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Phytochemical Characterization, TLC Fingerprinting, and Evaluation of the Laxative Activity of *Putranjiva roxburghii* Seed Extracts in Loperamide-Induced Constipation

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ABSTRACT

Background: Constipation is a common gastrointestinal disorder characterized by infrequent bowel movements, hard stools, and difficulty in defecation. Although several synthetic laxatives are available, their prolonged use is often associated with undesirable adverse effects. Putranjiva roxburghii Wall. seeds have traditionally been used for the management of constipation; however, scientific evidence supporting their efficacy and safety remains limited.

Purpose: The present study aimed to investigate the phytochemical composition, thin-layer chromatography (TLC) fingerprint profile, acute oral toxicity, and laxative potential of successive seed extracts of Putranjiva roxburghii Wall. in a loperamide-induced constipation model.

Methods: Successive extraction of Putranjiva roxburghii Wall. seeds was performed using petroleum ether (60–80°C), ethyl acetate, and hydroalcoholic solvent (ethanol:water (70:30 v/v)) in a Soxhlet apparatus. Preliminary phytochemical screening and TLC fingerprint analysis were carried out to characterize the extracts. Acute oral toxicity was evaluated according to OECD Guideline 420. Constipation was induced in rats using loperamide (3 mg/kg, p.o.), and the laxative activity of the extracts was assessed by measuring fecal output, wet fecal weight, fecal water content, and gastrointestinal transit. Bisacodyl (0.25 mg/kg) served as the standard reference drug.

Results: The hydroalcoholic extract (HAE) exhibited the highest extraction yield (7.9%) and contained flavonoids, alkaloids, tannins, saponins, steroids, and terpenoids. TLC fingerprint analysis demonstrated greater chromatographic complexity in HAE than in the petroleum ether (PEE) and ethyl acetate (EAE) extracts. No mortality or evident signs of acute toxicity were observed following oral administration of HAE and EAE at 2000 mg/kg body weight under the conditions of the acute toxicity study. Pharmacological evaluation revealed that HAE significantly improved fecal parameters and gastrointestinal transit, with generally greater effects observed at 400 mg/kg than at 200 mg/kg. At 400 mg/kg, HAE produced effects of a similar magnitude to those observed with bisacodyl (0.25 mg/kg).

Conclusion: The findings provide scientific evidence supporting the traditional use of Putranjiva roxburghii Wall. seeds for the management of constipation. The hydroalcoholic seed extract demonstrated significant laxative and gastrointestinal motility-enhancing activities while exhibiting a favorable acute toxicity profile, suggesting its potential as a natural therapeutic agent for the treatment of constipation.

1. Introduction

Constipation is a serious digestive ailment that is faced by people of all ages around the world. It is characterised by infrequent bowel motions, difficulty passing faeces, firm stools, prolonged straining during defecation, and a sensation of incomplete evacuation (Abhimanyu *et al.*, 2017). Although there are a number of pharmacological agents available for the management of constipation, including stimulant laxatives, osmotic laxatives, stool softeners and prokinetic agents, its long-term usage is often linked to negative side consequences, inconsistent efficacy and poor patient compliance. Thus, there has been increased interest in the search for medicinal herbs historically used for the treatment of constipation (Bijekar *et al.*, 2007).

Medicinal plants have served as a vital source of therapeutic substances since the dawn of civilisation (Ali *et al.*, 2025). A significant portion of the global population relies on conventional herbal treatments. Herbal medicines are often believed to be safer, cheaper and culturally acceptable alternatives to synthetic pharmaceuticals. Numerous medicinal plants have been examined scientifically to substantiate their traditional claims (Patil *et al.*, 2014).

Putranjiva roxburghii Wall. Putranjivaceae, often called Putranjiva or the Child Life tree, is an evergreen tree found extensively in the Indian subcontinent and several Southeast Asian countries (Reanmongkol *et al.*, 2009). Different components of the plant, mainly seeds and leaves, have been employed in the traditional medicinal systems for the treatment of various maladies such as inflammation, fever, infertility, dysuria, ophthalmic disorders and gastrointestinal disturbances. Traditionally the seeds of *Putranjiva roxburghii* Wall. are used as a laxative for the management of constipation (Balkrishna *et al.*, 2020).

Several pharmacological investigations have shown that various portions of the plant possess several pharmacological activities such as antibacterial, antioxidant, anti-inflammatory, antipyretic, antinociceptive, antiparasitic, anticancer and fertility-enhancing activities. *Putranjiva roxburghii* Wall. Seeds have been traditionally used as a cure for constipation, but scientific evidence confirming the laxative effect of *Putranjiva roxburghii* Wall. is limited. Seeds are still few (Dar *et al.*, 2019).

