

PHARMACOGNOSTIC STUDIES IN THE LEAF OF TYLOPHORA INDICA (BURM. f.) MERR

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ABSTRACT: The paper deals with a detailed investigation of the leaves of *Tylophora indica*, including its morphological, anatomical, histochemical and powder analysis. Contrary to the earlier observations, the leaf-lamina possesses emergences (squamellae), striated cuticle and amphistomatic condition. Stomata are of paracytic, anisocytic and anomocytic types, while trichomes are represented by the uniseriate macroform conical hair type. In t.s. the leaf-lamina is bifacial and petiole consists of three vascular bundles. Leaves are lactiferous with colourless latex. Lignin, starch, tannins and alkaloids were recorded in leaves. Microscopically the powder consists of fragments of epidermis, mesophyll, trichomes, latex tubes, tracheary elements and sphaero-crystals.

INTRODUCTION

The leaves of *Tylophora indica* are used as emetic, diaphoretic expectorant in respiratory affections, chronic bronchitis, whooping cough and in over-loaded states of stomach (Nadkarni, 1976). Besides, it is highly reputed in varied systems of medicine for curing asthma, allergic rhinitis and rheumatism (Shivapuri *et al.* 1978; Chopra *et al.* 1950; Haranath and Shyamalakumari, 1975; Nadkarni, 1976). The leaves have been reported to contain several biologically active compounds (Govindachari *et al.* 1954, 1959, 1961, 1973; Govindachari, 1973). Early anatomical and pharmacognostic studies on the plant include those of Solereder (1908), Metcalfe and Chalk (1950), Krishnamurthy and Kannabiran (1970), Chaudhury and Majumdar (1977). However, the present authors find the information so far available on the species is too scanty, to be adequate in identification of the different forms of drug prepared from the leaf. Hence, the present study was taken up, which includes the morphological, anatomical, histochemical, chemical and powder characteristics, to bring out the salient features which might help in the identification of the different drug preparations.

MATERIALS AND METHODS

Leaves of *Tylophora indica* (Burm. f.) Merr. were collected from plants growing wild in the Osmania University campus, Hyderabad, A.P. Varied micropreparations, viz. slides of epidermal peels, whole mounts, hand and microtome sections, macerations and powder presently studied were prepared as in the following. Epidermal peels were obtained by scraping the leaf with a blade as well as following the 'double-treatment method' of Leelavathi and Ramayya (1975). Following standard procedures (Johansen,

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1940), microtome and maceration slides of leaf-lamina and petiole were made staining with safranin and fast green. Statistics of the pharmacognostic characters determined include, palisade ratio, stomatal index, stomatal frequency, epidermal cell frequency and dimensions of varied cell elements in t.s. (Trease and Evans, 1978). For histochemical studies, hand sections were examined following Johansen (1940) and Gibbs (1974). The leaves were shade-dried, powdered and sieved with 70 mesh following Wallis (1950).

Extracts of powders (uniformly 0.1g of powder boiled with 1ml water) were tested for tannins, starch, anthroquinones, cyanogenetic glucosides following Gibb's schedule (1974). The powder (0.1g of powder boiled with 2ml of water) was examined under 15×10 magnification for noting the cell elements (Table 1). The terms used in the text are after Metcalfe and Chalk (1950), Stace (1965), Ramayya (1975) and Leelavathi (1976).

Abbreviations used in the text are: E.C.F. = epidermal cell frequency; S.F. = Stomatal frequency; S.I. = Stomatal index; Ad = Adaxial surface of the organ; Ab = Abaxial surface of the organ; P.R. = Palisade ratio; T.F. = Trichome frequency.

OBSERVATIONS

Tylophora is a palaeotropical genus represented by 50 species (Airy Shaw, 1966) of which *T. indica* (*T. asthmatica* W.&A.) is reputed for its medicinal uses. *T. indica* is widely distributed having been recorded from Deccan peninsula, North East Bengal, Assam, Chittagon, Burma to Malacca, Sri Lanka, Siam and Malay Island (Hooker, 1885).

