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Pharmacognostical Evaluation of *Clerodendrum viscosum* (Vent.)

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Abstract – This study presents a detailed pharmacognostical study of the root and leaf of the crude drug *Clerodendrum viscosum* Vent. (Verbenaceae), an important plant in the Indian system of medicine. The root and leaf samples were studied using light and confocal microscopy, WHO recommended-physicochemical determinations, and authentic phytochemical procedures. The physicochemical, morphological, and histological parameters presented in this paper may be proposed as parameters to establish the authenticity of *C. viscosum* root and leaf, and may possibly help to differentiate the drug from its other species.
Keywords – *Clerodendrum viscosum,* Verbenaceae, pharmacognosy, root and leaf

Introduction

Clerodendrum viscosum Vent. (Verbenaceae) known as Bhandirah in Sanskrit, is an important plant in the Indian system of medicine. It is a large gregarious tawny-villous shrub found throughout India as undergrowth in forests up to 1800 m and as a weed along the roadsides and wastelands (Warrier *et al.*, 1994). The plant has been used as an antiseptic, anti-inflammatory, antipyretic, vermifuge, expectorant and in the treatment of tumors, leprosy, and skin diseases.

The aerial part of the plant contains sterols; the root contains lupeol, β-sitosterol, and steroidal glycosides; the leaf contains a diterpene clerodin; the seed oil contains fatty acids, and the flower contains glycoside acetoside, fumaric acid esters of caffeic acid, lupeol, β-sitosterol, β-sitosterol glucoside, cleridine, and hentriacontane (Yogana-rasimhan, 2000). Essential oil analysis of the leaf and root bark of the plant *C. viscosum* revealed the presence of fatty acids, their esters, monoterpene, limonene, α-pinene, β-pinene, p-cymene, myrcene, and sesquiterpenes (Jirovets *et al.*, 1999).

Traditionally *C. viscosum* has been used in the treatment of snake bites and scorpion sting (Nadkarni, 1954). The oil fraction of *C. viscosum* possesses insecticidal property while the leaf has shown encouraging results when rubbed on the lesions of alopecia patients (Pawan and Ojha, 1993).

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In spite of the numerous medicinal uses attributed to this plant, however, there is no pharmacognostical report on the leaf or root of the plant to determine the anatomical and other physicochemical standards required for the quality control of the crude drug. Hence, the present investigation includes morphological and anatomical evaluation, determination of physicochemical constants and the preliminary phytochemical screening of the different extracts of *C. viscosum*.

Experimental

Plant material – The root and leaf of *Clerodendrum viscosum* were collected from Shirva, Udupi district, Karnataka, India in the month of April 2002. The plant was authenticated by Dr. Gopalakrishna Bhat, Professor, Department of Botany, Poorna Prajna College, Udupi, Karnataka, India. A voucher specimen (PP 515) has been deposited in the herbarium of the Department of Pharmacognosy, Manipal College of Pharmaceutical Sciences, Manipal, India.

Chemicals and instruments – Compound microscope, stage micrometer, camera lucida, drawing sheets, glass slides, cover slips, watch glass and other common glass wares were the basic apparatus and instruments used for the study. Microphotographs were taken using a Leica DMLS microscope attached with Leitz MPS 32 camera. Solvents *viz.* ethanol (95%), hexane, petroleum ether, diethyl ether, chloroform, acetone, n-butanol and reagents *viz.*

