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ORIGINAL ARTICLE

Development of quality standards of medicinal mistletoe – *Helicanthes elastica* (Desr.) Danser employing Pharmacopoeial procedures

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Quality control

Abstract *Helicanthes elastica* (Desr.) Danser (Loranthaceae), commonly known as Indian mango mistletoe, is a parasitic shrub found widely growing on mango trees in southern India. Development of monographic quality standards is need of the hour for Pharmacopoeial/extra-Pharmacopoeial and folk medicinal plants. Systematic pharmacognostical evaluation of leaves of *H. elastica* has been carried out employing Pharmacopoeial procedures of testing herbal drugs. Macro–microscopic features of *H. elastica* leaf were recorded. Ethanolic extract was tested positive for alkaloids, steroids, carbohydrates, tannins, saponins and phenols. HPTLC fingerprint profile was developed for the identification of extracts using reference standard β-sitosterol glucoside. Results of the present investigation would serve as a source of pharmacognostical information and a document to control the quality of *H. elastica* (Desr.) Danser.

1. Introduction

Before any further tests are undertaken, medicinal plant materials are authenticated based on sensory, macroscopic, microscopic, physico-chemical and chromatographic fingerprint characteristics as the first step towards establishing quality and the degree of purity. As macroscopic identity of medicinal plant materials is based on subjective parameters like shape, size, colour, surface characteristics, texture, fracture characteristics and appearance of cut surface, it is often necessary to re-confirm the findings by microscopy. Microscopic inspection of medicinal plant materials is indispensable for broken or powdered materials and if necessary specimens should also be treated with chemical reagents. Any additional useful information such as vein-islets and palisade ratio should also be included in the test procedures for leaves (Anonymous, 1992). Physico-chemical examination by determining moisture,

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ash values, extractive values etc. is another quality determination procedure for seeing chemical quality of herbal drugs. Phytochemical examination and HPTLC characterisation of extracts are a few other Pharmacopoeial procedures to obtain quality indicating constants.

Mistletoes are hemiparasitic plants of family Loranthaceae, with exception of genera like *Nuytsia*, *Atkinsonia*, and *Gaiadendron*, producing parasitic roots on aerial shoots of other higher plants (Kuijt, 1969). Mistletoes are proved to be highly active against human cancer and its usage has reportedly improved the quality of life of those affected patients (Piao et al., 2004). *Helicanthes elastica* (Desr.) Danser (Syn. *Loranthus elasticus* Desr.) is a one such less studied common Indian mistletoe species growing widely on mango trees (hence the name mango mistletoe), and several other hosts species (Sunil Kumar, 2011; Sunil Kumar et al., 2015a). Differences have been observed in the genomic DNA of *H. elastica* growing on different hosts (Sunil Kumar et al., 2016). Leaves are claimed to be anti-abortifacient, useful in vesical calculi and kidney affections (Kirtikar and Basu, 1935). Aqueous extract of *H. elastica* is reported to produce significant increase in the volume of urine and excretion of sodium, potassium and chloride (Aleykutty et al., 1991). Diuretic activity of methanol extract of *H. elastica* in rats is also reported (Jadhav et al., 2010). Biologically active extracts prepared from *H. elastica* growing on *Mangifera indica* and *Citrus maxima* (host trees) were found to be cytotoxic *in vitro* and reduce solid and ascites tumours in mice (Mary et al., 1994). The plant is reported to be a good antimicrobial (Sunil Kumar et al., 2014a) and antioxidant agent (Sunil Kumar et al., 2014b) and a good source of many nutritional supplements (Sunil Kumar et al., 2014c). Acute toxicity of ethanolic and aqueous extract of the plant is found to be nil up to 2000 mg/kg and the extracts possess hepatoprotective activity and immunomodulatory activity (Sunil Kumar, 2011). The plant is found to contain friedelin, epifriedelinol, β-amyrin, β-sitosterol, ethyl gallate, gallic acid and β-sitosterol-3-β-D-glucopyranoside as major constituents (Sunil Kumar et al., 2015b).

Argentine mistletoe, *Ligaria cuneifolia*, is regarded as a substitute for *Viscum album* and a close relative of *H. elastica* both morphologically and chemically (Fernandez et al., 2004). Another Indian species, *Dendrophthoe falcata* (L. f.) Ettingsh. (Syn. *Loranthus falcatus* Linn. f.) known as Vrikshadani, Bandaka or Vanda in Ayurveda, is many times confused with *H. elastica* by plant collectors due to morphological resemblances among these species. There are no pharmacognostical studies available on *H. elastica* for authentication of its botanical source and to differentiate it from related species. In this paper an attempt has been made to study the pharmacognostical characters of *H. elastica* growing on mango trees in detail so as to have monographic quality standards for this species of Indian mistletoes.

2. Materials and methods

2.1. Collection

Fresh leaves of the mistletoe found growing on *M. indica* were obtained while flowering in the month of December, 2013 from Kasaragod District of Kerala and morphological features were noted referring to a number of regional floras (Gamble, 1967; Cooke, 1967). Identity of the specimens was authenticated by Dr. S. Amerjothy, retired HOD of Plant Biology and Biotechnology department, Presidency College, Chennai. Voucher specimen (00637) of the plant collected for the study was deposited at the Pharmacognosy department of CSMDMR Institute for Ayurveda, Chennai.

2.2. Macro–microscopic study

Photographs from natural habitat and plant parts were taken. The leaf was evaluated for morphological and organoleptic characteristics like taste, odour, colour and touch. Leaves preserved in formalin acetic acid alcohol (FAA) were dehydrated with graded series of tertiary-butyl alcohol as per the schedule (Sass, 1940). Microscopic slides were prepared after staining the sections with metachromatic toluidine blue (O'brien et al., 1964), Safranin and Fast green following standardised methodologies (Johansen, 1940).

Maceration: Cellulosic elements like trichomes, oil cells, tannin cells, mucilage cells, etc. were isolated by boiling the leaves in 4% KOH solution. Lignified elements were isolated by Schultz and Jeffery's maceration procedure (Johansen, 1940). The separated elements were studied under microscope.