Vernacular names: BENGALI—Antamul; Dukhini—Pitakari; HINDI—Jangli Pikvan; MARATHI—Kharaki—Rasana, Anthamul, Pitakari; MALAYALAM—Vallippala, SANSKRIT—Anthrachaka; SINHALESE—Binnuga; TELUGU—Vattipala, Kukkapala; TAMIL—Tayappali, Peyppalai, Naypalai (Chopra *et al.* 1956; Nadkarni, 1976).

Morphology: Leaves simple, opposite, decussate, exstipulate, petiolate with colourless latex. Lamina ovate to elliptic, cordate at base, entire, acute, mucronate, green in colour, 3 to 9cm long, 3 to 5cm wide with unicostate reticulate venation. Young lamina adaxially hairy, becoming glabrous or sub-glabrous at maturity but abaxially hairy. Adaxially laminar base with a transverse row of multiseriate conical squamellae, 5 to 8 in number, 90-270-300 μ m long, 60-90-130 μ m wide (Figs. 4, 9). Petiole short, flattened adaxially, 0.5 to 1.5cm long and hairy.

Anatomy: (a) Surface view of the leaf-lamina. Epidermal cell complex: Adaxial and abaxial epidermal cells 5 to 7-sided, mostly polygonal-anisodiametric, few polygonal-isodiametric; sides mostly straight, in few straight to curved; cytoplasm scanty; walls slightly thick; surface striated. Dist: irregularly arranged, variously oriented, E.C.F. AD/AB. 535/2182 (base), 525/1196 (middle), 1129/2182 (apex) per cm^2 ; P.R. 5 or 6.

Costal cells: Cells similar to the above, but narrow, mostly polygonal-

TABLE—1
MICROSCOPIC OBSERVATIONS OF *Tylophora Indica* LEAF AND PETIOLE POWDER (15×10)

Field No.	Epidermal cells	Costal cells	Cuticle	Stomata	Trichomes (full or bits)	Tracheary elements	Mesophyll		Crystals	Laticiferous tubes
							Palisade	Spongy		
1.	6 pieces	—	2 pieces	6	8	—	—	—	3	—
2.	8 pieces	1	1 piece	10	7	1	—	—	2	—
3.	9 pieces	—	1 piece	5	7	—	—	10 cells	2	—
4.	13 pieces	—	1 piece	10	8	—	—	—	1	—
5.	8 pieces	1	1 piece	6	4	—	—	—	12	—
6.	7 pieces	1	1 piece	10	10	1	—	5 cells	5	1
7.	4 pieces	1	1 piece	5	12	1	6 cells	—	10	—
8.	5 pieces	—	—	10	14	—	—	—	4	—
9.	6 pieces	1	1 piece	10	11	—	—	4 cells	—	—
10.	12 pieces	—	1 piece	15	6	8	—	—	6	—

linear and anisodiametric, few squarish. Abaxial costal cells slightly larger than the adaxial. Dist: On primary and secondary veins, irregularly arranged, parallelly oriented (Figs. 1-3).

Stomatal complex: Stomata on adaxial and abaxial surfaces mostly paracytic and tetracytic, few anisocytic, anomocytic. Subsidiaries 2-7, monocyclic, mostly indistinct, few distinct, mostly f-type, in few 1 of a-or c-type. Dist: Few on either sides of primary and secondary veins and apex of the adaxial surface, irregularly arranged, parallelly oriented near veins and variously oriented near apex; S.F. 71 per cm², S.I. 5.62 (apex); on the abaxial all over except on primary and secondary veins, irregularly arranged, variously oriented. S.F. 360 (base), 330 (middle), 324 (apex) per cm², S.I. 16.22 (Figs. 2, 3, 7).

Trichome complex: Uniseriate macroform conical hair. *Foot:* 1-celled; cytoplasm scanty; walls thin. *Body:* Unicellular to uniseriate, 1 to 12-celled in length, conical, tapering above, pointed or obtuse at apex; cells of varied lengths; cytoplasm scanty; lateral walls slightly thick; cross walls thin, straight or oblique; surface verrucose. Dist: All over on both surfaces (Figs. 5, 6). T.F. Ad/Ab 3/32 (base & middle), 2/33 per sq.mm (apex).

