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Detailed micrometrical, pharmacognostical and analytical evaluation of *Rhododendron arboreum Sm.* leaves

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Abstract
Rhododendron arboreum Sm. is an ethnomedicinal plant of Himachal Pradesh belonging to family Ericaceae. It is locally known as *Barah*, *Brass* and its leaves are used in the treatment of fever, headache, diarrhoea, gout and rheumatism. The present study is designed to investigate the pharmacognostical profile of leaves including powder microscopy, detailed micrometric evaluation and analytical study. Transverse section of petiole is circular to heart shape in outline showing central arc shaped vascular bundle and one - two meristeles on its either side. Transverse section of leaf shows upper epidermis and lower epidermis followed by collenchyma, ground tissue, central vascular bundle. Covering trichomes and sessile glandular trichomes are present on lower surface. Leaf powder shows the presence of trichomes, epidermal cells, stomata and rosette crystals. Loss on drying of leaves found to be 4.29 ± 0.16 % w/w. Qualitative tests showed the presence of tannin, alkaloids and flavonoids. HPTLC study reveals presence of 9 and 8 peaks at 254 nm and 366 nm respectively. This study would be useful in the identification and authentication of the plant material.

Keywords: *Burans*, *Rhododendron arboreum*, pharmacognostical, tannin

1. Introduction
Rhododendron is the largest genus in the plant family Ericaceae. It consists of nearly one thousand mostly evergreen, terrestrial or epiphytic species ranging from tiny plants to shrubs and to giant trees. *Rhododendron* is native to the temperate areas of northern hemisphere and is most abundant in southeastern Asia from the Himalayas, China and Tibet to Malaysia and the Philippines. There are about 80 species of *Rhododendron* found throughout in India [1].
Among all Indian *rhododendron* species, *Rhododendron arboreum* Sm. is widely distributed from the western to eastern Himalayan region and other neighbouring countries [2]. It is locally known as *Barah*, *Brass* in Himachal Pradesh and *Burans*. It has simple leaves and pinkish-brown, exfoliating bark, blood red colored flowers crowded in large rounded corymbs [3]. Its leaves are used in diarrhoea, fever, headache [4], gout and rheumatism [5]. Young leaves are chewed to get relief from headache [6] and its paste is applied over forehead as febrifuge [7]. The present study includes detailed microscopical as well as micrometric evaluation of leaves and its powder and phytochemical screening, physicochemical and quantitative analysis. This study could be used to establish standards of pharmacognostical and analytical characters for proper identification and standardization of drug.

2. Materials and methods
2.1 Collection and Authentication
Leaves of *Rhododendron arboreum* Sm. were collected from its natural habitat Jhatingri (distt Mandi), Himachal Pradesh during month of March 2018. A specimen of the sample herbarium has been deposited in Pharmacognosy Laboratory, Institute for Post Graduate Teaching and Research in Ayurveda, Gujarat Ayurved University (Specimen No. Ph. M: 6263/ 2018-19**)** (Fig. 1). Fresh leaves are used for taking transverse section. Remaining leaves were shade dried, powdered and stored in airtight container for powder microscopy and phytochemical study.

Fig 1: (A) Natural habitat of plant; (B) Plant herbarium

2.2 Pharmacognostical study
Morphological characters of *Rhododendron arboreum* leaves were observed through naked eyes.
Thin transverse sections of petiole, midrib and powder of leaves powder are observed under Quasmo binocular compound microscope and photographs were taken by using Nikon Coolpix L 19 camera. Histochemical study and micrometric evaluation of observed cellular characters also done. For surface study, epidermal layers from upper and lower surfaces of leaves were peeled out separately by means of forceps and kept on slide to mount in glycerin water [8]. All determinations were performed in triplicate and the results are presented as mean ± standard deviation (SD).

2.3 Analytical study

Physicochemical parameters like loss on drying, ash values, extractive values were determined according to the standard procedures mentioned in API [9]. Methanolic and aqueous extract of leaves was prepared and used for detection of different phytochemicals [10].

