Journal of Pharmaceutical Sciences and Research
www.jpsr.pharmainfo.in

Pharmacognostic and Preliminary Phytochemical Investigations on the Leaves of *Viburnum punctatum* Buch.-Ham.ex D.Don

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ABSTRACT

The genus *"Viburnum"* includes about 200 species under the family "Caprifoliaceae" (Viburnaceae). There are nearly 17 species in India, especially, in the hill stations at an altitude of more than 1500 m. Scientific information on their pharmacognosy, phytochemistry and pharmacology are very scant. Hence, the current study describes some pharmacognostical and preliminary phytochemical investigations undertaken on the leaves of one of those species namely *Viburnum punctatum* Buch.-Ham.ex D.Don. The samples for research were collected from Nilgiri hills, Tamil Nadu, India and authentificated by Dr.V.Chelladurai, Ex. Prof, Botany, and then subjected for morphological, microscopical and physicochemical analysis. The parameters from the above were recorded with an objective of drawing an attention on those populations as well as a reference for further scientific investigations.

Keywords - Caprifoliaceae, Druses, Pharmacognosy, Preliminary phytochemical, *Viburnum punctatum*

INTRODUCTION

Viburnum punctatum Buch.-Ham.ex D.Don is a medicinal plant belonging to the family Caprifoliaceae, under Dipsacales order. It is a medium sized tree or shrub, growing at an altitude not less than 1500 m; profusely with other plants in Himalaya, Nilgiri and Coimbatore. The leaves were traditionally used for the treatment of fever, stomach disorder and mentioned to possess anti-periodic effect.

The phyto-constituents such as flavonoids, terpenoids, iridoid glycosides, sterols, proanthocyanidine and predominantly tannins and other polyphenolic compounds and their derivatives are reported to be present with this species [1]. A few Viburnum species, such as *Viburnum acuminatum* [2], *Viburnum awabukii* [3], *Viburnum foetidum* [4], *Viburnum nervosum* [5], *Viburnum luzonicum* [6] and *Viburnum chinshanense* [7] have been already investigated and reported in literatures for their medicinal values. It may best suit to state that the phytochemical based pharmacology of those species is very inconsistent or obscure. Hence, it may an absolute necessity to create a profile in regards to their identification and then standardization which may lead to further scientific investigations. This paper encampasses some of the pharmacognostical investigations carried out on the leaves of one of the species namely *Viburnum punctatum* Buch.-Ham.ex D.Don. The assignment such as macroscopy, anatomical studies, micro measurements and preliminary phytochemical screening were performed since the species was not noted for its pharmacognosy and bioactivity in the past.

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MATERIALS AND METHODS

Plant materials

The leaf specimens for the proposed study were collected in the month of February-07 from Nilgiri hills, Tamil Nadu, India at an altitude of 1800 m and authenticated as *Viburnum punctatum* Buch.-Ham.ex D.Don by Dr.V.Chelladurai, Ex. Professor (Botany), Medicinal plant survey for

Siddha, Government of India. A voucher specimen (V181) has been deposited at the Museum of the Department of Pharmacognosy, Nandini Nagar Mahavidyalaya College of Pharmacy, Nawabganj, Gonda, Uttar Pradesh, India. Care was taken to select healthy plants and for normal organs, the required samples of different organs were cut and removed from the plant and fixed in FAA (Formalin – 5 ml + Acetic acid – 5 ml + 70% Ethyl alcohol – 90 ml) Formalin Aceto-alcohol in fresh form . After 24 h of fixing, the specimens were dehydrated with graded series of tertiary-butyl alcohol. Infiltration of the specimens was carried by gradual addition of paraffin wax (melting point 58-60º C) 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. Since Toluidine Blue is a polychromatic stain, the staining results were remarkably good; and some cytochemical reactions were also obtained [8]. 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. Wherever necessary sections were also stained with safranin and fast-green and IKI (for starch).

For studying the stomatal morphology, veination pattern and trichome 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 [9] were prepared. 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 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 bi-refringent property, under polarized light they appear bright against dark background. Magnifications of the figures were indicated by the scale-bars [10, 11 and 12].

