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Pharmacognostical & phytochemical investigation of *Dichrostachys cinerea* W. & A. (Mimosaceae)

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Abstract

The present study deals with the macroscopical, microscopical & preliminary phytochemical investigation of leaves & roots of *Dichrostachys cinerea* W. & A. Some of the diagnostic features of leaves & roots are presence of non-glandular trichomes, stomatal complex, pitted vessels, tracheids, fibers and scanty cellular contents.

Key words: *Dichrostachys cinerea*, microscopy, Russell's viper venom.

1. Introduction

Dichrostachys cinerea (1) (2) W. & A. (Mimosaceae) is small thorny shrub about 7 to 8 meters tall with lateral dense shoots. Locally known as 'Hiver' & it is found throughout this region. Ethno medical investigations revealed that tribals of this Satpura range forests viz. Kokani, Pawara, Bhil, Vasave etc. use decoction of its roots to treat snakebite victims. (3) In our previous work (4) (5) (6) we have reported promising anti snake venom as well as anti inflammatory activity of the crude methanolic extracts of the roots & leaves of Dichrostachys cinerea against Russell's viper (Vipera rusellii) & Indian cobra (Naja naja) venom .It is also observed that tribals of this region prescribe this drug for the treatment of ailments like urinary & ophthalmic problems. Present study was undertaken to extend & support our previous research work further by its histological studies.

2. Materials & Methods

Ethno botanical information was collected by interviewing local medicine men, tribal chief& bhagats, which prescribe their own herbal

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medicine. Fresh leaves & samples of the roots of the plant D. cinerea (7) W. & A. (Mimosaceae) were collected from the wild sources in the month of October. A voucher specimen has been deposited at the Pharmacognosy department, K.E.S's College of Pharmacy, Amalner. Collected root samples were made free from aerial parts & wiry rootlets and thoroughly washed with running water to remove the adherent soil & dried. Free hand sections of the roots & single leaflets were taken from the preserved material & observed under microscope. Microscopical drawings were made with the help of camera lucida after clearing the sections. Macroscopic & microscopic characters were studied as described by Metcalf et al (8) & Datta *et al* (9)

2.1 Macroscopy

Leaves are pinnate, 1.5 to 2 inches long. Rachis is mostly pubescent, with a small erect gland between each pair of pinnae total8 to 14 pairs; leaflets are minute, mutijugate, sessile, 12 to 20 in pairs, oblique, and sub acute. Stipules are subulate from a triangular base. Branchlets end in spines.

2.2 Microscopy of leaf

T.S. of rachis: It is cylindrical with lateral wings and a median upper ridge. (Fig.1). The epidermis is single layered. Cells are thick-walled small, variable in size with moderate cuticle. The stomata are few in number. Epidermis is followed by 2-3 layered chlorenchymatous tissue interrupted by a few layered collenchyma at lateral wings. It is then followed by parenchymatous cortex. Vascular tissue forms an interrupted ring of bundles accompanied by two lateral ones and surrounded b sclerenchyma. The pith is narrow and parenchymatous. secretory cells occur as solitary in ground tissue.

T.S. of leaf: Leaves are dorsiventral and amphistomatic. Upper epidermis is of slightly larger cells. Outer wall of the cells is thick while lower epidermis is of comparatively smaller cells. Some epidermal cells occur with mucilaginous contents. Uniseriate trichomes occur on both the surfaces. Mesophyll is composed of palisade and spongy tissue. The former is two layered while latter has loosely arranged cells with spaces in between. Vascular bundles are inversely oriented and extend through the mesophyll tissue. Bundles are collateral with xylem oriented towards the upper side. They are also surrounded by single layered parenchymatous bundle sheath. In the midrib region to the ad axial side, epidermis is followed by 2-3 layered collenchyma and parenchyma. Secretory cells are of common occurrence in the ground tissue (Fig. 2).

Epidermal cell complex: (Leaf abaxial) Epidermal cells: Polygonal, or irregular, undulate to slightly sinuous, sides moderately thick, surface mainly smooth, contents scanty. Distributed all over, diffuse & variously oriented.

Costal cells: Rectangular to linear with straight sides and straight or oblique cross walls. Their surface is found to be smooth or faintly striated. Distributed on primary, secondary and tertiary veins and oriented parallel to the vein axis.

