

PHARMACOGNOSTIC STUDIES OF THE *CUSCUTA REFLEXA* ROXB AND *CASSYTHA FILIFORMIS* LINN

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ABSTRACT

The present study was carried out to establish the pharmacognostic characteristics of *Cuscuta reflexa* Roxb. and *Cassythia filiformis* Linn., two traditionally used medicinal plants. The investigation focused on the morphological and microscopic anatomical characteristics of the selected plant materials, which are important parameters for authentication and quality assessment of crude drugs. Macroscopic evaluation was performed with respect to the observable characters such as colour, shape, size, texture, surface, branching pattern, and other diagnostic features. Microscopic studies were conducted using transverse sections of the plant materials to investigate their internal anatomical organization and diagnostic tissue characteristics. The microscopic examination revealed characteristic features of the epidermis, cortex, vascular tissues, ground tissue, and other cellular structures, which can be useful in distinguishing the two species. The observed morphological and anatomical characters provide valuable reference data for the identification, authentication, and standardization of *C. reflexa* and *C. filiformis* and may support further pharmacognostic investigations of these medicinal plants.

KEYWORDS: *Cuscuta reflexa*; *Cassythia filiformis*; Pharmacognosy; Morphological evaluation; Microscopic anatomy.

INTRODUCTION

Most of the raw materials used by the herbal industry are procured from wild sources and this result in inconsistency in quality, adulteration and substitution. Hence, pharmacognostical evaluation of crude drugs is most important in herbal drug industry. Pharmacognostical studies include authentication, determination of foreign matter, organoleptic evaluation, macroscopic and microscopical examination, volatile oil content determination and evaluation of ash values and extractive values.

Crude drugs when supplied in intact form can be identified by morphological characteristics. The same can be investigated for histological characteristics to

confirm the identity of the supplied drugs. Microscopical techniques provide detailed information about the crude drugs by virtue of their two main analytical uses. Firstly, their property of magnification permits the fine structures of minute objects to be visualized and thereby confirm the structural details of the plant drugs under evaluation. Secondly, these techniques can be used in the determination of the optical as well as micro-chemical properties of the crude drug specimens under study. The powdered crude drugs can be identified based on the form, the presence or absence of different cell types based on their cytomorphological characters e.g. parenchyma, collenchyma, fibres, stone cells, trichomes, secretory cells, epidermal cells etc. The same may sometimes be achieved by evaluating the cell inclusion

characteristics for some unorganized crude drugs like starch grains, aleurone grains, gums, mucilage, calcium oxalate crystals etc.

Cuscuta reflexa Roxb. is a leafless, twining, parasitic medicinal plant belonging to the family Convolvulaceae. It is commonly known as giant dodder and is widely distributed in tropical and subtropical regions, including India. The plant is characterized by slender, yellowish-green to orange, thread-like stems that twine around host plants and obtain water and nutrients through specialized structures called haustoria. Leaves are highly reduced and inconspicuous, while the plant produces small, clustered flowers. Traditionally, *C. reflexa* has been used in various indigenous systems of medicine for managing conditions associated with hair, skin, inflammation, and other ailments. Its distinctive morphology makes it an important subject for pharmacognostic identification.^[1]

Cassytha filiformis Linn. is a slender, perennial, parasitic or semi-parasitic plant belonging to the family Lauraceae. Commonly known as love-vine or devil's guts, it is distributed in tropical and subtropical regions and is frequently found growing over shrubs and other vegetation. The plant possesses long, slender, green, thread-like stems that form dense tangled masses over host plants. Leaves are greatly reduced and scale-like, reflecting its parasitic habit. Small, inconspicuous flowers occur in clusters, followed by small globose fruits. The plant has a history of traditional medicinal use in several regions. Its characteristic external and internal anatomical features provide useful diagnostic parameters for pharmacognostic authentication and identification.^[2]

MATERIALS AND METHODS

Plant Anatomical Studies

(i) Preparation of specimens

The fresh, healthy samples were cut and removed from the plant and fixed in FAA (formalin 5 ml + acetic acid 5 ml + 70% ethyl alcohol 90 ml). After 24 hrs of fixing, the specimens were dehydrated with gradual series of TBA (tertiary butyl alcohol) as per the schedule given by Sass.^[3] Infiltration of the specimens was carried out by gradual addition of paraffin wax (m.p. 58-60°C) until TBA solution attained super saturation. The specimens were cast into paraffin blocks.