Photomicrography and description: In order to supplement the descriptive part, photomicrographs in different magnifications of all necessary cells, their contents and tissues were taken in Zeiss AxioLab trinocular microscope. For normal histological purposes, sections were photographed under bright field light microscope. For the purpose of studying crystals, starch grains and lignified walls, photographs were taken under polarised light. Magnifications of figures are indicated by scale-bars. Adobe Photoshop was used to compare/edit microscopic characters. Descriptive anatomical terms were used as per the terminologies found in popular anatomy books (Easu, 1979; Fahn, 1987).

Venation pattern: To study the venation pattern, leaf fragments were first immersed in warm alcohol to remove chlorophyll, followed by treating with 10% sodium hydroxide and staining by safranine (Sass, 1940).

Quantitative microscopy: A few of leaf constants such as stomatal number, stomatal index, palisade ratio and vein islet number were attempted using micrometry (Wallis, 1967).

2.3. Physico-chemical examination

Loss on drying at 105 °C (LOD), total ash, acid insoluble ash, ethanol and water soluble extractive and successive extractive values were determined as per the Pharmacopoeial protocol (Anonymous, 1992).

2.4. Preliminary phytochemical examination

Preliminary phytochemical investigation was done to detect the presence of alkaloids, steroids, carbohydrates, tannin, flavanoids, saponins, triterpenoids, coumarins, phenols, resins and carboxylic acid in total ethanol extract (Harborne, 1998).

2.5. HPTLC fingerprinting

Extraction: Four grams of air dried *H. elastica* plant material was successively extracted with chloroform, ethyl acetate and ethanol respectively using Soxhlet apparatus. The chloroform soluble portion was concentrated to dryness and 100 mg of dried residue was dissolved in 5 ml of chloroform in a standard flask. As chloroform extract (CHE) has given optimum

Figure 1 *Helicanthes elastica*. (A) Mistletoe growing on trunk of *Mangifera indica*. (B) Upper and lower surfaces of leaf.

separation of bands, it is used for HPTLC fingerprint analysis. Marker β-sitosterol glucoside (BSG) was used as a phytochemical reference standard (Sunil Kumar et al., 2013).

Methodology: 4, 8 and 12 µl of CHE was applied on aluminium plates precoated with silica gel 60 F_{254} of 0.2 mm thickness (Merck, Darmstadt, Germany) using a CAMAG LINOMAT 5 applicator. Plate was developed in CAMAG glass twin trough chamber previously saturated with mobile phase toluene: ethyl acetate (10:1.5 v/v). Developed plate was visualised using CAMAG visualising chamber and scanned

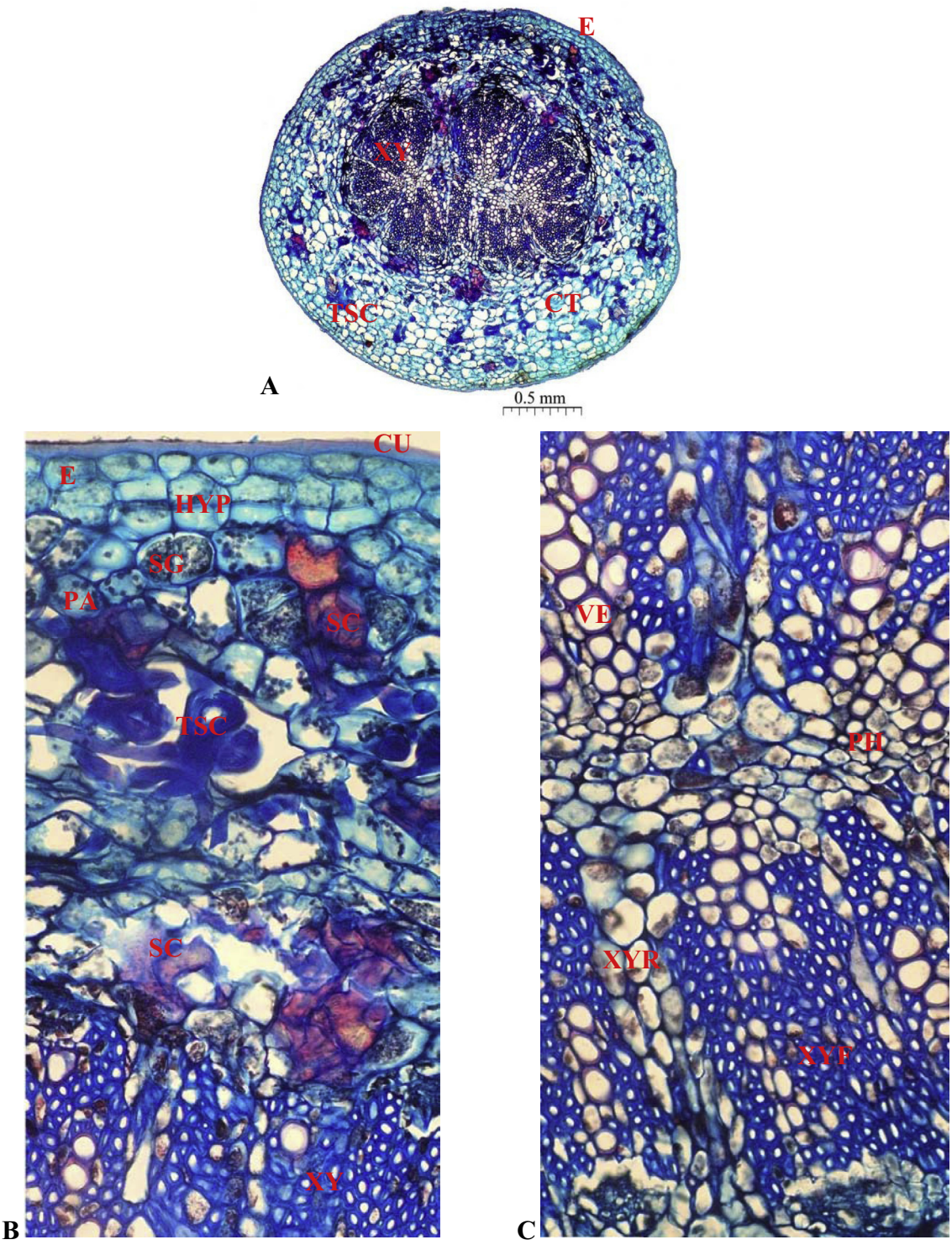

Figure 2 Microscopy of petiole of *Helicanthes elastica*. (A) Detailed TS of petiole to scale. (B) Outer region enlarged. (C) Inner region enlarged. CT, cortex; CU, cuticle; E, epidermis; HYP, hypodermis; PA, parenchyma; PH, phloem; SC, sclereids; SG, starch grains; TSC, trichosclereid; VE, vessel; XY, xylem; XYF, xylem fibre; XYR, xylem ray.

