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The anatomy and histochemistry of *Grewia lasiocarpa* E. Mey. ex Harv. (Malvaceae)

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Grewia lasiocarpa E. Mey. ex Harv is commonly known as the forest raisin(s) due to its edible red-brown 4-lobed fruit(s), which turn black as they age. The genus is easily recognised by its distinctive morphology (edible fruits, crystals, trichomes) and abundant medicinal properties (antimicrobial, anti-inflammatory, antioxidant). In order to distinguish this species from other *Grewia* species, this study aimed to describe the morphology, ultrastructure of the leaves, stem bark and secretory structures of *G. lasiocarpa* using microscopic techniques and histochemical tests. he morphological and anatomical studies revealed the presence of glandular trichomes. One type of peltate, three types of capitate and non-glandular (simple, stellate, multangulate-stellate) trichomes were found on the leaves and stem bark of *G. lasiocarpa*. The histochemical investigation revealed that certain primary and secondary metabolites such as starch, protein, mucilage, lignin (polyphenols) and alkaloids are present *in the leaves, stem bark and indumentum.* This is the first report on the morphological, ultrastructure and histochemical studies of leaves and stem bark of *Grewia lasiocarpa*. This structural characterisation of the leaves and stem bark would help to distinguish this species from others in this genus, its ascertain authenticity, contribute to the pharmacognostic usage and general knowledge.

Keywords: *Grewia lasiocarpa*; capitate trichome; peltate trichome; mucilaginous idioblasts; histochemistry

Die anatomie en histochemie van *Grewia lasiocarpa* E. Mey. ex Harv. (Malvaceae): *Grewia lasiocarpa* E. Mey. ex Harv. staan in die algemeen bekend as bosrosyntjie vanweë sy eetbare, rooi-bruin, 4-lobbige vrugte, wat swart word soos hulle verouder. Die genus *Grewia* word maklik herken aan hul uitsonderlike morfologie (eetbare vrugte, kristalle, trigome) en oorvloedige medisinale kenmerke (antimikrobies, anti-inflammatories, anti-oksidant). Om hierdie spesie van die ander *Grewia*-spesies te onderskei, is met die studie beoog om die morfologie, ultrastruktuur van die blare, stingelbas en sekresie-strukture van *G. lasiocarpa* te beskryf aan die hand van mikroskopiese tegnieke en histochemiese toetse. Die morfologiese en anatomiese studies het die teenwoordigheid van klieragtige trigome getoon. Een tipe skildvormig, drie tipes knobbelvormig en nie-klieragtige (enkelvoudig, stervormig en veelkantig-stervormig) trigome kom op die blare en stingelbas van *G. lasiocarpa* voor. Die histochemiese ondersoek het getoon sekere primêre en sekondêre metaboliete soos stysel, proteïene, slym, lignien (polifenole) en alkaloïede kom in die blare, stingelbas en haarkleed voor. Hierdie is die eerste verslag oor die studie van die morfologie, ultrastruktuur en histochemie van die blare en stingelbas van *Grewia lasiocarpa*. Die kenmerke van die blare en stingelbas sal geskik wees om hierdie spesie van die ander in hierdie genus te onderskei, sy outentisiteit te bevestig en by te dra tot die farmakognosiese gebruike en algemene kennis.

Sleutelwoorde: *Grewia lasiocarpa*; knobbelvormige trigome; skildvormige trigome; slymerige idioblaste; histochemie.

Introduction

The family Malvaceae, commonly known as mallow [formerly Sparrmanniaceae or Tiliaceae] (Johnson 1990; Baser 1995; Ibrahim et al. 2018). They are found in tropical and temperate regions and comprises of approximately 3020 species in 192 genera with fourteen subtribes (Morton 1987; Nep et al. 2013). A previous taxonomic classification scheme assigned *Grewia* as the only genus that produces edible fruits (Morton 1987), however there are several genera in the Malvaceae family that produce edible fruits e.g. *Abelmoschus, Azanza, Corchorus* and *Dombeya* species.

The genus *Grewia,* named after Nehemiah Grew (1641–1712), is predominant in South Africa, Arab, Madagascar, Himalayan regions, India, Pakistan, China, Myanmar, Pacific islands (Tonga and Samoa), Malaysia, Thailand, northern Australia and Bangladesh, with a total of 690 published binomials (Bayer and Kubitzki 2003; Wahlert et al. 2015). In South Africa, there are 27 indigenous species *e.g. G. occidentalis, G. flavescens* and *G. bicolor* (Boon and Pooley 2010).

Grewia lasiocarpa is usually found on rock outcrops, along the coast to the mist-belt forest of Eastern Cape to KwaZulu-Natal and also in tropical forests and margins (Boon and Pooley 2010).

The microscopic examination of the structural characterisation of plants during growth, development, genetic manipulations and breeding is vital because the observations derived from these examinations have been remarkable (Payne 1978; Svoboda et al. 2000). Amongst the important structural features are trichomes which could be glandular or non-glandular (Naidoo et al. 2011; Raghu et al. 2019; Bantho et al. 2020). The importance of a comprehensive morphological analysis of plants cannot be overruled as this is useful in the verification of authenticity, ascertainment of the economic importance, determination of the best processing method and detection of foreign bodies (Svoboda et al. 2000; Ibrahim et al. 2018). Geographical location influences the phytocompounds present in plants, because of abiotic factors such as varied climate, temperature, rainfall, altitude, day length and UV-radiation. These factors directly affect the biosynthesis of secondary metabolites (Khalid et al. 2012).

In general, plants cannot survive without the primary metabolites but the absence of certain secondary metabolites will not result in instantaneous death but rather long-term damage to the physiological functions (Agostini et al. 2012). The presence of primary metabolites has been reported in the leaves and stem bark of *G. lasiocarpa* (Akwu et al. 2019; Akwu et al. 2019). There are few reports on the morphology and ultrastructure of the leaves, flowers and stem bark of *Grewia* spp. *e.g. G. laevigata* Vahl (Sankhyan et al. 2008), *G. multiflora, G. huluperakensis, G. polygama* and *G. laevigata* (Chung 2005). These species exhibit morphological and anatomical variances, hence a number these species are scientifically distinct.

The main aim and objective of this study is to investigate the morphology, ultrastructure and histochemistry of the leaves and stem bark of *G. lasiocarpa* using conventional compound light, stereomicroscopy, fluorescence and electron microscopy (Scanning electron microscopy [SEM] and transmission electron microscopy [TEM]) and comparing our results with other reported *Grewia* species.

Materials and methods

Material used

Healthy plant organs (leaves and stem bark) of *Grewia lasiocarpa* were harvested from the Umdoni Trust Park area, southern KwaZulu-Natal, South Africa. The material was stored in air-tight bags on ice. The taxonomic identification was done by Dr. Syd Ramdhani, curator of the School of Life Sciences, University of KwaZulu-Natal and voucher specimens were deposited at the Ward Herbarium, School of Life Sciences, Westville Campus with herbarium number *Nneka 002*.

Stereomicroscopy

The morphology and density of the various trichomes present on the adaxial and abaxial surfaces of the randomly selected fresh whole leaves of emergent (< 1 cm), young (1–5 cm) and mature leaves (5–10 cm) as well as randomly selected photosynthetic/green, herbaceous youngish stem pieces were examined. Images were captured with a Nikon AZ100 stereomicroscope equipped with an AZ-LED ring, a Nikon Fibre illuminator and NIS-D Elements Software.

Sample preparation: electron and light microscopy

Scanning electron microscopy (SEM)

A scanning electron microscope was used to reveal the morphology and distribution of the trichomes present on the leaf surfaces (adaxial and abaxial sides). The leaves were randomly selected with respect to their relative sizes *viz.,* of *Grewia lasiocarpa*.

The fresh leaves from the three developmental stages of growth and stem bark portions were cut into small pieces approximately 2–3 x 4.0 mm^2 and a similar technique of scanning electron microscopy (SEM) described by Naidoo et al. (2013) was used with slight modifications in the use of 2.5% glutaraldehyde and 0.5% osmium tetroxide (OsO_4) for primary and secondary fixation respectively.

Freeze drying

Another set of fresh pieces from the three developmental stages of *G. lasiocarpa* leaves and stem bark were placed in liquid nitrogen (-196°C), after which the sections were further frozen in an Edwards Modulyo freeze dryer (Edwards High Vacuum International Ltd., UK), at -40 to -60°C in a vacuum of 10^{-1} Torr for 72 h. The samples were stuck to brass stubs with carbon conductive tape, gold sputter coated twice using a Polaron SC500 Sputter Coater (Quorum Technologies Ltd., UK) in 0.1 Torr vacuum. The

leaves and stem bark segments were viewed using a Zeiss Ultra-Plus FEG-Scanning electron microscope operating at 20 kV.

Trichome and stomatal index (quantitative microscopy)

The micrographs from the SEM analysis of the mature leaves were used to determine the trichome (Stace 1965) and stomatal index (Salisbury 1928) using the equation 1 and 2 respectively:

(1) *Stomatal index* (*SI*)

$$= \frac{Stomatal\ density\ (SD)}{Stomatal\ density\ (SD) + Epidermal\ cell\ density\ (ECD)} *100$$

Where SD = No. of stomata per mm^2; ECD = No. of epidermal cells within the mm^2 unit area

(2) *Trichome index* (*TI*)

$$= \frac{Trichome\ density\ (tD)}{Trichome\ density\ (TD) + Epidermal\ cell\ density\ (ECD)} *100$$

Where TD = No. of trichomes per mm^2; ECD = No. of epidermal cells within the mm^2 unit area

If half a section of either an epidermal, stomatal (stomatal pore and flanked by two guard cells) cell or a trichome falls within the unit area, the cells were counted. The analysis was done for five replicates and the average count recorded.

Transmission electron microscopy (TEM)

Fresh leaves of *G. lasiocarpa*, at different stages of development (emergent, young and old) were cut into pieces of approximately 2–3 x 5 m^2. A modified version of the technique described by Naidoo et al. (2011) was employed. The modifications were in the use of 2.5% glutaraldehyde and 0.5% osmium tetroxide (OsO_4) for primary and secondary fixation respectively.

Light microscopy

Randomly selected leaves and stem bark sections obtained from the embedded TEM tissues were stained with 0.05% toluidine blue. The samples were examined and photographed with a Nikon Eclipse 80i compound light microscope coupled to a Nikon DS-Fil camera and NIS-Elements imaging software package.

