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Research Paper

Pharmacognostic investigations on the leaves of *Viburnum coriaceum* Blume

K Prabhu[1]*, K Ponnudurai[1], S Hemalatha[2] and P K Karar[1]

[1]Department of Pharmacognosy, Nandini Nagar Mahavidyalaya College of Pharmacy, Nawabganj, Gonda-271 303, Uttar Pradesh, India

[2]Department of Pharmaceutics, IT-BHU, Varanasi - 221 005, Uttar Pradesh

*Correspondent author, E-mail: prabhu.cognosy@gmail.com

Phone: +91-9935745590 (Mob.); Fax No: +91-5260-275000

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Abstract

***Viburnum coriaceum* Blume** (syn. *V. cylindricum* Buch.-Ham. ex D. Don) of family Caprifoliaceae is of medicinal value. In the present study pharmacognostical investigations such as morphology, microscopy including leaf constant, measurements and other applicable physico-chemical parameters are carried out on its leaves. The findings may provide useful information with regards to its identification and standardization in future.

Keywords: *Viburnum coriaceum*, Viburnum, Caprifoliaceae, Pharmacognostic studies, Leaves.

IPC code; Int. cl.[8] — A61K 36/35, A61K 127/00

Introduction

The family Caprifoliaceae under the order Dipsacales contains about 12 genera including ***Viburnum* Linn.** which consists of more than 150 species. About 17 species have been reported from India, especially, at an altitude of not less than 1800 m in the Himalayan tracts and sub-Himalayan areas, West Bengal, Arunachal Pradesh and Tamil Nadu.

This paper describes some pharmacognostical and other physico-chemical characteristics studied on the leaves of ***V. coriaceum* Blume** (syn. *V. cylindricum* Buch.-Ham. ex D. Don). The leaves, stem, bark and roots were reported to possess wide range of ethno-medical applications. The biological activities (especially, uterine relaxant) may be attributed to their constituents such as terpenoids, flavonoids, sterols and simple and polyphenolic compounds[1-6].

The main objective of this study is to supplement some information with regards to its identification and standardization. Except for a few, all the species under this genus lack for an authentic protocol on their pharmacognosy.

Materials and Methods

Collection of specimens

The plant specimens for the proposed study were collected from Nilgiri hills, Tamil Nadu, India, and authenticated by Dr.V.Chelladurai, Ex. Professor (Botany), Medicinal Plant Survey for Siddha, Government of India. Care was taken to select healthy plants. The required samples of the leaves were cut and removed from the plant and fixed in FAA (Formalin - 5 ml + Acetic acid - 5 ml + 70% Ethyl alcohol - 90 ml). After 24h 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 58-60ºC) until TBA solution attained super saturation. Then the specimens were cast into paraffin blocks.

Sectioning

The paraffin embedded specimens were sectioned with the help of Rotary Microtome. The thickness of the sections was 10-12 µm. Dewaxing of the sections was performed by customary procedure[7]. Since, Toluidine Blue is a polychromatic stain, the staining results were remarkably good and some cytochemical reactions were also obtained. The dye rendered pink colour to the cellulose walls, blue to the lignified cells, dark green to suberin, violet to the mucilage and blue to the protein bodies, wherever necessary sections were also stained with safranin and fast-green and IKI (for starch). For studying the stomatal morphology, venation pattern and trichrome distribution, paradermal sections (sections taken parallel to the surface of leaf) as well as clearing of leaf with 5% sodium hydroxide or epidermal peeling by partial maceration employing Jeffrey's maceration fluid were prepared[8-10]. Glycerin mounted temporary preparations were made for macerated/ cleared materials.

Photomicrographs

Microscopic descriptions of tissues were supplemented with micrographs wherever necessary. Photographs of different magnifications were taken with Nikon labphot 2 microscopic units. 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 bi-refringent property, under polarized light they appear bright against dark background. Magnifications of the figures were indicated by the scale-bars[11].

