



Research Article

COMPARATIVE MORPHO-ANATOMICAL AND PRELIMINARY PHYTOCHEMICAL STUDIES OF *CUSCUTA REFLEXA* AND *CASSYTHA FILIFORMIS*

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ABSTRACT

The Morpho-anatomical characteristics of the stem of *Cuscuta reflexa* and *Cassytha filiformis* were investigated to ascertain the relevance of these characters in establishment similarities and differences in these two taxa. Transverse section of both plant were prepared with hydrochloric phloroglucinol to reveal lignified element. The morphological features of stem are studied in detail. In anatomical studies, transverse section and macerated tissue has been examined. From present evaluation it is evident that certain characters such as color of stem, stomata in epidermal layer, numbers of vascular bundles, pith, cortex and xylem vessels can provide useful information for differentiation of *Cuscuta reflexa* from *Cassytha filiformis*. In preliminary phytochemical evaluation, different solvents systems were tried for developing a TLC system for comparative study of *Cuscuta reflexa* and *Cassytha filiformis*.

Keywords: Morphology, anatomy, *Cuscuta reflexa*, *Cassytha filiformis*, TLC

INTRODUCTION

The plant *Cuscuta reflexa* Roxb. Coron is a perennial, parasitic herb of Convolvulaceae family, commonly known as Akashbela in Hindi. The plant is distributed throughout India, Ceylon and Malaya. The *Cuscuta reflexa* has been investigated for antispasmodic, hemodynamic, bradycardia¹, antisteroidogenic², antihypertensive, muscle relaxant, cardiotoxic³, psychopharmacological⁴ and antiviral and anticonvulsant⁵ activities. Many chemical constituents have been isolated from *Cuscuta reflexa* such as, cuscutin, amarbelin, beta-sterol, stigmaterol, kaempferol, dulcitol, myricetin, quercetin, coumarin and oleanolic acid⁶

Cassytha filiformis Linn., is perennial, parasitic, herbaceous and leafless plant belonging to family Lauraceae. This plant is distributed throughout India and used medicinally in China, Indochina, Madagascar and South Africa. *Cassytha filiformis* is medicinally used as antiplatelet, vesorelaxant⁷, alpha-adrenoreceptor antagonist⁸ and antitrypanosomal⁹. Some of the isolated compounds from this plant are aporphine alkaloid, oxo-aporphine alkaloid, cassyformine, filiformine, cathaformine, lignan, actinodophine, and octenine¹⁰

In ayurveda, *Cassytha filiformis* is used as substitute for *Cuscuta reflexa*¹¹. Literatures regarding anatomical and comparative phytochemical details are not available to distinguish *Cuscuta reflexa* from *Cassytha filiformis*, hence it was decided to establish the morpho-anatomical characters and comparative phytochemical studies of these two plants.

MATERIAL AND METHODS

Plant material

The areal part of *Cuscuta reflexa* was collected from the field of Charthaval, U. P. and authenticated by Mr. Mohan Kumar, professor, Dept of Botany, R.K.P.G.College, Shamli, U.P. The areal part of *Cassytha filiformis* was collected from the field of Tirupati, Andhra Pradesh and authenticated by Dr.K.Madhva Chetty, Dept of Botany, Venkateswara University, Tirupati. Herbarium specimen of both plants *Cuscuta reflexa* and *Cassytha filiformis* were deposited in the dept. of Pharmacognosy (Voucher specimen No. NCP/08-08/002 and NCP/08-09/003).

Anatomical studies

For histochemical reaction the transverse section of stem of both plants were prepared on treatment with different chemical reagents. This was carried out using the standard technique of clearing in chloral hydrate, treatment with hydrochloric phloroglucinol to reveal lignified elements for surface studies and microscopical observation. For detailed description, the stem of both plants were macerated with the mixture of 10% chromic acid and 10% nitric acid by conventional Jeffery's method¹². The presence or absence of features like cork cells, starch sheath, scleroids, vascular bundles, fibers, stomata calcium oxalate crystal as well as xylem vessels were observed.

