



## Comparative morphological and anatomical studies of leaves, stem, and roots of *Acalypha indica* L. and *Micrococca mercurialis* (L) Benth

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### Abstract

The basic morpho-anatomical characters of *Acalypha indica* L. & *Micrococca mercurialis* (L.) Benth. were compared to distinguish their macro and micro characteristic features. The difference in the morphological traits such as leaf shape, leaf margin, petiole length, leaf and flower arrangement and fruit type and anatomical features such as epidermal cell shape, type of trichomes, number and distribution of vascular bundles, cellular inclusions etc. were inferred between *A. indica* and *M. mercurialis*. The observations found in the present study can be used in the identification, delimitation and classification of these two species as well as, as a tool for the development of regulatory documents in the studies of raw drug materials identification.

**Keywords:** morphology, anatomy, *Acalypha*, *Micrococca*, siddha medicine, skin diseases

### Introduction

Euphorbiaceae (Spurge family) is the sixth largest family of Angiosperms next to Orchidaceae [1]. Euphorbiaceae includes around 8000-9000 species with 340 genera and is widespread throughout the World [2]. Members of this family exhibit diversity in growth forms varying from small ephemerals to herbaceous annuals or perennials, shrubs, small trees and cactus-like succulents [3]. The diversity of the genus can be summarized from Webster's (1994) [4] classification which has reported 20 tribes, 116 genera, and 2000 species found all over the world. The family is characterized by the presence of milky sap, unisexual flowers, superior trilobular (usually carunculate) ovary with axile placentation, pendulous and ventral raphe [5]. The economically important plants such as cassava (*Manihot esculenta*), Para rubber (*Hevea brasiliensis*), castor oil plant (*Ricinus communis*) and Barbados nut (*Jatropha curcas*) and ornamental plants such as poinsettia (*Euphorbia pulcherrima*) and leafy spurge (*Euphorbia esula*) are members belong to Euphorbiaceae [6].

The genus *Acalypha* Linn has reported with 462 species, distributed mostly in tropical and warm temperate regions of Africa, America and Asia [7]. Genus name '*Acalypha*' is derived from the Ancient Greek word '*Akelephe*' and was inspired by the nettle-like leaves. A large numbers of species of this genus are weeds while the others are ornamental plants. Some of them are well known for their usage in the traditional medicine and have recorded in the pharmacopoeia of Ayurveda [8], Siddha [9] and Homeopathy [10]. *Acalypha wilkessiana* (fire dragon or copper leaf) is important in the treatment of headache, common cold and skin infections [11]. Onocha *et al.* (2011) [12] reported that different parts of *A. hispida* have been used in the treatment of debilitating diseases like leprosy, Gonorrhoea, Asthma, pulmonary problems and kidney ailments.

*Acalypha indica* L. is well known for its multifarious medicinal uses in traditional medicinal system of India especially in Siddha and Ayurveda [13, 14]. The *A. indica* is commonly known as 'Indian Copper leaf', 'Indian Mercury' in English; 'Kuppi' in Hindi; 'Kuppaimeni' in Tamil and

'Harita manjari' in Sanskrit; 'Kaphajri' or 'Kaphkaturi' in Ayurveda. *A. indica* leaf and whole plant are used in many important formulations of Siddha System of Medicine (SSM) such as Parankippatti patankam, Kadugu ennai, Sankankuppi ennai, Peruvayuru melugu, Panchapadana and Chendrum used in the treatment of Bronchial asthma (Cuvācakācam), Bleeding haemorrhoids (Iratta mūlam), cold (Kapa nōykal), Gastro-intestinal disorders (Kunmam), sinusitis (Pīnacam), tooth ache (Tantamūla nōy), burn (Ticcuttapun), Pruritus (Tiṅavu), Rheumatoid arthritis (Vāta nōy) etc. [8]. In Ayurveda, the plant is used as a single drug. It is mainly used in the treatment of digestive impairment (Agnimandya), tooth ache (Dantasula), Otagia (Karnasula), cough (Kasa), arthritis (Sandhisotha), asthma (Svasa), constipation (Vibandha) [9].