(ii) Sectioning

The paraffin embedded specimens were sectioned with the help of Rotary Microtome. The thickness of the sections was 10-12 µm. De-waxing of the sections was done by customary procedure given by Johansen.^[4] The sections were stained with Toluidine blue method of O'Brien.^[5] Since Toluidine blue is a polychromatic stain, the staining results were remarkably good; and some cytochemical reactions were also obtained. The dye rendered pink colour to the cellulose mass, blue to the lignified cells, dark green to suberin, violet to the mucilage, blue to the protein bodies etc. Wherever

necessary, sections were also stained with safranin and fast-green and potassium iodide (for starch).

For studying the stomatal morphology, venation pattern and trichome distribution, paradermal sections (sections taken parallel to the surface of leaf) as well as clearing of leaf with 5% sodium hydroxide or epidermal peeling by partial maceration employing Jeffrey's maceration fluid^[3] were prepared. Glycerin mounted temporary preparations were made for macerated / cleared materials. Powdered materials of different parts were cleared with sodium hydroxide and mounted in glycerine medium after staining. Different cell component were studied and measured.

Photomicrographs

Microscopic descriptions of tissues are supplemented with micrographs. Photographs of different magnifications were taken with Nikon labphoto 2 microscopic unit. For normal observations bright field was used. For the study of crystals, starch grains and lignified cells, polarized light was employed. Since these structures have birefringent property, under polarized light they appear bright against dark background. Magnifications of the figures are indicated by the scale-bars. Descriptive terms of the anatomical features are given as in the standard anatomy books.^[6]

RESULTS AND DISCUSSION

Pharmacognostical studies play an important role in the standardization of plant material. In the present study, two plants were selected based on their ethnomedical uses and was authenticated. Their morphological and microscopical characters were determined. A detailed study of microscopic characters of the two plants along with the photographs serves in identifying the special characters of the plants.

(i) *Cuscuta reflexa* Roxb

Macroscopic Characters

The genus *Cuscuta* Linn is a leafless, twining parasitic Vine. The branchlets are much branched, glabrous, yellowish (achlorophyllous) and slender. According to Gamble there are three species in South India. Henry recorded four species in Tamil Nadu, South India. *Cuscuta reflexa* Roxb. can be differentiated on morphological grounds by the following features.

The plant is a parasitic vine with soft, thick, branched, yellow coloured stem. The plant selected for the present study, is a parasite growing on the host *Morinda Pubescences*. It may also be self-parasitic on its own stem. The flowers are subsessile racemes and creamy white in colour. Calyx is gamosepalous, five lobed and thick. Corolla has five petals, white or cream coloured, gamopetalous, tubular campanulate. Stamens are five, epipetalous. Ovary has two stout styles which are divergent. Fruit is a succulent capsule.

Microscopic Features

Microscopic profile of young stem

The stem varies in thickness from 700 μm to 2 mm. The thin stem is roughly circular in outline with shallow ridges (Fig. 1). It consists of a continuous fairly thick epidermal layer of 30 μm thick; the cells are rectangular with prominent cuticle.

The cortex is 150 μm wide comprising of 4-6 layers of wide, angular, thin walled compact parenchyma cells.

The pith is wide, parenchymatous, the cells being thin walled, angular and compact. The pith is 400 μm wide.