(b) Surface view of the petiole. **Epidermal cell complex:** Adaxial and abaxial epidermal cells similar to laminar ones but, mostly polygonal-linear and parallelly oriented on both surfaces (Figs. 8, 10).

Stomatal complex: Stomata as on the lamina adaxial but, tetracytic and anomocytic types are dominant and parallelly oriented on both surfaces (Figs. 8, 11, 12).

Trichome complex: Uniseriate macroform conical hair. As on lamina adaxial. Dist: All over on both surfaces, common.

(c) T.S. of leaf-lamina: Flat, bifacial (ribbed abaxially on the midzone), Fig. 13.

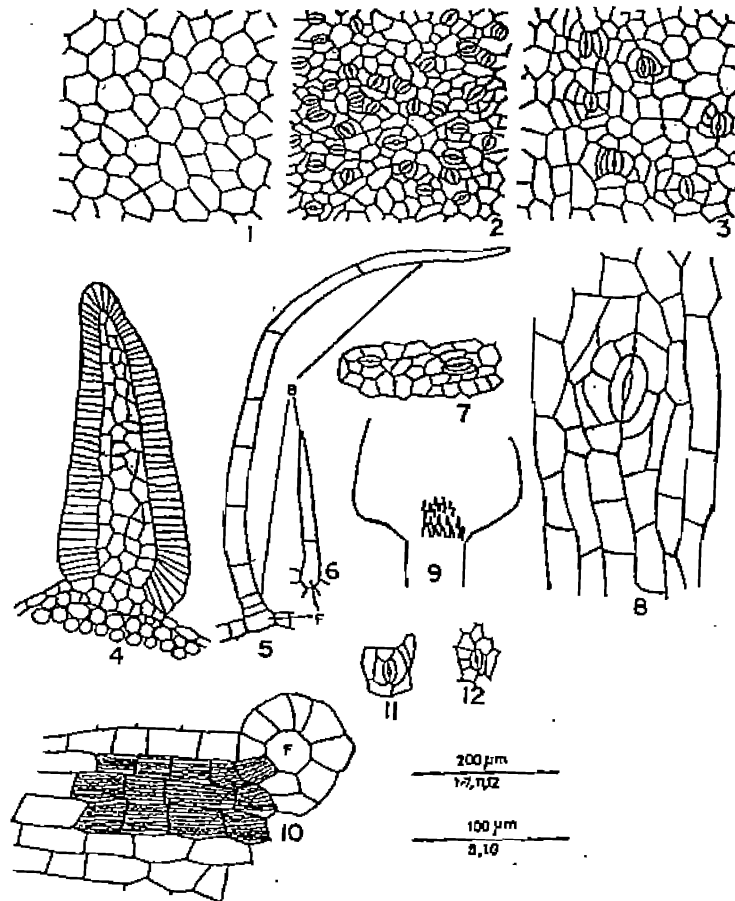
Epidermis: One-layered; adaxial cells oval to circular, radially 17-20-40 μ m long, tangentially 23-37-52 μ m wide; abaxial cells smaller, radially 17-20-22 μ m long, tangentially 13-22-37 μ m wide; cuticle slightly thick. Stomata at the same level as epidermal cells, outer wall of the guard cells ledged (Fig. 14).

Mesophyll: Consists of palisade and spongy mesophyll. Palisade cells hypodermal, adaxial, 1 or 2-layered, large, rectangular, 44-56-82 μ m long, 12-16-22 μ m wide with small intercellular spaces, oriented at right angle to adaxial epidermis, chloroplasts abundant. Spongy mesophyll parenchymatous, 7 or 8-layered; cells of varied shapes, 15-24-35 μ m long, 13-19-38 μ m wide, with large intercellular spaces; chloroplasts few, few cells with single sphaerocrystal; crystals 17-24-26 μ m in diameter (Fig. 14).