Spots of methonalic extract of leaves are applied on precoated Silica Gel GF254 Plates using Camag Linomat V. The solvent system used for the study is toluene: ethyl acetate: formic acid (9:2:1v/v/v). Camag twin trough chamber is saturated for 30 minutes. Plates were scanned at 254 nm and 366 nm using Camag Scanner III equipped with Win cats software [11].

3. Results and discussion

3.1 Pharmacognostical study

Leaves are simple, lanceolate narrowed at both ends, about 7.5cm -15 cm long and 3cm-5.5 cm wide, crowded towards the ends of the branches, lanceolate or oblong, narrowed at both ends, glabrous and glossy green above, pale beneath form a film of small white scales, the midrib and nerves prominent beneath. Petiole is stout, 1.3-2.5 cm long, 0.1 cm - 0.3 cm in diameter, clothed with white scales when young. (Fig 2: A, B)

Diagrammatic transverse section of petiole is oval to cordate in shape. Detailed transverse section of petiole shows outermost single layered epidermis consisting of rounded to oval shaped cells covered with thick cuticle. Outer zone of the ground cortex forms 2-3 layers collenchymatous hypo-dermis. Collenchyma is more prominent at the grooved region. Rest of the cortex is made of thin-walled chlorenchyma and aerenchyma cells with large number of air chambers. Some of the cells contain stone cells and starch grains. Vascular system comprises of centrally located main arc shaped vascular bundle and one to two meristele on its either sides. It is surrounded by discontinuous ring of pericyclic fibre. Inner to the xylem region sclerenchyma layer is present. (Fig. 2)

Diagrammatic transverse section of leaf passing through midrib shows that leaf is dorsiventral in nature. Transverse section passing through midrib shows single layered, papillose shaped epidermal cells forming epidermis covered with thick layer of cuticle. Both epidermis are followed by collenchymatous hypodermis, less no. of layers observed at lower epidermis. Some of the collenchyma cells are lignified. Rest of the section is composed of parenchyma cells. Some of the parenchyma cells contain isolated stone cells, starch grains, cluster crystals. Inner to the parenchyma cells followed by single layered bundle sheath. 6-8 layers of lignified pericyclic fibres forming ring like structure. Vascular bundles are radially, and circular arranged in the form of a ring around the sclerenchymatous central ground tissue. Xylem is present towards the centre and phloem towards the periphery. Outer phloem made up of phloem fibers and sieve elements, inner xylem made up of xylem vessel, xylem parenchyma, and its fiber. Central ground tissue is sclerenchymatous with lignin and pitted form.

Detailed transverse section through lamina shows 2-3 layered upper epidermis covered with thick cuticle and devoid of trichomes. Uppermost epidermal layer has small, squarish cells while second and third layer is composed of tangentially elongated, cuboidal shaped epidermal cells. Lower epidermis is single layered composed of oval shaped cells covered with thick cuticle and often, interrupted by stomatal openings (paracytic stomata), covering trichomes; sessile, glandular trichomes; multicellular, non-glandular trichomes filled with brown content. Mesophyll comprises of upper palisade parenchyma and lower spongy parenchyma. Palisade parenchyma is composed of 3-4 layers of longitudinally elongated, compactly arranged cells followed by 4-7 layers of loosely arranged, irregular, thin walled spongy parenchyma. Few cells contain rosette crystals of calcium oxalate. Both spongy and palisade parenchyma contain chloroplast. Starch grains are found all over the lamina region (Fig 3). Vascular strands obliquely cut between the palisade and spongy parenchyma layers. Micrometric evaluation (Mean ± Standard deviation) of cells and cellular components are also performed and presented in table 1.