using CAMAG Scanner 4 at 254 nm and 366 nm. The plate was dipped in vanillin-sulphuric acid (VSA) and heated at 105 °C till spots appeared (Wagner and Bladt, 1996; Sethi, 1996). Plates were scanned again at 610 nm. R_f values, colour of spots and densitograms were recorded with help of CAMAG WinCATS software.

For the identification of marker compound, 10 and 15 µl of CHE and 10 µl (1 mg/1 ml) of marker compound β-sitosterol

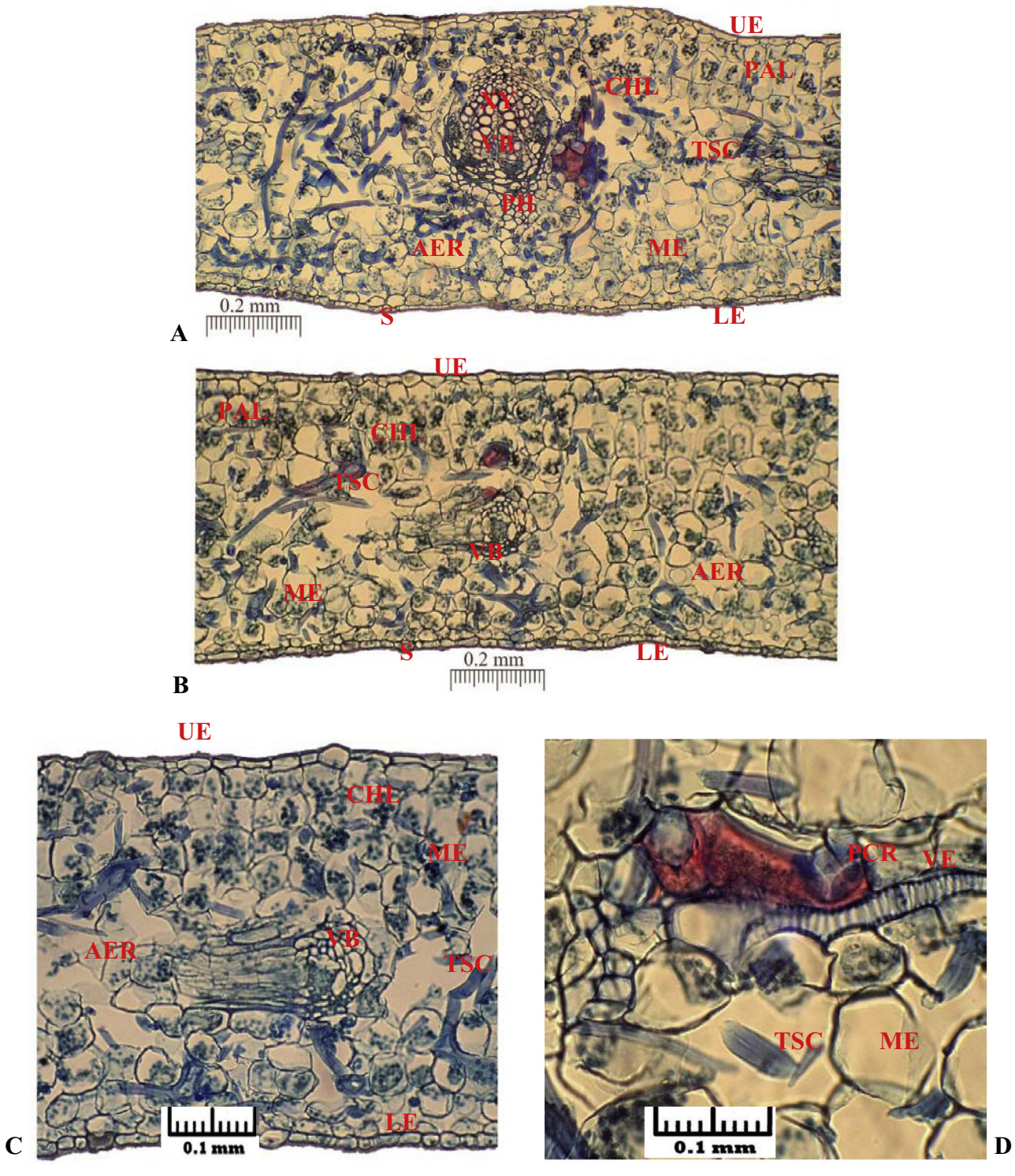

Figure 3 Microscopy of lamina of *Helicanthes elastica*. (A) TS through midrib. (B) TS through lamina. (C) Lamina portion enlarged. (D) A vascular bundle of lamina enlarged. AER, aerenchyma; CHL, chlorophyll; LE, lower epidermis; ME, mesophyll; PAL, palisade; PCR, prismatic crystals; PH, phloem; S, stomata; TSC, trichosclereids; UE, upper epidermis; VB, vascular bundle; VE, vessel; XY, xylem.

glucoside were applied and developed in chloroform: methanol (8.5:1.5) solvent system. Superimposable spectrum of BSG with extract was recorded at a wavelength of 375 nm.

