



Pharmacognostical standardisation of an epiphytic orchid, *Luisia tenuifolia* Blume

[Estandarización farmacognóstica de una orquídea epífita, *Luisia tenuifolia* Blume]

Sakthi P. Sethuraman, Kumar P. Ramachandran*

Department of Pharmacognosy, SRM College of Pharmacy, Faculty of Medicine and Health Sciences, SRM Institute of Science & Technology, SRM Nagar, Kattankulathur, 603203, Kanchipuram, Chennai TN, India.

*E-mail: kumarp1@srmist.edu.in

Abstract

Context: Pharmacognostical standardization of crude drugs is essential before carrying out its scientific validation as it furnishes the identity and authenticity of the plant. *Luisia tenuifolia* Blume also known as 'slender leaved *Luisia*', has been found to be traditionally practised in treating cuts, boils, wounds and abscesses.

Aims: To perform the pharmacognostical investigation of *L. tenuifolia* and thereby reporting the morpho-anatomical, histochemical and physico-chemical characteristics of the epiphyte.

Methods: Anatomical characteristic features were observed from the transverse sections of the plant specimens while staining of the transverse sections by using suitable chemical reagents was performed for the histochemical analysis. Moreover, the powder microscopy, physicochemical parameters and fluorescence analysis were also carried out on the pulverised whole plants of *L. tenuifolia*.

Results: The morphological and anatomical characteristics revealed in the study will remain as valuable diagnostic features in the botanical authentication of this orchid. The powder microscopical characters revealed the presence of epidermal fragments and paracytic stomata, fibres, vessel elements and sclereids. The histochemical profile of different parts of the epiphyte showed the localisation of tannins, starch, lipids, lignin, mucilage, protein, cutin and calcium oxalate crystals in the tissue system. The physico-chemical parameters and fluorescence observations determined the pharmacopoeial standards of the plant in powdered form.

Conclusions: Thus, the study records the morpho-anatomical, histochemical and physicochemical standards of the whole plants of *L. tenuifolia* in order to achieve pharmacognostical standardization of this epiphytic orchid. Further, phytochemical standardization and pharmacological validation are under pipeline owing towards an effective phyto-pharmacological research.

Keywords: histochemistry; *Luisia tenuifolia*; physicochemical constants; powder microscopy; *Orchidaceae*; whole plant.

Resumen

Contexto: La estandarización farmacognóstica de los medicamentos crudos es fundamental antes de llevar a cabo su validación científica, ya que proporciona la identidad y autenticidad de la planta. Se ha descubierto que la *Luisia tenuifolia* Blume, también conocida como "Luisia de hojas delgadas", se practica tradicionalmente en el tratamiento de cortes, forúnculos, heridas y abscesos.

Objetivos: Realizar la investigación farmacognóstica de *L. tenuifolia* y así informar las características morfoanatómicas, histoquímicas y fisicoquímicas de la epífita.

Métodos: Se observaron rasgos anatómicos característicos de las secciones transversales de los especímenes de plantas mientras se realizaba la tinción de las secciones transversales mediante el uso de reactivos químicos adecuados para el análisis histoquímico. Además, la microscopía de polvo, los parámetros fisicoquímicos y el análisis de fluorescencia también se llevaron a cabo en las plantas enteras pulverizadas de *L. tenuifolia*.

Resultados: Las características morfológicas y anatómicas reveladas en el estudio permanecerán como características diagnósticas valiosas en la autenticación botánica de esta orquídea. Los caracteres microscópicos de polvo revelaron la presencia de fragmentos epidérmicos y estomas paracíticos, fibras, elementos vasculares y esclereidas. El perfil histoquímico de diferentes partes de la epífita mostró la localización de taninos, almidón, lípidos, lignina, mucílagos, proteínas, cutina y cristales de oxalato de calcio en el sistema tisular. Los parámetros físico-químicos y las observaciones de fluorescencia determinaron los estándares farmacopoeicos de la planta en forma de polvo.

Conclusiones: De esta manera, el estudio registra los estándares morfoanatómicos, histoquímicos y fisicoquímicos de todas las plantas de *L. tenuifolia* para lograr la estandarización farmacognóstica de esta orquídea epífita. Además, la estandarización fitoquímica y la validación farmacológica están en proceso debido a una investigación fito-farmacológica eficaz.

Palabras Clave: constantes fisicoquímicas; histoquímica; *Luisia tenuifolia*; microscopía de polvo; *Orchidaceae*; toda la planta.

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AUTHOR INFO

ORCID: 0000-0002-2737-0365 (KPR)



INTRODUCTION

Although there remains a co-evolutionary relationship between plants and mankind, plants stand as the main reason behind human existence. Plants play the dual role of being both food as well as medicine for mankind. The nature research evolved as food science, flourished as an ethnomedicine, and later established itself as Pharmacognosy. India stands as a predominant shelter of an enormously diversified ecosystem and rich heritage of traditional knowledge. From time immemorial, natural products justify their vital role in therapeutic research with their promising and never exhausting novel leads (Cragg and Newman, 2013).

Orchidaceae remains as a family with miraculous therapeutic properties and comprise nearly 25 000 to 35 000 species. It has about 750-850 genera that were widely distributed in tropical and subtropical regions (Hossain, 2011; Pathak et al., 2001). The orchid plant family is known for its pretty, colourful, fragrant complex flowers and stands as the second most popular cut flowers in floriculture (USDA, 2008). In addition to their ornamental significance, the orchids also hold a rich traditional usage against several ailments (Hossain, 2011). This might be due to the presence of promising phyto-compounds that demands further scientific validation.

Luisia tenuifolia Blume (*Orchidaceae*) is also known as 'slender leaved *Luisia*' and is found in Southern India at high elevations of above 1500 m. The epiphyte exists as large clusters on tree trunks in tropical, wet, evergreen forests. It is also distributed in Sri Lanka and Andaman and Nicobar Islands (Teoh, 2016). The whole plants are traditionally used as an emollient. It is applied in the form of poultice on boils, abscesses and tumours. It is also used to treat kidney diseases, leucorrhoea and gonorrhoea (Chopra et al., 1956; The Wealth of India, 1965; Karuppusamy and Kumuthakalavalli, 2002; Hossain, 2011).

The present study encompasses the macroscopical and microscopical features of the leaf, stem, root, flower and fruit parts of *L. tenuifolia*. Also, the histochemical analysis was carried out in order to attain a better insight into the localisation of its metabolites. Additionally, powder microscopical characters and physicochemical constants including ash and extractive values, loss on drying, foaming index, and fluorescent characteristics of the powdered whole plant of *L. tenuifolia* were also determined owing to a proper identification and standardization of this orchid.

MATERIAL AND METHODS

Chemicals and reagents

All the chemicals and reagents used in the study were procured from certified suppliers and were of the highest analytical grade.

Plant collection

Fresh healthy samples of whole plants of *Luisia tenuifolia* were collected from Yercaud (Dt), (11.40°N, 78.22°E), Tamil Nadu, during February 2019. The plant specimens were further identified and authenticated in the Botanical Survey of India (BSI), Coimbatore, with voucher BSI/SRC/5/23/2019/Tech/3443.

Fixation and sectioning of *Luisia tenuifolia*

These specimens were fixed in formalin-acetic acid-ethanol (1:1:18) for 24-48 h, dehydrated using graded series of t-butyl alcohol. The specimens were further subjected to infiltration with paraffin wax at a melting point of 58-60°C and were cast into paraffin blocks. The paraffin embedded samples were sectioned with the help of a rotary microtome (Weswox rotary microtome, ARMT-1090a, Haryana) to a thickness of 10 µm. The sections after dewaxing were stained and observed for microscopical studies (Johansen, 1940; Sass, 1940; O'Brien et al., 1964).

