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COMPARATIVE EPIDERMAL MICROMORPHOLOGY AND PHYTOCHEMICAL LOCALIZATION IN FIVE CULTIVATED CITRUS SPECIES OF WEST BENGAL

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ABSTRACT: This present study investigates the morphological, anatomical, and phytochemical diversity among five *Citrus* species—*C. aurantifolia*, *C. sinensis*, *C. maxima*, *C. limonia*, and *C. limetta*—collected from lateritic regions of Birbhum District, West Bengal, India. Significant variation was observed in crown architecture, growth habit, leaf area, petiole wing, and thorn providing key traits for taxonomic identification and horticultural selection. *C. maxima* exhibited the tallest stature and largest leaf area, suggesting superior photosynthetic efficiency, while *C. aurantifolia* showed a compact morphology suited to drought tolerance. Stomatal analysis revealed diversity in type and index, with *C. limonia* possessing the highest stomatal frequency and *C. sinensis* the largest stomatal size, indicating species-specific gas exchange strategies. Anatomically, dorsiventral leaf structure, variation in epidermal cells, presence of sheath cells, and occurrence of megastomata were noted, supporting interspecific differentiation. Histochemical screening confirmed the presence of metabolites such as alkaloids, starch, tannins, proteins, flavonoids, amino acids, and lipids, with tissue-specific localization patterns. Notably, the xylem and sclerenchyma tissues showed a higher concentration of phytochemicals, especially in *C. sinensis* and *C. limetta*, underlining their medicinal potential. These findings highlight the adaptive strategies and taxonomic relevance of key morphological and biochemical traits among *Citrus* species. The observed diversity offers valuable insights for cultivar selection in breeding programs, ecological suitability, and pharmaceutical applications. This comprehensive characterization enhances our understanding of *Citrus* biology and supports the sustainable utilization of genetic resources in agro-horticultural and ethnobotanical contexts.

Keywords: Citrus, Phytochemical, Stomata, Micromorphology.

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1. INTRODUCTION

Citrus is a genus of flowering trees or shrubs under the family Rutaceae. The plants known for their economic and nutritional value, play a significant role in global horticulture. Immense attention has given in cultivation due to their rich content of vitamins, antioxidants, and phytochemicals, contributing to health benefits and disease prevention [1]. The strategic importance of citrus cultivation in regions such as West Bengal highlights the need for detailed studies on their morphological and biochemical profiles. The Key lime or acid lime (*Citrus aurantifolia*) native to Southeast Asia is grown mainly in tropical and subtropical regions. It is widely used because of its antibacterial, anticancer, antidiabetic, antifungal, antioxidant properties [2,3,4,5]. The cultivated species *Citrus sinensis* (L.) Osbeck is a seasonal citrus fruit valued in the hilly Northern regions of India. It is popular for its sweet-sour taste and rich vitamin content. The species commonly grown in kitchen and home gardens, but remains underutilized commercially. Another cultivated species *Citrus limonia* known as "Citron" or Gondhoraj lime looks like a lemon but has rough skin. It is a small to medium citrus, with a round to oval shape and pointed ends, low on juice but rich in aroma, The sweet, citrusy aroma of Gandhoraj lime fills the air with an unforgettable fragrance. The pomelo (*Citrus maxima*) or Batabi lemon also called shaddock, is the largest citrus fruit and an ancestor of several cultivated citrus species, including bitter orange (*Citrus auruntium*). Mosambi or sweet lime (*Citrus limetta)* is small and round like a common lime in shape. It is a cross between the citron, *Citrus medica* and a bitter orange, *Citrus aurantium*. Mosambi contains dietary fibre and natural acids that aid digestion and prevent constipation. High in vitamin C, Mosambi helps strengthen the immune system [6]. Epidermal micromorphology, which examines the structure and features of the epidermis, offers valuable insights into the adaptation mechanisms of plants in their specific environments [7]. The lateritic belt of West Bengal, characterized by its unique soil properties and climatic conditions, poses both challenges and opportunities for citrus cultivation. Understanding the epidermal characteristics of cultivated citrus species can provide clues about their resilience and cultivation practices in these lateritic regions [8]. Histochemical analysis enables the localization of phytochemicals within plant tissues, facilitating a deeper understanding of their distribution and function. Phytochemicals such as flavonoids, alkaloids, tannins are crucial for plant defense mechanisms and contribute to the antioxidant properties of citrus fruits [9]. Investigating these compounds in conjunction with micromorphological features can reveal correlations between

structure and biochemical content that are critical for breeding and conservation efforts. This study aims to explore the comparative epidermal micromorphology and histochemical localization of key phytochemicals in five important cultivated species of citrus in the lateritic belt of West Bengal. By bridging the gap between morphology and biochemistry, this research will enhance our understanding of citrus adaptability and nutrition while providing fundamental knowledge for future agricultural practices.