Hence, the present investigation was undertaken to evaluate the phytochemical profile and laxative activity of EAE and HAE of *Putranjiva roxburghii* Wall. seeds. The present study findings may provide scientific legitimacy to the traditional use of *Putranjiva roxburghii* Wall. seeds in the management of constipation and help in the development of plant-based medicinal medicines for gastrointestinal problems (Isdoni *et al.*, 2025).

2. Materials and Method

2.1. Plant Material Collection and Authentication

Seeds of *Putranjiva roxburghii* Wall. were procured in June 2021 from a local herbal raw drug supplier in Haridwar, Uttarakhand, India. The seeds were cleaned to remove extraneous matter and stored under appropriate conditions until further processing. The taxonomic authentication of the plant material was carried out by Dr Ashok Kumar, Department of Botany, IFTM University, Moradabad, Uttar Pradesh, India. A voucher specimen was made and deposited in the herbarium of the Department of Botany with voucher number 2021/SOS/BOT/122 for future reference. All the phytochemical and pharmacological studies used the verified seed material.

2.2. Preparation of Extracts

The collected seeds were sun-dried at an ambient temperature of approximately 30–35°C for 7–10 days, until a constant weight was achieved, and were subsequently mechanically ground into a coarse powder. The coarse powder was then subjected to successive Soxhlet extraction using petroleum ether (60–80°C), ethyl acetate, and hydroalcoholic solvent (ethanol:water, 70:30 v/v) (Uddin *et al.*, 2024). Extraction continued until the plant material was fully depleted. The extracts obtained were concentrated at decreased pressure in a rotary vacuum evaporator, and residual solvents were removed by drying in a vacuum oven at temperatures not exceeding 50°C. The dried extracts were kept in desiccators until further use. The PEE, EAE and HAE were labelled accordingly. Figure 1 given below shows the crushed seeds of *Putranjiva roxburghii* Wall. and the Soxhlet extraction process (Mehra & Sah, 2026).

Figure 1: *Putranjiva roxburghii* Wall. Crushed Seeds and Soxhlet Extraction Process

2.3. Determination of Percentage Yield

The extraction yield of each extract was evaluated on a dry weight basis. The percentage yield was calculated using the following equation:

$$Percentage\,Yield\,(\%) = (Weight\ of\ Dried\ Extract\ /\ Weight\ of\ Crude\ Powdered\ Drug) \times 100$$

The yield and physical parameters of the resulting extracts were then recorded and compared (Haidara *et al.*, 2026).

2.4. Preliminary Phytochemical Screening

A preliminary phytochemical test of PEE, EAE, and HAE was conducted using standard qualitative procedures. The extracts were analyzed for various secondary metabolites. The presence or absence of particular phytoconstituents was determined by the characteristic color responses or development of precipitate by the standard phytochemical procedures (Muthukumaran *et al.*, 2016).

2.5. TLC Fingerprinting

Putranjiva roxburghii Wall. seed extracts were carried out on silica gel 60 F254 precoated plates (0.2 mm thickness). A TLC applicator from Camag Linomat-5 was used for band application, and a photo documentation unit (Camag Reprostar-3: 140604) was used for documentation of chromatographic fingerprints (Kumar *et al.*, 2026).

The test solutions (8 µL each) were applied as 6 mm bands, and the plate was developed in a solvent system consisting of toluene and ethyl acetate (7:3) to a distance of 9 cm. The developed plate was air-dried and examined under ultraviolet light at 254 nm and 366 nm before derivatization (Saji *et al.*, 2026). The plate was derivatized using a 5% methanolic–sulfuric acid reagent and heated at 105 °C until the bands became clearly visible. The plate was subsequently examined under UV light at 366 nm. The retention factor (Rf) values were determined using the following equation:

$$Rf = \text{Distance traveled by the compound} / \text{Distance traveled by the solvent front}$$

The number of spots, color features, and corresponding Rf values were recorded to establish characteristic chromatographic fingerprints of the samples (Kumar *et al.*, 2026).

