



Morphological and anatomical studies of two medicinal plants: *Harrisonia abyssinica* Oliv. (Simaroubaceae) and *Spathodea campanulata* P. Beauv. (Bignoniaceae) and their systematic significance

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ABSTRACT

The anatomy, morphology and trichome distribution of leaves of two medicinal plants: *Harrisonia abyssinica* Oliv. and *Spathodea campanulata* P. Beauv. from Nigeria were studied in order to understand the usefulness of these characteristics for systematic purposes as well as provide useful pharmacopoeial information in addition to previous identification tests available on the species. Some anatomical characters such as the number and shape of epidermal cells, number of palisade and spongy parenchyma layers of the leaf blade, and the trichome types provide information of taxonomical significance. The species showed variations in stomata types, epidermal cell shape, size and trichomes morphology. Most of the characters especially trichomes were diagnostic and used for distinguishing taxa. Trichomes were mostly multicellular and glandular on adaxial surface of *H. abyssinica*, on the two surfaces of *S. campanulata*, they were glandular and non-glandular. Stellate hairs were observed in *S. campanulata*. Trichomes were well segmented in *H. abyssinica*. Trichomes of *S. campanulata* on abaxial surface had aggregates of 5 basal-cells, in *H. abyssinica* there was just one basal cell. Leaf epidermal anatomy was reported for the first time in these species and was found taxonomically useful in the identification at the generic level.

Key Words: Epidermis; Trichomes; Anatomy; medicinal plants; Nigeria.

INTRODUCTION

The genus *Harrisonia* comprises 3 species, 2 of which occur in tropical Asia. *Harrisonia abyssinica* Oliv. is widely distributed in most tropical regions of Africa. *H. abyssinica* is variable, especially in size, shape and hairiness of the leaves. It is an evergreen, much-branched shrub or small tree, sometimes climbing, up to 6 (-13) m tall; bole and larger branches with up to 2 cm long thorns on conical corky outgrowths; bark pale brown to grey; branches long and flexible. Leaves alternate, imparipinnately compound with 2-7 pairs of leaflets, up to 25 cm long, glabrous or hairy; stipules absent; petiole up to 3 cm long, with 2 recurved spines at base, petiole and rachis with 1-3 mm wide wings; petiolules 0-2 mm long; leaflets elliptical or broadly obovate to almost circular, 0.5-9 cm × 0.5-4 cm, base asymmetrical, cuneate to rounded, apex rounded to acuminate, margins variably toothed or entire. *H. abyssinica* occurs in dry evergreen forest, forest edges, wooded grassland, riverine forest and coastal areas, from sea-level up to 1700 m altitude. It may form dense thickets on eroded soils. Annual rainfall in its area of distribution ranges between 150 mm and 2000 mm [1]. Medicinal applications of the plant include being used as treatment against a number of diseased conditions such as venereal diseases, fever, malaria, diarrhea, urinary problems and intestinal worms. Leaf sap is drunk for treatment in general body pains [2]. The methanolic and water extracts from the stem bark showed moderate antiplasmodial activity *in-vitro* and low toxicity in the brine shrimp toxicity test [3].

Spathodea is a monotypic genus in the flowering plant family Bignoniaceae. The single species is commonly known as the African tulip tree, Flame-of-the-forest. The species is found throughout tropical Africa where it grows

naturally in secondary forests in the high forest zone and in deciduous, transition, and savannah forests. It colonizes even heavily eroded sites, though form and growth rate suffer considerably on difficult sites. It is a tree that grows between 7–25 m (23–82 ft) tall and is native to tropical dry forests of Africa. This tree is planted extensively as an ornamental tree throughout the tropics and is much appreciated for its very showy reddish-orange or crimson (rarely yellow), campanulate flowers. It has become an invasive species in many tropical areas. The bark, flowers and leaves are used in traditional medicine in Western Africa. Medicinal applications of *S. campanulata* include: the bark as laxative and antiseptics and the seeds, flowers and roots are used as medicine. The bark is chewed and sprayed over swollen cheeks. The bark may also be boiled in water used for bathing newly born babies to heal body rashes. It is used in southwestern Nigeria for malaria treatment by drinking the decoction of its stem bark [4].

Leaves of *S. campanulata* have been reported to possess antiplasmodial activity, astringent properties and relief for painful inflammatory conditions [5-7]. The leaf extract was also reported to possess both analgesic and anti-inflammatory properties and could be beneficial in alleviating painful inflammatory conditions [8].

