg doses significantly reducing diarrhea frequency and severity. Among the fractions, F3 (2 mg/kg) displayed the most potent antidiarrheal activity, evidenced by the lowest fecal counts (1.26 ± 0.20), wet feces (0.45 ± 0.00), and fecal weight (20.6 ± 20.60 mg). A charcoal-induced diarrhea model further confirmed the antidiarrheal effects of the crude extract at both 200 mg/kg (43.68 ± 1.27 cm) and 400 mg/kg (34.89 ± 1.82 cm) doses, significantly reducing charcoal movement ($P<0.01$). Remarkably, F3 (2 mg/kg) demonstrated the most substantial reduction in charcoal movement (29.34 ± 1.68 cm) at a considerably lower dose. These results highlight the potential of *P. zeylanica* and its fractions, especially F3, as promising sources for developing natural antidiarrheal agents.

**Keywords**: *Plumbago zeylanica*, Antidiarrhoeal activity, Plumbagin, Bioactivity-guided fractionation, Extraction, Charcoal induced diarrhea model.

## INTRODUCTION

Diarrhea, a prevalent gastrointestinal condition marked by increased stool frequency and loose or watery consistency, presents a substantial global health concern, particularly in developing countries [1]. It is a major contributor to morbidity and mortality, notably among children under five, where it ranks as the second leading cause of death, accounting for an estimated 525,000 deaths each year, according to the World Health Organization [2]. Infections from viral, bacterial, and parasitic sources are primary drivers of diarrhea, often exacerbated by inadequate sanitation, unsafe water, and malnutrition [3].

Standard diarrhea treatment combines oral rehydration therapy with antidiarrheal medications for symptom management [4]. However, many synthetic antidiarrheals carry the risk of side effects such as constipation, abdominal discomfort, and potential toxicity [5]. This has spurred growing interest in exploring natural remedies, especially those derived from medicinal plants, as potentially safer and more affordable alternatives [6].

*Plumbago zeylanica*, commonly known as Ceylon leadwort or Chitrak, is a medicinal plant

widely used in traditional medicine systems like Ayurveda, Unani, and Siddha [7]. It is recognized for its diverse pharmacological properties, including antimicrobial, anti-inflammatory, and gastrointestinal effects [8]. While traditionally employed to manage gastrointestinal disorders, including diarrhea, scientific evidence validating its efficacy and pinpointing the active compounds responsible for these effects remains limited [9].

This study aimed to investigate the antidiarrheal potential of P. zeylanica through an activity-guided fractionation approach, with the goal of identifying the bioactive constituents contributing to its traditional use in treating diarrhea.

## MATERIALS AND METHODS

### Materials

*Plumbago zeylanica* roots, collected from Neemuch and its surrounding regions, were authenticated by the Department of Botany, Oriental University, Indore. All solvents (methanol, ethyl acetate, hexane, and n-butanol) were of analytical grade and purchased from Merck. Castor oil was sourced from a local pharmacy, while charcoal powder was procured from Himedia Laboratories. Chromatographic and spectroscopic analyses utilized silica gel and deuterated solvents along with HPLC-grade acetonitrile, all supplied by SD Fine Chemicals, Indore.

### Animal Selection

Wistar albino rats (150-200 g, both sexes) were obtained from the animal house of PBRI, Bhopal. Following a one-week acclimatization period under standard laboratory conditions (25°C, 60 ± 5% relative humidity, 12-hour light-dark cycle), the animals were housed in appropriate cages and provided with standard pellet diet and water *ad libitum* [10]. All experimental procedures and animal care were conducted in accordance with CPCSEA guidelines under approved protocol (No-PBRI/IAEC/15-12-2023/017) from the Institutional Animal Ethics Committee of PBRI, Bhopal, India (CPCSEA Registration No: 1824/PO./ERe/S/15/CPCSEA).

