ORIGINAL ARTICLE

Morpho-anatomy of leaf, stem and root of *Alternanthera sessilis* (L.) R. Br. ex DC and *Alternanthera pungens Kunth (Amaranthaceae)* and its significance in drug identification

H.C. Gupta¹, J. Raj², A. Rathi^{3*}, E. N. Sundaram³, S. Kumar³ and R.K. Manchanda¹

- ¹ Central Council for Research in Homoeopathy, New Delhi
- ² H-58, South Extn, New Delhi
- ³ Central Research Institute (H), Noida

The present communication deals with morpho-anatomical characters of leaf, stem and root of *Alternanthera sesilis* and *Alternanthera pungens* and its signifinace in identification and differentiation of raw drug originated from these species. The plant characters of diagnostic importance are for example colour of stem is green in *Alternanthera sesilis* and brown in *Alternanthera pungens;* flower of *Alternanthera sesilis* possess three stamens while *Alternanthera pungens* possess five stamens. The study will be useful in confirming the authenticity of raw drug and also serve as a reference for advanced studies of these two such closely related species.

Keywords: Alternanthera sessilis; Alternanthera pungens; pharmacognosy.

INTRODUCTION

The genus Alternanthera (family Amaranthaceae) includes approximately 80 species, native to tropical and sub-tropical regions of Australia and South America¹. In India, five species have been recorded, out of which Alternanthera sessilis (L.) R. Br. ex DC and Alternanthera pungens Kunth are utilized extensively as raw drug sources worldwide in many traditional systems of medicine. 1,2 As drugs are collected by traditional practitioners who have inherited herbal practices by tradition, the identification is mostly based on morphological features or other artificial and traditionally known characteristics. However, in such cases, there is a chance of misidentification resulting in spurious quality of medicine. Therefore, it is essential that anatomical details of authentic drug material should be prepared as diagnostic characters in identification and differentiation between closely related species to avoid any ambiguity.3

Alternanthera sessilis (Syn. Alternanthera triandra Lam., Alternanthera prostrata Don., Achyranthus triandra

*Address for correspondence:

Dr. Anshu Rathi, Senior Research Fellow (H) Central Research Institute (H), A1/1 sector 24, Noida-201 301, Uttar Pradesh, Email: anshurathi@rediffmail.com

Received: 30th August 2012 Accepted: 29th January 2013 Roxb.), commonly known as 'matsyakshi' in Hindi, is a small prostrate herb with several spreading branches bearing short petiolate, simple leaves and small white flowers. It grows throughout the warmer parts of India and is frequently found in wet places especially around tanks and ponds. $^{2-7}$ In indigenous systems of medicine, it is used as a galactagogue, cholagogue, febrifuge and "cure" for snake bite. 2,6,8,9 The plant is reported to have hydrocarbons, enecycloartanol, cycloecucalenol, stigmasterol, campesterol, β -sistosterol, α -spinasterol, oleanolic acid rhamnoside, 24-methylene cycloartenol, cycloeucarlenol, lupeol, $5-\alpha$ -stigmasta-7-enol, and its palmitate, nonacosane, 16-hentriacontane, and handianol. 2

Alternanthera pungens (Syn. Alternanthera repens L.), commonly called as 'Chaff-flower' or 'khaki-weed' is a creeping herb, with small paired leaves, bearing flowers with whitish bracts in small cylindrical or almost round heads. It is native to South Carolina to Florida and California, widely spreading on road sides and in waste places. ¹¹ In India, it was introduced during in 1918 and the first report of its occurrence was from southern India. ¹² The plant is reported to contain azulene, borneol, camphene, eudesmol, geraniol, limonene, linalool, pinene, terpineol and thujone. ¹³ It is said to possess diuretic properties and its decoction is taken in gonorrhoea. ^{14,15} In Homoeopathic system of medicine the drug was proved by Dr. Manuel M. De

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Legarreta of Mexico, in 1911 and found mention in the Homoeopathic Pharmacopoeia of United States under official name *Paronichia illeceberum*. ^{16,17}

In view of the medicinal uses of *Alternanthera* sessilis and *Alternanthera* pungens, an attempt is made to study morpho-anatomical data of the leaf, stem and root which would provide data for differentiation and identification of these two closely related species.

