



## Anatomical studies on critically endangered *Inga cynometroides* (Bedd.) Bedd. ex Baker

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### Abstract

*Inga cynometroides* (Bedd.) Bedd.ex Baker is a critically endangered species unknown to science anatomically as well as in other fields of science. The aerial parts of the plant were anatomically evaluated by the double staining method. Anatomical features of the young stem and stem after secondary growth were described. Similarly, the petiole and leaflets were examined. Further, features of stomata such as stomal size and stomatal index were also determined to help the identification of *Inga cynometroides*.

**Keywords:** *Sanjappa cynometroides*, *Inga cynometroides*, anatomy, stem, petiole, leaflet, stomata

### Introduction

Plants contain several chemical compounds useful to man in many ways like food, medicine, various raw materials for daily life etc. Many of the plants were screened chemically and several thousands of chemicals were separated and their structures are also known to science. Plants like *Curcuma longa*, *Azadirachta indica*, *Piper nigrum*, *Rauwolfia serpentina*, *Cinchona officinalis* etc., are well known for their variable chemical compositions. Similarly, myriads of plant species possess many useful properties are yet to be studied regarding their chemical composition in a comprehensive manner. Most of the popular and common plants are studied and their chemistry is somewhat known to us. Many of the rare, endangered, and threatened (RET) plants are unknown chemically due to their rarity and non-accessibility. Only plant taxonomists with professional field experience can identify and locate such plants from nature.

*Inga cynometroides*, recently elevated as a genus *Sanjappa*, with a single species *S. cynometroides* is a small tree of the subfamily Mimosoideae (presently treated as a clade under the subfamily Caesalpinoideae), endemic to Kerala, growing in evergreen forests areas of the Western Ghats of southern Kerala. Beddome first collected the original specimens in 1870 from Rosemala forests, Kollam District, Kerala (Beddome, 1873) <sup>[1]</sup>. The specimen has not been collected for a long time since the type collection and is placed under indeterminate threat category (Nayer and Sastry, 1990) <sup>[6]</sup>. This species was relocated from its type locality after 100 years of its original type collection (Sasidharan, 1998) <sup>[10]</sup>. Considering the rarity in distribution, this species is placed under critically endangered category in the revised IUCN Red list categories (Somen *et al.*, 2014) <sup>[11]</sup>.

The studies on the population status of *I. cynometroides* were carried out through extensive field surveys during 2010-2013. With the aid of global positioning system (GPS) and GIS software, the population sites of *I. cynometroides* were geographically marked and subsequently, a distribution map was prepared. The study evidenced that the population size of *I. cynometroides* is considerably small with low dominance and ecological success in the study site. A declining population of the species *I. cynometroides* is

newly located from Kallar in Trivandrum forest division and is the very first report from other than the type locality after the relocation of the species *I. cynometroides* from the type locality, Rosemala, Sendurney WLS of Kollam district, Kerala (Somen *et al.*, 2014) <sup>[11]</sup>.

### Taxonomy of the species

*Sanjappa* E.R. Souza & Krishnaraj (Fabaceae *sensu lato* - Mimosoideae: Ingeae), a new genus described to accommodate the species *Inga cynometroides* (Bedd.) Bedd.ex Baker and is monotypic (De Souza *et al.*, 2016). Since the name *Inga cynometroides* (Bedd.) Bedd.ex Baker was popular among the scientific community and scientific literature that name is followed here.

*Sanjappa cynometroides* (Bedd.) E.R. Souza & M.V. Krishnaraj, Rheedeia. 26(1): 6. 2016. *Inga cynometroides* (Bedd.) Bedd. ex Baker in Hook. f., Fl. Brit. India 2:308.1876; Gamble, Fl. Pres. Madras 435(309). 1919; Sasidharan, Biodiv. Doc. Kerala pt.6. Fl. Pl.163. 2004; Somen *et al.* <sup>[11]</sup>, Res. J. Agriculture and Forestry Sci. 2(4): 13-18. 2014. *Calliandra cynometroides* Bedd., Fl. Sylvat. 317. 1873; Sasidh., Fl. Senthurny W.L.S. 112. 1997.