Result and Discussion

Macroscopic and Organoleptic features

The leaves are simple, petiolate, 3-5 pairs of lateral nerves joining together just prior to the margin, entire, compound in venation; acuminate apex; and obovate, oblong and acute base giving rise to symmetric nature. The adaxial side of the fresh leaves was green and the dried leaves were blackish green. The abaxial side was pale green. The leaves measured 8-20 cm length and 4-6 cm width; the petiole being 1-2.5 cm in length, showing proximal wings; significant feature was the presence of leaf hairs (Fig. 1.1) only on the abaxial sides and arising from the region where lateral veins branched from the midrib. The leaves were slightly bitter in taste and had no specific odour. The abaxial side was pubescent and adaxial was waxy. The midribs and lateral veins were flat above and rose below. The dried leaves were folded enclosing the adaxial surface and in course of time turning blackish.

Anatomy

In cross sectional view, the leaf exhibits distinct dorsi-ventral symmetry with prominent midrib and their wing like lamina (Fig. 1.2). Midrib has wide "U" shaped adaxial groove and hemi-circular quite thick and prominent abaxial part. It is 1 mm in vertical plane and 1.05 mm in horizontal plane. The midrib consists of a prominent epidermis of semicircular cells with prominent cuticle. The ground tissues are homogeneous comprising of thin walled, circular, compact, parenchymatous cells measuring 30-60 µm in diameter. The vascular system consists of a wide, bowl shaped main strand and a horizontal band of small, less prominent xylem strands (Fig. 1.3).

The main strand has closely arranged radial files of thin walled, angular xylem elements and small nests of phloem all along the outer border of the xylem band. The main strand is 550 µm wide and 150 µm thick. Lamina is smooth and even on both surfaces (Fig. 2.1), and is 350 µm thick. The adaxial epidermis has rectangular thick walled cells with thick cuticle; the cells are 40 µm thick. The abaxial epidermis is thinner than the adaxial layer and the abaxial cells are cylindrical or spindle shaped measuring up to 30 µm thickness. The mesophyll tissue consists of an adaxial zone of single layer of shoot, cylindrical undulate palisade cells which are 70 µm in height. The spongy parenchyma zone is wide consisting of about six layers of large, lobed parenchyma cells with crystals (Fig. 2.2).

Petiole

Petiole was studied along the basal or proximal portion (Fig. 3.1) and upper or distal portion. The proximal part of the petiole is circular in sectional view with slight, flat adaxial side. It is 1.5 mm horizontally and 1.6 mm vertically. It consists of narrow, thick walled, small epidermal cells with thick cuticle. The ground tissue is differentiated into outer zone of two or three layers of squarish collenchyma cells and the remaining portion has dilated circular or angular, compact thin walled parenchyma cells. The vascular system consists of a wide, bowl shaped main strand and a horizontal plate of adaxial strand. The vascular strands are collateral, close, radial rows of xylem elements and thin zone of phloem. The adaxial plate has inner xylem and outer phloem. Tannin-filled cells are seen in the phloem tissue. Crystals are common in the ground cells. The distal part of the petiole differs from the proximal part both in cross sectional outline and vascular pattern. The distal part has semicircular, wide basal part and two thick horn shaped adaxial wings with deep groove in between (Fig. 3.2). The wings are 600 µm high and 200 to 400 µm thick. The epidermal layer consists of papillate epidermal cells with prominent cuticle. The outer ground tissue has two or three layers of collenchymatous cells and inner portion has small circular, thin walled compact parenchymatous cells. The vascular system has wide, horse-shoe shaped main strand, U shaped adaxial strand and two pairs of wing strand. The vascular strands are collateral and have uniseriate radial rows of xylem and continuous zone of phloem on the outer part of the xylem band. Tannin is abundant in the phloem and xylem parenchyma cells. The wing strands are circular with central mass of xylem unsheathed by tannin-filled cells.