Optimization of TLC solvent system

The powdered plant material of both plants extracted with 70% ethanol in soxhlet apparatus separately for 2 hr, filtered and concentrated to complete dryness. The marc obtained from ethanolic extract was subjected to

maceration in chloroform water for 12 hr, filtered and concentrated to complete dryness. Different solvents systems were tried for developing a TLC system for comparative study of *Cuscuta reflexa* and *Cassytha filiformis*. Solvents systems were tried for identification of constituents in the alcoholic and aqueous extracts of both plants based on the literature survey and the one showing maximum separation was selected as mobile phase for the study. The solvent system Toluene: Ethyl acetate: Glacial acetic acid (5:7:0.1 ml) was used for the development of the TLC system

The extract were dissolved in respective solvents and then spotted on the pre-coated silica gel G F-254 plates with the help of fused capillary tubes. TLC plates were developed in suitable solvent system. Spots were identified under UV-light at 365nm and Iodine chamber.

RESULTS AND DISCUSSION

Morphological details

Stem of *Cuscuta reflexa* is very long, rather stout, closely twining, branched, glabrous, pale greenish yellow, sometimes dotted with red. The flowers are small, about 2 mm in diameter and are borne singly along the stem. The fruit is dry, spherical, thin-shelled structure containing several small black seeds. The stem powder is very bitter in taste.

Stem of *Cassytha filiformis* is green to orange, filiform, and glabrous. Leaves are reduced to minute Scale 1mm long, near the tips of stem. Flowers are sessile and borne in small panicles. The fruit is small, fleshy, and berry-like, bearing a single, spherical seed. The stem powder is slightly bitter in taste (Fig. 1 and 2).

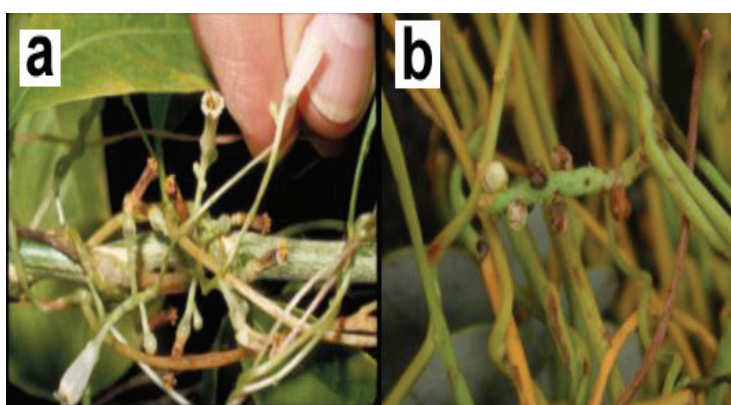


Fig. 1: a) Field photograph of *Cuscuta reflexa* b) Field photograph of *Cassytha filiformis*

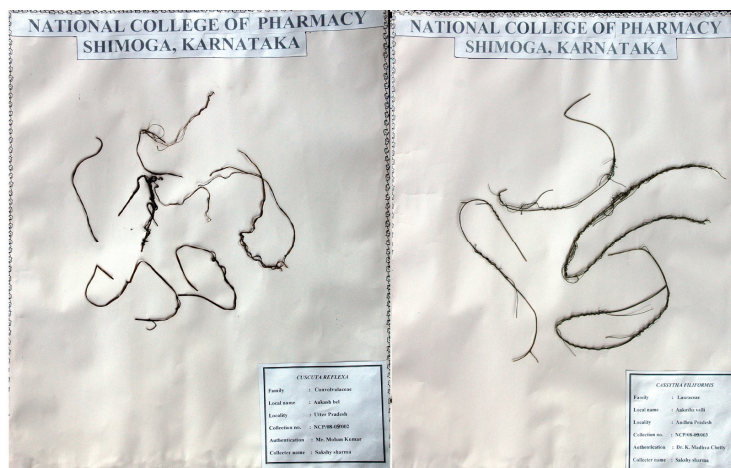


Fig. 2: a) Herbarium sheath of *C. reflexa* b) Herbarium sheath of *C. filiformis*

Anatomical characters

***Cuscuta reflexa*:** Transverse section taken from the middle part of the stem was observed: the epidermis is composed of single layer of paranchymatous cells. The paranchymatous cork tissue located immediately under the epidermis, is 7-10 layered. Stellar region start with starch sheath, but not very distinct. Conjoint,

collateral and open type vascular bundles are arranged in a ring, consisting about 8-10 in numbers. Pith relatively large and made up of paranchymatous tissue. Results are given in Fig-3 and (Table-1)