Morphologically and anatomically, the leaf is the most variable plant organ and the difference such as trichome is occasionally specific for species, genera or even families [9]. Information on foliar micromorphology has been reported of capable of shedding more light on plants structural features and their possible functional attributes [10]. Several studies have reported the significance of foliar micromorphological features used to evaluate taxonomical delimitations of many plants [11-16]. This in effect has helped in correct identification and authentication of many plants hence finding usefulness in standardization of herbal products obtainable from various medicinal plants indigenous to most communities. Micromorphological characters of the leaf that have been used in some studies include: epidermal cell type, stomata, trichome and vascular bundle pattern and arrangement [17-21]. However, no such information is available on the anatomy of *H. abyssinica* and *S. campanulata*. The study was therefore undertaken to obtain information on micromorphological features of these two important medicinal plants which would help in their identification and authentication.

EXPERIMENTAL SECTION

Leaf epidermal anatomy of *Harrisonia abyssinica* and *Spathodea campanulata* were studied. Both specimens were collected in July 2011. *H. abyssinica* was collected from Ifesowopo market, a few kilometers to Iseyin, and *S. campanulata* from Ilaju village along Eruwa road in Ido local government area both in Oyo state, Nigeria. The specimens were identified and authenticated by the second author at the Forest Herbarium, Ibadan (FHI), Nigeria where vouchers were also deposited as *Harrisonia abyssinica*-FHI 109474 and *Spathodea campanulata*-FHI 109471 respectively. For epidermal studies Shultze's method of maceration with improved technique was followed [22]. Leaves were taken in petri dishes, covered with 4 mL of concentrated nitric acid and kept under sun for 30 mins. Adaxial and abaxial epidermal peels obtained with the use of camel hair brush were rinsed in distilled water, bleached with one to two drops of Chloral hydrate for 30 sec. to remove chlorophyll, stained in 1 % aqueous Safranin O solution for 3 mins. and mounted in dilute glycerol.

Leaf transverse sections (8-10 μm) were made by the histological technique, using a rotary microtome Leitz Wetzlar and inclusion in Eukitt [23]. The cross-sections were stained using Astra blue and counter stained with Safranin O reagents. The sections were studied using an optical microscope Leitz Diaplan Germany 543669 and photographed with a Leica DFC 420 C camera.

RESULTS AND DISCUSSION

Macroscopy

Sensory characters observed in the two medicinal plants are recorded in Table 1. In *H. abyssinica* leaf was fresh and green in colour at the time of analysis; leaf is compound and shortly petiolate; lamina 3-5 cm long, 1-2 cm broad; oblanceolate to elliptic in shape; margin entire; apex is round or slightly acuminate, leaf base is attenuate and venation is reticulate, leaf surface is glabrous above but pubescent beneath, texture is papery with a prominent midrib. In *S. campanulata* leaf was fresh and green in colour at the time of analysis; leaf is compound with a long petiole; lamina 10-15cm long, 6-8cm broad; elliptic to oblong in shape; margin entire; apex is acuminate, leaf base is slightly cuneate and venation is reticulate, leaf surface is glabrous, texture is papery with a prominent midrib.

Microscopy

Leaf epidermal studies of the taxa *Harrisonia abyssinica* and *Spathodea campnulata* were carried out to find the anatomical characters of taxonomic value. Epidermal and stomatal characters of the plants are presented in Table 2 while anatomical features are recorded in Table 3.

Table 1. Morphological features of *Harrisonia abyssinica* and *Spathodea campanulata*

Sensory characters	Taxa	
	<i>H. abyssinica</i>	<i>S. campanulata</i>
Composition	Compound	Compound
Lamina		
Size	3-5 cm long; 1-2 cm wide	10-15 cm long; 6-8 cm wide
Shape	Oblanceolate-elliptic	Elliptic to oblong
Margin	Entire	Entire
Apex	Round to slightly acuminate	Acuminate
Base	Attenuate	Slightly cuneate
Venation	Reticulate	Reticulate
Surface	Glabrous (pubescent beneath)	Glabrous
Mid-rib	Prominent	Prominent
Texture	Papery	Papery
Petiole		
Occurrence	Petiolate	Petiolate
Character	Short	Long

Table 2. Qualitative epidermal and stomata characters of *Harrisonia abyssinica* and *Spathodea campanulata*

