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Original Article

Pharmacognostic Standardization of Stem of *Cissus vitiginea* L.: A Traditional Siddha Drug

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ABSTRACT

Background: The present study deals with the detailed pharmacognostic investigation of Sidhha single drug, the stem of *Cissus vitiginea* L. It is a climber that belongs to the family 'Vitaceae' with a pantropical distribution throughout India and Sri Lanka. In dried form, identification of the specific plant is difficult at present due to the lack of authentic pharmacognostic data related to this specific plant part. **Materials and Methods:** In order to prevent the adulteration of medicinal components, the stem of *C. vitiginea* was subjected to different pharmacognostic studies, physicochemical analysis, FT-IR and HPTLC fingerprinting. **Results and Discussion:** In the microscopical studies, the unique and elaborated anatomical structure is detailed with evidence of powder microscopic characters like trichomes, elongated fibres, raphide sacs, acicular crystals and different arrangements like xylem vessels with perforation plates observed. The results from the HPTLC fingerprint scanned at wavelengths 254 nm, 366 nm, and 575 nm for alcohol extract of stem of *C. vitiginea* L. confirmed the presence of different phytoconstituents revealed by the FT-IR analysis. **Conclusion:** All the pharmacognostic characters and physicochemical parameters of stem of *C. vitiginea* L. could be useful in the identification and standardization of a crude drug.

Keywords: *Cissus vitiginea* L., Pharmacognosy, Physicochemical analysis, FT-IR, HPTLC fingerprinting.

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INTRODUCTION

Siddha is an ancient Indian treatment practice dating back to five thousand years and originated in South India. Siddha medicine aims to make the body perfect and differs vastly from the other conventional forms of medicine. In this system of medicine, the dried stem of *Cissus vitiginea* L. is known as "Panadiappan" and is used to arrest bleeding and promote healing.

The species of the *Cissus* genus are often used as medicinal plants because they contain valuable phytochemicals like vitamins, proteins, carbohydrates and polyphenols. Its medicinal applications increased in recent years as a result of the studies of many researchers. The genetic relationship and chemical contents of eight *Cissus* species available in Thailand were studied earlier.[1,2] Medicinal uses and biological properties of *Cissus quadrangularis* were well explored in India. But *Cissus vitiginea* L. (Syn.-*Vitis vitiginea* Kuntze, *Cissus angulata* Lam, *Vitis vitiginea* (L.)W.L. Theob., and *Vitis vitiginea* (L.) Haines) is one of the less explored medicinal climbers of the genus *Cissus*.

The plant is a common folk medicine, which can be applied all over the body to control excessive swellings (whole plant paste) by Irula Tribals of Coimbatore (Tamil Nadu, India).[3] Tribal people of the Pachamalai hills of Tamil Nadu use this plant paste to set bones.[4] Some tribal communities of Andhra Pradesh named Yanadi, Chenchu and Nakkalas used to apply the root powder of *C. vitiginea* to heal the wounds and some others use leaf paste for the same condition.[5] Paste of stem bark is used to cure conjunctivitis by the tribals of the West Vidarbha region in Maharashtra, India.[6] The fruit juice of *C. vitiginea* mixed with the stem bark of *Chloroxylon swietenia* has been administered with goat milk once a day to treat asthma.[7] Oral aphthous ulcers and rashes are cured by the sap of the hollow stem of this plant.[8]

Even though the plant is used for multiple therapies, there is no comprehensive monographic standardization report of the stem of *Cissus vitiginea* L. Hence the present work was undertaken to explore the pharmacognostic studies which will help in setting a suitable monograph for this particular plant part.

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MATERIALS AND METHODS

Plant material and authentication

Cissus vitiginea L. plant material was collected from the local region of Thiruvananthapuram district of Kerala, India. Authentication of the collected material was carried out at the Department of Pharmacognosy, Sidhha Regional Research Institute (SRRI), Thiruvananthapuram and a voucher specimen of the plant was pressed and kept as a herbarium for future reference.

Morphological evaluation

The morphological studies show the important characteristics of the plant, the structure of the stem, the hairy surface of the stem, the typical tongue sensation and the odour of the plant. The fresh stem was evaluated for its morphological features like shape, size, colour, odour and type.

Microscopical characterization

Transverse Sections (TS) of the stem, nodal region and tendril of *Cissus vitiginea* L. were made using a sharp section cutter. Transverse sections were stained with safranin and mounted with glycerin on a glass slide and observed under the 10X and 40X objective of the Brightfield microscope attached to an analyser. Photographs of observed microscopic characteristics of the stem and tendril were captured and stored for further referencing.

Powder microscopy

The stem of *C. vitiginea* L. was shade-dried. The dried stem with tendril was powdered using a mixer grinder and 0.5 g powder was mounted in glycerin at room temperature for 24 hr and observed under 10X and 40X objectives of a bright field microscope for the identification of plant parts.

Proximate analysis

The proximate analysis aids in setting up certain standards for dried crude drugs to avoid batch-to-batch variation and also to judge their quality. Proximate analysis of powder of stem of *C. vitiginea* L. was carried out[9] by subjecting them to various determinations like total ash, acid insoluble ash, water soluble ash, alcohol soluble extractive value, water soluble extractive value, loss on drying/moisture content.

FT-IR Analysis

The FTIR spectra of *C. vitiginea* L. were recorded in an FTIR instrument (INFRA 3000-50, Analytical Technologies), with PC-based software-controlled instrument operation and data processing. A small amount of powdered stem samples were made into pellets using KBr for FTIR analysis and a thin film was prepared by applying pressure. The data of infrared transmittance was collected over a wave number ranging from 4000 cm^{-1} to 500 cm^{-1}.

HPTLC fingerprinting

Ethanol extract of *C. vitiginea* L. was taken by refluxing the material at a temperature of 75°C for 10 min. The extract was filtered and concentrated to the desired volume. The instrument employed was the CAMAG HPTLC system (Muttenz, Switzerland) equipped with a sample applicator TLC autosampler 4 with Win CATS software version 1.4.4. The volume of samples applied was Track 1-5 µL; Track 2-7 µL; and Track 3-10 µL. The plate was developed using solvent systems (Toluene: Ethyl acetate: Formic acid (4: 6: 0.1 v/v) in a twin trough chamber. The plate was developed up to 7 cm, removed from the chamber and allowed to dry. The developed plate was scanned using TLC Scanner 3 and analyzed with Win CATS software version 1.4.4. at λ_{max} 254 nm using a deuterium light source, the slit dimensions were 6.00 mm×0.40 mm. Densitometric documentation was done. After scanning, the plate was observed under 254 nm and 366 nm and TLC chromatograms were recorded. Then the plate was dipped in vanillin-sulfuric acid reagent and dried at 105°C on a hot plate till the colour of the spots appeared. The plate was visualized under white light and scanned at 575 nm. TLC chromatograms, R_f values and fingerprint data were recorded.