Histochemical staining

The deparaffinised and hydrated sections were stained with specific chemical reagents, and the localization of metabolites in leaf, stem, root, flower, and fruit were studied using the standard procedure. Accordingly, the sections were treated with a saturated solution of aqueous phloroglucinol in hydrochloric acid for the detection of lignins. For the detection of proteins, the sections were stained in 0.02% Coomassie brilliant blue R 250 in Clark's solution (pH 2). Further, iodine in potassium iodide solution was used for the detection of starch. Mucilage was detected using toluidine blue. Tannins were detected using dilute ferric chloride solution. Lipids were stained with Nile blue in acetic acid solution, and crystals were observed under polarized light (Gramwell, 1955; O'Brien et al., 1964; Krishnamurthy, 1988).

Powder microscopical studies

The whole plants of *L. tenuifolia* were pulverised after drying under shade and passed through 100 mesh

sieve. A powdered material was transferred to a microscopic slide and treated with chloral hydrate in order to clear the chlorophyll. Then the powdered drug was mounted in 50% v/v glycerine and observed under a microscope, and the powder characteristic features were recorded (Brain and Turner, 1975; Kokate, 1986).

Physico-chemical evaluation

The ash values, extractive values, loss on drying, crude fibre content, swelling index, and foaming index of the dried and powdered whole plants of *L. tenuifolia* were determined as per the official methods given in Indian Pharmacopoeia and WHO guidelines on quality control methods for medicinal plant materials (WHO, 1992; Indian Pharmacopoeia, 1996).

Fluorescence analysis

Fluorescence analysis was carried out in accordance with the methods prescribed by Chase and Pratt (1949) and Kokoshi et al. (1958). Accordingly, the powdered plant material was treated with various solvents and reagents and observed under daylight, and ultra-violet lamps of shorter (254 nm) and longer (365 nm) wavelengths and the fluorescent colour changes were noted.

RESULTS AND DISCUSSION

Very few research works were found to have been carried out in *L. tenuifolia*. A study by Balachandar et al. (2019) has reported the comparative vegetative anatomy and mycorrhizal morphology of various species of *Luisia*. Also, another study on the dermal anatomy of two corticolous orchids was investigated by Mulgaonkar (2005). Even now, there remains a growing demand in the transformation of unexplored plants from their ethnobotanical usage towards therapeutic validation. Therefore, proper pharmacognostical standardization of medicinal plants is necessary to become a safe and effective drug (Zhu et al., 2015; Marandi et al., 2016). In the present study, pharmacognostical standardization of whole plants of *L. tenuifolia* was established by documenting the morphological, microscopical, powder microscopical, histochemical, physicochemical and fluorescence studies.

Morphological description

The morphological studies revealed that the plant possesses terete leaves, woody stems, and racemose inflorescence with vermiform roots as its characteristic features (Fig. 1A). The leaf is thick, smooth, cylindrical, and light to dark green in colour. The leaves are often 6-

10 in number, 8-9 cm × 1-2 cm variable in thickness, straight tailed at apex forming dense clusters (Fig. 1B). The stem appears long, slender, dark brown to green, usually 10-30 cm in length and 3-5 mm thick, stout and uniform (Fig. 1C). The roots are strong, penetrate the bark, and get attached to the host tree (Fig. 1D). Flowers are few and form extra-axillary inflorescence with a single flower facing downwards (Fig. 1E). Fruits are spindle-shaped, green, minutely 6-ribbed, hexagonal, 4-6 cm long, with persistent calyx lobes (Fig. 1F).

Microscopical features

The leaf is modified into a cylindrical, vertically elongated body. In the cross-sectional view, it is elliptical in outline. The anatomy of the leaf showed a distinct epidermal layer followed by a homogenous parenchymatous ground tissue with small and large vascular bundles occurring in inner and outer rings (Fig. 2A), respectively. The epidermal cells consist of small, rectangular, thin-walled cells ensheathed by a thick cuticle. Collateral vascular bundles with endarch protoxylem and circular units of phloem on the outer part were also observed (Fig. 2B). The paradermal section of the leaf showed vertically elongated and six-sided epidermal cells and paracytic stomata (Fig. 2C-D).

The stem is wide, thick, and circular in cross-sectional view. It is ensheathed by a thick, parenchymatous, foliaceous sheath. The stem proper has thin epidermal layers of angular cells with thick cuticles followed by parenchymatous ground tissue (Fig. 2E-F). The vascular bundles are monocotyledonous and collateral type ensheathed by sclerenchymatous tissues. The xylem elements are 5-10 in number, angular, thick-walled and lignified. Adjoining the xylem elements, there are prominent units of phloem with small, darkly stained sieve elements (Fig. 2G-H).

The root is circular with frequent depressions all around. The root consists of a continuous outer layer of velamen tissue, an inner homogeneous cortex and in the centre occur circular stele (Fig. 2I). The velamen tissue consists of a layer of T-shaped epidermal cells with their lateral and inner walls heavily thickened (Fig. 2J). Outer to this layer is a layer of radially oblong parenchyma cells with their radial walls having conical thick lignified cells (Fig. 2K). The central part of the root is occupied by atactostele type of stele. The stele is circular with an outer endodermal layer of slightly oblong thick-walled cells, which is followed by an inner small, less prominent pericycle layer. The xylem elements are in a separate ring with exarch protoxylem points. The phloem elements are alternating with xylem elements

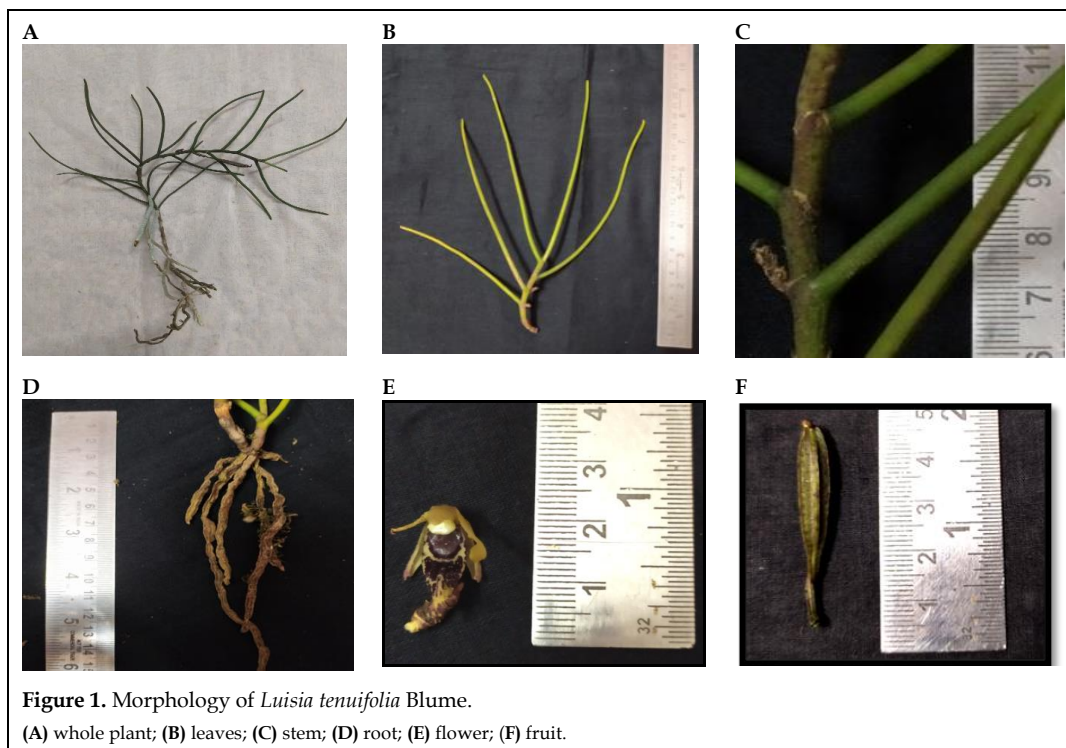


Figure 1. Morphology of *Luisia tenuifolia* Blume.

