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Berbris aristata DC: Pharmacognostical Standardization and Phytochemical Studies of its Leaves

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ABSTRACT

Berberis aristata DC. (Fam: Berberidaceae) commonly known as Daruharidra, Indian Barberry or tree turmeric. Leaves of this plant are traditionally used in the treatment of inflammation, wound healing, skin disease, menorrhagia, diarrhea, jaundice and infection of eyes etc. Micromorphology and physicochemical analysis of the leaves of *B.aristata* were performed as per WHO and Pharmacopoeial methods. Leaves (4.9cm × 1.8cm) are deep green on dorsal and light green on ventral side. Leaves are in tufts of 5 to 8, phyllotaxy verticillate, simple spiny, lanceolate, toothed, leathery, sessile, acuminate apex and reticulate pinnate venation. Microscopic evaluation of leaves showed biconvex midrib and thick lamina. The epidermal layers of the midrib are thick with small, less conspicuous cells and thick cuticle. The vascular system consists of three large vascular bundles; the median one is small than the two lateral bundles; the bundles are collateral and wedge shaped. Lamina is made of epidermal layer on the adaxial side with spindle shaped thick walled cells and papillate cuticle. The abaxial epidermis has squarish or rectangular epidermal cells with prominent spiny cuticular outgrowths. Powder microscopy showed the presence of cuticular papillae, anomocytic stomata and spiny outgrowth. Preliminary phytochemical screening of appropriate solvent extracts showed the presence of alkaloids, sterols, tannins, proteins and amino acids, flavonoids, terpenoids, saponin, carbohydrates and absence of glycosides and volatile and fixed oil. Microscopic analysis and other parameters were informative and provide valuable information in the identification, standardization of *B.aristata* leaves.

Keywords: *Berberis aristata,* Berberidaceae, leaf, Microscopical evaluation.

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INTRODUCTION

Berberis aristata DC. commonly known as Daruharidra. It is also known as Indian Barberry or tree turmeric, belongs to the family Berberidaceae [1]. Berberis has about 650 species worldwide of which 54 have been reported from Indian Himalaya. It is ever green shrub, found in Nilgiri hills (South India), temperate and sub-tropical regions of Asia, Europe and America [2]. *Berberis aristata* has been used in Ayurvedic medicines for very long time for treating various ailments. An important ayurvedic preparation namely Rashut is prepared from this plant [3]. Traditionally this plant is used in the treatment of inflammation, wound healing, skin disease, menorrhagia, diarrhea, jaundice and infection of eyes [4, 5]. Leaves of this plant have been reported as hepatoprotective [6], antidiarrhoeal [7] and antioxidant [8].

Taxonomical classification [9]

Kingdom	:	Plantae
Division	:	Magnoliophyta
Class	:	Magnoliopsida
Order	:	Ranunculales
Family	:	Berberidaceae
Genus	:	*Berberis*
Species	:	*aristata*

Vernacular names [10]

Sanskrit	-	Darvi, Katamkateri
Bengali	-	Daruharidra
Hindi	-	Daruhaldi, Darhald
Tamil	-	Gangeti
Malayalam	-	Maramannal, Maramanjnal
Marathi	-	Daruhalad
Nepali	-	Chitra
Himachal	-	Kashmal
Punjab	-	Sumalu

Various phytoconstituents has been reported in this plant such as berberine, oxyberberine, berbamine, aromoline, a protoberberine alkaloid - karachine, palmatine, oxycanthine and taxilamine [11, 12].

As mentioned earlier several reports have been published regarding chemical constituents and different biological activities *in-vitro* and *in-vivo*. An investigation to explore its pharmacognostic examination is inevitable. The present work was undertaken with a view to lay down standards which could be useful to detect the authenticity of this medicinally useful plant *Berberis aristata* DC leaves to treat various diseases and disorders.

2. MATERIALS AND METHODS

2.1: Chemicals: Formalin, acetic acid, ethyl alcohol, chloral hydrate, toludine blue, phloroglucinol, glycerin, hydrochloric acid and all other chemicals used in this study were of analytical grade.

2.2: Collection and authentication of Plant

The leaves of the selected plant were collected from in and around Sunder Nagar, Mandi, Himachal Pradesh, with the help of local tribal and field botanist. Care was taken to selected healthy plant and for normal leaves. It was authenticated by Dr. Manoj Joshi, Ph.D (forestry), HP State Forest Department, Himachal Pradesh, India.