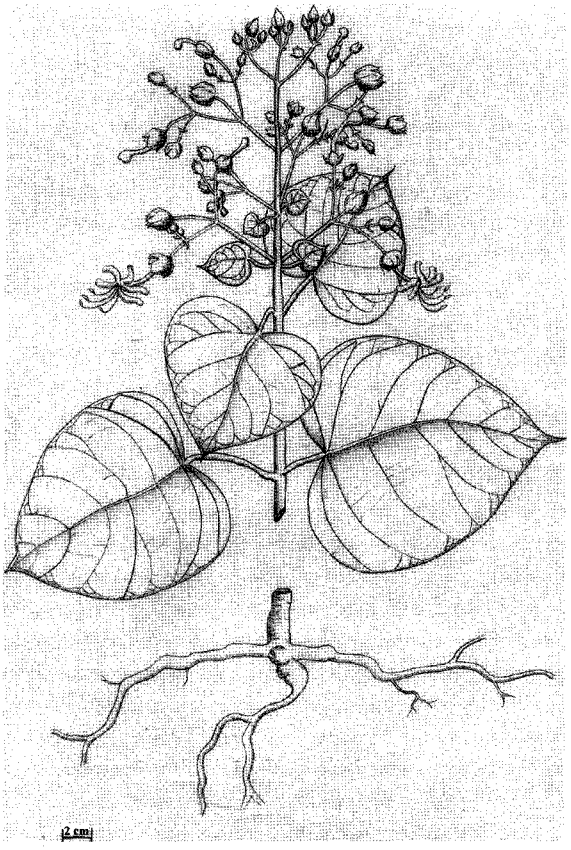

Fig. 1. Macroscopy of the aerial aspect and root of *Clerodendrum viscosum.*

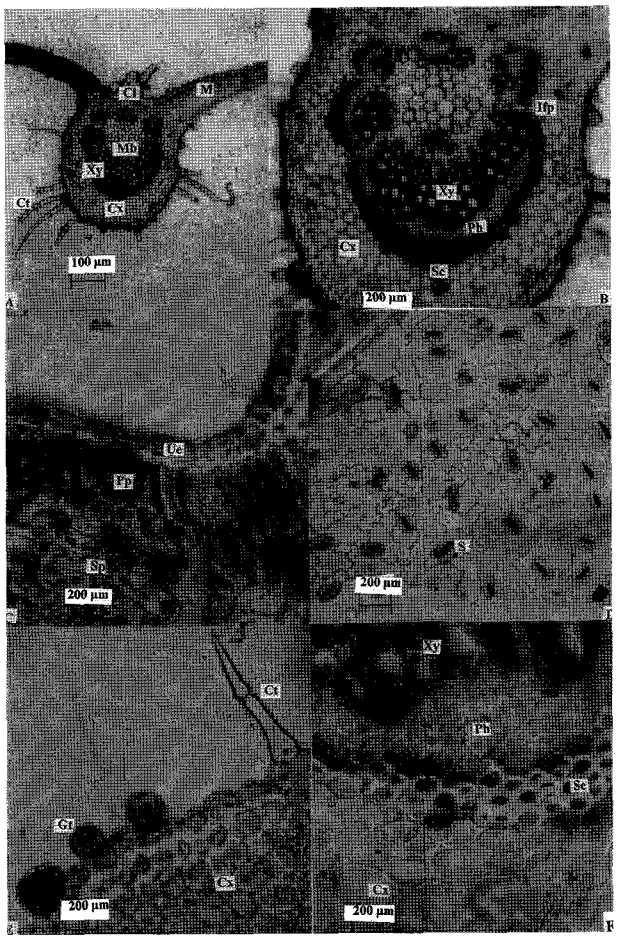

Fig. 2. Microscopy of the leaf of *Clerodendrum viscosum.*
A. Photomicrograph showing transverse section of the leaf of *Clerodendrum viscosum.*
B. A portion of midrib (enlarged).
C. A portion of mesophyll region (enlarged).
D. A section showing stomata.
E. A portion of leaf showing trichomes.
F. A section showing vascular elements and the sclereid layer.
Cl-collenchyma, **Ct**-covering trichome, **Cx**-cortex, **Gt**-glandular trichome, **Ifp**-interfascicular parenchyma, **M**-mesophyll, **Mb**-midrib, **Ph**-phloem, **Pp**-palisade parenchyma cells, **S**-stomata, **Sc**-sclereid layer, **Sp**-spongy parenchyma, **Ue**-upper epidermis, **Xy**-xylem.

phloroglucinol, glycerin, HCl, chloral hydrate and sodium hydroxide were procured from Ranbaxy Fine Chemicals Ltd., Mumbai, India.

Macroscopic and microscopic analysis – The macroscopy and microscopy of the plant were studied according to the method of Brain and Turner (1975a). For the microscopical studies, cross sections were prepared and stained as per the procedure of Johansen, (1940). The micropowder analysis was done according to the method of Brain and Turner (1975b) and Kokate (1986a). Leaf constants *viz.* vein islet number, veinlet termination number; palisade ratio and stomatal index were studied according to the method of Evans, 2003.

