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Pharmacognostic evaluation of *Rivea hypocrateriformis* Desr.

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Abstract

Rivea hypocrateriformis choisy plant belongs to convolvulaceae family, reported therapeutically as antioxidant, anti-arthritis and antioxidant and popular in Odisha and Gujarat. The leaf part of the plant was subjected to various pharmacognostical studies like, morphology, quantitative microscopy, powder microscopy and sectioning studies to identify authentically. Morphological characters were studied by observing the stem and root as such and also with the help of the dissecting microscope. Free hand thin transverse sections of Root and stem were taken for detailed microscopical observation. The Powder characters were studied according to the guidelines Ayurvedic Pharmacopoeia of India. The histochemical tests were carried out as per the standard guidelines. In microscopical studies, the leaf exhibits dorsiventral symmetry with abaxially prominent midrib and differentiation of adaxial and abaxial tissues of the lamina, Vascular bundle, Xylem and phloem. Powder microscopy consists of non-glandular epidermal trichomes, calcium oxalate and stomata. Epidermal Fragments exhibits epidermal trichomes, epidermal cells and stomata

Keywords: Powdered material of Plant *Rivea hypocrateriformis*, FAA (formalin + acetic acid + alcohol), glycerine, Paraffin blocks.

Introduction

Rivea hypocrateriformis (Desr).is a large climbing shrub found almost throughout India, belongs to the family convolvulaceae leaves orbicular-cordate, mucronate; flowers white fragrant clove scented, opening in the evening and closing in the day. Fruit is a globose, reddish brown capsule, usually 1-4 seeded (figure 1) ^[1].



Fig 1: Entire plant with flowers of *Rivea hypocrateriformis*

Synonyms: Convolvuls hypocrateriformis (Midnapore creeper)

Systematic Position ^[2]

Kingdom	-	Plantae
Subkingdom	-	Magnoliophyta
Division	-	Magnoliophyta
Class	-	Magnoliopsida
Subclass	-	Dilleniidae
Order	-	Convolvulales
Family	-	Convolvulaceae
Genus	-	<i>Rivea</i>
Species	-	<i>hypocrateriformis</i>

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Vernacular Names

Hindi	-	Phang
Tamil	-	Bhoddkirai, musutaikodi
Telugu	-	Niruboddi
Bengali	-	Thorki-bel
Marathi	-	Saiyvel

1.2 Geographical distribution

Northern Circars, Deccan and Carnatic, especially near the sea, in the hedges and dry open forests. Midnapore creeper is robust woody climbing shrub, found in dry subtropical forests of India to Assam and Pakistan [3].

2. Material and Method

The plant specimens for the proposed were collected from Coimbatore bharathiyar university campus, was taken to select healthy plants and normal organs. The present study was undertaken to establish certain botanical standards for identification and standardization of *R. hypocrateriformis*. Plant identification was done with the help of Forest flora of Gujarat state [4]. Macroscopical characters were studied by observing the leaf, stem and root as such and also with the help of dissecting microscope. Microscopical studies were characterized by free hand thin transverse sections of Root and stem. The Powder characters were studied according to the guidelines Ayurvedic Pharmacopoeia of India [5]. The histo-chemical tests were carried out as per the standard guideline.

3. Result and Discussion

3.1 Macroscopical studies of the plant *Rivea hypocrateriformis* (Figure 5)

Leaves: The Leaves are ovate-orbicular, appressedly-silky-hairy beneath, rarely broad than long, shallow basal sinus, lateral nerves about 6 on either half and petiole with a green on either side at junction with the midrib. Clove-scented creeper or midnapore creeper dorsal and lateral view of leaves were measured (Figure 2, 3).



Fig 2: Ventral View of *Rivea hypocrateriformis* *Rivea hypocrateriformis*



Fig 3: Dorsal View of *Rivea hypocrateriformis* *Rivea hypocrateriformis*

Flower: Flowers are white, fragrant, clove scented, opening in the evening and closing in the day. It bears axillary on 1-3 or sometimes more flowered peduncles; bracts 2-3, narrow. Calyx consists of 5 ovate or oblong sub equal sepals. Corolla is large, white, and salver-shaped; tube narrow, cylindric; lobes of the limb rounded, plicate and annular. Stamens are present near the middle of the tube; Filaments are slender, villous at the base; anthers are narrow oblong; pollen grains spinulose, consists of four celled ovary with four ovules. Style filiform; stigmas 2, linear-oblong, rugose (Figure 4) [6, 7].