(A) whole plant; (B) leaves; (C) stem; (D) root; (E) flower; (F) fruit.

and consist of small, darkly stained sieve elements. The pith is fairly prominent, comprising of angular, thin-walled, compact parenchyma cells (Fig. 2L).

The anatomy of various parts of the flower is displayed in Fig. 3A-H. The flower bud consists of a central column with upper lateral wings and lower prominent lateral wings. In the centre of the column, there is a wide, elliptical hollow chamber. The column is surrounded by two lateral thick perianth members, a single wide upper perianth with folded margins. There is also a pair of the outer perianth. One perianth is present on either side of the flower bud. The perianth members and the central column have well-developed vascular strands (Fig. 3A).

The perianth members are differentiated into sepal and petal. The sepal is flat and thin with two lateral incurved margins. The midrib of the sepal is 250 μm thick. It consists of a thin, small epidermal layer of cells on the adaxial and abaxial sides. There is a small, collateral vascular strand in the midrib portion. The marginal part of the sepal is deeply curved down, and the lower epidermal cells are modified into wide, thick, conical cells, which appear papillate (Fig. 3B-C). The petal is flat with slightly upwardly curved margins. The surface of the petal has ridges and furrows. The petal has compact parenchyma cells with small vascular strands arranged in a horizontal row—the midrib of the petal

measures 250 μm thick. The midrib part consists of one small, collateral vascular strand, small air chambers, and compact parenchymatous ground tissue. The epidermal cells are either smooth or conical in shape (Fig. 3D). The marginal part of the petal becomes highly reduced in size, forming a slightly raised platform-like figure. The marginal part consists of three layers of cells, which are reduced to a single cell at an extreme margin (Fig. 3E). The upper part of the gynoecium is a solid cylinder with deep lobes of three smaller and three larger club-shaped lobes. The lobes appear as free wings. Each wing consists of an epidermal layer, compact parenchymatous ground tissue, and radially elongated collateral vascular strands (Fig. 3F). The stigmatic part of the gynoecium consists of six lobed ovaries. Of the six lobes, three are umbrella-shaped, and the remaining three lobes are thickly conical. In the centre occurs tricarpellary, syncarpous, unilocular chamber. From the outer part of the chamber, arise three thick receptacles, which bear the ovules. The receptacle is dilated at the apex and bears many spherical ovules. The cells of the receptacle have prominent nuclei. The ovule also has darkly stained cells with spherical bodies (Fig. 3G-H).

As the ovary matures, the outline of the ovary becomes triangular. The larger lobes become conical and slightly curved. The smaller lobes remain umbrella-shaped. The receptacles become thick and bear two

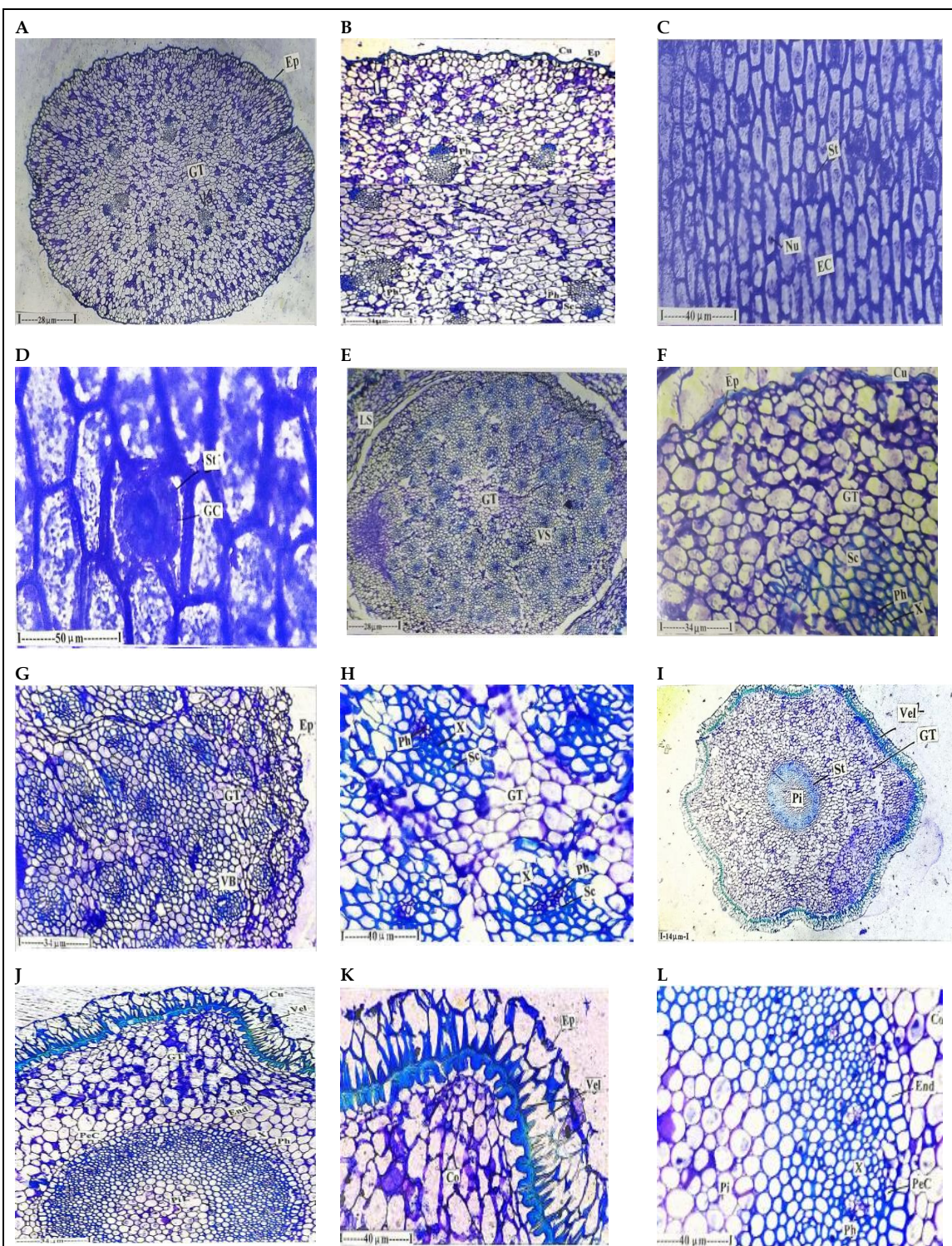


Figure 2. Anatomical characteristics of leaf, stem and root of *Luisia tenuifolia* Blume.

Leaf: (A) cross-section; (B) vascular bundle enlarged; (C) paradermal section; (D) paracytic stoma with guard cells. **Stem:** (E) cross-section, (F) a sector enlarged; (G) inner stem enlarged; (H) scattered vascular strands. **Root:** (I) cross section; (J) a sector enlarged; (K) velamen tissue and cortex; (L) ground tissue.

Cu: cuticle; Co: cortex; End: endodermis; Ep: epidermis; GT: ground tissue; LS: leaf sheath; Pec: pericycle; Ph: phloem; Pi: pith; Sc: sclerenchyma; Scc: sclerenchyma cap; St: stomata; SVS: small vascular strand; Vel: velamen; VB: vascular bundle; VS: vascular strand; X: xylem.

