

ORIGINAL RESEARCH ARTICLE

Pharmacognostical, Physicochemical, and Phytochemical Evaluation of Pullaas (*Rhododendron arboreum* Sm): A Holistic Approach to its Medicinal Potential

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ABSTRACT

Introduction: *Rhododendron arboreum* Sm., locally known as Pullaas (Burans), is conspicuous for its traditional use across the globe. The present study offers a comprehensive investigation into *R. arboreum* Sm., commonly known as Pullaas, integrating pharmacognostical, phytochemical, and physicochemical approaches to ensure accurate botanical identification and support its standardization in traditional medicine.

Materials and Methods: Classical Ayurveda texts, including commentaries and Nighantus, are the primary data source. The published articles related to phytopharmacognosy and physicochemical parameters available in PubMed, Google Scholar, etc., have also been referred for this review. Organoleptic and microscopic evaluations were conducted on the flower, leaf, and bark, revealing key diagnostic markers at Rajiv Gandhi Ayurvedic College and Hospital, Papprola. Furthermore, the study incorporated physicochemical parameter analysis, qualitative phytochemical screening, thin layer chromatography profiling, and ultra-performance liquid chromatography to evaluate the chemical constituents of the plant; at CSIR - IHBT, Palampur.

Discussion and Conclusion: Organoleptic, macroscopic, and microscopic assessments were conducted on the flower, leaf, and bark, revealing key diagnostic markers such as paracytic stomata. Phytochemical screening confirmed the presence of key secondary metabolites. The physicochemical parameters demonstrated acceptable limits for all parameters. The integrative analysis provides a scientific foundation for the pharmacognostical validation, quality control, and potential therapeutic use of *R. arboreum* Sm., contributing to the global acceptance of Ayurvedic plant-based medicine. Thus, the present paper serves as a foundation for selecting *R. arboreum* Sm. from the Himalayan region, where its rich traditional use shows promising results for the development of evidence-based herbal formulations.

1. INTRODUCTION

Herbal medicines have been a cornerstone of traditional healthcare systems for centuries, offering a natural reservoir of bioactive compounds with therapeutic potential. Among these, *Rhododendron arboreum* Sm. – commonly known as “Burans” or “Pullaas”^[1] – holds a significant place in the ethnobotanical traditions of the Himalayan Region. Rhododendron, meaning “red (or rose) tree,”^[2] This evergreen shrub, belonging to the family *Ericaceae*, is valued

not only for its eye-catching red blossoms^[3] as well as for its diverse medicinal applications documented in folk and Ayurvedic practices. Conventionally,^[4-6] it is used for a wide range of diseases such as dysentery with blood stains, nasal bleeding, asthma, stomachache, leukorrhea, fever, heart problems, diabetes, gout, and liver disorders. Its therapeutic utility is attributed to a wide range of phytoconstituents, including flavonoids, phenols, tannins, and glycosides, which exhibit anti-inflammatory, antioxidant, and antimicrobial properties.^[7-10] Despite its extensive traditional use, comprehensive scientific validation of *R. arboreum* remains limited, particularly in terms of its pharmacognostical identity, physicochemical parameters, and phytochemical composition.^[11] Therefore, this study was designed

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to provide an integrative pharmacognostical and phytochemical evaluation of *R. arboreum* to ensure accurate botanical identification, authentication, and standardization of the plant material. The investigation involved assessing organoleptic characters of the flower powder, conducting macroscopic and microscopic examinations of leaves and flowers, and performing powder microscopy to identify diagnostic markers. Standard physicochemical parameters were analyzed for quality control, while qualitative phytochemical screening was carried out to detect key secondary metabolites. In addition, thin-layer chromatography (TLC) was employed for preliminary phytochemical fingerprinting, and ultra-performance liquid chromatography (UPLC) was used for advanced profiling and quantification of selected bioactive compounds. This holistic approach aims to bridge traditional knowledge with scientific evidence, thereby supporting the therapeutic relevance and standardization of *R. arboreum* in herbal medicine.