### Preparation of Extract and Fractionations

One hundred grams of powdered *P. zeylanica* root material underwent Soxhlet extraction with ethanol for 72 hours at a temperature range of 55–60°C [11]. Post-extraction, the mixture was filtered, and the resulting filtrate was concentrated under reduced pressure at a temperature below 50°C using a rotary evaporator. After complete ethanol evaporation, the concentrated extract was lyophilized and stored at 4°C [12]. Ten grams of the ethanolic *P. zeylanica* root extract was fractionated using column chromatography. A glass column (60 cm × 3 cm) was packed with silica gel (60–120 mesh) [13]. The extract was loaded onto the column, and elution was carried out using a gradient solvent system of n-hexane, n-hexane-ethyl acetate, 100% ethyl acetate, and 100% methanol [14]. The elution process continued until approximately 90% of the loaded extract was recovered. Twenty-three fractions were collected initially, and those with similar TLC profiles were combined, resulting in four distinct fractions (F1–F4) [15]. The percentage yield of each fraction was determined relative to the initial weight of the ethanolic extract. Fractions displaying a single spot on TLC (n-hexane:ethyl formate, 9:1; Rf 0.37) were combined and evaporated to dryness to yield plumbagin [16]. TLC analysis was performed using precoated silica gel 60 F254 plates [17].

### Acute Toxicity Testing of *Plumbago zeylanica* Extract and Isolated Fractions

Acute oral toxicity was assessed in adult female Wistar albino rats using the fixed-dose method according to OECD Guideline No. 420, Annex 2d [18]. Following an overnight fast, the rats received a single oral dose of 2000 mg/kg body weight of *Plumbago zeylanica* extract suspended in 5% v/v Tween 80. Animals were closely monitored for the first three hours for any changes in general behavior, neurological, or autonomic responses [19]. Observations continued at 30-minute intervals for an additional three hours, followed by mortality checks at 24 hours and up to 14 days post-administration, in compliance with OECD guidelines [20].

**Antidiarrheal Activity of Extract and Isolated Fractions**

The *Plumbago zeylanica* extract was administered orally at doses of 200 and 400 mg/kg, prepared as a 1% Tween 80 suspension in distilled water [21]. Fractions F1, F2, and F4 were administered at 200 mg/kg, while F3 was administered at 2 mg/kg. The control group received the same experimental handling but received the dosing vehicle instead of the extract. Loperamide (3 mg/kg) in 1% Tween 80 served as the positive control and was also administered orally [22]. All extracts and controls were administered using a glass syringe fitted with an 18-gauge microsuction cannula.

**Castor Oil Induced Diarrhoea (Model - I)**

The castor oil-induced diarrhea model works by the hydrolysis of castor oil into ricinoleic acid. This fatty acid causes changes in water and electrolyte transport, leading to a hypersecretory response and diarrhea [23]. Following the method described by Bose et al., Wistar albino rats (150-200 g, both sexes) were divided into eight groups of six animals each. After a 24-hour fast with free access to water, the rats were orally administered the vehicle, the ethanolic extract of *Plumbago zeylanica* root, its fractions, or the standard loperamide. One hour post-treatment, each rat received 1 mL of castor oil orally [24]. The animals were then individually housed in cages lined with clean filter paper, and diarrheal episodes were observed over a 4-hour period. During this time, the total number of fecal pellets, the number of wet fecal pellets, and the total weight of feces were recorded [25].

**Table 1: Treatment protocol Castor Oil Induced Diarrhoea Model**

| Groups | Treatment |
|---|---|
| **Group I** | Normal control i.e. Normal saline solution |
| **Group II** | Standard (Loperamide 3mg/kg), orally |
| **Group III** | Treated with ethanolic extract of *Plumbago zeylanica* (200 mg/kg) |
| **Group IV** | Treated with ethanolic extract of *Plumbago zeylanica* (400mg/kg) |
| **Group V** | Treated with fraction F1 (200mg/kg) |
| **Group VI** | Treated with fraction F2 (200mg/kg) |
| **Group VII** | Treated with fraction F3 (2mg/kg) |
| **Group VIII** | Treated with fraction F4 (200mg/kg) |

**Charcoal Meal Test (Model - II)**

Wistar albino rats (150-200 g, both sexes) were fasted for 18 hours with free access to water. The rats were divided into eight groups of six, mirroring the grouping in the castor oil-induced diarrhea model. One hour after administration of the extracts, each animal received 1 mL of charcoal meal (3% deactivated charcoal in 2% aqueous Tween 80) via oral gavage [26]. Thirty minutes post-charcoal administration, the animals were euthanized, and the distance traveled by the charcoal meal from the pylorus to the cecum was measured and expressed as a percentage of total intestinal length [25].

**Statistical analysis**

Results were expressed as mean ± standard error of the mean. One-way analysis of variance followed by Dunnett's test was used to determine statistically significant differences between means, with $p < 0.05$ considered significant [27].