Table 1: Comparative micrometric evaluation of cells/cellular components in transverse section of leaf

Cell / Cellular components	Petiole [Length (in µm) X Breadth (µm)]	Midrib [Length (in µm) X Breadth (µm)]
Epidermal cell	21.42 ± 3.57 X 15.47 ± 4.12	27.37 ± 8.98 X 20.23 ± 5.45 (upper epidermis) 15.47 ± 5.45 X 11.9 ± 2.06 (lower epidermis)
Thickness of cuticle	8.33 ± 2.06	8.33 ± 2.06
Covering trichome	---	39.27 ± 7.14 X 9.52 ± 2.06
Sessile glandular trichome	---	20.23 ± 2.06114 X 16.66 ± 2.06114
Multicellular covering trichome	---	22.61 ± 2.06114 X 9.52 ± 2.06114
Parenchyma cell (ground tissue)	23.8 ± 5.45 X 19.04 ± 10.3 (Chlorenchyma)	38.08 ± 14.42 X 26.18 ± 4.12
Collenchyma cell (hypodermis)	21.42 ± 3.57 X 17.85 ± 3.57	24.99 ± 3.57 X 19.04 ±4.12
Central sclerenchyma cell	---	18.93 ± 2.16 X 14.28 ± 3.57
Pericyclic fibre	15.47 ± 2.06 X 11.9 ± 5.45	17.85 ± 3.57 X 10.71 ± 3.57
Xylem vessel	16.66 ± 5.45 X 8.33 ± 2.06	28.56 ± 16.35 X 20.23 ± 11.47
Phloem	4.76 ± 2.06 X 3.57 ± 0	5.95 ± 2.06 X 4.76 ± 2.06
Palisade cell	---	33.32 ± 8.89 X 8.33 ± 2.06
Spongy parenchyma cell	---	21.42 ± 3.57 X 15.47 ± 2.06
Cluster crystal	26.18 ± 2.06 X 20.23 ± 2.06	34.51 ± 5.46 X 29.75 ± 4.12
Oil globule	9.52 ± 4.12 X 8.33 ± 2.06	9.52 ± 4.12 X 8.33 ± 2.06
Starch grain	8.33 ± 2.06 X 5.95 ± 2.06	36.90 ± 4.13 X 33.32 ± 2.06
Prismatic crystal	---	13.09 ± 4.12 X 9.52 ± 2.06
Rosette crystal	32.13 ± 3.57 X 26.18 ± 5.45	38.08 ± 4.12 X 33.32 ± 2.06
Stone cell	69.02 ± 24.30 X 59.5 ± 26.79	48.79 ± 8.96 X 41.65 ± 5.45
Air spaces	69.02 ± 5.45 X 58.31 ± 5.45	---

Surface study of lower epidermis of leaf shows paracytic type of stomata. Stomata are absent at upper surface (Fig 3).

Results of surface study are depicted in table 2.

Table 2: Surface study of leaf

Cell / Cellular components	Values
Stomata [Length (in µm) X Breadth (in µm)]	13.09 ± 4.12 X 7.14 ± 0
Epidermal cell [Length (in µm) X Breadth (in µm)]	24.99 ± 3.57 X 13.09 ± 2.06
Stomatal index	20.58 ± 2.18
Palisade ratio	¼

