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MICROSCOPICAL AND PHYSICO-CHEMICAL INVESTIGATIONS OF THE LEAVES OF *PUTRANJIVA ROXBURGHII* WALL.

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ABSTRACT

Keywords:

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World Health Organization (WHO, Geneva) has appreciated the importance of medicinal plants for public health care for use in developing nations. *Putranjiva roxburghii* Wall. is having important role in the traditional Ayurvedic and Unani systems of holistic health and herbal medicine of the East. The leaves of *Putranjiva roxburghii* Wall. are reported to have good medicinal values in traditional system of medicine. The present study highlights the pharmacognostical studies including parameters such as powder analysis by microscopical evaluation, color reaction, and study of physicochemical parameters of the *Putranjiva roxburghii* Wall. leaves. These observations will help in the pharmacognostical identification and standardization of the drug in the crude form and can be used to distinguish the drug from its adulteration.

INTRODUCTION: After decades of serious obsession with the modern medicinal system, people have started looking at the ancient healing systems like Ayurveda, Siddha and Unani. This is because of the adverse effects associated with the synthetic drugs. Herbal drugs play an important role in health care programs especially in developing countries¹.

However a key obstacle, which has hindered the acceptance of the alternative medicines in the developed countries, is the lack of documentation and stringent quality control methods. There is a need for documentation of research work carried out on traditional medicines².

With this backdrop, it becomes extremely important to make an effort towards standardization of the plant material to be used as medicine. The process of standardization can be achieved by stepwise pharmacognostic and physicochemical studies.

These studies help in identification and authentication of the plant material. Correct identification and quality assurance of the starting materials is an essential prerequisite to ensure reproducible quality of herbal medicines which will contribute to their safety and efficacy. Simple pharmacognostic techniques used in standardization of plant material include its morphological, anatomical and biochemical characteristics³.

Putranjiva roxburghii Wall. is a moderate sized evergreen tree. The height is up to 12m with pendent branches and dark grey bark having horizontal lenticels. Leaves are simple, alternate, dark green, shiny, elliptic-oblong. *Putranjiva roxburghii* Wall. is found wild or cultivated in almost all parts of India⁴.

Leaves of *Putranjiva roxburghii* Wall. are bitter, astringent, refrigerant and procreant. The leaves are useful in the treatment of catarrh, skin disease, fever and sterility. The leaves are given in decoction for cold and fever, and are also used in rheumatism⁵.

The leaves of *Putranjiva roxburghii* Wall. possess analgesic, antipyretic and anti-inflammatory activity⁶. No systematic studies have been reported for its pharmacognostical study.

Hence, an effort has been made to establish the pharmacognostical as well as physicochemical study of *Putranjiva roxburghii* Wall. leaves.

MATERIAL AND METHOD:

Plant material: *Putranjiva roxburghii* Wall. leaves were collected from Keshav shrushti, Mumbai, India. The leaves were washed with water to remove soil particles, dried in the shade, and finely powdered. The powder was passed through the 85 mesh sieve and stored in an airtight container at room temperature ($28 \pm 2^\circ\text{C}$).

Authentication: A herbarium of *Putranjiva roxburghii* Wall. was prepared and authenticated from Botanical Survey of India, Pune.

Powder analysis:

- 1. Microscopy:** The powder of leaves of *Putranjiva roxburghii* Wall. was boiled with conc. HNO_3 to remove coloring matter and mounted on glass slide using glycerin, covered with cover slip and viewed under microscope. The powder was also stained with saffranin and examined under microscope.
- 2. Color Reactions:** The powdered drug materials were treated with different chemical reagents so as to aid in detection of chemical constituents under ordinary day light by standard methods. To study the behavior of drugs a pinch of each drug was treated with different chemical reagents viz Conc. HCl, Conc. HNO_3 , Conc. H_2SO_4 , acetic acid, 5% NaOH solution, saturated solution of picric acid, 5% ferric chloride solution followed by 10N ammonia solution and colors were observed. The different colors obtained with these reagents are listed in **Table 1**.
- 3. Fluorescence behavior:** To study the fluorescence nature of powder, a pinch of powder after bleaching with 5% chloral hydrate was treated with different chemical reagents viz. 1N NaOH in methanol, 1N NaOH in water, 50% HCl, 50% HNO_3 , 50% H_2SO_4 , acetic acid, petroleum ether,

chloroform, picric acid, 5% ferric chloride solution, 5% iodine and methanol and observed under UV light. The different colors obtained with these reagents are listed in **Table 2**.

Physicochemical parameters: The determination of various physicochemical parameters such as total ash, acid insoluble ash, water soluble ash, moisture content and loss on drying were calculated as per Indian Pharmacopeia.

- 1. Total ash:** About 2.0 g of dried powder of *Putranjiva roxburghii* Wall. were accurately weighed and transferred to different pre weighed silica crucibles and were ignited with a flame of Bunsen burner, for about 1 hour. The ignition was completed by keeping in muffle furnace, at $555^\circ\text{C} \pm 20^\circ\text{C}$, till a white carbon free ash was formed. The silica crucibles were then cooled in desiccators and weighed, and the result obtained is given in **Table 3**.
- 2. Acid insoluble ash:** About 2.0 of dried powder of leaves of *Putranjiva roxburghii* Wall. was accurately weighed and transferred to different pre weighed silica crucible and was ignited with a Bunsen burner, for about 1 hour. The silica crucible was then kept in a muffle furnace at $555^\circ\text{C} \pm 20^\circ\text{C}$, till a white carbon free ash was formed. The ash obtained was moistened with concentrated HCl and evaporated to dryness, after which it was kept in an electric air oven, maintained at $135^\circ\text{C} \pm 2^\circ\text{C}$, for 3 hours. After cooling, the ash was taken in beaker and 25 mL of dilute hydrochloric acid (2N HCl) was added, and crucibles was kept covered and heated on a water bath, for 10 minutes.