In the midvein region hypodermal cells collenchymatous; collenchyma towards abaxial, 2 or 3-layered, mostly angular, few lacunar, diameter 23-33-48 μ m; parenchyma 10 to 14-celled in radius; cells circular to oval, 22-42-87 μ m in diameter with small intercellular spaces. Non-articulate branched

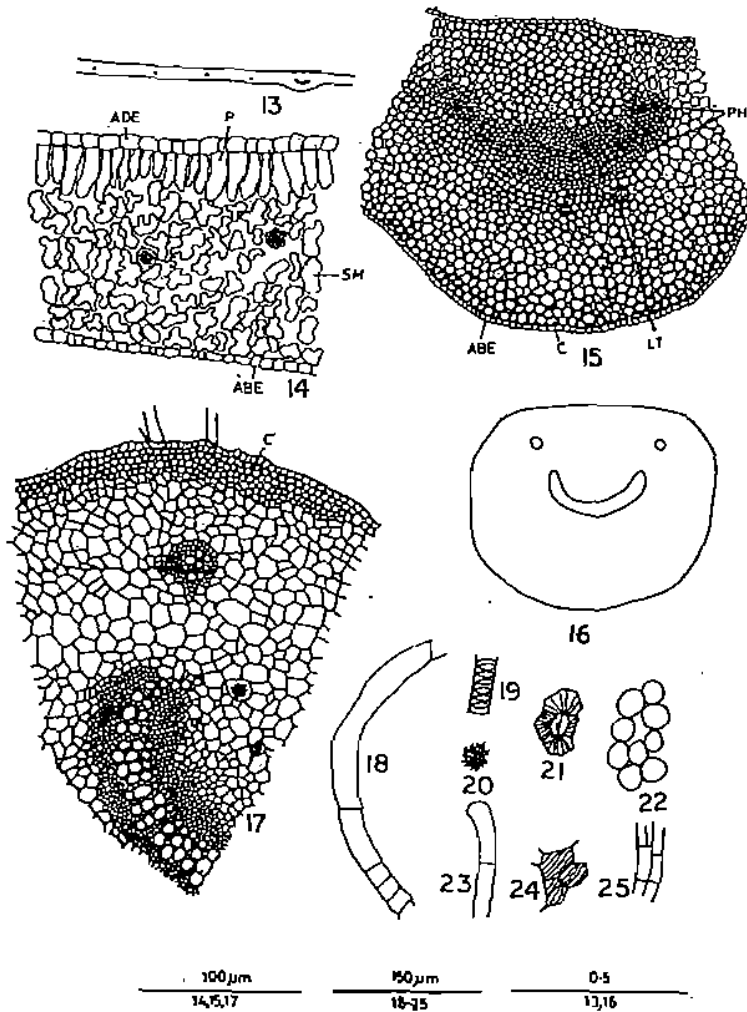
laticifers are present on abaxial and adaxial sides of the major veins in the mesophyll. Starch-sheath absent (Fig. 15).

Vascular system: Primary and secondary veins each with a single shallow u-shaped bundle, minor veins with oval-shaped bundles, all bundles situated slightly towards the adaxial, bicollateral and aperiocyclic. Tracheary elements numerous in primary and secondary veins, few in others, in radial rows, polygonal to circular, 4-15-26 μ m in diameter. Secondary walls of



FIGURES 1-12. *Tylophora indica*: 1 & 3. Adaxial surface views of leaf-lamina from middle region and apex respectively; 2. Abaxial surface view of leaf-lamina from middle region; 4. L.s. of squamellae; 5 & 6. Uniseriate macroform conical hairs from leaf-lamina abaxial; 7. Adaxial surface view of leaf-lamina near midvein showing stomata; 8 & 10. Surface views of petiole adaxial and abaxial respectively; 9. Diagrammatic representation of adaxial leaf base showing squamellae; 11 & 12. Types of stomata from petiole.

