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Research Article

Pharmacognostical Studies of the Leaves of *Cayratia carnosa gagnep.* in South India

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ABSTRACT
The plant Cayratia carnosa gagnep. is a rare medicinal plant belongs to the family vitaceae is commonly known in India as Amalbel in Hindi, heggoli in Kannada and amarakkoti or kattuperanta in Malayalam. It is a fleshy climbing shrub distributed through out India and Asian countries. Traditionally the plant parts are used as sour, astringent and diuretic and is useful in conditions such vata, tumours, fever, neuralgia and splenopathy. It purifies the blood and is given for hepatopathy, cardiac disorders, ulcers, wounds, dropsy, haemorrhoids and strangury. The presence of narrow rectangular and thick walled epidermimal cells, trichomes and vascular bundles connected to the abaxial epidermis in the lamina, rosette cells, raphide, petioles without wings etc. are very important characters. The combinations of these characters provide reliable characters for species identification. The present study deals with the micro morphological studies carried out on the leaves of *Cayratia carnosa,* one of the World Health Organization (WHO) accepted parameter for identification of medicinal plants.
Key words: *Cayratia carnosa*, macroscopical, microscopic

INTRODUCTION

The plant *Cayratia carnosa* is a vine-like shrub growing through out Asian tropics[1]from India and Sri Lanka to southern China. In India, it is found as a vine growing on fences as weed and distributed through out on the hills of west coast and Western Ghats [2]. The plant can also be found in open habitats and hedges which start flowering and fruiting in the month of August to November of every year. The aerial parts are densely pubescent. Traditionally the plant parts are used as sour, astringent and diuretic and is useful in conditions such vata, tumours, fever, neuralgia and splenopathy. It purifies the blood and is given for hepatopathy, cardiac disorders, ulcers, wounds, dropsy, haemorrhoids and strangury. A poultice prepared from leaves is used in the treatment of yolk sores on the neck of bullocks [3]. But no report is available on the micro- morphological study of this plant. Hence the present study was aimed to explore the pharmacognostical investigation of the leaves of *Cayratia carnosa*

MATERIAL AND METHODS

The plant materials were collected from Trivandrum district of Kerala in the months of May and June 2008 (Figure1). It was identified and authenticated by Dr. Jayraman, botanical Survey of India, Chennai India. The healthy plants were selected carefully and the different parts of the samples were cut into suitable sizes and washed well to remove the adhered impurities. Selected samples of the plant parts such as leaves were fixed in formaldehyde, acetic acid and 70% v/v ethyl alcohol in the ratio 5:5:90. After 24 hours of fixing, the specimens were dehydrated with graded series of tertiary butyl alcohol as per the schedule given by Sass [4]. Infiltration of the specimens was carried by gradual addition of paraffin wax (50-60 ^{0}C m.p.) until tertiary butyl alcohol solution attained super saturation. The specimens were caste into paraffin blocks.

Sectioning of leaves: The paraffin embedded specimens were sectioned with the help of Rotary Microtome, RMT 30 (Radical Instruments India). The thicknesses of the sections were kept between 10 and 12 µm. The dewaxing of the sections was carried according to the procedure of Johansen [5]. The sections were stained with toluidine blue as per the method published by O' Brien [6]. The dye rendered colours like pink to cellulose walls, blue to the lignified cells, dark green to suberin, violet to mucilage and blue to the protein bodies. The sections were also stained with saffranin, fast green and iodine potassium iodide reagents for starch. The stomatal morphology were studied based on venation pattern and trichome distribution, paradermal sections as well as clearing of leaf with 5% sodium hydroxide or by epidermal peeling by partial maceration employing Jeffrey's maceration fluid[4]. Glycerine mounted temporary preparations were made for macerated/cleared materials. Powdered materials of the different parts were cleared with sodium hydroxide and mounted in glycerine after staining . Different cell component were studied and measured [7,8,9,10] .