2.6. Experimental Animals

Pharmacological tests were performed using healthy Wistar albino rats weighing 150–200 g of both sexes (Ali *et al.*, 2025). The animals were sourced from the Central Animal House Facility at IFTM University, Moradabad, Uttar Pradesh, India. They were housed in polypropylene cages under standard laboratory conditions, which included a 12-hour light/dark cycle, a temperature of 25 ± 2°C, and a relative humidity of 55 ± 5%. Water and a standard pellet diet were provided ad libitum. Animals were acclimatized to the laboratory environment for one week prior to experimentation (Wang *et al.*, 2025). All experimental procedures involving animals were reviewed and approved by the Institutional Animal Ethics Committee (IAEC), IFTM University, Moradabad, Uttar Pradesh, India (Approval No. IAEC/2022/43; dated 27 January 2022), in accordance with the guidelines of the Committee for the Purpose of Control and Supervision of Experiments on Animals (CPCSEA), Government of India (CPCSEA Registration No. 837/PO/ReBiBt/S/04/CPCSEA).

2.7. Acute Oral Toxicity Study

The fixed-dose procedure was employed to assess the acute oral toxicity of EAE and HAE, following the Organization for Economic Co-operation and Development (OECD) guideline 420 (Rao, 2019). Experimental animals received an oral dose of 2000 mg/kg body weight. Animals were continuously observed during the initial four hours after treatment. For fourteen days, abnormal behavior was observed. The absence of mortality and overt signs of acute toxicity at the administered dose indicated that the extracts were well tolerated under the conditions of the acute toxicity study and provided a basis for selecting lower doses for subsequent pharmacological evaluation (Dibaba *et al.*, 2026).

2.8. Evaluation of Laxative Activity

2.8.1. Loperamide-induced Constipation Model

Experimental constipation was induced by oral administration of the peripherally acting opioid receptor agonist loperamide (3 mg/kg, p.o.), which is known to reduce intestinal motility and intestinal fluid secretion (Inatomi & Honma, 2021). A total of 42 animals were randomly divided into seven groups, with six animals in each group (n = 6). Group I served as the normal control and received the vehicle (10 mL/kg, p.o.), while Group II served as the constipated control and received loperamide with the vehicle. Groups III and V received the ethyl acetate extract (EAE) at doses of 200 and 400 mg/kg, p.o., respectively, whereas Groups IV and VI received the hydroalcoholic extract (HAE) at doses of 200 and 400 mg/kg, p.o., respectively, along with loperamide. Group VII received bisacodyl (0.25 mg/kg, p.o.) as the standard laxative treatment. Following drug administration, the animals were housed individually in metabolic cages for fecal collection. The total number of fecal pellets, wet fecal weight, and dry fecal weight (after drying to a constant weight) were recorded over a 12-hour period. The percentage of fecal water content was subsequently calculated to evaluate the laxative activity of the test extracts (Fadholly *et al.*, 2026).

The following equation was used to compute the fecal water content:

$$(Faecal\,Water\,Content\,\%) = \left[(Wet\,Faecal\,Weight - Dry\,Faecal\,weight)/Wet\,Faecal\,Weight\right]\times 100$$

The laxative activity was evidenced by an increase in fecal output and fecal water content compared with the constipated control group (Jang *et al.*, 2024).

2.9. Gastrointestinal Motility Study

The extracts were investigated for their effect on gastrointestinal transit using the charcoal meal technique in loperamide-induced constipated rats. After treatment, rats were given an oral suspension of charcoal (Na *et al.*, 2021). At the end of the experimental period, the rats were killed, and the small intestine was meticulously dissected out. The entire length of the small intestine and the distance covered by the charcoal tracer were measured (Padmanabhan *et al.*, 2013). The intestinal transit time was determined using the following calculation:

$$Intestinal\,Transit\,(\%) = (Distance\,Travelled\,by\,Charcoal\,Marker / Total\,Length\,of\,Small\,Intestine) \times 100$$

Increased intestinal transit compared to the constipated control group was suggestive of an increase in the gastrointestinal motility (Feder *et al.*, 2018).

2.10. Statistical Analysis

Data were expressed as mean ± SEM. Statistical analysis was performed using one-way ANOVA followed by Dunnett's multiple comparison test. $P < 0.05$ was considered significant.

3. Results and Discussion

3.1. Extraction Yield and Physical Characteristics of Seed Extracts

Different extraction efficiencies of *Putranjiva roxburghii* Wall. Seeds with petroleum ether, ethyl acetate, and hydroalcoholic solvent systems by successive Soxhlet extraction were observed. HAE gave the maximum extraction yield (7.9%), followed by EAE (5.8%), while the lowest yield was obtained by PEE (2.0%). The PEE was greenish yellow and oily in consistency, the EAE was light yellow and greasy, and the HAE was blackish brown and sticky in nature (Table 1). Figure 2 given below shows different types of extracts.