Small trees, 7-13m tall, young stem sometimes scandent. Stipules spinescent; spines in pairs, 6-20 mm long. Leaves pinnately 2-foliolate; petioles 0.5-3 cm long; leaflets 6-18×1.5-6 cm, obliquely obovate or oblanceolate, obtusely acuminate at apex, attenuate at base, sub coriaceous, glabrous; 6 pairs of lateral veins prominent beneath. Axillary heads 7-13-flowered; peduncle 2.5-3cm long, greenish-white, sessile. Calyx minutely pubescent; tube 1mm long, lobes 3, 0.25×0.25 mm, deltoid. 3 petals combined to a long tubular corolla; tube 8 mm long, lobes ovate, 2.5×2 mm. Stamens 20-40, filaments fused at base; tube 7-8 mm long; filaments 1.5-2cm long, filiform, anthers small, oblong. Ovary sub sessile, 1mm long, glabrous; style 2.5cm long, stigma capitate. Pods linear-oblong, 8-13×1.5-1.8 cm, flat, reticulate, glabrous, valves twisted after dehiscence, 2-5- seeded. Seeds 1-1.2 cm long, ellipsoid, black, compressed. Flowering and fruiting: March-December.

*Inga cynometroides* (*Sanjappa cynometroides*) is a critically endangered species unknown to science chemically as well

as in other fields of science such as anatomy, cytology, ecology etc. So the present attempt has undertaken to study the details of anatomy, micro morphology and phytochemistry of the plant.

## Materials and methods

### Plant Material

The materials used for the study were collected from the plants growing in Rosemala, Kollam District, Kerala. For anatomical studies, specimens were taken from cultivated plants raised from the seeds of the plant (Fig. 1).



**Fig 1:** Habit views of *Inga cynometroides* (Bedd.) Bedd. ex Baker

### Anatomical studies

Freehand sectioning of plant parts such as leaflet, petiole and different portions of the stem (young portion, mature portion and portion after secondary thickening) was used for the anatomical study. For dehydration, the sections made were passed through a series of alcohol solutions in the order 25%, 50%, 75%, 90% and absolute alcohol, allowing them for 3 minutes in each. In which safranin O stain was placed between 25% and 50% of alcohol, which stained the sections. After dehydration, the sections were counter-stained with fast green for 2 minutes. For clearing of natural wax, the sections were passed through different proportions of alcohol and xylene series in the following ratios (3:1, 1:1, 1:3) v/v for 3 minutes in each, and finally in pure xylene (100%). The section was then placed on a clean glass slide with a drop of glycerol and covered with clean coverslips for microscopic evaluation and microphotographs were taken (Retamales and Scharaschkin, 2014) [8].

### Analysis of stomata

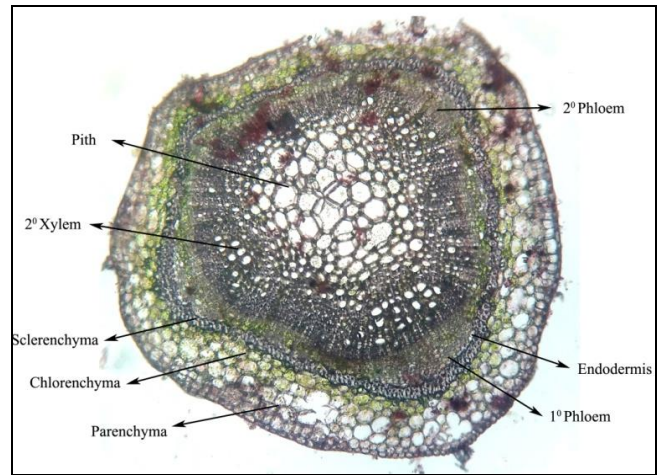
The upper and lower epidermis of the middle part of the leaf was peeled off and used for studying stomatal features. Micrometric method was employed for determining the size of stomata (Chattopadhyay *et al.*, 2011) [2]. Stomatal index was calculated using a standard protocol (Chattopadhyay *et al.*, 2011) [2]. For determining the stomatal index, the number of stomata and the total number of epidermal cells of 0.4mm<sup>2</sup> areas of 4 microscopic (40x) fields were noted. An average of stomatal numbers and epidermal cells from the fields were used for determining the stomatal index.