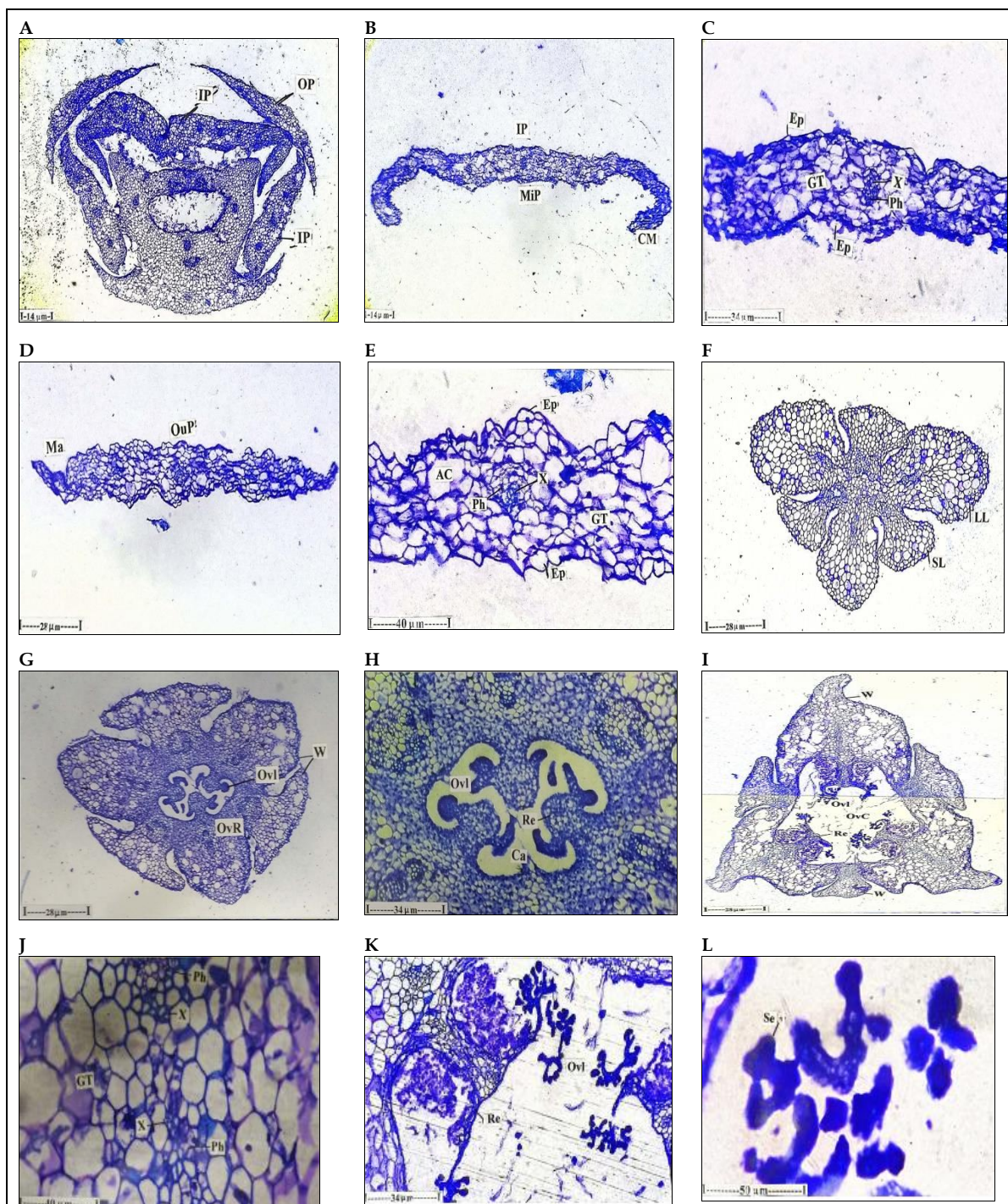


Figure 3. Anatomical characteristics of flower and fruit of *Luisia tenuifolia* Blume.

Flower: (A) flower bud; (B and C) sepal; (D and E) petal; (F) sterile stigmatic lobes; (G) ovary with outer wings and central carpels; (H) tricarpellary ovary with parietal placentation. **Fruit:** (I) mature fruit; (J) ovary lobes; (K) seeds; (L) seeds enlarged.

AC: air chamber; Ca: cavity; CM: curved margin; Co: cortex; Ep: epidermis; End: endodermis; GP: ground parenchyma; GT: ground tissue; IP: inner part; Ma: margin; MiP: middle part; Op: outer part; Ovc: ovule chamber; OvL: ovule; OVR: ovary rib; Pec: pericycle; Ph: phloem; Pi: pith; Re: receptacle; Se: sepal; SL: small lobe; VB: vascular bundle; W: wings; X: xylem.

clusters of black spherical minute seeds (Fig. 3I). The larger lobe of the ovary has well-developed collateral

vascular strands. The ground tissue consists of wide, angular, thin-walled, compact parenchyma cells (Fig.

3J). The seeds occur in dense bunches at the tip of the receptacle (Fig. 3K). The seeds are spherical, darkly stained, and consist of a thick-walled seed coat. Each seed has a small, short stalk. The seeds measure 18 μ m in diameter (Fig. 3L).

Histochemical characteristics

Histochemistry is essential for the detection and localisation of different classes of phytochemical compounds within the cells. Also, an exact analysis of the deposition of metabolites in a cellular gland can be achieved only by systematic histochemical analysis. Several studies report the histochemical investigation of medicinal plants and provide valuable information on the presence of primary and secondary metabolites and their localization in the plant tissue system (Edeoga and Okoli, 1995; Edeoga and Ikem, 2002; Mbagwu and Edeoga, 2006; Kadam et al., 2013).

In our study, histochemical analysis was performed through the transverse sections of different parts of *Luisia tenuifolia*. Fig. 4 indicates the various metabolites present in the leaf when subjected to histological staining with different reagents. The starch grains were found in the mesophyll tissue as indicated by the development of bluish-black colour with iodine in potassium iodide solution (Fig. 4A). The tannins were located in the subepidermal layers of the leaf (Fig. 4B). Lipids were present in the mesophyll tissue showing blue color upon treatment with Nile blue and acetic acid (Fig. 4C). Lignins were found deposited in the vascular bundles, mainly in the xylem elements and fibres, as indicated by the development of pink colour when treated with phloroglucinol and HCl (Fig. 4D). The mucilage is found localized in the mesophyll tissue (Fig. 4E). The presence of proteins was indicated by the development of bluish-black colour upon treatment with Coomassive brilliant blue reagent and was found in the mesophyll tissue (Fig. 4F). The presence of cutin was indicated by the development of green colour when treated with ferric chloride (Fig. 4G). The raphide crystals and polarized lignins were detected under polarized light and were found localized in the epidermal cells (Fig. 4H).

Fig. 5 indicates the histochemical characteristics of the stem of *Luisia tenuifolia*. Starch, lipids, and tannins were found localized in the ground parenchyma of stem and turned blackish, dark bluish, and reddish, respectively, upon treatment with corresponding chemical reagents (Fig. 5A-C). Lignin appeared as pink-colored cells in the perivascular fibres (Fig. 5D). Mucilage turned dark violet in the cortical zone upon treatment with toluidine blue (Fig. 5E). Protein was detected

bluish-black in the parenchyma cells of the ground tissue with Coomassive brilliant blue (Fig. 5F). The cuticle of the epidermal cells of the stem turned light red color indicating the presence of cutin (Fig. 5G). Calcium oxalate crystals of druses type were observed bright white under polarized light due to birefringent property (Fig. 5H).

The phytochemicals localized in the aerial roots of *L. tenuifolia* were represented in Fig. 6. The results revealed that the starch grains were localized in the grain cortical tissue and tannins in the velamen tissue (Fig. 6A-B). The lipids were deposited in the ground parenchyma, and the lignins were localized in the fibres of a vascular cylinder (Fig. 6C-D). Mucilage appeared in the ground parenchyma of root sections (Fig. 6E). Moreover, the proteins were detected in the outer cortical tissue, whereas cutin was localized in the velamen layers (Fig. 6F-G). Elongated cylindrical crystals were seen in the ground parenchyma and appeared bright white (Fig. 6H).

