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Pharmacognostic studies of an ethnomedicinal plant: *Cissus latifolia* Lam

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Abstract

Cissus latifolia Lam. commonly called in Malayalam as Chunnambuvalli is a large climbing shrub belonging to the family Vitaceae. *Hortus malabaricus*, the book on medicinal plants of Western Ghats describes its use for the treatment of pleuritis, fever, cough, ulcers etc. by the traditional healers of Kerala. Owing to its ethnomedicinal importance, proper identification and evaluation is vital for drug development and to prevent adulteration. The present paper discusses the macroscopic, microscopic and powder microscopic study of *Cissus latifolia*. Since minimal data on the plant is available, the information gathered here will be highly valuable. This study also provides the information for proper identification and standardization of the drug.

Keywords: *Cissus latifolia* Lam., ethnomedicine, *Hortus malabaricus*, pharmacognosy

Introduction

Medicinal plants can play an important role in drug discovery. World Health Organisation (WHO) has estimated that in India about 65% of the population depends on traditional medicine for their primary healthcare needs [1]. Studies in the recent years are focussing on drug discovery from unexplored ethno botanical resources around the world.

Cissus latifolia Lam. commonly called in Malayalam as *Chunnambuvalli* is a climber belonging to the family Vitaceae that has ethnic significance. It is found in Peninsular India and Sri Lanka. In India, it is distributed in Kottayam, Kollam, Alappuzha, Idukki, Thrissur, Coimbatore, Madurai, Kanyakumari and Thirunelveli. It is a large climbing shrub; stem is thick and glaucous with leaf opposed tendrils. The tendril of the species shows a morphological difference as bearing two branches or three branches. Leaves are simple, alternate, 8 to 16 cm long, 6 to 13 cm wide, broadly ovate-cordate or orbicular with serrated margins. Flowers are very small, somewhat compact thyrsoid cymes, which are leaf opposed and greenish white in colour. Fruits are berry, 1 cm in diameter. First oblong-round, green; then round, black in colour, shiny, soft, filled with succulent, sticky flesh. One seed is present which is ash coloured and round. It is commonly seen flowering and fruiting throughout the rainy season [2].

In the family Vitaceae, *Cissus* genus consists of 350 species and is distributed throughout the tropical and temperate areas of the world [3]. Though the ethno medical studies of other species of *Cissus* genus viz. *Cissus quadrangularis*, *Cissus elongata*, *Cissus repens* have been conducted widely, fewer studies have been conducted on *Cissus latifolia*. *Hortus malabaricus*, the book on medicinal plants of Western Ghats has a detail description of the plant. The 7th volume of *Hortus malabaricus* describes the plant in detail and explains its therapeutic effects in fever, pleuritis, cough and ulcers [4]. Its descriptions are also found in some ethnobotanical studies [5]. Phytochemical investigations of the plant revealed the presence of flavonoids, tannins, alkaloids, steroids, saponins, quinines, anthraquinones and phenols [6]. *Cissus latifolia* Lam. and *Cissus quadrangularis* L. is used in the treatment of weak bones, bone fractures, cancer, scurvy, peptic ulcer disease, haemorrhoids, malaria, pain and asthma [7]. Dose-dependent cytotoxic and genoprotective effect of plant extract had been proved [8].

Owing to its ethnomedicinal importance, proper identification and evaluation is essential for drug development and to prevent adulteration. The present paper discusses the macroscopic and microscopic evaluation of *Cissus latifolia* Lam.

Materials and Methods**Collection and authentication of plant material**

Stem and leaf samples of *Cissus latifolia* Lam. were collected from Kerala Agricultural University Campus, Vellanikkara, Kerala.

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The samples were authenticated for its botanical identity by Dr V B Sreekumar, Scientist, Botany Department, Kerala Forest Research Institute, Peechi. A voucher specimen (18030) was also deposited in the institute.

Macroscopic and Microscopic study

The fresh stem and leaves of *Cissus latifolia* were visually examined. The organoleptic properties such as colour, odour and taste of the plant material were observed and recorded. For microscopic analysis, transverse section of stem, leaf and petiole were carried out as per standard procedure^[9, 10]. Both stem and leaf were dried and powdered for powder microscopy. Photographs of sections and powder analysis were made by using Olympus Microscope (Model CX 41; Tokyo, Japan) with CCD camera 2 mega pixel and measurements were taken using Olympus Image-Pro Plus, version 5.1 software. Epidermal peels were taken using standard procedure and were stained with Safranin and were mounted in glycerin. The number of stomata and epidermal cells were calculated per square millimetre of leaf area. Stomatal index was calculated using the formula $(S/S+E) \times 100$. S and E are the number of stomata and epidermal cells per unit area respectively^[9].