2. MATERIALS AND METHODS

2.1. Plant Material Collection and Authentication

Fresh leaves and flowers of *R. arboreum* Sm. were collected from the Bir Billing region (altitude 1,300–2,400 m) located in Kangra district, Himachal Pradesh. Botanical authentication of the plant material was performed using standard floras and herbarium references.

2.2. Processing and Preservation

The collected samples were thoroughly cleaned to remove debris and dried in the shade. The dried plant material was powdered using a mechanical grinder and stored in airtight containers for further pharmacognostical and phytochemical analysis.

2.3. Pharmacognostical Studies

All pharmacognostical evaluations, including macroscopic, microscopic, and powder microscopy, were conducted at the Department of Dravyaguna, Rajiv Gandhi Ayurvedic College and Hospital, Paprola.

2.4. Macroscopic (Organoleptic) Evaluation

The flower powder of *R. arboreum* was assessed for its organoleptic properties, including color, odor, taste, and texture. Morphological characteristics of leaves and flowers, such as size, shape, surface features, and arrangement, were documented.

2.5. Microscopic Examination

Transverse sections (T.S.) of leaves and floral parts (style, ovary, peduncle) were prepared by standard freehand sectioning. Sections were stained and mounted for observation under a compound microscope. Diagnostic anatomical features were recorded.

2.6. Powder Microscopy

Powdered flower samples were examined under a microscope to identify diagnostic features such as trichomes, stomata, pollen grains, calcium oxalate crystals, and oil globules.

2.7. Physicochemical Analysis

Standard physicochemical analyses of *R. arboreum* Sm. were carried out at CSIR-IHBT, Palampur, to ensure the quality and authenticity of the plant material. The parameters assessed included foreign matter, loss on drying, total ash, acid-insoluble ash, and water-soluble

ash, which help determine the purity and cleanliness of the crude drug. Extractive values in both alcohol and water were evaluated to estimate the presence of soluble active constituents. In addition, pH measurements of 1% and 10% aqueous solutions, swelling index, and foaming index were recorded to assess the physicochemical behavior of the sample. Fluorescence analysis under visible and ultraviolet light provided further confirmation of the identity and quality of the drug. These evaluations are essential for establishing reliable quality control standards and ensuring the consistency and safety of herbal formulations derived from *R. arboreum*.

2.8. Phytochemical Analysis

2.8.1. Qualitative screening

Qualitative phytochemical screening of the flower powder was performed using standard chemical tests to detect major secondary metabolites such as alkaloids, flavonoids, tannins, saponins, phenolics, glycosides, and steroids.

2.8.2. Quantitative analysis and chromatographic profiling

Phytochemical profiling of *R. arboreum* Sm. was performed using TLC and UPLC to evaluate the presence of bioactive compounds. In the TLC procedure, silica gel 60 F₂₅₄ was employed as the stationary phase, and a chloroform: Methanol solvent system in the ratio of 70:30 served as the mobile phase. The developed chromatograms were visualized under ultraviolet light to obtain a preliminary phytochemical fingerprint. For advanced profiling, UPLC coupled with a photodiode array (PDA) detector was utilized for qualitative and quantitative analysis. Standard phytochemical markers used for identification included gallic acid, protocatechuic acid, vanillic acid, syringic acid, caffeic acid, epicatechin (EC), epigallocatechin gallate (EGCG), *p*-coumaric acid, ferulic acid, rutin, quercetin, luteolin, and kaempferol. The use of UPLC enabled high-resolution separation and accurate quantification of these bioactive compounds, supporting the standardization and therapeutic validation of the plant material.

3. RESULTS

3.1. Macroscopic and Microscopic Evaluation

The macroscopic characteristics of *R. arboreum* Sm. confirmed its identity as a small evergreen tree, reaching up to 10 m in height. The plant exhibits distinctive morphological features, including stout young shoots covered in white scales and a branched, crooked trunk with pinkish-brown exfoliating bark. Leaves are alternate, leathery, and typically clustered at the ends of branches. They are lanceolate to oblong, with an acuminate apex and silvery-scaled abaxial surface, measuring 7–15 cm in length and 3–4 cm in width. The flowers are bisexual, actinomorphic, and arranged in large, rounded corymbs. Each flower displays a crimson red campanulate corolla and hairy, pale yellow calyx. Stamens are ten, free, and unequal in length, while the ovary is densely woolly and multi-ocular. Capsules mature into dark brown woody structures containing numerous winged seeds. The flowering period spans from February to April, with fruiting from April to May [Figures 1 and 2; Table 1].

