

PHYTOCHEMICAL AND PHARMACOGNOSTIC INVESTIGATION AND DIURETIC ACTIVITY OF *PUTRANJIVA ROXBURGHII*

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ABSTRACT

Background: *Putranjiva roxburghii* Wall. (family: Putranjivaceae) is a medicinal plant widely used in traditional systems of medicine for the treatment of various ailments, including urinary disorders, inflammation, fever, and other health conditions. Despite its ethnomedicinal importance, limited scientific evidence is available regarding its pharmacognostic characteristics, phytochemical profile, and diuretic potential. The present study was undertaken to establish pharmacognostic standards, investigate the phytochemical constituents, and evaluate the diuretic activity of the methanolic leaf extract of *P. roxburghii*. **Methods:** Fresh leaves of *P. roxburghii* were collected, authenticated, shade-dried, powdered, and extracted with methanol by the maceration method. Pharmacognostic evaluation included

organoleptic, microscopic, fluorescence, and physicochemical analyses. Preliminary phytochemical screening was performed using standard qualitative chemical tests. The diuretic activity of the methanolic leaf extract was evaluated in Wistar albino rats at doses of 100 and 200 mg/kg using furosemide (20 mg/kg) as the standard drug. Urine volume, urinary sodium, potassium, chloride excretion, and diuretic index were determined to assess the diuretic effect. **Results:** The methanolic extraction yielded 48.5 g of crude extract (9.7% w/w). Pharmacognostic studies established characteristic organoleptic features, microscopic diagnostic characters, fluorescence behaviour, and acceptable physicochemical parameters, supporting the identity and quality of the plant material. Preliminary phytochemical screening confirmed the presence of alkaloids, flavonoids, tannins, glycosides, saponins, terpenoids, steroids, carbohydrates, and reducing sugars. In vivo evaluation demonstrated a dose-

dependent increase in urine output and urinary electrolyte excretion. The higher dose (200 mg/kg) exhibited greater diuretic activity, producing a urine output of 7.1 ± 0.31 mL with a diuretic index of 2.21, compared with 8.6 ± 0.35 mL and 2.68, respectively, for the standard drug furosemide. **Conclusion:** The findings indicate that the methanolic leaf extract of *Putranjiva roxburghii* possesses significant phytochemical constituents and exhibits promising dose-dependent diuretic activity. The pharmacognostic parameters established in this study may serve as useful standards for authentication and quality control of the plant. The observed diuretic effect supports the traditional use of *P. roxburghii* in the management of urinary disorders and suggests its potential as a natural source for the development of safe and effective herbal diuretic agents.

KEYWORDS: *Putranjiva roxburghii*, Pharmacognostic evaluation, Phytochemical screening, Medicinal plant, Diuretic activity, Methanolic extract, Wistar rats, Herbal medicine.

INTRODUCTION

Medicinal plants have served as one of the primary sources of healthcare since ancient times and continue to play an indispensable role in modern drug discovery. According to the World Health Organization (WHO), nearly 80% of the population in developing countries depends on traditional herbal medicines for primary healthcare. Medicinal plants contain a wide variety of secondary metabolites such as alkaloids, flavonoids, tannins, terpenoids, glycosides, saponins, steroids, and phenolic compounds that exhibit diverse pharmacological activities including antioxidant, antimicrobial, anti-inflammatory, antidiabetic, hepatoprotective, cardioprotective, nephroprotective, and diuretic effects.^[1,4]

The increasing prevalence of chronic diseases and adverse effects associated with synthetic drugs have renewed scientific interest in medicinal plants as safer and cost-effective therapeutic alternatives. Herbal medicines possess complex mixtures of bioactive constituents that often act synergistically to produce therapeutic benefits while minimizing toxicity. Consequently, systematic scientific evaluation of medicinal plants through pharmacognostic, phytochemical, and pharmacological investigations has become essential for validating their traditional uses and establishing quality standards for future drug development.^[5,8]

Pharmacognosy is an important branch of pharmaceutical sciences that deals with the identification, authentication, quality control, and standardization of crude drugs obtained

from natural sources. Pharmacognostic evaluation involves macroscopic and microscopic characterization, powder microscopy, fluorescence analysis, and physicochemical studies such as moisture content, ash values, and foreign matter determination. These parameters serve as reliable diagnostic tools for detecting adulteration, ensuring purity, and maintaining consistency in herbal raw materials used for medicinal purposes.^[9,12]

Phytochemical screening provides valuable information regarding the chemical constituents present in medicinal plants and serves as the foundation for understanding their biological activities. Preliminary phytochemical analysis identifies major classes of secondary metabolites responsible for various pharmacological effects and facilitates further isolation and characterization of active compounds. Numerous studies have demonstrated that flavonoids, phenolic compounds, lignans, terpenoids, saponins, and alkaloids contribute significantly to antioxidant, anti-inflammatory, antimicrobial, nephroprotective, and diuretic activities observed in medicinal plants.^[13,16]