RESULTS

Macroscopic characters

The stem bark is blackish to reddish, densely covered with grey hairs (pubescent), swollen at the nodes and the tendrils are simple, stout and grow up to the length of 30-50 cm, nodal length 5-6.5 cm (Figure 1A and 1B).

Microscopic characteristics

Anatomy of Internode and development of successive cambia

Freehand Transverse Sections (T.S) of the fresh stem of *C. vitiginea* L. was taken and histological characters were studied. The young stem is circular in outline (3.5 mm thick) with a smooth and shiny appearance and shows abundant multicellular uniseriate to biseriate non-glandular and glandular trichomes (Figure 2A and E-H). The young stems possess thin-walled, barrel-shaped epidermal cells enclosed by striated uniseriate cuticle followed by a hypodermis comprising a few layers of chollenchymatous cells (Figure 2I). It is followed by a thin-walled oval to polygonal

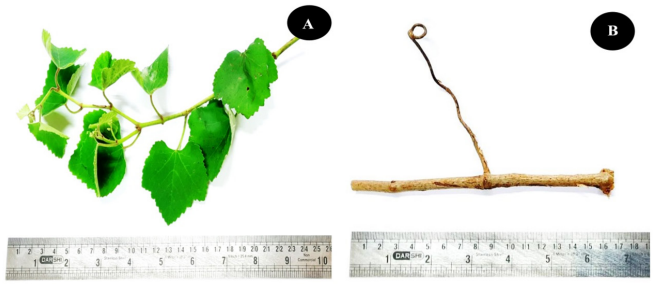

Figure 1: A) *C. vitiginea* L. twig with fresh leaves. B) Dried stem with tendril.

parenchymatous cortex with large mucilaginous cells (Figure 2B). The endodermis and pericycle appear indistinct (Figure 2A), but they can be identified by the presence of pericyclic fibre bundles (Figure 2M). Several radial collateral vascular bundles get interconnected by interfascicular cambium and form a complete cylinder of vascular cambium. The cambium produces secondary xylem centripetally and phloem centrifugally (Figure 2C). As evidenced by Figure 2A, a certain portion of the stem shows a complete absence of wide vessels, which alternates with a wide patch of cambial meristematic tissues. The pith is much wider and composed of thin-walled parenchymatous tissues with morphologically diverse starch grains (Figure 2B).

As the secondary growth progresses (in 5-6 mm thick stems), 4 to 5 layers of prominent sclerenchymatous layers start to develop just beneath the chollenchymatous hypodermal layer and the size of mucilaginous cells becomes much wider. The distribution and size of the trichome in the mature stem are antagonistic to each other. The proto-xylem elements started to invade the pith region as a result of the vessel development in the biseriate to triaseriate pattern with multiple perforation plates (Figure 2B). The initiation of the formation of single discrete units of bundles results in a narrow pith region (Figure 2C).

Secondary structure of Internode and nodal regions

The thick section of the mature stem is mostly circular with a crenate border (Figure 2D). Outer brown, internally beige in colour, and slightly aromatic in odour. The bark is well differentiated into phellem, phellogen and phelloderm (Figure 2J). The phellem is many-layered, thin-walled, small and rectangular. Phellogen is 3 to 5 layered with broad cells. And followed by phelloderm arranged in many layers and composed of thin-walled, tangentially elongated parenchymatous cells filled with chlorophyll (Figure 2K). The cortex has outer stretch masses of 3 to 4 layers of sclerenchyma masses containing rosette crystals and the inner cortex region is represented by thin-walled parenchymatous layers with idioblast and raphide sacs (Figure 2K and L). Large-size mucilage cells are frequently observed in the cortex. Semicircular discrete masses of fibres are located in a regular circle around the vascular cylinder. The fibres have thick lignified walls (Figure 2M). Radially arranged vascular bundles are separated by multi-seriate (5-8 layers) medullary rays with pitted parenchyma (Figure 3H). The phloem is well-developed and composed of small thin-walled cells (Figure 3D). A prominent cambial ring is present in between the phloem and xylem tissues. The xylem cylinder is thick and continuous (Figure 3F). Short rows of primary xylem occur along the inner border of the xylem cylinder (Figure 3E). The secondary xylem includes wide, circular, thin-walled, mostly solitary vessels with perforation plate and tylosis formations enclosed in a well stretch of tracheids (Figure 2I). Each xylem vessel is arranged in a zig-zag manner in each bundle and surrounded by paratracheal axial parenchyma (Figure 3F and G). Wide pith with thin-walled parenchymatous cells filled with clusters of starch grains was also observed (Figure 3J and K).

In the nodal region, the stem shows a periderm with a rough surface and lenticels (Figure 3L). A significant stretch of vascular tissues (Spiral vessels) in the wide cortex is responsible for the leaf trace. And fused fibre bundles are frequently seen in the cortex just above the phloem elements of individual bundles making a peculiar character for this portion (Figure 3M). The anatomy of the stellar region of the nodal region is completely similar to a mature stem.

Cellular structure of tendril

The tendril superficially resembles a young stem (Figure 4). In the young tendril, the epidermal layer consists of a thin continuous of small semicircular cells with prominent striated cuticles (Figure 4A and F). The hypodermal region is composed of 3-4 layers of thin-walled wavy parenchymatous cells with starch grains. The cortex consists of outer wide patches of sclerenchyma cells (Figure 4G) and the inner cortex consists of many layers of parenchyma cells and fibre bundles are absent (Figure 4A and B). The vascular cylinder is thin (150 µm thick), wide and hollow enclosing a wide parenchymatous pith. It comprises thick-walled lignified radial vessels restricted to a few elements situated along the inner border of the xylem cylinder (Figure 4B). Phloem is arranged in a thin layer on the outer region of the xylem cylinder (Figure 4A-D). The growth of tendrils is well evidenced by the development of the vascular system (Figure 4H-K). In the young tendril, vascular system development is in its initial stage and shows uniseriate xylem vessel and phloem elements (Figure 4H). Gradually it developed into a vascular bundle with triseriate xylem vessels (Figure 4I). In the next stage of development, xylem trachiery elements started to develop in between phloem and xylem vessels (Figure 4J) and finally became well developed vascular bundle with prominent trachiery elements in mature tendril (Figure 4K).