2. MATERIALS AND METHODS

Specimen Collection: The study was conducted using living plant specimens from five species of the genus Citrus like *Citrus aurantifolia* i.e. Key lime, *Citrus sinensis* i.e. Malta, *Citrus maxima* i.e. pomelo, *Citrus limonia* i.e. Aroma King Lemon, *Citrus limetta* i.e. sweet lime were collected from Rathindra Krishi Vigyan Kendra (RKVK), Palli Siksha Bhavana (Institute of Agriculture), Visva-Bharati, Sriniketan at lateritic regions of Birbhum District, West Bengal at 23.66° North latitude and 87.66° East longitude. The site enjoys an average annual rainfall of 1400-1500 mm, and the average annual relative humidity of this area is about 67%. The mean maximum and minimum temperature are 37.0 ^{0}C and 12.8 ^{0}C, respectively. The soils were classified as slightly acidic with 5.65 pH. The specimens were identified and authenticated by the scientists of Rathindra Krishi Vigyan Kendra (RKVK), Palli Siksha Bhavana (Institute of Agriculture), Visva Bharati

Plant type: Branching pattern was observed based on the position of branch origination from the stem, plant growth habit was recorded, Crown shape was classified depending on the observed crown structure. Foliage density was assessed visually. All observations were recorded and tabulated**.**

Leaf morphological characters

The different parameters of leaf were studied. The leaf length-to-breadth ratio was calculated using the measured leaf length and width. Leaf area was determined by counting the number of squares covered on millimeter graph paper, following the method described by Faur and Ianovici (2004) [10]. Fresh leaf weight was recorded immediately after bringing the samples to the laboratory. The leaves were then air dried until a constant dry weight was obtained.

Recording of Stomatal Characters

Stomatal observations were carried out using a Labomed Vision 2000 compound microscope. The number of stomata, length and width of the stomatal aperture, and the size and number of epidermal cells were recorded. For each species, ten replicates (n = 10) were analyzed. Stomatal index (percentage of stomata relative to the total number of epidermal cells) and stomatal frequency (number of stomata per mm^2) were calculated following the method of Salisbury (1927) [11].

Anatomical Study

Leaf anatomical studies were conducted by clearing leaf sections with 10% sodium hydroxide

(NaOH). The specimens were then stained with safranin and dehydrated through a graded ethanol series, following the protocol of Lersten and Curtis (2001) [12].

Pharmacognostic Study

Histochemical Localization of Phytochemicals in Plants

Histochemical techniques allow the *in-situ* identification and localization of various phytochemicals in plant tissues. These methods involve treating thin sections of fresh or fixed plant materials with specific reagents that produce color reactions upon interacting with targeted compounds. The sections were typically examined under a light microscope to determine the distribution and intensity of the reaction products.

Localization of Alkaloids: Plant sections were stained with Wagner's reagent stain and gave orange to reddish-brown colouration. Plant sections were treated with Potassium Iodide (KI) solution. Starch granules stained blue-black. When treated with 5% ferric chloride, tannins gave a bluish-black or green coloration depending on their type. When treated with Benedict's reagent, reducing sugar gave a bluish-black coloration. Lugol's reagent, proteins gave a dark brown coloration. Plant sections were stained with 10% NaOH, which stained flavonoids bright yellow colouration. Plant sections were stained with Ninhydrin, which stained amino acid Ruhemann's purple colour. Plant sections were stained with lipid-soluble dyes like Sudan III which stained lipids red colouration.

Statistical Analysis

The data obtained from the anatomical measurements were subjected to statistical analysis to calculate the mean and standard error (SE) for each parameter. The standard error was used to indicate the variability of the sample mean and to provide an estimate of the precision of the mean values. The results are expressed as mean ± SE, this method follows the standard approach for descriptive statistics as recommended by Zar (1999) [13].

3. RESULTS AND DISCUSSION

Branching pattern: The five *Citrus* species studied exhibited significant morphological diversity in crown shape, growth habit, leaf area, and petiole wing presence, which are vital for taxonomic classification and horticultural practices [14] (Fig. 1). *C. maxima* showed the greatest height (20–22 ft) and leaf area (56.63 cm^2), supporting its high photosynthetic potential and vigorous growth, while *C. aurantifolia* had the smallest dimensions (3-4 ft), reflecting a more compact growth habit [15] (Table 1). Presence of thorns were observed in most species except *C. maxima*, indicating its potential advantage in cultivation due to ease of handling [16]. Petiole wings, an important taxonomic trait, were absent in *C. limonia*, suggesting possible phylogenetic divergence [17] (Fig. 2). These morphological traits can guide species selection for breeding and cultivation suited to specific agro-ecological zones.

Table 1. Branching pattern of Citrus species from different lateritic regions.