It has been reported that *A. indica* possess hepato-protective, anti-inflammatory, antitussive, antibacterial, antifungal and wound healing properties [15, 16, 17]. Leaves are used in jaundice, piles, rheumatism, ulcers, skin eruptions, ring worms, eczema and leaves extract are applied to pustules, insect bites [18]. Leaf decoction is used in treatment of fever, heart diseases, retained excretions and biliousness [19]. The plant extract or decoction is useful in treating asthma, pneumonia and rheumatism [20]. The phytochemical such as acalyphine (alkaloids), sterols, kaempferol (flavonoids) and glycosides corroborating the innumerable medicinal properties and therapeutic uses of *A. indica* prove the value as an inevitable medicinal plant [21].

*Micrococca mercurialis* (L) Benth. commonly called 'Pulladi' or 'Kunuku thooki' also belongs to Euphorbiaceae family appear like *A. indica* at a first look. It grows well in saline soil and distributed throughout Africa, Malaysia, North Australia and India [22]. The medicinal properties of *M. mercurialis* have not been explored well. It possesses many important secondary metabolites such as saponins, flavonoids, anthroquinone [23] and traditionally it has used to treat sores, rheumatic pain, constipation, etc. [24].

Adulteration and substitution are one of the major threat facing in the raw drug industry that affecting the quality and

therapeutic effect of the herbal formulations. Adequate knowledge pertaining to the identification and authentication of the raw drugs are mandatory to prevent the adulteration and substitution of herbal drug with inferior quality material as well as by distantly related plants of the same family. Accurate analysis on the morphology and detailed examination of the anatomy are basic parameters to evaluate the identity and quality of the herbal drugs. Macroscopic and microscopic evaluation of the pharmacognostic indices is an important thing to establish the quality of the herbal drugs [25]. As there is a high probability for misinterpretation between *A. indica* and *M. mercurialis* due to similarity in their habit and closeness in the morphology, the present study has designed to compare their macroscopic and microscopic morpho-anatomical characters.

## Materials and Methods

### 1. Sample collection

The fresh plant materials of *Acalypha indica* L. and *Micrococca mercurialis* (L.) Benth. was collected from suburbs of Mettur Dam, Mettur Taluk, Salem District, Tamil Nadu. It lies between 11°14'. 46" N latitude and 77° 32'.52" E longitude. Collected plants were identified by using regional floras and processed for herbarium [26]. Voucher specimens were stored in the herbarium section of Siddha Medicinal Plants Garden, Mettur Dam, Salem District, Tamil Nadu for further reference.

### 2. Morphology

The nature of habit and detailed morphology of stem, leaves, inflorescence, flower, Fruit and seed were recorded. The detailed traits for each characters were authenticated by using the 'Flora of Presidency of Madras' [27], 'Flora of Tamil Nadu' [28, 29], 'Flora of Tamil Nadu Carnatic' [30] and 'An Excursion Flora of Central Tamil Nadu, India' [31].

### 3. Anatomy

The anatomy of stem, root and leaf were analysed by taking hand sections using stainless razor blade (*Superior quality Supermax*). The uniformly thin sections were stained with 'safranin O' and Fast green. The stained materials were mounted in glycerine and observed under various magnifications (10x, 20x, 45x) under MX 21i LED Trinocular microscope and pictures captured accordingly.

### 4. Epidermal Peeling and Stomatal Indexing

The pieces of leaves were soaked in concentrated Nitric acid (HNO<sub>3</sub>) in a clean cavity slide. The slides were covered and allowed to stand for between 3 and 5 hours. The epidermal surfaces were separated from the macerated leaf materials and transferred into petri dishes containing water for cleaning the surfaces. Staining was done with 'safranin O' for about five minutes before mounting in glycerine. Stomatal index was calculated by using the formula;

$$\text{Stomatal index (S.I)} = [S/(S+E)] \times 100$$

Where, 'S' is the Number of stomata per unit area and 'E' is Number of epidermal cells in the same area.

## Results

### 1. Morphological studies

*Acalypha indica* L. (Fig.1a) herb to 50(75) cm; branchlets striate, thinly pubescent. Leaves broadly ovate, 2-6×1-5 cm, base rounded to shortly attenuate, margin crenate to serrate,

apex acute; petiole to 7 cm, longer than leaf blade. Spikes axillary, to 7 cm; male flowers from above the middle terminating in an allomorphic flower; female flowers (9)12-15 at the lower half, bracts folded, campanulate, shortly dentate. Anthers vermiculiform. Pistils 2, one sterile; Ovary 2mm across, hispid; styles 3mm. Allomorphic flowers long-stalked, with 2, narrow, toothed, tubular whorls. Fruit single seeded, ovoid, smooth, pale brown and 1-2mm long.