(B = Body; F = Foot)



FIGURES 13-15; *Tylophora indica*: 13. T.s. of leaf-lamina showing vascular bundles; 14 & 15. T.s. of leaf-lamina and midrib respectively; 16. Diagrammatic representation of t.s. of petiole showing the distribution of vascular bundles; 17. Portion of petiole in t.s., 18-25. Cell elements in powder; 18 & 23. Portions of the trichomes belonging to uniseriate macroform conical hair; 19. Portion of the tracheary element showing annular rings; 20. Sphaero-crystal; 21. Tetracytic stoma; 22. Group of palisade cells; 24. Polygonal-anisodiametric epidermal cells of adaxial leaf-lamina showing striations; 25. Linear epidermal cells from petiole.

(ABE = Abaxial epidermis; ADE = Adaxial epidermis; C = Collenchyma; LT = Laticiferous tubes; P = Palisade; PH = Phloem; S = Sphaero-crystal; SM = Spongy mesophyll)

tracheary elements of midvein with annular, helical, reticulate, scalariform and pitted types; annular rings mostly free, few directly united, helices single or double, helical-reticulate, reticulate and scalariform; pits elongate, bordered, alternate. Perforation plate simple (Figs. 14, 15).

(d) T.S. of petiole: Semi-circular. Epidermis: One-layered; cells circular to oval, radially 17-19-22 μ m long, tangentially 13-17-22 μ m wide. Ground tissue: Heterogeneous, consisting of collenchyma and parenchyma, 19 to 25-celled in thickness. Collenchyma hypodermal, circular, 3 or 4-layered, mostly angular, few lacunar, diameter 17-22-23 μ m, with small intercellular spaces. Parenchyma 16 to 22-layered, cells oval to circular, 17-38-52 μ m in diameter with small intercellular spaces; cytoplasm scanty. Laticifers as in t.s. lamina, but distributed all over except in the few outer layers. Vascular system: Bundles 3, the median larger, shallow u-shaped, whereas laterals small, oval-shaped, bicollateral. Tracheary elements as in t.s. of lamina but 7-17-26 μ m in diameter (Figs. 16, 17).

(e) Histochemistry: Leaf-lamina and petiole showed negative results for sberin, cyanogenetic glucosides, while lignin was observed in walls of trichomes, tracheary elements; tannin, starch seen in few mesophyll cells of leaf-lamina and ground tissue of the petiole.

(f) Powder analysis: Powder grayish-green in colour with pleasant odour.

Microscopically bits of epidermis consisting of epidermal cells, costal cells, stomata, trichomes; mesophyll tissue consisting of palisade and spongy cells; isolated trichomes in full or pieces; tracheary elements showing annular and helical thickenings; sphaero-crystals of varied sizes and few laticiferous tubes were observed (Figs. 18-25; Table 1).

Water extracts of the powder showed negative colour reaction with 10% iodine, while pale yellow and black colours were observed with 50% KOH and 10% FeCl₃ respectively. Powder was negative for tests of saponins, anthroquinones, cyanogenetic glucosides but positive for tannins, starch and alkaloids.

DISCUSSION

The squamellae known in the family have been morphologically controversial (Solereder, 1908; Woodson, 1930; Woodson and Moore, 1938; Metcalfe and Chalk, 1950; Rao and Ganguli, 1963; Ramayya and Bahadur, 1968). Ontogenetically they are similar to emergences as shown by Ramayya and Bahadur (1968). In *Tylophora indica* they occur near lamina base on the adaxial side (Figs. 4, 9) and represent the emergences of glandular nature. Krishnamurthy and Kannahiran (1970) described the surfaces of the lamina and petiole to be smooth and also reported spherides in laminar epidermal cells. But, present study reveals striated cuticle on both surfaces of the lamina and petiole and absence of spherides (Figs. 1-3, 8, 10). According to Chaudhury and Majumdar (1977) adaxial epidermal cells of the lamina

are slightly bigger than abaxial ones which is presently confirmed (compare Figs. 2, 3, 14).

Recently foliar costal cell distribution due to numerous and consistent variation, has been shown to be taxonomically valuable (Leelavathi *et al.*, 1981). In *T. indica* costal cells are expressed only on primary and secondary veins, which is a useful pointer for identification of leaf material on epidermal basis.

