

Macro-Microscopic and HPTLC Atlas of *Argyreia sericea* Dalzell

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ABSTRACT

Background: *Argyreia sericea* Dalzell (Convolvulaceae) is a densely hirsute stout climbing shrub found throughout the dry deciduous forests of India. *A. sericea* is morphologically related to *Argyreia nervosa* (Burm.f.) Bojer and *Argyreia involucreta* C.B. Clarke which are used in traditional systems of medicine for treatment of various diseases. As related species look similar in dried form. **Objectives:** A macro-microscopic and HPTLC study was planned on whole plant parts of *A. sericea* to analyze key distinguishing characters. **Materials and Methods:** Standard pharmacopoeial procedures were employed to obtain all the results of the study. **Results:** Surface characters, odour and taste; transverse section of leaf, stem, root and stomata of epidermal cells, cluster crystal, trichomes, mesophyll tissue, laticiferous content, brownish content, fibres, starch grains, tracheids, fibres tracheids, pitted vessels with scalariform perforation along with pitted, spiral and annular thickening are the unique diagnostic characters observed. Fingerprint profile with unique R_f obtained by HPTLC will aid in correct identification of the species. **Conclusion:** The findings of the present study will be helpful in identification and differentiation from related species as whole drug or in powder form.

Keywords: *Bidhara*, Controversial drugs, Monograph, Pharmacognosy, Plant anatomy, Standardization.

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INTRODUCTION

Argyreia sericea Dalzell (Syn. *Argyreia sericea* St.-Lag.; *Argyreia leschenaultii* Thw.; and *Ipomoea bracteata* J. Grah.) family Convolvulaceae is an endemic, densely hirsute stout climbing shrub found throughout the dry deciduous forests of India especially Tamil Nadu, Andhra Pradesh, Karnataka, Madhya Pradesh, Maharashtra, Punjab and Rajasthan (Figure 1A and B).^[1-4] The whole plant is used as a medicine in traditional system of Indian medicine and in folklore.^[5]

The *A. sericea* called in Marati as *gavel*, *gavli vel*, *khujlowel*,^[6,7] in Rajasthani as *Tamesar*,^[8] in Gujarati as *sambarvali*, *samudrasos*,^[9,10] in Tamil as *Onan kodi*, *Kattu kodi*, *Samuththira palaikodi*,^[11,12] in Malayalam as *samudrastokan*, *samudrastjogai*,^[13] and in Kannada

as *emme balli*, *samudra balli*.^[14] Due to similarity in external appearances, in earlier classical literature, *A. sericea* is confused with closely related species like *A. nervosa*. *A. nervosa* is official source of *Virdharuka* in Ayurveda, but, according to Ayurvedic materia medicas, *Bidhara* (Syn. *Virdharuka*) characters are related to *A. sericea* Dalz.^[13,14] Rajasthan tribal peoples use root decoction of *A. sericea* for purifies of blood, leaves poultice used for body cooling and reduce the swelling and root paste applied for externally in snake bite.^[15] The *Bhilla* tribe of Maharashtra root paste is applied on the boils and other burn wounds.^[16] *Pawra* Tribe of Satpura Hills, Maharashtra use 2 to 5 g of raw root and leave powder for lactation purposes; seed powder along with cow's milk is taken for fever and tender leaves cooked as vegetable.^[17-20] The morphological features of *Bidhara* and therapeutically other classical literatures denote with *A. sericea* and *A. nervosa*.^[14]

As there are no pharmacognostical studies in report for this plant this study was planned with objectives of carrying out anatomy, powder microscopy and HPTLC for authentication purpose of this plant material.



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MATERIALS AND METHODS

Authentic whole plant of *A. sericea* was collected from *Anaikatty* and *Maruthamalai* hills, Tamil Nadu, India. The voucher specimen (B/AP/86) was preserved in the museum of Pharmacognosy department of Siddha Central Research Institute, Chennai.

Macro-microscopy

The Morphology, organoleptic characters and quantitative microscopy studies were documented by pharmacopeias standards. Habit and morphology of the whole plant photo were taken with NIKON-D 5600 Digital SLR Camera; Magnifications of figures were indicated by scale bars. The whole plant is submerged in water for 2 hr and free hand sections were taken following standard procedures. Microscopic features were recorded using Zeiss Axiolab 5 trinocular microscope having AxioCam 208 color camera attachment. Powder microscopy illustrations were made under 200X magnifications using Olympus BX43 trinocular microscope drawing attachment. [21,22]

Chemicals, Solvents and Reagents

AR grade (Merck) chemicals and solvents were used. VSA reagent (vanillin-1 g, sulphuric acid-5% in ethanol) was used for visualizing the developed spots. The mobile phases used was toluene: chloroform: methanol: formic acid (4.5:4.5:1:0.5) and for development pre-coated aluminium plate (Merck) Silica gel 60 F₂₅₄ of 0.2 mm thickness was used. [22,23]

HPTLC

Extracts applied on TLC plate using ATS4 Automatic sampler; plates develop on twin trough chamber (10×10 cm); photo documentation under UV-visible lights were done with visualizer; densitograms obtained by winCATS Scanner 4 software; derivatization was done using TLC plate heater (all from CAMAG, Switzerland).



Figure 1: A. Fruiting twig; B. Flower.