Table 1: Extraction Yield and Physical Characteristics of *Putranjiva roxburghii* Wall Seed Extracts

Extract	Yield (%)	Appearance	Consistency
PEE	2.0	Greenish yellow	Oily
EAE	5.8	Light yellow	Greasy
HAE	7.9	Blackish brown	Sticky

Note: PEE: Petroleum Ether Extract; **EAE:** Ethyl Acetate Extract; **HAE:** Hydroalcoholic Extract

The increased yield of HAE could be ascribed to the capacity of hydroalcoholic solvents to solubilise a wider variety of polar and moderately polar phytoconstituents.

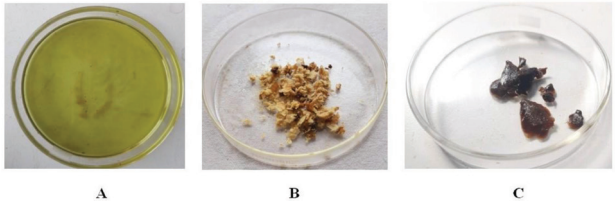


Figure 2: Extracts (A-PEE, B-EAE, and C-HAE)

3.2. Preliminary Phytochemical Screening

Qualitative phytochemical analysis showed significant variation among the extracts. The PEE contained predominantly fats and fixed oils. The EAE contained carbohydrates, proteins, amino acids, alkaloids, flavonoids, saponins and fixed oils. The HAE displayed the broadest phytochemical spectrum and was positive for carbohydrates, proteins, amino acids, alkaloids, flavonoids, tannins, saponins, steroids, terpenoids and fixed oils. The results of the preliminary phytochemical screening are presented in Table 2.

Table 2: Preliminary Phytochemical Screening of *Putranjiva roxburghii* Wall. Seed Extracts

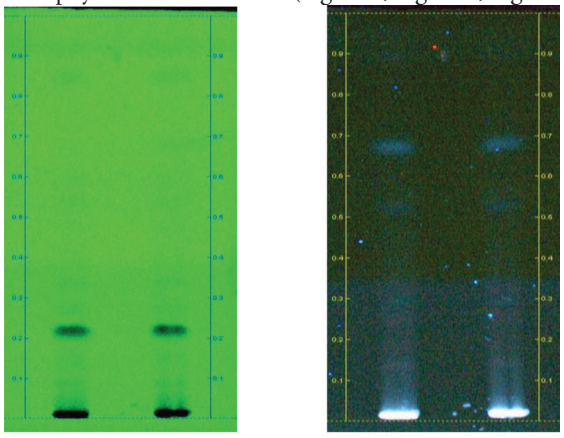
Secondary Metabolites	PEE	EAE	HAE
Carbohydrates	–	+	+
Proteins	–	+	+
Amino acids	–	+	+
Fixed oils and fats	+	+	+
Steroids	–	–	+

Glycosides	–	–	–
Flavonoids	–	+	+
Alkaloids	–	+	+
Tannins	–	–	+
Saponins	–	+	+
Terpenoids	–	–	+
Phenolics	–	–	–

Note: PEE: Petroleum Ether Extract; **EAE:** Ethyl Acetate Extract; **HAE:** Hydroalcoholic Extract (+ Present; – Absent)

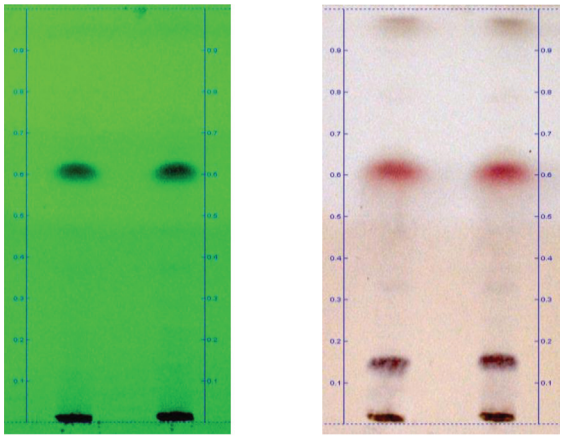
3.3. TLC Fingerprint Analysis

TLC fingerprinting was used to establish the characteristic chromatographic profiles of the extracts. Different chromatographic profiles were found for each extract using solvent system toluene: ethyl acetate (7: 3) indicating variances in their phytochemical content (Figure 3, Figure 4, Figure 5).