## Results and discussion

### Anatomical studies

#### C.S of a young portion of the stem

C.S of young portion of the stem is sub spherical in outline. Epidermis is found to be composed of a uniseriate layer of rectangular cells with uniform deposition of thick cuticle on the outer surface. Epidermis is followed by the multiseriate differentiated cortex (Fig. 2).

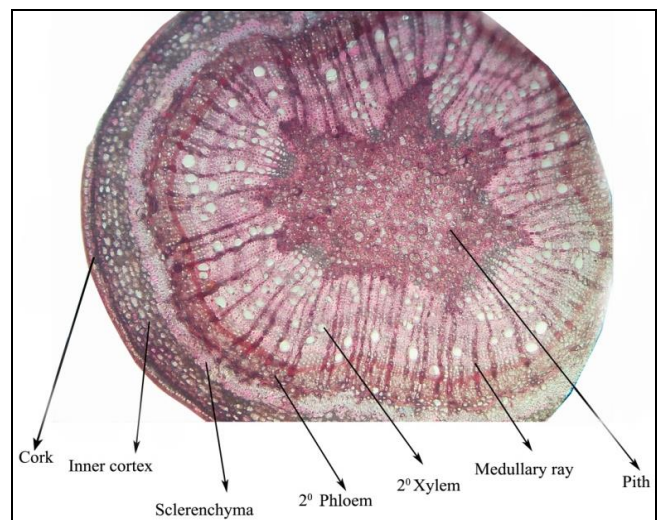


**Fig 2:** Anatomical features of young stem of *Inga cynometroides* (Bedd.) Bedd. ex Baker

Cortex is differentiated into outer 3-5 layered parenchymatous cortex and inner 2-3 layered chlorenchymatous cortex. Cortex is bordered internally by sclerenchymatous cells which is uniform in size and 2-3 layers in thickness. Sclerenchyma patches also evident in the secondary phloem. Beneath sclerenchymatous patches, single layered endodermis is present. Secondary xylem is composed of thick walled tracheids and vessels. Primary phloem is seen as crushed mass due to early secondary thickening. Pericycle, primary xylem etc., are not clear. Secondary phloem formed as a ring, having a varying thickness, some patches appeared as larger and some were smaller, 4-5 such patches on one side were comparatively larger in size, whereas as the other side contains secondary xylem having a smaller area. Central large pith is composed of parenchymatous cells with intercellular spaces.

#### C.S of a mature portion of the stem

C.S of the mature stem is sub spherical in outline. Epidermis and inner cortex are replaced with outer cork, composed of phellem, phellogen and phelloderm (Fig. 3). Phelloderm containing two rows of small sized and thin walled cells, cork cambium (phellogen) in single layered and phellem (secondary cortex) with 4-6 layers of thin walled parenchymatous cells.

