

Pharmacognostical Evaluation of *Rhododendron arboreum* Sm. from Uttarakhand

Devesh Tewari, Archana Negi Sah*, Sweta Bawari

ABSTRACT

Objective: *Rhododendron arboreum* Sm. commonly known as Burans is an important plant of the Himalayan region. It is the national flower of Nepal and state tree of Uttarakhand. The present study pertains to the pharmacognostical evaluation of *R. arboreum* from Uttarakhand. **Methods:** Macroscopy, microscopy by free hand section cutting and with the help of scanning electron microscopy (SEM) were done along with the physicochemical analysis. Preliminary phytochemical studies and fluorescence analysis was also carried out. **Results:** Results revealed that the leaves contain paracytic or amphiparacytic stomata in cluster form. The epidermal cells were long and spongy. The cells also contain some grooves and papillae throughout the surface. Preliminary phytochemical analysis showed the presence of different secondary metabolites like alkaloids carbohydrates, phenols, proteins, saponins, and tannins. **Conclusion:** *R. arboreum* from Uttarakhand region was investigated for its morphological and cytological characteristics with the help of scanning electron microscopy for the first time. The results provide details on the presence of several specific characters which are important for the identification of the genuine crude drug.

Key words: Burans, SEM, Microscopy, Himalaya, Phytochemical.

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History

- Submission Date: 10-12-2017;
- Review completed: 30-12-2017;
- Accepted Date: 25-01-2018

DOI : 10.5530/pj.2018.3.86

Article Available online

<http://www.phcogj.com/v10/i3>

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INTRODUCTION

Himalayan region is the treasure of biodiversity and consists of diverse flora and fauna. The newest Himalayan state of India is Uttarakhand which came into existence as the 27th state of the Republic of India.¹ The state is endowed with rich cultural heritage and natural resources with potential bioactive properties. Several less explored plants are available in the Himalayan region. One such plant is *Rhododendron arboreum* Sm. (family *Ericaceae*) which is a popular plant especially for its beautiful flower and flower juice which is consumed as a popular beverage in the Himalayan region. The plant is commonly known as “Burans” and “*Laligurans*” and is an important contributor to the economy of the rural areas of Uttarakhand. *R. arboreum* is the national flower of Nepal and state tree of Uttarakhand.² The plant is supposed to be described in Ayurveda as *Kurabaka* which is kept in the Kashaya group.³ and sold in the local market of Nepal in the name of “*Rohitaka*”.²

Different parts of this small tree possess important therapeutic activities. The leaves of this plant showed hepatoprotective effects in rats.⁴ Apart from the leaves of this plant, other parts like flowers and bark are also used for various therapeutic purposes. The plant contains numerous phytoconstituents like ursolic acid, lupeol, sitosterol, taraxerol and betulin (Figure 1).⁵

The pharmacognostic evaluation parameters are useful in the identification and standardization of a crude

drug.⁶ Considering the therapeutic importance of the plant, this study pertains to the pharmacognostical evaluation of *R. arboreum* leaves. To the best of our knowledge, the leaves of *R. arboreum* from Kumaun region of Uttarakhand were investigated for its morphological and cytological investigation with the help of scanning electron microscopy for the first time.

MATERIAL AND METHODS

Plant material

The fresh leaves and flowers of *R. arboreum* were collected from Ramgarh region of Nainital district, Uttarakhand, India. The herbarium specimens were prepared and authenticated from Regional Ayurveda Research Institute (RARI), Jhansi and the voucher specimen was deposited in the respective Herbarium for future reference (Accession no. 25277).

Organoleptic Evaluation

Organoleptic study of *R. arboreum* was carried out to evaluate its sensory characters such as colour, odour, taste, shape, size as per the reported method.^{7,8} The leaves of the plant material were placed separately on a watch glass and observed carefully for their apparent characteristics like general appearance, colour, odour and taste. The observations were recorded as accurately as possible that provided information on obvious physical properties.^{7,8}

Cite this article: Tewari D, Sah AN, Bawari S. Pharmacognostical Evaluation of *Rhododendron arboreum* Sm. from Uttarakhand. *Pharmacogn J.* 2018;10(3):527-32.