The stele (vascular tissues) consists of about eight small, discrete collateral vascular bundles which occur in a ring. Each bundle has four to eight xylem elements which are thick walled, lignified and angular in outline, they are 20-40 μm wide; phloem occurs in small nests on the outer part of the xylem mass (Fig. 2).

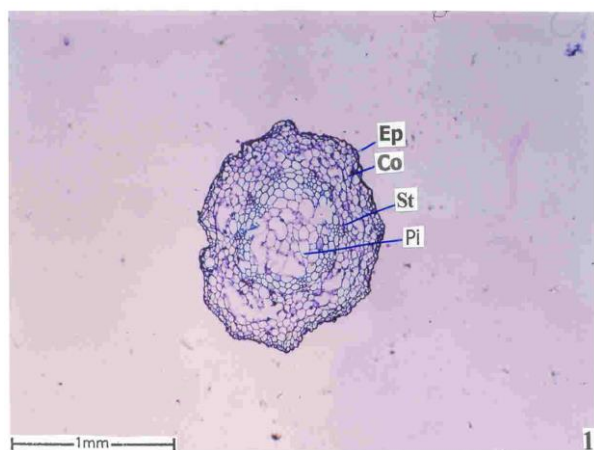


Fig. 1 TS of young (thin) stem - entire view.

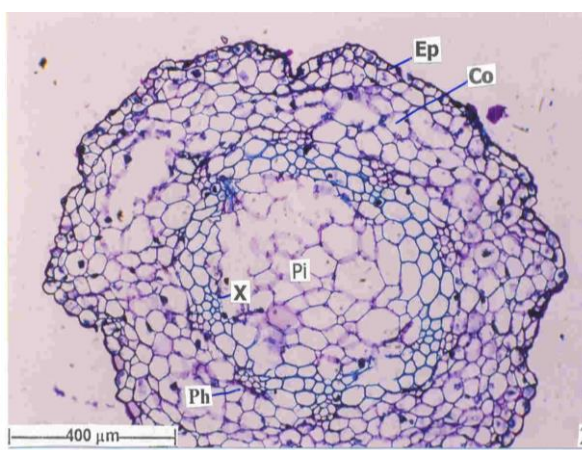


Fig. 2 Enlarged section of the stem.

(Co-Cortex; Ep-Epidermis; Ph-Phloem; X-Xylem; St-Stele; Pi-Pith)

Microscopic profile of thick (old) stem

Thick stem differs from a thin stem in some essential features. The thick stem is also circular in transectional view with irregular ridges and furrows. The epidermis is 30-40 μm thick; the epidermal cells are barrel shaped with thick cuticle. The cortex is 240 μm wide with six to eight layers of thin walled, compact, angular parenchyma cells (Fig. 3). Three layers inner to the epidermis there is a thin, circular line of crushed cells. The pith is 800 μm wide and the cells are large, thin walled, angular and compact (Fig. 4).

The vascular system consists of a thin closed cylinder formed by 10-12 small collateral vascular strands which are interlinked by a thin sclerenchyma cylinder (Fig. 4). Each vascular bundle has a small cone of xylem elements and fairly prominent mass of phloem elements.

The xylem elements are narrow, angular, thin walled and compact. The interconnecting sclerenchyma band has tangentially oblong thin walled, wide, lignified cells.

Cell inclusions such as starch grains or calcium oxalate crystals are not evident in the cells.

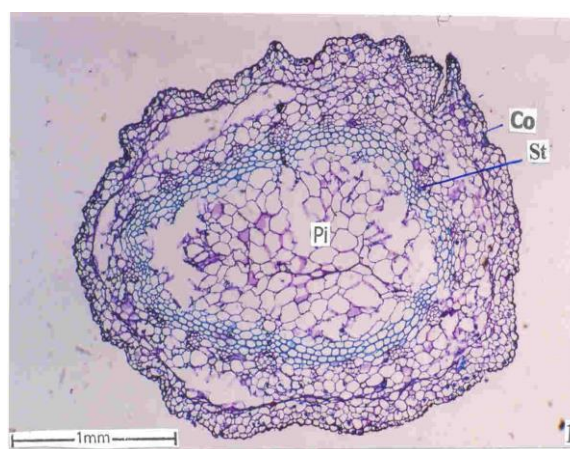


Fig. 3: TS of the stem - ground plan.