Fruit: Fruit is subglobose, dry, indehiscent or breaking up irregularly often one-celled by the absorption of the septa. Seeds are, glabrous, surrounded by mealy pulp; cotyledon is much folded; the radical are thick. A climbing shrub with large white flowers which opens at sunset and close at sunrise and are very fragrant (Figure 4).

Bark: Bark is thin, bears grey color and the wood is concentric.



Fig 4: Fruit And Flower Bud of *Rivea hypocrateriformis*

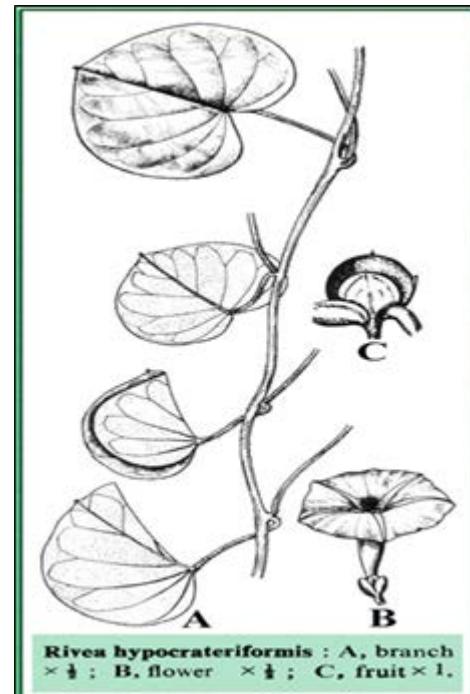


Fig 5: Plant of *Rivea hypocrateriformis*

3.2 Microscopic Characters of the Leaves of *Rivea hypocrateriformis*

3.2.1 Collection of Specimen: The required samples of leaf were cut and removed from the plant and fixed in FAA (formalin + acetic acid + alcohol). After 24 hours of fixing, the specimens were dehydrated. The specimens were cast into paraffin blocks. The dewaxing of sections were done,

wherever necessary sections were also stained. Powdered materials of different parts were cleared and mounted in glycerine medium after staining. Different cell component were studied and measured.

3.2.2 Leaf: [8-11] the leaf exhibits dorsiventral symmetry with abaxially prominent midrib and differentiation of adaxial and abaxial tissues of the lamina (figure 6).The midrib is planoconvex in transactional view, the adaxial side semicircular. It consists of a thin epidermal layer of small,

thick walled cells. The ground tissue is homogenous and includes circular, thin walled parenchyma cells. Some of the ground cells are laticifers which are smaller, thick walled with dense latex content (figure 7).The vascular consists of a thick, cup shaped vascular strand. The xylem elements occur in parallel rows of 2-6 cells in each row; the cells are angular and thin walled. Phloem occurs in discrete clusters distributed both along the lower as well as upper sides of the xylem are (figure 7,8).