Fig. 1.1: Fresh leaves and tops showing fruiting twigs and Leaf hairs

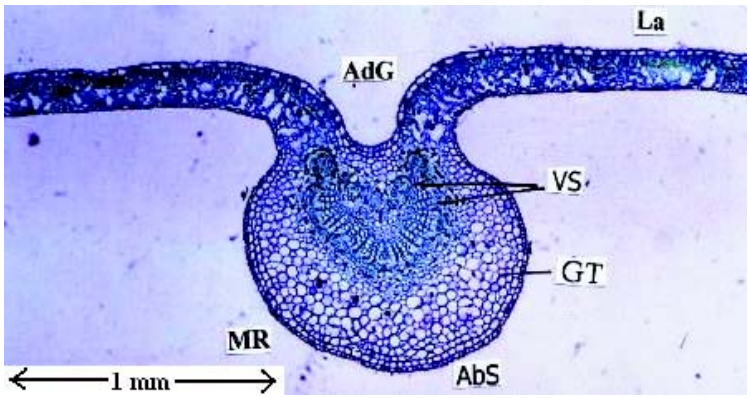

Fig. 1.2: T.S. of the leaf showing entire view

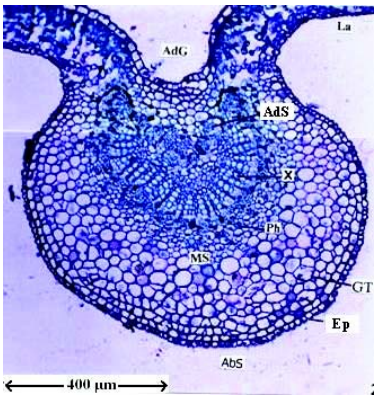

Fig 1.3 T.S. of the leaf through midrib (Magnified)

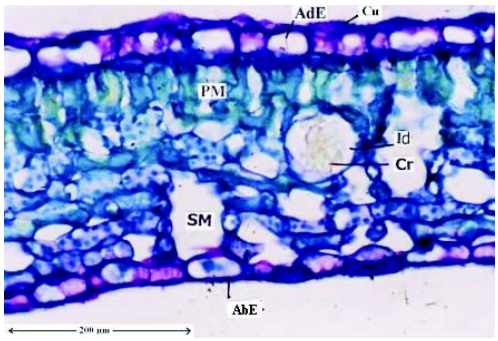

Fig 2.1: T.S. of Lamina showing Cuticle, Palisade mesophyll, Idioblast and Spongy mesophyll

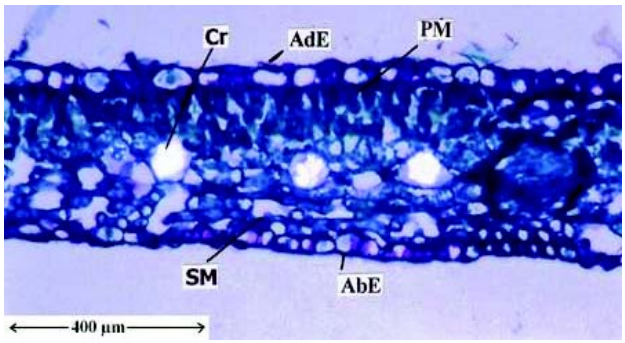

Fig. 2.2: T.S. of Lamina showing crystals (Under Polarized Light Microscope)

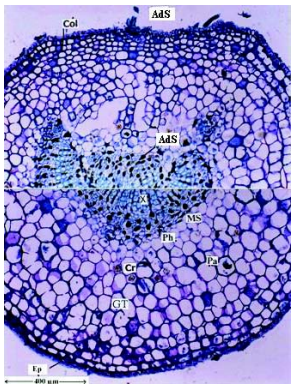

Fig. 3.1: Anatomy of petiole (Basal region)