Procedure

Ethanol extract (20 µL) as 8 mm bands on 8x10 cm plate using was applied with a space of 10 mm on left, right and bottom of the plate. The chamber was pre-saturated with mobile phases. The plates were air dried post development to 90 mm from the bottom, photo documented under short and long UV followed by scanning at 254 nm (D₂ lamp, absorption mode) and 366 nm (Hg lamp, fluorescence mode) with a slit dimension of 6×0.45 mm at scanning speed of 20 mm/s. Plate dipped in a dip tank having VSA and heated at 100°C or till the colour development, photo documented under white light and then scanned at 520 nm (W lamp, absorption mode).

RESULTS

Macroscopy

Leaf

Dried leaves occurs crumpled, when expanded by boiling in warm water is up to 13x10 cm, deltoid, oblong to lanceolate, apex acute, base truncate, pubescent above, densely hirsute below, margin entire, up to 11 pairs of lateral veins and almost running alternatively parallel to each other; upper and lower surface glabrous, dark greenish brown on upper surface and dark greenish brown white, tomentose on lower surface; petiole up to 4 cm long and 0.2 to 0.4 mm thick in diameter, twisted, densely white tomentose, longitudinally ridged (Figure 2); odour nil, taste slightly astringent bitter.

Stem

Dried cut pieces of stem is of varying size, about 10 to 15 cm in length and 0.3 to 2 cm in thickness; cylindrical, branched, irregularly bent at places, slightly twisted, externally rough, brown in color, densely white tomentose in young stem and longitudinally ridged with lenticels on mature stem, longitudinally wrinkled with exfoliating cork at some places, grayish black in color, internally yellowish brown in color and there is a central



Figure 2: Dried whole plant.

hollow pith; fracture short and splintery (Figure 2); odour nil; taste slightly astringent bitter.

Root

Dried cut pieces of root vary in size about 10 to 15 cm long, 0.1 to 0.7 cm in diameter; branched, cylindrical, irregularly bent and slightly twisted, externally rough with lenticels, fissured at places due to exfoliating cork; fracture short; young roots are yellowish brown in color, matured root bark grayish black in color and wood yellowish brown in color (Figure 2); odour nil; taste slightly astringent bitter.

Microscopy

Petiole

Diagrammatic TS of petiole is more or less heart shaped with wavy outline having single layered, epidermal cells with numerous unicellular covering trichomes; followed by a band of thick walled hypodermis and central parenchyma pith encircled by collateral vascular bundles.

Detailed TS of petiole shows single layer epidermis having numerous, densely covered, unicellular, pointed, covering, glandular and a few stellate trichomes; underneath the epidermis there are 6 to 10 rows of thick walled angular collenchymatous hypodermis embedded with a few calcium oxalate cluster crystals; there is a wide thin-walled ground tissue formed by parenchyma having intercellular spaces; parenchyma cells are embedded with starch grains, oil globules, yellowish brown content, laticiferous cavities and cluster crystal of calcium oxalate; the ground tissues possess prominent arc of collateral vascular bundles on lower side and 4 small vascular bundles located at the upper side, bundles are composed of usual phloem and xylem elements, a few xylem vessels and phloem tube cells contain latex (Figure 3).

Midrib

Diagrammatic TS of midrib is slightly wavy in outline having single layered epidermal cells on upper and lower epidermii with numerous unicellular trichomes; beneath epidermis there is a thick walled hypodermis followed by an arc of collateral vascular bundles at the centre.

Detailed TS of midrib is wavy in outline having single layered, thin walled, epidermal cells on both upper and lower epidermii covered by thin cuticle, densely numerous unicellular, pointed covering and glandular trichomes are seen on epidermis; inner to epidermis 4 to 6 rows of thick walled angular collenchymatous hypodermis seen followed by polygonal thin-walled parenchymatous ground tissue having intercellular spaces and centrally located crescent-shaped collateral, open vascular bundle composed of phloem and xylem elements; parenchyma cells are embedded with starch grains, oil globules, yellowish brown content, laticiferous cavities; phloem parenchyma, ground tissues

and few collenchymas cells are embedded with cluster and rosette crystal of calcium oxalate; xylem consists of radially arranged vessels, uni and bi-seriate xylem rays, a few xylem parenchyma and vessel tylosis embedded with latex content (Figure 4).

Lamina

Transverse section of lamina is dorsiventral having single layered, thin walled epidermal cells with thick cuticle on both upper and lower side, epidermii are densely covered by numerous unicellular pointed covering, glandular and a few stellate trichomes; underneath the upper epidermis two rows of palisade and 8 to 10 layers of spongy parenchymatous mesophyll having brownish content is seen; few cluster and rosette crystal of calcium oxalate, laticiferous canals and fibro-vascular bundles occur in middle region of the lamina (Figure 5).

Stem

Diagrammatic TS of stem shows circular in outline, young stem shows single layered epidermal cells with numerous unicellular trichomes and mature stem shows narrow cork with lenticels; followed by narrow parenchymatous cortex, at the centre there is a wide zone of alternating bands of phloem and xylem with central hollow parenchymatous pith (Figure 6A).