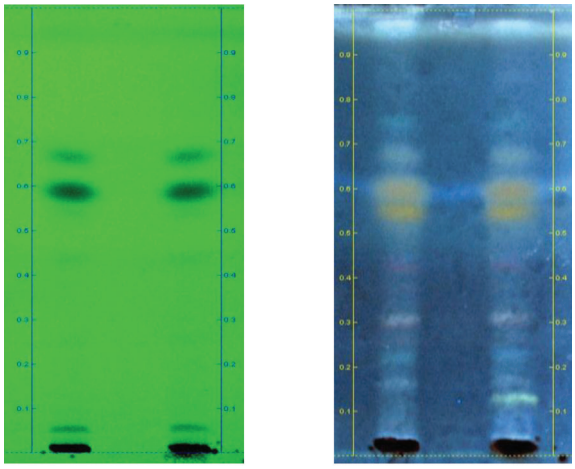
254nm **366nm**

Figure 3: TLC fingerprints profile of PEE of seeds of *Putranjiva roxburghii* Wall.



254nm **366nm**

Figure 4: TLC fingerprints profile of EAE of seeds of *Putranjiva roxburghii* Wall.



254nm **366nm**

Figure 5: TLC Fingerprints Profile of HAE of Seeds of *Putranjiva roxburghii* Wall.

Table 3: TLC Fingerprint Profile of *Putranjiva roxburghii* Wall. Seed Extracts

Extract	Spot No.	Rf Value (Before Derivatization)	Rf Value (After Derivatization)	Colour
PEE	1	0.22	0.30	Sky blue
PEE	2	–	0.68	Sky blue
EAE	1	0.62	0.14	Brown
EAE	2	–	0.60	Reddish brown
EAE	3	–	0.95	Brown
HAE	1	0.04	0.13	Sky blue
HAE	2	0.11	0.18	blue
HAE	3	0.30	0.32	Light band
HAE	4	0.41	0.43	Purple
HAE	5	0.60	0.60	Yellow
HAE	6	0.68	0.74	Sky blue

Note: PEE: Petroleum Ether Extract; **EAE:** Ethyl Acetate Extract; **HAE:** Hydroalcoholic Extract

As shown in Table 3, HAE displayed the most complex chromatographic pattern with six spots over a wide range of Rf values, while PEE and EAE showed relatively fewer spots. The greater chromatographic complexity of HAE was consistent with its broader qualitative phytochemical profile and suggested the presence of a greater diversity of extractable constituents.

TLC fingerprinting is a significant quality-control tool for herbal medicines since it provides a characteristic

chemical profile for identification and standardization. The chromatographic fingerprints obtained in the present investigation could be used as reference profiles for future authentication and quality control of *Putranjiva roxburghii* Wall. seed extracts (Balkrishna *et al.*, 2020).

3.4. Selection of Extracts for Pharmacological Evaluation

The selection of extracts for pharmacological evaluation was made on the basis of extraction yield, phytochemical content, and TLC fingerprint analysis. Three extracts were produced from the seeds of *Putranjiva roxburghii* Wall.; however, only the EAE and HAE were selected for further biological studies. The PEE showed the lowest extraction yield (2.0%) and was found to include mostly fats and fixed oils, while most of the pharmacologically relevant secondary metabolites such as alkaloids, flavonoids, tannins, saponins, steroids, and terpenoids were lacking.

This was further supported by TLC fingerprinting. The chromatographic profile of PEE exhibited only two spots, reflecting the low phytochemical complexity. On the other hand, chromatographic profiles of EAE and HAE were more complex, with several well-resolved spots across a larger range of Rf values. For instance, the HAE showed the highest number of spots, which indicated the most diversity of phytochemical contents. TLC fingerprint analysis is a useful approach for qualitative assessment of phytochemical variety and gives vital information on the complexity and chemical richness of plant extracts. Thus, the higher number of TLC spots observed in EAE and HAE suggested greater chemical complexity and the presence of a wider range of extractable constituents.

Furthermore, phytochemical screening found both EAE and HAE contained alkaloids, flavonoids, and saponins, while HAE further contained tannins, steroids, and terpenoids. The classes of phytoconstituents found to have gastrointestinal stimulatory, secretory, and laxative activities. Most of these secondary metabolites were not present in PEE, which exhibited minimal chromatographic variety and was less likely to be responsible for the traditionally claimed laxative effect of the plant. Thus, PEE was not included in the following pharmacologic experiments, and only EAE and HAE were selected for the acute toxicity, laxative action, and gastrointestinal motility tests.

3.5. Acute Toxicity Study and Dose Selection

The safety profile of EAE and HAE of *Putranjiva roxburghii* Wall. seeds was assessed by an acute oral toxicity study using OECD Guideline 420 (Fixed Dose Procedure). The two extracts were delivered orally at a limited dose of 2000 mg/kg body weight, and the experimental rats were monitored continuously for the first four hours after delivery and regularly for the next fourteen days (Rao, 2019).