2.3: **Macroscopic analysis**: Macroscopic observation of the plant was done. The shape, size, surface characters, texture, colour, odour, taste etc were noted [13].

2.4: **Microscopic analysis:** The leaves were fixed in FAA (Formalin - 5 ml + acetic acid - 5 ml + 70% ethyl alcohol - 90 ml). After 24 hrs of fixing, the specimens were dehydrated with graded series of tertiary-butyl alcohol (TBA). Infiltration of the specimens was carried by gradual addition of paraffin wax (melting point 58-60°C), until TBA solution attained super saturation. The specimens were cast into paraffin blocks [14].

Sectioning: The paraffin embedded specimens were sectioned with the help of rotary microtome. The thickness of the sections were about 10-12 µm. After de-waxing the sections were stained with toludine blue. Since toludine blue is a polychromatic stain, the staining results were remarkably good and some cytochemical reactions were also obtained. The dye rendered pink color to the cellulose walls, blue to the lignified cells, dark green to suberin, violet to the mucilage, blue to the protein bodies etc [15].

Photomicrographs: Photographs of different magnifications were taken with Nikon lab-photo 2 microscopic Unit. For normal observations bright field was used. For the study of crystals, starch grains and lignified cells, polarized light was employed. Since these structures have birefringent property, under polarized light they appear bright against dark background.

2.5: Powder microscopy: Coarse powder of the leaf was used to study the microscopical characters of the leaf powder [16].

2.6: **Physicochemical analysis**: Total ash, acid insoluble ash, water soluble ash, loss on drying and extractive values was determined [17].

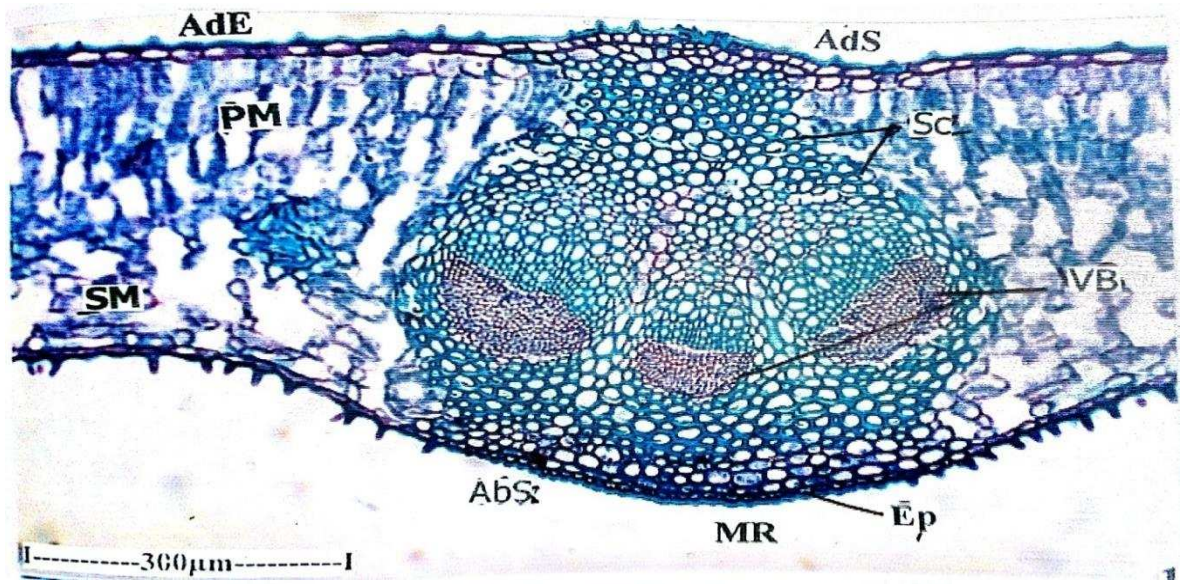
2.7: **Preliminary phytochemical screening:** Preliminary phytochemical screening of ethanolic and aqueous extract carried out to find out the presence of various phytoconstituents using standard procedure [18, 19].

3. RESULTS

3.1: Macroscopy: leaves are arranged in tufts of 5-8cm and are approximately 4.9cm long and 1.8 cm broad. The leaves are deep green on the dorsal surface and light green on the ventral surface and obovate or elliptic, entire, base gradually narrowed with reticulate nerves and glossy dark green color and simple with pinnate venation (Fig 1).