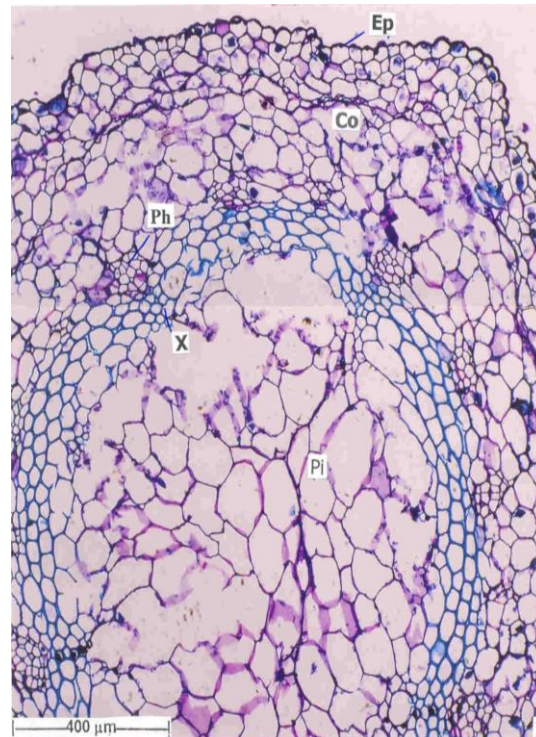


Fig. 4: Enlarged section of the stem.

The stem twines over other plants developing haustoria to derive the nutrition from the host plant. Sometimes the stem may twine with other branch of same *Cuscuta* plant forming what is known as “self-parasitism.” The stem that functions as the parasite develops a broad cup shaped portion with which it clasps over the other branch which acts as the ‘host’. The epidermis of the clasping part of the parasitic branch becomes much wider, the cells elongate considerably into a compact palisade like

zone (Fig. 5, 6). The cortical cells of the parasitic branch develop radially elongated bundle of **haustorium** which penetrates into the cortex of the host branch which later traces into the vascular tissues. The haustorial cells are darkly staining.

The vascular system of the stem that acts as the host is well developed; the vascular bundles are prominent with thick walled larger cluster of xylem and phloem (Fig. 6).

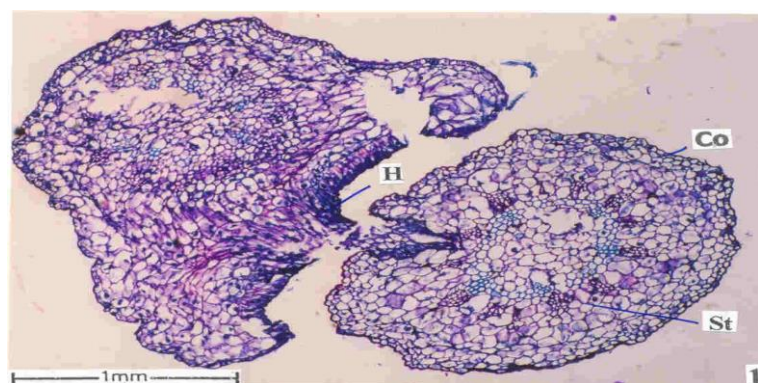


Fig. 5: TS of host stem and parasitic stem.