3. Results

3.1. Macroscopic

Natural habitat of the plant is depicted in Fig. 1A. Leaves are opposite, sessile to nearly so, thickly coriaceous, brittle, ovate, elliptic, sub-orbicular or oblong–lanceolate, obtuse, 5–15 cm in length, 5–10 cm in width, perfectly glabrous, base usually cuneate, young leaves often red; nerves 3–5, obscure, dark green and smooth above, glaucous beneath (Fig. 1B); odour not characteristic and taste slightly astringent. Leathery and elastic leaves are earmarked as diagnostic morphological markers for macroscopic identification of *H. elastica*. The morphological features may slightly vary depending upon the hosts on which plants grow.

3.2. Microscopic

Petiole: Cross sectional outline of petiole is circular. Epidermis is thin and conspicuous consisting of small circular cells which are papillate. Peripheral wall of these cells is heavily cutinised. Ground tissue consists of wide thin walled circular or polygonal parenchyma cells (Fig. 2A–C). Vascular system consists of three radially arranged distinct vascular bundles. All the bundles are collateral with five to ten radial multiples of xylem elements followed by a broad zone of phloem. All three bundles have thick sclerenchymatous caps on both sides (Fig. 2A and B).

Midrib: Midrib is not prominent and slightly projecting. Epidermal cells are squarish or rectangular and compact. Palisade zone of parenchyma is continuous without adaxial collenchyma layers. Meristele has a single strand vascular system, comprising of discrete, collateral vascular bundle. A hemispherical sclerenchyma cap is seen peripheral to phloem cells which are 3–5 layers in thick. Vascular bundle has radial parallel rows of xylem elements and a broad band of phloem elements.

Adaxial and abaxial lamina has stomata with prominent wide guard cells enclosing elliptical stomatal opening. Epidermal cells are polygonal, small and have thick, straight or slightly wavy anticlinal walls. Lateral veins are uniformly thin forming distinct, fairly wide vein islets. Veins are rendered prominent by wide, hyaline bundle sheath cells which occur all along the veins (Fig. 3A).

Lamina: Transverse section is dorsiventral, well differentiated into adaxial and abaxial side by mesophyll differentiation and slightly projecting midrib. Lamina is uniformly thin, measuring 400–650 μm in thickness. Both upper and lower sides of lamina is thickly cutinised. Adaxial epidermis is thin consisting of narrowly oblong barrel shaped epidermal cells with thick cuticle; abaxial layer is flat with compactly arranged rectangular cells. Upper epidermal layers are sparingly stomatiferous.

Mesophyll tissue is often differentiated into adaxial palisade and abaxial 8–10 layered spongy parenchyma cells; they are small, lobed and loosely disposed forming wide air spaces. Lateral veins and veinlets are well differentiated. Major veins do not project much beyond surface level of lamina. Vascular bundle of the major lateral vein is collateral with a small core of xylem elements and small group of phloem elements; vascular strand is surrounded by a single layer of dilated, hyaline bundle-sheath parenchyma with bundle sheath extension (Fig. 3B–D). A comparative account of macro–microscopy of leaf of *H. elastica* and a relative species *D. falcata* (Anonymous, 2006) is given in Table 1.

Macerated leaf shows parenchyma cells from petiole of different types, sizes and shapes; group of lignified stone cells with narrow lumen and pitted wall; plenty of thin-walled stone cell

Table 1 Comparative account of macro–microscopy of leaf of *Helicanthes elastica* and *Dendrophthoe falcata*.

H. elastica	*D. falcata*
Macroscopy	
Opposite; sessile to nearly so; ovate, elliptic, sub-orbicular to oblong–lanceolate; cuneate base; apex obtuse; thickly coriaceous, brittle; perfectly glabrous; margin entire; young leaves often red; 5–15 cm in length, 5–10 cm in width; dark green and smooth above, glaucous beneath; odour nil; slightly astringent taste	Opposite – decussate; petiolate; ovate to oblanceolate; decurrent base; apex acute; soft and leathery, brittle; perfectly glabrous; margin entire; young leaves green; 7.5–18 cm in length, 2–10 cm in width; pale green at both the sides; odour resembling those of tea leaves; slightly astringent taste
Microscopy	
Epidermis	
Epidermis cells with straight wall in surface view showing stomata, stomata paracytic, lower epidermis shows plenty of stomata	Epidermis cells with straight wall in surface view showing stomata, stomata paracytic, present on both surfaces
Lamina	
Mesophyll tissue is often differentiated into adaxial palisade and abaxial spongy parenchyma, cells are 8–10 layered; they are small, lobed and loosely disposed forming wide air spaces; lot of trichosclereids present	Mesophyll consisting of 2–4 layers of compactly arranged short rectangular cells and irregularly arranged parenchyma cells of middle layers but possessing a few intercellular spaces; trichosclereids absent
Midrib	
A hemispherical sclerenchyma cap is seen peripheral to phloem cells which is 3–5 layers in thick	Bundle sheath absent; each vascular bundle associated with patch of collenchymatous cells outside phloem
Calcium oxalate	
Prismatic crystals of calcium oxalate in sclereids and trichosclereids present in lamina region	Isolated sclereids containing prismatic crystals in parenchyma

with narrow lumen; branched sclereids with very narrow lumen and finger like branches; stone cells with thin-wall, lignified and non-lignified types, few branched; annular vessels in groups; pitted vessel of different dimensions; tiny tracheids; parenchyma of mesophyll with content; epidermis cells with straight wall in surface view showing stomata; highly branched thick-walled trichosclereid; pitted tracheidal fibres, interwoven each other in groups; few simple starch grains and prismatic crystals of calcium oxalate; few thick- and thin-walled fibres; network of mesophyll tissue in surface view; scalariform vessels; pairs of tannin cells; thick-walled highly branched trichosclereids; fragment of phloem tissue; and epidermis of petiole with striated cuticle (Fig. 4).