Detailed TS of stem shows young stem having single layered, thin-walled, epidermal cells externally covered by thin cuticle with numerous uni-seriate, unicellular, covering, glandular and a few stellate trichomes; mature stem shows upto 15 layers of cork cells exfoliating at the place of lenticels; cortex is narrow, formed of compressed, parenchymatous cells with starch grains, yellowish brown inclusions, laticiferous cavities, cluster and rosette crystal of calcium oxalate; beneath the cortex is an amphiphloic siphonostele consists a continuous primary phloem on the outside and a tertiary cambium arises in the secondary phloem and give rise to tertiary phloem and xylem in alternative band; xylem is represented mostly by radiating rows of xylem vessels, xylem parenchyma and uni-seriate medullary rays. Central thin-walled

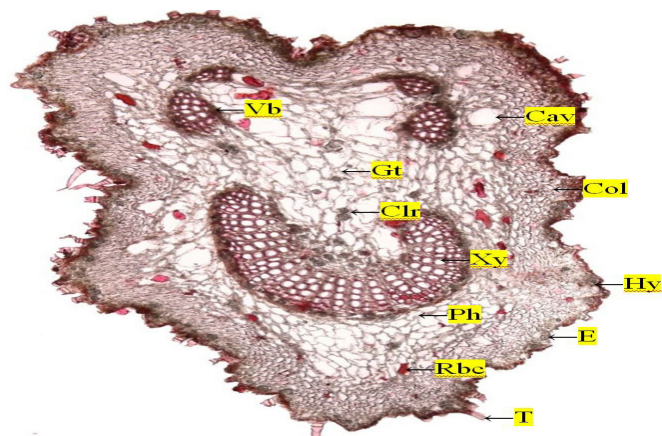


Figure 3: Detailed TS of petiole of *A.sericea*.

parenchymatous pith is embedded with group of phloem tissue, yellowish brown content, laticiferous cavities, cluster and rosette crystal of calcium oxalate (Figure 7).

Root

Diagrammatic TS of root is circular to oval in outline; young root shows single layered epidermal cells with root hairs; mature root shows narrow band of cork, cortical tissue, phloem region and wide central porous wood (Figure 6B).

Detailed TS of mature root shows upto 15 layers of exfoliating thick-walled cork cells embedded with blackish brown content followed by 10 to 15 cells layer of non-storied, lignified cork cells and narrow compressed parenchymatous secondary cortex embedded with reddish brown content; underneath cortex there is narrow phloem embedded with isolated and a few group of thick walled narrow lumened fibre, parenchyma cells with reddish

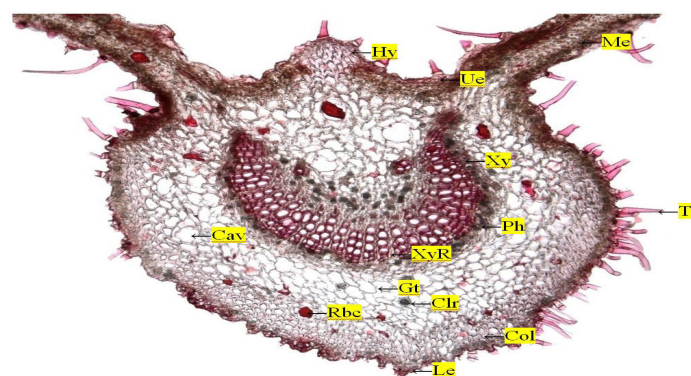


Figure 4: Detailed TS of leaf passing through midrib of *A.sericea*.

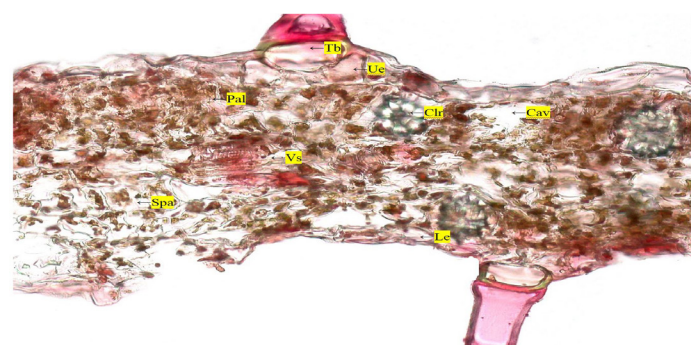


Figure 5: Detailed TS of leaf passing through lamina of *A.sericea*.

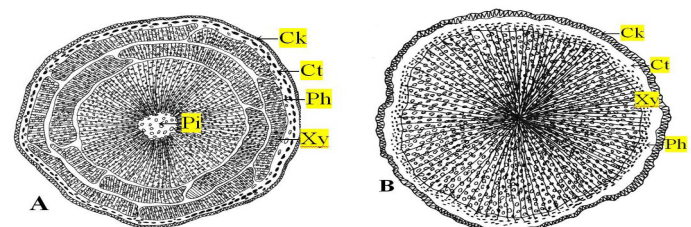


Figure 6: Diagrammatic TS of *A.sericea*. A. Stem; B. Root.

brown inclusions, starch, calcium oxalate prismatic and cluster crystals; xylem consist diffuse porous vessels with scalariform perforation plates about 20 to 40 bars, group of fibres, uni and bi-seriate xylem ray cells (Figure 8).

Powder microscopy

Leaf

Powder is greenish brown in colour with no characteristics odour; slightly bitter and astringent taste; shows upper and lower epidermis fragments having paracytic type of stomata; lamina in sectional view with glandular trichome; parenchymatous cells possessing calcium oxalate cluster crystals; surface view of petiole and midrib epidermal cells; upper epidermis in surface view underlie palisade head, fragment of covering and glandular trichomes, crystal fibre, few simple round to oval starch grains having concentric hilum, latex canals, spiral and annular vessel (Figure 9).