Microscopical evaluation

The leaves of the *R. arboreum* were subjected to microscopical studies. The material was dipped in FAA (Formalin–acetic acid–alcohol) solution.^{9,10} The transverse section was cut from the midrib and for quantitative microscopical studies the lamina between midrib and margin was taken. Leaves were soaked in 70% v/v ethanol followed by free hand section cutting and then viewed under the microscope. Moreover, quantitative microscopical studies such as determination of stomatal index, vein islet number and veinlet-termination number were also carried out as per the standard protocols.^{11,12} The material was placed on the slide and stained with different staining reagents and examined under the microscope (Leica DM 2500 LED).¹²

Scanning electron microscopy

Leaf sample for *R. arboreum* was subjected to scanning electron microscopy (SEM) with the help of scanning electron microscope. Electron microscope (ZEISS EVO 40 EP) was used and images were analyzed by QUANTAX 200 analytical software. The sample was properly dried with an electric lamp and then separately mounted on stubs using double-sided adhesive tape. After that the samples were sputtercoated with 5 nm cathodic spraying (Polaron gold) using a separate coating machine to make the sample more conductive and suitable for SEM analysis and then observed under the scanning electron microscope (ZEISS EVO 50). SEM was carried out under the following analytical conditions: EHT = 20.00 kV, WD = 9.5 mm and signal A = SE1. The SEM photographs were captured on the Zeiss EVO 50 scanning electron microscope at the required magnification (ranging between 100x to 8000x) at room temperature.¹³

Physicochemical evaluation

Evaluation of physicochemical parameters viz. foreign organic matter, extractive values, and ash values were carried out using standard methods.^{14,15}

Preliminary phytochemical screening

Different extracts (methanolic, ethyl acetate and petroleum ether) of *R. arboreum* were subjected to qualitative evaluation for the presence or absence of different groups of phytoconstituents.^{11,12} such as alkaloids,

flavonoids, saponins, carbohydrates, glycosides, lipids, phenols and tannins.

Fluorescence analysis

Fluorescence analysis is an important qualitative evaluation parameter of herbal drugs that is helpful in the detection of the adulteration in the raw material or finished products. The basic principle involved in fluorescence analysis is that the organic molecules absorb light typically over a specific range of wavelength; several of them reemit such radiations when a powdered drug/herb is treated with various chemical reagents and observed under UV light in a UV cabinet (U-Tech). Varying range of coloured fluorescence produced in short-wavelength (254 nm) and long-wavelength (366 nm) were prudently scrutinized for the detection of fluorescent compounds as per the suggested method.¹⁶

RESULTS AND DISCUSSION

Organoleptic Evaluation

Organoleptic evaluation is an important initial step for the identification of the plant material. *R. arboreum* was evaluated for its basic botanical and organoleptic characteristics. The plant has dark glossy green, broad leaves with size ranging between 6-20 cm, with a brown coating beneath. The leaves are oblong-lanceolate, 4-6 cm wide, glabrous, white or rusty brown-tomentose beneath. The leaves are present in abundance towards the ends of the branches. Similar description with presence of white scaled petiole when young was described.¹⁷ The flowers of *R. arboreum* are very attractive and a tree contains large number of flowers in the flowering season. Flowers of *Rhododendron* exhibit coloration ranging from deep scarlet, to red with white markings, pink to white. The plant species collected for the present study had dark red flowers in dense globose cymes as presented in Figure 2 and the details of the organoleptic characters are presented in Table 1. Forests with *Rhododendron* tree covers are of astonishing sight when in full bloom.

Microscopic evaluation

The transverse section of *R. arboreum* leaf through midrib depicted a normal dicot leaf. The outer most layer of cuticle was followed by upper epidermis and then collenchyma. Long palisade layer was present. The inner circle consisted of the xylem and phloem cells. Vacuole was also