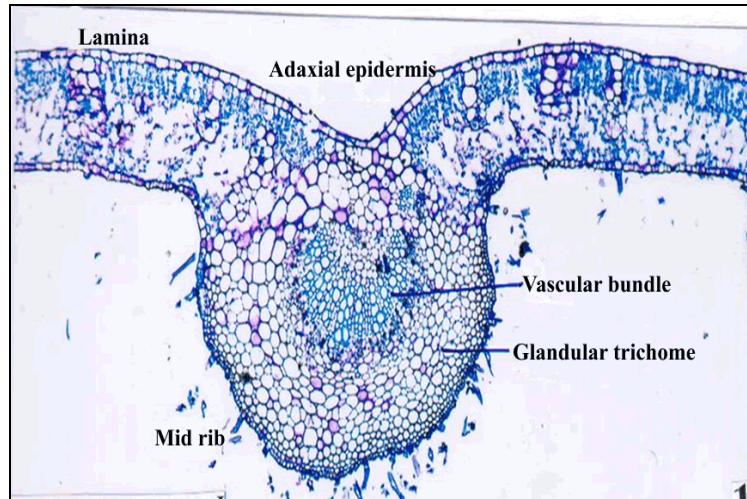


Fig 6: t.s of leaf through midrib

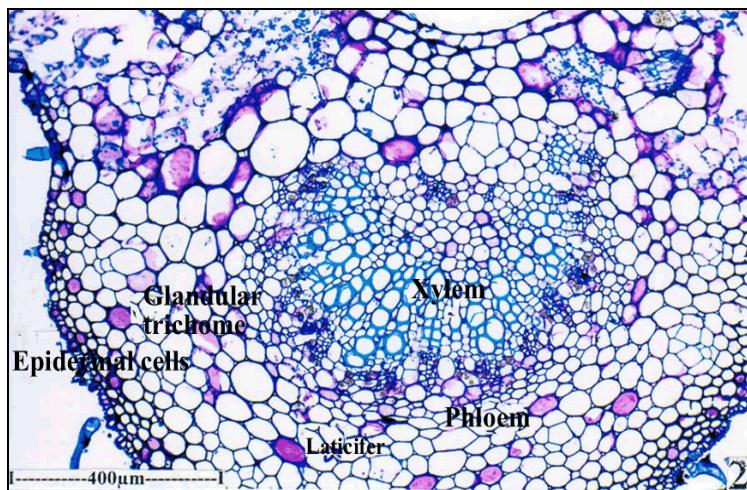


Fig 7: Midrib enlarged

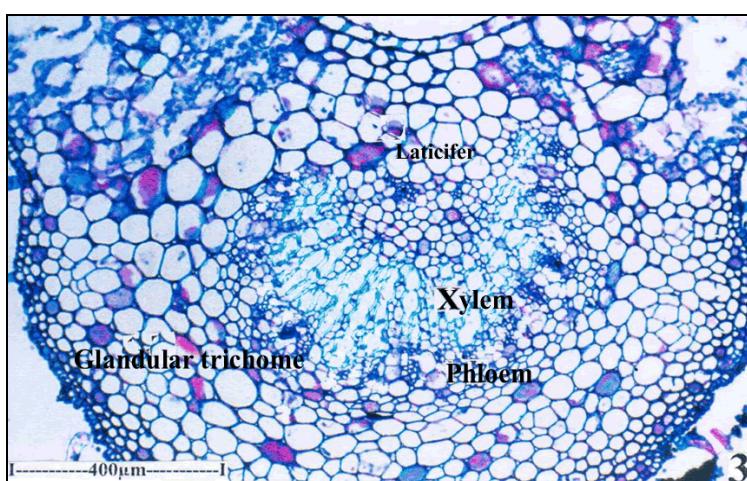


Fig 8: Midrib enlarged

3.2.2.1 Lamina: The lamina is dorsiventral, amphi stomatic and smooth on both surfaces (fig.9). The adaxial epidermis consists of thick cylindrical cells with thin walls. The abaxial epidermis is thinner and have squamish or rectangular thin walled cells. The mesophyll consists of upper band of two layers cylindrical palisade cells and lower zone of 6-8 layers of lobed loosed arranged spongy parenchyma cells. The vascular strands of the veinlets are located in the median

position of the lamina surrounded by circular dilated bundle sheath cells with adaxial and abaxial extension (fig.9). Epidermal trichomes of non-glandular type are often seen on the abaxial surface (fig 10).

Calcium oxalate druses are abundant in the mesophyll tissue (fig 11). The druses are diffuse in distribution. They are located in the ordinary parenchyma cells.