Microscopic examination revealed a dorsiventral leaf with distinct upper and lower epidermis. The upper epidermis comprises polygonal cells without stomata or trichomes, while the lower surface contains paracytic stomata and unicellular glandular trichomes. The transverse section of the leaf displayed collenchyma, spongy parenchyma, and a centrally located vascular bundle enclosed by a sclerenchymatous sheath. Flower anatomy included a circular cross-section of the style and ovary, with axile placentation and multilocular structure. The peduncle

showed a nearly circular section with defined vascular bundles and central pith, while the longitudinal section of the filament exhibited parenchymatous cells. Powder microscopy revealed tetrahedral tetrad pollen grains, calcium oxalate crystals, paracytic stomata, and oil-laden parenchyma [Figures 3 and 4]. These morphological and anatomical markers provide critical parameters for pharmacognostical standardization and authentication of the species.

3.2. Physicochemical and Phytochemical Analysis

Physicochemical parameters, including loss on drying, ash values, extractive values, pH, swelling and foaming indices, and fluorescence behavior, were evaluated according to standard protocols and are summarized in Table 2. These parameters serve as benchmarks for the identification and purity assessment of the raw drug.

Preliminary phytochemical screening indicated the presence of key secondary metabolites such as flavonoids, phenolics, tannins, and glycosides [Table 3]. These compounds correlate with the traditional therapeutic uses of the plant and warrant further phytochemical profiling.

3.3. Chromatographic Profiling

TLC was performed using silica gel 60 F₂₅₄ as the stationary phase and a chloroform: methanol (70:30) mobile phase. Under UV light, the chromatogram revealed multiple bands corresponding to the presence of diverse phytoconstituents, facilitating fingerprint analysis [Table 4 and Figure 5].

UPLC with a Photodiode Array Detector (PDA) was employed for quantitative profiling of major bioactive compounds. The analysis confirmed the presence of multiple polyphenolic and flavonoid compounds such as gallic acid, protocatechuic acid, vanillic acid, syringic acid, caffeic acid, EC, EGCG, p-coumaric acid, ferulic acid, rutin, quercetin, luteolin, and kaempferol. These constituents were identified based on their retention times and UV spectra compared with authenticated standards [Table 5 and Figure 6].

4. DISCUSSION

A pharmacognostic study proves that the sample was genuine and comprises a microscopic study of the leaf and flower that may prove helpful for further work. Phytochemical analysis reveals that Pullaas (flowers) show the presence of carbohydrates, reducing sugar, alkaloids, triterpenoids, flavonoids, tannins, phenols, and coumarins.^[12-17] The physicochemical parameters serve as crucial indicators for assessing the identity, purity, and quality of crude plant drugs. In the present study, *R. arboreum* Sm. demonstrated acceptable limits for loss on drying, ash values, and extractive values, indicating good quality and minimal adulteration or degradation. The pH, swelling index, and foaming index further contributed to evaluating the physical stability and solubility characteristics of the plant material. Fluorescence analysis under UV light revealed distinct color changes, aiding in preliminary authentication. Phytochemical screening confirmed the presence of key secondary metabolites such as carbohydrates, flavonoids, tannins, alkaloids, phenols, and triterpenoids, which support the traditional use of this plant for therapeutic purposes. Furthermore, various studies showed that these metabolites possess antioxidants,^[18] cardioprotective,^[19] hypolipidemic effect,^[20,21] hepatotoxicity,^[22,23] immunomodulatory,^[24,25] antibacterial, and cytotoxic activities.^[26-30] These results collectively provide a scientific foundation for the standardization and future pharmacological validation of *R. arboreum*.