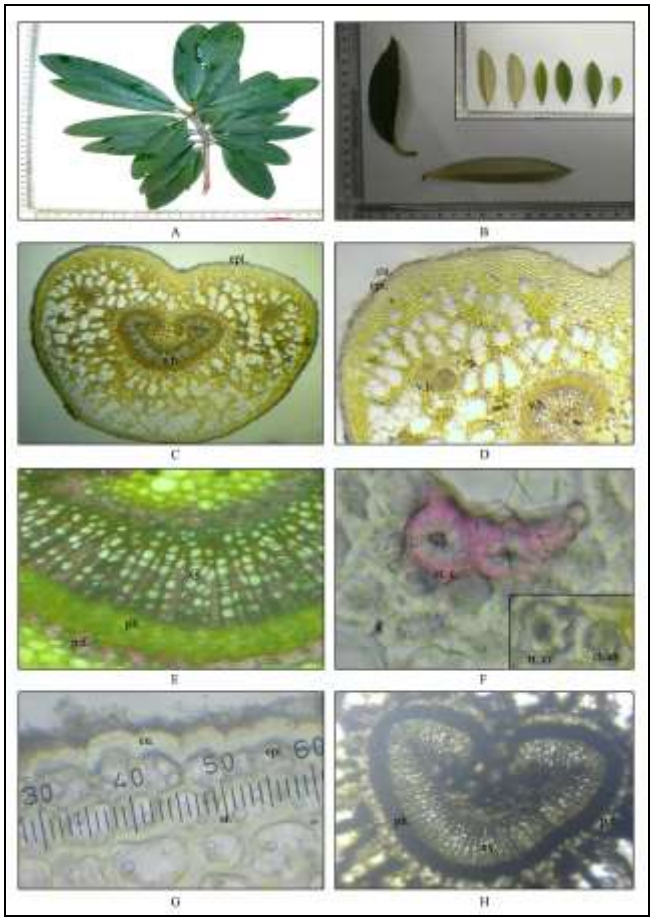

Fig 2: (A) Morphology of leaves; (B) Measurement of leaves; (C) Diagrammatic TS of petiole; (D) Aerenchymatous ground tissue; (E) Lignified xylem, phloem, lignified pericyclic fibres; (F) Lignified stone cells; rosette crystal, cluster crystal; (G) Epidermis with thick cuticle; (H) Tannin filled cells after staining with $FeCl_3$. Abbreviation: epi.- epidermis; c. cr.- cluster crystal; cu.- cuticle; epi.- epidermis; ph.- phloem; r. cr.- rosette crystal; vb.- vascular bundles; p.f.- pericyclic fibres; ph.- phloem; l. epi.- lower epidermis; st.- starch grain; st. c.- stone cell; u. epi.- upper epidermis; xy.- xylem

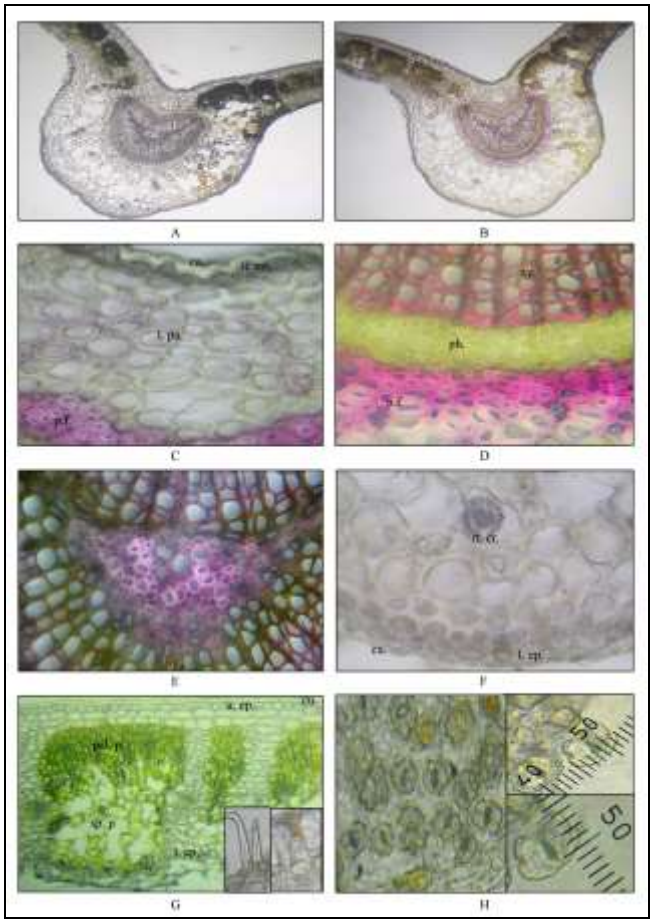

Fig. 3: (A) Diagrammatic transverse section of midrib through lamina; (B) TS after staining with phloroglucinol and HCl; (C) Epidermal cells covered with thick cuticle; (D) Vascular bundle; (E) Lignified xylem and central sclerenchyms; (F) Lower epidermis; (G) TS of lamina; (H) Stomata and its measurement epi.- epidermis. Abbreviation: cu.- cuticle; ph.- phloem; r. cr.- rosette crystal; p.f.- pericyclic fibres; pal. p.- palisade parenchyma; l. ep. - lower epidermis; l. pa.- lignified parenchyma; sp. p.- spongy parenchyma; u. epi.- upper epidermis; xy.- xylem.