The anatomy of the base of a mature tendril is more or less similar to a mature stem. And which indicates the stem-modified ontogeny of the tendril. The similarities in anatomical features of the stem and tendril are presence of multicellular uniseriate to biseriate non-glandular trichomes, striated cuticles in young structures, collenchymatous hypodermis, well-developed sclerenchymatous layers and mucilage cells in the cortex region, semicircular discrete masses of lignified fibres around the vascular cylinder in matured structures, radially arranged vascular system, and thin-walled parenchymatous cells form the pith with starch grains. The differences are the absence of glandular trichomes, rosette crystals, medullary rays, and trachiery element dominant vascular system. However, to confirm the origin of this organ an ontogenetic study is needed.

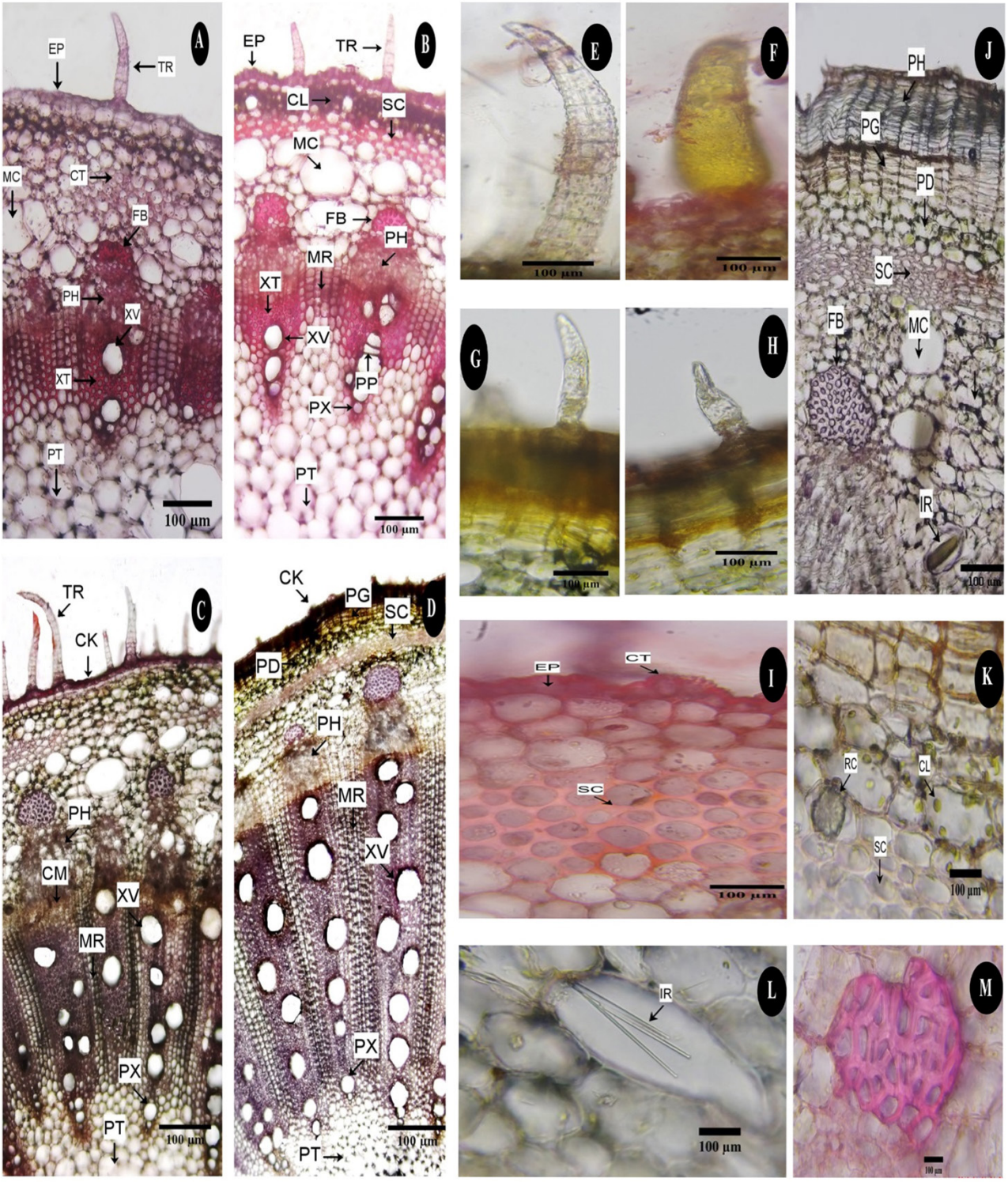

Figure 2: *C. vitiginea* L. stem structure. (Transverse sections). A-B: Primary structure; A. Young stem showing primary development of vascular bundles with wide cortex and pith, B. Thick layered epidermal formation with large mucilaginous canals; C-D: secondary structure; C. Cork layer formation and xylem secondary growth; D. Radial growth of vascular bundles. E-M: Enlarged view of epidermal and cortical portion. E-H: Trichomes; E. Multi-seriated non-glandular elongated trichomes on young stem; F. Unicellular glandular trichome on young stem; G and H. Morphologically diverse unicellular non-glandular trichomes seen on mature stem; I. Epidermal and subepidermal portion of young stem showing striated cuticle and sclerenchymatous layer; J-M: Details of mature stem; J. General aspects of matured stem; K. Subepidermal layers showing rosette crystals and chloroplast; L. Idioblast with raphides; M. Fibre bundle. (CT-Cuticle; EP-Epidermis; PH-Phellem; PG-Phellogen; PD-Phelloderm; SC-Sclerenchymatous layers; MC-Mucilaginous canal; FB-Fibre bundle; IR-Idioblast with raphides; CL-Chloroplast; RC-Rosette crystal; TR-Trichome; CK-Cork; CLCollenchymatous layer; CT-Cortex; PH-Phloem; CM-Cambium; MR-Medullary rays; XV-Xylem vessel; XT-Xylem tracheids; PX-Primary xylem; PT-Pith; PP-Perforation plate).