Histochemistry

Free hand sections of the fresh leaves and stem bark of *G. lasiocarpa* were subjected to various histochemical tests, to reveal the presence and location of certain phyto-metabolites such as tannins, lipids, alkaloids. The following tests were conducted: Wagner's and Dittmar reagents for alkaloids (Johansen 1940; Furr and Mahlberg 1981); ruthenium red for acidic polysaccharides e.g. unesterified pectins and for mucilage (Johansen 1940; Jensen 1962; Bornman et al. 1969); NADI reagent for terpenoids of essential oils (David and Carde 1964); ferric trichloride for phenolic compounds (Johansen 1940; Gabe 1968); toluidine blue for carboxylated polysaccharides and polyphenol, bromophenol blue for total proteins; phloroglucinol for lignin aldehydes; III and IV (Sudan red) for lipids (Lison 1960), Nile blue for lipids (Cain 1947; Ascensão and Pais 1987) and fluorescence analysis using acridine orange for deoxyribonucleic acid (DNA) (Martin and Ortiz 1967) and calcofluor-white for cellulose (Mitra and Loqué 2014). The unstained sections served as controls (results not shown). The sections were examined and photographed using a Nikon Eclipse 80i Compound Light Microscope (Nikon, Japan).

Statistical data analysis

The quantitative data (mean and standard deviation) were analysed using Microsoft Excel spreadsheet functions and Statistical Package for the Social Science (SPSS) 25 computer programmes.

Results

Observations of the leaves with the stereomicroscope and the SEM showed that the emergent and young leaves are more densely pubescent than the mature leaves (Fig. 1A–C). On the abaxial surface of the leaves, there are five distinct veins with a cluster of trichomes on them (Fig. 1D). Gum is secreted by the leaves and stem bark of *Grewia lasiocarpa* (Fig. 2A and B). Non-glandular (indicated with arrows) (Fig. 3A) and glandular trichomes (brown spots) (Fig. 3A) are present on the leaves. The presence of non-glandular trichomes (Fig. 3B) are also evident on the stem bark. Similarly, with the abaxial surface of the leaves (Fig. 1D), there are clusters of non-glandular trichomes along the mid-rib on the adaxial surface (Fig. 3A).

The scanning electron micrograph also revealed that the adaxial and abaxial surfaces of the three developmental stages of the leaves studied have varying degrees of trichome clusters, in the order emergent > young > mature (Fig. 4A–C). The non-glandular trichomes were also clustered along the mid and lateral veins of the leaves (Fig. 4A–C) and on the stem bark (Fig. 4D). There are different types of structural trichomes on the leaves and stem bark of *G. lasiocarpa viz.,* simple trichomes which are long and tapered at the tip (Fig. 5Ai), armed tufted trichomes with different subtypes (Fig. 5Aii and iii – 6Bi and ii), stellate trichomes without a central cushion (Fig. 5Ci–Fi) and stellate trichomes with a central cushion (Fig. 5Gi–Ii) with subtypes. Multivariate stellate trichomes with numerous arms (Fig. 5Ji) and multangulate trichome (Fig. 5Ki). On the leaf blade and stem bark there are two types of glandular trichomes *viz.,* capitate and peltate glandular trichomes (Fig. 6A and B). Three types of capitate trichomes were observed on the leaves of *G. lasiocarpa viz.,* short unicellular stalk with the multicellular rounded glandular head (Fig. 6Ai), short unicellular stalk with the multicellular elliptic glandular head (Fig. 6Aii), and short unicellular stalk with the multicellular oblong glandular head (arrow) (Fig. 6Aiii). The head of the capitate glandular trichomes on the leaf adaxial surfaces are directed towards the leaf apex (Fig.

7A and B). Two epidermal cells were observed to be parallel to the long axis of the stomata guard cells (Fig. 8A and B). The stomatal and trichome density on the adaxial and abaxial surfaces of the mature leaves were measured using the stomatal (SI) and trichome (TI) indices and a variation in the density on both surfaces was observed. A higher percentage of the SI and TI was observed on the adaxial surface (Fig. 9).

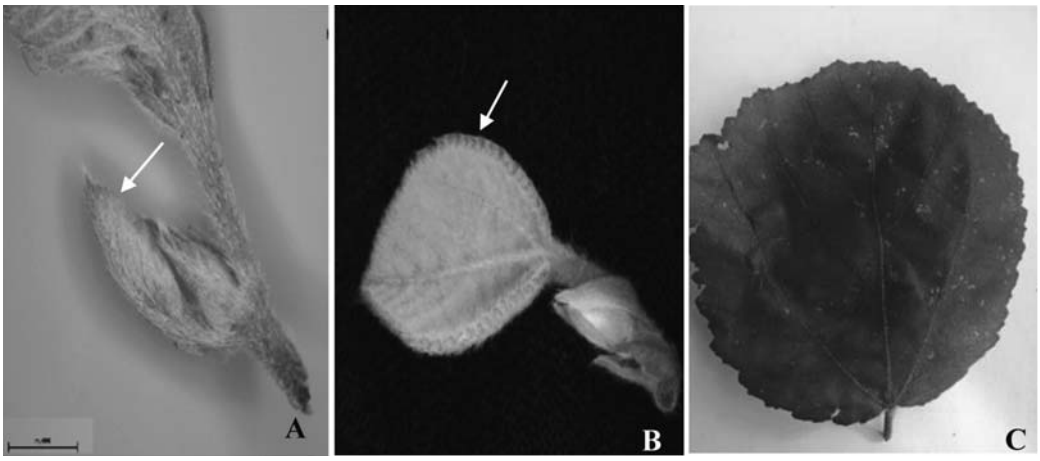


FIGURE 1 (A–C): The leaves of *Grewia lasiocarpa*: (A) emergent, (B) **y**oung, (C) mature. **e**mergent and young leaves densely hairy (trichomes), mature leaves sparsely hairy (trichomes).

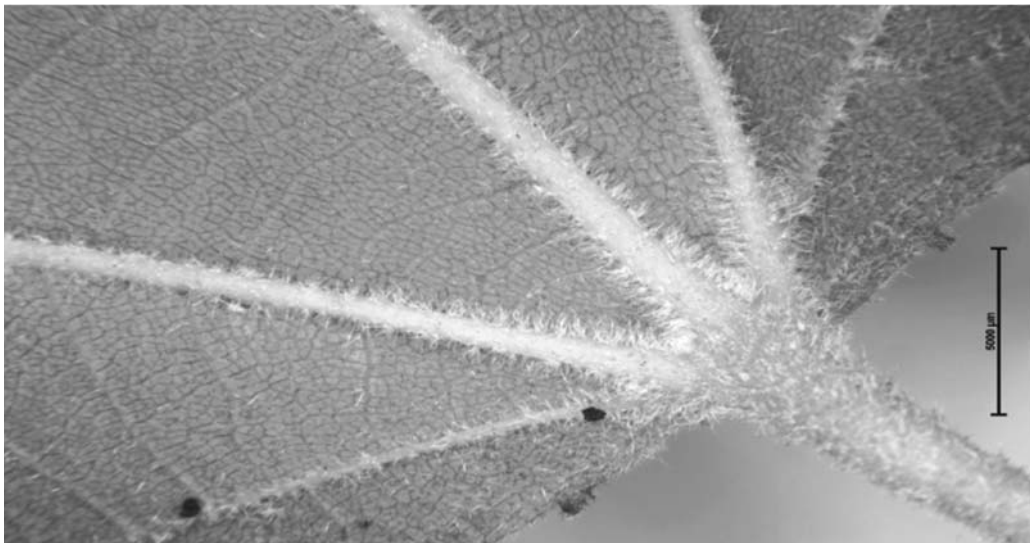


FIGURE 1D: Abaxial side of *Grewia lasiocarpa* leaf base showing the main veins and dense clusters of trichomes along the veins.

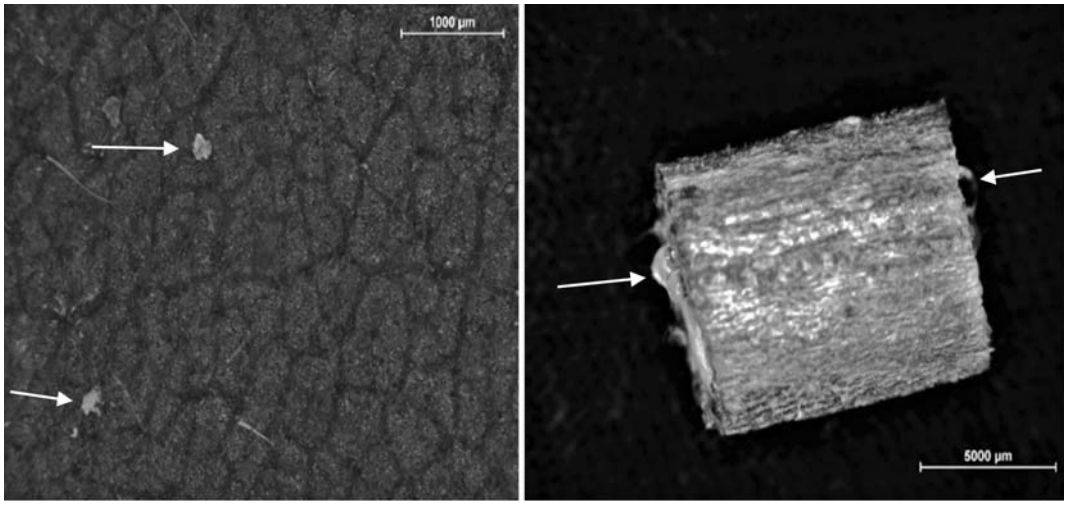


FIGURE 2 (A and B): (A) The adaxial surface of a mature leaf with epidermal cells, arrows showing the gum; (B) A chunk of the stem bark of *Grewia lasiocarpa* (arrows showing the gum)

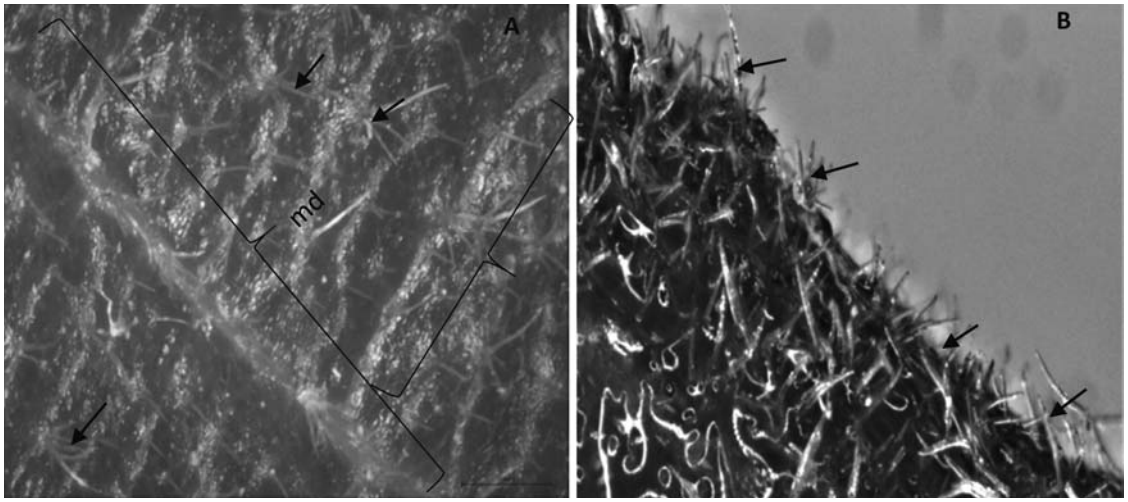


FIGURE 3 (A and B): (A) Adaxial surface of a mature leaf, and (B) photosynthetic/green region of the stem of *Grewia lasiocarpa*. (md) mid rib, (v) vein.