The histochemical profile of *L. tenuifolia* flowers was exhibited in Fig. 7. Tannins were found in masses in the parenchyma cells of the ovary (Fig. 7A). Starch depositories were observed in the pericarp of the flower (Fig. 7B). Further, the lipids, mucilage, and proteins were found located in the ovary wall (Fig. 7C-E). The epidermis indicated the presence of cutin on the cuticle cells (Fig. 7F). Both raphides, as well as styloid-type crystals, were found. Raphides were located in the cells of the ovary, whereas the styloid crystals were in the sepal (Fig. 7G-H).

Fig. 8 indicates the histochemical features of *L. tenuifolia* fruit. Starch and proteins were found located in the ovary wall (Fig. 8A-B). Lipids and mucilage depositions were observed in the ovary wall as well as in the seeds (Fig. 8C-D). The cuticle of the epidermal layer confirmed the presence of cutin (Fig. 8E). Further, when the sections were viewed under polarized light, calcium oxalate crystals were found densely distributed in the ovary walls (Fig. 8F).

Powder characteristics

The powder microscopical evaluation was carried out, and the diagnostic characteristics of the whole plant powder of *Luisia tenuifolia* were determined. The stomata are the paracytic type, with vertically elongated subsidiary cells lying parallel to the long axis of the guard cells. Short thick vessel elements, in addition to long, narrow, cylindrical vessel elements, are commonly seen in the powder. The anticlinal walls of the epi-

dermal cells are straight and thin (Fig. 9A-B). The vessel elements have wide, circular or oblique, elliptical perforations at the end walls. The lateral wall pits are less distinct (Fig. 9C-D). It also consists of fibres, which are narrow fibre types. The fibres are long, narrow with tapering and pointed ends. Some of the narrow fibres are attached to each other (Fig. 9E). Typical sclereids, which are long and rectangular with wide cell lumen, are seen in the powder. The sclereid has numerous wide simple pits (Fig. 9F).

Physicochemical constants

Identification of genuine drug is a major challenge during the commercial supply of medicinal plants in crude form, and pharmacognostical standardization remain as a valuable solution in this regard (Nayak and

Patel, 2010). Physico-chemical constants of the whole plant powder of *L. tenuifolia* were determined as per the WHO guidelines (WHO, 1996), and the results were tabulated in Table 1. This is essential as the physico-chemical parameters help in the correct identification of drugs and detection of adulterants. Also, inappropriate values may represent the improper collection and handling of crude drugs (Fiaz and Qazi, 2015). The ash values provide information on the inorganic composition of the plant drug. Higher ash values may represent the presence of impurities. The total ash value indicated the composition of metals, salts, and silica and was found to be 8.83 %w/w. While silica impurities can be detected from higher acid insoluble ash values, the water-soluble ash values indicate the cellulosic content (Musa et al., 2006).

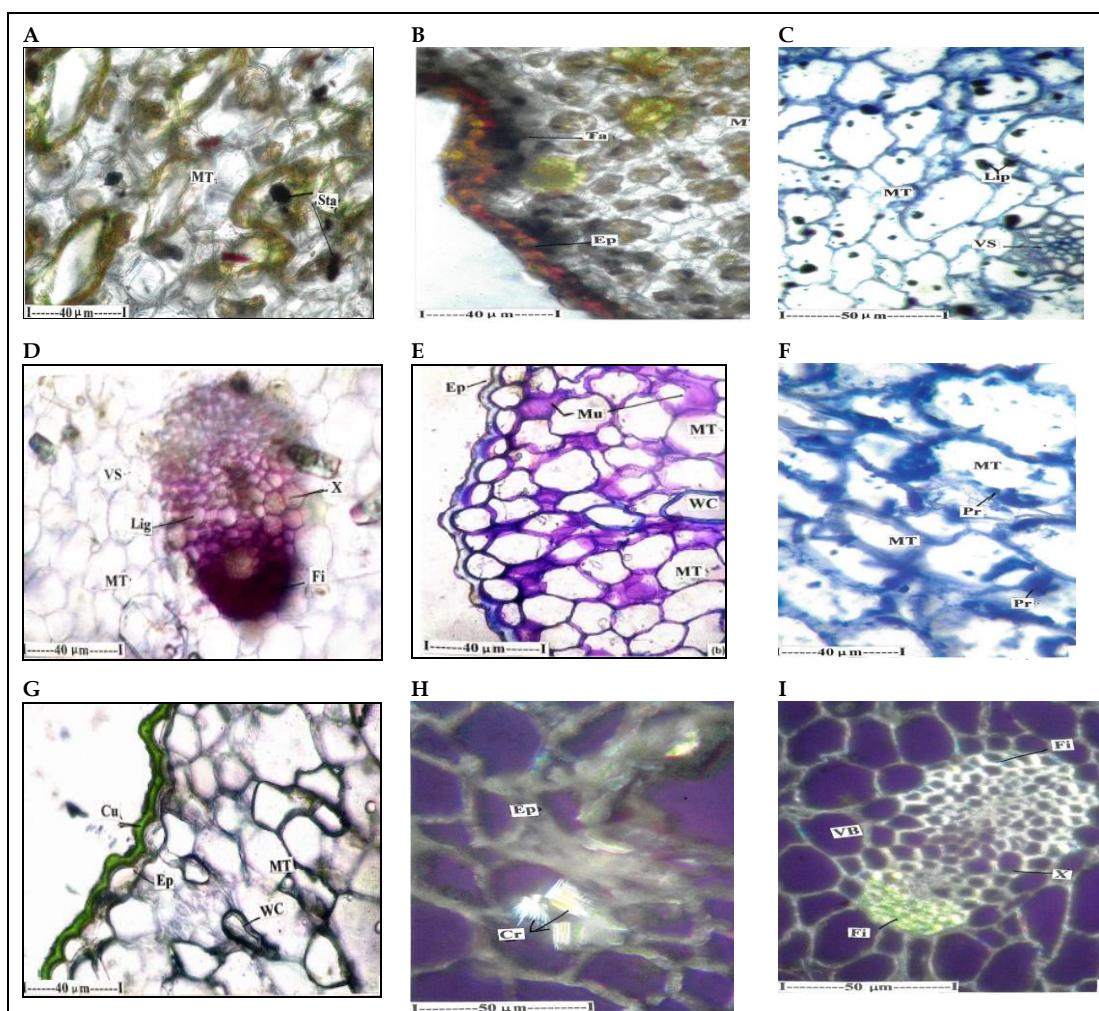


Figure 4. Histochemical characteristics of the leaf of *Luisia tenuifolia* Blume.

Transverse section of a leaf showing the localization of (A) starch; (B) tannins; (C) lipids; (D) lignin; (E) mucilage; (F) proteins; (G) cutin; (H) raphides; (I) polarised xylem bundle sheath.

Ep: epidermis; Fi: fibre; Lig: lignin; MT: mesophyll tissue; Mu: mucilage; Pr: protein; Sta: starch; Ta: tannin; VB: vascular bundle; VS: vascular strand; WC: water cell; X: xylem.