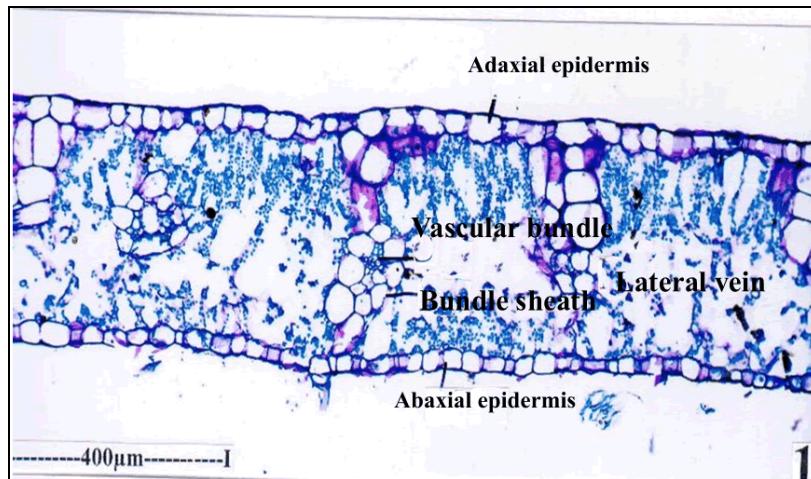


Fig 9: T.s of lamina with lateral veins

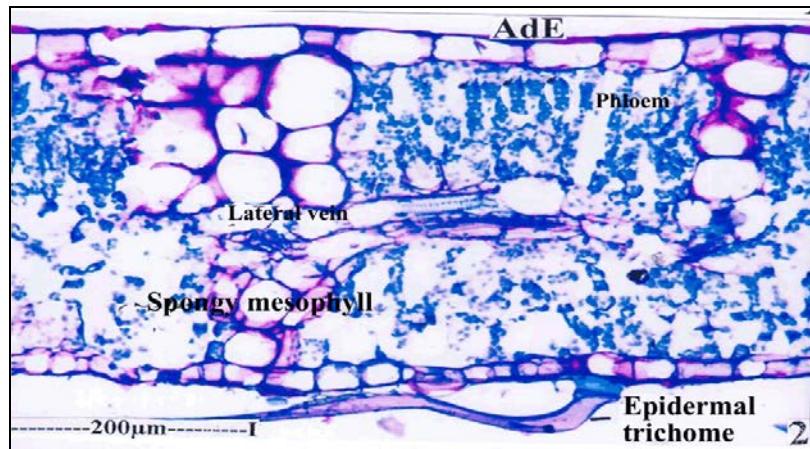


Fig 10: T.s of lamina further enlarged (with abaxial trichome)

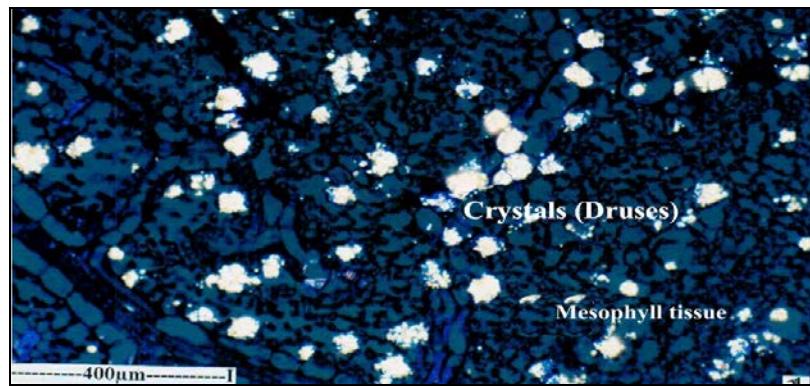


Fig 11: Paradermal section of the lamina showing calcium oxalate (as seen under the polarised light microscope)

3.2.2.2 Venation pattern (fig.12): The venation is densely reticulate. The veins are ensheathed by hyaline bundle sheath cells. The vein-islets are small, polygonal with distinct

boundaries. The vein-terminations are short thick and unbranched. The terminations possess spherical terminal tracheids (fig 13, 14, & 15).