Results

Macroscopy of stem

Macroscopic features of stem are depicted in table 1 and figure 1.

Table 1: Macroscopic features of stem

Parameter	Observation
Shape	Cylindrical
Size	1 cm in diameter
Internodal distance	9-10 cm
Colour	Greenish
Surface	Glaucous
Texture	Smooth
Odour	Not characteristic
Taste	Slightly bitter
Fracture	Slightly fibrous

Macroscopy of leaf

Macroscopic features of leaf are depicted in table 2 and figure 1.

Table 2: Macroscopic features of leaf

Parameter	Observation
Type of leaf	Simple
Leaf arrangement	Alternate
Petiole/Non-Petiole	Petiolate
Stipule/Non-Stipule	Stipulate
Shape of lamina	Broadly ovate cordate orbicular
Size	Length : 8 - 16 cm Width : 6 - 13 cm
Apex	Acuminate
Base	Cordate
Margin	Serrate, serrations with bristly tip
Venation	Reticulate
Mid-rib	Flat on the upper surface, raised at the lower surface
Colour	Green
Texture	Adaxial surface: coarse Abaxial surface: slightly coarse
Odour	Non distinct
Taste	Slightly bitter

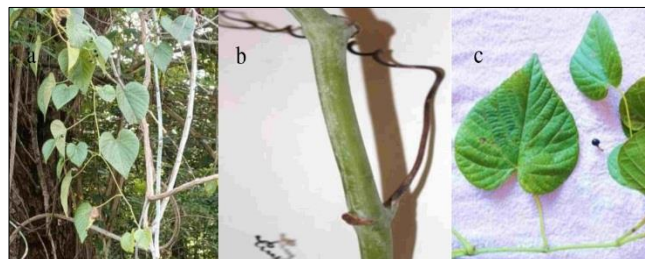


Fig 1: a- Habit & Habitat, b- Macroscopy of stem, c- Macroscopy of leaf

Microscopy of stem

Transverse section of stem is circular in shape. The epidermal cells are rectangular shaped, single layered and covered with a thin cuticle. Cortex consists of parenchyma, chlorenchyma, collenchyma and mucilaginous cells. Below epidermis there are 2-3 layers of parenchymatous cells followed by 2-4 layers of chlorenchymatous cells. Then 4-5 layers of collenchymatous cells are present followed by parenchymatous cells. Mucilaginous cells are frequently observed in the inner cortex. Calcium oxalate crystals like rosette crystals, raphides and acicular crystals are also present. Vascular bundle is collateral, open and endarch. Sclerenchymatous bundle cap is present above the phloem of each bundle. Inner to the phloem, cambium and 5-6 xylem vessels are noted. 6-10 rows of radially elongated medullary rays are seen between each vascular bundle. Pith is very large and consists of parenchymatous cells. Abundant starch grains which are oval shaped are also present in the cortex and pith. (Fig. 2)

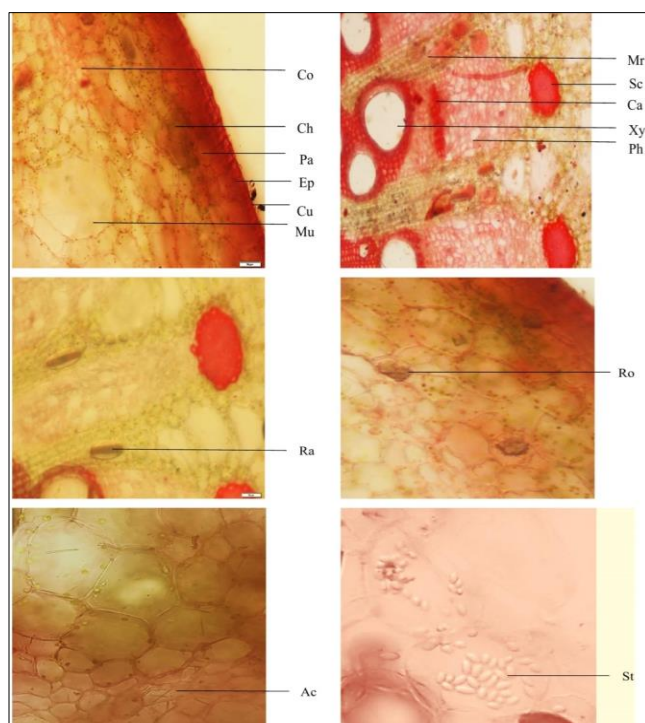


Fig 2: TS of stem of *Cissus latifolia*: Co-collenchyma, Ch-chlorenchyma, Pa-parenchyma, Ep-epidermis, Cu-cuticle, Mu-mucilaginous cells, Mr- medullary rays, Sc- sclerenchymatous bundle cap, Ca-cambium, Xy-xylem, Ph-phloem, Ra-raphide, Ro-rosette crystal, Ac-acicular crystal, St-starch grains

