

Macro and micromorphological study of *Canscora decurrens* Dalz. Found in Deolapar area near Nagpur Maharashtra

V Yadav

Department of Botany, Hislop College, civil lines, Nagpur India

Abstract

Macro and micromorphology study of *C. decurrens* was conducted with a view to elucidating their taxonomic significance and presenting complementary data to aid in the identification of the plant. Macromorphological characterization includes study of visible characters (habit, branching, colour, shape, texture of stem and leaves, root morphology) and reproductive characters namely inflorescence, morphology of different parts of flowers and pollen grain and seeds while micromorphology studies included anatomical details of stem, leaf and root as well as structure of stomata and trichome which are stable characteristic features of the given species and hence they help in the identification of plant at microscopic level.

Keywords: *Canscora decurrens*, Gentianaceae Macromorphology, micromorphology, ethanobotany

1. Introduction

Genus *Canscora decurrens* (2n =38) (Syn. of *C. diffusa*) belongs to family *Gentianaceae*. This genus is included in the famous 'Shankpushpi' group of plants including *Convolvulus microphyllus*, *Evolvulus alsinoides* and *Clitorea ternatea* which are used as ingredient in formulation used to improve intelligence, memory and other higher mental function [8, 13]. It is such a potential medicinal plant known to cure large number of disorders of central nervous system. They are not only used to boost memory and intellect but they show analgesic, anxiolytic, antidepressant, antistress, antioxidant, immunomodulatory activities etc [2]. Although its medicinal uses are well documented in old literature [4]. Identification and description of exact taxonomic status of *Canscora* plant created lots of confusion. This plant being an ethanobotanical medicinal plant, its references are found from ancient period. With renewed interest in herbal medicine, different species of this plant have been worked out by modern pharmacist also. The practices of traditional medicine predate the development and spread of modern medicine. Medicinal plants are moving from periphery to main stream [15]. However a key obstacle in both, traditional plants and their acceptance in modern medicine is the lack of scientific documentation and stringent quality control. It becomes extremely important to make judicious efforts towards standardization of desired plant material [3]. The first step of taxonomic identification is the most critical task which is based on the study of cognitive characters like, structure, shape, colour, size and other morphological features as well as anatomical details of vegetative and reproductive parts of plants [16]. Therefore in this investigation various studies were undertaken which helped the identification and authentication of plant material. In the present study of habit, branching, colour, shape, texture of stem and leaves, root morphology and reproductive characters namely inflorescence, morphology of different parts of flower, pollen grain and seeds included morphological description and measurement of all plant

parts which are seen by naked eye and dissecting, or compound microscope [11]. Anatomical studies include detailed give information regarding tissue and its distribution in stem, leaf, root etc [6].

2. Material and Methods

1. Collection of plant material

Preliminary survey in local areas indicated that *C. decurrens* is available in wild condition around Nagpur. Mature and entire (stem, leaf, root, flower and fruit) plants of *C. decurrens* were collected in the month of March from Deolapar area near Nagpur, Maharashtra. The plant was identified and authenticated from Department of Botany, Rashtrasant Tukadoji Maharaj Nagpur University and deposited bearing voucher numbers 1044, 2470, 973.

2. Processing of plant material

1. Entire plants were collected and thoroughly cleaned to remove soil, and dust spoiled and decaying leaves and processed for following studies.
2. Fresh plants were used for study of structural attributes of leaf, stem, roots, flower, inflorescence and fruit. Anatomical studies were carried out by entire mature plants were fixed suitably in Formalin: Glacial acetic acid: Absolute alcohol (5:5:90) for 24hrs, transferred and preserved in 70% alcohol and used for further studies.

3. Macromorphology

Detailed study of visible characters like habit, branching, colour, shape, texture of stem and leaves, root morphology and reproductive characters namely inflorescence, morphology of different parts of flower, pollen grain and seeds.

4. Micromorphology

Micromorphological studies involved following investigation:

1. Leaf: Type of stomata and trichomes were studied. For that, preserved leaves were dechlorophyllised by 10% Potassium hydroxid (KOH) which rendered transparency to the leaves. Epidermal layers were peeled off with the help of forceps and stained with saffranin (1%) and temporary slides were prepared in 15% glycerin (Evans *et al.* 2002). Thin transverse sections were taken by free hand sectioning for the detailed study of anatomy of leaf and observed under light microscope.
2. Stem: For anatomical study of stem and type of trichome, Transverse sections were taken by free hand sectioning and observed under light microscope.
3. Root: T.S were taken by free hand sectioning and observed under light microscope.

