PHARMACOGNOSTIC STUDIES ON CENTELLA ASIATICA (L) URBAN

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Received: 20 March, 1992

Accepted: 3 July, 1992

ABSTRACT: The paper deals with pharmacognosy of Centella asiatica (L.) Urban, including its morphological, anatomical, chemical constituents and powder analysis. Stomata are mostly anisotricytic, isotricytic and few tetracytic. Sphaero-crystals of calcium oxalate are observed in palisade, spongy and ground parenchyma of leaf lamina, petiole and rhizome and absent in roots. Uniseriate flagellate conical hairs present only on abaxial surface of leaf and all over the petiole. The venation is palmatous actinodromous with six primary veins. Midrib consists of a single vascular bundle while petiole consists of 7 – 8, rhizome with numerous bundles are observed and root consists of tetrarch vascular bundle. Powder microscopically show fragment of epidermis, mesophyll, sphaero-crystals, trichomes, collenchyma and parenchyma of leaf, petiole, rhizome and root. Tracheary elements show annular helical and pitted types.

INTRODUCTION

Centella asiatica (L.) Urban, used as brain tonic, diuretic, emmenogogue, stupefyer, narcotic, spasmolytic and stimulant. It is a curative in several diseases viz., genytourinarytract, cutaneous system, urinary and ovarian irritation. itching, headache. giddiness, coma, affections of cellular tissues, bruises, inflamed and swollen parts dysentery, bowl complains, skin tuberculosis, eruptions due to heat or blood and mental weakness. It is an internal and external remedy to various skin diseases, chronic callus scrofulous, ulcerations. syphilitic with gummatous infiltration. chronic and obstinate eczema, psoriasis, leprosy, epilepsy, insanity, enlargement of glands, abscess, chronic rhumetism, infantile diarrhoea, ozoena and amenorrhoea, in various sorts of fevers viz., intermittent, chronic, malarial, also in horseness of phthisis, hypochondriasis. It is a nervine tonic and used as ghritham to improve body colour, youth, memory and long life (Kirtika & Basu, 1933; Anonymous,1966; Nadkarni, 1976).

Glycosides, sterols, tannins. sugars, alkaloids, inorganic salts, amino acids, viz. aspartic acid, glycine, glutamic acid 2 phenyl-alanine alanine and ,flavones, quercetin were recorded in the plant (Bhattacharya, 1956a,b; Datta and Basu, 1962, 1967, 1968; Rastogi and Dhar, 1962,1963; Mukerjee and Dey, 1966; Singh and Rastogi, 1968, 1969), while Rao and Seshadri (1969) have determined some of the chemical structures of glycosides, asiaticosede, medicassocide, drahnocide (c.f. Shah and Qadry, while 1971). pharmacological and clinical properties have been determined by (Dhar et al, 1968; Ramaswamy et al., 1970; Appa Rao et al., 1967, 1969, 1973). Though vegetative twig along with roots are used in drug

preparation, the anatomical and pharmacognostic analysis are scanty (Solereder, 1908; Metcalfe and Chalk, 1950; Aiyer and Kolamal, 1964) and confined only to lamina and petiole. Hence the present study was taken by which includes morphology, anatomy, histochemistry and powder analysis to bring out the salient features of the drug.

MATERIALS AND METHODS

Twigs of *Centella asiatica* were collected from plants growing at Osmania University campus, Hyderabad, Andra Pradesh. The methodology and the terms used in this study are after Prabhakar et al., (1984) Leelavathi et al (1981), Prabhakar and Leelavathi (1989). Abbrevations used in the text are Ab: abaxial surface of the leaf; Ad: adaxial surface of the leaf; D: diameter; Dist: distribution; L/W: length and width; a: abutting type; f: free type and c:common type of subsidiaries (Ramya and Rajagopal, 1980).

OBSERVATIONS AND DISCUSSION

The genus *Centella* is represented by 40 species (Airy Shaw, 1966) of which *C.asiatica* (*Hydrocotyle asiatica* L., *H.wightiana willd.*) is reputed for its medicinal uses. *C.asistica* is widely distributed throughout India from tropical to sub-tropical regions, ranging from 0-2000 feet altitude, from Himalaya to Ceylone and Malaya (Hooker, 1879).