Photomicrograph: Microscopic descriptions of the selected tissues were supplemented with micrographs. Photographs of different magnifications were taken with Nikon Lab Photo 2 microscopic unit. Bright field was used for normal observations where as polarized light was employed for the detailed study of crystals, starch grains

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Fig 1. *Cayratia carnosa(*entire plant*)* and Ovoid fleshy white berries

Table1. Macromorphological characters of *Cayratia carnosa*

Plant parts (*Cayratia carnosa*)	Description
Inflorescence	Umbellate or corimbose cyme
Flowers	Regular, bisexual, hypogynous, pentamerous,
Calyx	Four sepals, gamosepalous
Corolla	Four petals, polypetalous and greenish white in colour
Stamens	Four in number
Ovary	Two celled and ovules are two per cell
Fruit	Ovoid fleshy white berries
Seeds	Pyriform or triangular, rounded and rugose on the back

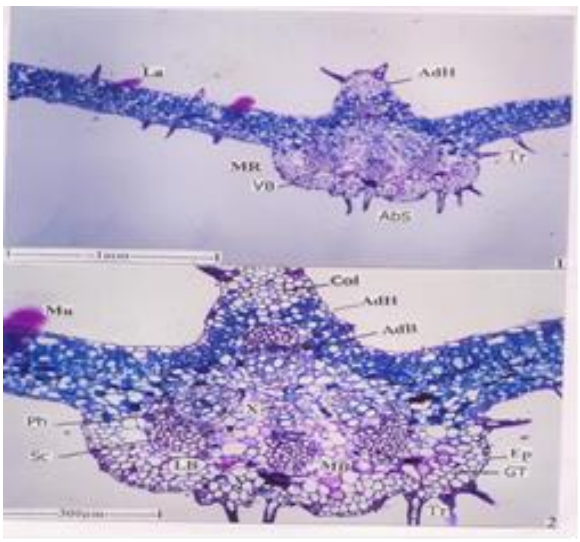

Fig 2: Anatomy **of the leaf of *Cayratia carnosa***
1. T.S of leaf through midrib with lamina;
2. Mibrib with lamina enlarged
Abs:Abaxial side, AdB:Adaxial bundle, AdH:Adaxial hump,Col:Collenchyma,
Ep: Epidermis,GT:Ground tissue,La:Lamina,LB:Lateral bundle,MB:Median bundle,MR:Midrib,MU:Mucilage,Ph:Pholem,Sc:Sclerenchyma,Tr:Trichome,VB:Vascular bundle, X:Xylem

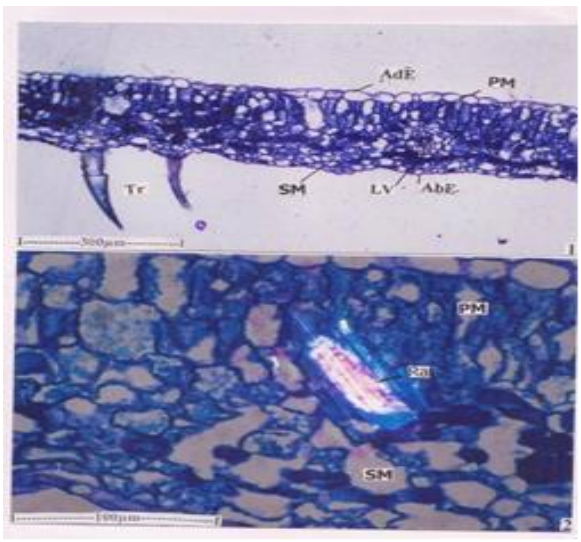

Fig 3.Anatony of the lamina of *Cayratia carnosa*
1.T.S of lamina showing Non-glandular epidermal trichomes
2.Raphide in the mesophyll tissue (under polarized light microscope)
AbE:Abaxial epidermis,AdE :Adaxial epidermis,LV: Lateral Vein, PM:Palisade mesophyll,
Ra:Raphide,SM:Spongy mesophyll,Tr:Trichome.