**Results and Discussion**

The roots of *Plumbago zeylanica* were processed using Soxhlet extraction with ethanol as the

solvent. This method efficiently extracted non-polar to moderately polar bioactive constituents, yielding approximately 11.45 g of crude brown residue. The resulting extract was stored at 4°C in airtight containers to maintain stability for further analysis. The ethanolic extract was fractionated using silica gel column chromatography. A stepwise gradient elution was performed using n-hexane and increasing proportions of ethyl acetate. Fractions of 100–150 mL were collected, guided by the appearance of colored bands and the total elution volume. The column was eluted until approximately 90% of the loaded extract was recovered. Twenty-three fractions were collected initially and pooled based on similar TLC profiles. This process resulted in four distinct fractions (F1-F4). The percentage yield of each fraction, relative to the starting weight of the ethanolic extract, is presented in Table 2.

**Table 2: Percentage yields of various fractions from the ethanolic extract of *P. zeylanica***

| Fraction | Colour | % Yield (W/W) |
|---|---|---|
| F1 | Yellowish | 28.13 |
| F2 | Yellowish brown | 42.55 |
| F3 | Orange | 2.81 |
| F4 | Yellowish brown | 22.37 |

Thin-layer chromatography analysis revealed a single spot with an Rf value of 0.37 (n-hexane:ethyl formate, 9:1) in fractions 17, 18, and 19. These fractions were combined and evaporated to dryness, yielding plumbagin. A yellow spot with an Rf value of 0.86 was observed under UV-visible light (Figure 1). While this Rf value is slightly lower than the reported value of 0.90 for plumbagin, the similarity suggests the isolated compound is indeed plumbagin. The minor discrepancy may be attributed to experimental variation.

**Figure 1: TLC Analyzing Plumbagin in Extract**

**Acute Toxicity of *Plumbago zeylanica* root Extract**

Acute oral toxicity studies in female rats administered a single 2000 mg/kg dose revealed no mortality during the 14-day observation period. This suggests a lack of acute lethality at this dose. Animals were monitored for behavioral, neurological, and autonomic changes for six

hours post-administration, with no adverse effects observed. This indicates the substance did not induce significant alterations in normal physiological or behavioral function during the monitoring period. These findings demonstrate the lack of acute toxicity of the *Plumbago zeylanica* extract at a dose of 2000 mg/kg in rats, supporting its safety at this concentration. Based on these results, doses of 200 and 400 mg/kg were selected for further investigation. The absence of mortality and adverse effects at the higher dose provides a strong indication of safety for the chosen lower doses. The acute toxicity results are summarized in Table 3.

**Table 3: Determination Acute Toxicity Testing of Ethanolic Extract of *Plumbago zeylanica***

| Treatment | Dose | No of animal used | Parameter | No of animal recovered | | |
|---|---|---|---|---|---|---|
| | | | | 24 hrs | 72 hrs | 14 days |
| Ethanolic extract of *Plumbago zeylanica* | 2000 mg /kg | 6 | Wt loss | Nil | Nil | Nil |
| | | | Death rate | Nil | Nil | Nil |
| | | | CNS toxicity | Nil | Nil | Nil |
| | | | Neurological disorder | Nil | Nil | Nil |

**Acute Toxicity of fractions from *Plumbago zeylanica* root Extract**

Acute toxicity assessment revealed that fractions F1, F2, and F4 exhibited no toxicity or mortality up to 2000 mg/kg. Consequently, a dose of 200 mg/kg was selected for subsequent experiments, ensuring a safe margin while facilitating detailed pharmacological investigation. In contrast, fraction F3 (containing plumbagin) displayed toxicity at doses exceeding 10 mg/kg. Therefore, a lower dose of 2 mg/kg was chosen for further studies involving F3 to maintain animal safety and minimize potential adverse effects. This cautious dose selection allows for continued investigation of plumbagin's therapeutic potential while prioritizing safety. The observed differences in toxicity among the fractions likely reflect variations in their chemical composition and bioactive constituents.