Vernacular names: Arabic artanivaehindi. Assam Jharniba: _ Bengal Manimuni; _ Brahamanduki, Tholkuri; Bombay – Karinga, Karivana; Burma – Minkuabin; Cambodia – Trachiek kranh; Canarese - Vondelaga; Dacca -Bhikapurni; Deccan - Vallari; Dutch Indies - Antanon, Kakikoeda, pegagan; English -

Indian Penny wort, Thick leaved penny wort; French - Bevilaque, Cotyliole, asiatique, Hydrocote d' Asie; German Waaernabel; Gujarat - Barmi; Hindi -Brahmamanduke, Khulakhudi: Hova – Loviantsahonantanenty, Raviolesoka, Vilantsahonantanenty; Indochina - Ban dai uyeh, Rau ma, Tich huyet thao; Kashmiri -Brahm bhuti; Kwang tung – Loui kong ken; La Reunion - Cochlearia du pays; Malaya -Pegaga; Malayalam - Kodagam, Kutakam, Muthal; Marathi - Brahmi; Mundari -Cokeara; New Caldonia - Volari; Persian -Sardeturkastan; *Philipines* Rabasa; _ Bhekaparni, Sanskrit Bheki. Brahmamanduki, Darduchada, Divya, Mahaushandi. Mandukaparni, Mandukaparnika, Manduke, Supriya, Tvashti; Sinhalese – Hingotukola; Tagalong - Taguipcuhol, Taguipsosa, Taquipcohol, Taquipsuso; Tamil – Babassa, Vallari; Telughu – Babasa. Bekaparnamu, Bokkudu; Urdu – Barhmi, Visayan, Yahonyahon (Kirthikar & Basu, 1933).

Morphology

A slender, prostrate or creeping Habit : perennial aromatic herb with long creeping runners. Branches long, slender, slightly succulent, greenish or dark reddish with long internodes and rooting at every node. Tap root, fleshy, a bunch of Root: adventitious roots at each node. Leaf: Simple, alternate, exstipulate, petiolate, all leaves arising from rhizomes, 7 - 8 in number. Variable in size. Lamina reniform or orbuscular - reniform; marginal shallow crenate to denate, green in colour, 1.2 to 5.0 cm in diameter, pubescent abaxially, venation palmatous actinodromous with six primary veins. Petiole: Quite long, 3 to 5 cm with a sheathing base, cylindrical above, adaxially groved, hairy, feebly hairy above. Inflorescence: Axillary, umbel, each umbel consists of 3 or 4 flowers; peduncle 1 to 1.5

cm long, pale green, glabrous. *Flower:* Incomplete bisexual, irregular and bracteate, pedicellate, pedicel short. *Bract:* Bracts 2, ovate, apex acute, glabrous. *Perianth:* Five, united, green to pink in colour. *Androcium:* Stamens 5, epiphyllous, anthers, bilobed, filaments short. *Gynoecium:* Ovary superior, bicarpellary syncarpus, locules 2, styles 2, terminal, stigma simple. *Fruit:* Cremocarp, longer than broad, 0.5 cm long, hard. *Flowering and Fruiting:* Throughout the year.

Surface view of leaf lamina: Laminar epidermal cells were reported to be polygonal - anisodiametric with straight andticlinal walls (Datta and Mukerji, 1952). However in the present study the adaxial and abaxial epidermal cells are recorded to be mostly polygonal - anisodiametric, some polygonal – linear and trapezoidal, anticlinical walls are mostly straight with few straight to curved on both adaxial and abaxial sides; walls thin, contents scanty, few cells with mucilage; surface striated. Adaxial / Abaxial cells 81,600/1, 20,000 per cm². *Dist:* All over the leaf lamina except on primary, secondary and tertiary veins; irregularly arranged; variously oriented. Costal cells: Cells are polygonal to linear, few rectangular linear; contents scanty surface striated. Dist: On primary, secondary and tertiary veins; irregularly arranged, parallel oriented.

Stomatal Complex: Stomata are described to be mostly rubeaceous (Dutta and Mukherji, 1952). Presently they are found to be mostly anisotricytic, isotricytic, some tetracytic and are monocyclic. Subsidiaries indistinct, of 'a' – and 'c' – type on adaxial, mostly of 'a' – some of 'c' – and 'f' – type on abaxial. *Dist:* Allover, except on veins, irregularly arranged, variously oriented; stomatal frequency ad/ab. 1200 / 24000 per cm² and stomatal index 12 / 16. Mucilage present in adaxial and abaxial of spidermal cells and only in abaxial subsidiaries and costal cells.