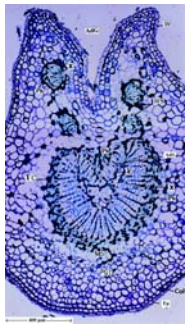

Fig. 3.2 : Anatomy of the petiole (Distal region)

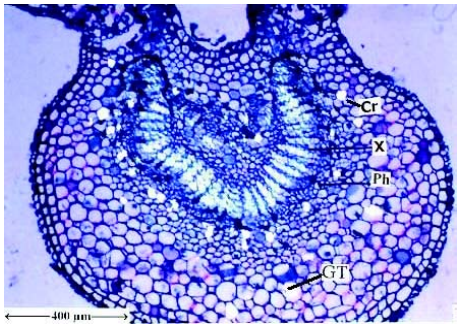

Fig. 4.1: T.S. of leaf showing crystals distribution in the ground tissue

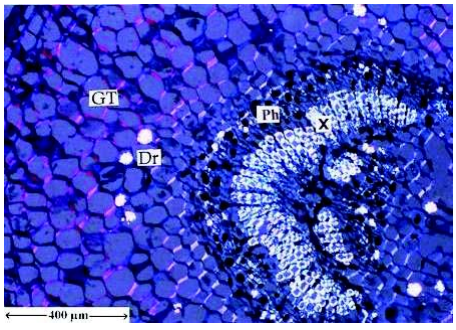

Fig. 4.2: T.S. of petiole showing Druses in the ground tissue

FT – Fruiting Twigs; LH – Leaf Hairs AdG – Adaxial Groove; VS – Vascular Strand; GT – Ground Tissue; AbS – Abaxial Side; MR – Midrib ; AdS – Adaxial Side; Ep – Epidermis; La – Lamina; MS – Median Strand; Ph – Phloem; X – Xylem; AbE – Abaxial Epidermis; AdE – Adaxial Epidermis; Cr – Crystals; Cu – Cuticle; Id – Idioblast; PM – Palisade mesophyll; SM – Spongy mesophyll; Col – Collenchyma; Pa – Parenchyma; PGT – Parenchymatous Ground Tissue; TC – Tannin-filled Cells; W – Wing ; WS – Wing Strand; Dr – Druses.

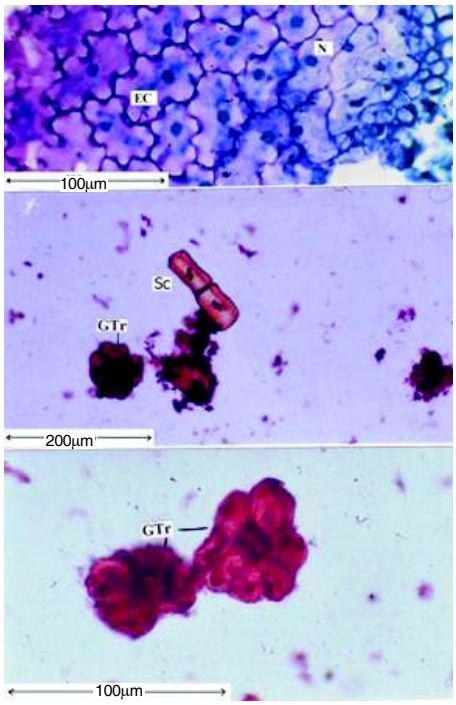

Fig. 5.0: Epidermal morphology showing adaxial surface sclereids and trichomes
EC – Epidermal Cells; GTr – Glandular Trichomes; N – Nucleus; Sc - Sclereids

Cell inclusions

Calcium oxalate crystals are abundant in the midrib, petiole and lamina. The crystals are druses. In the midrib, the druses occur in the phloem parenchyma as well as in the lower ground tissue (Fig. 4.1). In the petiole druses of smaller dimensions are located in the phloem and larger druses are seen in the ground parenchyma (Fig. 4.2). Smaller druses are 10 µm and larger ones are 30 µm in diameter. The epidermal cells of the leaf, as seen in surface view, are amoeboid outline with wavy anticlinal walls, the walls are thick with cuticular striations, visible on the cell surface. The nuclei are prominent. The leaf has abundant glandular trichomes on the abaxial side. The trichomes are sub-sessile having a short stalk cell and a circular plate of the rosette cells at the tip of the stalk. The rosette consists of 5-8 content. The glandular trichomes are up to 80 µm in diameter (Fig. 5.0).