Free hand sections of stem, leaf and root were cut from fixed material which were processed by standard double staining method.

Protocol used for double staining

Thin section → 30% alcohol: (1min) → 50% alcohol:

(1min) → Saffranin stain:

(10sec) → 70% alcohol: (1min) → Light green: (10sec) → 90%: alcohol

1min → Absolute alcohol: (1min) → Mount in Canada balsam

Seed 4: Shape, surface and colour of seed, were observed under comound microscope

Pollen grain 5: Pollengrains fixed in 70% alcohol were taken in to acetocarmin (1min), mounted in 15% glycerin and observed under microscope.

4. Results

1 Macromorphology

Macromorphological study included morphological description and measurement of all plant parts which are seen by naked eye and dissecting or compound microscope. The data is compiled in Table 1. The characteristic features of plant can be highlighted as quadrangular stem, decurrent branching, zygomorphic corolla, pink in colour and persistent calyx without wings (fig.1).

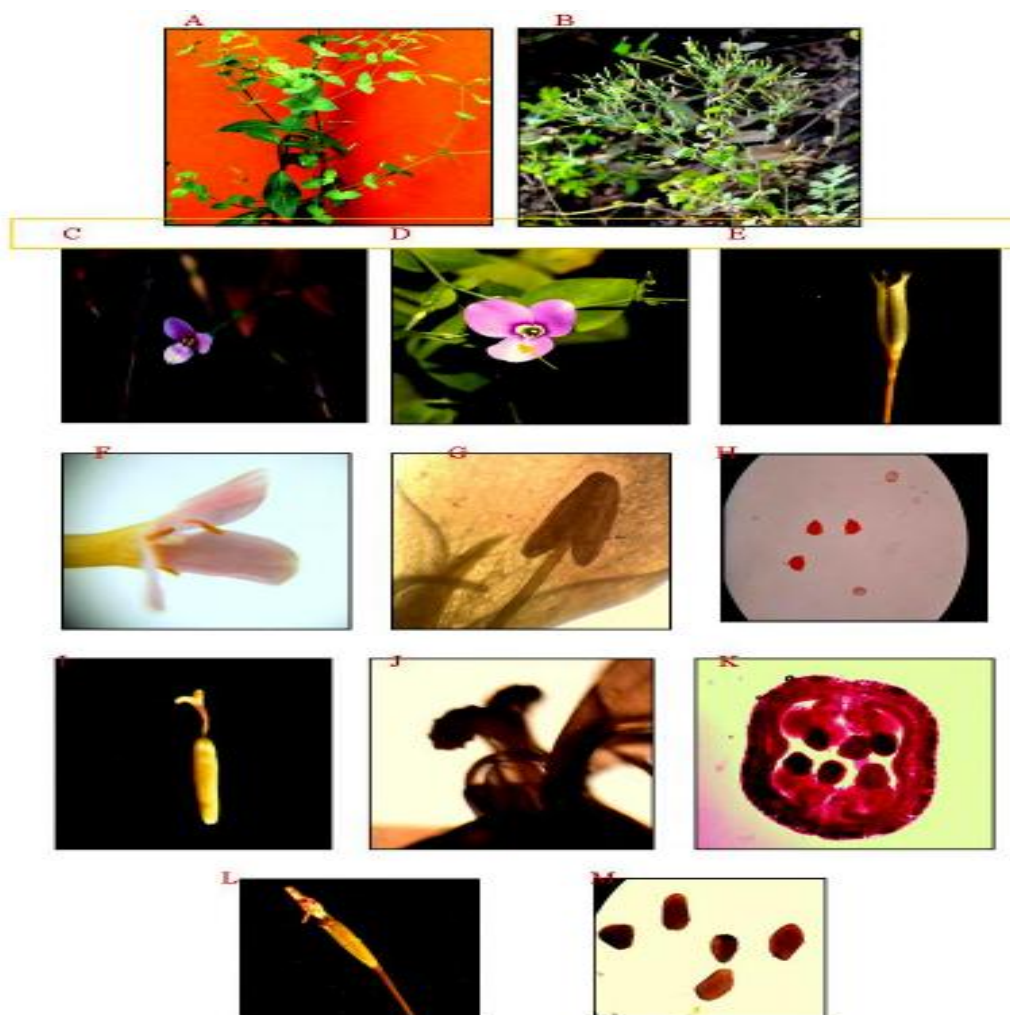


Fig 1: Vegetative and reproductive characters of *C. decurrens*. A-Vegetative growth with a and opposite, decussate leaves; B-Terminal cyme inflorescence; C and D-Zygomorphic, pink fused petals (arrow); E- Calyx; F, G-Unisomorphic anthers; H-Pollen grains; I-Gynoecium; J. T.S of ovary, Parietal placentation; L:Fruit; M- seeds.