MATERIALS AND METHODS

The plant materials, obtained from Survey of Medicinal Plants & Collection Unit, Nilgiris District, Tamil Nadu, India, were preserved in F.A.A. and used for anatomical studies following the method of Wallis. ¹⁸ Epidermal peels were taken by scraping with razor blade. Free hand sections were cut, stained in safraninfast green combination, mounted and subjected for microscopical studies by following the method of Johansen. ¹⁹

OBSERVATIONS

External morphology: Leaf: green, simple, sessile, 1.3-7.5 cm long, 0.3-2.0 cm broad, linear, oblong or elliptic, obtuse or subacute, tapering towards the base. **Stem:** yellowish-brown to light-brown, cylindrical, occasionally sub-quadrangular, fracture short. **Root:** creamish to grey, cylindrical, 1-6 mm in diameter, numerous roots arising from the main tap root as lateral rootlets: fracture short.



Fig. 1A: Alternanthera sessilis: Whole plant

Anatomy

Leaf: The adaxial surface shows polygonal epidermal cells and abaxial cells are sinuous (Fig. 1B) with rosette aggregates of calcium oxalate crystals. Anomocytic and diacytic stomata are

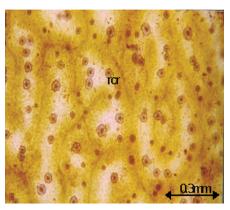


Fig. 1B: Adaxial surface view of leaf

seen on both surfaces (Fig. 1C), the later type is predominant on the abaxial surface. Trichomes are of two types: (a) multicellular, non-glandular, uniseriate with characteristic interlocking cells and (b) capitate trichomes having 3-4 celled stalk and unicellular,

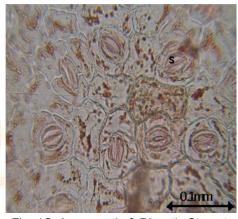


Fig. 1C: Anomocytic & Diacytic Stomata

spherical ellipsoid head. Both types of trichomes are covered with a conspicuous papillose cuticle (Fig. 1D, E). On the epidermis, the trichome detachment areas are distinguishable. Transverse section of leaf through midrib shows single layer of upper epidermis and double

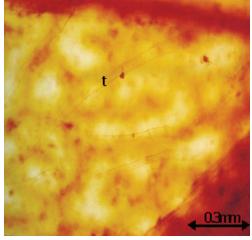


Fig. 1D: Trichomes

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Fig. 1E: Trichomes

layer of lower epidermis covered with a smooth and thin cuticle. Mesophyll is dorsiventral and consists of a single layer of palisade parenchyma and about four layers of spongy parenchyma, the latter occupy 50-60% of the mesophyll. Idioblasts with druses are prominent in the palisade parenchyma. Both upper and lower epidermis is underlain by 4-5 layers of chlorenchyma. The midrib, in transverse section, shows a biconvex shape, prominent on the abaxial face followed by 3-4 layers of angular collenchyma on the adaxial side and 4-7 on the opposite side. Collateral vascular bundles. accompanied by thick-walled parenchymatous cells adjoining the phloem, are embedded in the ground parenchyma. These vascular bundles vary in number and arrangement, from the crescent-shaped central vascular bundle (Fig 1F).

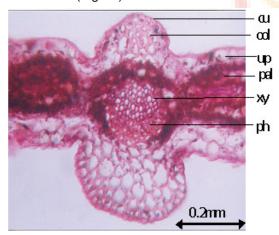


Fig. 1F: TS of leaf
Cu-Cuticle, Col-Collenchyma, up-Upper epidermis,
pal-Palisade, xy-Xylem, ph-Phloem

Stem: The stem in transverse section shows circular outline. The epidermis is single-layered and consists of polygonal cells. The trichomes present on the stem are non-glandular. The cortex is composed of 6-7 layers of loosely arranged parenchymatous cells with alternate strands of chlorenchyma and angular collenchyma. In the vascular cylinder the first cambium forms phloem centrifugally and xylem centripetally.

Vascular bundles are conjoint, collateral arranged in a ring. Each vascular bundle is encapped by a patch of pericyclic fibres. Pith is wide and consists of thin-walled large parenchymatous cells. Idioblast containing druses are found in the cortex and in the pith (Fig.1G).

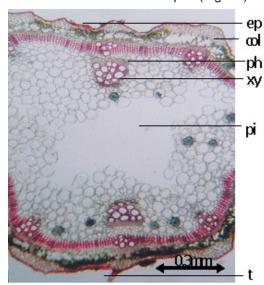


Fig. 1G: TS of stem ep-Epidermis, col-Collenchyma, ph-Ploem, xy-xylem, pi-Pith

Root: The root in a transverse section shows circular outline. The cork is composed of 4-5 layers of tangentially elongated suberised rectangular cells followed by 4-5 layers of loosely arranged parenchymatous cortex. Pericycle and endodermis are indistinct. Phloem is narrow and consists of phloem fibres, sieve tubes, companion cells and phloem parenchyma. Xylem is composed of radially arranged tracheary elements. Xylem is exarch, and consists of vessels, tracheids, fibres and xylem parenchyma (Fig.1H, Table.1).