Microscopy of lamina

Transverse section of leaf lamina is dorsiventral consisting of upper and lower epidermal cells which are rectangular in shape and single layered. The mesophyll tissue is

differentiated into single layer of upper cylindrical palisade cells and 3-4 layers of lower spherical spongy parenchyma cells. Multicellular trichomes were present on upper epidermis. (Fig. 3a)

Determination of stomatal index

Stomata are anomocytic (irregular-celled) in nature. The stoma is surrounded by a definite number of cells that are not different from the remaining epidermal cells. Upper stomatal index was 7.86% and lower stomatal index was 9.79 %.(Fig. 3b)

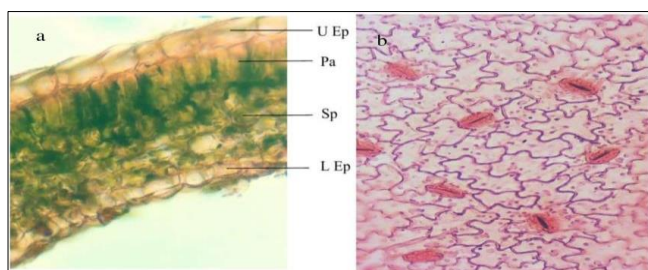


Fig. 3a: TS of lamina of *Cissus latifolia*: U Ep-upper epidermis, Pa-palisade mesophyll cells, Sp-spongy mesophyll cells, L Ep-lower epidermis;

Fig 3b: Stomata

Microscopy of midrib

Transverse section of midrib is broader on the abaxial side and has a small protuberance in the adaxial side. The midrib has an outer epidermis, followed by collenchyma zone, parenchymatous ground zone and centrally located vascular zone. Multicellular trichome and glandular trichomes are present. Cortex consists of 2-3 layers of outer collenchyma cells and a broad region of parenchyma. 5-6 centrally located vascular bundles are present which are collateral and endarch. Each vascular bundle consists of an outer phloem and an inner xylem. A sclerenchymatous cap is present above each vascular bundle. The central region is made up of sclerenchymatous cells. Calcium oxalate crystals like rosette crystals and acicular crystals are also present. (Fig. 4)

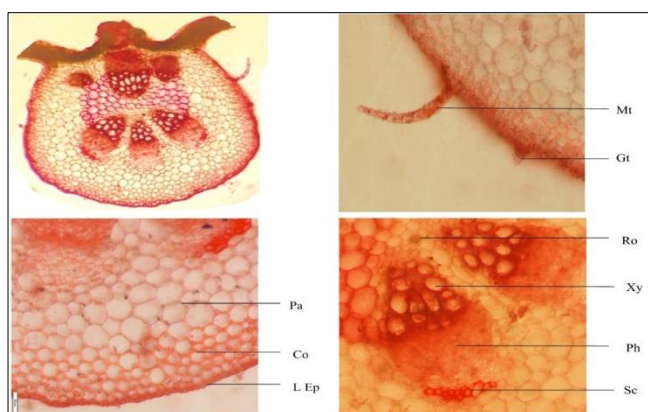


Fig 4: TS of leaf midrib of *Cissus latifolia*: Mt-multicellular trichome, Gt-glandular trichome, Pa-parenchyma, Co-collenchyma, L Ep-lower epidermis, Ro-rosette crystal, Xy-xylem, Ph-phloem, Sc-sclerenchymatous bundle cap.