Diuretics constitute an important class of therapeutic agents widely used in the management of hypertension, congestive heart failure, renal disorders, edema, liver cirrhosis, nephrotic syndrome, and electrolyte imbalance. These agents increase urine production by promoting the excretion of water and electrolytes through the kidneys. Although synthetic diuretics such as furosemide, hydrochlorothiazide, and spironolactone are clinically effective, their prolonged use is frequently associated with adverse effects including hypokalemia, hyperuricemia, dehydration, metabolic alkalosis, renal dysfunction, and electrolyte disturbances. Therefore, there is a growing need to identify plant-derived diuretic agents possessing comparable efficacy with improved safety profiles.^[17,20]

Putranjiva roxburghii Wall., belonging to the family Putranjivaceae, is an evergreen medicinal tree widely distributed throughout the Indian subcontinent and several tropical Asian countries. The plant has been extensively utilized in Ayurvedic and traditional systems of medicine for the treatment of fever, inflammation, skin disorders, rheumatism, infertility, urinary ailments, and various gastrointestinal disorders. Different parts of the plant, including leaves, bark, seeds, and fruits, have been traditionally employed because of their reputed therapeutic properties.^[21,22]

Phytochemical investigations of *P. roxburghii* have revealed the presence of numerous biologically active constituents including flavonoids, tannins, glycosides, lignans,

triterpenoids, steroids, phenolic compounds, and saponins. These phytoconstituents have been reported to exhibit antioxidant, antimicrobial, anti-inflammatory, hepatoprotective, analgesic, antidiabetic, and cytoprotective activities. However, despite its extensive traditional use, scientific evidence regarding its pharmacognostic standards and diuretic potential remains limited, necessitating comprehensive investigation.^[23,24]

The present study was therefore designed to perform detailed pharmacognostic and phytochemical evaluation of *Putranjiva roxburghii* leaves along with assessment of their diuretic activity using an established experimental animal model. Standardization of the plant material through organoleptic, microscopic, fluorescence, and physicochemical studies, followed by qualitative phytochemical screening and pharmacological evaluation, is expected to generate scientific evidence supporting its traditional medicinal claims. The findings of this investigation may contribute to the development of standardized herbal formulations and provide a scientific basis for future research on novel plant-derived diuretic agents.^[25]

MATERIALS AND METHODOLOGY

Experimental Requirements

The present study was carried out using various chemicals, reagents, instruments, and laboratory equipment required for the extraction, phytochemical investigation, pharmacognostic evaluation, and pharmacological assessment of the methanolic leaf extract of *Putranjiva roxburghii*. Fresh leaves of *P. roxburghii* were used as the plant material, while methanol, ethanol, distilled water, Freund's adjuvant, indomethacin, and paraffin were employed as solvents, reagents, and standard chemicals during the experimental procedures. All chemicals and reagents used throughout the study were of analytical grade and procured from standard commercial suppliers to ensure the quality and reproducibility of the results. The experimental work was performed using standard laboratory instruments and apparatus, including a digital weighing balance, round-bottom flask, Soxhlet apparatus, condenser, rotary vacuum evaporator, clinical thermometer, plethysmograph, digital pH meter, hot air oven, UV chamber, microscope, and other essential glassware and laboratory accessories. Before commencement of the experiments, all instruments were properly calibrated to ensure the accuracy, precision, and reliability of the experimental observations.

Collection, Authentication and Extraction of the Plant

The plant material used in the present study was authenticated by the Department of Botany, Christ Church College. The collected plant specimen was identified as *Putranjiva roxburghii*

belonging to the family Putranjivaceae. The authentication was carried out by Dr. Navin K. Ambasht, Head, Department of Botany. An authentication certificate bearing reference number Botany/2025-26/0690 dated 27-12-2025 was issued and preserved for future reference. The collected leaves were thoroughly washed with tap water followed by distilled water to remove adhering dust and foreign matter. The cleaned leaves were shade-dried at room temperature (25–30°C) for about 10–15 days until a constant weight was obtained. The dried leaves were then coarsely powdered using a mechanical grinder and passed through sieve No. 40 to obtain a uniform fine powder. The powdered material was stored in airtight containers protected from moisture and light until further use.

Extraction Procedure

About 500 g of the dried powdered leaf material of *Putranjiva roxburghii* was extracted with methanol by the maceration method. The powdered drug was soaked in 2.5 L of methanol in a clean glass container and kept at room temperature for 15 days with occasional stirring to ensure maximum extraction of phytoconstituents. The container was tightly closed to prevent solvent evaporation. After completion of the extraction process, the mixture was filtered first through muslin cloth and then through Whatman No. 1 filter paper to remove plant debris. The filtrate obtained was concentrated under reduced pressure using a rotary vacuum evaporator at a temperature not exceeding 40–45°C to obtain a semisolid crude methanolic extract. The concentrated extract was further dried in a desiccator to remove residual solvent and stored in an airtight container at 4°C for further phytochemical and pharmacological studies. The percentage yield of the extract was calculated using the following formula:

Powder Studies

Powder analysis of the crude drug was carried out to evaluate its organoleptic and microscopic characteristics. The powdered leaf material of *Putranjiva roxburghii* was examined for various organoleptic properties such as colour, odour, taste, texture, fineness, degree of uniformity, and smoothness. These characteristics were recorded carefully as they are important parameters for the identification and standardization of crude herbal drugs.