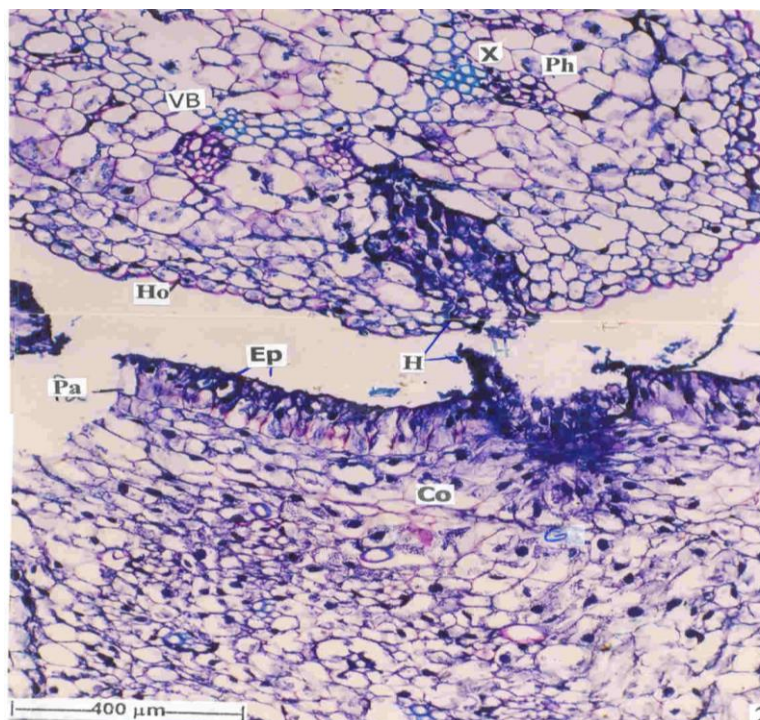


Fig. 6: Haustorium between host and parasitic stems.

(Co-Cortex; Ep-Epidermis; Ph-Phloem; X-Xylem; St-Stele; Vb-Vascular bundle; Ho-Host stem; Pa-Parasitic stem; H-Haustorium)

When *Cuscuta* is parasitic on other host plants of different species, the **haustorium** is well organized comprising of tracheary elements (xylem elements) (Fig.

7). The haustorium penetrates the cortex and reaches the xylem cylinder of the host plant. The cortical tissue of the host stem develops thick pad of sclerenchyma around the haustorium. The haustorium and the host tissue develop organic connection so that the parasite gets flow of nutrients from the host tissue.

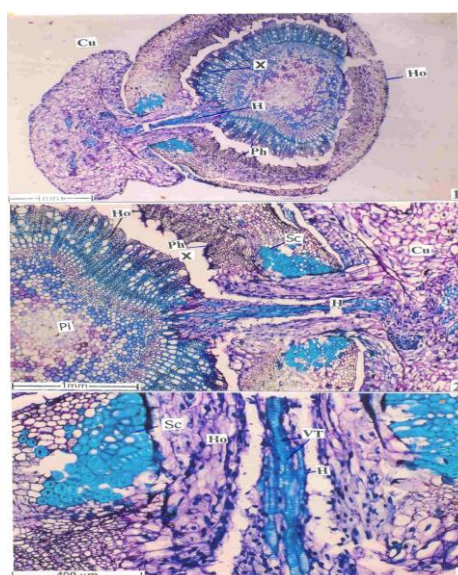


Fig. 7: *Cuscuta* parasiting on *Morinda* in TS view.

Salient Diagnostic Features on Microscopic Characters

- The young stem has a single layer of distinct epidermis, narrow zone of parenchymatous cortex, wide parenchymatous pith and a ring of discrete vascular bundles.

- The xylem elements are not prominent; they are narrow, thin walled and reduced in number. Phloem is comparatively well developed.
- In a thick stem, a thin ring of collapsed cells is seen in the cortex.

- The stele consists of more number of vascular bundles which are fairly prominent, collateral and closed.
- No secondary growth is evident.
- The vascular bundles are interconnected by a thin continuous cylinder of sclerenchyma cells, so that the stele appears as a closed cylinder.
- Cell inclusions such as crystals and starch grains are lacking.

The microscopic characters of *Cuscuta* revealed that the following features are identified as anatomical markers for authentication of this species.