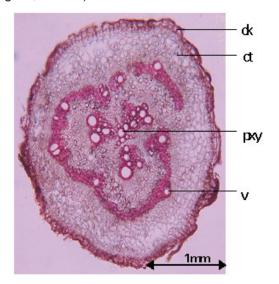


Fig. 1H: TS of root ck-Cork, ct-Cortex, pxy-Primary xylem, v-Vessel

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 Table 1: Diagnostic features of two Alternanthera species: Alternanthera sessilis and Alternanthera pungens

Parts	Alternanthera sessilis	Alternanthera pungens
	Macroscopic cha	aracters
Stem	Prostrate, cylindrical but slightly ridged, branched, solid, green.	Similar but, stem clothed with shaggy hair and brown.
Leaves	Opposite, linear – lanceolate, oblong or ovate, sessile, exstipulate, margin entire, measuring up to 20mm long and 8 mm broad.	Opposite pairs unequal, obtuse, entire, tapering at the base, orbicular or ovately orbicular, measuring up to 35mm long 28 mm broad.
Flowers	Bracteoles scarious.	Bracteoles with spinscent awns.
Perianth	Lobes 5, 2.5-3 mm long, segments not spine-tipped.	Tepals very dissimilar, glochidiate hair present, outer 2 tepals similar to spinscent bract, 2 innermost deeply keeled concave, hairy on the back becoming spiny
Androecium	Stamens 3.	Stamens 5.
Fruits and seeds	Utricle with winged margins. Seed, suborbicular.	Utricle. Seeds round and brownish.
Microscopic characters		
Leaf:		
Stomata	Anomocytic and diacytic on both surfaces, although the later type is predominant on the abaxial face.	Only anomocytic stomata are seen on both surfaces
Trichomes	Non glandular uniseriate with characteristic interlocking cells; multicellular with 3-4 celled; glandular, capitate, 3-4 celled stalk and unicellular, spherical ellipsoid head all coated with a conspicuous papillose cuticle	Non glandular uniseriate to multicellular trichomes with characteristic interlocking cells; all coated with a conspicuous papillose cuticle, glandular type of trichomes absent.
Epidermis	Uniseriate layer of upper epidermis while biseriate layer of lower epidermis	Uniseriate layer of on both the surface
Midrib	Palisade parenchyma single layer and 4 layers of spongy parenchyma.	Palisade parenchyma and about 3 layers of spongy parenchyma.
Stem:		
Cortex	6-7 layer of loosely arranged, oval to circular parenchymatous cells.	2-6 layers of thick-walled parenchymatous cells.
Root:		
Cortex	4-5 layers, of compactly arranged parenchyma in cortex region.	8-10 layers of compactly arranged thin-walled parenchyma in cortex.
Vascular bundle	Phloem narrows, parenchymatous followed by xylem. Xylem is composed of radially arranged vascular bundle. Xylem exarch, metaxylem vessels meet in the centre.	Primary xylem triarch cambium forms a complete ring of xylem surrounded phloem at the centre which results in anomalous secondary growth.



Fig. 2A: Alternanthera pungens whole plant

External morphology: Leaf: green, simple, 3.5x2.8 cm, orbicular or ovately orbicular, opposite pairs unequal, obtuse, entire, tapering at the base. **Stem:** yellowish-brown to light-brown, cylindrical, occasionally sub-quadrangular, clothed with shaggy hairs, fracture short. **Root:** cream to grey, cylindrical, 1-6 mm in diameter, numerous roots arising from the main tap root as lateral rootlets; fracture short.

Anatomy

Leaf: The surface view of leaf has adaxial epidermal cells with sinuous anticlinal abaxial cells. Anomocytic stomata are present on both surfaces (Fig.2 B). Uniseriate, multicellular trichomes with characteristic



Fig. 2B: Surface of leaf S-Stomata

interlocking cells, all covered with a conspicuous papillose cuticle are seen (Fig.2 C). Vertical section



Fig. 2C: Trichome

of leaf passing through midrib shows a single layer of epidermis, covered with thin cuticle on both lower and upper surfaces. The mesophyll is dorsiventral, comprising one layer of palisade parenchyma and about three layers of spongy parenchyma. Idioblast containing druses are present in the ground spongy parenchyma. The midrib, in vertical section, shows a prominent ridge on both abaxial and adaxial surfaces followed by 3 layers of chlorenchyma which lie below the upper and above the lower epidermises. The meristele consists of crescent-shaped vascular bundle embedded in parenchymatous ground tissue (Fig.2 D).