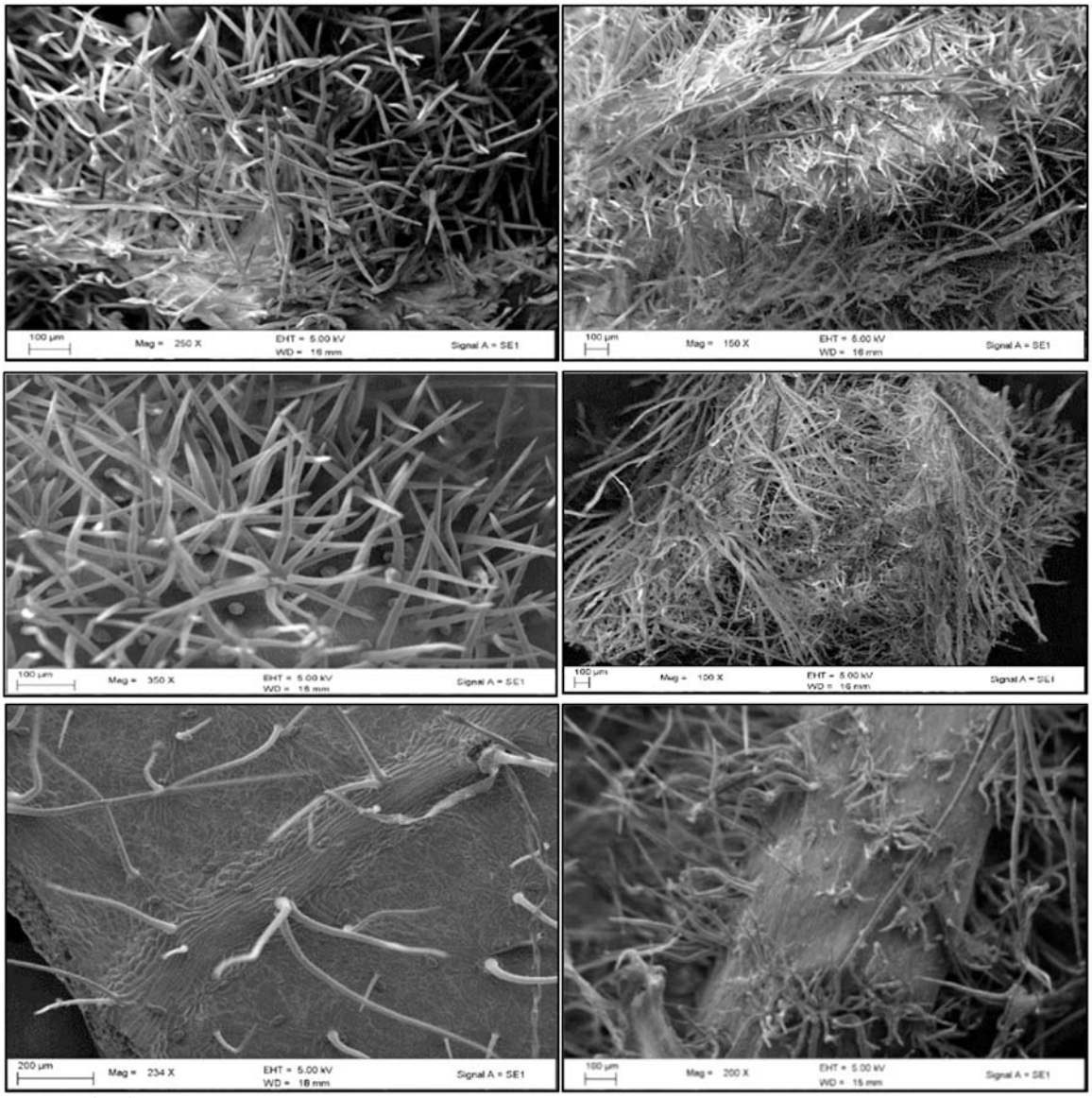


FIGURE 4 (A–C): Adaxial (i) and abaxial surfaces of (A) emergent; (B) young; and (C) mature leaves of *Grewia lasiocarpa.*

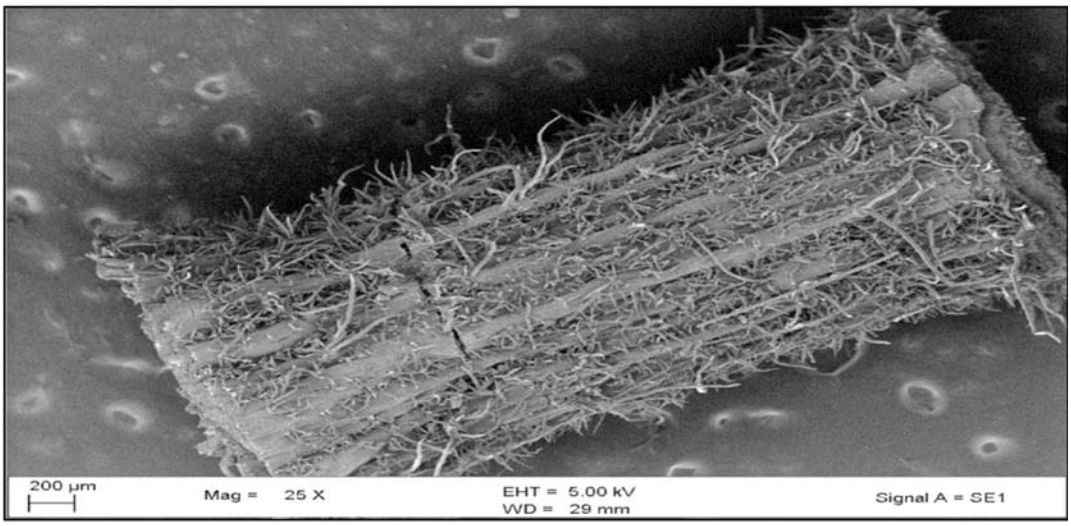


FIGURE 4D: SEM of the stem bark of *Grewia lasiocarpa.*

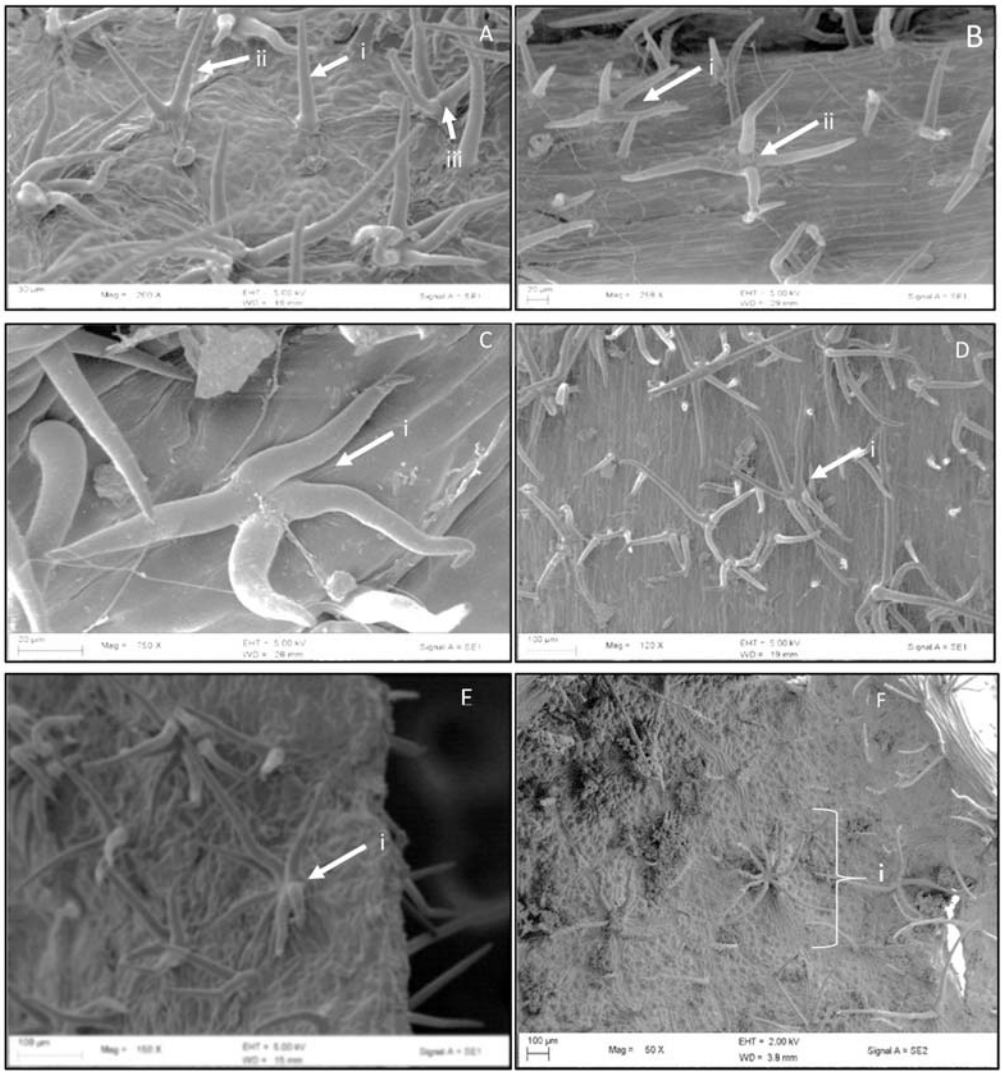


FIGURE 5 (A–K): Different types of structural (non-glandular) trichomes on the leaves and stem bark of *Grewia lasiocarpa.* Simple (6Ai); 2-armed (6Aii), 3-armed (6Aiii), 4-armed (6Bi and ii) tufted; 4-armed (6Ci), 6-armed (6Di), 7-armed (6Ei), 8-armed (6Fi), 11-armed (6Gi) stellate without a central cushion; 8-armed (6Hi), 13-armed (6Ii) stellate with central cushion; multiradiate (6Ji) and multangulate (6K) trichomes.