Powder microscopy

Leaf powder is fibrous, greenish in colour having astringent taste and aromatic odour.

Powder microscopy shows the presence of trichomes, epidermal cells, rosette crystal, prismatic crystals, simple starch grains, starch grains with hilum, oil, spiral vessel, sclereids, stone cells, simple fibres, stomata (Fig. 4). Micrometric evaluation (Mean±SD) of leaf powder characters are depicted in table 3.

Table 3: Micrometric measurement of powder characters

Powder characters	Length / Diameter (in µm)	Breadth/ Diameter (in µm)
Starch grain	36.90 ± 4.13	33.32 ± 2.06
Prismatic crystal	26.28 ± 7.48	20.23 ± 8.24
Rosette crystal	36.89 ± 5.45	34.51 ± 4.13
Oil globule	28.56 ± 3.57	23.8 ± 5.45
Stomata	15.47 ± 2.06	8.33 ± 2.06
Epidermal cell	28.56 ± 6.18	13.09 ± 2.06
Trichome	65.45 ± 12.53	8.33 ± 2.06
Xylem vessel	15.47 ± 2.06	---
Stone cell	63.07 ± 23.21	52.36 ± 19.66

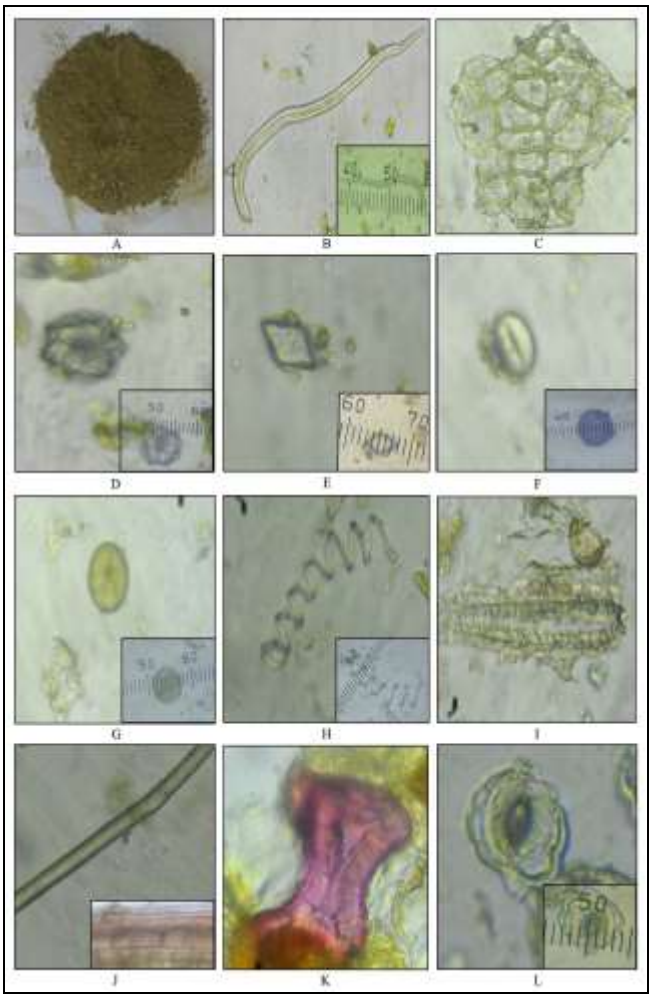

Fig 4: (A) Leaf powder; (B) Covering trichome; (C) Epidermal cells; (D) Rosette crystal; (E) Prismatic crystal; (F) Starch grain; (G) oil globule; (H) Spiral vessel; (I) Sclreid; (J) Fibre; (K) Lignified stone cell; (L) Stomata

3.2 Analytical study

3.2.1 Physicochemical Parameters

Powder of dried leaves is subjected to physico-chemical evaluation and their results are shown in table 4.