Dermal features: Upper epidermis (Fig. 5A and B) shows polygonal epidermal cells with normal cuticle with rare occurrence of paracytic stomata. Lower epidermis (Fig. 5C and D)

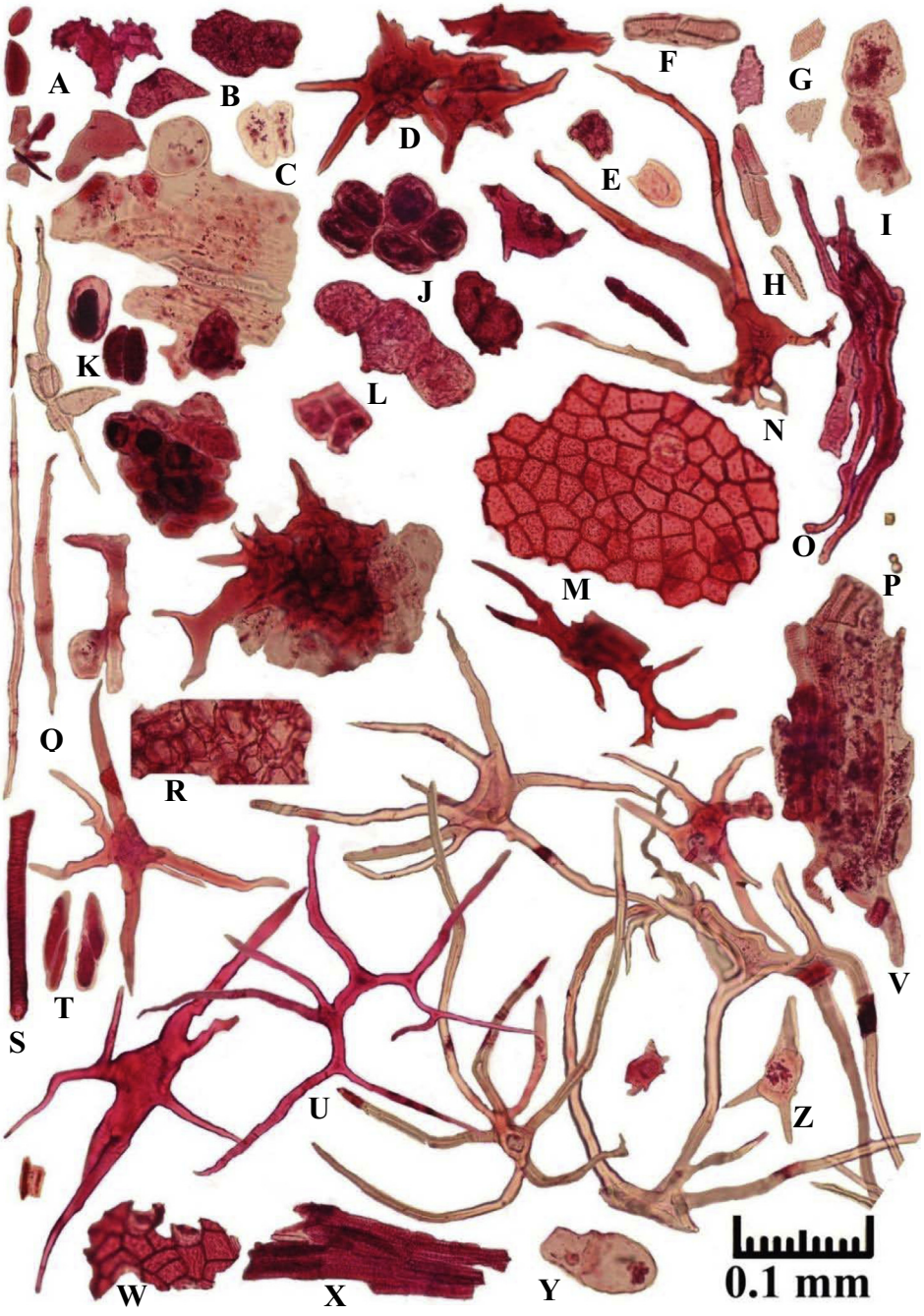

Figure 4 Microscopic characters of macerated leaf. (A) Parenchyma cells. (B) Lignified porous stone cells. (C) Non-lignified stone cell. (D) Branched sclereids. (E) Stone cells. (F) Annular vessels. (G) Pitted vessel. (H) Tracheid. (I) Non-lignified stone cell group. (J) and (K) Parenchyma with content. (L) Lignified porous stone cells. (M) Epidermis and stomata in surface view. (N) Branched sclereid. (O) Tracheidal fibres. (P) Starch and prismatic crystals. (Q) Thick-walled fibres. (R) Mesophyll in surface view. (S) Scalariform vessel. (T) Tannin cells. (U) Trichosclereids. (V) Phloem tissue. (W) Epidermis with striated cuticle. (X) Tracheidal fibres. (Y) Parenchyma. (Z) Branched stone cell.