For microscopic examination, a small quantity of the powdered drug was placed on a clean glass slide and treated with chloral hydrate solution. The preparation was covered with a cover slip and gently heated over a low flame for a short duration to clear the tissues. The slide was then observed under a compound microscope to identify characteristic cellular structures and diagnostic features of the powdered drug.

Fluorescence Analysis

Fluorescence analysis of the powdered drug was carried out under visible light and ultraviolet light (254 nm and 366 nm) according to the methods described by Chase and Pratt (1949) and Kokoski et al. (1948). Different chemical reagents were added separately to the powdered drug and the resulting colour changes and fluorescence characteristics were observed and recorded. Fluorescence analysis is considered an important pharmacognostic tool for the identification and quality evaluation of crude plant materials.

Physicochemical Parameters

Physicochemical evaluation of the powdered leaf material of *Putranjiva roxburghii* was carried out to determine the purity, quality, and standardization parameters of the crude drug. All determinations were performed in triplicate and the average values were recorded. The physicochemical parameters studied included determination of foreign matter, moisture content (loss on drying), and ash value.

Determination of Foreign Matter

About 100–400 g of the powdered drug sample was accurately weighed and spread in a thin layer on a clean surface. The sample was examined visually with the unaided eye and with the help of a magnifying lens (6×) to detect any foreign organic or inorganic matter such as soil particles, dust, insects, or other contaminants. The foreign matter was separated manually, weighed, and the percentage of foreign matter was calculated using the following formula:

Determination of Moisture Content (Loss on Drying)

Moisture content of the powdered drug was determined by the loss on drying method. About 10 g of the powdered sample was accurately weighed and placed in a previously dried and weighed evaporating dish. The sample was dried in a hot air oven at 100°C for 3 hours and cooled in a desiccator. Drying and weighing were continued until a constant weight was obtained. The percentage moisture content was calculated using the following formula.

Determination of Ash Value

Ash value determination was carried out to estimate the amount of inorganic and siliceous matter present in the crude drug. The ash value includes both physiological ashes derived from plant tissues and non-physiological ash originating from external contaminants such as sand and soil.

Total Ash Value

Approximately 2 g of air-dried powdered drug was accurately weighed and placed in a previously ignited and tarred silica crucible. The material was spread evenly and incinerated gradually by increasing the temperature, not exceeding 440°C, in a muffle furnace until the sample became white, indicating the absence of carbon.

The crucible was cooled in a desiccator for 30 minutes and weighed. If carbon-free ash was not obtained, the residue was moistened with distilled water, dried on a hot plate, and again ignited to constant weight. The percentage of total ash was calculated using the following formula:

Phytochemical Screening

Preliminary phytochemical screening of the methanolic leaf extract of *Putranjiva roxburghii* was carried out to identify the presence of various bioactive constituents such as alkaloids, glycosides, saponins, tannins, flavonoids, terpenoids, steroids, carbohydrates, and reducing sugars using standard qualitative chemical tests as described in the literature.^[24,26]

Detection of Alkaloids

For the detection of alkaloids, a small quantity of the plant extract was dissolved separately in dilute hydrochloric acid (HCl) and filtered. The filtrate obtained was subjected to the following tests:

(a) Mayer's Test

The filtrate was treated with Mayer's reagent (Potassium Mercuric Iodide solution). Formation of a cream or yellow coloured precipitate indicated the presence of alkaloids.

(b) Wagner's Test

The filtrate was treated with Wagner's reagent (Iodine in Potassium Iodide solution). Formation of a reddish-brown precipitate confirmed the presence of alkaloids.

(c) Hager's Test

The filtrate was treated with Hager's reagent (saturated picric acid solution). Formation of a yellow precipitate indicated the presence of alkaloids.

Detection of Glycosides**Fehling's Test**

The extract was diluted with distilled water and treated with freshly prepared Fehling's solutions A and B. The mixture was heated in a water bath for about 4 minutes. Formation of a brick-red precipitate indicated the presence of glycosides.

Detection of Saponins**Foam Test**

About 2 g of the plant extract was mixed with 10 mL of distilled water and shaken vigorously for several minutes. Formation of a stable and persistent froth indicated the presence of saponins.

Detection of Tannins**(a) Ferric Chloride Test**

Approximately 0.4 g of dried powdered sample was boiled with 20 mL of distilled water and filtered. A few drops of 0.1% ferric chloride solution were added to the filtrate. Development of a brownish green, blue-black, or greenish-black colour indicated the presence of tannins.