No mortality was observed in any of the treated rats during the observation period. Furthermore, no significant symptoms of toxicity, such as tremors, convulsions, salivation, diarrhea, lethargy, sleep disruptions, behavioral abnormalities, respiratory distress, change in locomotor activity, or change in feeding and drinking habits, were noted. During the entire trial time, the rats were active and healthy, indicating that both the extracts were well tolerated at the dose supplied (Intatham *et al.*, 2026).

At a dose of 2000 mg/kg body weight, neither fatalities nor severe toxic symptoms were observed. The absence of mortality at 2000 mg/kg suggests that the median lethal dose (LD_{50}) is greater than 2000 mg/kg under the conditions of this study. According to the Globally Harmonized System (GHS), compounds with an LD50 value greater than 2000 mg/kg are generally classified as having low acute toxicity. These findings indicate that EAE and HAE were well tolerated at 2000 mg/kg under the conditions of the present acute oral toxicity study and supported the selection of lower doses for subsequent pharmacological evaluation (Kala *et al.*, 2021).

The doses used for the subsequent laxative and gastrointestinal motility investigations were fractions of the greatest non-toxic dose based on the results of the acute toxicity trial. This is a frequent strategy in pharmacologic inquiry to guarantee that pharmacologically efficacious doses are used, but with a sufficient margin of safety. Based on the acute toxicity findings, doses of 200 and 400 mg/kg body weight, corresponding to one-tenth and one-fifth of the tested 2000 mg/kg dose, respectively, were selected for subsequent pharmacological evaluation.

The dose calculations were performed as follows:

$$Highest\ non-toxic\ dose = 2000\ mg\ /\ kg\ body\ weight$$
$$Low\ dose = 2000 / 10 = 200\ mg\ /\ kg\ body\ weight$$
$$High\ dose = 2000 / 5 = 400\ mg\ /\ kg\ body\ weight$$

Therefore, the EAE and HAE were tested at 200 mg/kg and 400 mg/kg body weight levels. The use of two dose levels permitted the evaluation of any dose-dependent pharmacological response and allowed the comparison of the efficacy of the extracts in experimental models of constipation.

The absence of mortality and overt signs of toxicity at 2000 mg/kg indicates a favorable acute toxicity profile for EAE and HAE under the conditions of the present study. These findings support further preclinical investigation of the extracts, including sub-chronic and chronic toxicity studies, to more comprehensively establish their safety profiles.

3.6. Laxative Activity in Loperamide-Induced Constipated Rats

The laxative potential of EAE and HAE of *Putranjiva roxburghii* Wall. seeds was studied using a model of loperamide-induced constipation in rats. Loperamide administration resulted in lower fecal pellet output, wet fecal weight, and fecal water content in the constipated control group than in the normal control group, indicating successful induction of constipation. Table 4 shows the effects of EAE and HAE on several fecal parameters.

Table 4: Effect of *Putranjiva Roxburghii* Wall. Seeds Extracts on Fecal Parameters in Loperamide-Induced Constipated Rats

Group	Dose	No. of faeces (12 h)	Wet faecal weight (g)	Dry faecal weight (g)	Faecal water content (%)
Normal Control	10 ml/kg	10.40 ± 1.30	1.82 ± 0.05	0.78 ± 0.02	57.14 ± 1.34
Constipated Control	10 ml/kg	5.80 ± 0.80	0.87 ± 0.04	0.50 ± 0.03	34.48 ± 0.50
EAE	200 mg/kg	8.80 ± 0.85	1.00 ± 0.04	0.63 ± 0.03	37.05 ± 1.34
HAE	200 mg/kg	10.02 ± 1.82	3.00 ± 0.03*	1.56 ± 0.02	48.00 ± 1.18*
EAE	400 mg/kg	9.90 ± 1.02	1.81 ± 0.02	0.90 ± 0.04	50.28 ± 1.48*
HAE	400 mg/kg	11.60 ± 1.18**	5.88 ± 0.04**	2.02 ± 0.02**	65.65 ± 1.50**
Bisacodyl	0.25 mg/kg	11.90 ± 1.00**	6.19 ± 0.09**	2.12 ± 0.04**	66.10 ± 2.76**

Note: Values are Mean ± SEM (n = 6). *P < 0.05, **P < 0.01 compared with constipated control.