2.3 Epidermal cell complex (Leaf ad axial)

Epidermal cells: Epidermal cells are polygonal or irregular, undulate to slightly sinuous with moderately thick sides and smooth surface having scanty content. (Fig.3a) distributed all over the surface.

Costal cells: Costal cells were found to be linear with straight sides and having scanty content. Cells are distributed on primary, secondary & tertiary veins, oriented parallel to the vein axis.

Table 1: Quantitative values of leaf epidermal features in Dichrostachys cinerea W. & A.

Sr. No.	Epidermal Features	Leaf	Around vein		Intercostal region	
1	Stomatal Frequency	Ad	27.00		442.00	
	(Per mm2)	Ab	42.00		579.00	
2	Stomatal index	Ad	01.40		07.87	
		Ab	01.80		10.39	
3	Trichome frequency	Ad	31.00		07.87	
	(Per mm2)	Ab	16.40		10.39	
4	Stomatal Size (µ)	Stoamata	Length	Mean	Breadth	Mean
		Ad	25.02 – 33.36	30.85	14 – 28	19.83
		Ab	20.85 – 27.10	22.10	12.51 – 25.02	18.72
		Pore	Length	Mean	Breadth	Mean
		Ad	08.34 –14.59	12.51	02.85 – 04.17	3.62
		Ab	11.46 – 16.68	13.38	02.40 – 05.20	3.92
5	Trichome Size (µ)		Length	Mean	Breadth	Mean
		Ad	91.74-437.85	384	16.68–25.60	21.37
		Ab	35.44-467.04	230.39	16.68-41.70	23.35

* AB = Abaxial, Ad = Adaxial
* Figure relates to a mean of minimum ten counts.
* Correct botanical & anatomical identification was carried by following Metcalf & Chack (1950) & Datta & Mukherjee (1956)

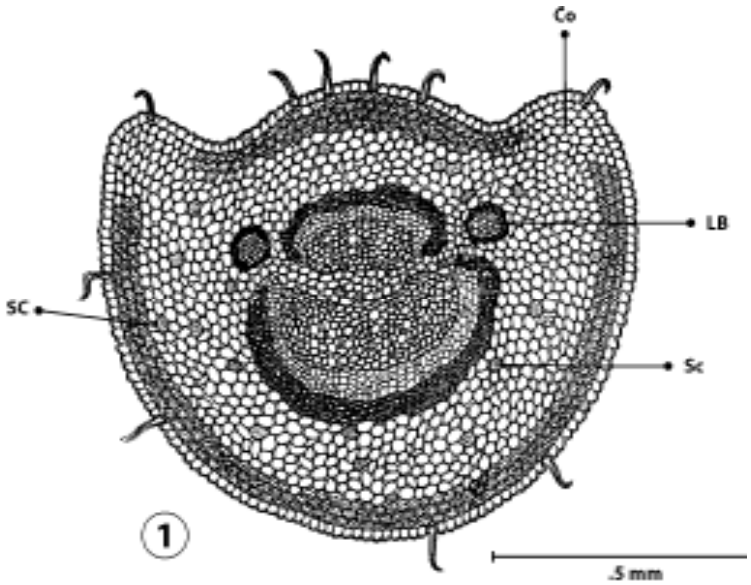

Fig. 1: T. S.of rachis:
Sc: sclerenchyma, **Co**: collenchyma, **Lb**: lateral vascular bundles.

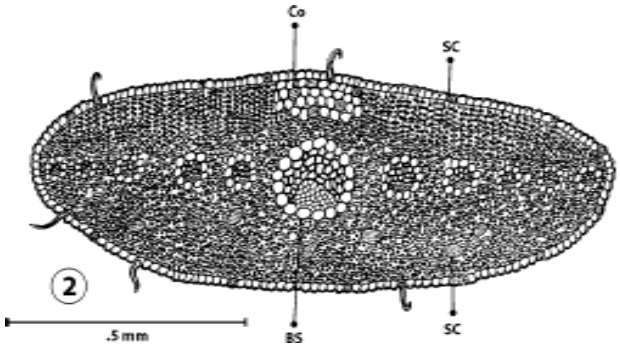

Fig. 2: T. S.of leaf:
Co: Collenchyma, **Sc:** Scelerenchyma, **BS:** Bundle sheath.