(b) Lead Acetate Test

About 2 mL of plant extract was mixed with 2 mL of distilled water followed by the addition of lead acetate solution. Formation of white turbidity or precipitate confirmed the presence of tannins.

Detection of Flavonoids**(a) Sodium Hydroxide Test**

A small quantity of the extract was treated with aqueous sodium hydroxide solution followed by dilute hydrochloric acid. Formation of a yellow to orange colour that disappeared on addition of acid indicated the presence of flavonoids.

(b) Sulphuric Acid Test

A portion of the extract was treated with concentrated sulphuric acid (H_2SO_4). Development of an orange colour indicated the presence of flavonoids.

Detection of Terpenoids

About 4 mL of aqueous plant extract was mixed with 2 mL of chloroform and evaporated to dryness on a water bath. The residue was then treated with 3 mL of concentrated sulphuric

acid and heated gently. Formation of a greyish coloration indicated the presence of terpenoids.

Detection of Steroids

Approximately 4 mL of aqueous plant extract was treated with 2 mL of chloroform and concentrated sulphuric acid. Appearance of a red colour in the lower chloroform layer indicated the presence of steroids.

Test for Reducing Sugars and Carbohydrates

(a) Molisch's Test

To 2–3 mL of plant extract, a few drops of alcoholic α -naphthol solution were added followed by careful addition of concentrated sulphuric acid along the sides of the test tube. Formation of a violet ring at the junction of the two liquids indicated the presence of carbohydrates.

(b) Fehling's Test for Reducing Sugars

Fehling's solution A was prepared by dissolving 34.66 g of copper sulphate in distilled water and making up the volume to 400 mL. Fehling's solution B was prepared by dissolving 40 g sodium hydroxide and 173 g potassium sodium tartrate in distilled water. Equal volumes of Fehling's solutions A and B were mixed freshly before use.

About 1 mL of the mixed Fehling's solution was boiled for one minute, followed by addition of an equal volume of the test solution. The mixture was heated in a boiling water bath for 4–10 minutes. Formation of yellow to brick-red precipitate indicated the presence of reducing sugars.

Experimental Animals and Ethical Approval

Healthy adult Wistar albino rats weighing between 130–160 g were procured from the animal house facility of Advance Institute of Biotech and Paramedical Sciences, Kanpur. The animals were housed in clean polypropylene cages under standard laboratory conditions at a temperature relative humidity of (40 \pm 2%), and a 12-hour light and dark cycle. The animals were provided with a standard pellet diet and water ad libitum throughout the study period. Prior to experimentation, the animals were acclimatized to laboratory conditions for one week. The rats were fasted overnight before the experiment, while water was made available up to one hour prior to the study.^[44]

All experimental procedures involving laboratory animals were carried out in accordance with the guidelines of the Committee for the Control and Supervision of Experiments on Animals (CCSEA), Government of India. The experimental protocol entitled “*Phytochemical & Pharmacognostic Investigation and Diuretic Activity of Putranjiva roxburghii*” was reviewed and approved by the Institutional Animal Ethics Committee (IAEC) of Advance Institute of Biotech and Paramedical Sciences, Kanpur. The study was approved under proposal number AIBS/IAEC/2025/11 with CCSEA Registration No.: 1122/PO/Re/S/07/CCSEA. A total of 25 Wistar rats were sanctioned for the study for a duration of six months.

Experimental Grouping and Treatment Schedule

The animals were randomly divided into four groups, each consisting of six rats ((n = 6)), and treated orally as follows:

Table 1: Experimental Animal Grouping and Treatment Protocol.

Group	Treatment	Dose	Route	Purpose
Group I	Normal Saline	24 mL/kg	Oral (p.o.)	Served as the control group for baseline comparison
Group II	Furosemide	20 mg/kg	Oral (p.o.)	Served as the standard/positive control group
Group III	<i>Putranjiva roxburghii</i> Methanolic Leaf Extract	100 mg/kg	Oral (p.o.)	Evaluated diuretic activity at lower dose
Group IV	<i>Putranjiva roxburghii</i> Methanolic Leaf Extract	200 mg/kg	Oral (p.o.)	Evaluated dose-dependent diuretic activity at higher dose

Evaluation Parameters for Diuretic Activity

The diuretic activity of the methanolic leaf extract of *Putranjiva roxburghii* was evaluated using different physiological and biochemical parameters in experimental Wistar rats. These parameters were used to assess the effect of the extract on urine formation and electrolyte excretion.

Measurement of Urine Output

After administration of the respective treatments, the animals were individually placed in metabolic cages for urine collection. Urine samples were collected over a specified period of time, and the total urine volume excreted by each rat was measured using a graduated measuring cylinder. An increase in urine volume in the treated groups compared to the control group was considered indicative of diuretic activity.