HAE improved the evaluated faecal parameters, with greater effects generally observed at 400 mg/kg than at 200 mg/kg. The wet faecal weight was considerably raised from 0.87 ± 0.04 g in the constipated control group to 3.00 ± 0.03 g and 5.88 ± 0.04 g after treatment with HAE at 200 and 400 mg/kg, respectively. The faecal water content was also increased from 34.48 ± 0.50% in the constipated control group to 48.00 ± 1.18% and 65.65 ± 1.50% after administration of HAE at 200 and 400 mg/kg, respectively. The highest dose of HAE produced an effect similar in magnitude to bisacodyl (66.10 ± 2.76%).

In comparison, EAE had relatively modest impacts. The extract did not show much rise in wet faecal weight except for a minor increase of faecal water content at 400 mg/kg, indicating less laxative activity than HAE. The superior activity of HAE suggests that the bioactive constituents responsible for the observed effects are likely to be present in HAE.

The increase in wet faecal weight and faecal water content observed suggests an increase in intestinal secretion and a decrease in the reabsorption of water from the intestinal lumen. Loperamide produces constipation by decreasing gastrointestinal motility and increasing intestinal fluid absorption. Hence, the reversal of these effects by HAE indicates a considerable laxative activity.

3.7. Effect on Gastrointestinal Motility

The effect of *Putranjiva roxburghii* Wall. seed extracts on gastrointestinal transit was studied by the charcoal meal transit technique. The gastrointestinal transit ratio is an essential index of intestinal motility and represents the ability of a test agent to promote intestinal content propulsion. The results showed that HAE increased intestinal transit, with a greater numerical effect observed at 400 mg/kg than at 200 mg/kg (Figure 6).

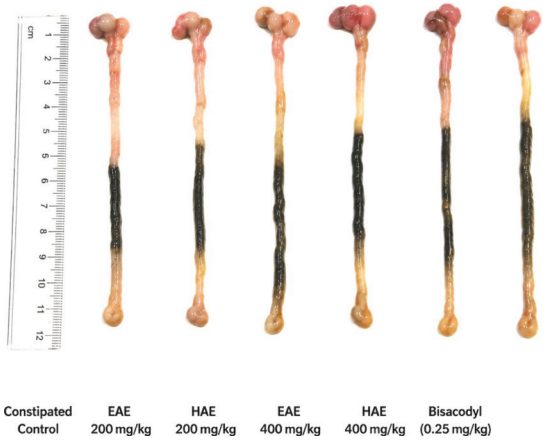


Figure 6: Effect of EAE and HAE on Gastrointestinal Motility in Albino Rats Using Charcoal Meal Transit Method. Dark Portion Indicates Distance Travelled by Charcoal Meal Through Intestine. Values Were Compared with Constipated Control Group

Table 5: Effect of *Putranjiva roxburghii* Wall. Seed Extracts on Gastrointestinal Motility in Loperamide-Induced Constipated Rats

Treatment Group	Gastrointestinal Transit Ratio (%)
Normal Control	67.01± 6.25
Constipated Control	44.52 ± 7.01

EAE 200 mg/kg	53.07 ± 7.27
HAE 200 mg/kg	63.57 ± 4.27**
EAE 400 mg/kg	57.02 ± 4.07
HAE 400 mg/kg	68.05 ± 8.81***
Bisacodyl (0.25 mg/kg)	69.12 ± 8.01***

Note: Values expressed as Mean ± SEM; **P < 0.01, ***P < 0.001 compared with constipated control.

Administration of HAE of *Putranjiva roxburghii* Wall. seeds significantly enhanced gastrointestinal motility in loperamide-induced constipated rats, with a greater numerical increase observed at 400 mg/kg than at 200 mg/kg (Table 5). The gastrointestinal transit ratio increased from 44.52 ± 7.01% in the constipated control group to 63.57 ± 4.27% and 68.05 ± 8.81% following treatment with HAE at doses of 200 and 400 mg/kg, respectively. At the higher dose, HAE produced an effect similar in magnitude to that of the standard laxative bisacodyl (69.12 ± 8.01%), indicating a marked improvement in intestinal propulsion.

In contrast, the EAE produced only modest improvements in gastrointestinal transit. Although both doses increased the transit ratio compared with the constipated control group, the response was considerably lower than that observed with HAE, suggesting that the hydroalcoholic extract possesses superior gastrointestinal motility-promoting activity.