Physicochemical analysis – Physicochemical values such as the percentage of ash values and extractive values were performed according to the official methods prescribed (Indian Pharmacopoeia, 1966) and the WHO guidelines on quality control methods for medicinal plant materials (WHO/QCMMPM guidelines, 1992). Fluorescence analysis was carried out by the method of Chase *et al.*, 1949 and Kokoski *et al.*, 1958.

Preliminary phytochemical screening – Preliminary screening was carried out by using standard procedures described by Kokate, 1986b and Harborne, 1984.

Results and Discussion

Macroscopic characteristics – A large gregarious tawny-villous shrub up to 9 m in height with bluntly quadrangular branchlets, the leaves large 10 ~ 25 cm in length and 9 ~ 20 cm in breadth, ovate, acuminate, base quadrate; hairy on both sides. Flowers white, tinged with pink in terminal panicles. Fruits somewhat globose drupes seated on the

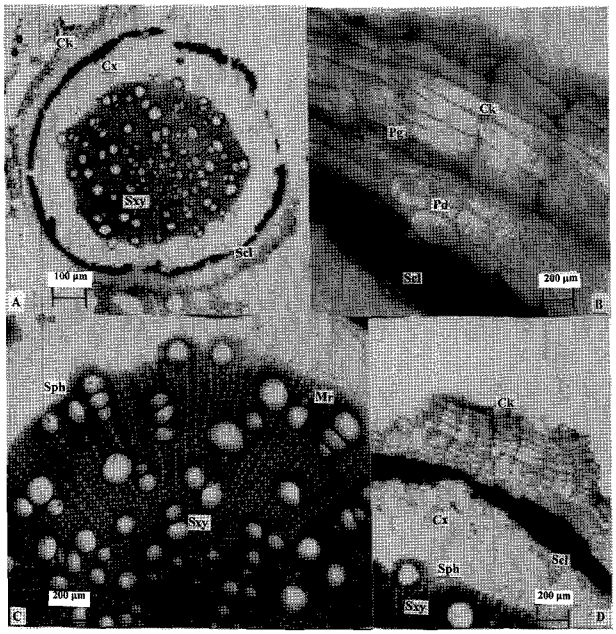

Fig. 3. Microscopy of the root of *Clerodendrum viscosum.*

A. Photomicrograph showing the transverse section of the root of *Clerodendrum Viscosum.*

B. A portion of root showing cork.

C. A section showing secondary xylem in root.

D. A portion showing secondary phloem and cortex in root.

Ck-cork, **Cx**-cortex, **Mr**-medullary ray, **Pd**-phelloderm, **Pg**-phellogen, **Scl**-stone cell layer, **Sph**-secondary phloem, **Sxy**-secondary xylem.

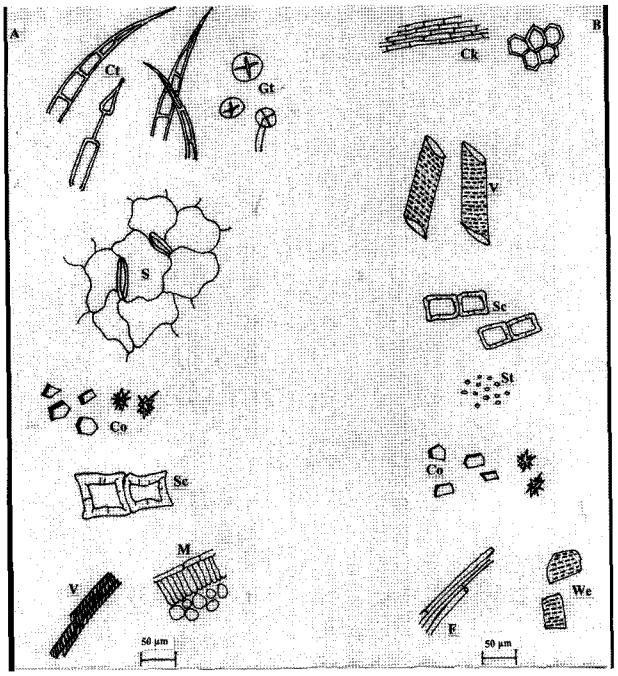

Fig. 4. Powder microscopy of the leaf and root of *Clerodendrum viscosum.*

A. Powder characters of the leaf of *Clerodendrum viscosum.*

B. Powder characters of the root of *Clerodendrum viscosum.*

Ck-cork, **Co**-calcium oxalate prisms, **Ct**-covering trichomes, **F**-fibres, **Gt**-glandular trichomes, **M**-mesophyll, **S**-stomata, **Sc**-stone cells, **St**-starch, **V**-vessels, **We**-wood elements.