Table 1: Macromorphological studies in *C. decurrens*.

Character	Description
Habit	Annual herb, erect, decurrent (Profusely branch), quadrangular stem(6-40cm in height and 0.4×0.6mm in width).
Leaf	Glabrous leaves, ebracteate, sessile acute-apex,3 veined, crenate- margin, elliptical-shape (15mm ×6mm in size).
Inflorescence	Terminal or axillary cyme
Flowers	Rosy pink in colour, funnel shaped(3-62 in no/plants),pedicellate..
Corolla	Zygomorphic, corolla tube(5-7mm),4 lobe,2upper lobes are equal (3.1×1.3mm) and lower lobe(4.2×1.5mm) fused at the apex forming deep slit.
Calyx	Green, sepals -4 in no., acute-apex, lanceolate, (3.2×1.4mm in size).
Stamens	4 –anisomorphic (1 big, and 3 small),large anther having long filament(1.4 mm) and small having small filament(0.7mm).
Anther	White in colour, 1.1mm long.
Pollen grain	Elliptical, tricolpate (21.8×17.3µm).
Ovary	Oblong, green (5.4mm in size), parietal-placentation.
Style	White in colour (2-3mm).
Stigma	Bilobed, white in colour.
Fruit	Capsule, brown in colour (6.21-6mm).
Seed	Brown in colour, irregular, (0.25mm).

2. Micromorphology

1. Anatomical studies: Anatomical studies include detailed information regarding tissue and its distribution in stem, leaf, root

etc. which are stable characteristic features of the given species and hence they help in the identification of plant at microscopic level.

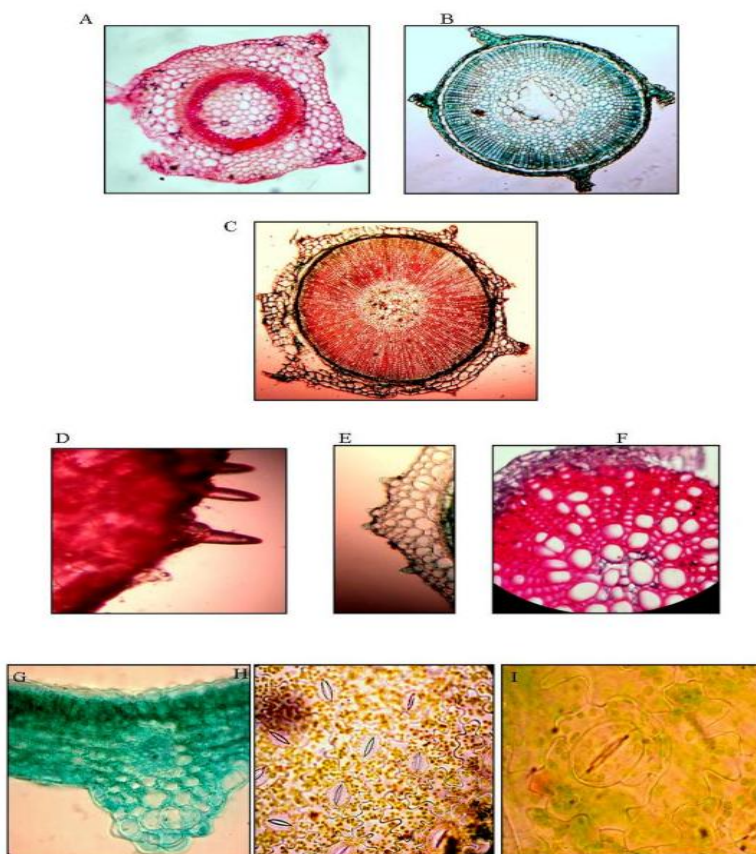


Fig :2-Anatomical features in *C. decurrens*. A,B,C– T.S. of stem at different stages of development; D and E-Trichome on stem; F– T.S of root; G-T.S of leaf; H and I-Stomatal type and distribution.