Trichome complex: Uniseriate flagellate conical hair: *Foot:* One celled; walls thin, contents scanty. *Body:* Uniseriate, 2 to 8 – celled, flagellate, conical, tapering above, pointed at apex; cells quite longer than broad, mostly of equal lengths; contents dense, walls thin; surface smooth. *Dist:* Abaxially allover, common more frequent on veins, mostly caduceus and adaxially absent (Fig. 10 & 11).

Transaction of leaf lamina: Flat. dorsiventral, abaxially ribbed at six major veins (Fig.4). Epidermis: One layered; epidermal cells mostly rectangular, some barrel - shaped, adaxially radially 26-32-40 μm long, tangentially 7-10-13 μm wide; walls thin; cytoplasm scanty, chloroplasts absent: cuticle thin. Stomata on both sides. at the same level as epidermal cells, outer wall of the guard cells ledged. Trichomes 1 as described in surface view. Mesophyll: Consists of palidade and spongy cell; palisade cells adaxial, 2 layered, cylindrical to rectangular, 20-31-41 µm long, 13-15-20 um wide, with small intercellular spaces, chloroplasts abundant, few cells in the second layers with a single sphaero-crystal of calcium oxalate; crystal 16-18-20 µm in diameter. Spongy mesophyll 3 to 5 layered, of varied shapes, mostly oval, 4-15-20 µm in diameter, parenchymatous with intercellular spaces, chloroplasts few, few cells with a sphearo – crystals.

In the six primary veinular zones, hypodermis composed of 5 or 6 – layered collenchyma adaxially, 1 or 2 – layered abaxially; cells mostly angular, few lacunar, diameter 7 – 16 – 27 μ m. Inner ground tissue parenchymatous, parenchyma 16 to 20 cells in radius, cells circular to oval, diameter $20 - 32 - 47 \mu m$ with small intercellular spaces, chloroplast few, few cells with sphaerocrystals, crystal 16 - 18 -20 µm in diameter. Secondary ducts present on both abaxial and adaxial sides of major veins; ducts surrounded by 5 to 8 - barrel shaped epithelial cells, inner diameter of the ducts $13 - 19 - 23 \mu m$, outer diameter $23 - 19 - 23 \mu m$ 32 – 47 µm. Starch- sheath absent. Vascular system: Each primary vein with one wedge shaped bundle, minor veins with circular to oval shaped bundles, all bundles situated in the centre of the ground tissue; bundles collateral. without pericycle. Tracheary elements many in primary veins, few in others, arranged in rows or in group of 3 or 4, polygonal to circular, 7 - 10 - 13µm in diameter (Fig.4). Secondary walls of tracheary elements with annular, helical, reticulate and pitted types; annular rings free, helices single or double, rarely pitted; pits elongated. Perforation plate simple (Figs. 23-25).

Venation pattern: Palmatous actinodromous. Primary vein; Six in number, branched. The course of median primary vein straight, lateral primaries uniformly curved, angle of divergence of lateral primaries acute to obtuse. Secondary *veins:* on either side of median and lateral primaries. some of the secondaries terminating at margin while others joined together to form prominent arches angle of divergence acute, moderately thick, straight to curved, loop forming branches present. Tertiary veins: Randomly reticulate. Vinlets: Mostly branched, few simple. Well developed, randomly Areoles: oriented, polygonal (Fig. 3).

Surface view of petiole: Epidermal cells mostly polygonal-linear, rectangular – linear throughout, with few squarish cells near base; sides mostly straight, few straight to curved, walls thin; contents scanty, dense in

some; surface smooth. Dist: Irregularly arranged, mostly parallely oriented, few variously oriented. Stomatal complex: Stomata tetracytic, anisotricytic, anomocytic, few paracytic; monocyclic. Subsidiaries indistinct of 'f' – type some are of 'a' - type. Dist: Irregularly arranged, parallely oriented. *Trichome complex:* Similar to those described on surface view of leaf lamina. Dist: Allover, common near base, few above.