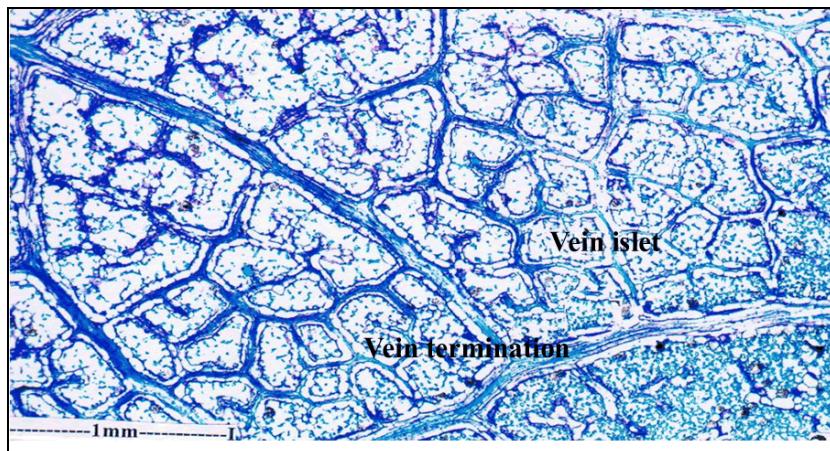


Fig 12: Paradermal section of the lamina showing venation pattern

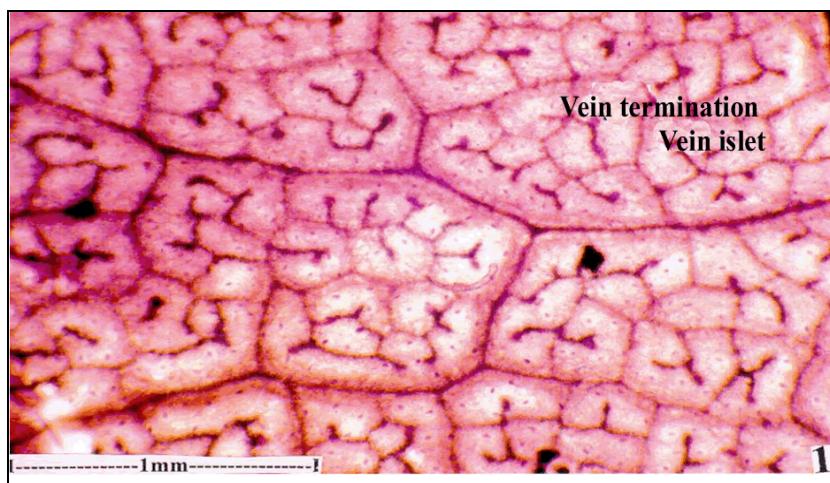


Fig 13: Leaf cleared showing venation pattern

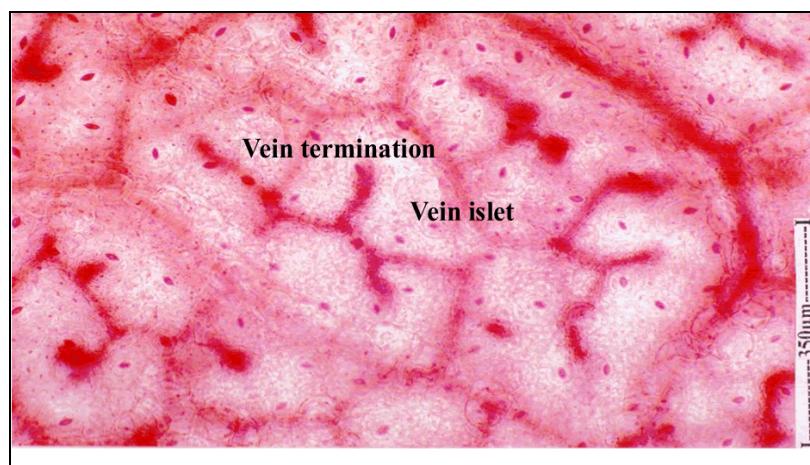


Fig 14: Vein islet and vein termination enlarged

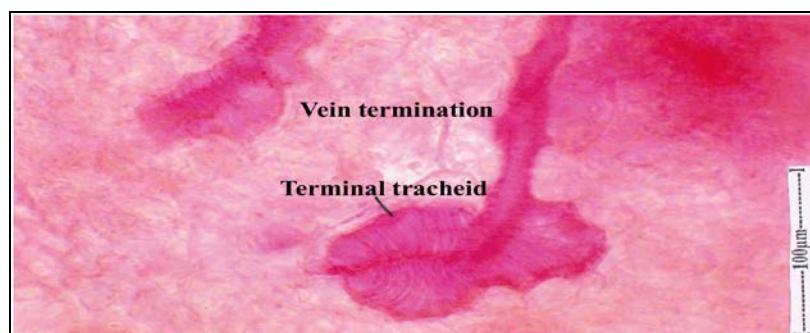


Fig 15: Terminal tracheid-enlarged

3.2.2.3 Epidermal cells and stomata (fig.16): Epidermal structure was studied from thin paradermal sections. The epidermal cells are small in size and are polygonal in shape. The anti clinal walls thin and straight. The stomata are

paracytic type. There are two equal or unequal subsidiary cells and parallel to the stoma. The guard cells are elliptical with wide stomatal pore. The stomata are $20 \times 30 \mu\text{m}$ in size.

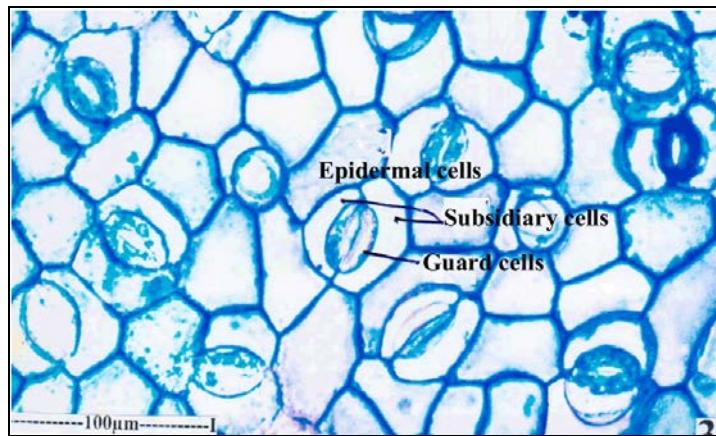


Fig 16: Epidermal cells and stomata in aerial view

3.2.2.4 Petiole (Fig. 17, 18): The petiole is circular in sectional view. The surface is clothed densely with epidermal trichomes. The epidermis is thick and cells are small with dark contents. The petiole is 1.5mm thick; the ground tissue is parenchymatous; the cells are circular and thin walled. The cells are circular and thin walled. The cells towards the periphery are small with thick walls; those towards the centre are larger and less compact. The vascular strand is deeply