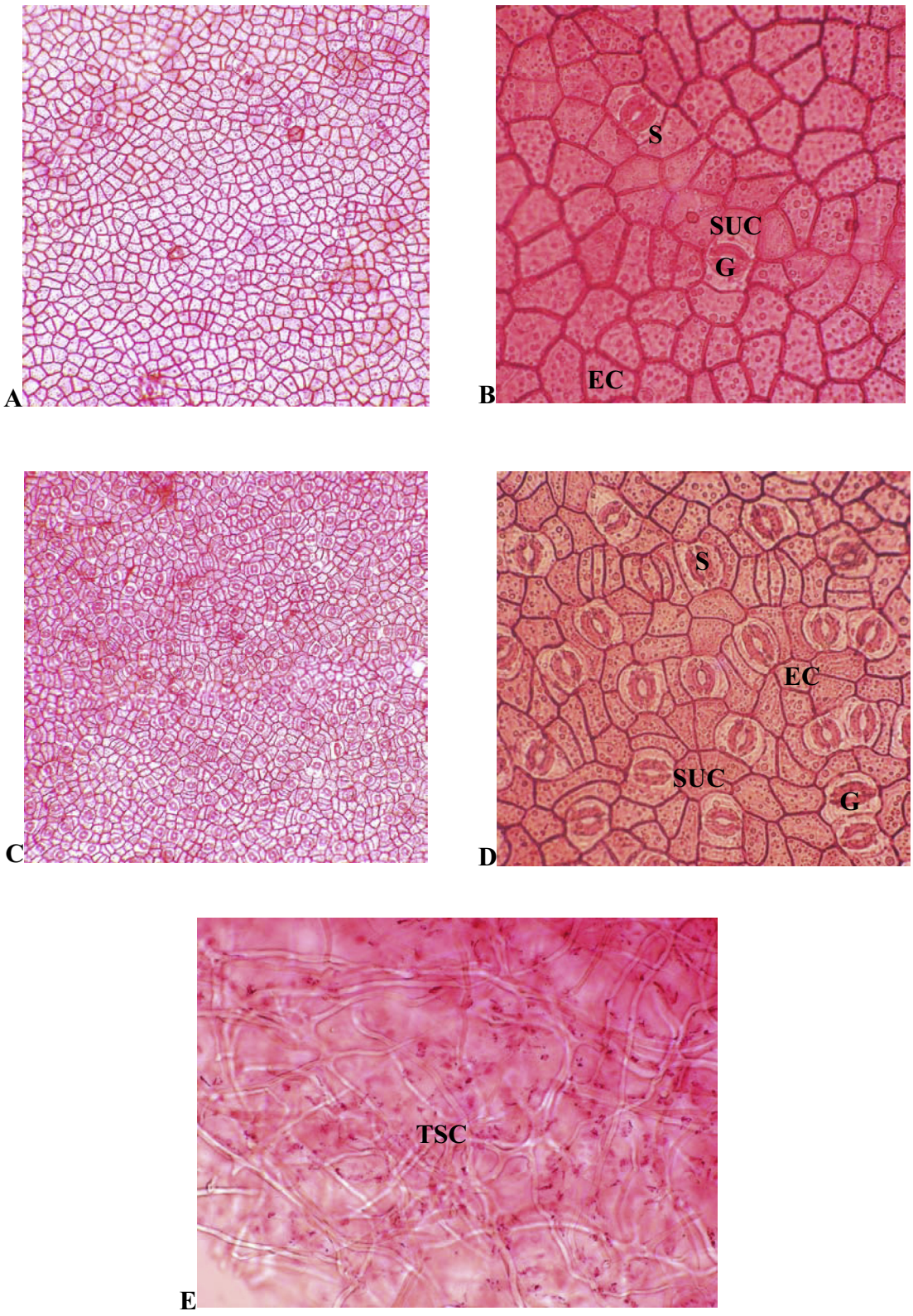

Figure 5 Epidermal features. (A) 1 mm^2 area to show frequency of stoma on upper epidermis. (B) A region of upper epidermis enlarged. (C) 1 mm^2 area to show frequency of stoma on lower epidermis. (D) A fragment of lamina in surface view after clearing. (E) A region enlarged. EC, epidermal cell; GC, guard cell; S, stomata; SUC, subsidiary cell; TSC, trichosclereid.

shows polygonal epidermal cells with normal cuticle and plenty of stomata. Trichomes are absent in both upper and lower epidermis.

Venation pattern: Piece of cleared lamina on surface view, does not show venation pattern, mesophyll is fully covered with plenty of trichosclereids which form dense network hiding veins in a cleared lamina fragment (Fig. 5E).

Quantitative microscopy: Palisade ratio could not be counted due to the presence of trichosclereids. The number of epidermal cells, stomata/mm^2 and stomatal index is shown in Table 2.

Histochemical tests: The presence of lignin, starch, and tannin was detected in all plant parts, whereas mucilage and volatile oil were absent.

3.3. Physico-chemical tests

Constants such as, loss on drying revealing moisture content; total ash indicating total inorganic content; acid insoluble ash revealing acid insoluble part of total ash, mainly silica; water soluble ash indicating water soluble part of total ash i.e. inorganic content without water insoluble inorganic salts like silica; alcohol and water soluble extractive indicative of percentage active constituents soluble in ethanol and water, were determined. Successive extraction of *H. elastica* was performed by Soxhlet apparatus using solvents chloroform, ethyl acetate and ethanol respectively, results were found to be 5.35%, 2.38% and 4.90% w/w respectively (Table 3).

Table 2 Quantitative microscopy of *Helicanthes elastica* leaf.

Parameter	Upper epidermis			Lower epidermis		
	Base	Middle	Tip	Base	Middle	Tip
Epidermal cells	1055	789	769	1257	1067	1327
Stomatal number	13	18	14	318	304	319
Stomatal index	1.2161	2.2305	1.7880	20.19	22.17	19.38
Stomatal percentage	1.23	2.28	1.82	25.30	28.49	24.04

$n = 3$; (/mm^2).

Table 3 Physico-chemical constants of *Helicanthes elastica* leaves.

Parameter	Mean ± SD% w/w
Loss on drying at 105 °C	9.18 ± 0.11
Total ash	15.49 ± 0.01
Acid-insoluble ash	1.45 ± 0.06
Water soluble ash	4.79 ± 0.01
Alcohol-soluble extractive	3.32 ± 0.24
Water-soluble extractive	16.92 ± 0.47
Successive extractive values	
Chloroform	5.35
Ethyl acetate	2.38
Ethanol	4.9

3.4. Preliminary phytochemical tests

Ethanolic extract of leaf showed the presence of steroid, carbohydrate, tannin, saponin, triterpenoid and phenols; while alkaloid, coumarin, carboxylic acid, resin, quinine and amino acid moieties were absent (Table 4).

3.5. HPTLC

Fingerprint of chloroform fraction of total ethanol extract of *H. elastica* was performed using toluene: ethyl acetate (10:1.5 v/v) as solvent system (Fig. 6A–C). At 254 nm it showed 7 spots with R_f 0.05, 0.22, 0.35, 0.47, 0.58, 0.65 and 0.85 (all green). At 366 nm there were 7 spots with R_f 0.38, 0.47, 0.53, 0.58, 0.65, 0.71 and 0.85 (all fluorescent red except spot with R_f 0.53 – fluorescent blue). After derivatisation with VSA, there were 8 spots with R_f 0.05, 0.10, 0.22, 0.41, 0.49, 0.58, 0.71 and 0.89 (all violet except 2 spots with R_f 0.49 – light blue and 0.89 – brown) (Table 5).