Transaction of petiole: *Distal part:* Shallow U-shaped (Fig.5) Epidermis: One layered; adaxial cells mostly polygonal to barrel-shaped and oval-shaped, radially 8 - $14 - 27 \mu m$ long, tangentially 8 - 21 - 40µm wide; abaxial ones slightly smaller, radially $6 - 10 - 17 \mu m$ long, tangentially 6 $-11 - 13 \,\mu\text{m}$ wide; cytoplasm scanty; walls Stomata few, sunken, thin, cuticle thin. outer wall of guard cells ledged. Trichomes described as in surface view. Ground tissue: Heterogenous, consisting of collenchyma and parenchyma. Collenchyma hypodermal, 1-layered, cells angular, circular to polygonal, $7 - 10 - 15 \ \mu m$ in diameter. Parenchyma 14 to 16 – layered in thickness, 1 or 2 – layered near margins, 7 - 27 - 47um in diameter, interspersed with large air cavities on either side of the vascular bundles, cavities 4 to 6 in number, middle ones larger, tangentially 368 µm long, radially 134 µm wide, gradually reducing towards margin; air cavities at margin tangentially 160 µm long, radially 10 µm wide; few cells with a single sphaero crystal of calcium oxalate, crystal 16 - 18 -20 µm in diameter; secretory ducts present on abaxial side of major vascular bundles, each duct surrounded by 3 to 8 - barrel shaped epithelial cells, $15 - 20 - 24 \mu m$ wide, $10 - 15 - 20 \mu m \log$. Vascular system: Vascular bundles 7 – 8 in number, larger ones towards centre, smaller ones towards margins; bundles oval to ovate;

maior veins surrounded bv sclerenchymatous sheath on abaxial side; sclerenchyma 1 to 3 – layered, cells polygonal, $3 - 7 - 11 \mu m$ in diameter, walls thick; contents scanty. Tracheary elements more in middle, few in laterals, arranged in rows or in groups, polygonal to circular in shape, $4 - 13 - 17 \mu m$ in diameter (Fig.5). Secondary walls of tracheary elements throughout the petiole are annular and helical types, annular rings, and free helices single or double. Perforation plate simple (Fig.18.)

Middle part: Similar to petiole proximal part except, oval to circular in shape, fistular (Fig. 6). Epidermal cells slightly smaller, radially $8 - 10 - 20 \,\mu\text{m}$ long, tangentially 8 -10 – 18 µm wide. Ground tissue: Heterogenous, collenchyma 1 or 2 layered, 6 $-13 - 20 \,\mu\text{m}$ in diameter; parenchyma 7 to 10 – celled in radius throughout, 20 - 45 – 60 µm in diameter; contents scanty with small intercellular spaces, air cavities and calcium oxalate crystals absent, but in pith region large cavity is present. Vascular system: Bundles six, all are wedge shaped arranged in a ring. Bundles collateral ovalshaped, apericyclic. Tracheary elements in radial rows, 4 - 6 elements in each row in larger bundles, in groups of 3 or 4 in smaller adaxial ones, $3 - 12 - 18 \ \mu m$ in diameter (Fig. 6).

Proximal part: Similar to petiole middle except, semicircular with in outline with lateral wings in outline (Fig.7). *Ground tissue:* Parenchymatous, 7 to 9 – layered adaxially, 6 to 10-layered abaxially; central hollow space gradually reduced to absent. Vascular bundles 5 – 6 in number, surrounded by sclerenchymatous bundle – sheath; circular non-modulated, bundles slightly larger, bundles are arranged in an arch form. Tracheary elements in radial rows, 2 to 4 in each row (Fig.7).

Transaction of Rhizome: Circular to ovalshaped. *Phellem:* Many – layered; cells squarish to rectangular, radially 20 - 37 - 68 μ m long, tangentially 20 – 24 – 27 μ m wide; cytoplasm scanty; walls thin (Fig.9) Phelloderm: Parenchymatous, many layered; cells circular to polygonal, 20 - 55– 75 µm in diameter, with small intercellular spaces; cytoplasmic contents scanty but mostly with numerous starch grains, few cells with a single sphaero-crystals to calcium oxalate, crystal $20 - 22 - 26 \mu m$ in diameter. Cortex: Similar to phelloderm but cells slightly smaller in size. Vascular system: Bundles numerous, collateral, strap shaped, arranged in a ring tracheary elements numerous, polygonal, 6 - 22 - 33µm in diameter, arranged in radial rows. Tracheary elements helical, sclariform and pitted types; helices single or double; pits elongate, bordered, alternate. Perforation plate simple. Medullary Rays: Rays 3 to 7 celled wide; cells polygonal, 20 - 36 - 67µm in diameter; cytoplasmic contents scanty, starch grains present; walls thin. Homogenous, 14 to 16 celled in Pith: diameter, paranchymatous. Cells circular to polygonal, $35 - 52 - 67 \mu m$ in diameter with small intercellular spaces; cytoplasmic contents scanty, mostly cells with numerous starch - grains, few with a single sphaerocrystal of calcium oxalate, crystal 20 - 22 -26 µm diameter; walls thin (Fig.9).