curved are with narrow opening on the adaxial side. The vascular strand consists of several parallel rows of xylem elements; each row consists of 3-6 elements which are circular, narrow and thick walled. Phloem occurs in small groups all along the outer zone and inner zone of the xylem. The outer phloem is well differentiated whereas the inner phloem is less distinct (fig.18).

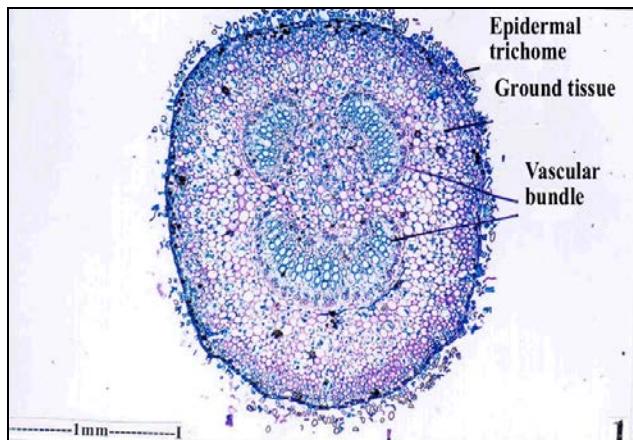


Fig 17: T.s of petiole entire view

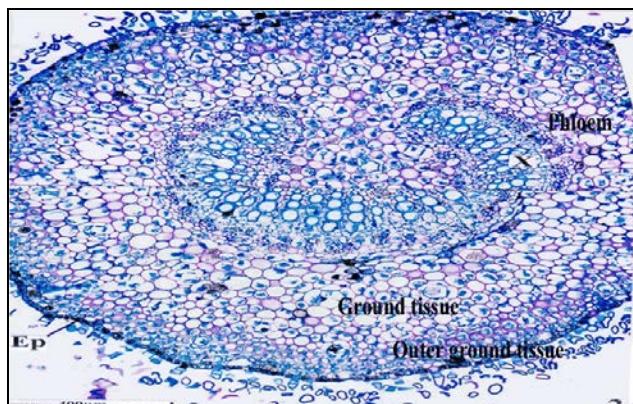


Fig 18: T.s of petiole entire view magnified

3.3 Powder Microscopy [12-14] The leaf powder shows the following inclusions under the microscope.

3.3.1 Epidermal Trichomes: Nonglandular epidermal trichomes are abundant in the powder. They are unicellular

and unbranched (fig.19,20).The cell walls are thin and the lumen is wide.The trichomes have tapering pointed ends.

They are 50-80 μ m long and 30 μ m wide.