Chloroform extract shows the presence of marker β-sitosterol glucoside under 620 nm (post derivatisation) with an R_f value of 0.49 (photo) (purple) in chloroform: methanol (8.5:1.5) after derivatisation with vanillin–sulfuric acid (Fig. 6D).

4. Discussion

Mistletoes are an important group of medicinal plants occurring worldwide. *H. elastica* (Desr.) Danser – Loranthaceae, Indian mistletoe, was chosen for the derivation of quality standards. The process of the derivation of standards of herbal drugs needs information from basic disciplines of plant sciences such as taxonomy, morphology, anatomy etc. for identifying plant drugs. At the same time, to evolve standards on quality specifications of a herb in terms of its chemical composition, analytical and phytochemical expertise is also required. Any material of medicinal interest must undergo assessment of quality standards employing all possible means of botanical or chemical analyses. According to Kunle et al. (2012), standardisation of herbal medicines is a process of prescribing a set of standards or inherent characteristics, constant parameters, definitive qualitative and quantitative values that carry an assurance of quality, efficacy, safety and reproducibility. It is the process of developing and agreeing upon technical standards. Specific standards are worked out by experimenting and observing, which would lead to the process of prescribing a set of characteristics exhibited by a particular herbal medicine. Hence standardisation is a tool in the quality control process.

Macroscopic and microscopic features recorded for a herbal drug help in the confirmation of its botanical source even when it is in dried form. Though plants can easily be identified in its fresh form, the same is difficult while it is dried as many features of plant parts change on drying. In case plant drugs are purchased from herbal drug stores, macroscopy will aid in quick confirmation of its botanical source. Microscopy of entire drug in transverse sections or as powder/isolated tissues will be helpful in further confirming botanical source and identifying the adulterants/substitutes if any. As observed by Metcalfe and Chalk (1957) in other Loranthaceae members, *H. elastica* shows the distribution of most rubiaceous stomata,

Table 4 Preliminary phytochemical tests for ethanolic extract of *Helicanthes elastica* leaves.

Tests	Colour if positive	Colour observed	Inference
Alkaloids			Negative
Dragendroff's test	Orange precipitate	Brown colour solution	
Wagner's test	Red precipitate	Reddish brown	
Mayer's test	Dull white precipitate	Light brown solution	
Hager's test	Yellow precipitate	Light yellow solution	
Steroids			Positive
Liebermann–Burchard test	Bluish green	Light green	
Salkowski test	Bluish red to cherry red	Reddish brown upper layer, green fluorescence in lower layer	
Carbohydrate			Positive
Molisch's test	Violet ring	No violet ring	
Fehling's test	Brick red precipitate	Brick red precipitate	
Benedict's test	Red precipitate	Brown precipitate	
Tannin			Positive
With $FeCl_3$	Dark blue or green or brown	Dark blue	
Flavanoids			Negative
Shinoda's test	Red to pink	No pink colour	
Saponins			Positive
On shaking with water	Stable froth	Stable froth	
Triterpenoids			Positive
Tin and thionyl chloride test	Pink	Pinkish brown precipitate	
Coumarins			Negative
With 2 N NaOH	Yellow	Brown solution	
Phenols			Positive
With ferric chloride	Blue to blue black, brown	Blue black	
Carboxylic acid			Negative
With water and $NaHCO_3$	Brisk effervescence	No effervescence	
Resin			Negative
With aqueous acetone	Turbidity	No turbidity	
Quinone			Negative
5% NaOH	Pink/purple/red	No red	
Amino acids			Negative
With Ninhydrin	Violet colour	No violet	

whole surface being perforated by the presence of stoma. Stomata on surface of assimilatory stems are often transversely orientated in relation to longitudinal axis. Mesophyll is dorsiventral, isobilateral, or wholly composed of isodiametric cells. Groups of silicified cells have been recorded in mesophyll, while stone cells also occur in the same region, as well as in cortex and pith axis. Crystals secreted, especially in old tissues, in solitary and clustered forms. Tanniniferous cells are present in the parenchymatous tissues, particularly of the Loranthoideae. This study is the first attempt to record microscopy of this mistletoe. A previous study on Iranian species, *Loranthus grewingkii* and *Lycopus europaeus*, revealed the type and distribution of calcium oxalate crystals as differentiating features. Irregular glandular as well as platelet crystalloid wax structures was observed in *L. grewingkii* while it was smooth in *L. europaeus* (Shavvon et al., 2012). *H. elastica* also showed the presence of prismatic crystals in sclereids and prominent cuticle on both petiole and lamina.

Examination of physico-chemical composition with some Pharmacopoeial analytical tests, which are employed normally for checking quality of herbal drugs as per international standards, is an effective method in evolving quality standards. The results obtained will infer quality in terms of its moisture content, ash content, extractive values which are normally found as standard values for a particular plant. According to WHO (Anonymous, 1992, 1996a,b), standardisation and quality control of herbals are the processes involved in the physicochemical evaluation of crude drug. The constants obtained in the current study will serve as an indication of chemical quality of *H. elastica* for quality control and standardisation of this herbal drug in future researches.