Physico-chemical and pharmacognostic studies

The research specimens were morphologically and organoleptically screened and subjected to physico-chemical and parameters such as Extractive values [13], Ash values [14, 15], Histo-chemical studies [16, 17], Fluorescence analysis [18] and Micrometrics including leaf constants [19, 20].

Powder microscopy

The leaves dried in shade were finely powdered and screened for the presence of its own and foreign vegetative matters (other than the organ selected for the research studies). The powder was passed through a sieve No.180 and a sieve No.125, separately, to obtain fine and very fine powder respectively and then subjected for microscopic examination. The sample was treated with following reagents and studied for their components of diagnostic value (50% glycerin as temporary mountant; phloroglucinol (2% W/V) in ethanol (90%) and Conc. HCl (1:1) for lignin; 5% W/V of alcoholic ferric chloride for phenolic compounds; 2% Iodine solution for starch grains; and Ruthenium red (0.08%) in 10% lead acetate for mucilage).

Preliminary phyto-chemical screening

The leaves were dried in shade at room temperature and screened for the presence of foreign matter. The leaves were ground to a moderately coarse powder in a

mechanical grinder. About 200g of the powder was extracted successively with petroleum ether (60 - 80º C bp), benzene, chloroform and ethanol (95%) using soxhlet apparatus. The extraction with each solvent was carried for 24 h. Finally, the marc left was extracted with water by digesting on a boiling water bath. The extraction was continued till a few drops of the last portion of the extract left no residue on drying. The extracts were taken in a tarred porcelain dishes and evaporated to dryness on a water bath and dried at 105º C to a constant weight. The percentage extractives were calculated with reference to air dried drug [21].

RESULTS AND DISCUSSION

Fresh leaves appear with greenish adaxial side and pale green abaxial side; dried leaves exhibit pale brown above and greenish orange below; petiolate; leaves appear with symmetric base, and shortly acuminate apex; veination prominent below and flat above; entire margin, petiole 1.5 – 2 cm long and with lateral wings; 6 - 8 pairs of lateral veins forming a compound veination; the matured leaf blade measured 4 – 6 cm in width and 6 – 13 cm in length, usually obovate; lateral veinations join just prior to the margin; leafy characteristic odour and slightly bitter in taste (Figure 1).

The leaf has uniformly even lamina and prominent midrib; the marginal part of the lamina is 250 µm thick in the midpart and 120 µm thick along the margin. The lamina has thick and prominent adaxial epidermis; the epidermal cells are squarish or rectangular with thick walls and prominent cuticle. The cells are about 30 µm thick. The abaxial epidermis is comparative and the cells are rectangular to square shape. The mesophyll consists of an adaxial zone of single lager palisade cells and abaxial zone of lobed spongy parenchyma cells. The palisade cells are thin and warmly cylindrical measuring 60 µm in height. The spongy parenchyma cells are up to seven cells in vertical plane and form wide aerenchymatous tissue. The marginal part of the lamina lacks palisade – spongy mesophyll differentiation. It has 2 or 3 layers of compact parenchyma cells and radially oblong thick walled epidermal cells (Figure 2).

The midrib has a broad short adaxial part and wide, a prominent abaxial part. It is about 1 mm in vertical plane and 600 – 800 µm in horizontal plane. The epidermal layer has thick walled squarish cells with prominent cuticle. The ground tissue has two or three layers of outer collenchyma cells and rest of the tissue has circular compact parenchyma cells with some air chambers. Calcium oxalate crystals are seen in the ground tissue around the vascular tissues. The vascular system has a thick bowl shaped median bundle and flat adaxial bundles. The xylem consists of long compact radial files of circular, thick walled cells and a narrow zone of phloem (Figure 3).

The upper (adaxial) epidermis is apostomatic (stomata absent). It consists of lobed, thick walled cells; the anticlinal walls are wavy (Figure 4).