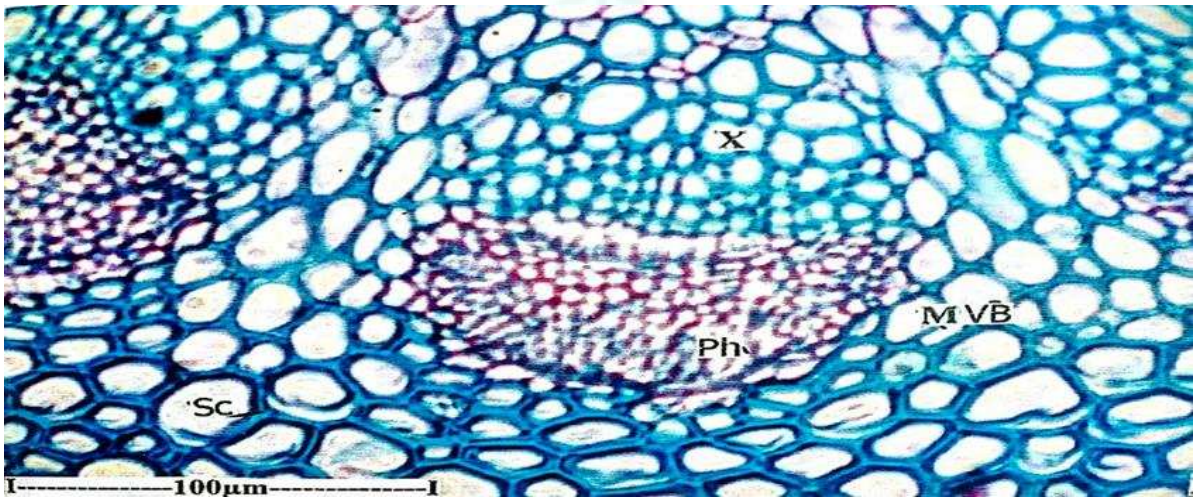
Fig 1: Leaves and flowers of *B.aristata*

3.2: Microscopy: T.S of leaf showed biconvex midrib and thick lamina. The midrib and lamina are more or less equal in thickness and the midrib does not project much beyond the leaf surface. The midrib is 550µm in thick. The epidermal layers of the midrib are thick with small, less conspicuous cells and thick cuticle. The vascular system consists of three large vascular bundles; the median one is smaller than the two lateral bundles; the bundles are collateral and wedge shaped. The xylem is triangular in outline with thick walled angular compact xylem elements and semicircular wide mass of phloem elements. The entire vascular system is surrounded by wide zone of sclerenchyma which extends both adaxially and abaxially (Fig 2 & 3).



(AdE-Adaxial Epidermis; Ads-Adaxial side, PM-Palisade Mesophyll, SC-Sclerenchyma, SM –Spongh Mesophyll; VB- Vascular bundle Ep-Epidermis, Abs -Abaxial side; MR-Midrib)

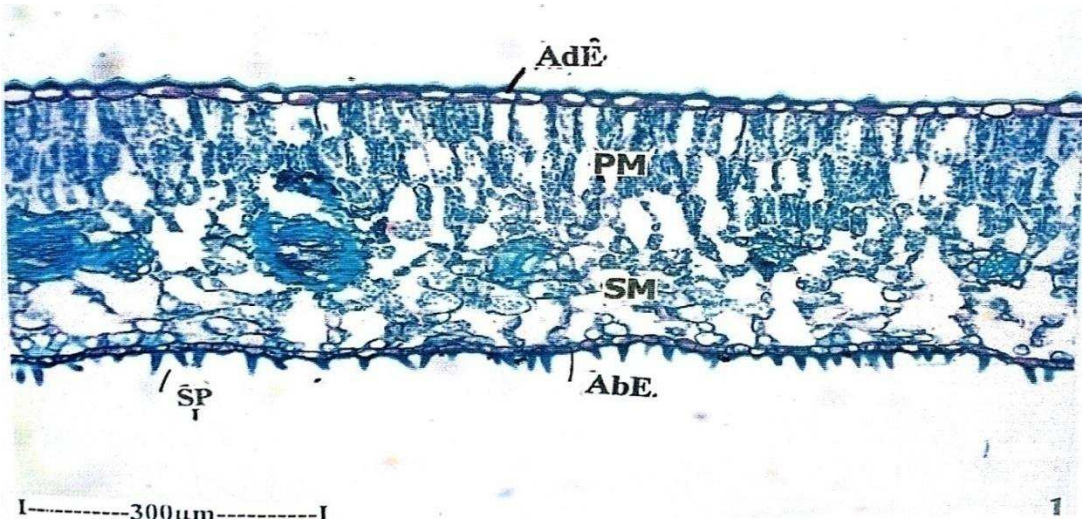
Fig.2 T.S of leaf through Midrib with Lamina