5. CONCLUSION

The present study provides a comprehensive pharmacognostical and phytochemical evaluation of *R. arboreum* Sm., supporting its traditional medicinal use. Detailed organoleptic, macroscopic, microscopic, and powder microscopy analyses established key diagnostic features essential for proper identification and authentication. Physicochemical parameters were found within acceptable limits, ensuring the quality and purity of the crude drug. Phytochemical screening revealed the presence of bioactive compounds such as flavonoids, tannins, alkaloids, and phenols, indicating potential therapeutic value. These findings contribute to the standardization, quality control, and scientific validation of *R. arboreum* for future medicinal applications.

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7. AUTHORS' CONTRIBUTIONS

Dr. C G: Conceptualization, Methodology/Study design, Validation, Writing - original draft preparation, Literature search related to study, Data gathering, Data interpretation Dr. R B: conduction of analytical study, Data gathering. Dr D K: Editing and Supervision. All authors have read and approved the content of the finalized manuscript and confirmed the accuracy and integrity of any part of the work

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The authors declare that no financial support was received from any organization for the submitted work. In addition, all authors declare that they have no financial relationships with organizations that might be interested in the submitted work.

9. ETHICAL STATEMENT

Ethical approval was not required for this study as it was a review article with data obtained through a literature search.

10. CONFLICT OF INTERESTS

The authors declare no conflicts of interest regarding the publication of this paper.

11. DATA AVAILABILITY STATEMENT

The data analyzed in this review were obtained from publicly available sources, including peer-reviewed articles, observational studies, and surveys accessible through databases.

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Figure 1: *Rhododendron arboreum* Sm. (a) Whole tree (b) Bark (c) Upper surface of leaf (d) Flower bud (e) Flower

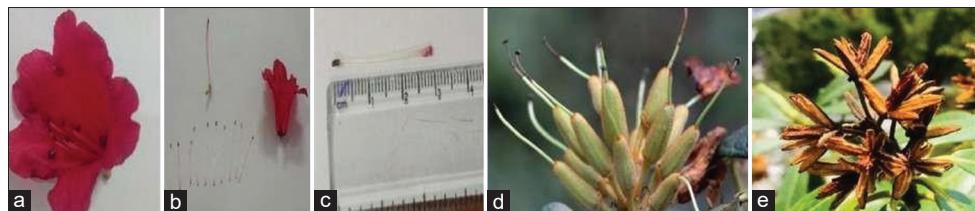


Figure 2: *Rhododendron arboreum* Sm., (a) Showing shape of flower showing 5 lobed corolla (b) Dissected flower showing no. free stamen and anthers (c) length of stamen (d) Young capsule (e) Mature dehisced capsule



Figure 3: *Rhododendron arboreum* Sm. (a) Lower epidermis showing stomata and epithelial cells (b) Lower epidermis showing trichomes (c-e) T.S. of Leaf depicting vascular bundle (f) T.S. of Style showing vascular bundle and sp oXn g y parenchyma (g and h) T.S. of Ovary showing