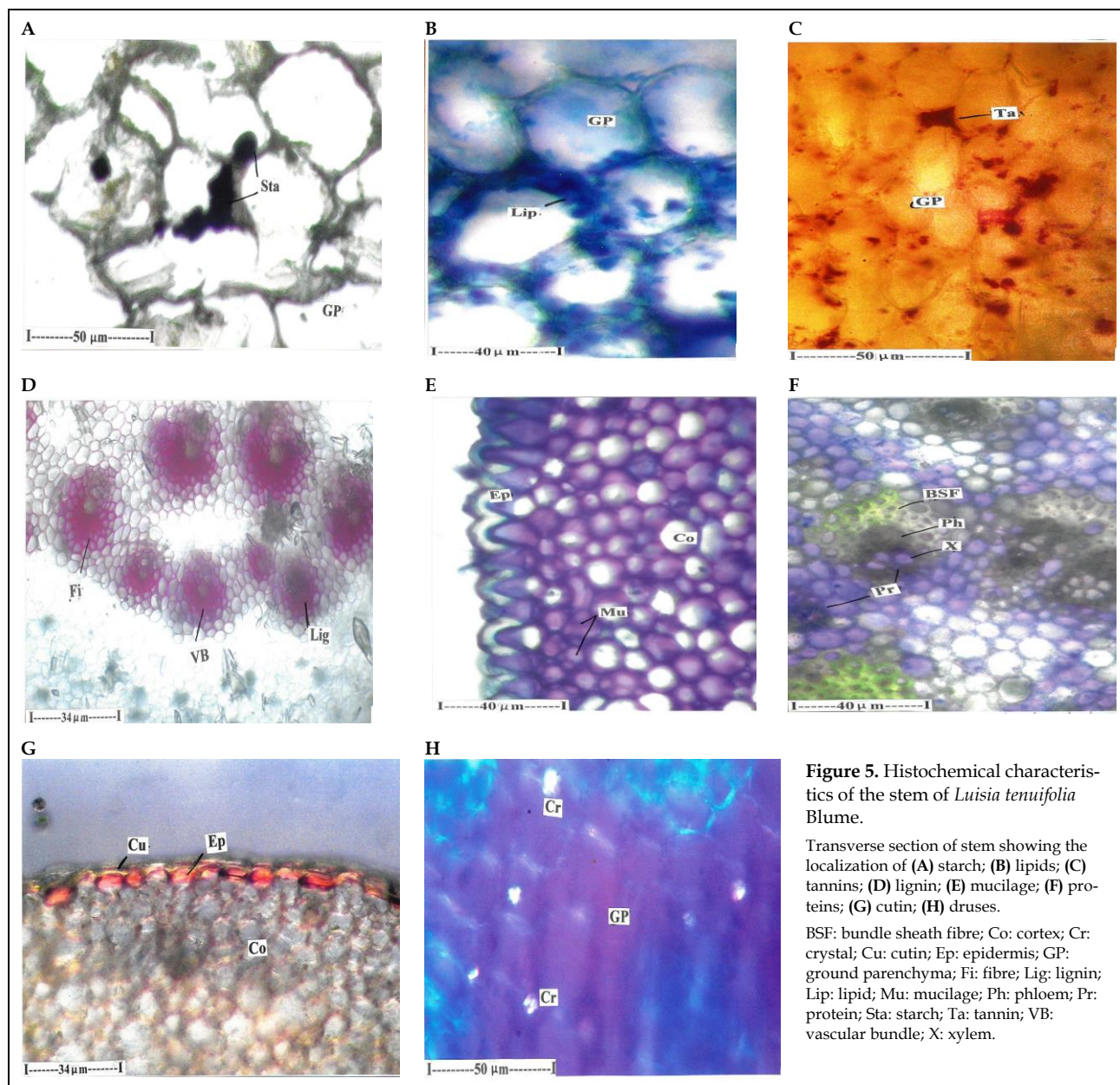


Figure 5. Histochemical characteristics of the stem of *Luisia tenuifolia* Blume.

Transverse section of stem showing the localization of (A) starch; (B) lipids; (C) tannins; (D) lignin; (E) mucilage; (F) proteins; (G) cutin; (H) druses.

BSF: bundle sheath fibre; Co: cortex; Cr: crystal; Cu: cutin; Ep: epidermis; GP: ground parenchyma; Fi: fibre; Lig: lignin; Lip: lipid; Mu: mucilage; Ph: phloem; Pr: protein; Sta: starch; Ta: tannin; VB: vascular bundle; X: xylem.

Thereby, the acid insoluble ash and water soluble ash values were found to be 1.52 and 4.62 %w/w in the pulverised whole plants of *L. tenuifolia*. The extractive values are yet another important parameter in determining the phytochemical content of the crude drug. In the study, the solvents, water, ethanol, and ether were used for the determination of extractive values and found that the extractives were more for ethanol compared to water and ether. Loss upon drying values represent the moisture content, and moreover, higher moisture paves the way for increased microbial growth (Okhale et al., 2010; Vidita et al., 2013).

Fluorescence observations

Fluorescent analysis demonstrates the chemical nature of phytoconstituents present in the drug, as shown by its reaction with several chemical reagents (Alam and Us Saqib, 2015). The fluorescent behaviour of the powdered whole plant of *L. tenuifolia* was displayed in Table 2. The results showed the colour change along with fluorescent properties were observed under daylight, short and long wavelength ultra-violet light. Further qualitative and quantitative phytochemical studies will ensure a better insight into the chemical constituents present in the plant.