Taxa	Leaf surface	Cell shape	Cuticular ornamentation	Anticlinal wall pattern	Stomata type	Presence of trichome	Striation
<i>H. abyssinica</i>	Adaxial	Cuboid-rectangular	Thick	undulating	absent	Present	Striated
	Abaxial	Cuboid-rectangular	Thin	Straight-wavy	Paracytic	Present	Striated
<i>S. campanulata</i>	Adaxial	Cuboid - rectangular	Thick	Wavy-undulating	Anisocytic	Present	Not striated
	Abaxial	Cuboid-rectangular	Thick	Wavy-undulating	Anisocytic (numerous)	Present	Not striated

Table 3. Anatomical features of transverse sections of leaves of *Harrisonia abyssinica* and *Spathodea campanulata*

Characters	Taxa	
	<i>H. abyssinica</i>	<i>S. campanulata</i>
Leaf type	Dorsiventral	Isobilateral
No. of epidermis	One	One
Lignification of cuticle		
Mesophyll differentiation	Differentiated	Undifferentiated
No. of palisade layer	2	-
Spongy parenchyma	Isodiametric, 3-4 celled	disjointed
Trichome type	Short and long unicellular and multicellular, stellate, 3-4 celled glandular	Glandular and non-glandular
Crystals		
Oil globules	many	many
Type of Xylem vessel		Lignified spiral vessels
Vascular bundle pattern	Central, 4-6 celled xylem (formed Convex arc)	Collateral, 3-4 celled xylem

In *H. abyssinica*, epidermal cells have undulating anticlinal walls on the adaxial surface and straight to wavy on the abaxial. Cells are striated (Fig. 1 A-D). Both surfaces have many oil globules; short unicellular trichomes with multicellular base as well as stellate trichomes. Some of the cells on the abaxial surface are lignified. In *S. campanulata*, epidermal strips on both surfaces possess wavy to undulating anticlinal walls and many oil globules. Different types of trichomes were seen on both surfaces; numerous unicellular non-glandular, multicellular non-glandular as well as multicellular glandular on the adaxial surface; short multicellular non-glandular trichomes on the abaxial (Fig. 2 E-H).

Transverse sections of leaf blades of *H. abyssinica* and *S. campanulata* are shown in Fig. 3 (I-J). In *H. abyssinica* lamina is dorsiventral with single-layered epidermis on both surfaces and thick cuticle (Fig. 3 I). Epidermal cells are cuboid-rectangular in shape and are compactly arranged. The lower epidermis is also single layered with thin cuticle and many stomata hence the leaf is hypostomatic. Mesophyll is differentiated into palisade and spongy parenchyma with 2 layers of palisade parenchyma adjoined to the upper epidermis. Spongy tissues are isodiametric, 3-4 cells thick and loosely connected. The T.S. of *S. campanulata* leaf is isobilateral having single-layered epidermis on both sides and thick cuticle. Epidermal cells are cuboid-rectangular in shape. Mesophyll is undifferentiated. Spongy cells are disjointed (Fig. 3 J). Mid-rib of *H. abyssinica* shows a narrow protuberance at the ventral side and 2 layers of epidermis (Fig. 3 K-L). The protuberance on the dorsal side is wide; 3-4 celled multicellular trichomes are present on the protuberances. Vascular bundles are centrally placed with 4-6 celled xylem forming a convex arc for the phloem. Mid-rib region in *S. campanulata* formed a prominent convex protrusion at the dorsal surface,

internally bearing bundles of lignified spiral xylem vessels which could be as a result of secondary thickening (Fig. 3 M-N). Multicellular trichomes with swollen basal cells are present at the protuberances of the mid-rib. 7 vascular bundles are collaterally arranged with phloem alternately arranged at the base of 3-5 celled xylem. Central pith bears large collenchymatous cells (Fig. 3 N).