***Cassytha filiformis*:** the epidermis is composed of single layer of paranchymatous cells with stomata. The paranchymatous cork tissue located immediately

under the epidermis, is 4-7 layered. Stellar region start with starch sheath. Conjoint, collateral and open type vascular bundles arranged in a ring, consisting about

13-15 in numbers. Pith relatively small and made up of paranchymatous tissue. Results are given in Fig-3 and (Table-1)

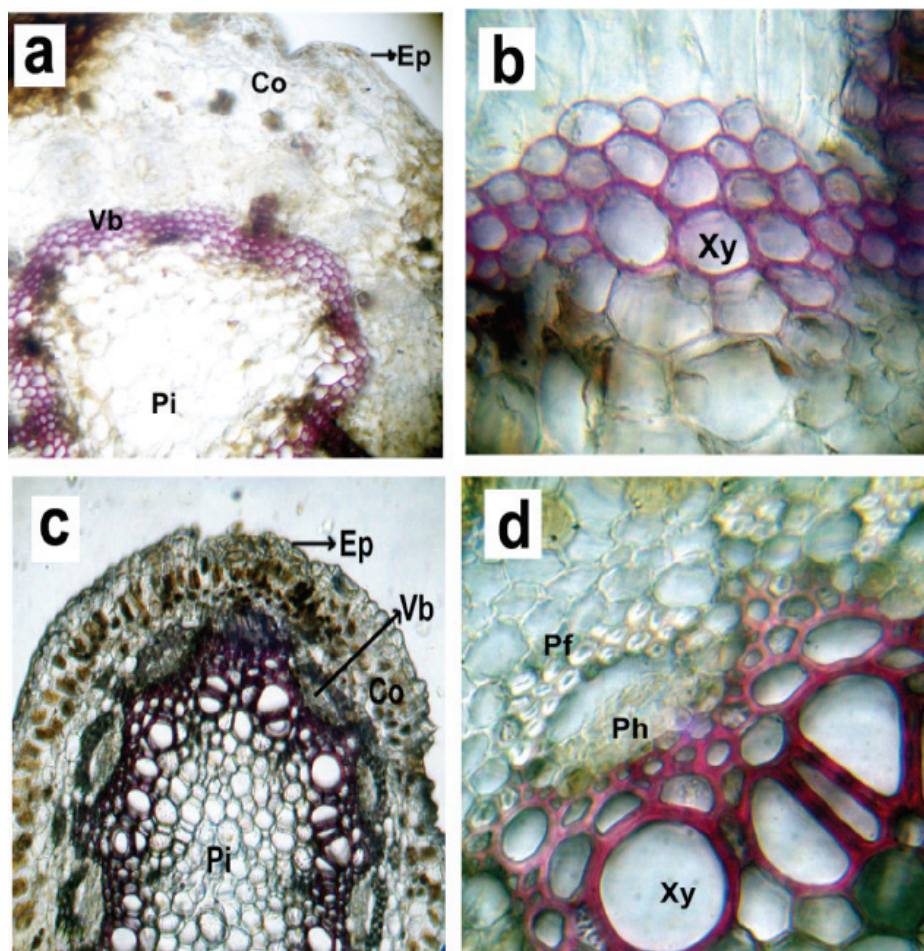


Fig. 3: a) T.S of *Cuscuta reflexa* b) Xylem vessel of *Cuscuta reflexa* c) T.S of *Cassytha filiformis* d) Xylem vessel of *Cassytha filiformis*. Ep-epidermis, Co-cortex, Vb-vascular bundles, Xy-xylem, Ph-phloem, Pf-pericyclic fiber, Pi-pith.