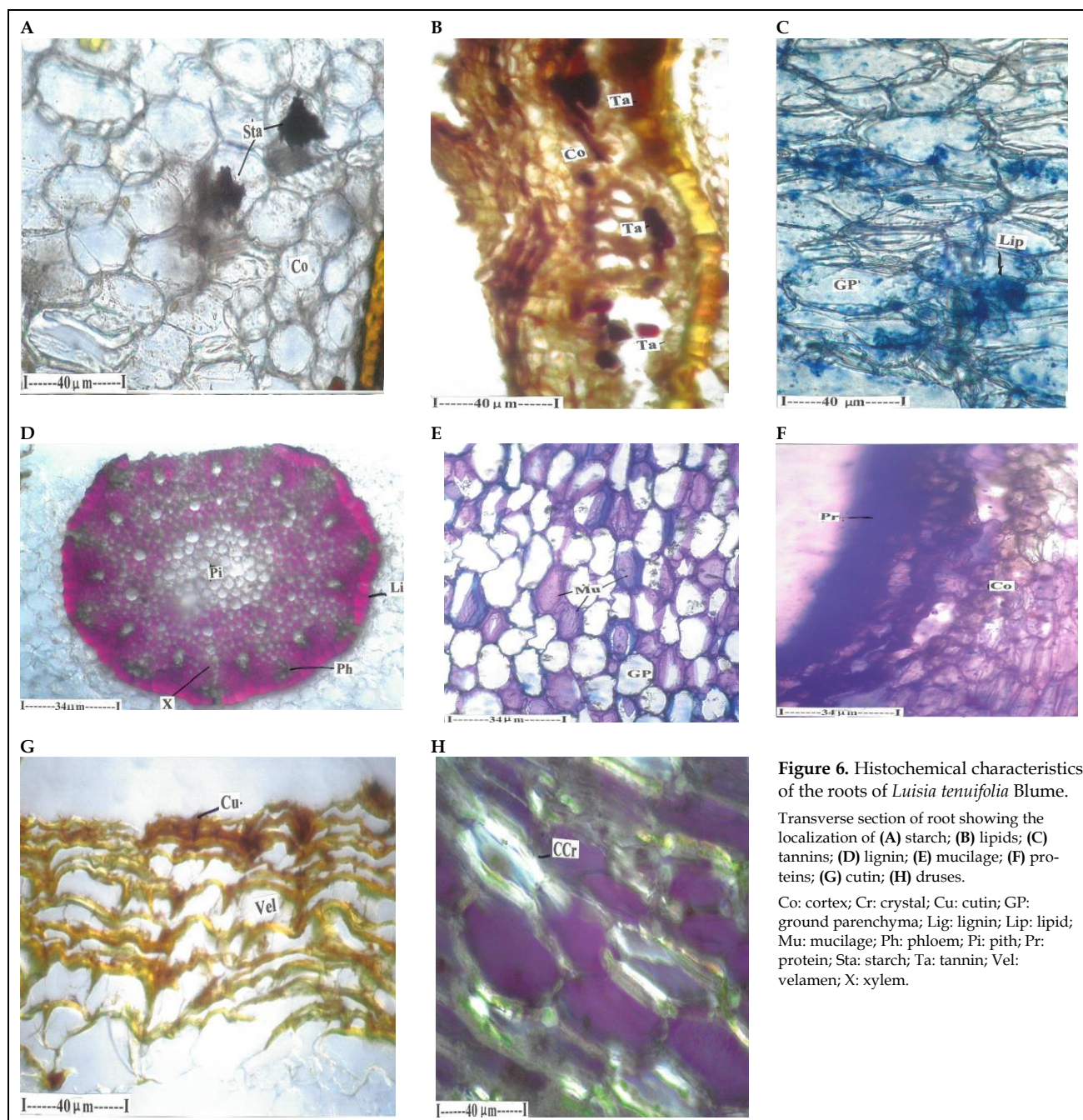
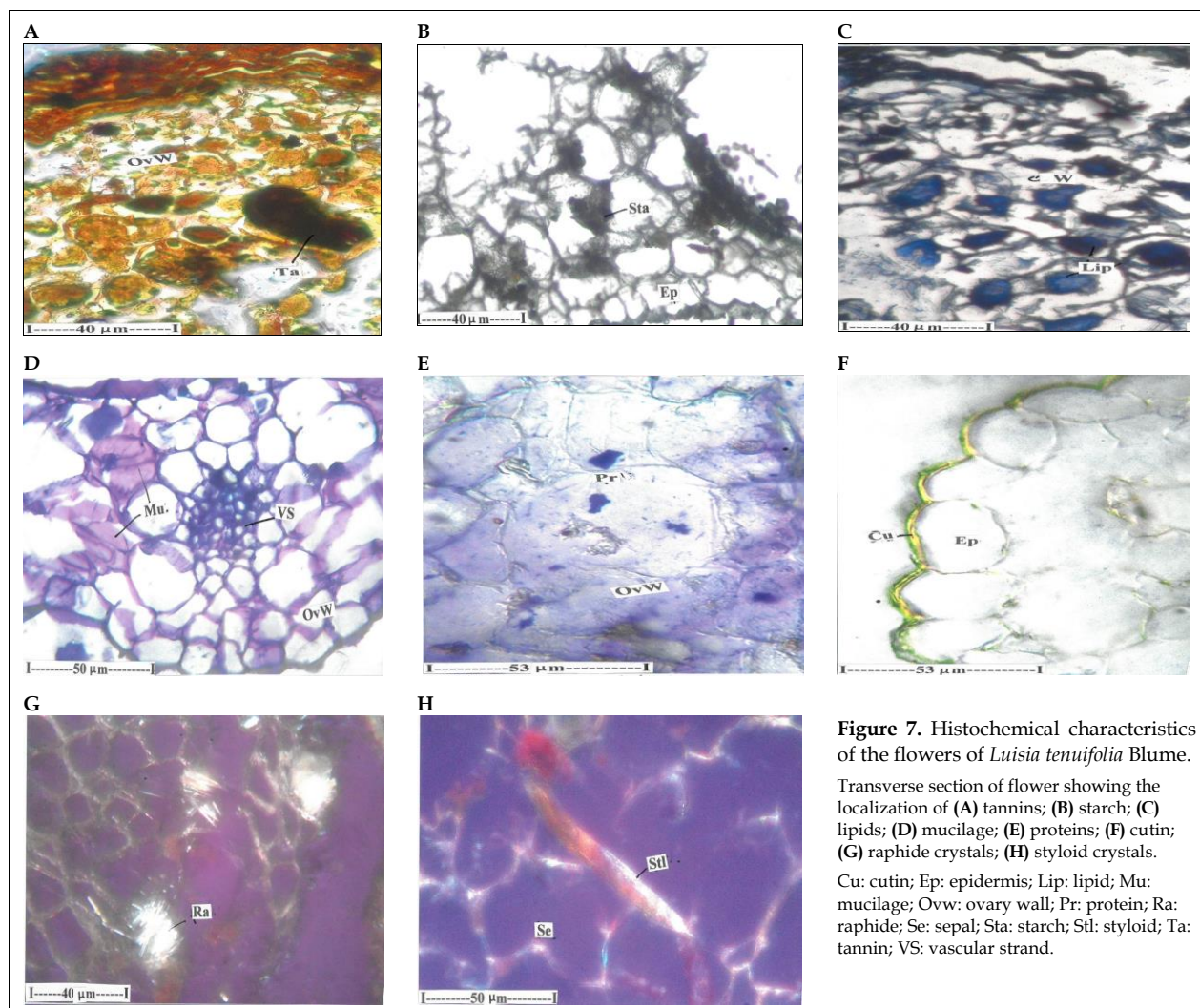


Figure 6. Histochemical characteristics of the roots of *Luisia tenuifolia* Blume.

Transverse section of root showing the localization of (A) starch; (B) lipids; (C) tannins; (D) lignin; (E) mucilage; (F) proteins; (G) cutin; (H) druses.

Co: cortex; Cr: crystal; Cu: cutin; GP: ground parenchyma; Lig: lignin; Lip: lipid; Mu: mucilage; Ph: phloem; Pi: pith; Pr: protein; Sta: starch; Ta: tannin; Vel: velamen; X: xylem.

**Table 1.** Physicochemical constants of whole plant powder of *Luisia tenuifolia*.

No.	Physico-chemical constants	Whole plant
1.	Total ash	8.83 ± 0.73 % w/w
2.	Acid insoluble ash	1.52 ± 0.13 % w/w
3.	Water soluble ash	4.62 ± 0.32 % w/w
4.	Sulphated ash	12.41 ± 1.05 % w/w
5.	Water soluble extractive	6.95 ± 1.14 % w/w
6.	Ethanol soluble extractive	14.30 ± 0.83 % w/w
7.	Ether soluble extractive	3.91 ± 0.25 % w/w
8..	Loss on drying	4.32 ± 0.71 % w/w
9.	Crude fibre content	28.36 ± 4.51 % w/w
10.	Foaming index	<100

Data are expressed as mean ± SEM of triplicates.