The lower end of the petiole is plano convex in sectional view with flat adaxial side and semicircular abaxial side. It is 1.55 mm vertically and 1.7 mm horizontally. The petiole has thin epidermal layer of thick walled squarish cells and ground tissue of circular, thin walled parenchyma cells with narrow intercellular spaces. The vascular system consists of a shallow, wide are of median bundle and two “U” shaped adaxial bundles. The bundles are collateral. Xylem elements are in these parallel lines, and are narrow, thin walled and squarish (Figure 5). Distal part of the petiole (near the lamina) has two lateral wings. The ground tissue is similar to that of the proximal region. However, the vascular system is much different. It consists of a deep cup with infolded margins forming an adaxial flat plate. These are too small, circular wing bundles, placed within the marginal part of the petiole (Figure 6).

Fig.1. Flowering and fruiting twigs with leaves

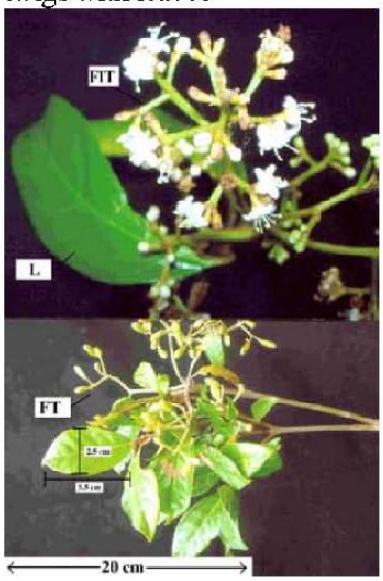
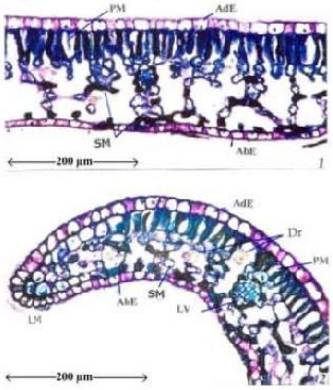


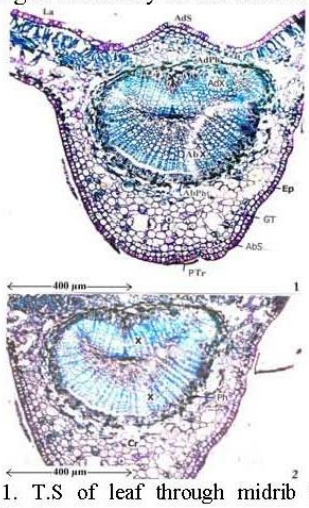
Fig.2. Anatomy of the Lamina



1. T.S of Lamina; 2. T.S of Leaf Margin

[Abe – Abaxial epidermis, Adc – Adaxial epidermis, Dr – Druses, LM – Leaf Margin, LV Lateral Vein, PM – Palisade Mesophyll, SM – Spongy Mesophyll]

Fig.3. Anatomy of the Midrib

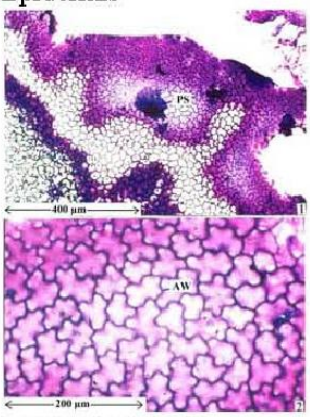


1. T.S of leaf through midrib with lamina

2. T.S of midrib under polarized light microscope showing crystal distribution.

[Abph – Abaxial phloem, AbS – Abaxial Side, AbX – Abaxial Xylem, Adph – Adaxial phloem, AdS – Adaxial Side, AdX – Adaxial Xylem, Cr – Crystals, Ep – Epidermis, GT – Ground tissue, La – Lamina, Ph – Phloem, PTr – Peltate Trichome, X – Xylem]