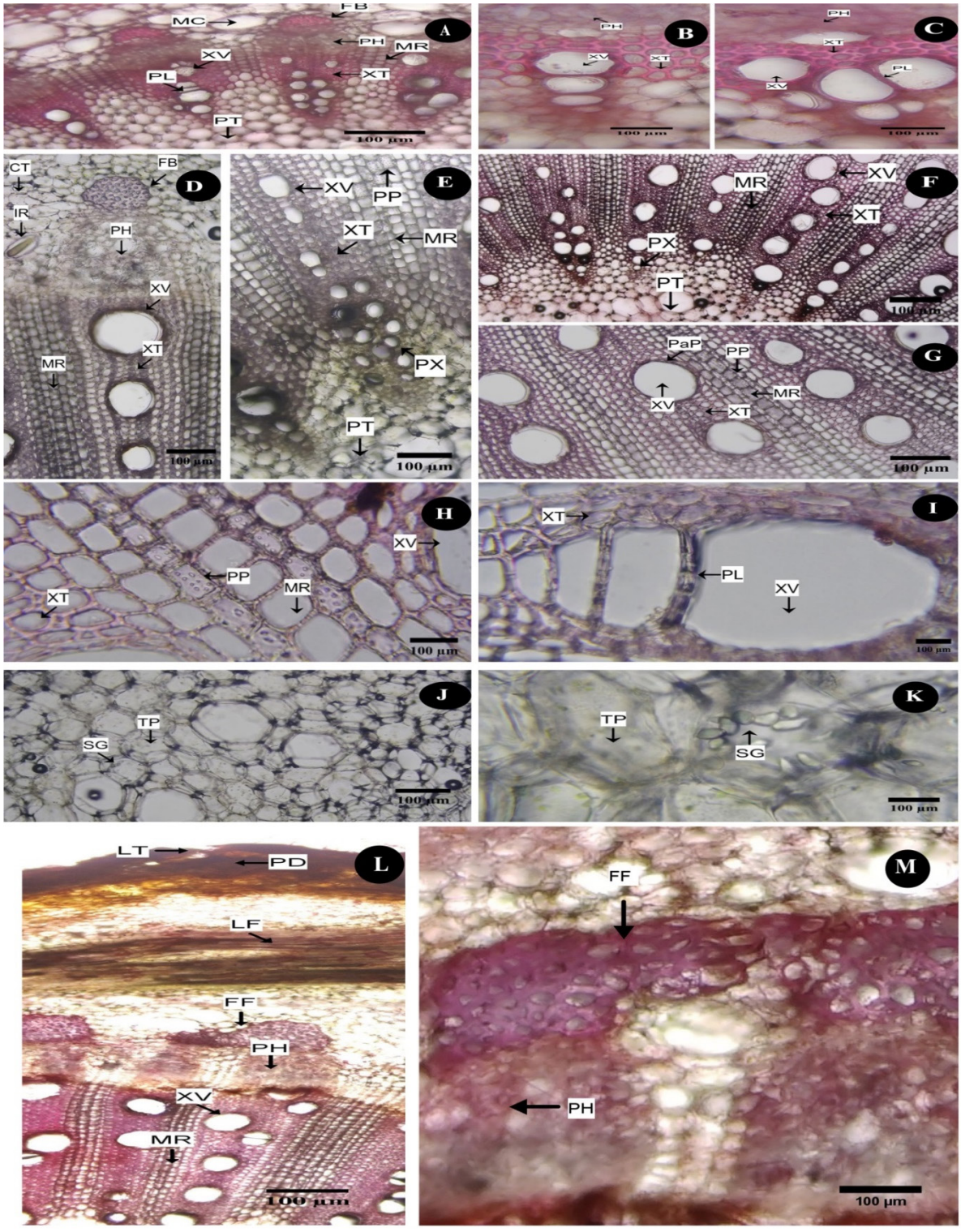

Figure 3: *C. vitiginea* stem-Vascular system and Nodal anatomy. A-C: Primary development of vascular bundle of young stem; A-Radially arranged vascular system showing smaller bundles with prominent xylem and phloem elements; B-Uniserite bundle; C-Multiseriate bundle; D-K: Details of vascular system and pith of mature stem; D-enlarged view of single vascular bundle with secondary growth; E-Enlarged view of the central portion of the transverse section of stem; F-Radially arranged xylem vascular bundles; G-Enlarged view of radially arranged xylem elements; H-Xylem elements showing pitted parenchyma; I-J: Xylem vessel with perforation; K-Pith cells contain clusters of starch grain, L-Transverse section of stem nodal region showing periderm with lenticel and cortex region with leaf trace and fused fibre bundles above the vascular bundles, M-Enlarged view of fused fibre bundles (MC-Mucilaginous canal; FB-Fibre bundle; IR-Idioblast with raphides; CT-Cortex; PH-Phloem; MR-Medullary rays; XV-Xylem vessels; XT-Xylem tracheids; PL-Perforation plate; PT-Pith; PP-Pitted parenchyma; PaP-Paratracheial axial parenchyma; PX-Protoxylem; TP-Thin-walled parenchyma; SG-Starch grains; LT-Lenticel; PD-Periderm; LF-Leaf trace; FF-Fused fibre).