All plant drugs act on targeted disease by virtue of their chemical constituents are produced from secondary metabolism. Composition of a plant will be usually connected with its phylogenetic relationships. A particular plant family or a selected genus under it will have certain chemical pathway which will produce only certain phytochemical entities. Phytochemical testing deals with screening, isolation, identification and purification of chemical components of a botanical drug. Therefore it is essential to evaluate potency of an herbal drug

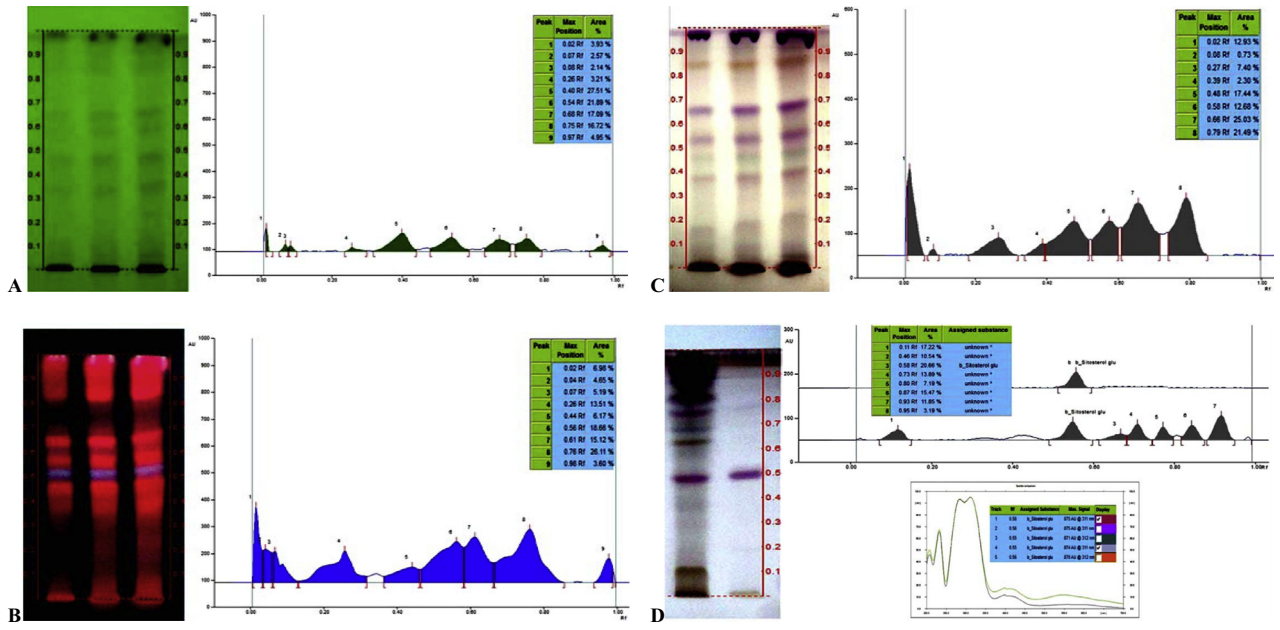

Figure 6 HPTLC fingerprint profile of chloroform extract of *Helicanthes elastica* leaves. (A) At 254 nm. (B) At 366 nm. (C) Under 620 nm (post derivatisation). (D) Extract showing presence of marker β-sitosterol glucoside under 620 nm (post derivatisation) – superimposable spectrum at 375 nm.

Table 5 R_f values of chloroform extract of *Helicanthes elastica* leaves.

Under short UV	Under long UV	Post derivatisation with VAS
0.05 (L Green)	–	0.05 (Violet)
–	–	0.10 (Violet)
0.22 (L Green)	–	0.22 (Violet)
0.35 (L Green)	–	–
–	0.38 (F Red)	–
–	–	0.41 (Violet)
0.47 (L Green)	0.47 (F Red)	–
–	–	0.49 (L Blue)
–	0.53 (FL Blue)	–
0.58 (L Green)	0.58 (F Red)	0.58 (Violet)
0.65 (L Green)	0.65 (F Red)	–
–	0.71 (F Red)	0.71 (Violet)
0.85 L (Green)	0.85 (F Red)	–
–	–	0.89 (Brown)

L, Light; F, Fluorescent; VAS, Vanillin/sulfuric acid reagent.

in terms of its active principles (Kunle et al., 2012). Mistletoes are sources of therapeutically important phytochemicals like flavonoids, polyphenols and lectins along with other phytochemicals such as sterols and triterpenes reported from it (Sunil Kumar et al., 2016). A preliminary examination for different phytochemical entities by colour tests indicated preliminary chemistry of this species for the first time. These phytochemicals may be held responsible for different medicinal activities of this mistletoe. *L. cuneifolia* – a morphologically closely related species of *H. elastica* – is reported to contain amino acids, flavonoids, and macromolecules like proteins (Fernandez et al., 2004). However, further studies are required for exploring detailed chemistry of this species.

Evaluation of botanical materials can be effectively assessed using high performance thin layer chromatography (HPTLC) as an important quality assessment tool. It is an efficient and cost effective way of analysing a broad number of chemical compositional variations. Multiple samples can be analysed on a single analysis thereby reducing significant time and other expenditures. It is also possible to view the compositional differences under different wavelengths of light providing fingerprint profile of a plant than other costlier chromatographic techniques like high pressure liquid chromatography (HPLC) (Kunle et al., 2012). TLC identity test is a part of every herbal monograph of international standards. HPTLC profile with β-sitosterol glucoside as marker can be used as a quality indicating fingerprint for *H. elastica*. Further, there is scope for undertaking research on quantification of compounds in mistletoe growing on different host species, though mango tree is the commonest one recorded.

Development of quality standards are an ongoing programme of Ministry of health in India and all over the world where herbal medicines are in practice. In India the Ayurvedic Pharmacopoeia committee has brought out several volumes of monographs under the umbrella of The Ayurvedic Pharmacopoeia of India – API (Anonymous, 2011). Indian Council of Medical Research (ICMR) is involved in prestigious project on the development of quality standards for Indian Medicinal Plants (Anonymous, 2015). Several Indian medicinal plants now have international standards for testing quality as a result of the efforts by ICMR and it is hoped that the output of findings of this paper would further help in the development of monographs for medicinal plants.

5. Conclusion

For the first time, several parameters were assigned to identity and develop quality standards of *H. elastica* which will be useful for laying down Pharmacopoeial standards. Plenty of sclerified tissues occurring as branched stone cells/trichosclereids in mesophyll have been found to be a diagnostic microscopic feature for its identification under microscope. Current study contributes to the quality standards of this unexplored mistletoe with respect to botanical and chemical standards which may go into the buildup of herbal pharmacopoeias. Results of the present study can be treated as a monograph on quality standards for *H. elastica* which may contribute significantly to mistletoe research world-wide.

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