Stem

Powder is ash brown in colour with no characteristics odour and slightly astringent taste; shows cork cells in surface and sectional view, parenchyma cells with prismatic crystals, fragment of epidermal cells with cicatrix, cortical fibre, tangential longitudinally cut vessels and tracheids crossing with xylem ray, tangential longitudinal cut xylem rays attached with fibre tracheids, parenchyma with pits from pith region, fragment of covering stellate and glandular trichome; simple round to oval starch grains with concentric hilum, xylem parenchyma, sclereids, fibre tracheids, latex canals, crystal fibre, cluster crystals

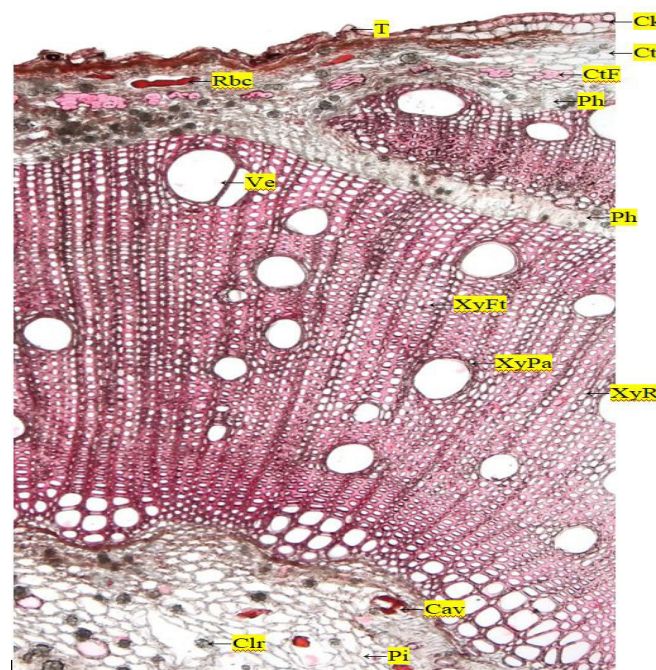


Figure 7: Detailed TS of stem.

of calcium oxalate, spiral and drum shaped pitted vessels with simple perforation, some vessels having long and few are tiled (Figure 10).

Root

Powder is dull yellowish brown in colour with no characteristics odour and slightly astringent bitter; shows cork cells in surface

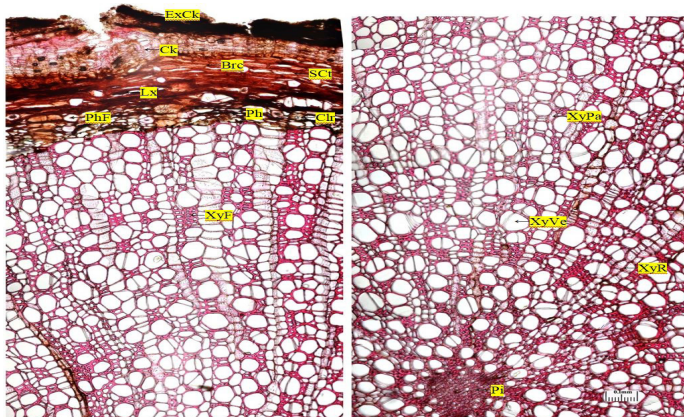


Figure 8: Detailed TS of root.

and sectional view, tangential longitudinally cut xylem axial parenchyma crossing with xylem ray parenchyma cells; pitted vessels with scalariform thickening perforation, xylem parenchyma, reddish brown content, fibre tracheids, cortical fibre, simple round to oval starch grains having concentric hilum, oil globule and cluster crystal of calcium oxalate (Figure 11).

Macroscopical and anatomical appearance of *A. nervosa*^[24] and *A. sericea* stem are same, but the differences can be seen in root, leaf anatomy and powder microscopy (Table 1).

Quantitative microscopy

The leaf lower side shows numerous paracytic stomata and they are very rare on the upper surface, only present adjacent to vein of epidermal region and absent in other area (Table 2).

HPTLC fingerprint of ethanol extract of whole plant of *A. sericea*

The solvent system was optimized to get distinguishable bands. The TLC plate applied with ethanol extract of the whole plant of *A. sericea* exhibited many bands at under different wavelength of light. There were 8 and 12 bands respectively under short and