enlarged pink calyx containing 1 ~ 4 pyrenes. Stem, grayish green and roots, yellowish brown in colour. Root is branched and posses longitudinal wrinkles and varies in length. The inner wood is yellow in colour.

Microscopic characteristics

i) Root – The transverse section of the root is more or less circular. The margin is prominently wavy. Cork, cork cambium, and cortex are the tissues present from the periphery to the center. The cork is thin, brown and made up of few layers of irregular parenchymatous cells. The cork cambium is indistinct. The cortex consists of 10 ~ 15 layers of parenchyma. Scattered, isolated sclereids and a continuous well-developed belt of sclereids occur in between the primary cortex and secondary phloem. Each sclereid is more or less rectangular, pitted and thickened with lignin. Some of the parenchymatous cells contain calcium oxalate crystals. The secondary phloem region comprises of mostly phloem.

The secondary xylem occupies about three fourths of the transverse section and is transversed regularly by rows of medullary rays whose cells are lignified. The xylem consists of vessels, wood fibres and lignified parenchyma. The vessels are either single or in pairs and show scalariform and bordered pitted thickenings. The xylem fibers appear as rounded or polygonal structures with thick lignified walls. Typical calcium oxalate prisms occur in the wood parenchyma region.

ii) Leaf – The transverse section of the leaf of *C. viscosum* shows a dorsiventral nature. The section is broadly divided into lamina and midrib region. The lamina of the leaf shows three distinct regions *viz,* upper epidermis, lower epidermis and mesophyll. The upper epidermis is single layered with more or less rectangular cells covered by a distinct cuticle. Abundant covering and glandular trichomes emerge from the upper epidermal layer. The covering trichomes are lignified or non-lignified, uniseriate, multicellular (2 ~ 5 cells) mostly straight and rarely warty with acute tips. The glandular trichomes are unicellular and sessile. Stomata are also seen occasionally in the upper epidermis.

The mesophyll is differentiated into palisade and spongy parenchyma. The palisade parenchyma is made up of a single layer of compactly arranged, radially elongated cells. The spongy parenchyma is multi-layered and loosely arranged with intercellular spaces. Microspenoidal crystals and vascular strands are found in the upper layer of the

Table 1. Preliminary phytochemical screening of the leaf powder of *C. viscosum*

test	hexane	benzene	chloroform	acetone	ethanol	water
carbohydrates	–	–	–	–	+	+
phytosterols	+	–	–	+	+	–
fixed oils and fats	+	+	–	–	–	–
saponins	–	–	–	–	+	+
phenolic compounds and tannins	–	–	–	+	+	–

+ denotes the presence of the respective class of compounds.

Table 2. Preliminary phytochemical screening of the root powder of *C. viscosum*

test	hexane	benzene	chloroform	acetone	ethanol	water
carbohydrates	–	–	–	–	+	+
phytosterols	+	–	–	+	+	–
saponins	–	–	–	–	+	+
phenolic compounds and tannins	–	–	–	+	+	+
gums and Mucilages	–	–	–	–	+	+

+ denotes the presence of the respective class of compounds.

spongy parenchyma. The lower epidermis which is identical to upper epidermis has stomata and numerous trichomes.

The epidermal layers of the lamina are continuous in the midrib region also. Strips of collenchyma appear below the upper and above the lower epidermis. This is followed by the cortical parenchyma containing prisms of calcium oxalate and microsphenoidal crystals. A prominent bicollateral vascular bundle occupies the central portion of the midrib with xylem towards the ventral surface and phloem towards the dorsal surface. The vascular bundle is surrounded by sclerenchymatous fibers with calcium oxalate crystals. Surface preparations revealed the presence of anomocytic or ranunculaceous type of stomata along with covering and glandular trichomes.