Microscopy of petiole

Transverse section of petiole is circular in outline. The epidermal cells are single layered and covered with a thin cuticle. Cortex is made up of 2-3 layers of chlorenchymatous cells, 5-6 layers of collenchymatous cells followed by parenchymatous cells. Vascular bundles are endarch,

collateral and with a sclerenchymatous bundle cap. The inner ground tissue is made up of parenchymatous cells. Large sized mucilage cells, calcium oxalate crystals and starch grains are frequently observed in cortex and pith. (Fig. 5)

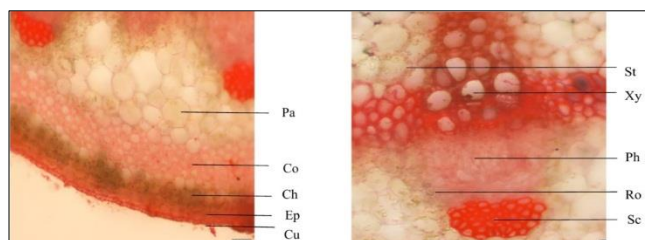


Fig 5: TS of petiole of *Cissus latifolia*: Pa-parenchyma, Co-collenchyma, Ch-chlorenchyma, Ep-epidermis, Cu-cuticle, St-starch grains, Xy-xylem, Ph-phloem, Ro-rosette crystal, Sc-sclerenchymatous bundle cap.

Powder microscopy

Powder microscopy of stem revealed abundant starch grains which were round or oval shaped. Many acicular crystals and raphides were seen. Presence of parenchyma cells which are spherical and in aggregates were also noted. Group of fibers, phloem fibers, spiral vessels were also seen (Fig. 6). Powder microscopy of leaf revealed the presence of acicular crystals, druse, prisms of calcium oxalate, group of sclereids and trichomes (Fig. 7).

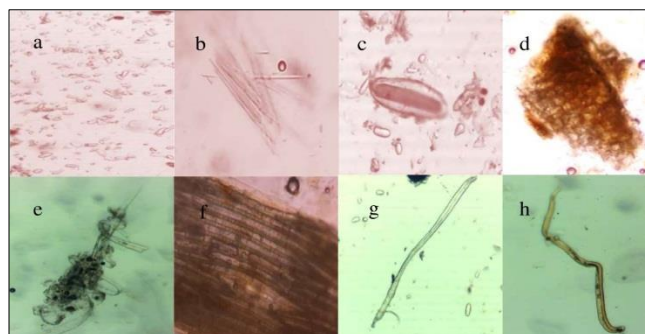


Fig. 6: Powder microscopy of stem: a-starch grains, b-acicular crystal, c-raphide, d-parenchyma cells, e-spiral vessels, f-group of fibres, g-fibre, h-phloem fibres

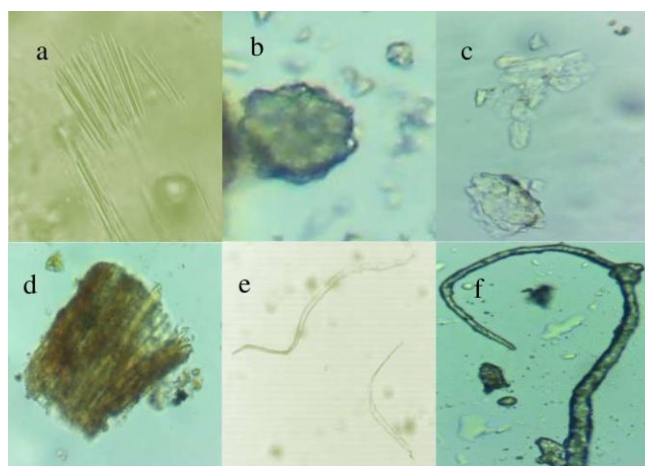


Fig. 7: Powder microscopy of leaf: a-acicular crystal, b-druse of calcium oxalate, c-prisms of calcium oxalate, d-group of sclereids, e-fibres, f-trichomes

Discussion

Although different species of *Cissus* genus have similar characteristics, *Cissus latifolia* can be identified by certain

diagnostic features. The glaucous and fibrous nature of stem, leaf opposed tendrils can be taken as a peculiar feature. Leaves are large, simple, arranged alternatively with cordate shape with serrations and acuminate apex.

Vitaceae family is characterized by the presence of raphides contained in sacs. Other crystals like acicular, rosette, prismatic crystals are also present [11]. Peculiar features of microscopy of stem are the presence of mucilaginous cells in inner cortex, collateral, open, endarch vascular bundles, calcium oxalate crystals like rosette, acicular and raphide crystals and abundant starch grains in pith region. Identifying features in microscopy of leaf are uniseriate multicellular trichomes, 5-6 vascular bundles and presence of acicular and rosette crystals. Presence of anomocytic stomata on both upper and lower epidermis can be taken as characteristic feature. Identifying features in powder microscopy are the presence of starch grains, abundant acicular crystals, raphides and druses of calcium oxalate.

Conclusion

The pharmacognostic standards of *Cissus latifolia* were determined and this will serve as the quality control parameters for its purity, identification and standardization.

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