**Table 4: Observation of Acute Toxicity of Fractions from *Plumbago zeylanica* root Extract**

| S. No. | Treatment | No of Animal Used | Mortality Rate | | | Safety Profile |
|---|---|---|---|---|---|---|
| | | | 24 hrs | 48 hrs | 14 day | |
| 1 | F1 (2000mg/kg) | 6 | 0 | 0 | 0 | Safe |
| 2 | F2(2000mg/kg) | 6 | 0 | 0 | 0 | Safe |
| 3 | F3(10mg/kg) | 6 | 0 | 0 | 0 | Safe |
| 4 | F4(2000mg/kg) | 6 | 0 | 0 | 0 | Safe |

**Antidiarrheal Activity of Extract and Isolated Fractions**

**Castor Oil Induced Diarrhoea (Model - I)**

This study investigated the antidiarrheal effects of the ethanolic extract and isolated fractions

of *Plumbago zeylanica* roots using a castor oil-induced diarrhea model. The results, including total fecal count, wet fecal count, and total fecal weight, are presented in Table 5.

The control group exhibited characteristic diarrheal symptoms, with a mean of 4.85 ± 0.73 fecal pellets, of which 2.42 ± 0.51 were wet, and a total fecal weight of 528.06 ± 138.16 mg. As expected, loperamide significantly reduced these parameters (1.42 ± 0.24 total pellets, 0.76 ± 0.37 wet pellets, and 166.58 ± 39.35 mg total weight), confirming its antidiarrheal efficacy. The crude *Plumbago zeylanica* extract also demonstrated significant antidiarrheal activity at both 200 and 400 mg/kg, although less potent than loperamide. Notably, fraction F1 (200 mg/kg) showed considerable efficacy (1.42 ± 0.25 total pellets, 0.82 ± 0.25 wet pellets, and 86.00 ± 39.97 mg total weight), suggesting it may be a primary contributor to the crude extract's overall activity. Fraction F2 (200 mg/kg) demonstrated promising antidiarrheal activity, significantly reducing fecal output (1.45 ± 0.40 pellets, 0.61 ± 0.00 wet pellets, and 51.2 ± 1.20 mg total weight). However, fraction F3 (2 mg/kg), containing plumbagin, exhibited the most potent antidiarrheal effect, with the lowest recorded values for all fecal parameters (1.26 ± 0.20 pellets, 0.45 ± 0.00 wet pellets, and 20.6 ± 20.60 mg total weight). While F4 (200 mg/kg) also showed activity, its effects were more moderate. These results suggest that both the crude extract and its isolated fractions, especially F2 and F3, hold significant antidiarrheal potential, warranting further investigation into their active compounds. The superior potency of F3, despite the considerably lower dose, highlights the potential of plumbagin as a key active constituent.

**Table 5: Antidiarrhoeal Activity of Extract and Isolated Fractions using Castor Oil Induced Diarrhea**

| Group | Treatment | Dose mg/kg | Mean total no umber of faeces | Mean total number of wet faeces | Total Weight of faeces |
|---|---|---|---|---|---|
| 1 | Control | - | 4.85 ± 0.7348 | 2.42 ± 0.5099 | 528.06 ± 138.16 |
| 2 | Loperamide | 3 | 1.42 ± 0.2449** | 0.76 ± 0.3742 ** | 166.58 ± 39.349 ** |
| 3 | Crude extract | 200 | 1.66 ± 0.2449** | 0.95 ± 0.2449 ** | 226.25 ± 56.217 ** |
| 4 | Crude extract | 400 | 1.48 ± 0.5099 ** | 0.86 ± 0.2449 ** | 184.83 ± 64.055 ** |
| 5 | F1 | 200 | 1.42 ± O.2449 ** | 0.82 ± 0.2449 ** | 86.15 ± 39.974 ** |
| 6 | F2 | 200 | 1.45 ± 0.4000 ** | 0.61 ± 0.000 ** | 51.27 ± 1.200 ** |
| 7 | F3 | 2 | 1.26 ± 0.2000 ** | 0.45 ± 0.000 ** | 20.62 ± 20.600 ** |
| 8 | F4 | 200 | 1.56 ± 0.2449 ** | 1.26 ± 0.2000 ** | 166.51 ±39.740 ** |

ANOVA followed by Dunnet test, Value are in Mean ± SEM (n=5); Significance v/s Control group: ***P<0.01, **P<0.05