2. T.S of stems: (fig 2 A, B and C)

Stem shows variable features during different growth phases. Young stem is quadrangular in shape having 4 winged projection at 4 corners. As the stem grows it attains circular shape and the wings are reduced to mere small projection (fig A and B). Young stem shows single layered cutinized epidermis followed by 4-5 layered cortex,

parenchymatous pith is prominent and enclosed by ring of vascular tissue.

In matured stem cortex is reduced and its place is occupied by abundant secondary xylem (tracheids and vessels) and scanty secondary phloem which in turn is surrounded by single layered distinct endodermis (fig B and C). The stem surface is covered by prominent unicellular trichomes (fig D

and E). Prominent pith region is interspersed with random patches of sclerenchyma (fig C).

3. T.S of Leaf

Single layered epidermis (upper and lower) is characterized by compactly arranged rectangular cells covered by thin cuticle. Cells of lower epidermis are larger than that of upper epidermis (fig G).

The leaf is amphistomatic where stomata are scattered on both the sides of leaf on the same level as the other epidermal cells. Stomata are anomocytic type in which the guard cells are surrounded by 4 subsidiary cells. Cell walls of subsidiary cells are undulate (fig. H and I)

Ground tissue consists of 2 layers namely palisade and spongy parenchyma. Palisade is single layered with elongated compactly arranged chlorophyll containing cells. Spongy parenchyma is 4-5 layered and is compactly arranged without intercellular spaces.

The vascular system of leaf includes 3 main vascular bundles (1 large and 2 small). The vascular bundles are collateral where xylem occurs on adaxial and phloem on the abaxial side. Unicellular trichome are present on abaxial surface mostly concentrated at mid vein region.

4. T.s of root: (fig. F)

The transverse section through mature root shows single layered epidermis followed by 2-3 layered cortex. Pith is absent instead, the central portion is occupied by prominent vascular tissue consisting of tracheids, vessels and phloem.

4. Discussion

1. Macromorphology

The diagnostic features of genus *Canscora* included branched, angular stem, oval pointed leaves 4 unequal petals and 4 unequal stamens^[17]. The taxonomic status of species *C. decurrens* was doubtful as this plant has been reported by many workers under different species names like *C. tenella*, *C. diffusa*, *C. khandalensis*^[12] *C. diffusa var tetraptera* and other. All of them are now considered to be conspecific^[14]. Hence the present experimental material is *C. decurrens* and is conspecific with *C. diffusa*. Similar attempts were made by defining the generic limits in the genus *Gentiana* (*Gentianaceae*)^[10]. A comparative account of leaf, calyx lobes, ratio of corolla tubes and lobes, stamens etc led to establish a new genus in this heteromorphic assemblage.

2. Micromorphology

There are no reports on the anatomical features of stem, leaf and root of *C. decurrens* so far. Stem show typical normal anatomical features except sclerenchymatous patches in pith region. This finding is reported for the 1st time in *C. decurrens* in 4 angled stem observed in *C. decurrens* ^[7,9]. Presence of unicellular trichomes on leaf and stem surface are reported for the 1st time. Stomata are majorly anomocytic, this type of stomata are also observed in *Gentiana kurroo* ^[1] and *Gentiana oliveri*^[18] of *Gentianaceae* family. However minor variation in stomata structure and distribution can be attributed to the variation in ecological condition prevailed during plant growth.

In leaf the mesophyll is not homogenous but is differentiated into palisade parenchyma and spongy parenchyma. The palisade layer is located at the upper (abaxial) side of blade

and spongy on lower (adaxial) hence leaf in *C. decurrens* is bifacial and this feature is characteristic of plants of temperate region with abundant soil water ^[5]. Vascular system in leaf of *C. decurrens* consist of 3 major veins (3-nerved) which are netted by minor veins. It is characteristic feature of *C. decurrens*.

5. Summary and conclusion

The present study summarizes macro and micromorphological features of the of *C. decurrens* which helped the identification and authentication of plant material. This study not only allows fingerprinting the plants but leads to establish biomarkers which are species specific. The plants is characterized by 4 angled stem, zygomorphic corolla (2 free + 2 fused), anisomorphic stamens (1 big+ 3 small), pink flower, tricolpate pollen grain, anomocytic stomata, unicellular trichomes and irregular shaped, tiny (0.25mm) seeds.

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