The leaf-lamina was considered to be hypostomatic (Krishnamurthy and Kannabiran, 1970; Chaudhury and Majumdar, 1977). But present study has shown both the lamina and the petiole to be amphistomatic (Figs. 2, 3, 7).

Earlier only paracytic stomata were recorded on the leaves (Solereeder, 1908; Metcalfe and Chalk, 1950; Krishnamurthy and Kannabiran, 1970; Chaudhury and Majumdar, 1977). But presently, paracytic, tetracytic, anisocytic, and anomocytic types have been recorded on leaf-lamina and petiole. Though paracytic and tetracytic types are dominant on leaf-lamina, the tetracytic and anomocytic are so on petiole.

The trichomes of *Tylophora indica* have been variedly described, viz. "Uniseriate clothing hairs" (Solereeder, 1908), "Uniseriate simple hairs" (Metcalfe and Chalk, 1950), "Simple unbranched hairs" (Krishnamurthy and Kannabiran, 1970), "Multicellular hairs" (Chaudhury and Majumdar, 1977). Present study reveals the trichomes to be of the uniseriate macroform conical hair type according to the classification proposed by Ramayya (1975).

Chaudhury and Majumdar (1977) have observed the following characters in t.s. of leaf-lamina and petiole respectively. Lamina bifacial, mesophyll with sphaero-crystals and laticifers; vascular bundles bicollateral, surrounded by pericycle and starch-sheath; midvein hundle crescent shaped. Petiole semi-circular, ground tissue with sphaero-crystals and laticifers; vascular bundles 3 in number, bicollateral, surrounded by pericycle and starch-sheath. Secondary wall thickenings of tracheary elements spiral, annular and scalariform. Presently, most of the above characters have been confirmed except that pericycle and starch-sheath are absent in leaf-lamina and petiole, though few cells surrounding the vascular hundle show presence of starch-grains. Besides, secondary and smaller grade veins of leaf-lamina possess oval-shaped bundles. Secondary wall thickenings of tracheary elements of leaf and petiole are annular, helical, reticulate, scalariform and pitted types; perforation plate simple in leaf-lamina and petiole.

In the microscopic examination of the powder, Chaudhury and Majumdar (1977) have reported epidermal fragments of leaf-lamina, crystals of calcium oxalate and isolated vessels with annular thickening which are all presently confirmed. Besides, fragments of laticifer tubes, epidermal pieces of petiole showing stomata, trichomes and costal epidermal cells of leaf-lamina were also observed.

Earlier starch, lignin, tannin, saponin, glucosides, alkaloids were re-

corded (Chaudhury and Majumdar, 1977). Presently they have been confirmed besides, the non-observation of anthroquinones.

In the light of the present investigation the salient features of the drug which would be useful in identification in its different states are as follows:

Lamina: Elliptic-oblong, lactescent, latex colourless; adaxial base with emergences (squamellae); surface striated; epidermal cells polygonal—anisodiametric, straight-sided; costal cells distinct only on midvein and lateral veins on both surfaces; stomata on adaxial mostly paracytic, tetracytic, confined to either sides of primary vein, secondary veins, apex and on abaxial all over except veins; uniseriate macroform conical hair type present on both surfaces. In t.s. bifacial, sphaero-crystals present in few spongy mesophyll cells; vascular bundles bicollateral; starch-sheath absent.

Petiole: Short, lactescent; epidermal cells polygonal-linear, straight-sided, parallelly oriented; surface striated; stomata anomocytic and tetracytic; uniseriate macroform conical hairs present. In t.s. semi-circular; sphaero-crystals absent; vascular bundles bicollateral, 3 in number.

Histochemically lamina and petiole were positive for lignin, starch, tannin and alkaloids.

Powder is grayish-green in colour and microscopically consists of fragments of epidermis, mesophyll, trichomes, latex tubes, sphaero-crystals and tracheary elements. Water extract of the powder showed pale yellow and black colours with 50% KOH and 10% FeCl₃, respectively.

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