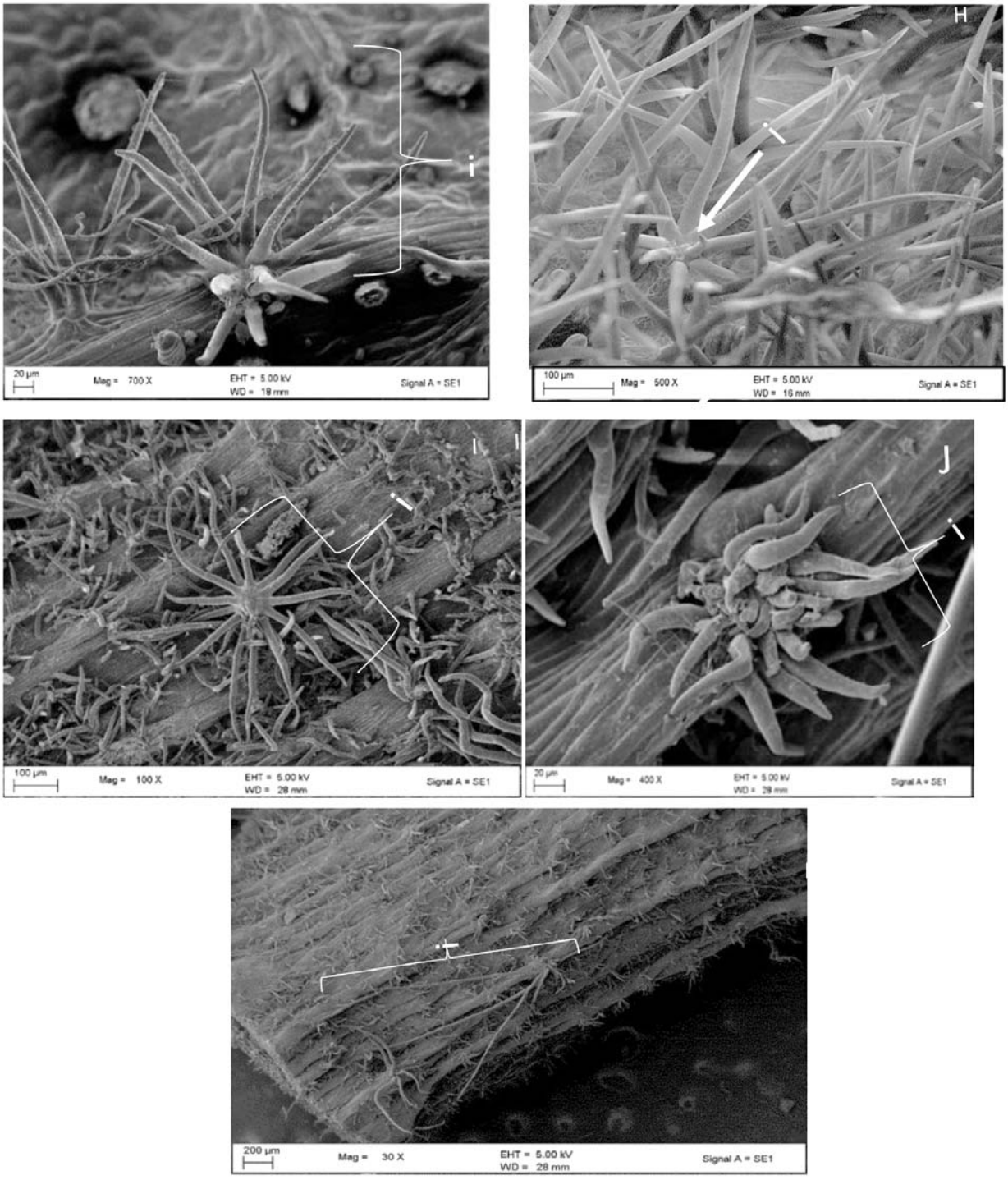


FIGURE 5 (A–K): Different types of structural (non-glandular) trichomes on the leaves and stem bark of *Grewia lasiocarpa.*
Simple (6Ai); 2-armed (6Aii), 3-armed (6Aiii), 4-armed (6Bi and ii) tufted; 4-armed (6Ci), 6-armed (6Di), 7-armed (6Ei), 8-armed (6Fi), 11-armed (6Gi) stellate without a central cushion; 8-armed (6Hi), 13-armed (6Ii) stellate with central cushion; multiradiate (6Ji) and multangulate (6K) trichomes.

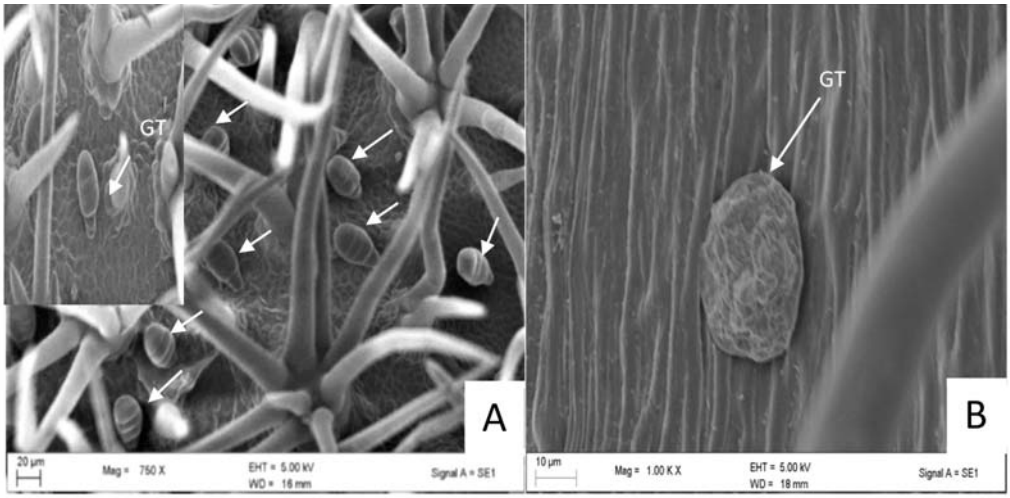


FIGURE 6 (A and B): Glandular trichomes on *Grewia lasiocarpa.*
(A) Adaxial surface with emphasis on the capitate trichomes (arrow) on the leaf blade immersed in the midst of non-glandular trichomes; (B) peltate trichome on the stem bark (freeze dried). (GT) glandular trichome.

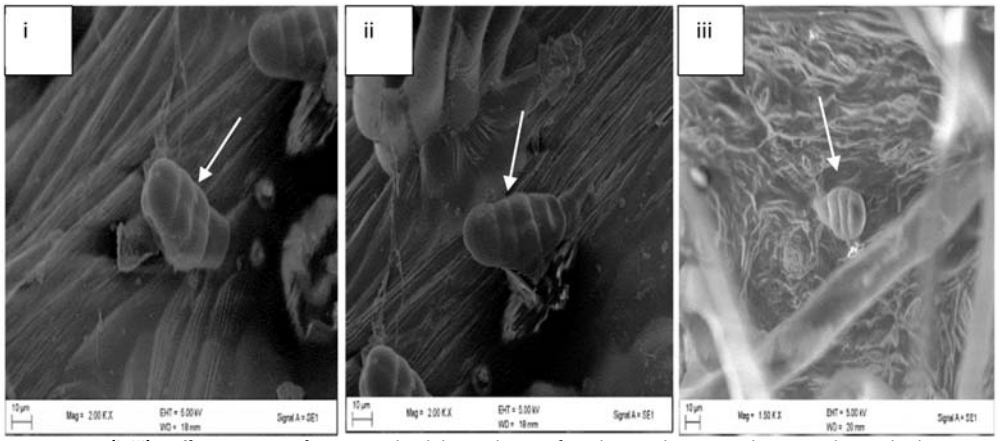


FIGURE 6A (i–iii): Different types of capitate glandular trichomes found on *G. lasiocarpa* leaves and stem bark, arrows showing the glandular trichomes.

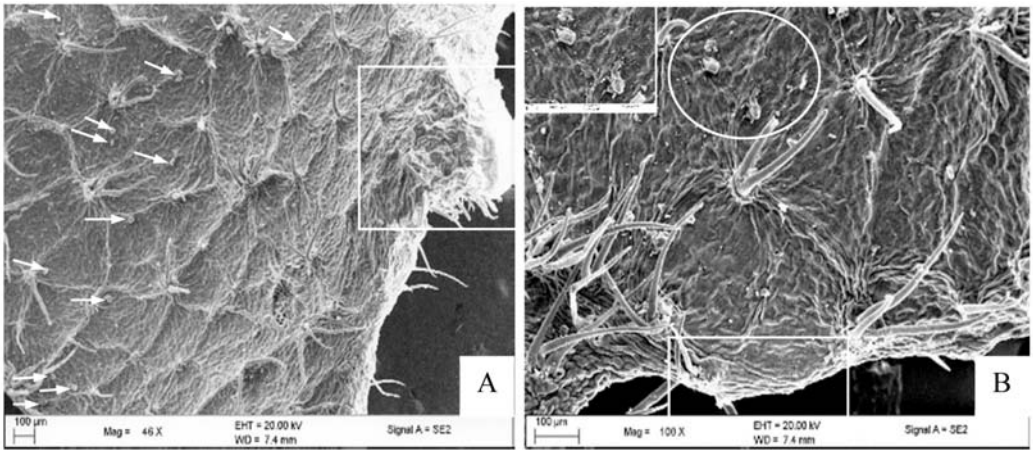


FIGURE 7 (A and B): Direction of glandular trichomes on the adaxial surface of *Grewia lasiocarpa*.
Rectangular box: apex of leaf, square and circle: glandular trichomes, glandular trichomes direction (arrow).