Table 1: Anatomical details of the stem of *Cuscuta reflexa* and *Cassytha filiformis*

Characters	<i>Cuscuta reflexa</i>	<i>Cassytha filiformis</i>
Epidermis	Uniseriate, paranchymatous	Uniseriate with stomata
Thickness	6-12µm	3-9µm
Cortical region	paranchymatous	paranchymatous
Thickness	90-129µm	30-60µm
Cork	7-10 layered	4-7 layered
Stellar region	conjoint, collateral and open type, 8-10 in numbers	conjoint, collateral and open type, 13-15 in numbers
Vascular bundles	27-45µm	24-48µm
Thickness	reticulate, spiral	pitted, spiral
Xylem vessel	paranchymatous	paranchymatous
Pith	90-126µm	72-90µm
Thickness		

In macerated tissue, the length and breadth of fibers was found to be almost similar but the vessels in *Cuscuta reflexa* found to be thin and long comparatively in *Cassytha filiformis*. The major

distinguish character of *Cassytha filiformis* is the presence of stomata where as no stomata in *Cuscuta reflexa*. , results are compiled in Fig-4, 5 (Table-2)

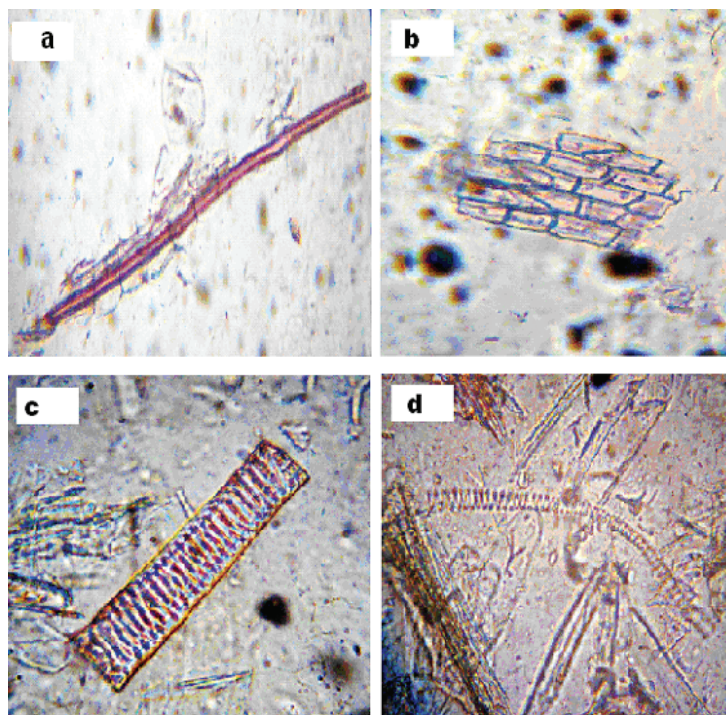


Fig.4: Macerated stem of *Cuscuta reflexa* a) fiber b) epidermal cell c) reticulate xylem vessel d) spiral xylem vessel

Table 2: Details of macerated tissue of *Cuscuta reflexa* and *Cassytha filiformis*

Macerated tissue	<i>Cuscuta reflexa</i>		<i>Cassytha filiformis</i>	
	Length (µm)	Breadth (µm)	Length (µm)	Breadth (µm)
Fibers	105-120	1.5-6	72-129	1.5-4.5
Vessels	261-345	3-15	93-111	6-15

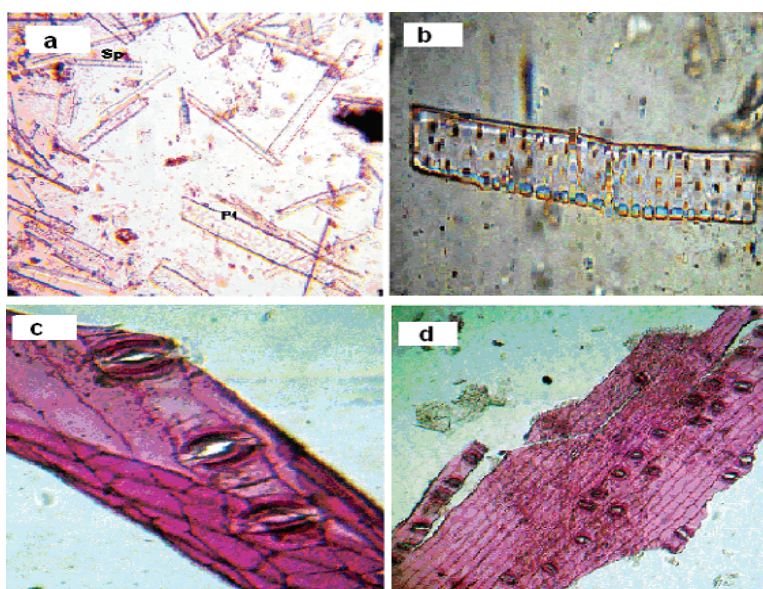


Fig. 5 Macerated stem of *Cassytha filiformis* a) spiral xylem vessel b) pitted xylem vessel c) stomata d) epidermal cell Sp-spiral, Pt-pitted