Estimation of Urinary Electrolytes

The collected urine samples were analysed for the concentration of electrolytes such as sodium ((Na⁺)), potassium ((K⁺)), and chloride ((Cl⁻)). Estimation of electrolyte levels was performed using standard laboratory procedures. The urinary electrolyte excretion pattern was evaluated to determine the effect of the extract on renal handling of electrolytes and to compare its activity with the standard diuretic drug, furosemide.

Determination of Diuretic Index

The diuretic index was calculated to quantify the diuretic potential of the methanolic leaf extract of *Putranjiva roxburghii*. The diuretic index was determined by comparing the urine volume of the treated groups with that of the control group using the following formula:

RESULT AND DISCUSSION

Extraction of *Putranjiva roxburghii* Leaf Extract

Approximately 500 g of dried powdered leaves of *Putranjiva roxburghii* were extracted with 2.5 L of methanol using the maceration method for 15 days. After filtration and concentration under reduced pressure using a rotary vacuum evaporator, a dark green semisolid crude methanolic extract was obtained. The extract was dried and preserved at (4° C) for further studies. The practical yield and percentage yield of the extract were calculated and are presented in Table 1.

Table 1: Yield of Methanolic Extract of *Putranjiva roxburghii* Leaves.

S. No.	Parameter	Observation
1	Plant material used	Dried powdered leaves
2	Quantity of powdered drug	500 g
3	Solvent used	Methanol
4	Volume of solvent	2.5 L
5	Extraction method	Maceration
6	Duration of extraction	15 days
7	Practical yield obtained	48.5 g
8	Percentage yield	9.7% w/w
9	Appearance of extract	Dark green semisolid mass

The methanolic extraction of *Putranjiva roxburghii* leaves yielded a satisfactory amount of crude extract, indicating effective extraction of phytoconstituents. Methanol acted as an efficient solvent for extracting polar and moderately polar bioactive compounds. The maceration method enhanced solvent penetration and extraction efficiency, while concentration under reduced pressure prevented degradation of heat-sensitive constituents.

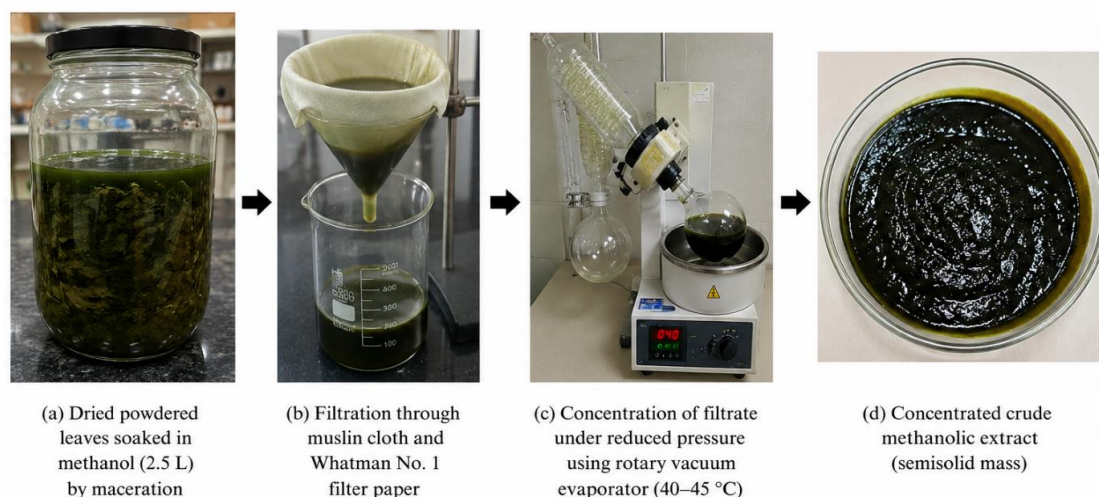


Figure 1: Methanolic extraction and concentrated crude extract of *Putranjiva roxburghii* leaves.

Powder Studies of *Putranjiva roxburghii* Leaves

The powdered leaf material of *Putranjiva roxburghii* was evaluated for its organoleptic, microscopic, and fluorescence characteristics. The powder was found to possess characteristic features useful for identification and standardization of the crude drug. The organoleptic properties of the powdered drug are presented in Table 2.

Table 2: Organoleptic Characteristics of Powdered Leaves of *Putranjiva roxburghii*.

S. No.	Parameter	Observation
1	Colour	Greenish brown
2	Odour	Characteristic
3	Taste	Slightly bitter
4	Texture	Smooth
5	Nature	Fine powder
6	Uniformity	Uniform

Microscopic examination of the powdered drug revealed the presence of characteristic plant tissues such as epidermal cells, fibres, stomata, xylem vessels, and calcium oxalate crystals, confirming the authenticity of the plant material. Fluorescence analysis of the powdered drug showed distinct colour changes under visible light and ultraviolet light (254 nm and 366 nm) after treatment with different chemical reagents. The fluorescence characteristics are summarized in Table 3.