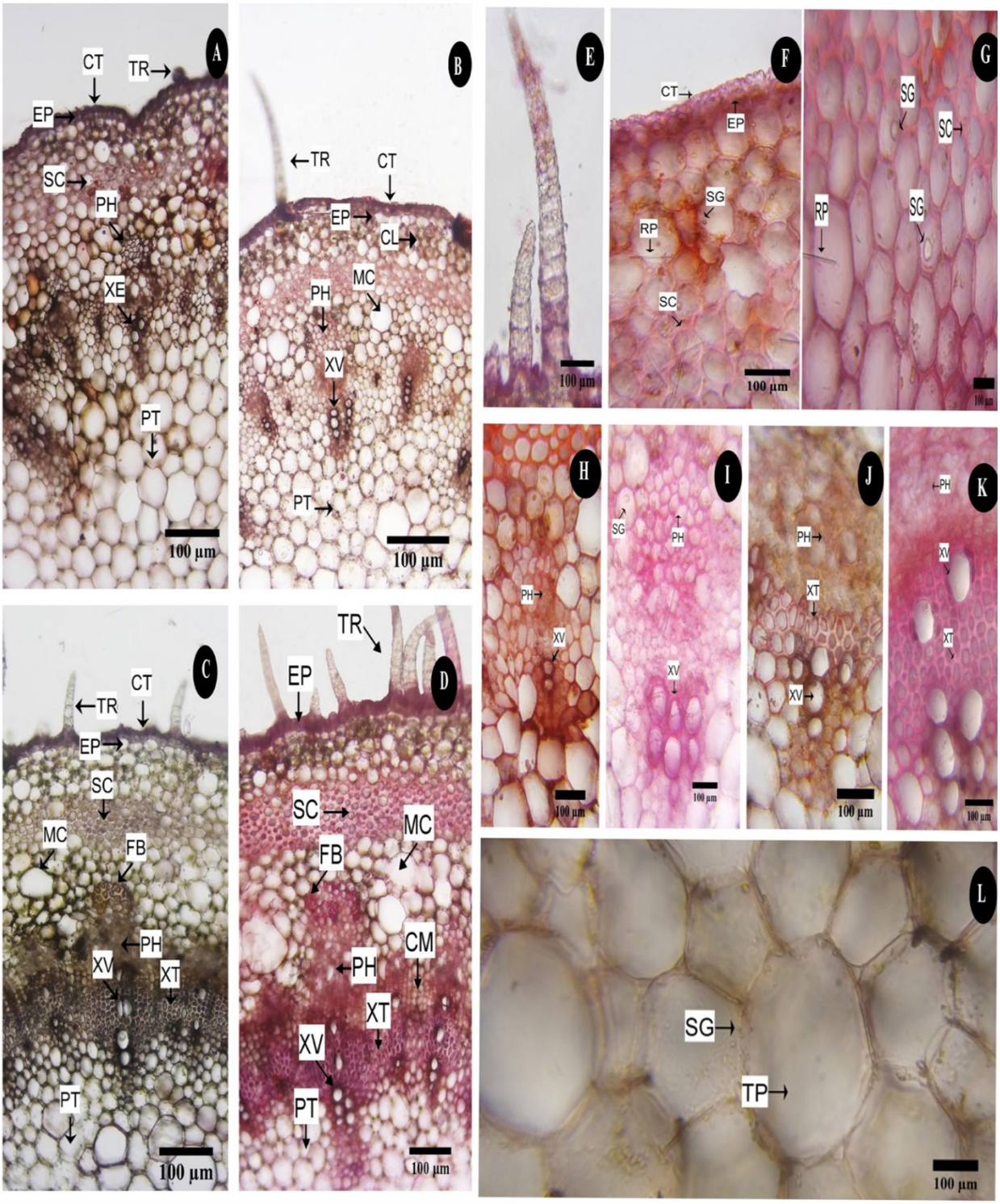

Figure 4: *C. vitiginea* L. tendril structure detailed with stages of vascular system development (Transverse sections). A-D: Anatomy of different parts of tendril showing growth stages. A-distal region of tendril; B-Median region of tendril; C-Mature tendril with prominent development of xylem elements; D-Base of a mature tendril showing anatomy similar to the secondary growth of stem, E-F: Epidermal details of young tendril; E-Elongated biserite non-glandular trichomes; F-Enlarged view of epidermal portion showing striated cuticle; G-Sub-epidermal portion showing sclerenchymatous layers with starchgrains and raphides; H-K: Developmental stages of vascular system; H-Initial stage of vascular system development showing a uniseriate xylem vessel and phloem elements; I-Vascular bundle with triseriate xylem vessel; J-Xylem trachiery elements started to develop in between phloem and xylem vessels; K-Well developed vascular bundle with prominent trachiery elements; L-Pith composed of thin-walled parenchyma with starch grains (TR-Trichome; CT-Cuticle; EP-Epidermis; CL-Chollenchymatous layer; CM-Cambium; FB-Fibre bundle; MC-Mucilaginous canal; RP-Raphides; SG-Starch grains; SC-Sclerenchymatous layer; PH-Phloem; XE-Xylem element; XV-Xylem vessels; XT-Xylem tracheids; PT-Pith; TP-Thin-walled parenchyma).

Powder microscopic characters

The powdered stem of *C. vitigine*a is light brown in colour with no characteristic odour or taste. And shows the presence of characters like thin-walled parenchyma fragments, xylem parenchyma cells, uniseriate non-glandular trichome, biseriate non-glandular trichome, warty trichome, idioblast with raphides, elongated acicular crystal, oil cell, rosette crystal, prismatic calcium oxalate crystal, tannin content, starch grains, reticulate