Micrometric observation showed some markable features such as glandular trichomes which were of sub-sessile, circular plate made of 5-8 rosette measuring from 74 to 109 µm. The leaf hairs were abundant and measuring from 468 to 1279 µm. The druses are abundant in mesophyllic region measuring from 15 to 25 µm (Table 1). Measurements of other leaf components are also given in Table 1.

Table 1: Measurement of leaf components of *Viburnum coriaceum*

S. No.	Characters	Values
		min mean max
1.	Adaxial epidermis	40 µm wide
2.	Abaxial epidermis	30 µm wide
3.	Stomatal number	20.4 – 30.5 – 40.6
4.	Stomatal index	16.2 – 18.5 – 20
5.	Palisade ratio	3 – 5 – 7
6.	Palisade cell	70 µm in height
7.	Collenchyma	65 µm
8.	Vein islet number	6 – 9.5 – 14
9.	Vein termination number	6 – 7.5 – 10
10.	Leaf hairs	[l]468 – 902 – 1279, [w]15.6 – 43.3 – 62.4
11.	Trichomes dimension (glandular)	[d]46.8 – 74.8 – 109.2
12.	Druses	15 – 20 – 25

d- diameter, l- length, w- width (at the point of broader region)

Table 2: Fluorescence analysis of solvent extracts of leaves

Solvent extracts [UV 366 nm]					Powdered specimen	
Petroleum ether (60-80 °C)	Benzene	$CHCl_3$	Alcohol	H_2O	Conc. H_2SO_4	Conc. HNO_3
Orange colour	Greenish brown colour	Reddish orange colour	Yellowish orange colour	Greenish blue colour	Black colour	Brown colour

Physico-chemical and pharmacognostic studies

The leaves were morphologically and organoleptically screened as per the references cited and subjected to physico-chemical studies including: extractive values[12], histo-chemical studies[13, 14], fluorescence analysis[15], ash values[16] and micrometrics[17, 18].

The aqueous extractive value (8.21 %) was higher than the alcoholic extractive value (7.47 %) revealing the presence of larger quantity of water soluble constituents such as sugars, glycosides, plant acids and tannins.

With aid of suitable chemical reagents, histo-chemical analysis was performed. The chemical nature of the metabolites and their location were traced out showing the presence of phenolic compounds and saponins in parenchymatous cells; cuticular layer on the surface; lignin with vascular region and abundant calcium oxalate in mesophyllic region. However, the presence of mucilage and starch grains was in the negative.

The fluorescence characteristics, being commonly applied parameters in the analysis of crude drugs, the solvent extracts of the leaves were observed under UV radiation. The colour change for the individual extract was distinctive and reproducible revealing the solvent properties to the phytoconstituents (Table 2).

The total ash value for a crude drug is not always reliable, since there is a possibility of presence of non–physiological substances such as earthy matters. So, the parameters such as acid insoluble, water soluble and sulphated ash values were performed. The sulphated ash value (9.20 %) was higher than the total ash value (4.88 %) due to the conversion of oxides, carbonates and oxalates in to sulphates. The acid insoluble and water soluble ash values were 3.40 and 2.10%, respectively.

Conclusion

From the above pharmacognostic studies of ***V. coriaceum*** leaves it can be concluded that the findings of the current study may be useful to differentiate this species from other ***Viburnum*** species.

Acknowledgements

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