Table 3: Fluorescence Analysis of Powdered Leaves of *Putranjiva roxburghii*.

S. No.	Treatment/Reagent	Visible Light	UV Light (254 nm)	UV Light (366 nm)
1	Powder as such	Greenish brown	Dark green	Brownish green
2	Powder + Water	Light green	Green	Fluorescent green
3	Powder + Methanol	Brownish green	Dark brown	Green fluorescence
4	Powder + HCl	Brown	Dark brown	Yellowish green
5	Powder + NaOH	Dark green	Greenish yellow	Bright green
6	Powder + H ₂ SO ₄	Brownish black	Black	Dark brown

Powder analysis is an important pharmacognostic parameter used for identification and standardization of crude herbal drugs. The organoleptic evaluation of *Putranjiva roxburghii* leaf powder showed characteristic colour, odour, texture, and taste, which can serve as preliminary identification markers.

Microscopic examination revealed the presence of diagnostic cellular structures that confirmed the authenticity and purity of the plant material. Fluorescence analysis demonstrated characteristic colour changes under visible and ultraviolet light, indicating the presence of different phytoconstituents. Such fluorescence behaviour is useful in detecting adulteration and ensuring quality control of herbal drugs.

The results obtained from powder microscopy and fluorescence analysis provide valuable pharmacognostic standards for the identification and quality assessment of *Putranjiva roxburghii* leaves.

Physicochemical Evaluation of *Putranjiva roxburghii* Leaves

Physicochemical evaluation of the powdered leaves of *Putranjiva roxburghii* was carried out to determine the purity, quality, and standardization parameters of the crude drug. The parameters studied included foreign matter, moisture content (loss on drying), and total ash value. The observations obtained are presented in Table 4.

Table 4: Physicochemical Parameters of *Putranjiva roxburghii* Leaf Powder.

S. No.	Parameter	Observation (% w/w)
1	Foreign matter	1.12%
2	Moisture content (Loss on drying)	6.45%
3	Total ash value	5.38%

The percentage of foreign matter was found to be within acceptable pharmacopoeial limits, indicating purity of the crude drug sample. The moisture content was comparatively low, suggesting reduced chances of microbial contamination and improved stability during

storage. The total ash value indicated the presence of a moderate amount of inorganic constituents within permissible limits. Physicochemical parameters are important quality control measures used for the standardization and identification of herbal drugs. In the present study, the foreign matter content of *Putranjiva roxburghii* leaf powder was found to be low, indicating that the collected plant material was free from excessive contamination such as dust, soil particles, and other extraneous matter. Moisture content is an important parameter that influences the stability and shelf life of herbal drugs. The low moisture content observed in the present study suggests minimal risk of microbial growth, enzymatic degradation, and spoilage during storage. Ash value determination provides information regarding the amount of inorganic matter and possible adulteration present in crude drugs. The total ash value obtained in this study was within acceptable limits, indicating the purity and quality of the powdered plant material.

Preliminary Phytochemical Screening of *Putranjiva roxburghii* Leaf Extract

Preliminary phytochemical screening of the methanolic leaf extract of *Putranjiva roxburghii* revealed the presence of various bioactive phytoconstituents including alkaloids, glycosides, saponins, tannins, flavonoids, terpenoids, steroids, carbohydrates, and reducing sugars. The observations obtained from different qualitative chemical tests are summarized in Table 5.

Table 5: Preliminary Phytochemical Screening of Methanolic Leaf Extract of *Putranjiva roxburghii*.

S. No.	Phytoconstituent	Test Performed	Observation	Result
1	Alkaloids	Mayer's Test	Yellow precipitate	Present (+)
		Wagner's Test	Reddish-brown precipitate	Present (+)
		Hager's Test	Yellow precipitate	Present (+)
2	Glycosides	Fehling's Test	Brick-red precipitate	Present (+)
3	Saponins	Foam Test	Stable persistent froth	Present (+)
4	Tannins	Ferric Chloride Test	Greenish-black colour	Present (+)
		Lead Acetate Test	White precipitate	Present (+)
5	Flavonoids	NaOH Test	Yellow-orange colour	Present (+)
		H ₂ SO ₄ Test	Orange colour	Present (+)
6	Terpenoids	Salkowski-type Test	Grey coloration	Present (+)
7	Steroids	Sulphuric Acid Test	Red colour in chloroform layer	Present (+)
8	Carbohydrates	Molisch's Test	Violet ring formation	Present (+)
9	Reducing Sugars	Fehling's Test	Brick-red precipitate	Present (+)