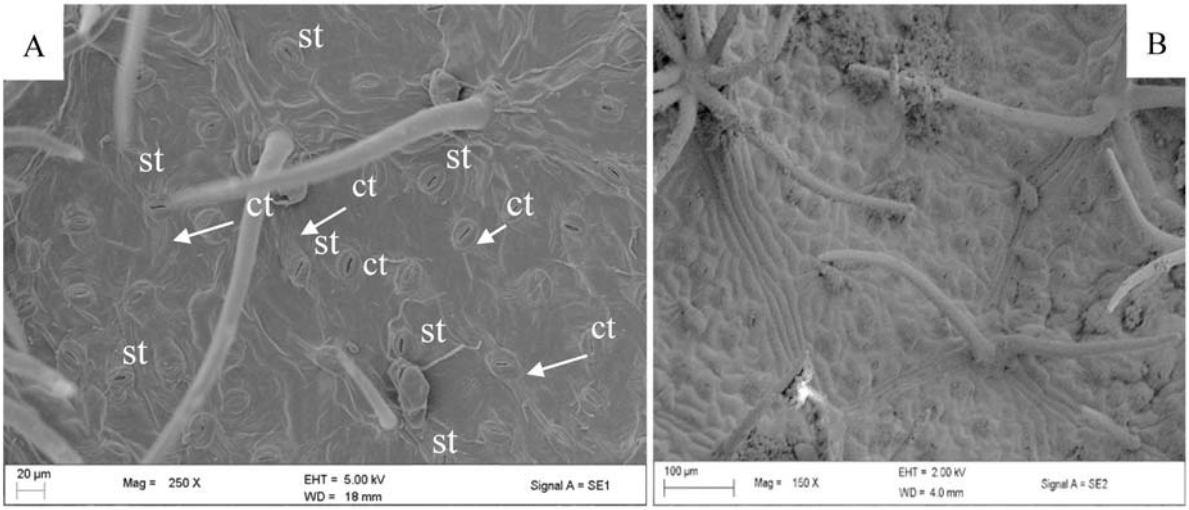


FIGURE 8 (A and B): Mature leaves of *Grewia lasiocarpa* showing the paracytic stomata on the abaxial surface of the leaf blade. (A) Chemically fixed leaf showing opened and closed stomata; (B) freeze dried leaf showing the epidermal cells on the leaf blade. (st) stomata, (ct) striate cuticle.

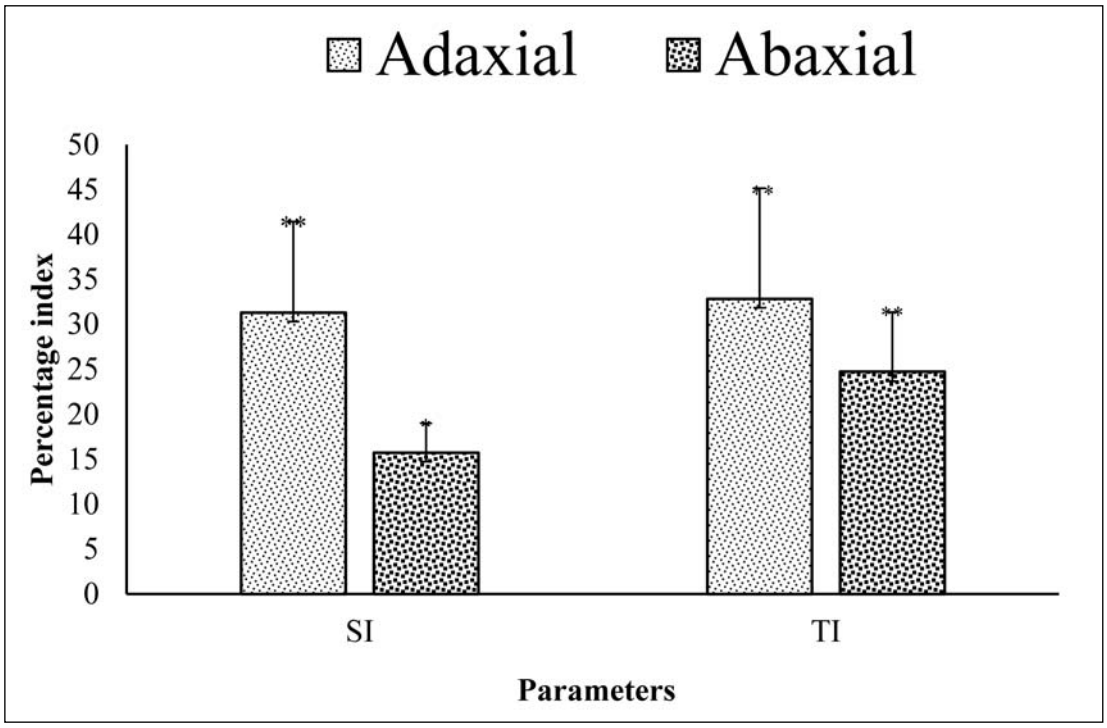


FIGURE 9: Average stomatal and trichome indices of the adaxial and abaxial surfaces of mature leaves of *Grewia lasiocarpa*. (SI = stomatal index, TI = trichome index)

Sections of the leaves stained with 0.05% toluidine blue and studied with the light microscope revealed that the number of trichomes decreases in the order emergent > young > mature (Fig. 10A–C). This observed development is associated with leaf expansion. The presence of mucilaginous cells is evident in the mid-rib and the stem of *G. lasiocarpa* (Fig. 10A and D), owing to the hardy nature of the stem bark the young photosynthetic/green herbaceous part of the stem were examined. The presence of structure and glandular trichomes (capitate and peltate) are also confirmed from the light microscopy study (Fig. 11A–D). Light microscopy also revealed the presence of tannins which appear green under the light microscope and mucilaginous cells in the stem bark of *G. lasiocarpa* (Fig. 12A and B). The leaves of *G. lasiocarpa* are rich in lipids (Fig. 13A and B). More plastids were present in the transverse section of the mature leaves than the young followed by the emergent (Fig. 14A–C).

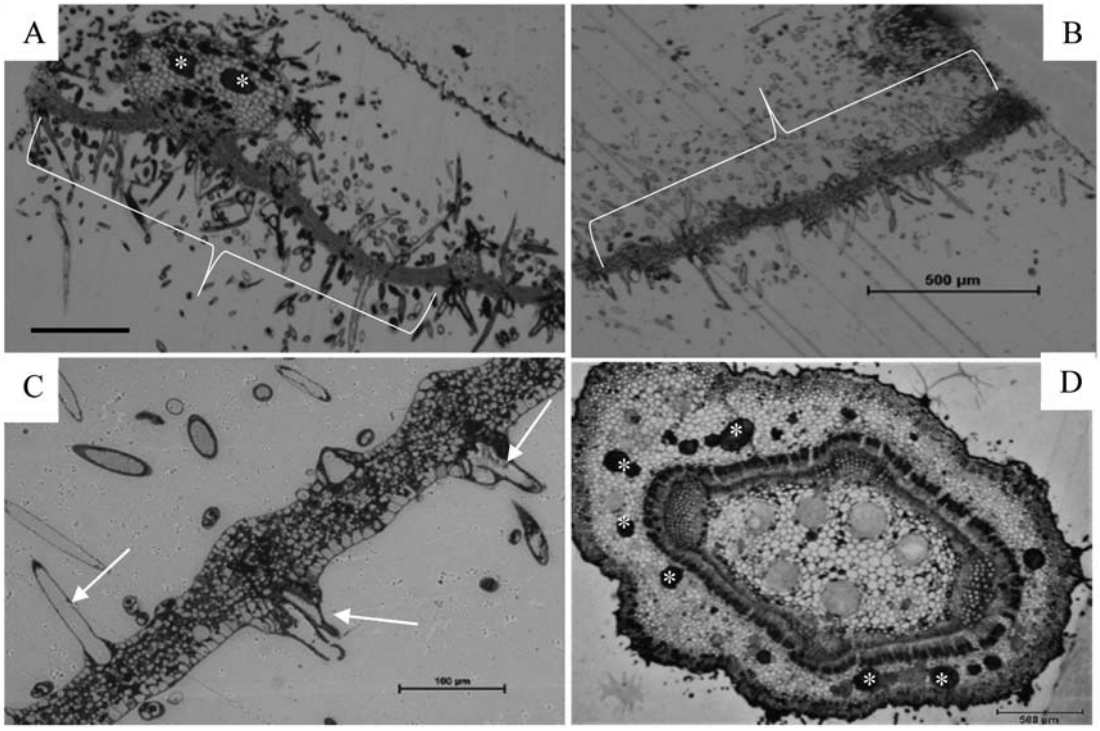


FIGURE 10 (A–D): Transverse sections of *Grewia lasiocarpa* leaves and stem bark. (A) emergent; (B) young; (C) mature leaves; (D) stem bark, arrow showing the non-glandular trichome, *mucilaginous cells.