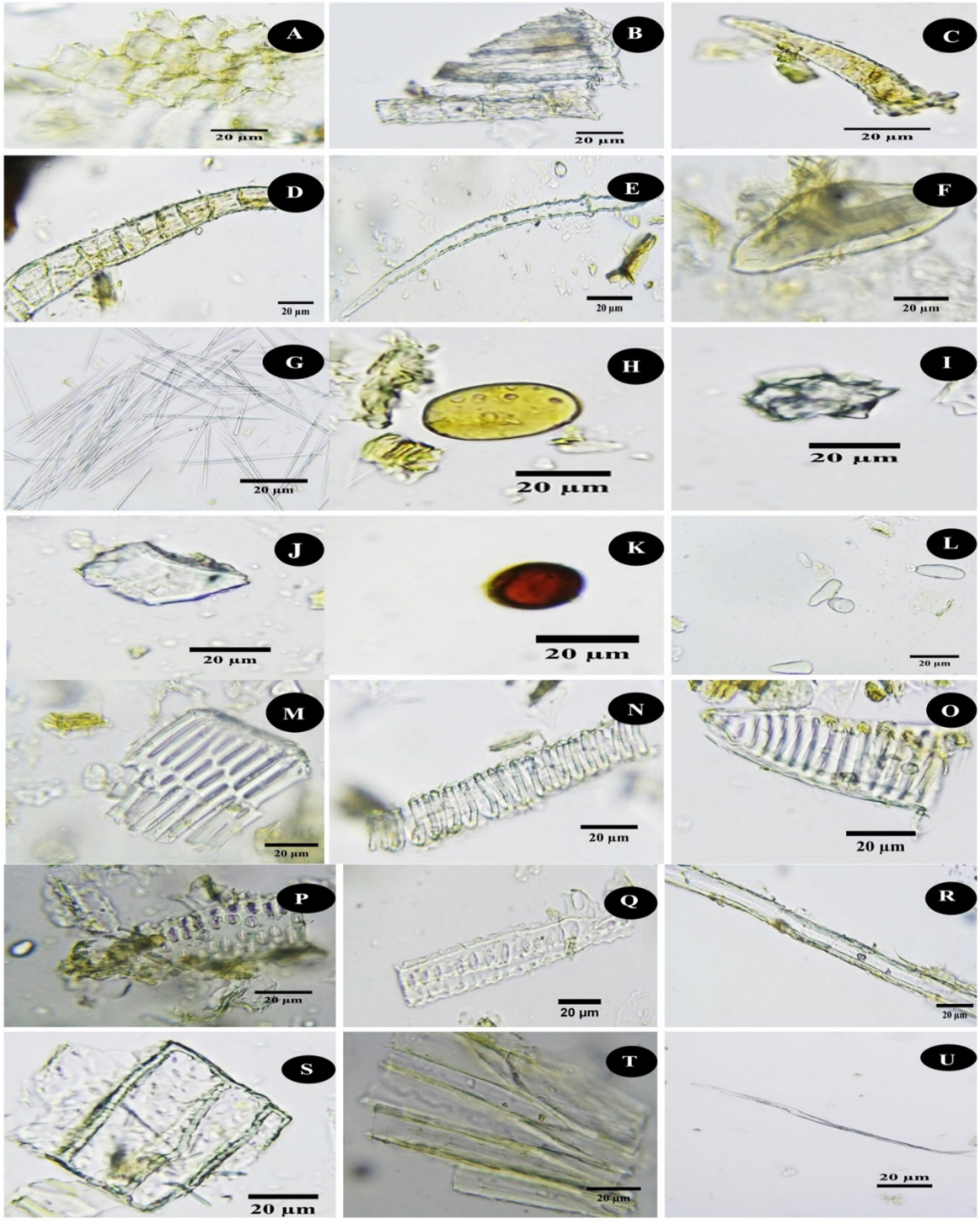

Figure 5: Powder microscopic characters of stem of *C. vitiginea* L. A: thin-walled parenchyma fragment, B: xylem parenchyma cells, C: uniseriate non-glandular trichome, D: biseriate non-glandular trichome, E: warty trichome, F: idioblast with raphides, G: acicular crystal, H: oil cell, I: rosette crystal, J: prismatic calcium oxalate crystal, K: tannin content, L: starch grains, M: reticulate vessel, N: spiral vessel fragment, O: annular tracheid, P: boardered pitted vessel, Q: simple pitted vessel, R: thick-walled tracheid with narrow lumen, S: xylem pitted parenchyma, T: lignified fibre bundle, and U: fibre.

vessel, spiral vessel fragment, annular tracheid, bordered pitted vessel, simple pitted vessel, thick-walled tracheid with narrow lumen, xylem pitted parenchyma, lignified fibre bundle, and fibre (Figure 5A-U).

Physico-chemical evaluation

The physicochemical parameters such as loss on drying at 105°C, ash content, acid insoluble ash, extractive values (water soluble extractive and alcohol soluble extractive), and volatile oil were evaluated and results were tabulated (Table 1).

FT-IR Analysis

In the present study, FTIR spectroscopy was used to identify the functional groups based on the peak values in the IR spectra of the stem of *Cissus vitiginea* L. (Figure 6). The result obtained indicated the presence of various functional groups are summarised in Table 2. Weak absorption peaks noted at frequencies of 3394 and 3340 cm^{-1} represent C-H stretching and O-H stretch. There is only one strong peak was observed in the entire IR spectra of the stem of *Cissus vitiginea* L. and that was attributed to C=C stretching at 1620 cm^{-1}. In this study, a range of weak peaks are observed in the regions with frequencies of 1092, 1045, 887, 820, and 781 cm^{-1}. The peak absorbance at 3340, 2852, and 1518 are characteristic of the hydroxyl functional group in alcohols and phenolic compounds. The presence of aliphatic and aromatic hydrocarbons was noted at the frequencies of 3394, 2920, 2368, 1736, 1441, 887 and 820 cm^{-1}, in which peaks at 2920, 2368 and 1441 represent saturated aliphatic hydrocarbons, i.e, alkanes (C-H bond/stretch) and peaks at 820 corresponded to unsaturated hydrocarbon, alkene (C=H bend) and 3394 for alkyne (C≡H stretch) respectively. Similarly, the jaggy peaks at the frequencies of 1736 (C-H bend) and 887 (=C-H bend) belong to aromatic hydrocarbons. A range of C-N stretch was observed at 1248, 1092, and 1045 cm^{-1} and a notable peak at 781cm^{-1} assigned for the halo compound.

Table 1: The physicochemical parameters of *C. vitiginea* L. Stem.

Parameters	Result (%)
Loss on Drying at 105°C	9.74
Ash Content	3.39
Acid Insoluble Ash	0.09
Water Soluble Extractive	12.33
Alcohol Soluble Extractive	8.25
Volatile oil	1.00

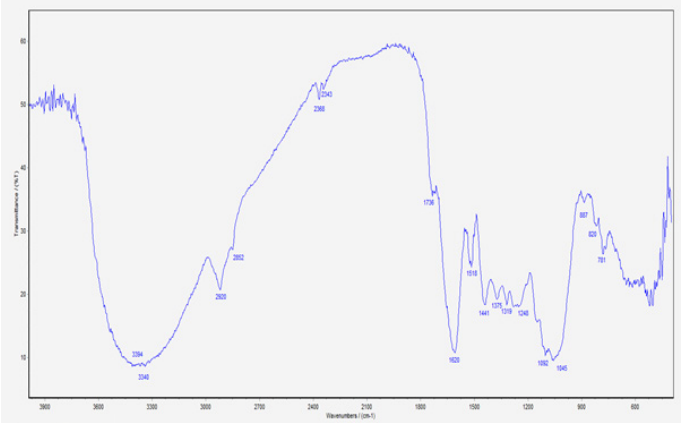

Figure 6: FTIR spectra of *Cissus vitiginea* L. stem.

HPTLC Analysis

The HPTLC fingerprint study of *C. vitiginea* L. stem carried out by using Toluene: Ethyl acetate: Formic acid (5: 7:0.1) as the solvent system at three wavelengths (254, 366, and 575 nm) is illustrated in Figure 7 and the Table 2 with peak values (Figure 8) showing the R_f values of different peaks which indicates diverse phytochemicals present in the sample in different concentrations. Analysis of alcohol extract at 254 nm revealed six peaks with R_f

Table 2: FTIR Peak Values of *Cissus vitiginea* L. stem.