(Ph-Pholem, MVB-Median Vascular Bundle; SC-Sclerenchyma, X-Xylem)

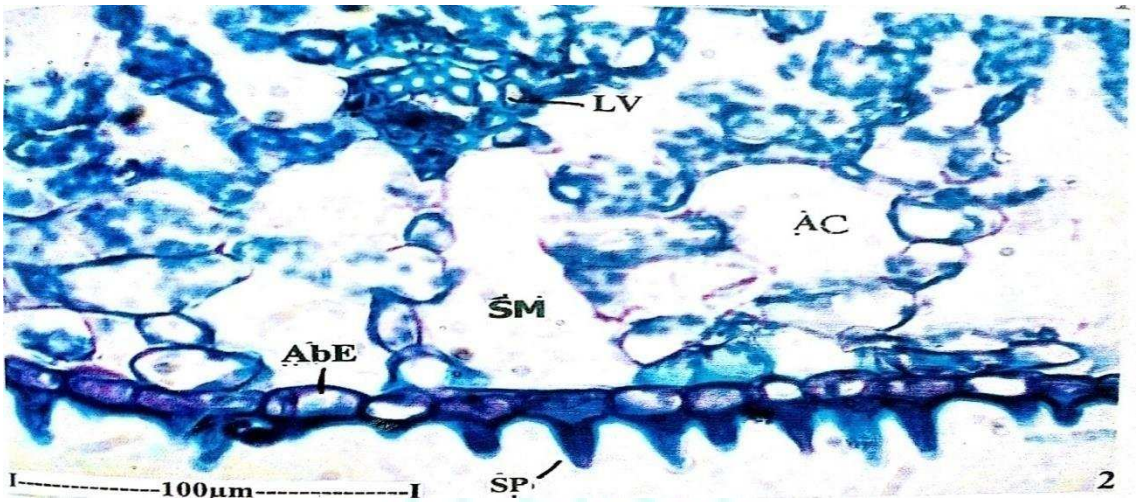
Fig 3: T.S of Midrib- Vascular Bundle enlarged

Lamina: lamina is 300µm in thick. It has epidermal layer on the adaxial side with spindle shaped thick walled cells and papillate cuticle. The abaxial epidermis has squarish or rectangular epidermal cells with prominent spiny cuticular outgrowths. The abaxial epidermis is 30µm thick including the spiny outgrowths. The mesophyll tissue is differentiated into three layers of palisade cells and six to eight layers of small lobed spongy parenchyma cells with wide air chambers. The lateral veins have small vascular strands that are placed in the median portion of the lamina.



(AbE-Abaxial Epidermis, AdE-Adaxial Epidermis, PM- Palisade Mesophyll; SM –Spongy Mesophyll; SP-Spindle shaped papillate cuticle)

Fig 4: T.S of lamina through lateral vein



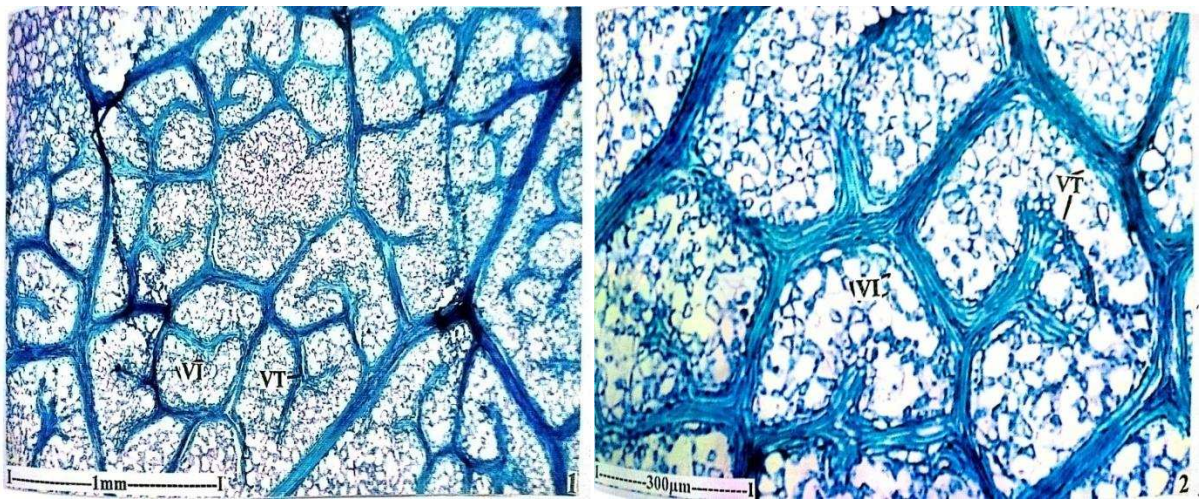
(Abe-Abaxial epidermis, Ao- Aerenchymatous tissue; LV - Lateral Vein; SM –Spongy Mesophyll; SP-Spindle shaped papillate cuticle)