Transaction of root: Circular, *Epiblema:* One layered; cells oval to circular, $3 - 8 - 10 \mu m$ in diameter; contents scanty; walls thin (Fig.8) *Cortex:* Homogenous, 6 to 9 – layered, parenchymatous. Parenchyma circular to oval shaped, some radially elongate, smaller ones towards, epiblema, larger ones towards centre, $6 - 30 - 65 \mu m$ in diameter with small intercellular spaces; contents scanty; walls thin. Interspersed with large air cavities (Fig. 8) *Endodermis:* One layered; cells barrel-shaped, radially 3 – 6-8 μm long, tangentially 3 to 4 μm wide; with casparian strips; contents scanty; walls thin (Fig.8). *Pericycle:* Solerenchymatous, 2 or 3 layered, discrete, alternating with xylem bundles, cells polygonal, small, 3-5-7 μm in diameter; contents scanty; walls thick (Fig.8). *Vascular system:* Vascular bundle tetrarch, radial, exarch. Tracheary elements polygonal, 3 - 15 - 30 μm in diameter. Secondary walls of tracheary elements annular to helical types; annular rings free, helices single or double. Perforation plate simple.

Histochemistry: Lignins was observed in tracheary elements of lamina, petiole and root, while tannin and cynogenetic glycosides in mesophyll of leaf and ground tissue of petiole and root. Suberin in the cuticle of leaf – lamina and petiole, while starch, calcium oxalate in mesophyll of lamina, ground tissue of lamina, rhizome and petiole while absent in roots.

Powder analysis : Powder is green and microscopically consists of bits of cuticle, epidermal cells and costal cells of leaf lamina showing striations; polygonal – anisodimaetric cells of leaf lamina, linear cells of costae and petiole, bits of epidermis with anisotricytic, isotricytic, tetracytic and anomocytic stomata (Fig. 12 & 13). Fragments of uniseriate flagellate conical hairs (Fig. 15), sphaerocrystals. (Fig. 19 & 21), palisade and spongy cells of leaf lamina (Fig. 16 – 18) and starch grains of rhizome (Fig. 22). Collenchyma and parenchyma of

petiole, root and rhizome (Fig. 20). Tracheary elements showing annular, helical and pitted thickenings (Figs. 23 – 25).

Water extracts of the powder showed dark yellow and black colours with 50% KOH and 10% FeCl₃, respectively while no colour change was observed with 10% iodine.

Explanation to figures

Figs. Centella asiatica (L.) Urban., Fig. 1 & 2. Adaxial and abaxial epidermis of leaf lamina (X 76); 3. Venation pattern of leaf lamina: 4. Transection of leaf lamina from midrib (X 33), lamina (X 55); 4 – 7. Sectional view of petiole, 4. Distal part (X 30); 5. Middle part of the petiole (X 21); 7. Proximal part of the petiole (X 11); 8. Sectional view of root (X 14); 9. Sectional view of rhizome (X 27); 10 & 11. Uniseriate flagellate conial hairs of adaxial leaf lamina; 12 - 13. Pieces of epidermal cells showing stomata; 14. Pieces of costal cells; 15. Bits of conical hairs; 16 & 17. Pieces of leaf lamina showing epidermis and palisade 18 Group parenchyma: of spongy parenchyma; 19. Ground tissue showing sphaero - crystal; 20. Pieces of ground showing collenchyma tissue and parenchyma; 21. Isolated sphaero – crystal; 22. Ground tissue of rhizome showing starch grains; 23 - 25. Pieces of tracheary elements (ac – air cavity; bs – bundle; lpb – lateral primary vein; lvb – lateral vascular bundle; mp – median primary vein; p – palisade parenchyma; pc – parenchyma).





ACKNOWLEDGEMENTS

The authors are thankful to the Head, Department of Botany for providing necessary facilities. One of the authors (S. Jelani) is thankful to CSIR for financial assistance.

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