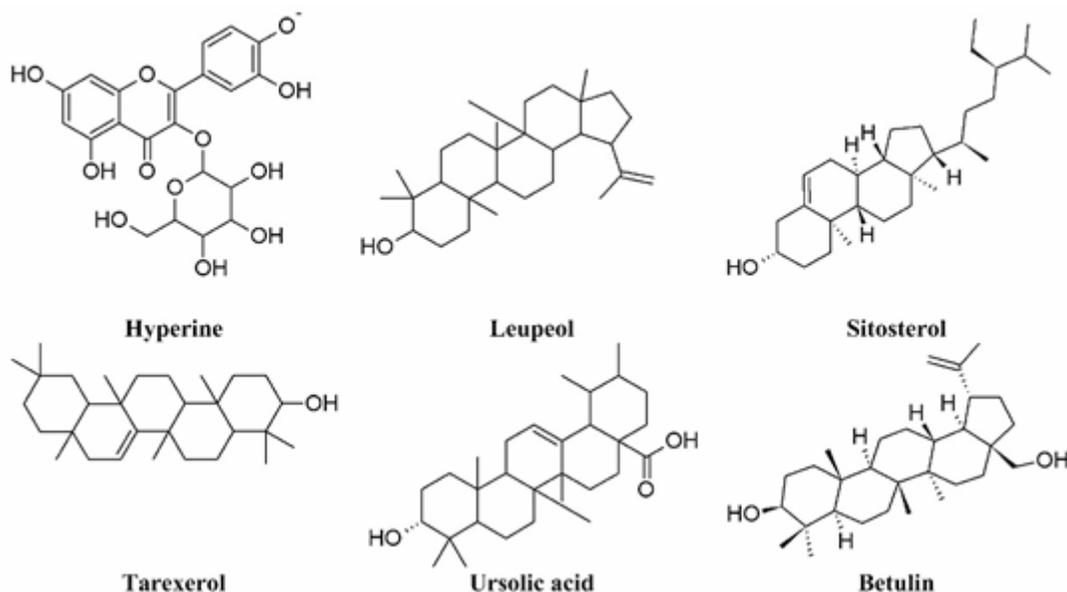


Figure 1: Chemical structures of phytoconstituents of *Rhododendron arboreum*.

present mainly in the lower epidermis part. The cells of upper epidermis were tightly closed as compared to the lower epidermis. Spongy parenchyma also contained vacuole. Microscopical features of the *R. arboreum* leaf are presented in Figure 3.

Stomatal index, vein-islet number and veinlet-termination number were determined as a part of quantitative microscopic evaluation. The mean values were calculated from different observations. Measurements of different tissues of leaf and stem were carried out, and the results are presented in Table 2.

Scanning electron microscopy

Use of powerful microscopical tools for the anatomical studies is gaining importance these days. SEM is one of the dominant methods for the surface structure elucidation of the plant based drugs. This technique is proving as one of the most exciting and momentous tool for the study and elevation of surface topography of solids.^{13,18} This study provides detailed information on anatomical characteristics of the plant pertaining to the

arrangement of different cells and tissues, details of abnormal cells or tissue arrangements.¹³ A typical structure of the stomata was observed by SEM studies of the leaves of the plant. The paracytic stomata were found in cluster form. The epidermal cells were long and spongy. The cells also contained some grooves and papillae throughout the surface. The details of the SEM studies of *R. arboreum* are presented in Figure 4.

Physicochemical analysis

The physicochemical evaluation of *R. arboreum* was carried out and the results are expressed in Table 3.

Preliminary phytochemical screening and fluorescence analysis

The preliminary phytochemical study revealed the presence of different phytoconstituents in different extracts of *R. arboreum* (Table 4). Highest chemical identities were present in the methanolic and ethyl acetate extracts whereas the petroleum ether extract showed the presence of only lipids and major constituents. The methanolic and ethylacetate



Figure 2: Morphological characteristics of *R. arboreum* (A, B: Flower; C: Tree; D: Reproductive organs of flower).

Table 1: Organoleptic evaluation of the plants.

Organoleptic characters	<i>R. arboreum</i> leaf	<i>R. arboreum</i> flower/juice
Colour	Green	Chrimson/amaranth Red
Odour	Odourless	Similar to that of beetroot
Taste	Bitter/Acidic	Acrid followed by astringent
Shape	Broad	NA
Size	6-20 cm	NA
Fracture	Fibrous	NA

Table 2: Details of quantitative microscopy.