Peak values	Bonds	Functional group
3394	C≡H stretch	Alkyne
3340	O-H stretch	Phenol
2920	C-H stretch	Alkane
2852	O-H stretch	Alcohol
2368	C-H bond	Saturated aliphatic ester
2343	C ≡ C bond	Carbonyl group
1736	C-H bend	Aromatic compound
1620	C=C stretch	α,β-unsaturated ketone
1518	C-C stretch	Phenyl group
1441	C-H bend	Alkane
1375, 1319	O-H bend	Phenol
1248, 1092, 1045	C-N stretch	Amine
887	=C-H bend	Aromatic ring
820	C=H bend	Alkene
781	C-Cl stretch	Halo compound

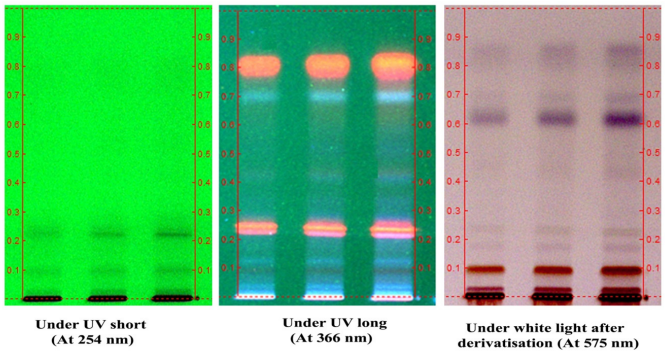

Figure 7: HPTLC profile of alcohol extract of the stem of *Cissus vitiginea* L. Solvent system-Toluene: Ethyl acetate: Formic acid (5:7:0.1); Volume applied; Track 1-5 µL: Track 2-7 µL: Track 1-10 µL.

At 254 nm

Peak	Start Position	Start Height	Max Position	Max Height	Max %	End Position	End Height	Area	Area %
1	0.01 Rf	36.6 AU	0.01 Rf	55.7 AU	19.88 %	0.04 Rf	0.1 AU	529.9 AU	6.51 %
2	0.06 Rf	0.3 AU	0.14 Rf	131.9 AU	47.08 %	0.24 Rf	0.7 AU	3561.4 AU	43.78 %
3	0.31 Rf	1.0 AU	0.34 Rf	14.1 AU	5.04 %	0.39 Rf	6.7 AU	438.8 AU	5.39 %
4	0.40 Rf	6.5 AU	0.46 Rf	20.8 AU	7.42 %	0.51 Rf	9.6 AU	967.4 AU	11.89 %
5	0.61 Rf	11.8 AU	0.70 Rf	33.4 AU	11.93 %	0.78 Rf	9.5 AU	2385.0 AU	29.32 %
6	0.90 Rf	4.3 AU	0.93 Rf	24.3 AU	8.65 %	0.94 Rf	0.5 AU	252.0 AU	3.10 %

At 366 nm

Peak	Start Position	Start Height	Max Position	Max Height	Max %	End Position	End Height	Area	Area %
1	0.12 Rf	8.2 AU	0.16 Rf	114.3 AU	48.26 %	0.22 Rf	4.5 AU	1886.6 AU	32.86 %
2	0.57 Rf	6.4 AU	0.63 Rf	21.4 AU	9.03 %	0.67 Rf	10.1 AU	755.6 AU	13.16 %
3	0.68 Rf	10.6 AU	0.74 Rf	101.1 AU	42.71 %	0.83 Rf	1.5 AU	3098.3 AU	53.97 %

At 575 nm

Peak	Start Position	Start Height	Max Position	Max Height	Max %	End Position	End Height	Area	Area %
1	0.01 Rf	193.5 AU	0.01 Rf	248.4 AU	18.90 %	0.02 Rf	11.5 AU	1694.6 AU	4.99 %
2	0.03 Rf	5.4 AU	0.04 Rf	106.2 AU	8.08 %	0.05 Rf	0.0 AU	759.8 AU	2.24 %
3	0.06 Rf	1.2 AU	0.10 Rf	333.2 AU	25.36 %	0.14 Rf	0.1 AU	6149.1 AU	18.11 %
4	0.15 Rf	0.0 AU	0.18 Rf	42.7 AU	3.25 %	0.21 Rf	5.9 AU	751.0 AU	2.21 %
5	0.21 Rf	6.4 AU	0.24 Rf	45.6 AU	3.47 %	0.26 Rf	0.2 AU	856.8 AU	2.52 %
6	0.39 Rf	6.1 AU	0.45 Rf	44.2 AU	3.36 %	0.48 Rf	31.8 AU	1727.2 AU	5.09 %
7	0.53 Rf	31.3 AU	0.63 Rf	277.9 AU	21.15 %	0.68 Rf	73.1 AU	11373.3 AU	33.50 %
8	0.68 Rf	73.2 AU	0.70 Rf	85.7 AU	6.52 %	0.76 Rf	32.5 AU	2809.2 AU	8.27 %
9	0.76 Rf	32.6 AU	0.87 Rf	130.3 AU	9.91 %	0.99 Rf	0.1 AU	7828.5 AU	23.06 %

Figure 8: Peak list of alcoholic extract of stem of *Cissus vitiginea* L. at different wavelengths (At 254 nm, 366 nm, and 575 nm).

values ranging from 0.01 to 0.90. Spots with R_f values 0.07 and 0.62 are the two intense peaks among the others indicating the highest concentration of phytoconstituents present in the sample that account for 56.45% and 45.61% area. At 366 nm, three peaks are observed. Among them, those with R_f values 0.68 and 0.67 are predominant with 53.97% and 49.66% area respectively. After the derivatisation with vanillin-sulfuric acid (575 nm), nine peaks are detected with a range of R_f values 0.01, 0.03, 0.06, 0.15, 0.21, 0.39, 0.53, 0.68, and 0.76. R_f values 0.53 and 0.76 correspond to the area percentage of 33.50% and 23.06% are dominant.

DISCUSSION

In this paper, the macro and microscopical characters and physicochemical studies of the stem of *Cissus vitiginea* L. are presented. Anatomy is an important taxonomic tool for the certification and quality control of medicinal plants and the localization of secretion and accumulation of biologically active compounds. The interest in the biology of vines and the functional significance of their anatomy has been started in the middle of 20th century.[10] Shared characters of members of family 'Vitaceae' were well studied earlier, i.e., exclusively simple perforation plates, large rays, vessel-ray parenchyma pits with reduced borders, storied imperforate elements, scanty paratracheal parenchyma, and septate fibres.[11] And all these points are corroborated by the result of the present study.