Fig 5: T.S of Lamina showing abaxial epidermis and spongy mesophyll tissue

Petiole: The lower end of the petiole is planoconvex is sectional view. It has flat adaxial side with lateral extensions. It has epidermal layer of small cells and thick smooth cuticle. The vascular system is wedge shaped collateral vascular bundles of unequal size. The bundles are 150 x 200m in size. They have dense parallel rows of xylem elements and wide semicircular phloem. All the bundles as surrounded by a thick arch of sclerenchyma. In addition to the main bundles, these are two wing bundles which are circular with collateral xylem and phloem and sclerenchyma sheath.

Venation pattern

The secondary and tertiary views are equally thick. The tertiary veins form distinct vein islets and vein terminations. The vein islets are variable in shape and size they are wide or narrow. The vein terminations are short and thick; they are simple or forked once (Fig 6).



(VI- Vein Islet, VT- Vein Termination)

Fig 6: Paradermal section showing vein-islets and vein-termination

3.3: Powder microscopy

The adaxial epidermal layer has polygonal cells with straight walls. The papillate cuticle of the outer tangential walls appears as circular thick dots on all cells (Fig 7). The abaxial epidermis has stomata which are anomocytic (Fig 8). The epidermal cells consist of pointed spiny outgrowths (Fig 9). These outgrowths are 20µm in height and 10µm in thickness.