The phytochemical screening demonstrated that the methanolic extract of *Putranjiva roxburghii* contains several secondary metabolites with potential pharmacological importance. Preliminary phytochemical screening plays an important role in identifying

biologically active constituents present in medicinal plants. In the present study, the methanolic leaf extract of *Putranjiva roxburghii* showed positive results for major phytoconstituents such as alkaloids, flavonoids, tannins, glycosides, saponins, terpenoids, steroids, carbohydrates, and reducing sugars. The presence of alkaloids suggests possible pharmacological activities such as analgesic, antimicrobial, and diuretic effects. Flavonoids and tannins are well known for their antioxidant and anti-inflammatory properties, while saponins and glycosides may contribute to the diuretic activity of the plant extract. Terpenoids and steroids also possess significant biological activities including anti-inflammatory and membrane-stabilizing effects. Methanol proved to be an effective solvent for extracting a broad range of phytoconstituents due to its polarity. The presence of these bioactive compounds may be responsible for the therapeutic potential and pharmacological activities exhibited by *Putranjiva roxburghii*.

Evaluation of Diuretic Activity of *Putranjiva roxburghii* Leaf Extract

The diuretic activity of the methanolic leaf extract of *Putranjiva roxburghii* was evaluated in Wistar albino rats by measuring urine output, urinary electrolyte excretion, and diuretic index. The extract-treated groups showed a significant increase in urine volume and electrolyte excretion compared to the control group. The higher dose ((200, mg/kg)) exhibited greater diuretic activity when compared with the lower dose ((100, mg/kg)).

Effect on Urine Output

The urine volume excreted by the animals was measured over the experimental period. The extract-treated groups demonstrated increased urine output compared to the control group. The maximum urine output was observed in the standard furosemide-treated group, while the higher dose of the plant extract showed appreciable diuretic activity.

Table 6: Effect of *Putranjiva roxburghii* Extract on Urine Output.

Group	Treatment	Urine Output (mL)
Group I	Normal Saline	3.2 ± 0.21
Group II	Furosemide (20 mg/kg)	8.6 ± 0.35
Group III	Extract (100 mg/kg)	5.4 ± 0.28
Group IV	Extract (200 mg/kg)	7.1 ± 0.31

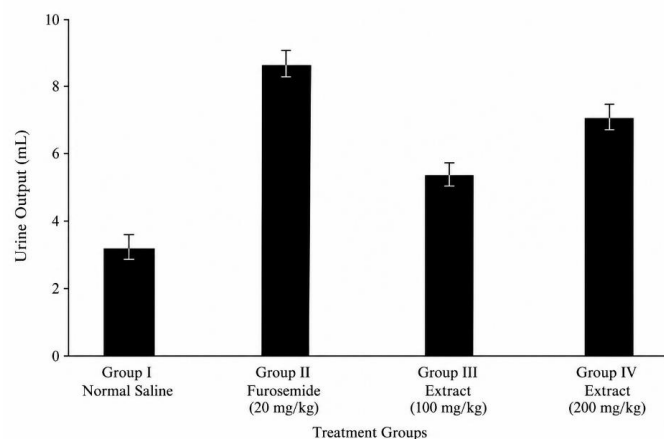


Figure 2: Effect of methanolic leaf extract of *Putranjiva roxburghii* on urine output in experimental Wistar rats.

Effect on Urinary Electrolyte Excretion

Urinary electrolyte analysis revealed increased excretion of sodium (Na^+), potassium (K^+), and chloride (Cl^-) ions in extract-treated animals compared to the control group. The higher dose of the extract showed electrolyte excretion closer to that of the standard drug furosemide.

Table 7: Effect of *Putranjiva roxburghii* Extract on Urinary Electrolytes.

Group	Sodium (Na^+) mEq/L	Potassium (K^+) mEq/L	Chloride (Cl^-) mEq/L
Control	58.4 ± 1.8	22.6 ± 0.9	49.2 ± 1.4
Furosemide	118.5 ± 2.4	46.3 ± 1.3	102.8 ± 2.1
Extract (100 mg/kg)	82.7 ± 2.1	31.4 ± 1.1	70.5 ± 1.7
Extract (200 mg/kg)	101.2 ± 2.3	39.6 ± 1.2	88.7 ± 1.9

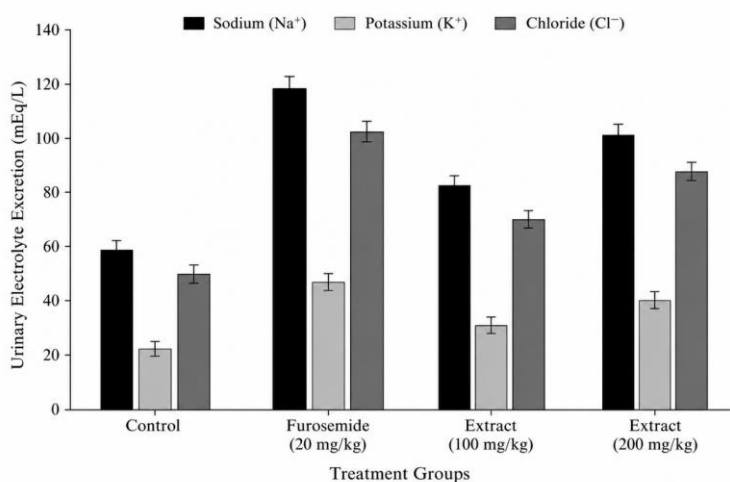


Figure 3: Effect of methanolic leaf extract of *Putranjiva roxburghii* on urinary electrolyte excretion (Na^+ , K^+ , and Cl^-) (Na^+ , K^+ , and Cl^-) (Na^+ , K^+ , and Cl^-) in experimental Wistar rats.