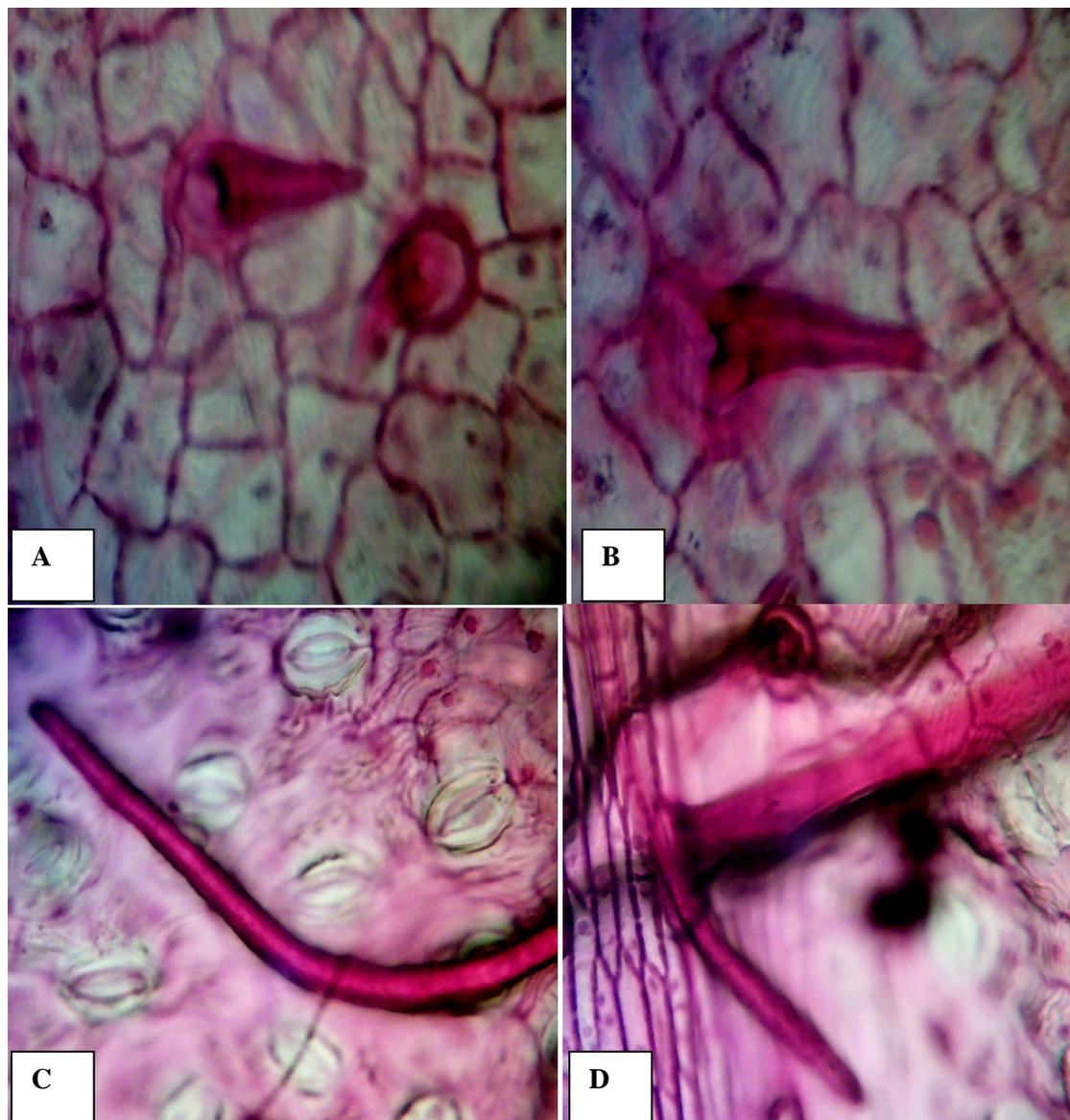


Fig. 1: (A-B) Adaxial epidermis of *H. abyssinica* (showing undulating cell wall pattern and many oil globules); (C-D) Abaxial epidermis of *H. abyssinica* (C showing long uniseriate trichome and many paracytic stomata; D shows straight and wavy epidermal cells)