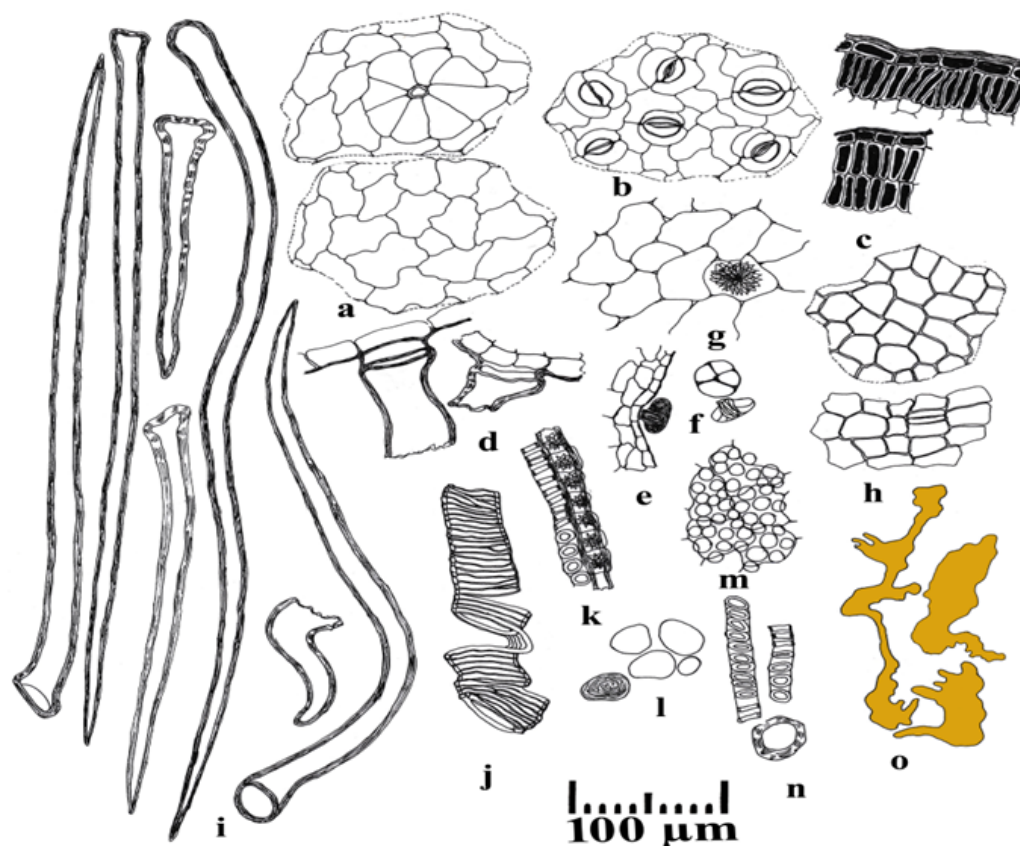


Figure 9: Powder microscopy of leaf of *A. sericea*. a: upper epidermis in surface view; b: lower epidermis in surface view with paracytic stomata; c: fragment lamina in sectional view; d: fragment of trichome base; e: transversally cut epidermal cells with glandular trichome; f: glandular trichome; g: parenchymatous cells with cluster crystal; h: petiole and midrib epidermal cells in surface view; i: fragment of trichome; j: spiral vessel; k: annular vessel associated with crystal fibre; l: starch grains; m: fragment of upper epidermis underlie palisade head; n: annular vessel; o: latex canals.



Figure 10: Powder microscopy of stem of *A. sericea*. a: cork cells in sectional view; b: cork cells in surface view; c: cluster crystals of calcium oxalate; d: parenchyma cells with prismatic crystals; e: stellate trichome; f: spiral vessel; g: fragment of epidermal cells with cicatrix; h: phloem fibre; i: tangential longitudinally cut vessels and tracheids crossing with xylem ray parenchyma cells; j: xylem fibre and fibre tracheids; k: tangential longitudinally cut xylem rays associated with fibre tracheids; l: fragment of covering trichome; m: starch grains; n: pith region pitted parenchyma; o: pitted vessels; p: xylem parenchyma; q: glandular trichome; r: sclereids; s: crystal fibre; t: latex canals.

long UV, when the plate was derivatized with VSA 9 bands were evident (Table 3).

The densitogram of ethanol extract showed 11 peaks at 254 nm with 5 appearing as major (area>10%) (Figure 12); 6 out of 14 peaks appeared major at 366 nm (Figure 13); on derivatization followed by scanned at 520 nm 2 out of 11 appeared major (Figure 14).

DISCUSSION

The botanical source of *Bidhara* is controversial due to difference of opinion among different writers of Indian Material Medica and Vaidyas; Raj Nighantu describes *Bidhara* as a perennial climber which spreads in all directions.^[25] *Bidhara* root is astringent to bitter in taste, growing in hilly terrain and cultivated in Belgaum as per another report.^[26] Pharmacognosy of indigenous drugs^[27] and Srivastava^[15] have discussed the controversy and possible

Table 1: Comparative microscopic features of *A. sericea* and *A. nervosa*.

Characters	<i>A. sericea</i>	<i>A. nervosa</i>
Leaf	TS of petiole shows heart shaped in outline with a crescent shaped large collateral vascular bundle and 2 to 4 trace vascular bundles on upper side, and, midrib shows crescent shaped large collateral vascular bundle at the centre. Powder Parasitic stomata with wavy epidermal cells; spiral and annular thickening vessels.	TS of petiole shows circular in outline with discontinuous ring of vascular bundles and midrib having crescent shaped bi-collateral vascular bundles. Powder Parasitic stomata with sinuous anticlinal walled epidermal cells; spiral thickening vessels. ^[29]
Stem	Powder Shows drum shaped pitted vessels with simple pits; vessels elongated and few are tailed; simple pitted fibre tracheids.	Powder Shows drum shaped bordered pitted vessels with simple pits; some vessels are long; bordered pitted fibre tracheids. ^[27]
Root	TS Isolated and group of thick-walled phloem and xylem fibre, diffused porous vessel and tracheids, uni and bi-seriate xylem rays. Powder Isolated thick walled narrow lumened phloem fibre and tertiary thickening xylem fibre tracheids, tailed pitted vessels with scalariform thickening.	TS Group of thin-walled phloem and xylem fibre, uni-seriate xylem ray at some places diverged patches of thin walled xylem parenchyma. Powder Thin walled xylem and phloem fibres, simple pits fibre tracheids, barrel shaped simple perforation bordered pitted vessel. ^[27,30]

sources for *Bidhara*. Srivashtava mentions that in his market research two types of stem parts, which is appears to be *Rivea hypocrateriformis* (Desr.) Choisy and *A. nervosa*, the article, when Daljeet Singh mentions *Samundar Soka*, the Sanskrit meaning of this name is spreading all direction like the sea or a plant that lives on coastal area.^[28]