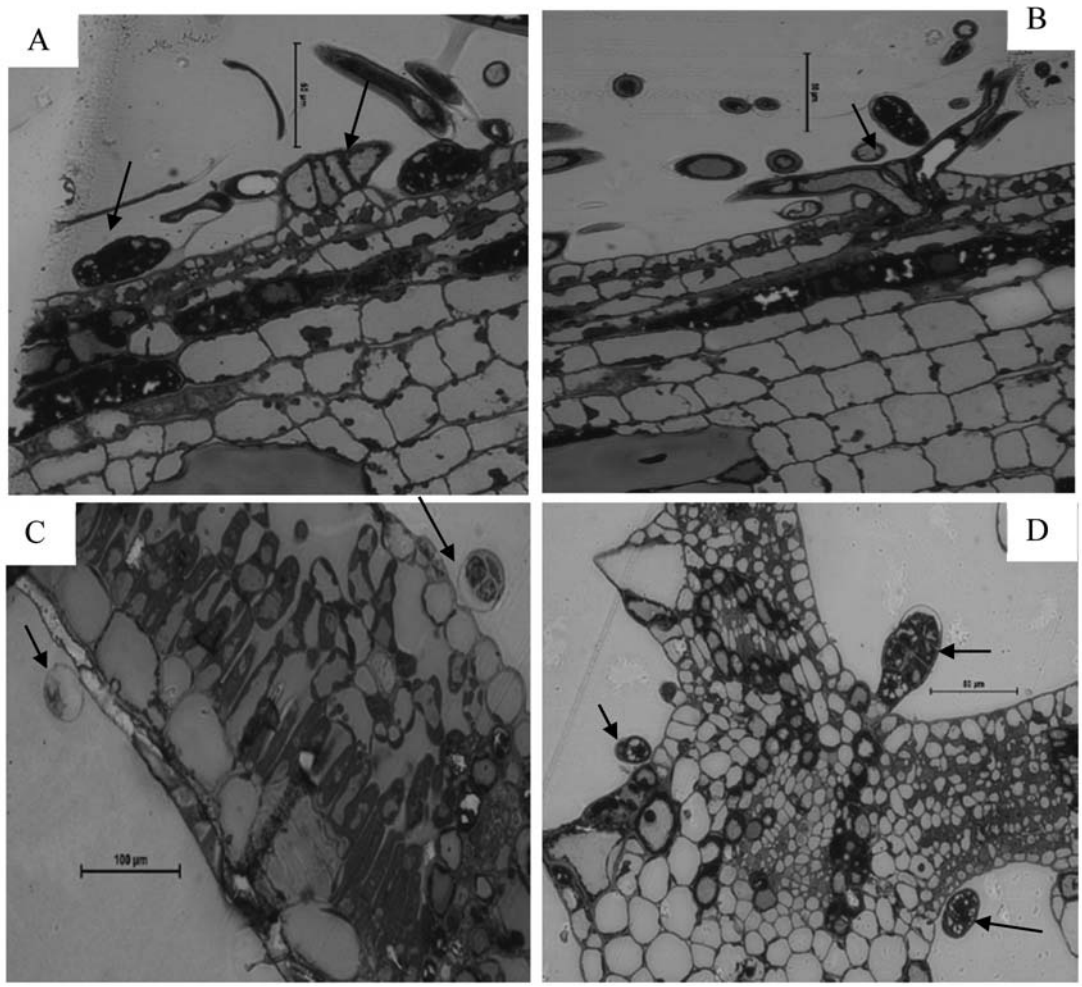


FIGURE 11 (A–D): Light microscopy sections of the leaves of *Grewia lasiocarpa,* (A) early developmental stage of a simple trichome; (B) a fully developed structural trichome; (C) peltate trichomes; (D) capitate and peltate trichomes (arrows).

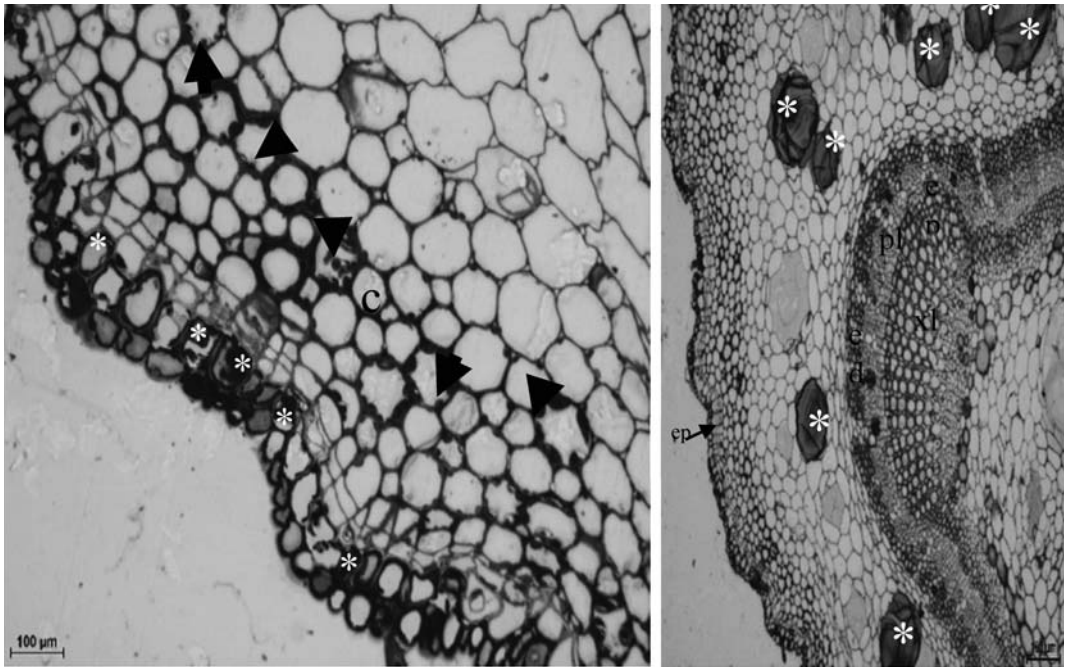


FIGURE 12 (A and B): (A) Transverse section through the stem bark of *Grewia lasiocarpa* green-blue colouration of tannins on the phellem-like layer (asterisks) and deposits of tannins (arrow heads) around the cortex (ct); (B) mucilaginous idioblast (*) stained with toluidine blue. (ep) epidermis with cuticle; (ct), cortex; (ed), endodermis; (pl), phloem; (xl), xylem.

The leaves of *G. lasiocarpa* are rich in lipids (Fig. 13A and B). More plastids were observed to be present in the transverse section of the mature leaves than the young followed by the emergent (Fig. 14 A–C).

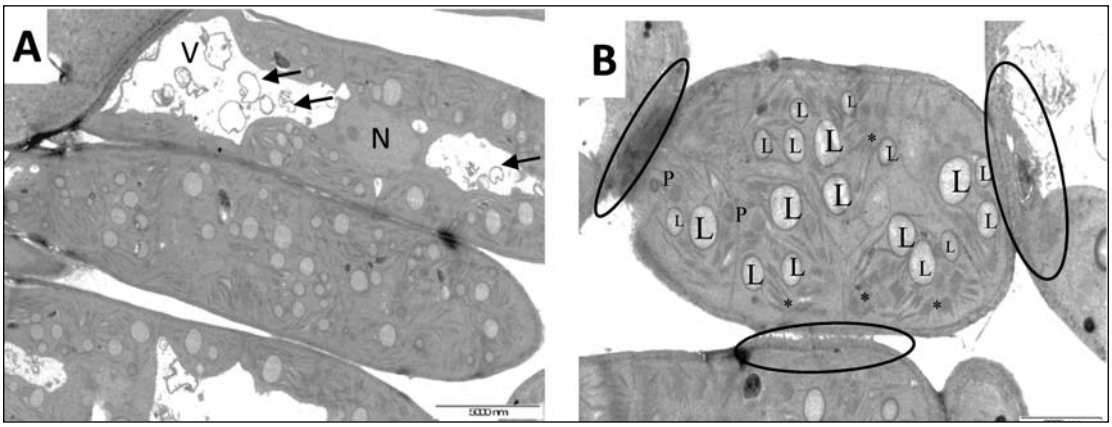


FIGURE 13 (A and B): Transverse sections (TEM) of a mature leaf of *Grewia lasiocarpa*. (arrows) provacuoles, (V) vacuoles, (N) nucleus, (L) lipid droplets, (P) plastids embedded with granules, (oval shape) region of cell-cell communication, (asterisks) thylakoids.

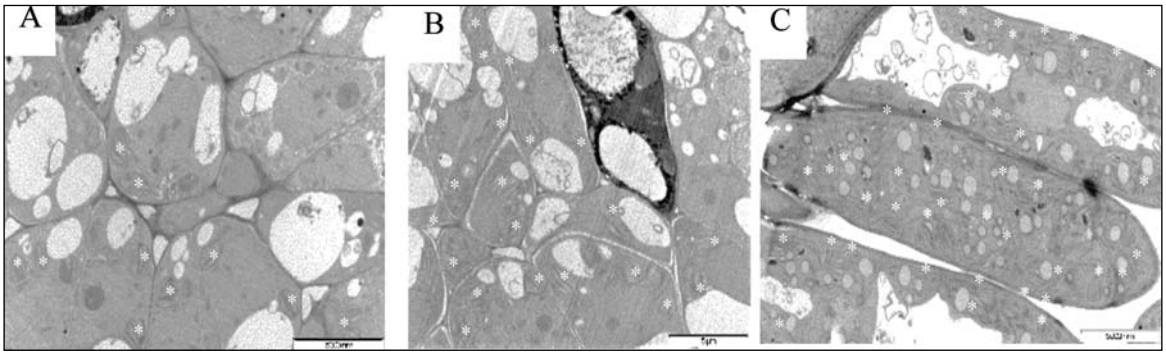
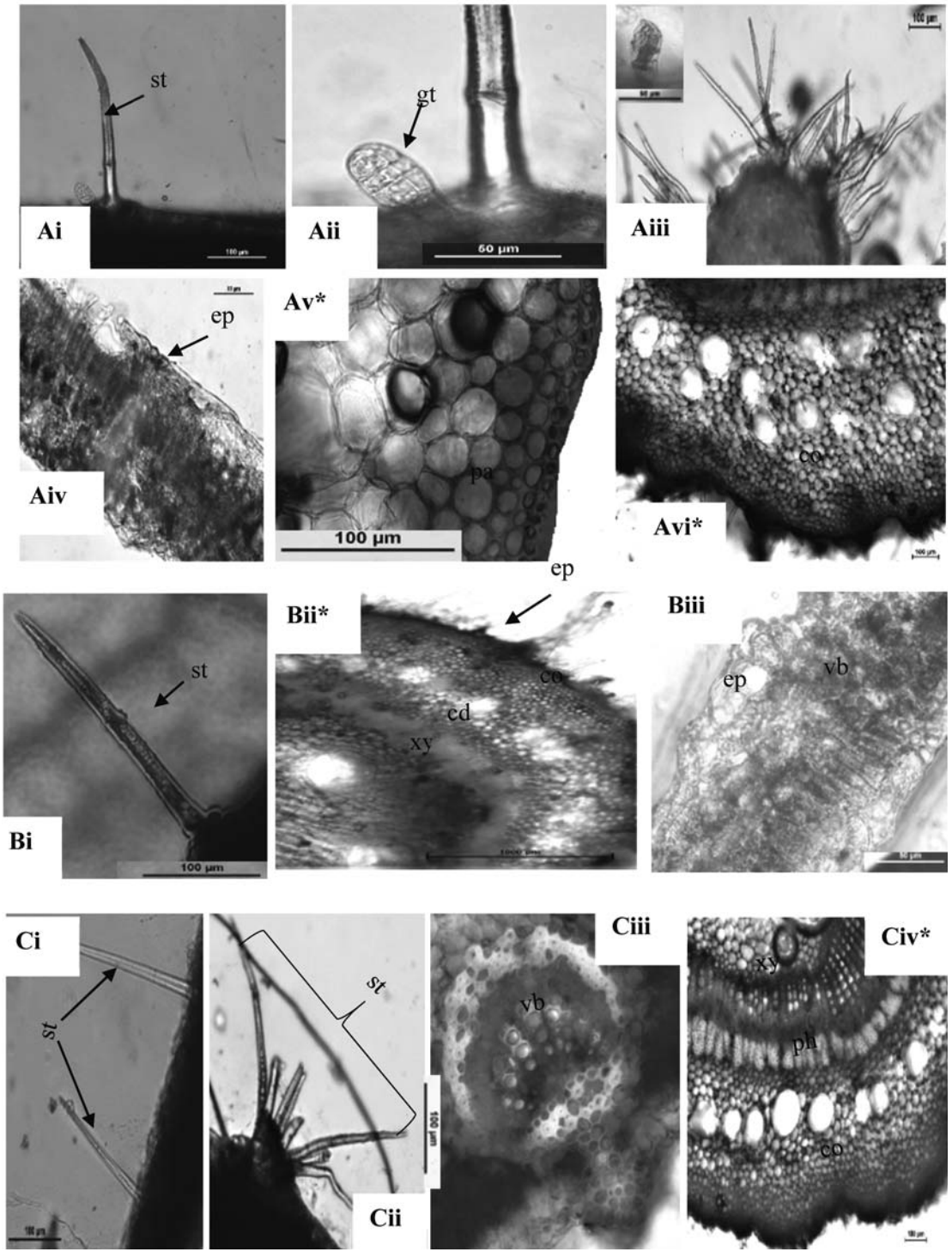