Powder characteristics

i) Leaf – The organoleptic evaluation of the leaf powder revealed the following characteristics. The leaf powder is pale green in color, with a characteristic odour and bitter taste. Both covering and glandular trichomes are seen and these are sometimes in fragments. The covering trichomes are multicellular, 2 ~ 5 celled uniseriate, both lignified and non lignified with sharp tips. Certain cells of the trichomes are often collapsed leaving behind only the cell walls. The glandular trichomes are numerous, both stalked and sessile with multicellular heads and unicellular stalks. Anomocytic or rananculaceous type of stomata are seen. Calcium oxalate crystals occur as clusters in the cells of the mesophyll and prisms are observed as a sheath of cells around the fibres. Stone cells are found at regular intervals.

Table 3. Ash values of the leaf and root powder of *C. viscosum*

parameters	values % (w/w)
root powder	
total ash	2.90
acid insoluble ash	0.49
water soluble ash	0.80
sulphated ash	7.96
leaf powder	
total ash	9.20
acid insoluble ash	2.63
water soluble ash	2.10
sulphated ash	13.5

ii) Root – The root powder is pale yellowish in colour, with a characteristic odour and bitter taste. The cork is thin-walled, irregular, polygonal or rectangular in surface and appears brown in colour. The parenchyma of the xylem and medullary ray cells are lignified. Fragments of xylem fibres cross the medullary ray cells at right angles. Vessels with numerous bordered pits are seen along with the wood elements. Fibres appear in bundles of 10 ~ 15, the tips of which are blunt. Stone cells are present in groups and are tangentially elongated with uniformly

Table 4. Extractive values of the root and leaf powder of *C. viscosum*

parameters	values % (w/w)
root powder	
a) water soluble extractive	6.4
b) ethanol soluble extractive	3.6
c) ether soluble extractive	1.2
leaf powder	
a) water soluble extractive	7.1
b) ethanol soluble extractive	6.0
c) ether soluble extractive	0.4

Table 5. Leaf constants of *C. viscosum*

parameters	values
vein islet number (1 mm^2 leaf surface)	12
vein termination number (1 mm^2 leaf surface)	2
palisade ratio (under 1 epidermal cell)	4.0
stomatal index (5 mm^2 leaf surface on lower epidermis)	4.2

Table 6. Fluorescence analysis of root and leaf powder of *C. viscosum*

treatment	day light	UV light (254 nm)
root powder		
powder as such	pale yellowish	brown
powder + 1N NaOH (aqueous)	yellowish brown	violet
powder + 1N NaOH (alcoholic)	yellow	pale violet
powder + 1N HCl	yellow	brownish violet
powder + 50 % H_2SO_4	brown	brownish violet
leaf powder		
powder as such	pale green	brownish green
powder + 1N NaOH (aqueous)	green	brown
powder + 1N NaOH (alcoholic)	light green	pale violet
powder + 1N HCl	pale yellow	pale violet
powder + 50 % H_2SO_4	emerald green	brown

thickened walls. Prisms and clusters of calcium oxalate are seen in the xylem parenchyma and scattered in the root powder. Starch is present in minute quantities and is simple or compound.

Preliminary phytochemical screening – Preliminary phytochemical screening mainly revealed the presence of carbohydrates, phytosterols, fixed oils, saponins and phenolic compounds (Table 1 and Table 2).

Physicochemical constants – Ash values of a drug give an idea of the earthy matter or the inorganic composition and other impurities present along with the drug. The ash values (Table 3) of the powdered *C. viscosum* leaf and root revealed a high concentration of sulphated ash.

The extractive values are primarily useful for the determination of exhausted or adulterated drug. The water soluble extractive (Table 4) was high in *C. viscosum*. The results of fluorescence analysis of the drug powder are presented in the Table 6.

Leaf constants – The leaf constants *viz.* the vein islet number, vein termination number, palisade ratio and stomatal index are presented in Table 5.

Conclusion

As there is no pharmacognostic anatomical work on record of this traditionally much valued drug, the present work was taken up with a view to lay down standards which could be useful to detect the authenticity of this medcinally useful plant. Macro and micro morphological standards discussed here can be considered as identifying parameters to substantiate and authenticate the drug.

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