Fig.4. Structure of the Adaxial Epidermis

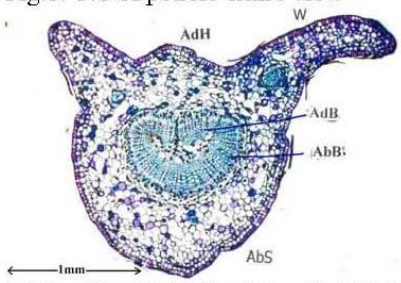


1. Adaxial Epidermis

2. Adaxial Epidermis showing anticlinal walls.

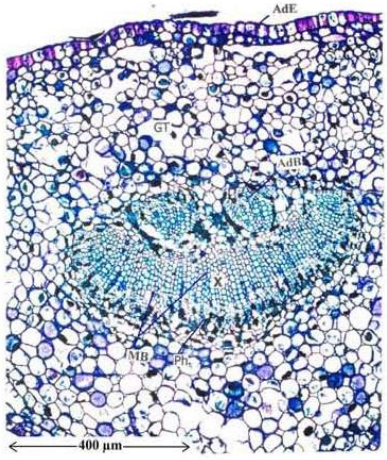
[AW – Anticlinal Walls, PS – Peltate Scale]

Fig.5. T.S of petiole entire view



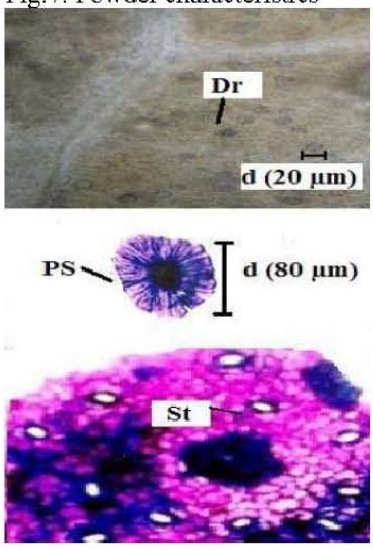
[AbB – Abaxial Bundle, Abs – Abaxial side AdB – Adaxial Bundle, AdH – Adaxial Hump W – Wing]

Fig.6. Structure of the Petiole (Enlarged) - [Proximal region]



[AdB – Adaxial Bundle, AdE – Adaxial Epidermis, GT – Ground Tissue, MB – Median Bundle, Ph – Phloem, X – Xylem]

Fig.7. Powder characteristics



[PS – Peltate Scale (Trichome); St – Stomata; Dr - Druses]

Table 1. Histo-chemical Analysis of Leaves of *Viburnum punctatum*.

Specimen	Reagents						Report
	2% Phloroglucinol and Conc. HCl (1:1)	Iodine solution (3%)	Alcoholic $FeCl_3$ (5%)	0.2% Sudan Red III in alcohol	Vannilin -Conc. H_2SO_4	Dragendorffs Reagent	
Leaves	(+) Pink colour	(-)	(+) Black colour	(+) Pink	(+) Reddish	(-)	Lignin Phenolic compounds Mucilage and Saponins
Histological Zone	Xylem region	(-)	Spongy Paren Chyma and Ground tissue	Epider mis	Cortical Parench yma	(-)	

(+) - Test positive
(-) - Test negative

Table 2. Physicochemical analysis and micrometrics of leaves of *Viburnum punctatum*

Physicochemical analysis	Leaf		
	min	mean	max
Percentage extractives*			
a) Ethanol		5.53±0.065	
b) Water		6.45±0.093	
Ash Values %W/W*			
a) Total ash		3.8±0.072	
b) Water soluble ash		1.1±0.124	
c) Acid insoluble		3.3±0.143	
d) Sulphated ash		8.1±0.171	
Micrometrics			
a) Dimension of Druses	*5	7	9
b) Stomatal number	*5.0	6.5	8.0
c) Stomatal index	*8.33	10.22	14.28
d) Vein-islet number	*18	21	24
e) Vein termination number	*20.0	22.5	25.0
f) Palisade ratio	*2.2	2.7	3.2
g) Glandular trichomes**	d 65	75	90