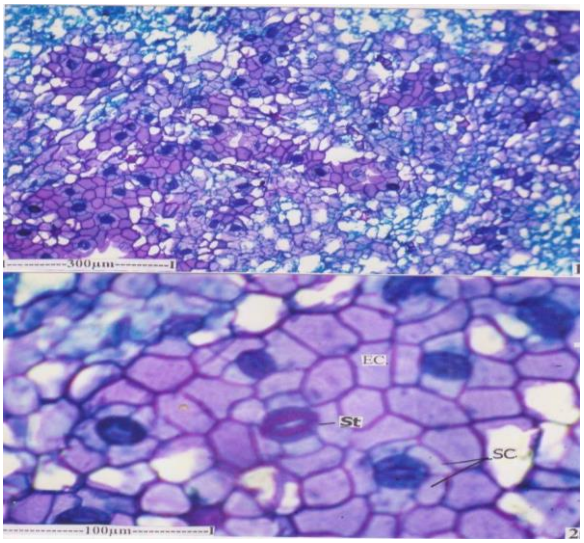

Fig 4. Stomatal Morphology of *Cayratia carnosa*
1.Abaxial epidermis with stomata
2. Stomata enlarged
EC: Epidermal cell,SC :Subsidiary cells,St:Stomata

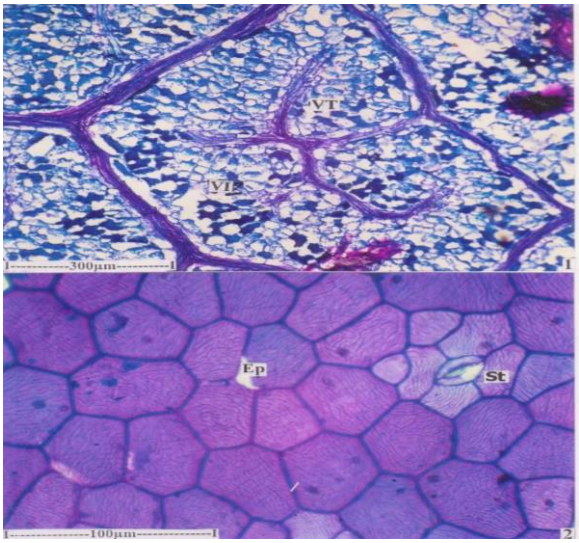

Fig 5. Venation pattern and epidermal morphology of *Cayratia carnosa*
1. Vein islets and vein termination;
2. Adaxial epidermis with stomata
Ep:Epidermis, St:Stoma, VI:Vein islets, VT:Vein termination

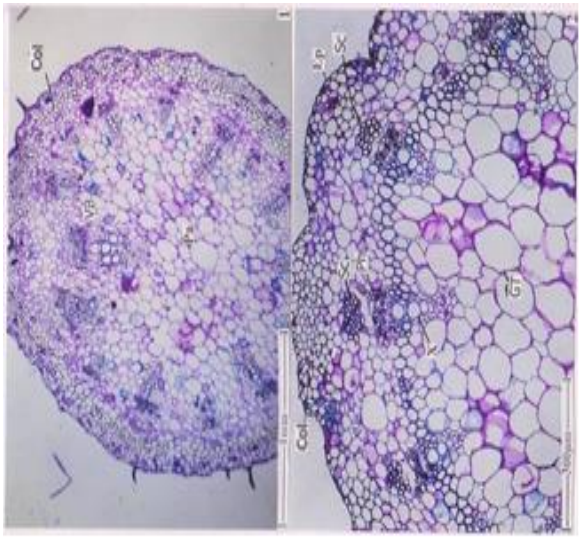

Fig 6. Anatomy of the petiolule of *Cayratia carnosa*
1. T.S of petiolule entire vie
2. T.S of petiolule half portion enlarged
AdG:Adaxial groove, AS:Acessory strand, Col:Collenchyma, Ep:Epidermis, GT:Ground tissue, Mu:Mucilage, Ph:Phloem, Sc:Sclerenchyma,W:Wing, X:Xylem.