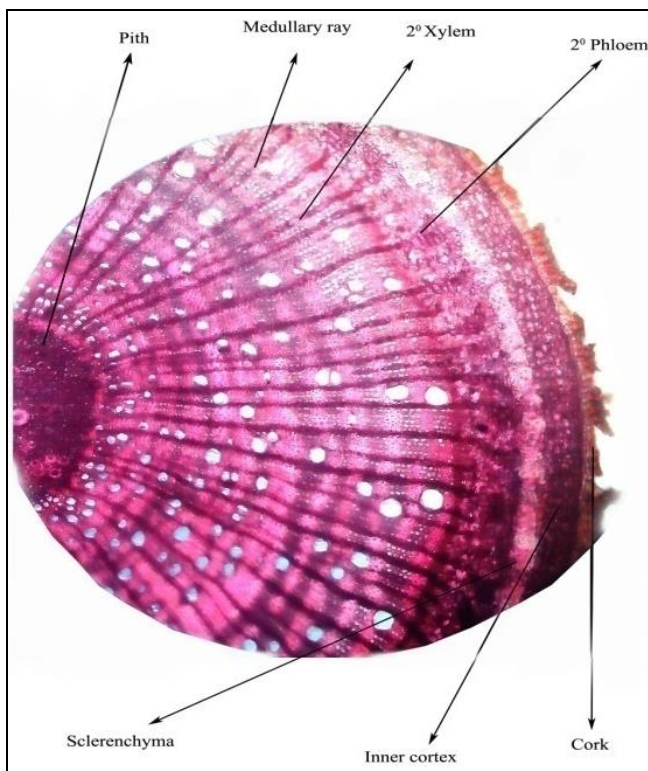


**Fig 3:** Anatomical features of the mature stem of *Inga cynometroides* (Bedd.) Bedd. ex Baker

Secondary cortex is bordered internally by 2-3 layers of sclerenchymatous cells and are uniform in size. Sclerenchyma patches are also present in the secondary phloem. Beneath sclerenchymatous patches, cambial ring is present. Primary phloem, primary xylem, etc., are not visible. Secondary xylem is composed of thick walled tracheids and vessels. In the secondary xylem zone, several cells have different kinds of depositions of polysaccharides. Secondary phloems are found as a ring of irregular shape with more or less even thickness. The cork is broken at certain places, due to secondary thickening. In the central pith, cells have a little lumen, almost all cells have depositions and inter cellular spaces are also found in certain places.

**C.S of the stem after secondary growth**

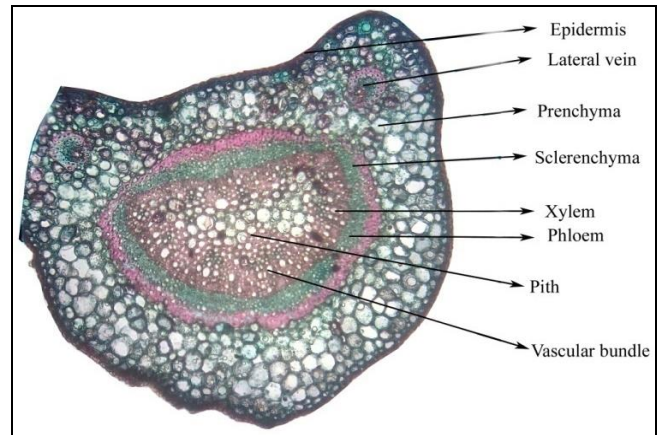
C.S of the stem after secondary growth is circular in outline. Cork is broken and only some regions are persistent beneath secondary cortex (Fig. 4). Secondary cortex is composed of 4-6 layers of thin walled parenchymatous cells. Secondary cortex is bordered internally by 2-3 layers of uniformly sized sclerenchymatous cells. Sclerenchyma patches are visible in the secondary phloem. Secondary xylem contains vessels and thick walled tracheids. Medullary rays and annual rings are also present in the secondary tissues. Secondary xylem possess different kinds of polysaccharide depositions. Secondary phloem is found as a ring of circular shape. In the central pith, almost all cells have depositions. The pith is compressed and cells have a little lumen.



**Fig 4:** Anatomical features of the stem of *Inga cynometroides* (Bedd.) Bedd. ex Baker after secondary thickening

**C.S of the petiole of *Inga cynometroides* (Bedd.) Bedd. ex Baker**

C.S of the petiole is omega shaped in outer line, having two lateral projections towards the dorsal side. Stele is fairly large in size and cortex is smaller (Fig. 5).