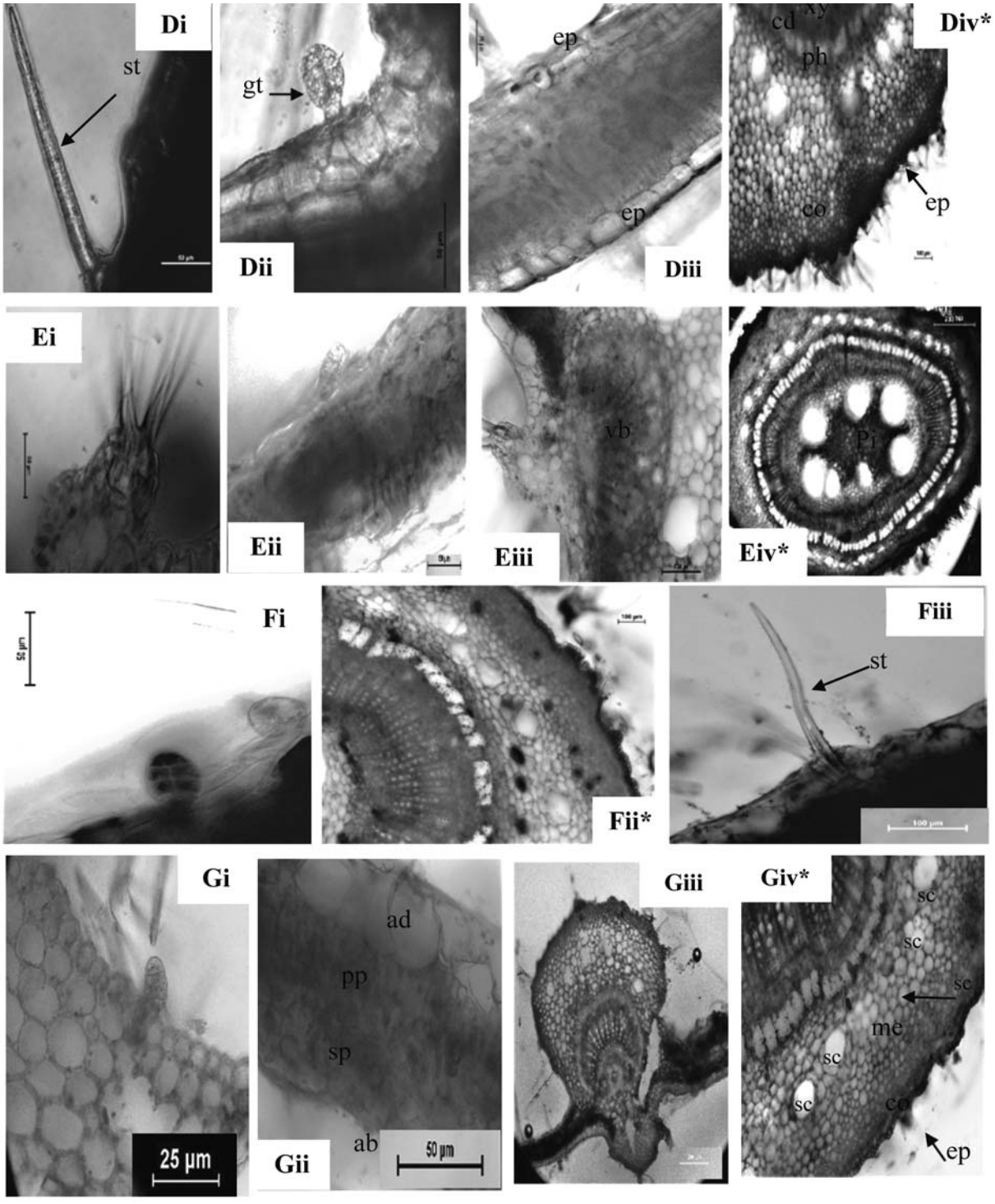
FIGURE 14 (A–C): The ultrastructure of (A) **e**mergent, (B) **y**oung, (C) mature leaves (asterisks) plastids.

The trichomes (glandular and non-glandular) were lignified (Fig. 15Ai and ii) and acidic (Fig.15Aiii), while the epidermal cells (adaxial and abaxial surfaces), vascular bundles (xylem and phloem) and parenchyma cells of the mid-rib, the cortex of the stem bark were also lignified. A positive reaction (red) was observed with 1-naphthol and N, N-dimethyl-p-phenylene diamine reagent indicating the presence of resinous acid in the non-glandular trichome (Fig. 15Bi); xylem, cambium, cortex, and epidermal cells (Fig. 15Bii); vascular bundles (Fig. 15 Biii). The non-glandular trichomes (Fig. 15Ci and ii) stained red for neutral acids and blue for acid resin as seen in Fig. 15Cii–iv i.e. some non-glandular trichomes, vascular bundles, xylem, phloem, cortex. Acidic polysaccharides were present in the trichomes, epidermal cells, cortex, phloem xylem and cambium as indicated with the ruthenium test (Fig. 15Di–iv). The iodine test showed black stains around the cuticles of all the non-glandular, and glandular trichomes (Fig. 15Ei and ii), the vascular bundle and pith also stained black. Staining with ferric chloride gave a positive reaction in the secreted substance present in the glandular trichome (Fig. 15Fi), confirming deposits of



ortho-dihydroxyphenols in the glandular trichome, cortex, phloem and xylem (Fig. 15Fii). The presence of protein in the structural trichome is confirmed in Fig. 15Fiii. The Sudan tests further confirm the presence of lipid inclusions in almost all parts of the leaves and stem bark (Fig. 15Gi–iv). The phloem of the stem bark stained orange while other parts stained brown with Wagner's reagents, confirming the presence of alkaloids (Fig. 15Hi), while the capitate and structural trichomes stained brown (Fig. 15Hii).

The fluorescence microscopy revealed that the non-glandular and glandular trichomes emitted a diffuse/intense and strong autofluorescence under UV excitation. DNA stained with fluorescent stain acridine orange revealed diffused fluorescence of the DNAs' present in the non-glandular trichome as shown in Fig. 15Ii and Fig. 15Iii and Iiii of the viable cells respectively. Calcofluor-white gave an intense blue fluorescence of the cell walls of the non-glandular (Fig. 15Ji) and glandular trichomes especially around the stalk and basal cells (Fig. 15Jii) in the leaves and stem bark.