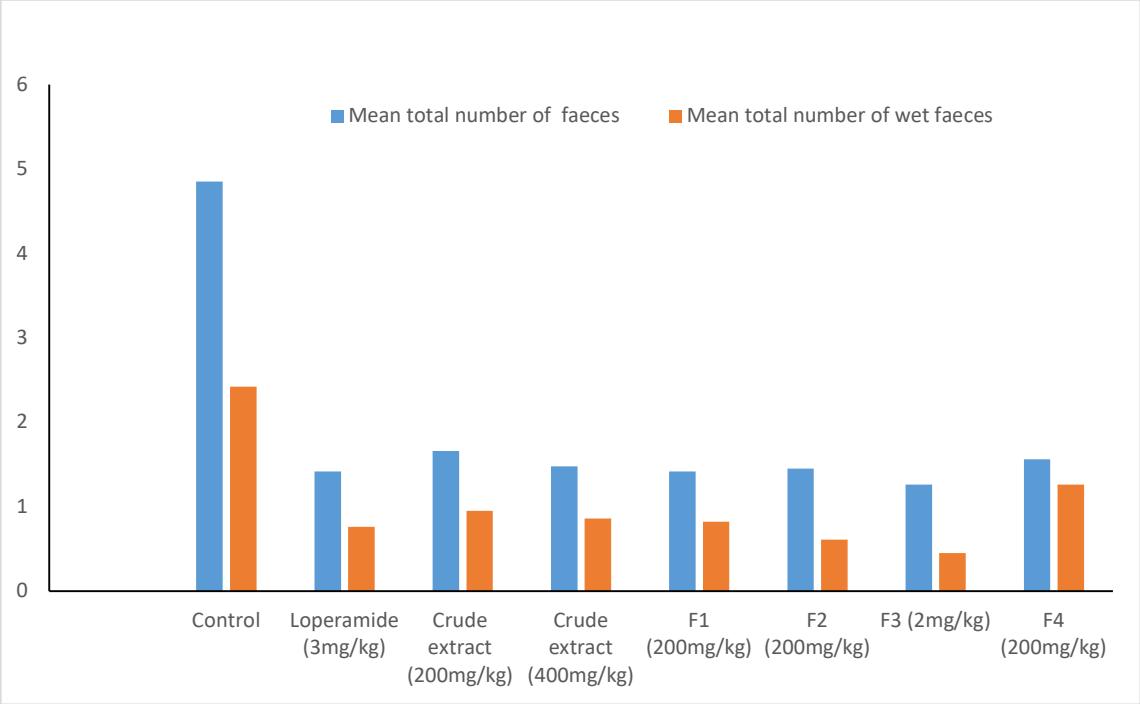


**Figure 2: Effect of Extract and Isolated Fractions on type of faeces in Castor Oil Induced Diarrhea**

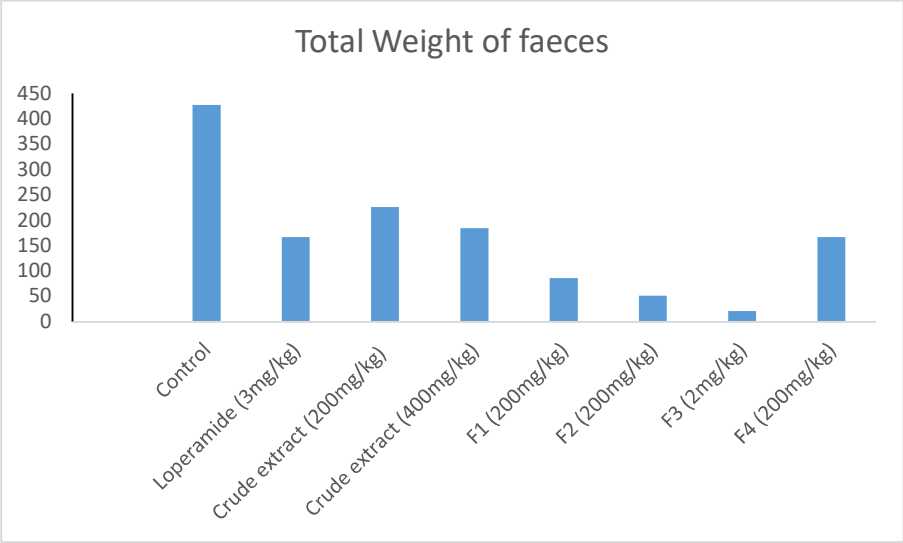


**Figure 3: Effect of Extract and Isolated Fractions on total weight of faeces in Castor Oil Induced Diarrhea**

This study demonstrates the antidiarrheal potential of *Plumbago zeylanica* root extract and its isolated fractions in a castor oil-induced diarrhea model. The crude extract showed dose-dependent efficacy, although less potent than loperamide. Fractions F2 and F3 exhibited the most significant antidiarrheal activity, with F3, containing plumbagin, displaying the greatest reduction in fecal parameters. These findings suggest plumbagin may be a key driver of the observed therapeutic effect, warranting further investigation. While F1 and F4 also demonstrated activity, their effects were less pronounced. Overall, these results support the potential of *Plumbago zeylanica* and its fractions, particularly F3, as candidates for natural

antidiarrheal agents.