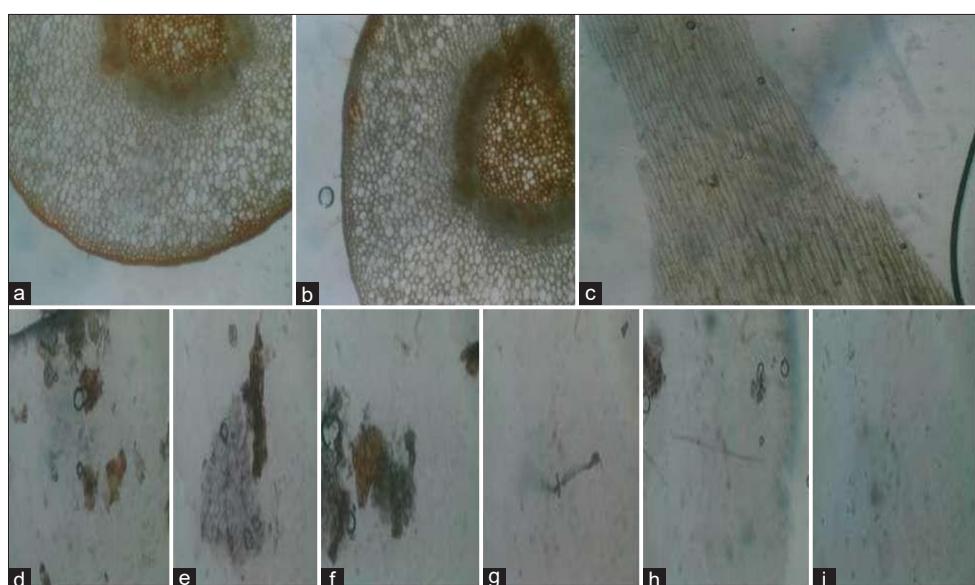


Figure 4: *Rhododendron arboreum* Sm. (a and b) TS of Peduncle (Cu- Cutical, Ep-Epidermis Gl Tr- Glandular Trichomes, NgTr - Non-glandular Trichomes, CC- Chollenchym, C- Cortex, Xy- Xylem, Ph- Phloem, Pi- Pith. (c) LS of filament showing Parenchmatous Cells (PC). (d-i) Powder microscopy of flower of *Rhododendron arboreum* showing tetrahedral tetrad pollen grains (PG), Parenchymatous cells (PC), Stomata (St), Oil containing Parenchymatous cells (OL), Gl Tr - Glandular Trichomes, NgTr - Non Glandular Trichomes, Oxalic acid crystals (Cr)

Table 1: Macroscopic study

Organoleptic character	Flower of <i>Rhododendron arboreum</i> Sm.	Leaf of <i>Rhododendron arboreum</i> Sm.	Bark of <i>Rhododendron arboreum</i> Sm.
Taste	Astringent followed by bitter	Bitter	Bitter
Odour	Similar to that of Beetroot	Odorless	Odorless
Color	Crimson red	Green	Light brown, pinkish
Touch	Thick, slightly fleshy and Smooth	Dorsal surface- smooth Ventral surface- Rough	The outer surface is rough, inner surface is slightly smooth
Fracture	NA	NA	Laminated fracture

Table 2: Physicochemical parameters

S. No.	Physicochemical parameters	Result (%)
1.	Foreign matter	2
2.	Loss on drying	5
3.	pH value 1%	4.51
	pH value 10%	4.15
4.	Total ash value	3
	Acid soluble ash value	1
	Water soluble ash value	2
5.	Aqueous extractive value	15
	Ethanoic extractive value	20
	Hydro-ethanoic extractive value	7.5
6.	Swelling index	8 mL
7.	Foaming index	<100

Table 3: Results of phytochemical screening

S. No.	Phytoconstituents	Test	Result
1.	Carbohydrates	Molisch's test	+ve
2.	Reducing sugar	Fehling test	+ve
3.	Proteins	Biuret's test	-ve
4.	Alkaloids	Dragendorff/Kraut's test	+ve
5.	Triterpenoids	Salkowski test	+ve
6.	Flavonoids	Lead acetate test	+ve
7.	Tannins	Braymer's test	+ve
8.	Phenols	Ferric chloride test	+ve
9.	Saponin glycosides	Foam test	-ve
10.	Coumarins	NaOH test	+ve

Table 4: Thin-layer chromatography

Solvent system	Visibility	No. of spots (3)	Colour	Rf
Chloroform:	UV	1 st (Above)	Yellow	0.61 cm
		2 nd (middle)	Pale yellow	0.35 cm
		3 rd (below)	Pinkish	0.16 cm

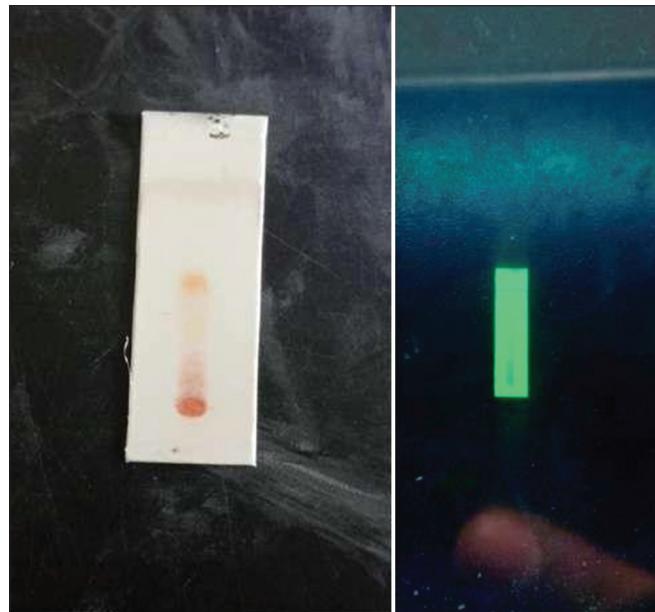
**Figure 5:** No. of spots- 3 at Rf- 0.61 cm, 0.35 cm, 0.16 cm