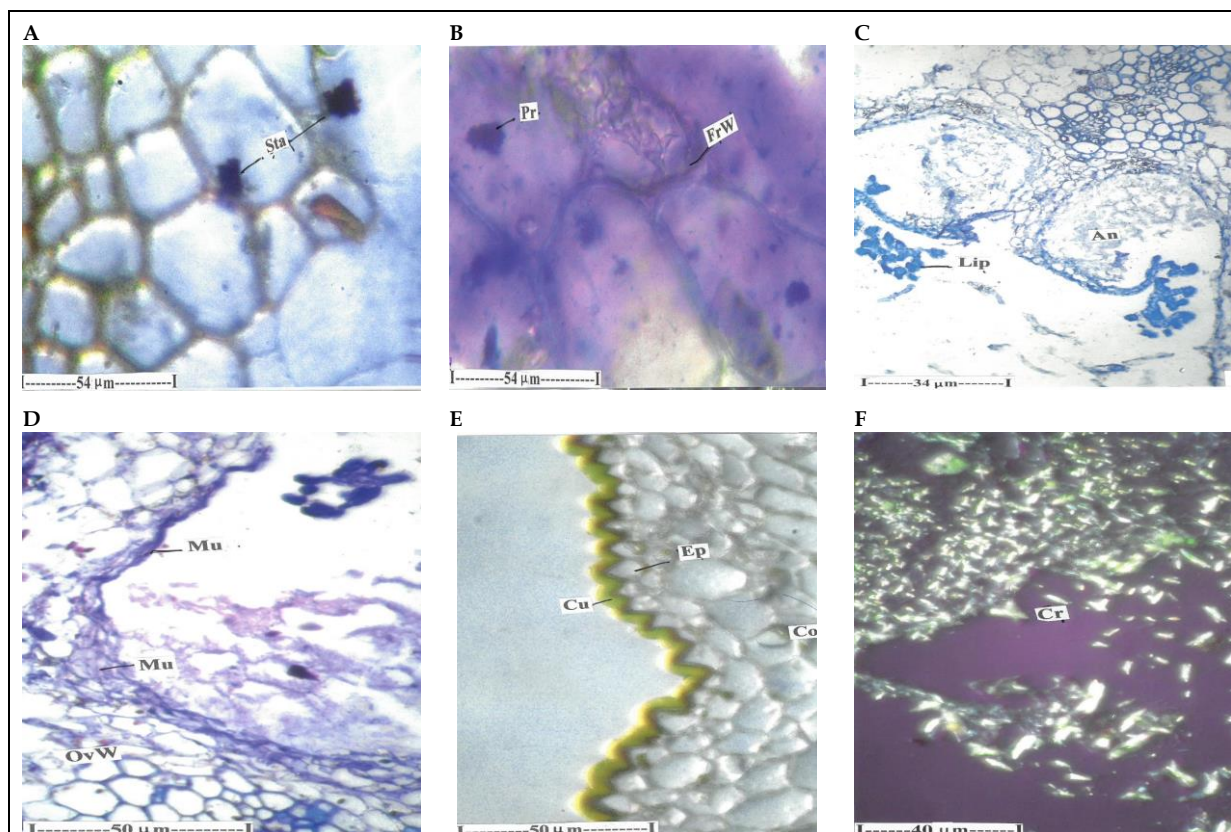


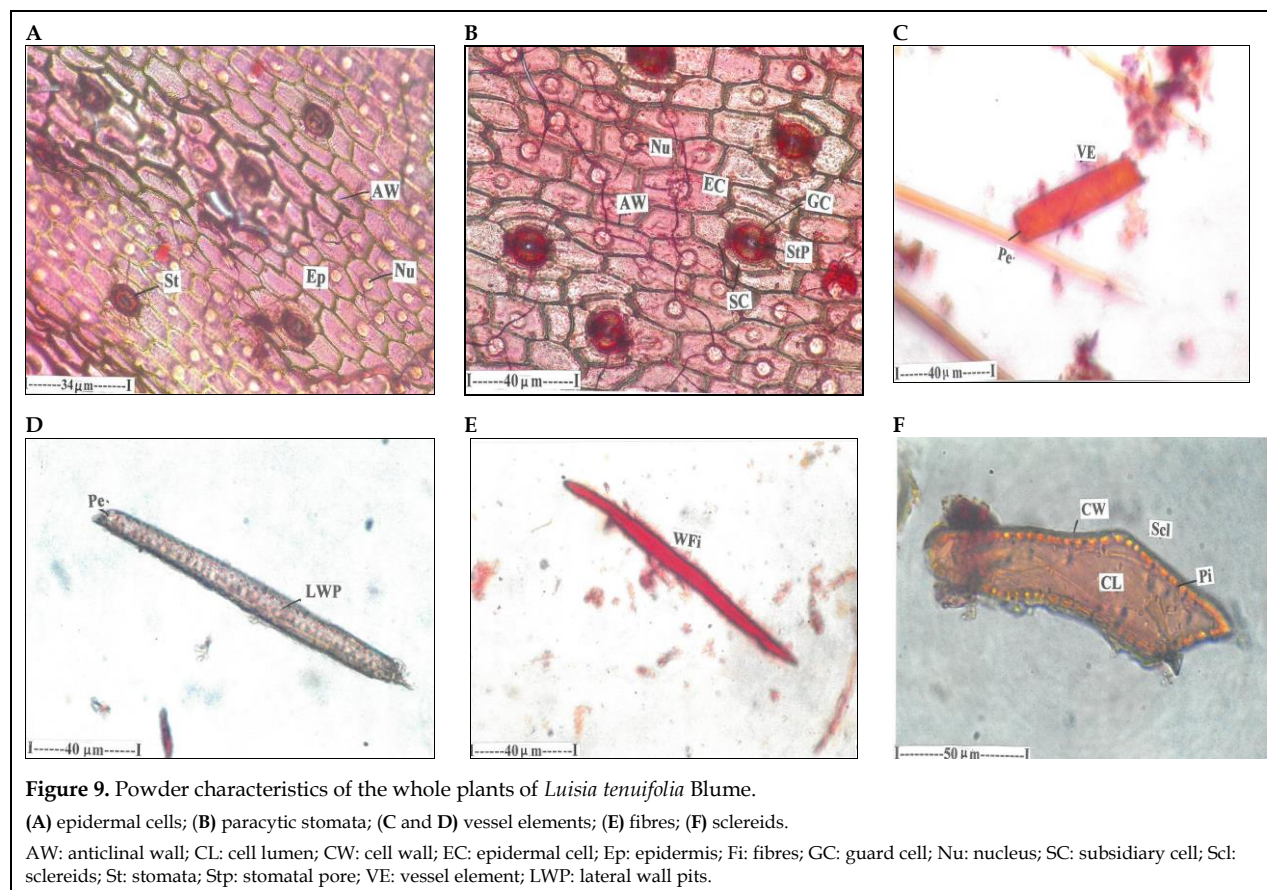
Figure 8. Histochemical characteristics of the fruit of *Luisia tenuifolia* Blume.

Transverse section of fruit showing the localization of (A) starch; (B) proteins; (C) lipids; (D) mucilage; (E) cutin; (F) calcium oxalate crystals.

An: anther; Co: cortex; Cr: crystal; Cu: cutin; Ep: epidermis; Frw: fruit wall; Lip: lipid; Mu: mucilage; OvW: ovary wall; Pr: protein; Sta: starch.

Table 2. Fluorescence analysis of powdered drug of *Luisia tenuifolia* whole plant in daylight and UV light after treatment with different reagents.

Treatment	Day light	Short UV (254nm)	Long UV (366nm)
Powder	Brown	Light green	Greenish brown
Powder + water	Light brown	Light green	Pale green
Powder +1 N HCl	Light Brown	Light green	Pale greenish brown
Powder +1 N H ₂ SO ₄	Brown	Light green	Pale greenish brown
Powder + nitric acid	Dark brown	Brownish black	Pale green
Powder + acetic acid	Brown	Green	Dark green
Powder + alc. NaOH	Dark green	Blue-green fluorescence	Dark green
Powder + alc. KOH	Brownish black	Blue-green fluorescence	Pale green
Powder + ammonia	Brownish black	Green fluorescence	Green
Powder + iodine	Brown	Black	Blackish green
Powder + FeCl ₃	Brown	Black	Pale green



CONCLUSION

The study was undertaken to establish the pharmacopoeial standardisation of whole plants of *L. tenuifolia*. The results of the study exhibit a valuable record of morphological, microscopical diagnostic characters aiding for botanical identification. While the epidermal fragments and stomata, fibres, vessel elements, and sclereids exist as the whole plant powder characteristic features, the localisation of tannins, starch, lipids, lignin, mucilage, protein, cutin, and calcium oxalate crystals were revealed from the histochemical profile of leaf, stem, root, flower, and fruit tissue system of *L. tenuifolia*. Furthermore, the physicochemical constants and fluorescence observations provide valuable data in establishing the pharmacopoeial standards. Additionally, studies will be directed on the phytochemical standardization and pharmacological validation of plant extracts in order to achieve effective phyto-pharmacological research.

CONFLICT OF INTEREST

The authors declare no conflicts of interest.

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AUTHOR CONTRIBUTION:

Contribution	Sakthi PS	Kumar PR
Concepts or ideas	x	x
Design	x	x
Definition of intellectual content	x	x
Literature search	x	x
Experimental studies	x	x
Data acquisition	x	x
Data analysis	x	x
Statistical analysis	x	x
Manuscript preparation	x	
Manuscript editing		x
Manuscript review	x	x

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