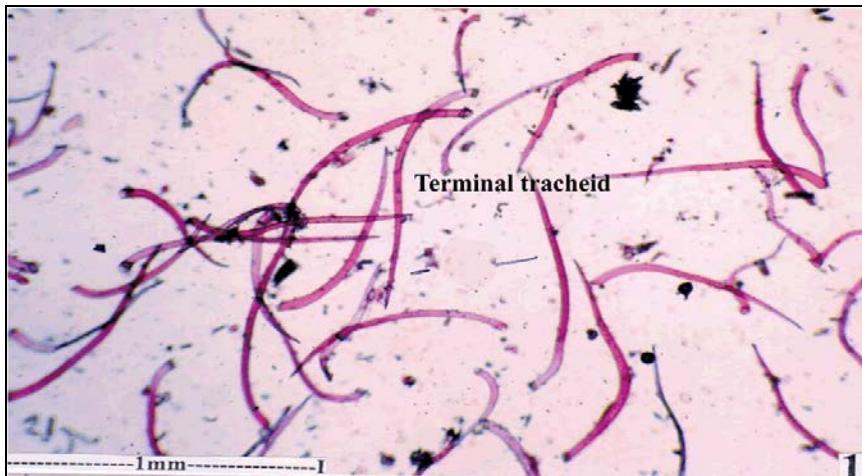


Fig 19: Epidermal trichomes removed from the lamina

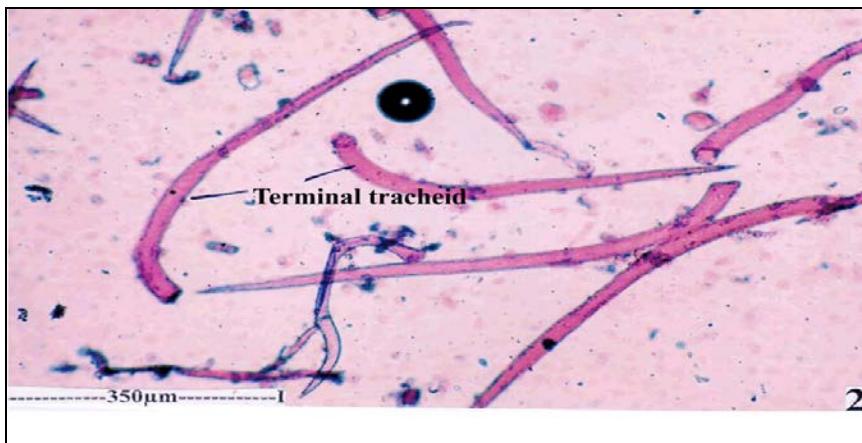


Fig 20: Epidermal trichomes removed from the lamina

3.3.2 Epidermal Fragments [15-18] Small pieces of epidermal peeling are common in the powder (fig.20, 21).The peelings exhibit epidermal trichomes, epidermal cells and stomata. The trichomes are unicellular, unbranched and non-glandular (fig.21).The epidermal cells are polygonal and have straight thin anticlinal walls. The stomata are paracytic type; the subsidiary cells are equal or unequal and are semicircular in

shape. The guard cells are elliptical in outline (fig.22). Apart from the epidermal peeling, fragments of entire leaf blade are also seen in the powder. The fragments exhibit the veinislets and vein termination (fig.23).The vein islets distinct and have thick well defined vein boundaries. The vein terminations are thick and prominent; they are unbranched; less frequently branched once.



Fig 21: Epidermal peeling showing the trichomes and stomata

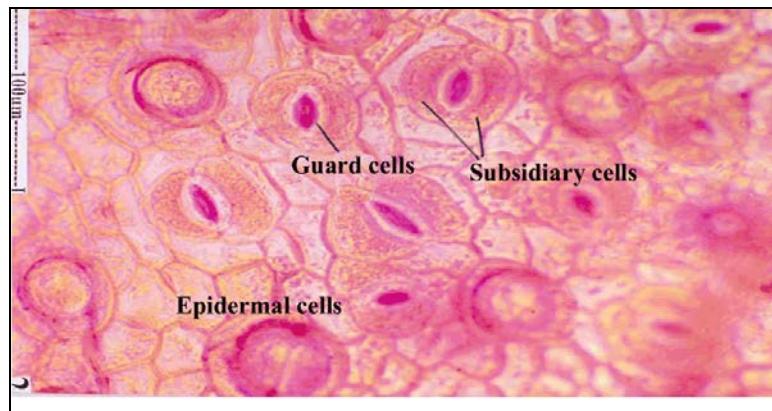


Fig 22: Epidermis showing stomata type

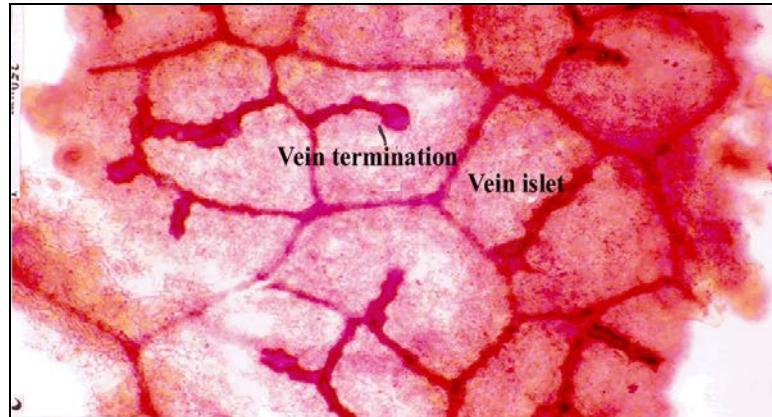


Fig 23: Leaf blade showing venation

3.4 Quantitative Microscopy [19, 20]

3.4.1 Determination of leaf constants: The vein islet number, vein termination number, stomatal number and stomatal index were determined on fresh leaves using standard procedure.