Quantitative microscopy	<i>R. arboreum</i>
Stomata index	13-15-18
Stomata density	180±59
Vein islet number	34-42-67
Veinlet termination number	32-36-45
Palisade ratio	2-4
Palisade layer	397.35±29 µm
Xylem vessels	148.9±36 µm

Table 3: Physicochemical parameters of the different plant studied.

Parameters	<i>R. arboreum</i> leaf
Foreign matter	Nil
Moisture content	0.78%
Total ash	1.6%
Acid insoluble ash	0.32
Sulphated ash	0.0214
Water soluble extractives	12%
Alcohol soluble extractives	9.65%

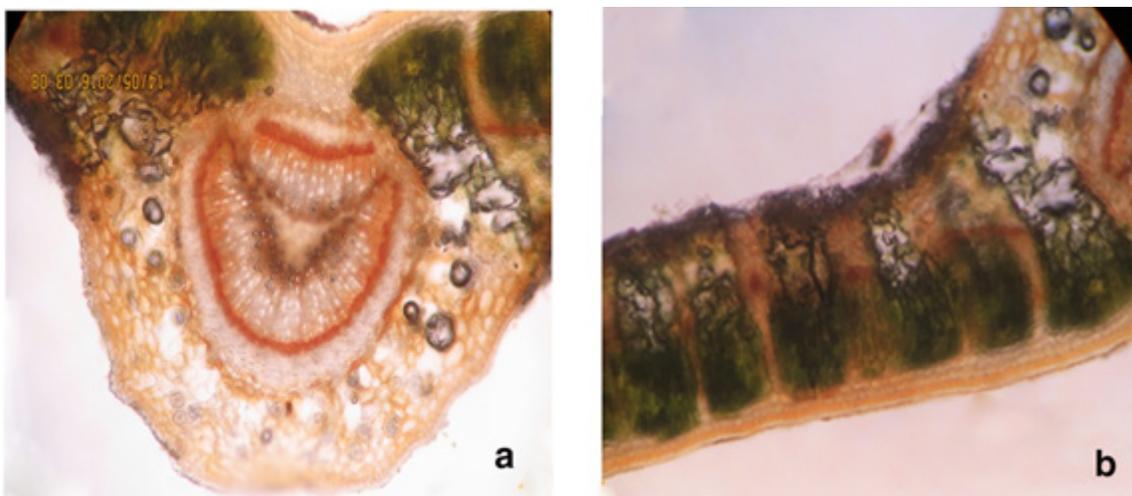
Table 4: Details of preliminary phytochemical screening of *R. arboreum*.

Phytoconstituents	<i>R. arboreum</i>		
	ME	EA	PE
Carbohydrate	++	--	--
Saponins	+++	++	--
Alkaloids	++	-	-
Reducing sugar	++	++	-
Lipids	--	--	+
Tannins	++	++	-
Phenols	++	++	-

ME: methanolic extract, EA: ethyl acetate extract, PE: petroleum ether extract

Table 5: Fluorescence analysis and reaction with different reagents.

Treatment	<i>Rhododendron arboreum</i> (Leaf)			<i>Rhododendron arboreum</i> (Juice)		
	Visible/Day light	UV254 nm	UV 366 nm	Visible/Day light	UV254 nm	UV 366 nm
Crude drug as such	Greenish brown	Dark green	Light green	Crimson Red	Dark red	Light pink
Powder + methanol	Dark green	Light green	Light green	Red	Light pink	Light pink
Powder+ Ether	Pale yellow	Pale green	Dark green	Pinkish red	Dark red	Pinkish red
Powder+ Chloroform	Light pink	Light pink	Light pink	Light pink	Dark green	Dark green
Powder+ Acetone	Dark green	Pale green	Dark green	Faint red	Light pink	Light pink
Powder +10% NaOH	Dark green	Light green	Light green	Faint red	Light pink	Light pink
Powder + dil. NH ₃	Greenish brown	Dark green	Light green	Pale yellow	Pale green	Pale green
Powder + Conc. HNO ₃	Dark green	Greenish brown	Light green	Transparent	Faint red	Dark brown
Powder + 1M H ₂ SO ₄	Dark green	Light green	Light green	Faint red	Light pink	Light pink
Powder+1M HCl	Light green	Dark green	Dark green	Red	Light pink	Light pink
Powder + 10% FeCl ₃	Dark green	Pale green	Dark green	Pinkish red	Dark red	Pinkish red
Powder + 10 % Iodine	Blue	Dark green	Dark green	Light pink	Dark green	Dark green