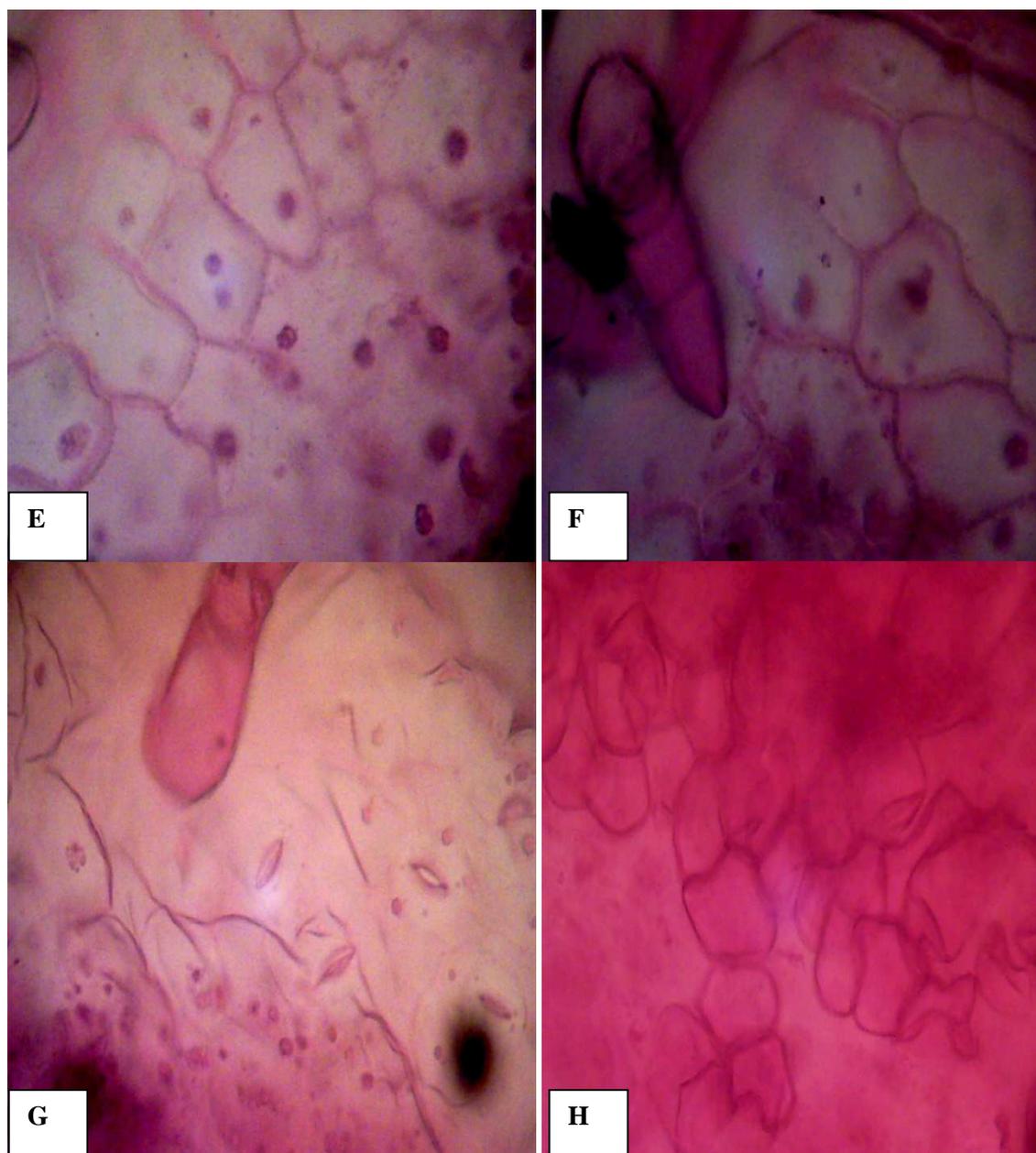


Fig. 2: (E-F) Adaxial epidermis of *S. campanulata* (showing wavy-undulating cell wall pattern, oil globules and short multicellular trichome); (G-H) Abaxial epidermis of *S. campanulata* (showing trichome and numerous anisocytic stomata)