Determination of Diuretic Index

The results indicated that the methanolic extract exhibited dose-dependent diuretic activity.

Table 8: Diuretic Index of *Putranjiva roxburghii* Extract.

Group	Urine Output (mL)	Diuretic Index
Control	3.2 ± 0.21	1.00
Furosemide	8.6 ± 0.35	2.68
Extract (100 mg/kg)	5.4 ± 0.28	1.68
Extract (200 mg/kg)	7.1 ± 0.31	2.21

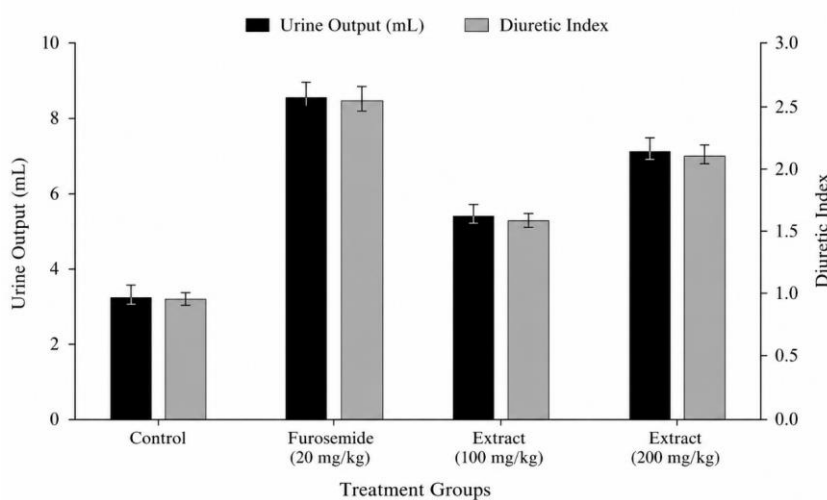


Figure 4: Effect of methanolic leaf extract of *Putranjiva roxburghii* on urine output and diuretic index in experimental Wistar rats.

The present study demonstrated significant diuretic activity of the methanolic leaf extract of *Putranjiva roxburghii* in experimental rats. The extract produced a dose-dependent increase in urine output and urinary electrolyte excretion. The higher dose ((200, mg/kg)) exhibited greater activity and showed results comparable to the standard diuretic drug furosemide. The increase in sodium, potassium, and chloride ion excretion suggests that the extract may act by influencing renal tubular reabsorption of electrolytes, thereby enhancing water excretion. The observed diuretic effect may be attributed to the presence of phytoconstituents such as flavonoids, saponins, glycosides, and alkaloids identified during preliminary phytochemical screening.

CONCLUSION

The present study successfully established the pharmacognostic standards, phytochemical profile, and diuretic potential of the methanolic leaf extract of *Putranjiva roxburghii*. Pharmacognostic evaluation, including organoleptic, microscopic, fluorescence, and

physicochemical analyses, confirmed the identity, purity, and quality of the plant material, providing reliable parameters for its authentication and standardization. Preliminary phytochemical screening revealed the presence of several important bioactive constituents, including alkaloids, flavonoids, tannins, glycosides, saponins, terpenoids, steroids, carbohydrates, and reducing sugars, indicating the medicinal significance of the plant.

The in vivo pharmacological investigation demonstrated that the methanolic leaf extract of *P. roxburghii* possesses significant dose-dependent diuretic activity. Administration of the extract at doses of 100 and 200 mg/kg produced a marked increase in urine volume as well as sodium, potassium, and chloride ion excretion compared with the control group. The higher dose exhibited greater efficacy and showed activity comparable to the standard diuretic drug, furosemide, suggesting that the extract may influence renal electrolyte handling and promote enhanced water excretion.

Overall, the findings of the present study provide scientific evidence supporting the traditional use of *Putranjiva roxburghii* in the management of urinary disorders. The established pharmacognostic parameters may serve as valuable quality control standards for future herbal formulations, while the demonstrated diuretic activity highlights the therapeutic potential of the plant as a natural source of diuretic agents. Further studies involving isolation and characterization of the active phytoconstituents, elucidation of the molecular mechanisms of action, detailed toxicological evaluation, and clinical investigations are warranted to validate its efficacy and facilitate its development into a safe and effective phytopharmaceutical product.

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