*Micrococca mercurialis* (L.) Benth. (Fig.1 b) monoecious herb to 75 cm. Leaves alternate, ovate to lanceolate, 3-5×1.5-3 cm, puberulous, base acute, margin serrate, apex shortly acuminate; petiole to 2 cm; stipules minute. Spikes lax, interrupted, axillary to 6 cm; bracts minute. Tepals 3, equal, ovate. Stamens 3-5, free, alternating with tepals, exserted. Glands ca.8, ciliate scales, free. Ovary 3-lobed, 3-locular; ovules laterally pendulous; style 3, linear, plumose-laciniate. Disc of linear glands. Capsule 5 mm across, 3 bivalved cocci, dehiscing both loculi and septicidally. Seeds globose, minutely arillate; testa pitted.

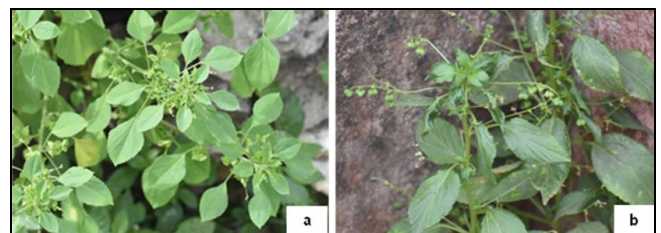


Fig 1: Habit, a- *Acalypha indica* L. b- *Micrococca mercurialis* (L.) Benth.

### 2. Anatomical studies

The anatomical characters of leaves, petiole, stem and roots of *Acalypha indica* and *Micrococca mercurialis* are described as follows.

#### 2.1 Anatomy of Leaf Epidermis

In *Acalypha indica*, the upper and lower epidermal cells are slightly polygonal in shape with undulating anticlinal cell wall. The stomatal indices are 22.77 and 26.97 for the upper and lower epidermis respectively. The upper and lower epidermis have paracytic stomata and glandular and non-glandular trichomes (Fig. 2h & 2i) Small spheroid calcium oxalate crystals are present while in *Micrococca mercurialis*, the upper and lower epidermal cells are irregular in shape having a wavy appearance on both upper and lower surfaces. The stomatal indices for the epidermis of *M. mercurialis*, are 18.32 and 21.69 for the upper & lower epidermis respectively and they are paracytic in nature. Non-glandular, unicellular, straight trichomes with pointed apex (Fig. 3e) and small spheroidal calcium oxalate crystals are found on both the epidermal layer. (Fig. 3f & 3g)

#### 2.2 Anatomy of leaf blade

The upper and lower epidermis in the midrib region of *A. indica* sinuous covered with thin cuticle bearing simple trichomes. Underneath the upper epidermis lies single layer of palisade cells discontinued over the midrib region where the space occupied by 4 to 5 rows of collenchymatous cells whereas 2 to 3 rows beneath the lower epidermis. Rosette crystals of calcium oxalate are embedded throughout the ground tissue of the leaf (Fig. 2d). *M. mercurialis* leaf consists of thick midrib and lateral veins. The epidermal layer of the midrib is thin and the cells are small and spindle shaped. The cortex region is made up of polygonal, compact

thin walled parenchyma cells with several storage regions. Vascular bundles are wedge shaped and amphicribal (Fig. 3d).

### 2.3 Anatomy of Petiole

The epidermal layer of the petiole of *A. indica* is somewhat plano-convex in outline. The outer layer of epidermis bearing large number of simple trichomes, a narrow band of collenchymatous hypodermis followed by a wide parenchymatous ground tissue embedded with clustered crystals of calcium oxalate and ring of vascular bundles around the central parenchymatous pith (Fig.2c)

*M. mercurialis* petiole is dorsiventrally differentiated. It consists of semicircular convex lower part and flat or slightly raised adaxial part. There are two short, very thick hemispherical wings on either side of the adaxial part. The epidermal layer of the petiole is wide, rectangular and thick walled cells. The ground tissue is parenchymatous with thin walled, angular and compact cells. There are three main bundles and prominent top shaped wing bundles with conjoint and bi-collateral nature (Fig.3c).