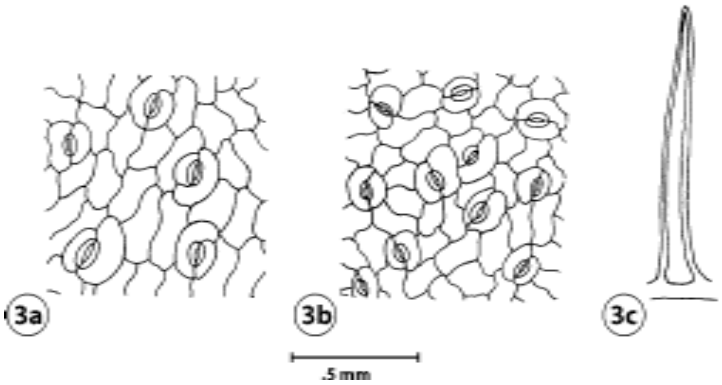

Fig. 3: a & b: Stoamatal complex
3a: Epidermal cells (Leaf ad axial) **3b:** epidermal cells (Leaf abaxial)
3c: Non glandular conical trichome

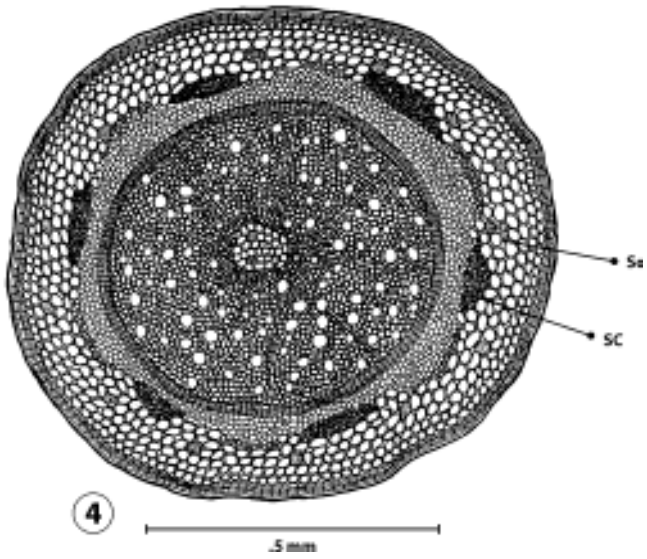

Fig. 4: T. S. of Root

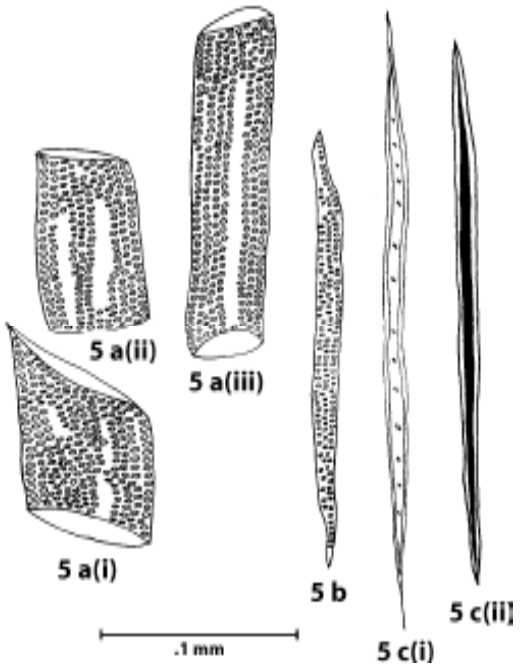

Fig. 5: a Xylem vessels
5a (i & ii): Broad Xylem vessels, **5a (iii):** Narrow & long xylem vessels.
Fig. 5b: Pitted tracheids **5c (i & ii):** Non septate libriform fibres.

Stomatal complex: Stomata paracytic with neighboring two cells. (Fig. 3 & 4) Distribution: Leaves are amphistomatic. Stomata are usually distributed in the intercostal area. A few stomata are also present in costal area. Stomatal frequency of the upper surface is lesser as compared to the lower surface. (Table 1) Stomatal index of lower epidermis is 10.39 and 7.87 of upper epidermis. Stomata are elliptic to oval one to three cells apart. Guard cells are typically reniform with differential cell walls. Stomatal size of the upper epidermis is larger than lower epidermis (Table 1)

Trichomes: Non-glandular trichomes of various sizes are present on both the surfaces of the species studied. Each trichome is a unicellular conical hair. (Fig. 3c) Morphologically trichomes are distinguished into two parts, the foot & the body.