The botanical source of *Vidhara* has been controversial indicating different plants for this drug like *Argyreia nervosa* (Burm.f.) Bojer (Syn. *A. speciosa* (L.f.) Sweet,^[31-33] *Ipomoea pes-caprae* (L.) R.Br. (Syn. *Convolvulus bilobatus* Roxb.) both belonging to the Family Convolvulaceae.^[33-35]

Table 2: Quantitative Microscopy of leaf of *A.sericea*.

Characters	Upper epidermis	Lower epidermis
Epidermal number	880-940	910-970
Stomatal number	8-12	140-170
Stomatal index	25-27	44-45
Palisade ratio	8-11	
Vein islet number	7-11	
Vein termination number	7-10	

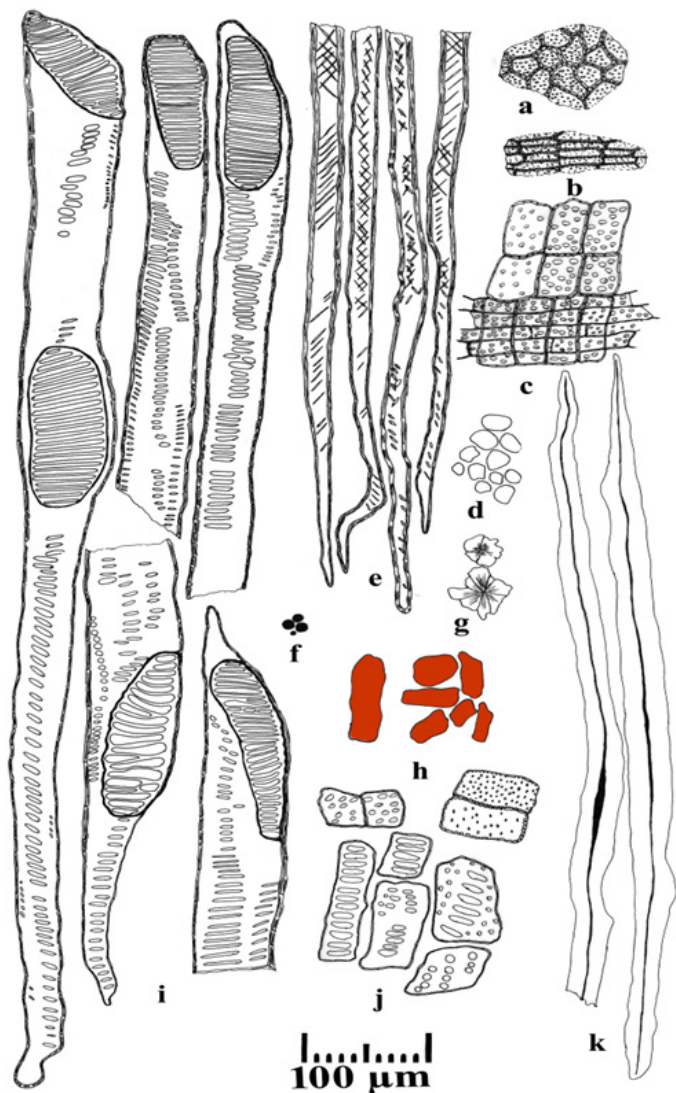


Figure 11: Powder microscopy of root of *A.sericea*. a: cork cells in surface view; b: cork cells in sectional view; c: tangential longitudinally cut xylem axial parenchyma crossing with xylem ray parenchyma cells; d: starch grains; e: fibre tracheids; f: oil globule; g: cluster crystal of calcium oxalate; h: reddish brown content; i: pitted vessels with scalariform perforation; j: xylem parenchyma; k: phloem fibre with very narrow lumen.

Many other Convolvulaceae plants possess similarity to *Vidhara* in Indian medical lexicons. They have been termed as '*Bidhara*' or assigned names similar to or synonymous with *Bidhara*.^[15] *Lettsonia atropurpurea* Clarke has been called '*Kala Bidhara*'

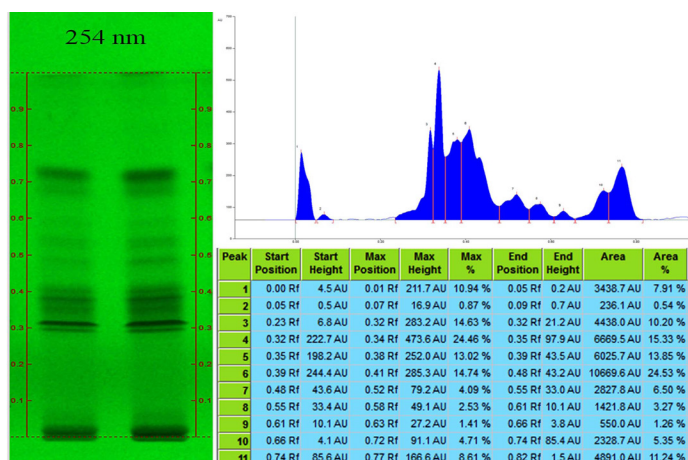


Figure 12: TLC plate and HPTLC densitogram of ethanol extract of whole plant of *A.sericea* at 254 nm.