It was allowed to cool, and contents was filtered through Whatman filter paper no. 41. The residue was then washed with water, till washings was free from chloride. The filter paper along with the residue of plant powder was placed in different silica crucible and ignited in a muffle furnace, at $550^\circ\text{C} \pm 20^\circ\text{C}$, for 1 hour. The crucible was cooled and weighed to a constant weight. The percent acid insoluble ash content was then calculated for dried powder of leaves of *Putranjiva roxburghii* Wall. and the result obtained is given in Table 3.

3. **Water soluble ash:** About 2.0 g of dried powder of leaves of *Putranjiva roxburghii* Wall. was accurately weighed and transferred to a pre weighed silica crucibles and was ignited with a Bunsen burner, for about 1 hour. The crucibles were then kept in a muffle furnace at $550^{\circ}\text{C} \pm 20^{\circ}\text{C}$, till a white carbon free ash was obtained. After cooling, the ash was taken in beaker and to it 2 mL of distilled water was added, and beaker was kept covered and heated on a water bath, for 10 min.

It was allowed to cool, and content was filtered through Whatman filter paper no. 41. The filter paper and the residue was placed in silica crucibles and ignited in a muffle furnace, at $55^{\circ}\text{C} \pm 20^{\circ}\text{C}$, for 1 hour. The crucible was cooled and weighed to a constant weight. The percent water soluble ash content was then calculated for dried powder of leaves of *Putranjiva roxburghii* Wall. and the result obtained is given in table 3.

4. **Moisture content:** About 100 mg of accurately weighed dried powder of leaf of *Putranjiva roxburghii* Wall. was transferred to the reaction vessel. Titration with Karl Fisher reagent was carried out as described in Indian Pharmacopeia and the result obtained is given in table 3.

RESULTS AND DISCUSSION

Microscopic character: The dried leaf powder of *Putranjiva roxburghii* Wall. showed the presence of straight walled and polygon epidermal cells. (Fig. 1) The vessels have annular thickening (Fig. 2) The stomata is anomocytic i.e. the stoma is surrounded by varying number of cells. (Fig. 3) The plant showed the presence of unicellular as well as multicellular trichomes (Fig. 4, 5).



FIG. 1: EPIDERMAL CELL 40X



FIG. 2: VESSEL 100X

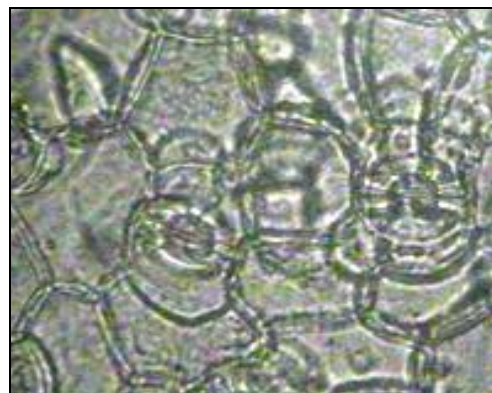


FIG. 3: STOMATA 100 X



FIG. 4: UNICELLULAR TRICHOMES 40X



FIG. 5: MULTICELLULAR TRICHOME 100X

Behavior of Powder Drug with different Chemical Reagents: The powder drug with different chemical reagents shows different color when seen on naked eye and the color obtained with different reagents is given in **Table 1**, and the fluorescence observed with different reagent is given in **Table 2**.

TABLE 1: POWDER ANALYSIS WITH CHEMICAL AGENTS

Powder + Reagent added	Color observed
Concentrated HCl	Dark Green
Concentrated HNO ₃	Yellowish green
Concentrated H ₂ SO ₄	Pale yellow
Glacial acetic acid	Dark green
5% NaOH solution	Brownish Green
5% KOH solution	Yellowish Green
5% Ferric chloride solution	Greenish Brown
Saturated solution of Picric acid	Greenish Yellow
10N Ammonia	Dark green

TABLE 2: FLUORESCENCE ANALYSIS OF POWDERED DRUG

Powder + Reagent added	Fluorescence observed
1N NaOH in methanol	Green
1N NaOH in water	Light green
50% HCl	Dark green
50% H ₂ SO ₄	Light green
50% HNO ₃	Green
Petroleum ether	Light green
Chloroform	Dark green
Saturated solution of Picric acid	Green
5% Ferric chloride solution	Dark green
5% Iodine solution	Light green
Methanol	Dark green
Powder + (HNO ₃ + NH ₃)	Green

Physicochemical Parameters: The physicochemical parameters are important for identifying adulterants and improper handling of drugs. **Table 3** reveals the result of physicochemical parameters of powdered drug, carried out by using standard procedures.

TABLE 3: PHYSICOCHEMICAL PARAMETERS OF LEAF POWDER OF PUTRANJIVA ROXBURGHII WALL.

Physicochemical parameters	Values
Total ash (%)	4.58
Acid insoluble ash (%)	1.09
Water soluble ash (%)	2.05
Moisture content (%)	5.06
Loss on drying (%)	8.66

CONCLUSION: The methods carried out in the present research work, like powder analysis and physicochemical studies will serve as standard reference for identification and distinguishing *Putranjiva roxburghii* Wall. leaves powder from its substitutes and adulterants. This report would assist in the identification of the crude drug in future.

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