(ii) *Cassytha filiformis* Linn

Macroscopic characters

Leafless twiner. Spikes terminal or axillary; peduncle 2-4.5 cm; bracteoles 3, ovate, 1.2 mm, ciliate. Flowers (sub) sessile, in spikes, 3-merous, bisexual, 3 mm across. Tepals 6, free, unequal, 3+3, truncate, obtuse; outer lobes ovate-orbicular, 1.2 mm, ciliate; inner lobes obovate, 2.5 mm. Fertile stamens 9; filaments 1.5 mm; glands sessile;

anthers 2-celled, 1.5 mm; staminodes 3 to 1 mm. Ovary 1.5 mm; style 0.5 mm; stigma capitate. Drupe globose, enclosed within inflated perianth, crowned by lobes; seed 1, globose, conform to fruit: testa thin.

Microscopical features

The stem is circular in cross sectional view with shallow ridges and furrows. The epidermal layer has small squarish cells and thick cuticle. The epidermis is stomatiferous with sunken stomata.

The cortex consists of two or three layers of small, circular air-chambers separated by radially elongated cells of the partitions. The cortex is 200 µm wide. Epidermal layer is 40 µm thick; the cuticle is 10 µm thick.

There is a thin layer of gelatinous fibers along the inner boundary of the cortex. The fibers are either in small discontinuous masses or in thin continuous cylinder.

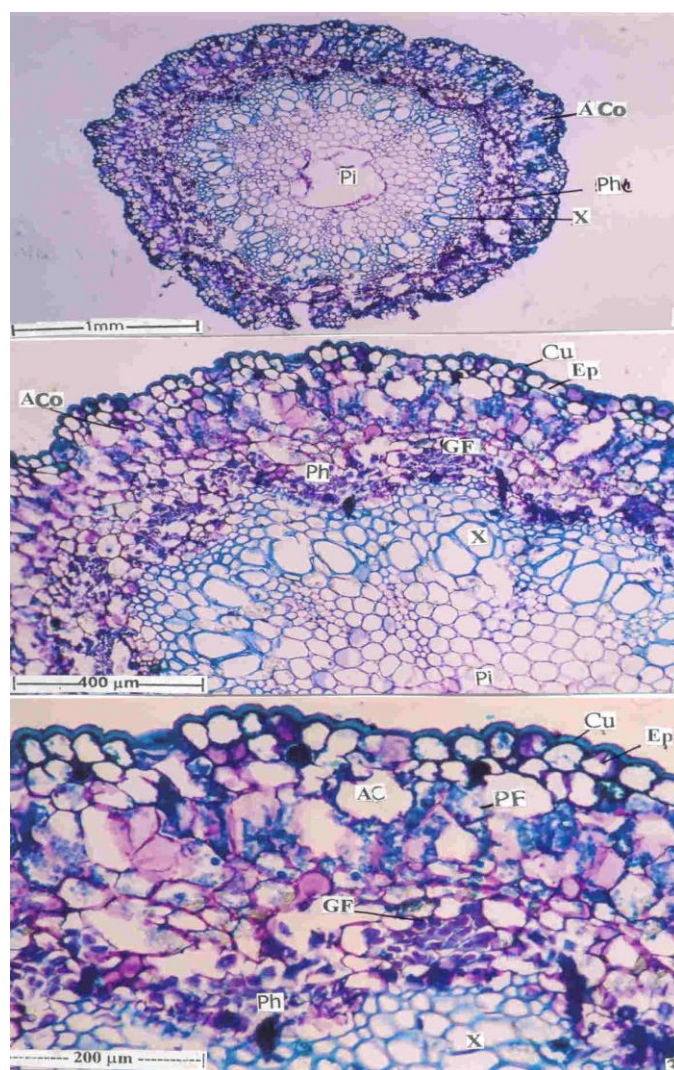


Fig. 8: TS of stem - *Cassytha filiformis*.