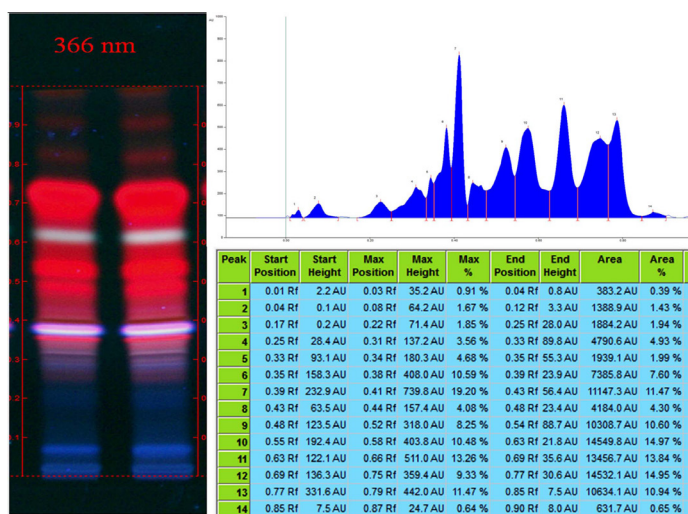


Figure 13: TLC plate and HPTLC densitogram of ethanol extract of whole plant of *A.sericea* at 366 nm.

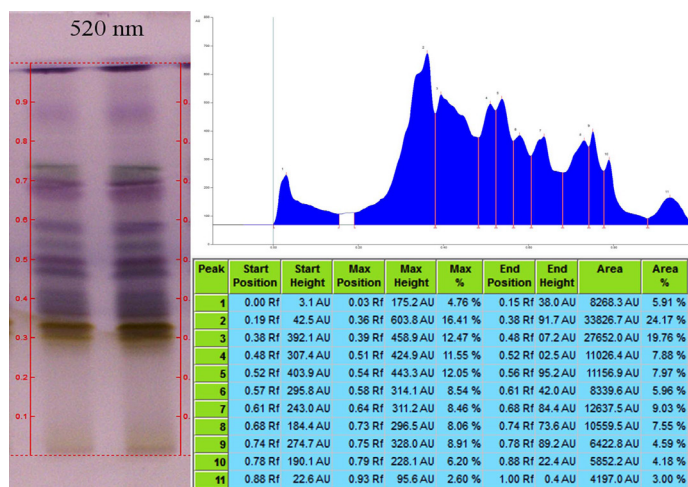


Figure 14: TLC plate and HPTLC densitogram of ethanol extract of whole plant of *A.sericea* at 520 nm (Derivatized with VSR).

Table 3: R_f values of TLC photo documentation (ethanol extract of whole plant) of *A. sericea*.

λ=254 nm		λ=366 nm		λ=520 nm (Derivitized)	
R _f	Colour	R _f	Colour	R _f	Colour
-	-	0.07	Blue	-	-
-	-	0.25	red	-	-
-	-	0.25	Bred	-	-
-	-	0.28	red	-	-
0.29	Dark	-	-	-	-
-	-	-	-	0.30	Yellow
0.32	Dark	-	-	-	-
-	-	-	-	0.33	Yellow
-	-	0.35	red	-	-
-	-	-	-	0.36	violet
-	-	0.37	F blue	-	-
0.38	Dark	-	-	-	-
0.40	Dark	0.40	red	-	-
-	-	-	-	0.47	violet
0.48	Dark	-	-	-	-
-	-	-	-	0.50	violet
0.53	Dark	-	-	-	-
-	-	0.54	red	-	-
-	-	-	-	0.58	violet
-	-	0.61	White	-	-
0.68	Dark	-	-	-	-
-	-	-	-	0.70	violet
0.73	Dark	0.73	red	-	-
-	-	-	-	0.74	green
-	-	0.82	red	-	-
-	-	-	-	0.88	violet
-	-	0.90	red	-	-

B-Bluish; F-Florescent.

in Dymock, Warden and Hooper. *Operculina turpethum* Linn. Silva Manso has been called 'Vidala' in Kirtikar and Basu.^[33] It is a strong purgative with a sharp taste and is mentioned in the Nighantus as 'Trivrit'.^[27] *Ipomoea pes-caprae* Roth has been mentioned as 'Vridhha daraka' in Nadkarni.^[34] Aiyer and Kolammal also recognize it is the true *Bidhara*; but this plant does not have cordate and white-velvety leaves, white tomentose branches.^[13,27] *Argyreia speciosa* Sweet has been termed *Bidhara* in a number of publications claim the properties of *Bidhara* to this plant.^[32,36] Aiyer and Kolammal^[13] and Pratap Singh^[37] identify it as the correct *Bidhara*. It has most of the physical features of the *Bidhara* plant of the Materia Medicas, excepting the trigonous or three winged stem mentioned in *Astanga sangrah* Tika^[38] and Dymock, Warden and Hooper^[35] say that it is not the true *Bidhara* but a substitute.