Table 4: Physicochemical evaluation of *Rhododendron arboreum* Sm. leaves

Parameters	Results (%w/w)
Loss on drying	4.29 ± 0.16
Total Ash value	3.68 ± 0.10
Acid insoluble ash value	0.69 ± 0.12
Water soluble extractive value	13.52 ± 0.11
Alcohol soluble extractive value	16.54 ± 0.13

3.2.2 Qualitative test

Phytochemical screening of water and alcohol soluble extracts of leaves show the presence of various secondary metabolites like carbohydrate, alkaloid, and tannin. Results of other tests conducted are mentioned in Table 5.

Table 5: Results of phytochemical screening of *Rhododendron arboreum* Sm. leaves

Phytoconstituents	Tests	Water extract	Alcohol extract
Carbohydrate	Molisch's	++	++
Protein	Biuret	++	++
	Ninhydrin	--	--
Steroid	Salkowski	++	++
Tannin	$FeCl_3$	++	++
Alkaloid	Dragendorff's	++	++
	Mayer's	++	++
Anthraquinone glycoside	Borntrager's	--	--
Saponin glycosides	Foam	++	--
Flavonoid	Lead acetate	++	++

'++' - present, '--' – absent

3.2.3 HPTLC study

HPTLC study of methanolic extract of leaves show 9 peaks and 8 peaks at 254 nm and 366 nm respectively. Values of retardation factor at 254 nm and 366 nm are mentioned in table 6.

Table 6: Peaks obtained in HPTLC study

254 nm	366 nm
0.05, 0.31, 0.36, 0.49, 0.61, 0.64, 0.81, 0.87, 0.98	0.04, 0.21, 0.32, 0.36, 0.44, 0.60, 0.64, 0.98

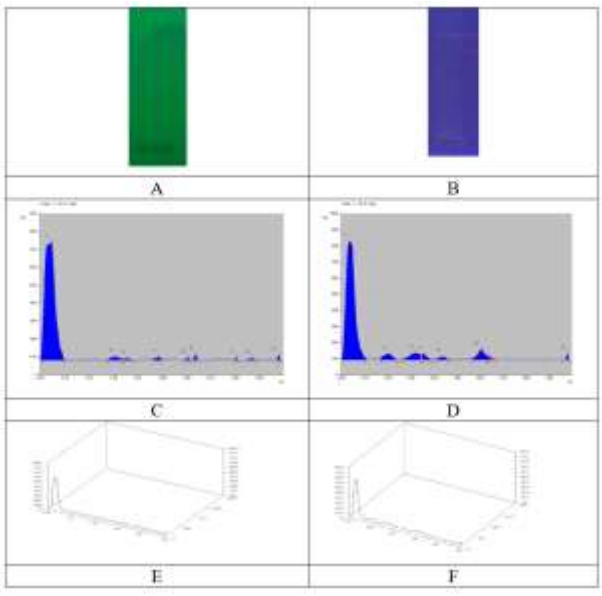

Fig 5: (A) HPTLC plate at 254 nm; (B) HPTLC plate at 366 nm; (C) Densitogram at 254 nm; (D) Densitogram at 366 nm; (E) 3-D graph at 254 nm; (F) 3-D graph at 366 nm

4. Conclusion

Leaves are simple, glabrous, alternately arranged. Transverse section showing one main central crescent shaped vascular bundles [12] with two-three meristeles, chlorenchymatous and aerenchymatous cells are the main characters of petiole. Presence of covering trichomes, sessile glandular trichomes, uniseriate-multicellular trichomes filled with brown content, thick papillose cuticle [13]. Tanniniferous cells and cluster crystals are diagnostic characters of *Rhododendron* genus [14]. Results obtained from pharmacognostical, physicochemical, qualitative test and HPTLC could be used in further standardization of the plant.

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