### 2.4 Anatomy of stem

*A. indica* stem is having an irregularly circular outline and having outer layer of epidermal cells embedded with globular masses of calcium oxalate crystals and stomata (Fig. 2a). It is covered with thick cuticle and bearing simple as well as sickle shaped multicellular trichomes (Fig. 2f & 2g). A ring of hypodermis with discontinuous oval patches

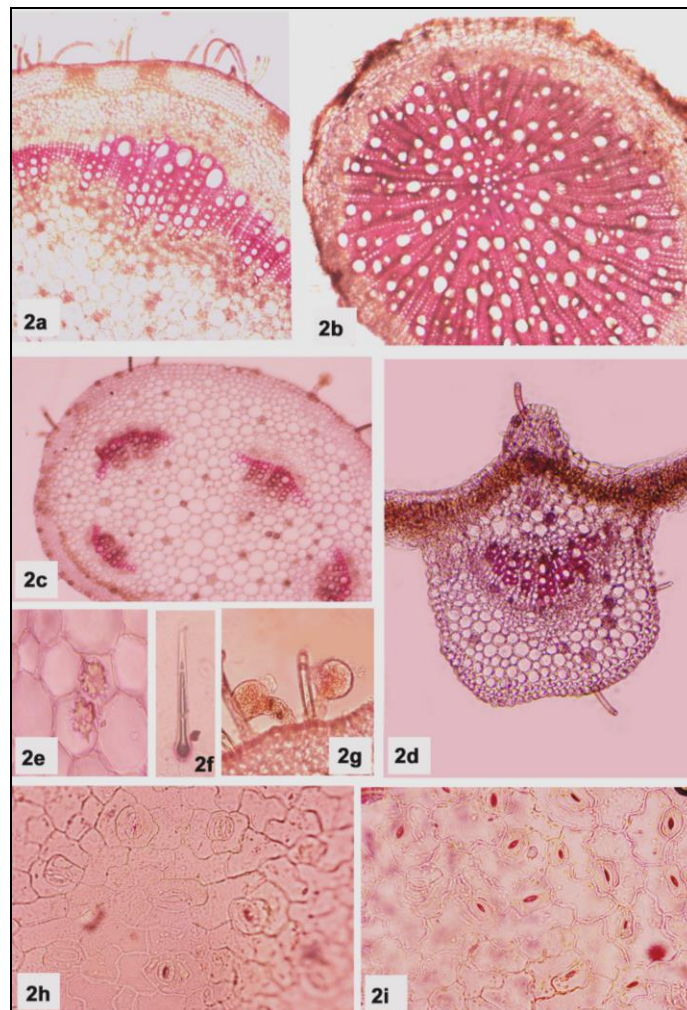
of collenchyma alternating with pigment cells. The parenchymatous cortex region showing clusters of prismatic crystals of calcium oxalate and starch grains. Pith is wide and parenchymatous. (Fig. 2e)

The stem of *M. mercurialis*, the stem is roughly circular in outline. The epidermal cell consists of rectangular thick walled cells bearing simple straight trichomes. A ring of hypodermis with discontinuous patches of parenchyma cells. Pith is wide and shows storage of starch grains (Fig. 3a).