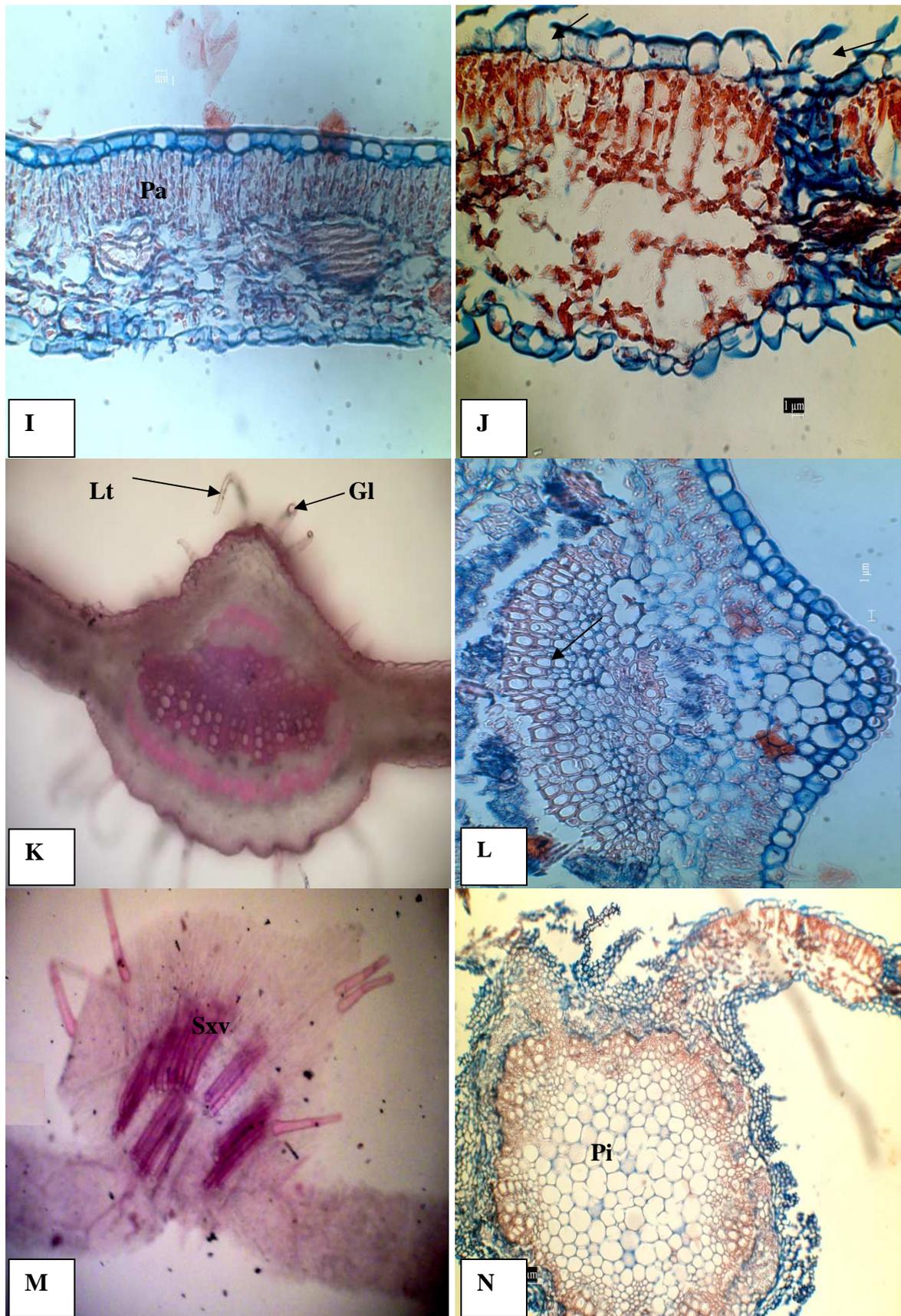


Figure 3 (I-J) Cross-sections of the leaf blades of *H. abyssinica* and *S. campanulata* (Pa: Palisade parenchyma, arrows in J show stomata); (K-L) Mid rib region of *H. abyssinica* (Lt: L-shaped trichome, Gl: glandular trichome, arrow in L shows 4-6 celled xylem); (M-N) Mid rib region of *S. campanulata* (Sxv: Spiral xylem vessels)

The presence of glandular and non-glandular trichomes on the two surfaces of *S. campanulata* is comparable to the observations made on foliar trichomes of *Tetradenia riparia* (Hochst.) Codd (Lamiaceae) where glandular and non-glandular trichomes were present in abundance on both the adaxial and abaxial surfaces [24]. Also in our study anisocytic stomata were found on both surfaces of *S. campanulata* (i.e. leaf is amphistomatic) but numerous on the abaxial surface.

In medicinal plants, trichome characters have been reported to act as biomarkers to identify the plant even in the raw material or powder form [25-27]. The presence of glandular trichomes in many of the medicinal plants is considered indicative on the concentration of secondary metabolites with pesticidal, pharmacological, and fragrant properties [28].

The results of our study in line with those reported in several other studies for other plants [18, 19, 27, 29-32] present morpho-anatomical features in *H. abyssinica* and *S. campanulata* for the first time. These micromorphological characters in addition to existing macro-characters of the genera would be useful in correct identification and authentication of the plants.

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