* – Results are presented as mean ± Standard Deviation, d – dimension in µm,
l – length in µm, w– width at the point of broader region in µm,
* – Range obtained from the average of 10 measurements,
**– Size range obtained from measurements of 25 individual components

Table 3. Percentage extractives, fluorescence analysis, and preliminary phytochemical screening leaves of *Viburnum punctatum*

Solvent Extracts	(% W/W)*	Fluorescence observed (UV 366nm)	Chemical constituents					
			Alkaloids	Sterols	Terpenoid	Sugar	Glycosides	Phenolics
Petroleum ether (60 - 80°C)	2.66±0.073	Orange colour	–	+	+	–	–	–
Benzene	1.13±0.060	Black	–	+	+	–	–	–
Chloroform	0.66±0.053	Reddish orange colour	–	+	+	–	+	–
Ethanol (95%)	3.21±0.064	Yellowish Orange	–	–	–	+	+	+
Water	7.08±0.064	Greenish Blue	–	–	–	+	+	+

*– Results are presented as mean ± Standard Deviation
+ – Test positive, – – Test negative,

Powder characteristics

Microscopic components of significant diagnostic value were studied under different magnifications; polarized light was subjected to study the starch grains and crystals. The powder was pale green in colour; with no any specific odour; slightly bitter; smooth and slippery in texture. The stomata were of anamocytic in nature. The epidermal cells were lobed with anticlinal walls. The trichomes were of peltate scales with short stalk. The scales were thin plates with a central stalk the scales were in average 80 µm in diameter. The calcium oxalate crystals were of druses ranging from 17 – 24 µm in diameter (Figure 7). Fragments vascular tissue appeared pinkish to phloroglucinol and HCl (1:1); cortical parenchyma fragment appeared blackish revealed the presence of lignin and phenolic compounds respectively. However, starch grains and mucilage were not evident against addition of Iodine solution and 0.2% Sudan red – III respectively.

Physico-chemical analysis

Histochemical analysis revealed the presence of lignin, phenolic compounds, mucilage and saponin. However, presence of alkaloids and starch grains were not evident (Table 1).

The aqueous extractive value was higher than the alcoholic extractive value revealing presence of larger amount of water soluble constituents in the leaves such as plant acids, carbohydrates and phenolic compounds.

The total ash value was higher than that of the acid insoluble and water soluble ash value and a decrease in the acid insoluble ash value may be due to presence of smaller quantity of siliceous matters. By conventional procedure, loss on drying was performed showing not less than 4% W/W volatile matters. However, volatile oil was screened to be absent on chemo-microscopic analysis.

The stomatal number is not a reliable parameter for a species. Hence the measurements were made at the base, midrib and apex of a leaf then the mean of the readings was accounted to render reproducibility. The peltate scale measured from 65 - 90 µm. The druses were abundant with size in average 20.4 µm. Palisade ratio was determined to be not more than 3.2. The vein islets and termination numbers were comparable.

Fluorescence analysis showed no any

specific fluorescence. However, a transition in colour was observed and was reproducible; a pale pink colouration was noted to be predominant (Table 2).

The percentage extractive of water was higher than the rest of the extractives. Petroleum ether, benzene and chloroform extracts showed the presence of phyto-constituents such as triterpenoids and sterols where as ethanolic (95%) and aqueous extracts showed phenolic compounds, glycosides, flavonoids, saponins and reducing sugar (Table 3).

CONCLUSION

The leaves of *Viburnum punctatum* collected from Nilgiri district, Tamil Nadu, India were subjected for macroscopy, microscopy, physicochemical and preliminary phytochemical analysis. The objective of investigations was to ease the identification of the species both in whole and powdered form. The presence of valuable phytoconstituents such as triterpenoid, glycosides and phenolic compounds also demand further phytochemical studies of the species.

Acknowledgements

We pay our respects to late Dr. Yogesh Mohan, Reader, Department of Pharmaceutics, IT-BHU, who suggested this species for the current assignment. Also, we express our gratitude to our management for the facilities provided.

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