TLC analysis

In the TLC evaluation, the various solvent systems tried for both plant extracts. Among of them the solvent system, Toluene: ethylacetate: glacial acetic

acid (5:7:0.1) was shown maximum separation. In comparative TLC, the both extract of *Cuscuta reflexa* was shown good separation where as none of extract of *Cassytha filiformis* shown separation, details are given in Fig 6 and (Table-3)

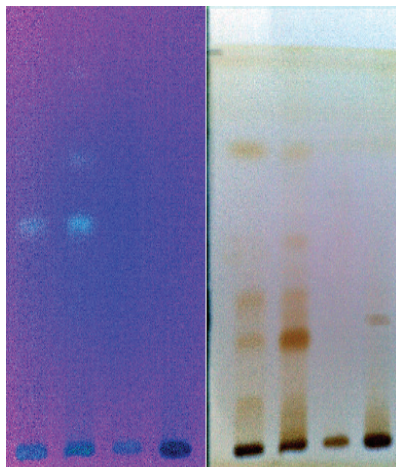


Fig.6 TLC- profile a) Extract in UV light and b) After Exposing to iodine vapors

1. CRAE, 2.CREE, 3.CFAE, 4.CFEE

Table-3: TLC analysis of *Cuscuta reflexa* and *Cassytha filiformis*

Extracts	Spot no.	R _f value
<i>Cuscuta reflexa</i> (ethanolic extract)	1	0.15
	2	0.32
	3	0.35
	4	0.4
	5	0.55
	6	0.76
<i>Cuscuta reflexa</i> (aqueous extract)	1	0.15
	2	0.29
	3	0.4
	4	0.52
	5	0.76
<i>Cassytha filiformis</i> (ethanolic extract)	1	0.38
<i>Cassytha filiformis</i> (aqueous extract)	-	-

DISCUSSION

In general the resemblance of the external morphology of the stem of all different population is obvious. In all the population studied, the stem height was between 40-70cm and a basal ligneous part is clearly developed and reaches the length of up to 30cm, however slight difference could be observed between the population groups growing in very different ecological condition.

In ayurveda, *Cassytha filiformis* is used as substitute for *Cuscuta reflexa*¹¹. These two plants can be distinguished based on the floral arrangement, but due to almost same physical appearance it becomes

difficult to distinguish them in dried condition. Hence diagnostic characters are evolved based on morphological and anatomical characters of the drug.

Morphologically the plant stem of *Cuscuta reflexa* is very long, pale greenish yellow, sometimes dotted with red. Where as stem of *Cassytha filiformis* is green to orange, filiform and glabrous.

In *Cuscuta reflexa*, no stomata in epidermal layer and cortex were found to be large compare to *Cassytha filiformis*. The another distinguish character is the area of vessels, which are thin and long comparatively in *Cassytha filiformis* and the pattern of vascular strips,

here the no. of vascular bundles are 8-10 and pith is relatively small than *Cassythia filiformis*.

Cassythia filiformis can be identified by the presence of stomata in epidermal layer, cortex relatively small than *Cuscuta reflexa*, vascular bundles consisting about 13-15, phloem are very distinct. Xylem vessels and pith are very large compare to *Cuscuta reflexa*.

In preliminary phytochemical evaluation the comparative TLC analysis is a good criteria for distinguishing two plants *Cuscuta reflexa* and *Cassythia filiformis*.

These data are required for the identification procedure to meet the quality standard. Even though some sophisticated chemical and molecular method are available for identification of the plant material. Morphological and anatomical identification is simplest among the qualitative methods. Hence it becomes a useful tool to determine characteristics of the plant material to avoid falsification and adulteration of the drugs.

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