**Figure 3:** Transverse section of *Rhododendron arboreum* Sm. leaf (a: dicot leaf; b: palisade layer).

extract revealed the presence of alkaloids, tannins, reducing sugar and phenolic compounds. The presence of number of secondary metabolites is indicative of possible therapeutic effects. Observations presented in Table 5 indicate identifiable traits of the plant material tested. Colour

variations from pale green to dark green and black were observed on treatment with the mentioned solvents and reagents under day light and UV light (short and long wavelength). Fluorescent study of *R. arboreum* using different chemical reagents exhibited various colouration under

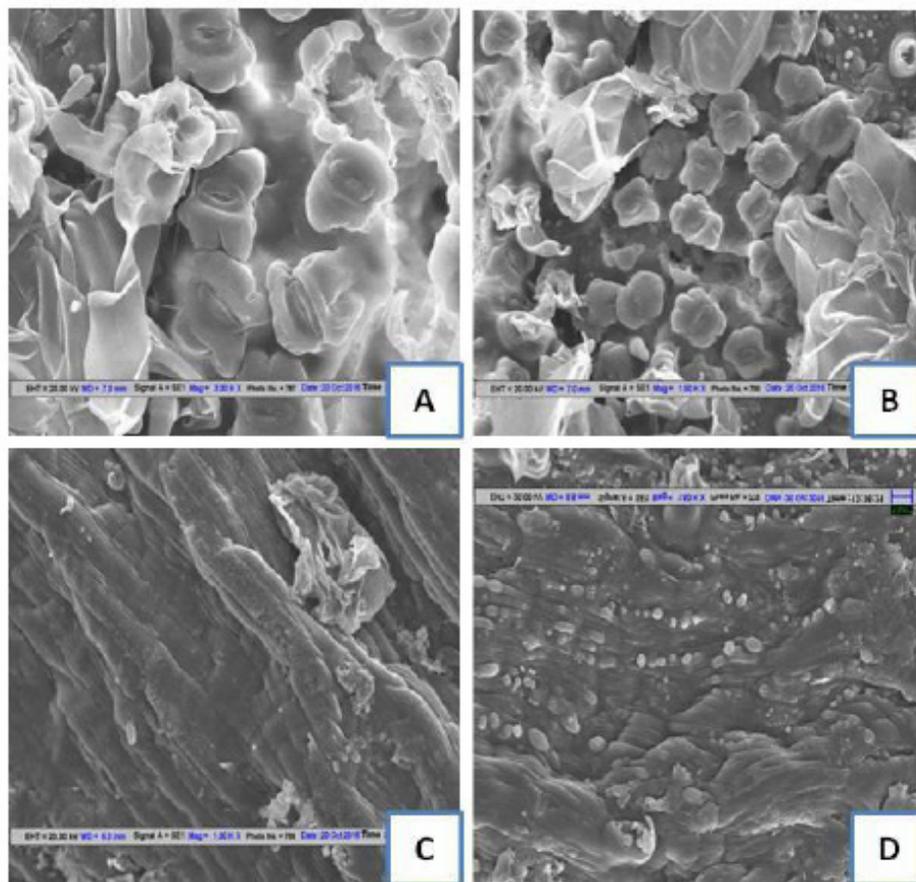


Figure 4: Scanning electron photomicrographs of the leaves of *R. arboreum* (A, B: Stomata, C, D: Papillae and palisade cells)

visible light and UV light. UV light induces several fluorescent components in natural products.