Loperamide induces constipation primarily by suppressing intestinal motility through activation of peripheral opioid receptors, thereby delaying intestinal transit and increasing the absorption of water from the intestinal lumen. The increase in gastrointestinal transit observed following HAE treatment indicates reversal of loperamide-induced impairment of intestinal motility, with transit values approaching those observed in the normal control group. The faster movement of intestinal contents reduces the time available for water reabsorption, thereby contributing to softer stools and increased fecal water content. Thus, the enhanced intestinal transit may partly explain the improvements in fecal output, wet fecal weight, and fecal water content observed in the laxative study.

The superior activity of HAE may be associated with the presence of multiple phytoconstituents, including flavonoids, alkaloids, saponins, tannins, steroids, and terpenoids, which were detected during preliminary phytochemical screening. These classes of secondary metabolites have been reported to influence gastrointestinal motility and intestinal secretion. However, further phytochemical characterization and mechanistic studies are required to identify the specific bioactive constituents responsible for the observed pharmacological effects.

4. Limitations of the Study

There are various limitations to the present investigation. Firstly, the pharmacological assessment was carried out only in experimental animal models. Secondly, phytochemical screening and TLC fingerprinting were carried out, but quantitative quantification of specific phytoconstituents was not carried out. Third, the precise chemical mechanism responsible for the laxative activity was not studied. Also, long-term toxicity and chronic safety assessment were not included in this investigation. Finally, the observed pharmacological effects were not clinically proven.

5. Conclusion

In the present investigation, it has been found out that the seeds of *Putranjiva roxburghii* Wall. have shown laxative potential. In case of successive extraction, HAE has maximum extraction percentage (7.9%) and diverse phytochemical composition, which includes alkaloids, flavonoids, tannins, saponins, steroids, and terpenoids. In TLC fingerprinting, HAE has more complex chromatogram than other extracts.

From the results of acute oral toxicity study, it can be said that both EAE and HAE were nontoxic at 2000 mg/kg body weight in the experimental condition. In the case of loperamide-induced constipation animal model, it was observed that HAE has shown better laxative activity in comparison to EAE, especially at 400 mg/kg dose in terms of fecal output, wet/dry fecal weight and fecal water content. In addition to this, HAE also showed a significant increase in gastrointestinal motility, which at 400 mg/kg was almost similar to bisacodyl. It has been seen that this study provides the experimental basis for the folk use of *Putranjiva roxburghii* Wall. seeds in the treatment of constipation, and also HAE is a better extract among all other extracts.

6. Future Scope

The present study shows preliminary evidence for laxative activity of *Putranjiva roxburghii* Wall. seeds. However, further studies are required to establish its medicinal potential. Future studies should address isolation and pharmacological evaluation of the individual bioactive compounds responsible for the observed laxative activity. Detailed mechanistic investigations, including intestinal secretion, electrolyte transport, modulation of neurotransmitters, and contractility of smooth muscle, should clarify the precise mode of action.

Long-term safety requires chronic toxicity, subchronic toxicity, and reproductive toxicity investigations. Additionally, formulation development and pharmacokinetic studies

may help to generate standardized herbal products. Finally, clinical trials in human subjects are needed to confirm efficacy, safety, dosage optimization, and therapeutic application in individuals suffering from persistent constipation.

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Authorship Contribution

Harpreet Singh: Writing – review & editing, Writing – original draft, Formal analysis, Supervision. Amit Kumar Rishi: Writing – review & editing, Writing – original draft, Visualization, Arvind Kumar: Formal analysis, Conceptualization. Arun Kumar Mishra: Writing – review & editing, Writing – original draft.

Conflict of Interest

The authors declare that there is no conflict of interest regarding this manuscript.

Ethical Approval

The animal experimental protocol was reviewed and approved by the Institutional Animal Ethics Committee (IAEC), IFTM University, Moradabad, Uttar Pradesh, India (Approval No. IAEC/2022/43; dated 27 January 2022). All animal experiments were conducted in accordance with the guidelines of the Committee for the Purpose of Control and Supervision of Experiments on Animals (CPCSEA), Government of India (CPCSEA Registration No. 837/PO/ReBiBt/S/04/CPCSEA), for the care and use of laboratory animals. Appropriate measures were taken throughout the study to minimize animal suffering and to use the minimum number of animals necessary to achieve the objectives of the investigation.

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Data Availability Statement

All data generated and analyzed during the present study are included in the published article. Additional data supporting the findings of this study are available from the corresponding author upon reasonable request.

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AI Declaration Statement

During the preparation of this manuscript, the author(s) used Grammarly language editing service for language editing and grammar improvement. Following that, the author(s) reviewed and verified all AI-generated content. The author(s) are solely responsible for the content and integrity of the final published article.

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