**Charcoal Meal Test: - (Model - II)**

Evaluation of the antidiarrheal activity of *Plumbago zeylanica* root extract and its isolated fractions using the charcoal meal test demonstrated significant impacts on gastrointestinal motility. The control group exhibited a mean charcoal transit of 65.18 ± 1.74 cm, indicative of normal intestinal motility (Figure 4). Charcoal transit is a standard measure of gastrointestinal transit time, with increased transit correlating with faster motility. Loperamide, the positive control, significantly reduced charcoal transit (33.54 ± 1.45 cm), confirming its anti-motility action. The *Plumbago zeylanica* crude extract also significantly decreased charcoal transit at both 200 mg/kg (43.68 ± 1.27 cm) and 400 mg/kg (34.89 ± 1.83 cm) ($p < 0.01$), supporting its antidiarrheal properties.

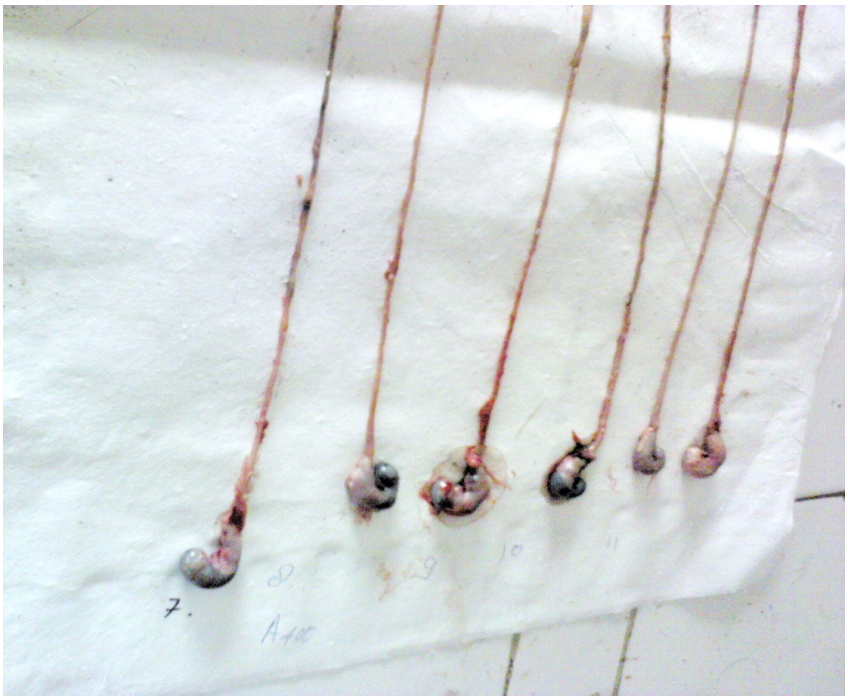

**Figure 4: Antidiarrhoeal activity of fraction F3 using Charcoal induced diarrhea model**

All isolated fractions demonstrated antidiarrheal activity in the charcoal meal test, albeit to varying degrees. F1 (200 mg/kg) and F2 (200 mg/kg) reduced charcoal transit to 40.72 ± 1.49 cm and 35.78 ± 1.64 cm, respectively ($p < 0.05$). Remarkably, F3 (2 mg/kg) exhibited the most substantial reduction (29.34 ± 1.68 cm) despite the significantly lower dose. F4 (200 mg/kg) also showed a reduction to 44.84 ± 1.37 cm. The superior potency of F3 reinforces the potential of plumbagin as a key active compound.