Non-glandular trichomes have already been reported for the *Cissus* species.[12,13] The presence of multicellular and uniseriate non-glandular trichomes in *C. vitiginea* agrees with the result of a previous study in *C. verticillata.*[14] The functions of the non-glandular trichomes vary according to their type, abundance, and position in which they occur.[15] The presence of glandular trichomes in the stem of *C. vitiginea* was supported by the presence of the same in some species of Vitis known as *V. romanetii, V. adenoclada* and *V. shenxiensis* respectively.[16] In general, they protected plants from several external factors, such as herbivores and pathogens, excessive light, high temperatures, and excessive water loss.[17]

The presence of large mucilage cavities in the cortex of *C. vitiginea* is supported by a previous study of *C. quadrangularis* L.[18] The inner cortex characters like two or three layers of parenchyma cells and prominent, discrete circular masses of thick-walled and lignified fibres were also found in another vitaceae member which is closely related to the *Cissus* species named '*Cayratia pedata*' (Lam.) Gagnep.[19] The presence of a thick cylindrical bundle comprising several thin pointed needles called 'raphides' located alongside the elongated wide parenchyma cells in *C. vitiginea* is supported by a study of *C. quadrangularis* L.[20] Even though the presence of raphides is common in the genus *Cissus*, the distribution is species-specific. Raphides were identified in the enlarged ray cells of *V. coignetiae, V. rotundifolia var. munsoniana,*

V. riparia, and *V. rotundifoli*a var. rotundifolia Moore while they were seen in the cortex region of *C. vitiginea.*

The vessels are mostly solitary, unlignified rays and ground tissue, vessels in the ring adjacent to the pith markedly narrower than in subsequently formed xylem in *Cissus neerii.*[11] This observation is supporting evidence for the xylem vessel arrangement in *C. vitiginea.* Many researchers already studied and reported the presence of parenchymatous pith with the starch reserve in the species of genus *Cissus*. The similar anatomical pattern between the stem and tendril of *C. vitiginea*, is particularly related to the vascularization as reported for another Vitis species named *C. verticillata.*[14] Even though it suggests a common origin, an ontogenetic study is needed to confirm the origin of this organ.

The most practical method for the primary authentication of a drug sample is powder microscopy.[21] The characters found in the sample help in the identification of the right variety and search for adulterants.[22] In this present study, different types of trichomes, morphologically diverse calcium oxalate crystals (Raphides, acicular crystals, rosette crystals, and prismatic crystals), and various kinds of thickening of vascular tissues were observed. So in conclusion we can state that the powder microscopic result of the present study substantiates the anatomical features of the stem of *C. vitiginea*.

Quantitative standards are used to ensure the therapeutic efficacy of herbal drugs.[23] In this study, physicochemical parameters like ash content, acid insoluble ash, extractive values (water soluble extractive and alcohol soluble extractive), loss on drying at 105ºC and volatile oil were used to determine the purity and quality of the crude drug. The ash of crude drugs consists of non-volatile inorganic materials which can be used to set quality control parameters to check the contamination of crude drugs. A notable ash value is indicative of contamination, substitution, and adulteration[24] which are 3.39% for *C. vitiginea*. The acid insoluble ash value represents the quantity of siliceous matter present in the sample[25] and it is 0.09 % for *Cissus vitiginea* indicating good quality. The extractive value in a particular solvent is the parameter used to estimate the active constituents in a given amount of crude drug.[26] In the case of *C. vitiginea*, water-soluble extractive was found to be higher than alcohol-soluble extractive. It hints at the high ability of water to extract the maximum components of *C. vitiginea* into it. Loss on drying at 105ºC of *C. vitiginea* indicates that only 9.74% of water and volatile components were lost when 4 g of *C. vitiginea* was kept at 105ºC. This moisture content helped to prevent deprivation of efficacy and degeneration of the sample. High moisture content can adversely affect the active principles of the drug. It may get an early infection of the drug. Thus, low moisture content could get maximum stability and long shelf life.[27] Aromatic compounds predominate in certain volatile oils. Because they are considered to be the "essence" of the plant material and often biologically active, they are also known as "essential oils". In *C. vitiginea*, volatile oil content is found to be 1.00%.

The differences in spectra are the reflection of componential differences.[28] By using the FT-IR spectrum, we can confirm the functional constituent's presence in the given parts and extract, identify the medicinal materials from the adulterate and even evaluate the qualities of medicinal materials. The results of the present study spectrum revealed the presence of functional constituents like phenol, alkanes, aldehyde, alcohol, carboxylic acids, aromatic and aliphatic amines. Which is already reported in the leaf of the same species.[29] In this study, there are some weak peaks observed in the fingerprint region of IR spectra ranging from 1200-700 cm^{-1}. Many different vibrations, including C-N single bond stretches, =C-H bending vibrations, C=C bend, C-Cl stretch and some bands due to benzene rings are found in this region. The fingerprint region is commonly the most complex region of the spectrum and the last section of a spectrum to be interpreted. However, the significance of the fingerprint region is that the many bands present there provide a fingerprint for a molecule.

HPTLC fingerprinting is a linear, precise, and accurate method for herbal identification[30] and is useful in quality control of herbal products and checking for adulterants.[31] Apart from that, HPTLC fingerprinting of particular plant species will provide basic information useful for the isolation, characterization and identification of marker compounds of the species.[32] There is a significant area of percentage observed of R_f values 0.06, 0.68, and 0.53 at 254 nm, 366 nm, and 575 nm respectively. The area of percentage corresponding to the specific R_f values indicates the presence of the relative amount of phytoconstituents in the sample.[33] The result of the present study suggests that *C. vitiginea* contains a notable amount of phytoconstituents and that may be responsible for its medicinal properties. The results confirm the preliminary observations driven from FT-IR for the presence of various phytoconstituents in the stem of *C. vitiginea*. The chromatograms generated after HPTLC study can be used to establish reference HPTLC fingerprints of the stem of *Cissus vitiginea* against which raw materials can be evaluated and finished products containing the plant material can be analyzed.

CONCLUSION

In the present study, the pharmacognostic and physicochemical characteristics of *Cissus vitiginea* L. (stem) were studied. Various parameters established in the present study will help in controlling the standards and quality of the raw drug material of Siddha medicine 'Panadiappan'. All the pharmacognostic characters and physicochemical parameters of the specific plant part have been reported in detail for the first time and could be useful in the identification and standardization of a crude drug. The data produced in the present investigation is also helpful in the preparation of the crude drug's monograph and inclusion in

various pharmacopoeias and offers scope for further research in the field of medical and pharmacological evaluation and development of a formulation for treating various ailments.

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CONFLICT OF INTEREST

The authors declare that there is no conflict of interest.

AUTHOR'S CONTRIBUTIONS

SK and MCN carried out the original research work in the laboratory experiments and wrote the manuscript. GKS and NKB were guided in performing the research and writing the manuscript. RVL helped in carrying out the FT-IR analysis and guided during the work and SMV contributed during the reviewing. LGS gave guidance and support.

ABBREVIATIONS

FTIR: Fourier Transform Infrared Spectroscopy; **HPTLC:** High-performance thin layer chromatography; **KBr:** Potassium bromide; **R_f value:** Retardation factor value.

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