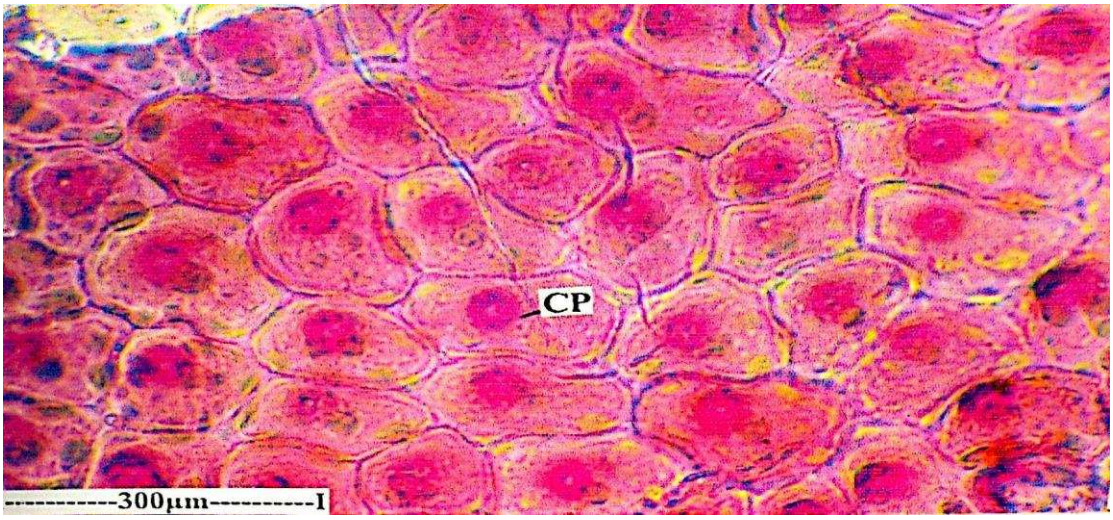


Fig 7: Cuticlar Papillae

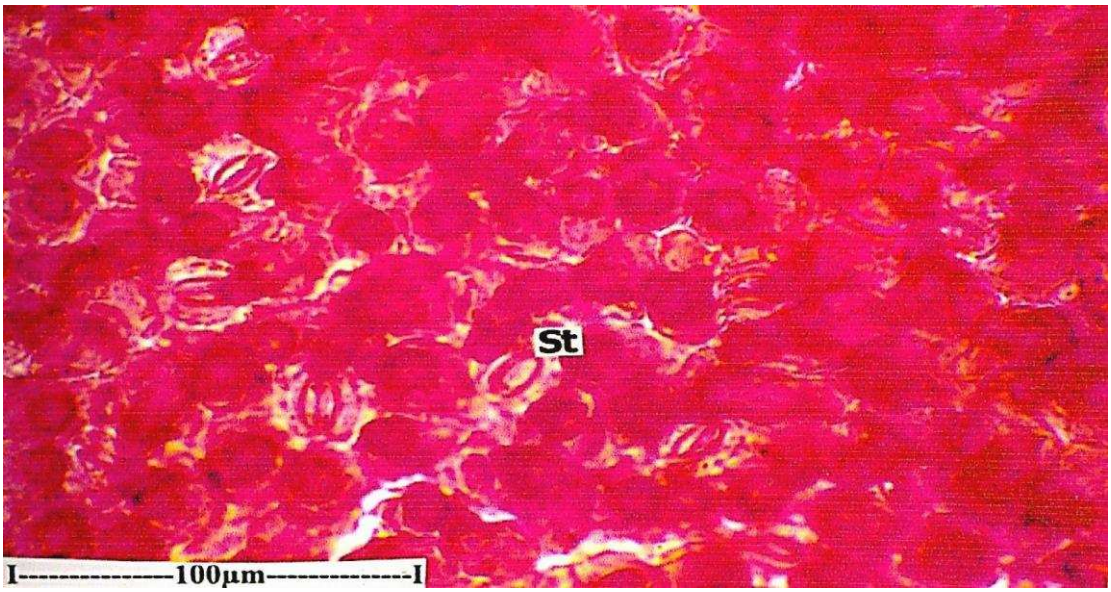


Fig 8: Anomocytic stomata

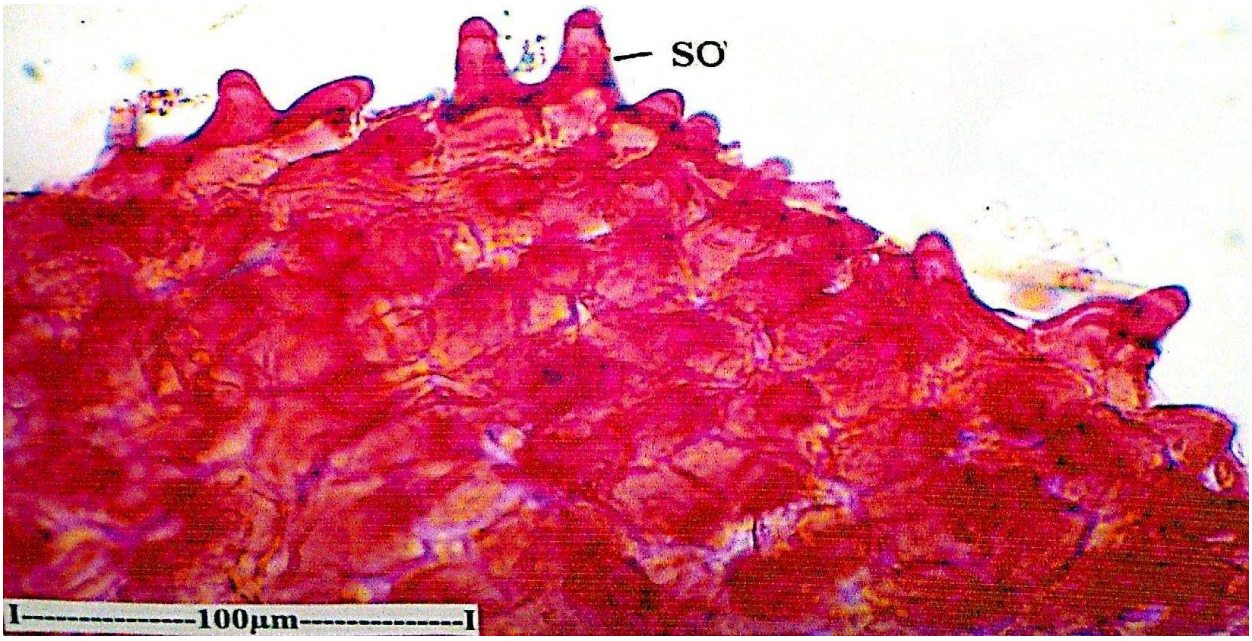


Fig 9: Spiny outgrowth

3.4: Physio-chemical parameter

3.4.1: Ash Value of leaves of *Berberis aristata* DC.