### 2.5 Anatomy of root

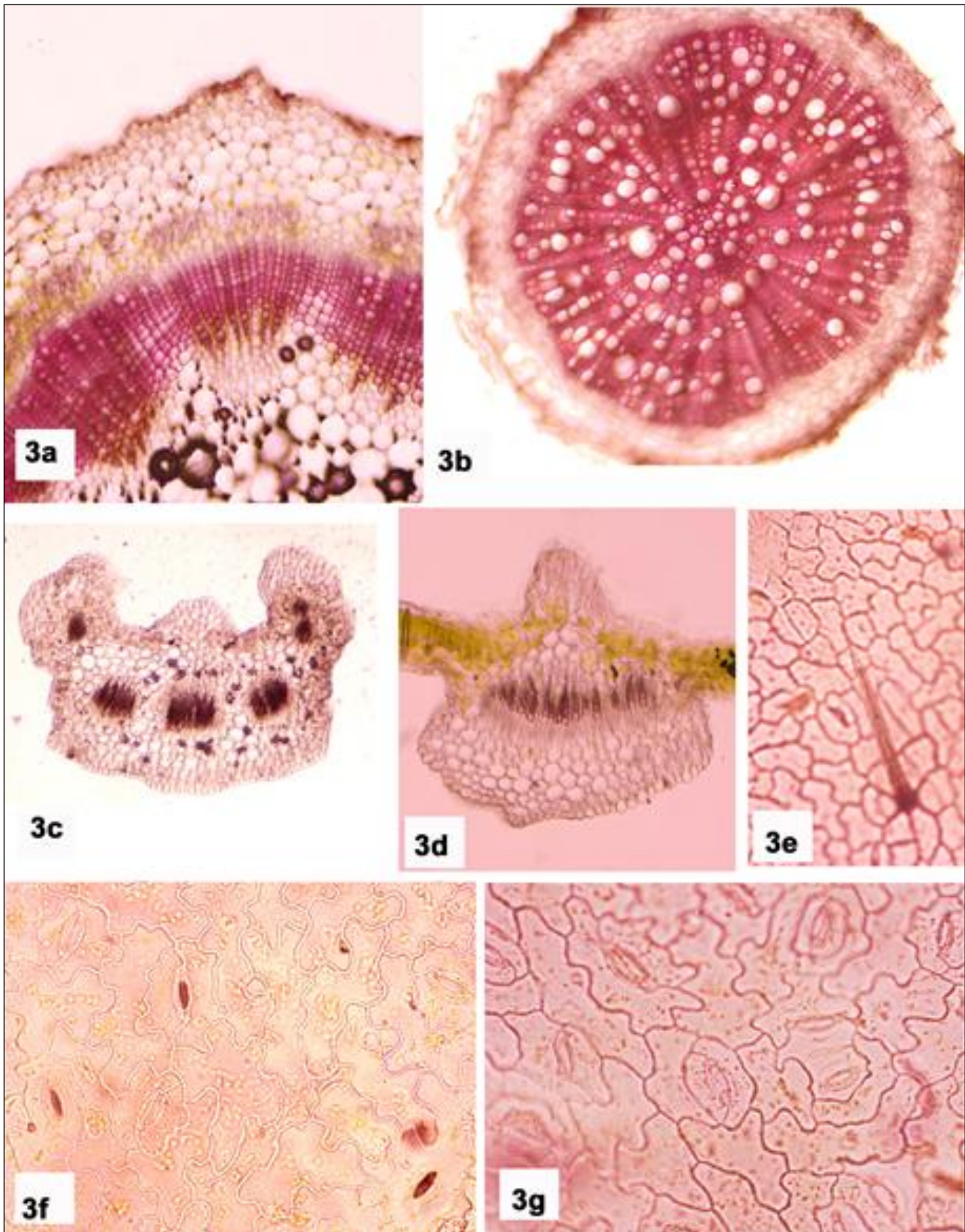
*A. indica* root is circular in outline with irregularly running cork. The cells are irregular, lignified and thick walled. Cortex is parenchymatous with few rosette and prismatic crystals of calcium oxalate cells. Phloem found to be traversed with uni-to biseriate medullary rays. Central xylem is very wide with isolated xylem vessels. The major areas of xylem are occupied by fibres and xylem parenchyma (Fig.2b)

In *M. mercurialis*, the root is circular in outline. The cells are lignified with thick walled. Cortex zone is not evidently visible and crushed into 3 or 4 layer of elongated cells. Starch grains were present in the cortical region. Secondary phloem is narrow with thin layer of small discrete sieve elements. The major area of xylem is occupied by vessels and fibres (Fig. 3b)



**Fig 2:** Anatomy of *A. indica*; 2a: Stem, 2b: Root, 2c: Petiole. 2d: Mid rib. 2e: Calcium Oxalate crystals, 2f: Simple trichome, 2g: Glandular trichome, 2h: Stomata - upper epidermis, 2i: Stomata-Lower epidermis





**Fig 3:** Anatomy of *M. mercurialis*; 3a: Stem, 3b: Root, 3c: Petiole, 3d: Mid rib, 3e: Simple trichome, 3f: Stomata - upper epidermis, 3g: Stomata-Lower epidermis

### Conclusion

The present study has been observed and documented the diagnostic key characters of macro and micromorphology of *Acalypha indica* L. and *Micrococca mercurialis* (L) Benth. in basic morphological and anatomical aspects. Considerable differences were observed between these two species in their morphological and anatomical attributes.

Morphological comparison revealed substantial variation especially in leaves, and stem. Anatomical studies explored the variation in nature of trichome, arrangements and type of vascular bundles, xylem, pith, medullary ray and types and distribution of cellular inclusions especially calcium oxalate and starch grains. The morphologic uniqueness of each species presented in this study will be helpful in

distinguishing *Acalypha indica* L. and *Micrococca mercurialis* (L) Benth. the anatomical features can be considered as key character in their identification in market sample to overcome the risks of adulteration or/and substitution.

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