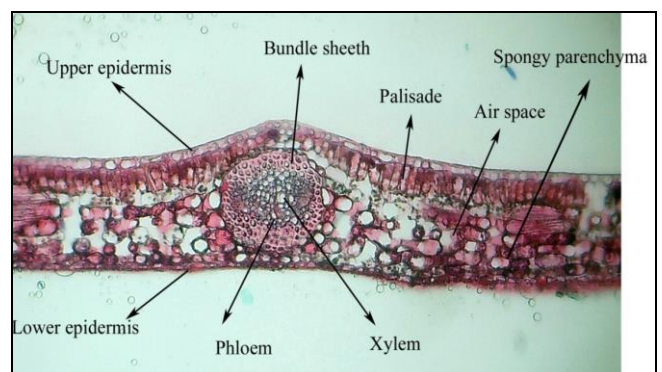


**Fig 5:** Anatomical features of the petiole of *Inga cynometroides* (Bedd.) Bedd. ex Baker

The outermost epidermis is with uniformly thickened cells and supported externally by the cuticle. Below the epidermis is the cortex having 6-7 layers of cells on the upper portion with smaller parenchymatous round cells. The lower portion of the cortex is slightly thicker and having 6-7 layers of larger parenchymatous cells. Some of the cells in the cortex contain colored depositions of various chemicals. There are two lateral projections on the upper portion, each containing a vascular strand with xylem surrounded by phloem and it is covered by a ring of sclerenchymatous tissue. The central portion occupied by stellar region, separated from the cortex by a small ring of sclerenchymatous tissue, which is 2-3 layered. Sclerenchymatous ring is followed by vascular tissue with phloem towards outer side and xylem towards the inner side. The middle portion is occupied by large, rounded parenchymatous cells. In the stellar area, some of the cells found in the phloem as well as the xylem region contain depositions and are darker in color.

**T.S of the leaflet of *Inga cynometroides* (Bedd.)**

T.S of the leaflet is flat at lamina region and bulged at midrib region in outline. Bulged midrib region has a semicircular adaxial side and a flattened abaxial side. The epidermis along the adaxial surface is smooth and has rectangular cells (Fig. 6).



**Fig 6:** Anatomical features of leaflet of *Inga cynometroides* (Bedd.)

Cells along the abaxial part are circular. In the midrib, the vascular bundles are surrounded by a ring of sclerenchymatous bundle sheath. It is thicker at adaxial and abaxial sides (3-4 layered) and thinner at lateral sides (1-2 layered). The vascular bundle is collateral with abaxial cluster of phloem elements and adaxial triangular mass of

xylem elements. The xylem elements are thick, compact, narrow and angular. The epidermal layer is with deposition of cuticle at both adaxial and abaxial surfaces. The abaxial epidermis is discontinuous with stomatal openings. The mesophyll tissue consist of palisade and spongy parenchyma. The adaxial part has a single layer of palisade cells and in the abaxial part round shaped and loosely arranged spongy parenchyma cells are present.

### Stomatal size and stomatal index

Stomatal analysis revealed that in *Inga cynometroides*, stomata are confined to the lower epidermis of leaf and is absent in the upper epidermis (hypostomatic leaves). The type of stomata is paracytic, in which the guard cells are surrounded by two parallel subsidiary cells. Epidermal cells are irregular in shape. The length and the breadth of stomata were determined micrometrically as  $96\mu m$  and  $48\mu m$ , respectively. The average stomatal index was determined as 11.89%.

Some of the general anatomical characters of the investigated plant confirmed the features identified earlier by different workers in other members of Leguminosae (Metcalf and Chalk, 1950) [5]. Anatomical studies on plant parts revealed the presence of different kinds of depositions on the cortex, secondary tissues and also in pith region. Different type of crystals are also present abundantly in pith region. The frequency distribution and size of crystals are used as diagnostic characters in microscopic studies of herbal drugs. Studies conducted on crystals in plants is mostly associated to the taxonomic studies of plants (Prychid and Rudall, 1999) [7]. Studies in stomata can have a great taxonomic as well as pharmacognostic value in proper identification of different plant taxa, including medicinal plants (Santhan, 2014; Kothari and Shah, 1975) [9, 4]. The investigated plant bear hypostomatic leaves, i.e., stomata present only at the abaxial surface of leaves. Metcalfe and Chalk (1950) [5] reported that the paracytic type of stomata and hypostomatic leaves of the members of the family Leguminosae. In Mimosaceae, most of the species showed paracytic stomata and hypostomatous leaves (Tripathi and Mandal, 2012) [12]. Stomatal index is used as marker characters for taxonomic identification of plant species. In this investigation stomatal index is 11.89%. Tripathi and Mondal (2012) [12] reported in a work that in Mimosaceae most of the investigated members bears stomatal index percentage from 11 to 20.

### Conclusion

In the present investigation, anatomical studies on plant parts showed the presence of different kinds of depositions on cortex, secondary tissues and also in pith region, which are considered taxonomically important. The studies on leaf revealed it's hypostomatic nature with paracytic stomata and stomatal index as 11.89%. These findings possess significant taxonomic value in plant identification. This is the first time work on *Inga cynometroides* based on anatomy. Further studies are needed to unravel therapeutical significances of *Inga cynometroides*.

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