Analytical parameter	(%)w/w
Total Ash	3
Acid Insoluble ash	1.5
Water soluble Ash	1

3.4.1: Extractive Values and LOD of leaves of *Berberis aristata* DC.

Solvent	Method of Extraction	Extractive value (% w/w)
Alcohol soluble extractive	Continues hot percolations (**Soxhlet** apparatus)	20.80
Water soluble extractive		15.26
LOD	105^0 C	20

3.5: Preliminary Phytochemical Screening of leaves of *Berberis aristata* DC.

Preliminary Phytochemical Screening of Different Solvent Extracts

Tests	Ethanolic extract	Aqueous extract
Alkaloids		
Mayers Reagent	+	-
Dragendorffs reagent	+	-
Hagers reagent	+	-
Wagners reagent	+	-
Carbohydrates		
Molishch's Test	+	+
Fehlings Test	+	+
Benedicts Test	+	+
Glycosides		
General Test	-	-
Anthraquinone	-	-
Cardiac	-	-
Cyanogenetic	-	-
Coumarin	-	-
Phytosterols		-
Salkowski Test	+	-
Libermann Burchard Test	+	-
Saponins	+	+
Tannins	+	+
Proteins & Free Amino Acid		
Millons test	+	+
Biuret test	+	+
Flavonoids		
Shinoda test	+	+
Alkaline Reagent test	+	+
Terpenoids	+	-
Fixed Oil	-	-

4. DISCUSSION

Organoleptic evaluation of a crude drug is mainly for qualitative evaluation based on the observation of morphological and sensory profile [20]. Hence we have undertaken this study to serve as a tool for developing standards for identification, quality and purity of leaves of *Berberis aristata*.

Adulteration and misidentification of crude drugs can cause serious health problems to consumers and legal problems for the pharmaceutical industries. The observation of cellular level morphology or anatomy is a major aid for the authentication of drugs [21]. Microscopic evaluation is one of the simplest and cheapest methods for the correct identification of the source of the materials [22].

Microscopic evaluation of leaves showed biconvex midrib and thick lamina. The epidermal layers of the midrib are thick with small, less conspicuous cells and thick cuticle. The vascular system consists of three large vascular bundles; the median one is small than the two lateral bundles; the bundles are collateral and wedge shaped. Lamina is made of epidermal layer on the adaxial side with spindle shaped thick walled cells and papillate cuticle. The abaxial epidermis has squarish or rectangular epidermal cells with prominent spiny cuticular outgrowths. A Pharmacognostical study on the stem and roots of *B.aristata* was previously published [23, 24]. No report available on therapeutically important rhizome portion. Powder microscopy showed the presence of cuticular papillae, anomocytic stomata and spiny outgrowth.

The ash values are particularly important to find out the presence or absence of foreign inorganic matter such as metallic salts and or silica (earthy matter) [25]. Acid insoluble ash provides information about non-physiological ash produced due do adherence of inorganic dirt, dust to the crude drug [26]. Phytochemical evaluation and molecular characterization of plants is an important task in medicinal botany and drug discovery [27]. Preliminary phytochemical screening of appropriate solvent extracts showed the presence of alkaloids, sterols, tannins, proteins and amino acids, flavonoids, terpenoids, saponin, carbohydrates and absence of glycosides and volatile and fixed oil.

5. CONCLUSION

B.aristata has a wide range of therapeutically active phytochemicals which could be useful for treatment of various ailments. Many reports were done on screening of leaves of *Berberis aristata* both *in-vivo* and *in-vitro* exhibited its potency to cure diseases. No report available on quality assessment of leaves of *B.aristata*. Keeping in this view an attempt was made for standardization of purity and quality of therapeutically valuable leafy portion of *Berberis aristata* DC.

Conflict of interest statement:

We declare that we have no conflict of interest.

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