Rhododendrons are believed to be amongst the widely grown ornamentals of great horticultural interest. The genus comprises of about 1000 species worldwide.¹⁹ Leaf anatomy of *Rhododendron* genus is of interest for various scientists worldwide. A critical comparison of the leaf structures in two *Rhododendron* species viz. *R. catawbiense* and *R. ponticum* propagated in USA was carried out using scanning electron microscopy. The results of the study indicated that *R. ponticum* has higher stomatal density than *R. catawbiense* leaves. The study also demonstrated the thermanasty (i.e., temperature-induced leaf movements) behaviours of *Rhododendron* species and their distinct characters.²⁰

The distribution of the species *R. arboreum* Sm. is restricted to a small number of South East Asian countries like India, Bhutan, Nepal, Sri Lanka, Northern Myanmar, Northern Thailand, South Western China, and Northern Vietnam. Different species of *Rhododendron* have been extensively studied by Indian researchers but most of the studies were focused on the pollen morphology.^{21,22} Sufficient studies have not been carried out on the pharmacognostical aspect of *R. arboreum* leaves. Although, one extensive study was recently carried out by Panda and Kirtania,²³ on the leaf anatomy and pollen morphology of *R. arboreum* based on herbarium and field based exomorphological data to evaluate the intraspecies variations in *R. arboreum* complex. In the study several leaf samples from Arunachal Pradesh, Sikkim, Meghalaya, Darjeeling, Shilong, Uttaranchal, Bhutan, Nepal, Himachal Pradesh, Tamil Nadu and China were presented and the stomata complex was evaluated on

Arunachal Pradesh, Sikkim, Bhutan, Sikkim, China, Kerala, and Tamil Nadu samples.²³ Our results on the leaf anatomy support the findings reported earlier.²³

CONCLUSION

The results of the present study provide an insight on the pharmacognostical evaluation of the *R. arboreum* leaves from Uttarakhand region. The details presented in the study confirms the qualitative presence of various secondary metabolites in the leaves and flowers of *R. arboreum*. Moreover, the data of this study could be useful in the preparation of the pharmacopoeial monograph of *R. arboreum*. Further phytochemical and pharmacological studies are required to unravel the safety and bio-efficacy of *R. arboreum*.

ACKNOWLEDGEMENT

We are grateful to Head D.O.P.S. Bhimtal for necessary facilities, Textile Department, Indian Institute of Technology, New Delhi (IIT) for providing their SEM facility, In-charge, RARI Jhansi and Dr. Sanjeev Lale for identification of plant, Dr. L. S. Rautela for the technical support.

CONFLICT OF INTEREST

The authors declare no conflict of interest.

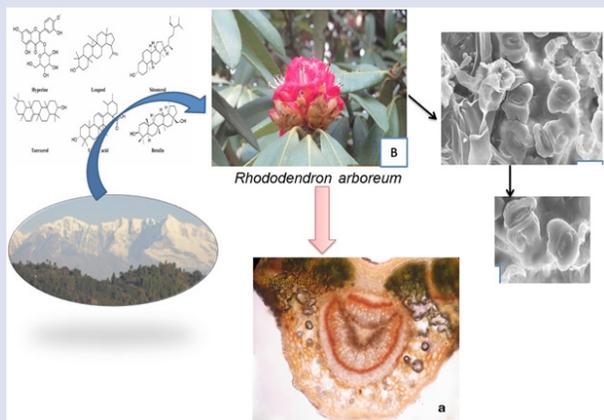
ABBREVIATIONS USED

SEM: Scanning electron microscopy; TS: Transverse section.