(Ac-Air chamber; Aco-Aerenchymatous cortex; Cu-Cuticle; Ep-Epidermis; Gf-Gelatinous fibers; Ph-Phloem; Pi-Pith; X-Xylem)

The stele consists of a continuous thin cylinder of xylem with external phloem. The xylem elements are wide, thin walled and angular in outline. The meta xylem elements are 50-60 μm wide. Phloem elements are in small nests.

Pith is wide and has a central narrow canal and outer intact parenchyma cells. The outer pith cells are wide, thin walled and compact. Starch grains and calcium oxalate crystals are abundant in outer pith cells. The crystals are prismatic type. The starch grains are circular, simple, concentric and have central hilum.

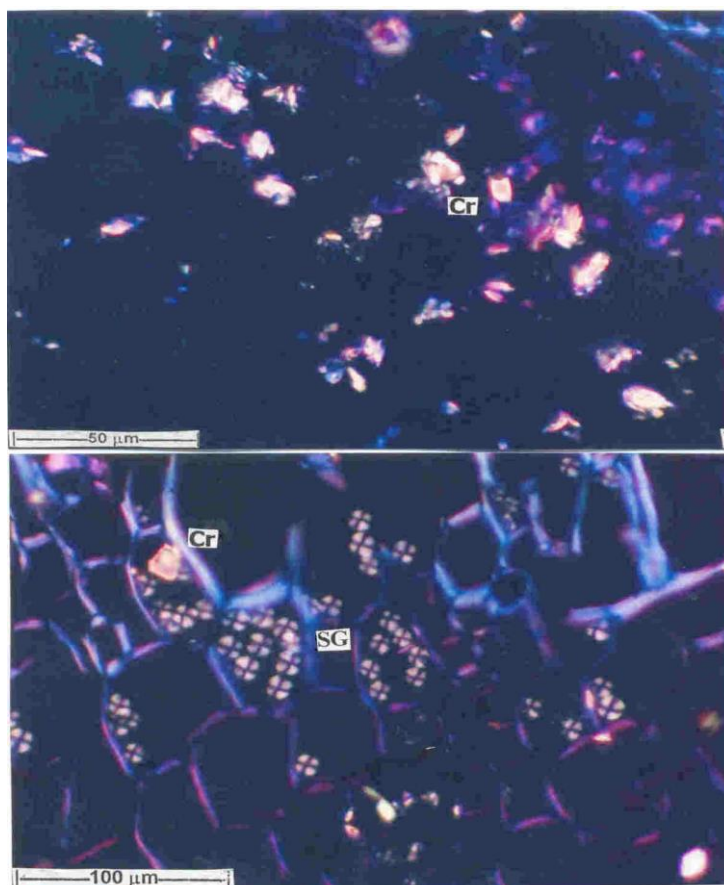


Fig. 9: Calcium oxalate prismatic crystals in the cortical cells; Concentric starch grains in the ground parenchyma cells.

(Cr-Crystals of prismatic type; SG-Starch grains with “+” mark due to double refringent nature of the starch grains)

The results obtained in pharmacognostical studies reported, herein established the macroscopic and microscopic parameters that characterize the genuine plant drug *Cuscuta reflexa* and *Cassythia filiformis*. These morphological characteristics can be utilized for quick identification of the drug and are particularly useful in the case of powdered materials¹²⁴.

CONCLUSION

The present pharmacognostic investigation established the characteristic morphological and microscopic features of *Cuscuta reflexa* Roxb. and *Cassythia filiformis* Linn. The macroscopic characteristics, together with the diagnostic anatomical features observed through microscopic examination, provide useful parameters for the identification and authentication of these medicinal plants. The distinctive structural features observed in the

investigated plant materials can help differentiate the two species and support the detection of possible adulteration or substitution. These findings contribute valuable baseline information for pharmacognostic standardization and quality assessment. Further studies involving physicochemical evaluation, phytochemical profiling, and pharmacological investigations may provide additional scientific evidence regarding their medicinal significance.

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