3.4.2 Vein islet number and vein termination number

The term vein islet is used to denote the minute area of photosynthetic tissue encircled by the ultimate division of the conducting strands. The number of vein islets per square mm area is called vein-islet number. Vein termination number may be defined as the number of vein terminations present in one square mm area of the

photosynthetic tissue.

3.4.3 Determination of vein islet number and vein termination number

Pieces of leaves were cut from various regions of the leaves between midrib and the margin, cleared in chloral hydrate and mounted on a slide. Camera Lucida and drawing board were arranged. With the help of a stage micrometer, camera Lucida and microscope, 1 mm square was drawn on the paper. Then the stage micrometer was replaced by the preparation and the veins were traced in that square. Then the vein islets and vein terminations were counted in the square. Ten such readings were taken and the average was calculated and the results were presented in Table 1.

Table 1: Vein islet number and vein termination number of leaves of *Rivea hypocrateriformis*

Observation Number	Vein islet Number	Vein termination number
1.	8	19
2	6	15
3	11	15
4	7	14
5	9	12
6	11	13
7	9	10
8	11	11
9	11	13
10	10	14
Range		
Minimum	6	10
Average	9.3	13.6
Maximum	11	19

3.4.4 Stomatal number [21]

The average number of stomata per square mm area of epidermis of the leaf is called stomatal number.

3.4.5 Determination of stomatal number

Pieces of upper and lower epidermal peelings were mounted on a slide with the help of camera Lucida and stage

micrometer 1 mm square was drawn on a paper. The stage micrometer was replaced by the preparation. Then the preparation was observed and the stomata marked in that unit area. Number of stomata present in those unit area was

calculated. Ten such readings were taken and the average of stomatal number was calculated and presented in the Table 2 for lower epidermis and table 3 for upper epidermis.

Table 2: Stomatal Number of Leaves *Rivea hypocrateriformis* (lower epidermis)

Observation Number	Lower Epidermis
1	19
2	14
3	23
4	17
5	22
6	18
7	19
8	13
9	23
10	11
11	26
12	24
Range	
Minimum	13
Average	19.08
Maximum	26

Table 3: Stomatal number of Leaves *Rivea hypocrateriformis* (upper epidermis)

Observation number	Upper epidermis
1	22
2	32
3	18
4	20
5	15
6	20
7	20
8	26
9	21
10	22
11	21
12	27
Range	
Minimum	15
Average	22
Maximum	32

3.4.6 Stomatal Index

It is the percentage which the numbers of stomata form to the total number of epidermal cells, each stoma being counted as one cell.

$$S.I = \frac{S}{E+S} \times 100$$

Where, S=number of stomata per unit area

E= number of epidermal cells in the same unit area

3.4.6.1 Determination of Stomatal Index

The procedure adopted in the determination of stomatal number was followed and then the preparation was observed under high power. The epidermal cells and the stomata were counted. From these values the stomatal index was calculated using the above formula and was given in Table 4.

Table 4: Stomatal Index of Leaves *Rivea hypocrateriformis*

Observation number	Lower epidermis
1	14.28
2	17.39
3	16.27
4	14.89
5	20.19
6	16.75
7	13.98
8	14.44
9	18.64
10	15.23
Range	
Minimum	14.28
Average	16.21
Maximum	20.19

Conclusion

The macroscopy of the leaves were discussed such as leaves are predominantly ovate rather orbicular in shape and appressedly-silky-hairs in the ventral surface. It is broader than longer and bears six lateral nerves on either half of the lamina.

The important microscopical characters observed in the leaves of *Rivea hypocrateriformis* are as follows. The leaf exhibits dorsiventral symmetry with abaxially prominent midrib and differentiation of adaxial and abaxial tissues of the lamina.

Midrib is planoconvex with flat adaxial and semicircular abaxial side. Epidermis layer consists of thick walled cells. Ground tissue with thin walled parenchyma cells. Vascular bundle with thick, cup shaped vascular strand. Xylem elements are angular and thick walled, phloem occurs in discrete clusters. The mesophyll region consists of upper band of two layers of cylindrical palisade cells and lower zone of loosely arranged spongy parenchyma cells. Epidermal trichomes of non-glandular type is present. Venation is densely reticulate, the veins are ensheathed by hyaline bundle sheath cells. Epidermal cells are small in size and are polygonal in shape Stomata is paracytic type. Powder microscopy consists of the following character Non glandular epidermal trichomes are abundant, they are unicellular, unbranched, and tapering pointed ends. They are 50-80 μ m long and 30 μ m wide. Stomata are paracytic. Calcium oxalate are abundant in mesophyll region, they are 20-30 μ m thick.

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