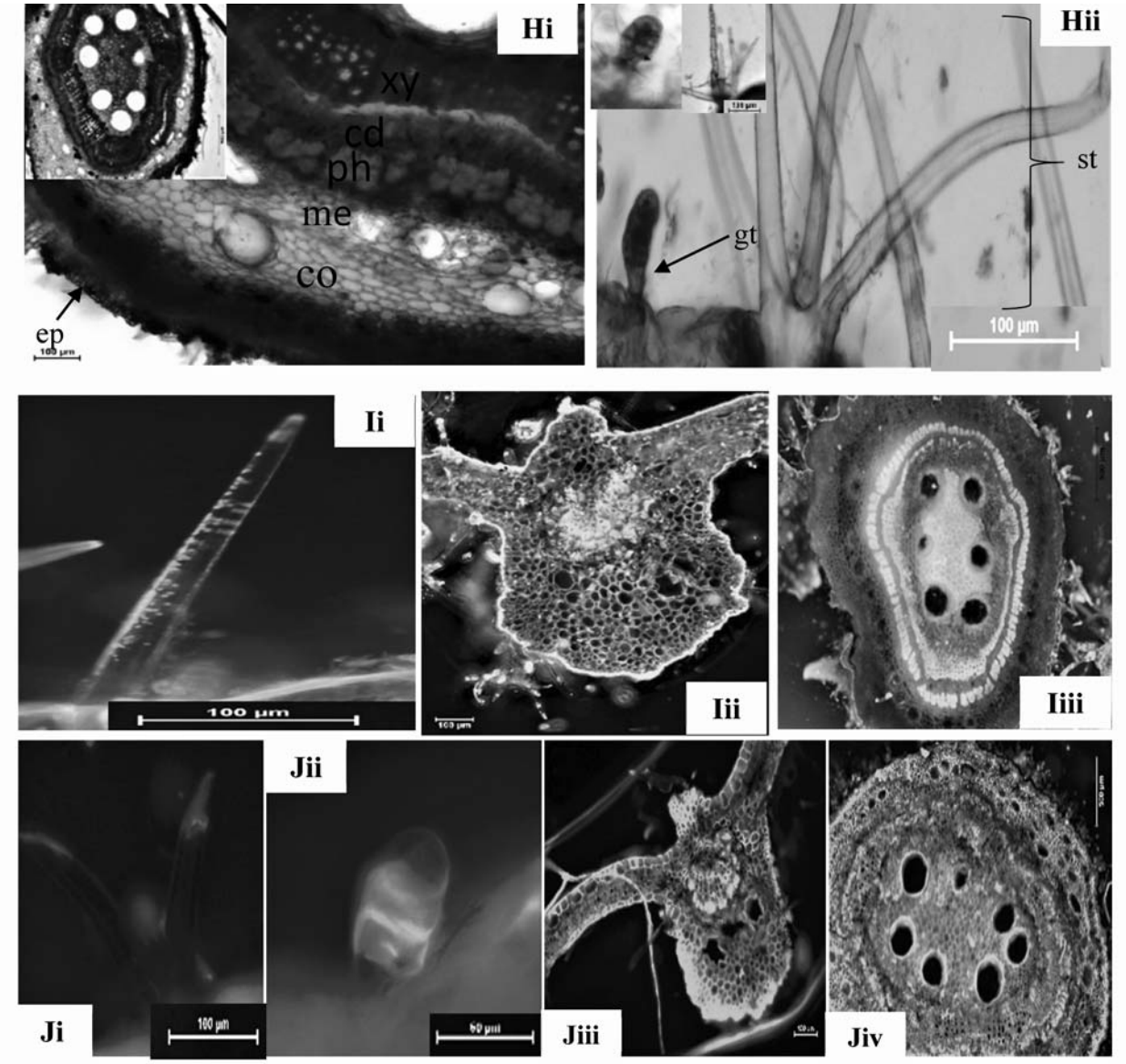


FIGURE 15 (A-J): Structural and histochemical characterisation of *Grewia lasiocarpa*.
(Ai, ii, iv,v, vi) lignins stained blue with toluidine blue, (Aiii) acidified lipids stained red-brown with toluidine blue (insert glandular trichome); (Bi–vii) resin acids stained red with NADI reagent; (Ci–ii) neutral and (Cii–iv) acidic lipids stained red and blue respectively with nile blue; (Di-iv) acidic polysaccharides such as mucilage and pectin stain red or rose pink; (Ei–iv) starch stained black with iodine; (Fi and ii) orto-dihydroxyphenols stained black with ferric trichloride; (Fiii) total proteins stained blue with mercuric bromophenol blue; (Gi–iv) lipid inclusions stained red with Sudan iii and iv; (Hi and ii) alkaloids stained brown or orange with Wagner's reagent [inserts complete section of stem bark showing alkaloids deposits and another glandular trichome]; (Ii–iii) DNA stained with fluorescent stain acridine orange; (Ji–iv) cellulose stained blue with fluorescent stain calcofluor-white. (st) structural trichome, (ad) adaxial surface, (ab) abaxial, (gt) glandular trichome, (pp) palisade parenchyma, (sc) secretory cell, (sp) spongy parenchyma, (me) mesophyll, (pa) parenchyma, (pi) pith, (vb) vascular bundle, (cd) cambium, (co) cortex, (ph) phloem, (xy) xylem. All sections are from the leaves except otherwise indicated with an asterisk.

Discussion

This study focussed on three leaf developmental stages, namely emergent, young and mature. The main leaf veins number between three and five emerging from the rounded or lobed base; this observation is in agreement with the report of Boon and Pooley 2010. Gums are present in the leaves (epicuticular) and in the rough hairy grey stem bark of *G. lasiocarpa*. The structural and glandular trichomes are present on the leaves and stem bark of *G. lasiocarpa.* Glandular trichomes are known to carry out specific metabolic functions of absorption of nutrients, biosynthesis and secretion of substances such as mucilage, digestive enzymes, salts, protective secondary metabolites (alkaloids, tannins), acyl lipids and nectar (Lange and Turner 2013; Naidoo et al. 2014).

The adaxial and abaxial surfaces of the emergent and young leaves were densely pubescent, while the trichomes on the mature leaves were observed to be sparse (Fig. 4C i and ii). Boon and Pooley (2010), reported that the mature stem bark of *G. lasiocarpa* is typically smooth, grey, and hairy and this is similar to our observation, while the stem bark of *G. mollis* is rough, hairy and black (Martins et al. 2008). In *G. lasiocarpa* the stem bark is also clustered with trichomes. Like other *Grewia* spp. e.g. *G. huluperakensis, G.*

polygama, G. multiflora, except *G. laevigata* (Chung 2002), simple structural and specialised glandular trichomes are also present on both sides of the leaves of *G. lasiocarpa.* Structural (non-glandular) and glandular trichomes were observed to be present on the stem bark of *G. lasiocarpa.*

About six main types with different sub-types of structural trichomes were observed on the leaves and stem bark. Chung (2002) reported the presence of these trichomes on other *Grewia* spp., except for the multiradiate stellate trichomes with numerous arm and central cushions, and multangulate trichomes. Ibrahim et al. (2018), also gave reference to the reported trichomes, and the T-shaped trichome. The presence of star-shaped (stellate) trichomes has been found on the outer part of the sepals of *G. lasiocarpa* (Boon and Pooley 2010). Stellate trichomes are also present on the leaves of *G. villosa* (Kumar and Paul 2015), *G. asiatica* (Joshi et al. 2013) and its fruits (Parul et al. 2013).

Although the presence of peltate trichomes was not reported by Chung, (2002), only one type of sessile peltate trichome was observed to be present on the leaves and stem bark of *G. lasiocarpa*. Based, on external structural features, three types of short unicellular stalk capitate trichomes were observed to be present with multicellular rounded glandular head, multicellular elliptic glandular head or multicellular oblong glandular head. These capitate trichomes were also reported by Chung (2002) for *G. occidentalis, G. huluperakensis, G. multiflora, G. laevigata* and *G. polygama*. The presence of dense non-glandular and glandular trichomes on the mid-rib and veins, compared to the leaf blade might be due to the functional role of food and water transportation played by these tissues (Rao 2009). A molecular signalling cascade might be responsible for the apex-directed capitate trichomes. Owing to the densely pubescent nature of the trichomes on the emergent and young leaves, only the mature leaves were evaluated for trichomes and stomatal indices for comparison with other reported *Grewia* spp. The epidermal cells are adjacent to the noticeable stomata cuticular striations. Chung (2002) found anomocytic stomata to be the major type of stomata on the *Grewia* spp. he studied, and the anisocytic and paracytic types were less common. Contrary to our findings, only the paracytic stomata, were observed to be present on the epidermis of the leaves (Fig. 8A). Similar to *G. polygama* (Chung 2002), the stomata on the mature leaves are amphistomatous i.e. stomata are present on both sides of the leaves. Although Chung (2002) did not specify the precise surface of the leaves examined, our percentage stomatal indices for the adaxial and abaxial surfaces were relatively similar to the ranges reported by Chung (2002). The reported ranges were between 11.3 and 29, with a mean of 14.1 in *G. laevigata* and 25.9–27.3 in *G. multiflora* and *G. polygama.*

Although the trichome density of the emergent and young leaves was not determined, the micrographs support the report of Werker (2000), which states that the initiation of the trichomes starts prior to the initiation of the leaf primordia i.e. the region that develops into leaves and also before stomatal development. This means that the trichomes are formed before leaf expansion.

On the adaxial surface of the mature leaves, the average number of stomata per mm^2 of the epidermis was evaluated to be lower than that of the abaxial, and this observation is similar to the findings of Hunsche et al. (2010) for *G. tenax*. However, Hunsche and his colleagues did not state the developmental stage of the leaves that they analysed. This difference in number may be influenced by one of the functional roles played by plant stomata, which is the reduction of transpiration rate caused by heat and air current (Royer 2001). Furthermore, the trichome index was also higher in the abaxial surface than the abaxial. This might be due to environmental factors such as heat and air current since an increase in any of these factors would alter the normal physiology of the plant's cells. In general, the presence of the trichomes would help to reduce heat effect from sunlight, the rate of transpiration, and protect the leaves from other external biotic factors. These observations may also be explained by the geographical location of the *G. lasiocarpa* plant examined for this study, which was located in a mist-belt forest with a flowing underground water source, close to a coast, hence the need for more stomata and trichome on the adaxial surface than the abaxial for a systematic maintenance of the osmotic and turgor pressure in the cells via interaction between the plant and the environment.

In the leaves and stem of plants that belong to the Malvaceae family, mucilaginous epidermis and druses are the main microscopic diagnostic features (Molares and Ladio 2014; Ibrahim et al. 2018). Mucilaginous epidermis and tannins are also present in some cortex cells (phellem-like layer).

In addition, anticlinal and not the periclinal cell wall divisions of the epidermal cells of the leaves were also observed, which has also been reported in other *Grewia* spp. (Chung 2002).

The mature leaves of *G. lasiocarpa* have quite a high number of lipid droplets, with varying sizes, because the smaller lipid droplets fuse to form larger droplets. The lipids are results of hormone metabolism, stress response, and pathogen resistance (Chapman et al. 2012). This high number implies that *G. lasiocarpa* has a high oil content and may have several metabolites that could be used to treat certain pathogenic diseases.

The greenish colour variation with respect to the intensity of the emergent, young and mature leaves (Fig. 1A–C), may be influenced by the number of plastids per cell. In correlation with this, the TEM micrographs show an increase in the number of thylakoids in the order emergent < young < mature, thus the mature leaves appear as darker green.

Based on our findings, glandular trichomes secrete protective secondary metabolites such as orto-dihydroxyphenols, alkaloids, lipids, and lignin. The presence of acidified lipids containing sulfatides (Sridharan and Shankar 2012) is confirmed in the structural and glandular trichomes. Acridine orange, a metachromatic fluorochrome, stains DNA fluorescent green and ribo-nucleic acid (RNAs) orange. Hence the red coloured regions might be indicative of the presence of endophytic bacteria or acidic organelles, and the conspicuous green spots the endophytic fungi (Guzaev et al. 2017).

Conclusion

Two types of glandular trichomes were found on the leaves and stem bark of *G. lasiocarpa.* However, there are various types of structural trichomes with the simple trichomes being more abundant. The presence of glandular trichomes on the leaves and stem bark of *G. lasiocarpa* indicates that it is a plant with ecological, economic and medicinal values. This is the first report on the micromorphology, histo-chemistry and ultrastructure of the leaves, stem bark and *indumentum* of *Grewia lasiocarpa* E. Mey. ex Harv.

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