Table 5: Ultra-performance liquid chromatography (UPLC)

Sample code	Gallic acid	Procatechuic acid	Epicatechin	<i>P</i> -coumaric acid	Rutin	Quercetin	Kaempferol
	mg/g						
V4	0.553	0.556	ND	0.651	1.879	2.416	ND
V5	0.060	0.159	0.330	0.944	4.863	5.927	ND
V6	0.055	0.136	0.279	0.742	4.488	4.925	0.026

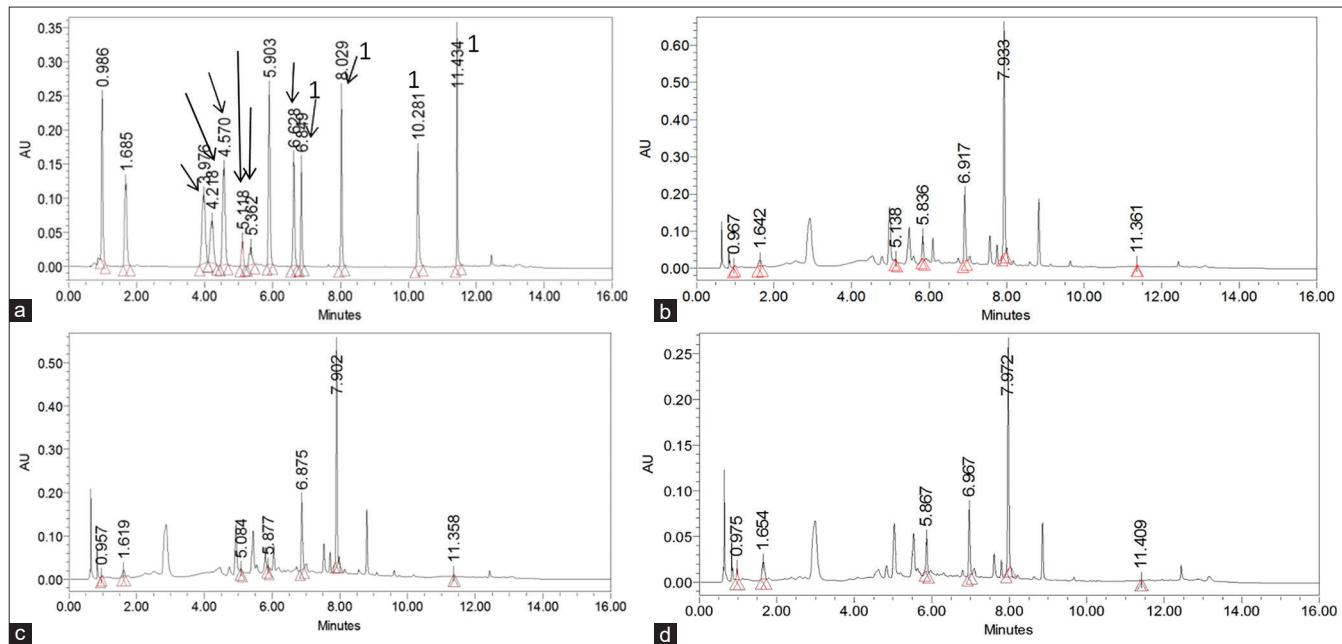


Figure 6: UPLC. (a) Standard mix (b) Aqueous extracts of *Rhododendron arboreum* Sm. (c) Ethanolic extracts of *Rhododendron arboreum* Sm. (d) Hydro-ethanolic extracts of *Rhododendron arboreum* Sm.