Foot: Consists of the basal end of the body cell, distinct from the body, embedded in the epidermis.

Body: Body represents extension of the foot, conical, apical pointed, contents absen, thick walled non lignified with nearly smooth surface. Trichome frequency is more on the upper surface as compared to the lower surface. (Table 1) They are of various sizes, dense on veins & lamina margin.

Microscopy of the root: The section almost appears circular in transverse planeand shows a small pith and of polyarch type (Fig. 4). Medullary rays run radially from centre to the cortex. Rays are observed to be two cells wide, 6-18 cells longer, exclusively biseriate with a few uniseriate exceptions. Frequently containing mucilage like deposits. Heterogeneous rays

consist of both vertical and radilly elongated cells as seen in tangential sections. Xylem vessels are found between the medullary rays. Protoxylem lies towards the periphery while metaxylem towards the centre. Phloem consists of sieve tubes, companion cells, parenchyma and fibres. Outermost part consists of the layer of cork cells, stratified cells and parenchymatous cortex. The cortex cells are oval to rounded in shape, thin walled and with undetermined but probably tannin like contents. In longitudinal section, cork and cortex cells are found elongated with the presence of secretory cells in between. Phloem fibers show longitudinal crossing. Xylem vessels are of two types broad and Short (Figs. 5a-i) and narrow & long (Fig. 5a-ii&iii); typically solitary with a few multiples of 2-3 cells; nearly circular in outline. Lateral walls of the vessels exhibit alternate to opposite pitted thickening. Pits simple, end wall transverse to slightly oblique, with simple perforation plate. Tracheids have small simple pits, more numerous on radial than tangential wall (Fig. 5b) Both types of xylem fibres viz fibre-tracheids and libriform fibres noted, they are uniformly non-septet (Fig.5c-i&ii). Root powder shows fragments of cork, which are pale yellow to brown with indistinct and darker outermost layers. Cells are polygonal and somewhat variable in size with intracellular spaces and the walls are moderately thick. Cork fragments consist of thin walled parenchymatous cells with colourless mucilaginous content, which appears as transparent dots. Tracheary elements of variable sizes i.e. simple pitted vessels (42.08 x 26.41µ - 126x11.20µ), tracheids (102x8.6 – 189x10.20) and fibres (129x9.8 – 221x10.20) are also noted.

Preliminary phyto chemical studies: Powdered fresh dried leaves & roots of Dichrostachys cinerea W& A were extracted separately with Petroleum ether at 50-600 in soxhlet apparatus for 72 hrs. Material was then dried thoroughly extracted with methanol by refluxing at 60-800 for 72 hrs. Leaf & root extracts were then concentrated to a semisolid, thick sticky mass & kept in the refrigerator until further use. All the extracts were subjected to the qualitative phyto chemical screening for identification of active constituents using standard methods. (10) (11)

3. Results & Discussion

Microscopic examinations of D.cinerea leaves & roots revealed the presence of outer epidermis with polygonal and curved cells, paracytic stomata on both the surfaces with thick walled, non glandular trichomes, and secretory cells in the ground tissues. It is observed that outer wall of the epidermal cells is slightly thicker in the upper epidermis. Mucilaginous content is found in some of the cells. Presence of two-layered palisade tissue in the mesophyll is also a specific feature observed. Inversely oriented vascular bundles are also observed in the section. Root section appeared almost circular in plane and has biseriate medullary rays running from the center to cortex. Phloem consists of sieve tubes and parenchyma. Longitudinal section of the roots shown presence of secretory cells in the cortical region. While xylem vessel are observed to be circular in outline exhibiting simple pits. Tracheids with simple pits and libriform fibres are also observed. Tannin contents are found in the cortex of the root. Preliminary phytochemical tests revealed the presence of glycosides, flavonoides, carbohydrates and tannins in the drug. Although we have described prominent anti snake venom & anti inflammatory activity of this plant against Russell's viper (Vipera russellii) venom & Indian cobra (Naja naja) venom, so far no systematic study has been reported about its anti snake venom mechanism. This work was undertaken to support our study & for accurate & precise identification & pharmacognostical profiling of D.cinerea W. & A. However more detailed phytochemical studies are necessary to identify the active principles & exact mechanism of action.

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