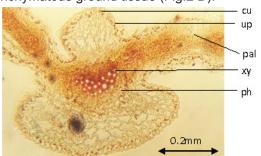


Fig. 2D: TS of leaf cu-Cuticle, up-Upper Epidemis, pal-Palisade, xy-xylem, ph-Phloem

Stem: In a transverse section the stem is circular in outline. The epidermis is single-layered and covered with a thin layer of cuticle and interrupted by stomata and uniseriate or multiseriate types of trichomes. Hypodermis is 3-4 layered and is composed of collenchymatous cells. The cortex comprises 2-6 rows of thick-walled parenchymatous cells. Endodermis is indistinct. The vascular bundles are conjoint, collateral and are arranged in a ring. Each vascular bundle is encapped by a patch of pericyclic fiber. Pith is wide and consists of thin-walled large parenchyma cells. Idioblast containing druses are found both in the cortex and pith (Fig.2 E).

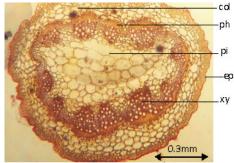


Fig. 2E: TS of stem col-Collenchyma, ph-Ploem, pl-Pith ep-Epidermis, xy-Xylem

Root: In a transverse section, root is circular in outline. The cork comprising of 4-5 layers of tangentially elongated suberised rectangular cells followed by 8-10 layers of compactly arranged thin-walled parenchymatous cortex. Primary xylem is triarch, and

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cambium forms a complete ring of xylem surrounded of phloem at the centre. Unusual secondary growth occurs by formation of cambium arising successively outward from the centre of root, each producing xylem towards the inside and phloem towards outside, thus exhibiting collateral vascular strands arranged in concentric circles and embedded in parenchymatous conjunctive tissue) (Fig.2 F, Table.1).

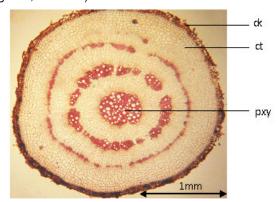


Fig. 2F: TS of root ck - cork, ct-cortex, pxy-Primary xylen

DISCUSSION

Earlier study showed that the leaf morphology of Alternanthera sessilis and Alternanthera pungens are similar, but the present investigation shows that leaves can be distinguished on the basis of size and stomata type. We found two types of stomata viz., anomocytic and diacytic in Alternanthera sessilis whereas only anomocytic type of stomata for Alternanthera sessilis was previously reported.20 In case of Alternanthera pungens only anomocytic type of stomata are predominant. The trichomes in the family Amaranthaceae have been reported to be of taxonomic value. 22, 21 We noted glandular capitate trichomes, 3-4 celled stalk with unicellular, spherical, ellipsoidal head in Alternanthera sessilis whereas Alternanthera pungens possess unicellular nonglandular trichomes along the veins of the epidermal surface of leaf. Concerning the stem organization in secondary growth, the Amaranthaceae family has an unusual development characterized by a sequence of concentrical extra cambium, formed outside the original cambium.21 In the present study, secondary growth pattern of Alternanthera sessilis was noted as first cambium formed a complete cylinder and differentiated bidirectionally, as described earlier.²³ However, unusual secondary growth of cambium was also evident in case of Alternanthera pungens. We observed anomalous thickenings in stem and roots of similar kind, the first accessory cambium arising in the pericambial region and the subsequent one in the secondary ground tissue. These data may be considered as standard for correct identification and differentiation in two closely related species of *Alternanthera*.

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सार: प्रस्तुत अध्ययन ऑल्टरनन्थेरा सेसिलिस एवं ए. पजेंस के पत्ते, तने और जड़ की आकार—शारीरिक संरचना और इस प्रजाति के पौधों से उत्पन्न अपरिष्कृत औषधियों के चिह्नीकरण और विभेदीकरण से संबंधित है। यह अध्ययन अपरिष्कृत औषधि की प्रमाणिकता को सिद्ध करने में एवं इन दो प्रजातियों से संबंधित अग्र अध्ययनों हेतु संदर्भ के रूप में उपयोगी सिद्ध होगा।

खोजराद्धः ऑल्टरनन्थेरा सेसिलिस, ए.पजेंस, भेषज गुणविज्ञान