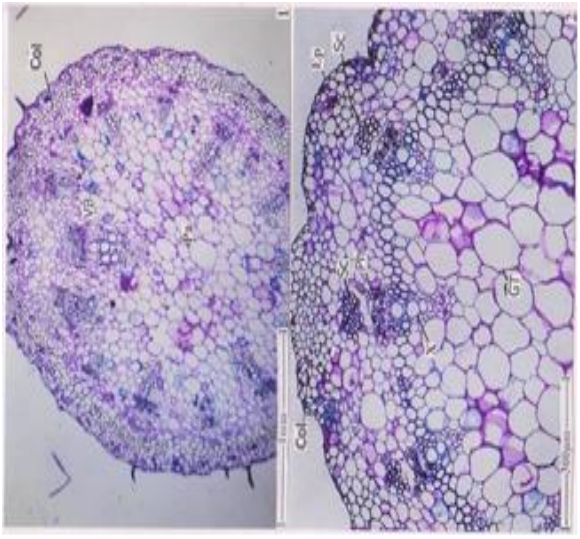

Fig 7.Anatony of the petiole of *Cayratia carnosa*
1. T.S of the petiole ground plan
2. T.S of petiole a sector enlarged
Col:Collenchyma, Ep:Epidermis, GT:Ground tissue, Pa:Parenchymatous ground tissue, Ph:Phloem, Sc:Sclerenchyma, VB:Vascular bundle, X:Xylem.

and lignified cells. Magnification of the figures is indicated by scale bars [11,12].

RESULTS AND DISCUSSIONS

Macroscopical studies: The leaves are trifoliate and leaflets are ovate orbicular, thin and coriaceous. The leaf surfaces are pubscent and margins are dentate, petiole about 7cm long and petiolate is 3mm long. Flowers are long peduncled branched cymes. Macroscopical characters are discussed in table1.

Microscopic study- 1. **Leaf:** The leaf has prominently projecting midrib and thin, densely pubescent dorsivental lamina (Fig 2.1). Midrib (Fig 2.2) has prominent adaxial conical hump and abaxial hemispherical wide part. The midrib is 950 µm in height, the adaxial hump is 350 µm in height and 200 µm in breadth, the abaxial midrib is

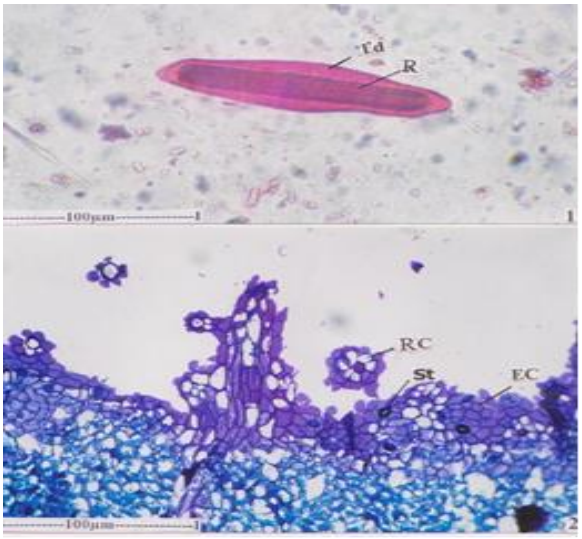

Fig 8 Powder microscopy
1 Raphide in the leaf powder,
2 Fragment of epidermal cells.
EC:Epidermal cells,Id: Idioblast,RC Rosette cells,R:Raphide,St:Stomata.

Fig 9 Trichome Morphology
9-1,2 Nonglandular covering trichome

1mm wide. The midrib consists of a single layer of small epidermal circular thin walled cells. The adaxial conical part and two or three layers of sub epidermal ground tissue have collenchymatous cells. The palisade tissue of the lamina extends up to the shoulders of the adaxial conical part. The inner ground tissue is parenchymatous and compact.

Vascular strands (Fig 2.2): There is an abaxial row of three vascular bundles, of which one is median and the other two are lateral bundles. There is an accessory adaxial bundle placed at the bottom of the adaxial cone. All the bundles are collateral, having one to three short radial rows of xylem elements and narrow strands of phloem. Xylem elements are narrow, circular and thin walled. A thick mass of sclerenchymatous cap is seen attached to the phloem of the vascular bundles.