The drug *Vidhara* is of specific commercial importance in many localities in Uttar Pradesh, Bengal, Bihar, Punjab, Bombay and Calcutta. The samples collected from eminent drug dealers, physicians and pharmacies as well as research centers were botanically compared and identified with naturally growing and cultivated plants and screened clinically for their purgative action.^[39] It was revealed that the above mentioned plants had no purgative activity. Furthermore, he reported that the drug *Bidhara* is not obtained from *Ipomoea biloba* as reported by many writers. The samples procured from Kanpur (actually obtained from Musanagar) in the name of *Vidhara* showed significant purgative activity. It was found to be the famous Turpeth or *Nisoth* of Ayurveda.

Four different plant species are being sold or used in this country under this name. They are *Ipomoea turpethum* (L.) R.Br., *I.*

petaloidea Choisy, *I. pescaprae* Roth and *Argyreia nervosa* (Burm) Boj (Syn. *A. speciosa* Sweet).^[40]

The writers on Unani materia medica did also recognise this drug in similar way where they have described the Arabic name *Sharf'* and *Vidhara'* in Hindi well as the popular name *Bidhara*. Therapeutically they have considered the drug highly as an aphrodisiac and *rasayan*.^[25] *Vidhara* enters into a number of Ayurvedic and Unani formulations, both compound as well as single drug preparations.^[40,41]

Due to the difficulty in finding out which is the correct *Bidhara*, they seem to have defined the synonym of the *bidhara* as *Virdharuka*. The Ayurvedic Pharmacopeia AFI volume III mention *A. nervosa* is an official botanical source of *Virdharuka*.^[42] But based on the classical literatures reviews, the *A.sericea* morphological characters are similar to *Virdharuka*. Khan mentions the *Bidhara* lives in hilly area and many classical literature mention identification of key distinguishing characters matched with *A.sericea*.^[43] Because of same time the looking morphology of *A.sericea* likes this *A. nervosa*.

The *A. sericea* whole plant anatomy, powder microscopy and HPTLC are not to be done, the *Bidhara* is a controversial source finding correct botanical names, hence to carry out herbal drug standardization purpose detailed studies and differentiated controversial sources of the *A. nervosa*.

CONCLUSION

Several methods of the distinguishing differentiated microscopical study to useful for the identification of the correct botanical source. The macroscopy, anatomy and powder microscopy of stem, leaf and root, leaf quantitative and whole plant of TLC/HPTLC studies was carried out and the results were documented as per the standard pharmacopeias methods. The present study will be helpful in carrying future scientific works, identification, and differentiated from related species as single parts or whole plant or drug used in the powder from any formulations.

AUTHORS' CONTRIBUTIONS

Susikumar S: Drafting the article, anatomical section and powder microscopy drawing. Sunil Kumar KN: Correction, analysis and interpretation of the data. Murugammal S: TLC and HPTLC analysis. Shakila R: Correction and supervision of TLC and HPTLC data. Elankani P: Study analyses were overseen and guide. Dayanand Reddy G: Analysing the work and supervision. Ganesan R: Conception and design, approval of the final version.

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CONFLICT OF INTEREST

The authors declare that there is no conflict of interest.

ABBREVIATIONS

Brc: Brownish content; **Cav:** Cavity; **Ck:** Cork; **Clr:** Cluster crystals of calcium oxalate; **Col:** Collenchyma; **Ct:** Cortex; **CtF:** Cortical fibre; **E:** Epidermis; **ExCk:** Exfoliating cork; **Gt:** Ground tissue; **Hy:** Hypodermis; **Le:** Lower epidermis; **Lx:** Latex; **Me:** Mesophyll; **Pa:** Parenchyma; **Pal:** Palisade parenchyma; **Ph:** Phloem; **PhF:** Phloem fibre; **Pi:** Pith; **Rbc:** Reddish brown content; **Spa:** Sponge parenchyma; **SCt:** Secondary cortex; **T:** Trichome; **Tb:** Trichome base; **Ue:** Upper epidermis; **Vb:** Vascular bundle; **Ve:** Vessels; **Vs:** Vascular strand; **Xy:** Xylem; **XyF:** Xylem fibre; **XyPa:** Xylem parenchyma; **XyR:** Xylem ray; **XyFt:** Xylem fibre tracheid; **XyVe:** Xylem vessels.

SUMMARY

This paper identified key distinguishing characters for whole plant of *A. Sericea* and to distinguish it from *A. nervosa* (*Virdharuka*). *Bidhara* is controversial sources finding correct botanical name, due to the difficulty in finding out which is the correct *Bidhara*, they seem to have defined the synonym of the *bidhara* as *Virdharuka*. The Macroscopy, transverse section tissue systems, powder microscopy of different shape and sizes of epidermal cells with stomata and cluster crystal, trichomes, mesophyll tissue, laticiferous content, brownish content, fibres, starch grains, tracheids, fibres tracheids, pitted vessels with scalariform perforation along with pitted, spiral and annular thickening are the unique diagnostic characters. The chromatographic differences in spots having fingerprint R_f value will aid in identification the species chemically.

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