REFERENCES

1. Tewari D. Ecopharmacognosy: With Special Reference to Indian Himalayan Region. Int J Pharm Sci Rev Res. 2014;28(2):147-9.
2. Keshari P, Pradeep, Prabhu SN. Pharmacognostical and chromatographic evaluation of market sample of *Rhododendron arboreum* stem bark as a source plant for Rohitaka in Nepal. J of Pharmacognosy and Phytochemistry. 2017;6(5):296-306.
3. Singh Thakur Balwant, Chuneekar KC. Glossary of vegetable drugs in Brihatrayee. 1st edition. Varanasi: The Chowkhamba Sanskrit Series. 1972;447-8.
4. Prakash T, Fadadu SD, Sharma UR. Hepatoprotective activity of leaves of *Rhododendron arboreum* in CCl₄ induced hepatotoxicity in rats. Journal Medicinal Plant Research. 2008;2(11):315-20.
5. Nisar M, Ali S, Muhammad N, Gillani SN, Shah MR, Khan H, Maione F. Antinociceptive and anti-inflammatory potential of *Rhododendron arboreum* bark. Toxicol and Industrial Health. 2016;32(7):1254-9.
6. Tewari D, Kumar P, Sharma P. Pharmacognostical Evaluation of *Elaeocarpus sphaericus* (Rudraksha) Leaves. Int J Pharmacognosy and Phytochemical Res. 2013;5(3):147-50.
7. Kokate CK. Practical pharmacognosy. Vallabh Prakashan, New Delhi. 1986;111.
8. Khandelwal KR. Practical Pharmacognosy. Techniques and experiments. 9th edn. Nirali prakashan India. 2008.
9. Johansen DA. Plant microtechnique. McGraw-Hill Book Company, Inc: London. 1940:530.
10. Berlyn GP, Miksche JP, Sass JE. Botanical microtechnique and cytochemistry. Iowa State University Press. 1976.
11. Trease GE and Evans WC. Pharmacognosy. 12th Edition, Bailliere Tindall, A division of Cassell Ltd., Eastbourne BN, 2130N. 1983;17-63.
12. Wallis TE. Text book of Pharmacognosy reprint. CBS Publication New Dehli. 2005;578-81.
13. Tewari D, Sah AN, Bawari S, Sharma H, Mangal AK. Pharmacognostical Evaluation and HPTLC Fingerprinting Identification of *Ficus palmata* Forssk. (Bedu) from Western Himalaya. Curr Bioactive Compounds. 2017;13:10.2174/1573407213666170126161111
14. Anonymous. Quality Control Methods for Medicinal Plant Materials, World Health Organization (WHO), Geneva. 1998.
15. Anonymous. Physico-chemical standards of Unani formulations, Part-IV, Central Council for Research in Unani Medicine (CCRUM), Dept. of AYUSH, M/o Health and Family Welfare, Govt. of India, New Delhi. 2006;157-60.
16. Ramashesan ST, Pitsaiah P, Maramreddy PR, Bharti V, Ramakrishana KK, Gaddam V, et al. Pharmacognostical Standardization of Goraksha pods: an important Nutritive and antidiabetic Plant. Pharmacognosy Journal. 2016;8(5).
17. Orwa C, Mutua A, Kindt R, Jamnadass R, Simons A. Agroforestry database: a tree species reference and selection guide version 4.0. World Agroforestry Centre ICRAF, Nairobi, KE. 2009.
18. Haywood V.H. Scanning electron microscopy in the study of plant materials. Micron. 1969;1(1):1-14.
19. Chamberlain D, Hyam R, Argent G, Fairweather G, Walter KS. The genus *Rhododendron*: its classification and synonymy. Royal Botanic Garden Edinburgh. 1996.
20. Wang X, Arora R, Horner HT, Krebs SL. Structural adaptations in overwintering leaves of thermonastic and nonthermonastic *Rhododendron* species. Journal of the American Society for Horticultural Science. 2008;133(6):768-76.
21. Nair PKK, Kothari S. Pollen morphology of Indian Heteromerae. Adv. Pollen-Spore Res. 1985;13:1-7.
22. Paria ND, Pal A. Quantitative and experimental studies on pollen morphology of some Indian *Rhododendron* (*Ericaceae*). J. Palynol. 1990;26:95-104.
23. Panda S, Kirtania I. Variation in *Rhododendron arboreum* Sm. complex (*Ericaceae*): insights from exomorphology, leaf anatomy and pollen morphology. Modern Phytomorphology. 2016;9:27-49.

GRAPHICAL ABSTRACT



SUMMARY

- *Rhododendron arboreum* Sm. commonly known as Burans is an important plant of the Himalayan region.
- *R. arboreum* from Uttarakhand region was investigated for its morphological and cytological characteristics with the help of scanning electron microscopy
- The results of the present study provide an insight on the pharmacognostical evaluation of the *R. arboreum* leaves from Uttarakhand region.

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Cite this article: Tewari D, Sah AN, Bawari S. Pharmacognostical Evaluation of *Rhododendron arboreum* Sm. from Uttarakhand. Pharmacog J. 2018;10(3):527-32.