Lamina (Fig 3:1,2): The lamina is 250µm thick. It has a wide epidermal layer consisting of cylindrical or spindle shaped thin walled cells on the adaxial side and narrow small cells on the abaxial side. The adaxial epidermis is 20µm thick while the abaxial epidermis is 10µm thick. The mesophyll is differentiated into an upper zone of palisade cells and lower spongy parenchyma. The palisade zone has two rows of short, narrow, compact rectangular cells. The spongy parenchyma has four or five layers of lobed cells with wide intercellular air spaces. Nonglandular, multicellular unbranched trichomes arise from a group of rosette of epidermal cells. When viewed under polarized light, thick bundles of pointed needles of calcium oxalate crystals called raphides are seen embedded in the mesophyll tissue (Fig 3.2).

Epidermal cells and stomata (Fig 4:1,2): The abaxial epidermal layer is densely stomatiferous .The stomata are of cyclocytic type. Each stomata has four or five subsidiary cells encircling the guard cells.The guard cells are circular, elliptical measuring 22 x 30 µm (Fig 4.2).The epidermal cells of the abaxial side are smaller, polyhedreal and straight walled. The adaxial epidermis has sparse stomata or is apostomatic. The epidermal cells are wider, polyhedral and have thick straight anticlinal walls. Thin lanimellate cuticular striations are seen in parallel lines within the cells. (Fig 5:1,2)

2. Petiolule (Fig 6.1,2): Petiolule stalk of the leaflet has two adaxial lateral wings and circular outline . It is 1.9 mm thick. It has a thin and distinct epidermal layer of small spindle shaped cells. The ground tissue is differentiated into outer collenchyma and inner parenchyma. Collenchyma is restricted to the adaxial wing and semicircular patches along the periphery (Fig 6.2).The parechymatous cells secrete copious amount of mucilage which is seen in large masses (Fig 6.1). A small nest of sclerenchyma cells is seen within the wings (Fig 6.2).The vascular system consists of about 15 discrete vascular bundles arranged in a ring. The adaxial vascular bundle is larger than the rest of the bundles. The vascular bundles are collateral; the bundles have wide, thick walled angular xylem elements, a thick arc of phloem and sclerenchyma caps (Fig 6.2).

3. Petiole (fig 7:1,2):The petiole, stalk of the compound leaf is circular in outline. The adaxial wings are reduced or absent. It is 2.2mm thick. The epidermal layer is thin and less prominent. The ground tissue is parenchymatous; the cells are large, circular or angular and compact. Thick patches of collenchymatous cells are seen beneath the ridged portions. There is a wide ring of about 15 vascular bundles of unequal size. The vascular bundles are collateral with sclerenchymatous cap. The xylem elements are wide, angular and thin walled (Fig 7.2)

Powder microscopy: The powdered material showed the following inclusions

i. Calcium oxalate crystals: Calcium oxalates in the form of raphides are abundant in the powders. They are thick, cylindrical bundles comprising of this pointed needles. The raphides are mostly associated with mucilage contents (Fig 8.1).

ii. Epidermal trichomes: Multicellular, uniseriate, unbranched, nonglandular trichomes are frequently seen in the powder. The trichomes are mostly curved; they have vertically oblong wide, uniwalled cells. The wall surface is smooth. The trichomes arise from an epidermal cell which is surrounded by a rosette of several cells. The trichomes tapers gradually into a pointed tip (Fig 9:1,2).

iii. Epidermal fragments (Fig 8.2).Epidermal cells from which the trichomes arises are surrounded by radiating rosette of cells. Stomata and normal epidermal cells (as seen in sectional view) are seen in the powder.

CONCLUSION

Anatomical characters are useful in the preliminary identification of herbarium material and they are also used to infer evolutionary trends in interrelationships of taxa or above species level [13]. According to Carlquist, [14] leaf is perhaps anatomically a most varied organ of angiosperms and it posses many anatomical features of taxonomic significance. The presence of narrow rectangular and thick walled epidermal cells, trichomes, vascular bundles connected to the abaxial epidermis in the lamina, petioles without wings etc. are very important characters. The combinations of these characters provide reliable characters for species identification. Herbarium materials can also be used for anatomical studies.

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