**Table 6: Antidiarrhoeal Activity of the Extract and Isolated Fractions in Charcoal Meal Test**

| Group | Treatment | Dose mg/kg | Mean % movement of charcoal (cm) |
|---|---|---|---|
| 1 | Control | - | 65.18 ± 1.741 |
| 2 | Lopermide | 3 | 33.54 ± 1.449 ** |
| 3 | Crude extract | 200 | 43.68 ± 1.273 ** |

| | | | |
|---|---|---|---|
| 4 | Crude extract | 400 | 34.89 ± 1.828 ** |
| 5 | F1 | 200 | 40.72 ± 1.489 * |
| 6 | F2 | 200 | 35.78 ± 1.640 * * |
| 7 | F3 | 2 | 29.34 ± 1.680 * * |
| 8 | F4 | 200 | 44.84 ± 1.372 * |

ANOVA followed by Dunnet test, Value are in Mean ± SEM (n=5); Significance v/s Control group: ***P<0.01, **P<0.05

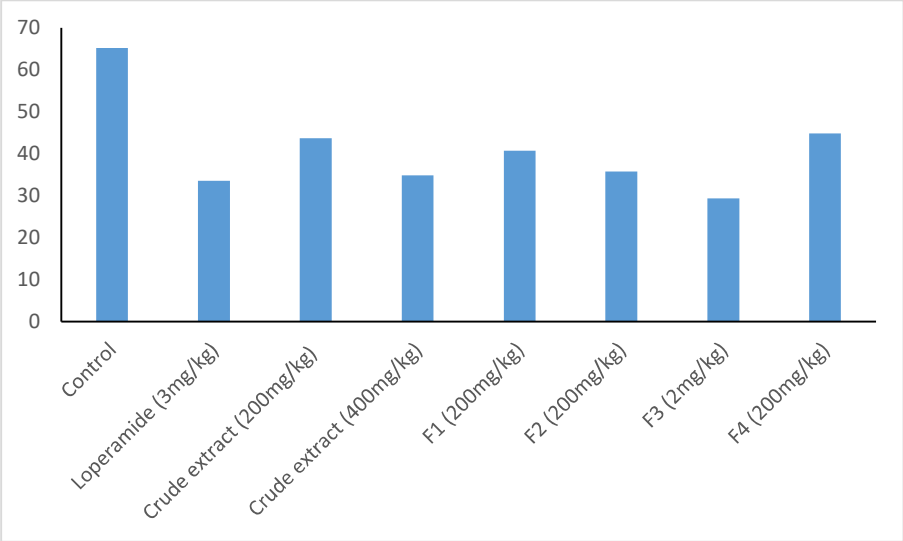


**Figure 5: Antidiarrhoeal Activity of the Extract and Isolated Fractions in Charcoal Meal Test**

This study's findings strongly support the antidiarrheal potential of *Plumbago zeylanica* root extract and its isolated fractions. The crude extract significantly reduced gastrointestinal motility at doses of 200 mg/kg and 400 mg/kg, suggesting a potential mechanism of action involving slowed intestinal transit. Isolated fractions, particularly F3 (containing plumbagin) at a lower dose of 2 mg/kg, exhibited even more pronounced effects, indicating the presence of potent bioactive constituents. The observed reduction in charcoal transit by these fractions suggests potential mechanisms involving the inhibition of intestinal motility or modulation of intestinal fluid dynamics, similar to the action of loperamide. Further research is warranted to elucidate the precise mechanisms of action and identify the specific bioactive compounds responsible for the observed antidiarrheal effects.

Fraction F3's potent antidiarrheal activity at a lower dose compared to the crude extract and other fractions highlights its potential as a source of key bioactive compounds. The significant reductions in charcoal transit observed with fractions F1, F2, and F4 indicate that multiple components within the *Plumbago zeylanica* root extract contribute to its overall antidiarrheal effect. These findings corroborate the traditional use of *Plumbago zeylanica* for gastrointestinal disorders. Further investigation into the specific active compounds, particularly within F3, is warranted and could lead to the development of novel antidiarrheal agents.

## Conclusion

This study demonstrates the antidiarrheal potential of *Plumbago zeylanica* root extract in a

castor oil-induced diarrhea model in Wistar rats. The extract's dose-dependent efficacy in delaying diarrhea onset, reducing fecal output, and decreasing fecal wet weight is comparable to loperamide, particularly at higher extract doses (400 mg/kg). The observed effects suggest potential mechanisms involving reduced intestinal motility, inhibited fluid secretion, and enhanced water and electrolyte absorption, aligning with traditional uses of *P. zeylanica* for gastrointestinal disorders. While these findings are promising, further research, including comprehensive toxicity studies and clinical trials, is essential to validate the safety and efficacy of *P. zeylanica* root extract as a potential therapeutic agent for diarrhea.

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**Conflict of Interest**

The authors declare no conflict of interest.

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