Citrus species	Crown shape	Tree growth habit and branching pattern	Height(ft)	Leaf area(cm^2)	Petiole wing
Citrus aurantifolia Key lime	Rounded	Slender, spreading branches, armed with short, stiff, sharp spines	3-4	10.15	Present
Citrus sinensis, Malta	Ovate	Medium sized, armed, profusely branched	15-17	15.63	Present
Citrus maxima, pomelo	Oval	Low, irregular branches, no thorns	20-22	56.63	Present
Citrus limonia, Aroma King Lemon	Round to oval shape	Slender, spreading branches, armed with short, stiff, sharp spines	8-9	39.69	Absent
Citrus limetta Risso, sweet lime	spreading Ovate canopy that provides excellent shade	Irregular branches and relatively smooth, it has numerous thorns,	10-12	37.72	Present

Fig 1 Branching pattern of five commercially cultivated mango found throughout lateritic belt. A. *Citrus aurantifolia* Key lime, B. *Citrus sinensis*, Malta, C. *Citrus maxima*, pomelo,

D. *Citrus limonia*, Aroma King Lemon,E. *Citrus limetta* Risso, sweet lime

Fig.2 Leaf and fruit morphology of *Citrus aurantifolia* Key lime, *Citrus sinensis*, Malta, *Citrus maxima*, pomelo, *Citrus limonia*, Aroma King Lemon, *Citrus limetta* Risso, sweet lime

Leaf morphological characters

The leaf morphological characteristics among the *Citrus* species (Table2, Fig. 2) exhibited notable interspecific variability, particularly in leaf size, shape, and apex, which are key indicators in species identification and adaptation strategies [9]. *C. limonia* exhibited the largest leaf area (56.63 cm²) with apiculate apex and undulated margins, suggesting higher photosynthetic efficiency and possibly greater drought tolerance [8]. In contrast, *C. aurantifolia* had the smallest leaves (4.8 × 2.5 cm, 15.63 cm²), with an acute apex and entire margins, typical of xeromorphic adaptations in compact cultivars [10]. Leaf shape varied from elliptical (*C. aurantifolia*, *C. limonia*) to oval (*C. sinensis*, *C. maxima*) and lanceolate (*C. limetta*), indicating species-specific developmental pathways and ecological preferences [11]. The diversity in leaf apex forms—acute, obtuse, cuspidate, and apiculate—also reflects taxonomic divergence within the genus *Citrus*.

Table 2. Quantitative leaf morphological characters of mango cultivars from different agro-climatic regions.

Citrus species	Leaf length cm)	Leaf width (cm)	Leaf area (cm2)	Leaf apex	Leaf margin	Leaf shape
Citrus aurantifolia Key lime	4.8	2.5	15.63	Acute	Entire	Elliptical
Citrus sinensis, Malta	8.2	4.6	10.15	Obtuse	Undulated	Oval
Citrus maxima, pomelo	9.3	3.2	39.65	Cuspidate	Undulated	Oval
Citrus limonia, Aroma King Lemon	9.2	4.1	56.63	Apiculate	Undulated	Elliptical
Citrus limetta Risso, sweet	9.4	4.5	37.72	Cuspidate	Wavy	Lanceolate

Leaf epidermal characters

In *C. auruntifolia* epidermal cells on adaxial surface were largely polygonal, rectangular to pentagonal, with straight anticlinal walls (Fig. 3A). Stomata and trichomes were generally absent

but there were prismatic crystals as well as secretory cavities distributed throughout the surface. On the abaxial surface (Fig. 3B), epidermal cells were largely polygonal. Prismatic crystals were distributed throughout the surface but with stomata and secretory cavity. Adaxial epidermal cells were largely polygonal, cell wall were 3 or 4 layers thick (Fig. 3C) in *Citrus sinensis*. Stomata and trichomes were absent. Prismatic shaped crystals and secretory cavity were present and they were distributed throughout the surface of the epidermis. Epidermal cells on the abaxial surface (Fig. 3D) were largely polygonal. Paracytic stomata were abundantly present, predominantly circular in shape, with a few being elliptical in *C. maxima*. On the adaxial surface of the epidermal cells were largely polygonal (rectangular to pentagonal) with straight anticlinal walls in *Citrus maxima* (Fig. 3E). The walls were thick, having 3 or 4 layers of cells. Stomata and trichomes were absent, but prismatic crystals and secretory cavities were present and they were distributed throughout the surface of the epidermis. The shape of the epidermal cells on the abaxial surface was the same as on the adaxial with straight anticlinal walls (Fig. 3F). In *C.limonia t*he cells were arranged in elongated rows, the walls were thick with 3 or 4 layers of cells. Prismatic crystals and secretory cavities were present and distributed throughout the surface on adaxial surface of *Citrus limonia* (3G). On the abaxial surface (Fig. 3H), epidermal cells were largely polygonal just like it was on the adaxial surface, stomata occurred in abundant, largely circular in shape. Prismatic crystals were distributed throughout the surface but without secretory cavity. In *Citrus limetta* epidermal cells on adaxial surface were largely polygonal, rectangular to pentagonal, with straight anticlinal walls (Fig. 3I). On the abaxial surface (3j) stomata were abundant.

Recording of Stomatal Characters

Paradermal observations revealed that the leaves of all examined *Citrus* species possess actinocytic, cyclocytic, laterocytic, amphilaterocytic, and stephanocytic stomata. These stomata were surrounded by 4 to 12 subsidiary cells arranged in a radial pattern and were confined to the abaxial surface, indicating a hypostomatic condition (Figure 5) [18,19,20]. Such an arrangement is commonly associated with reduced transpiration through the stomata, serving as an adaptive feature in water conservation [21,22]

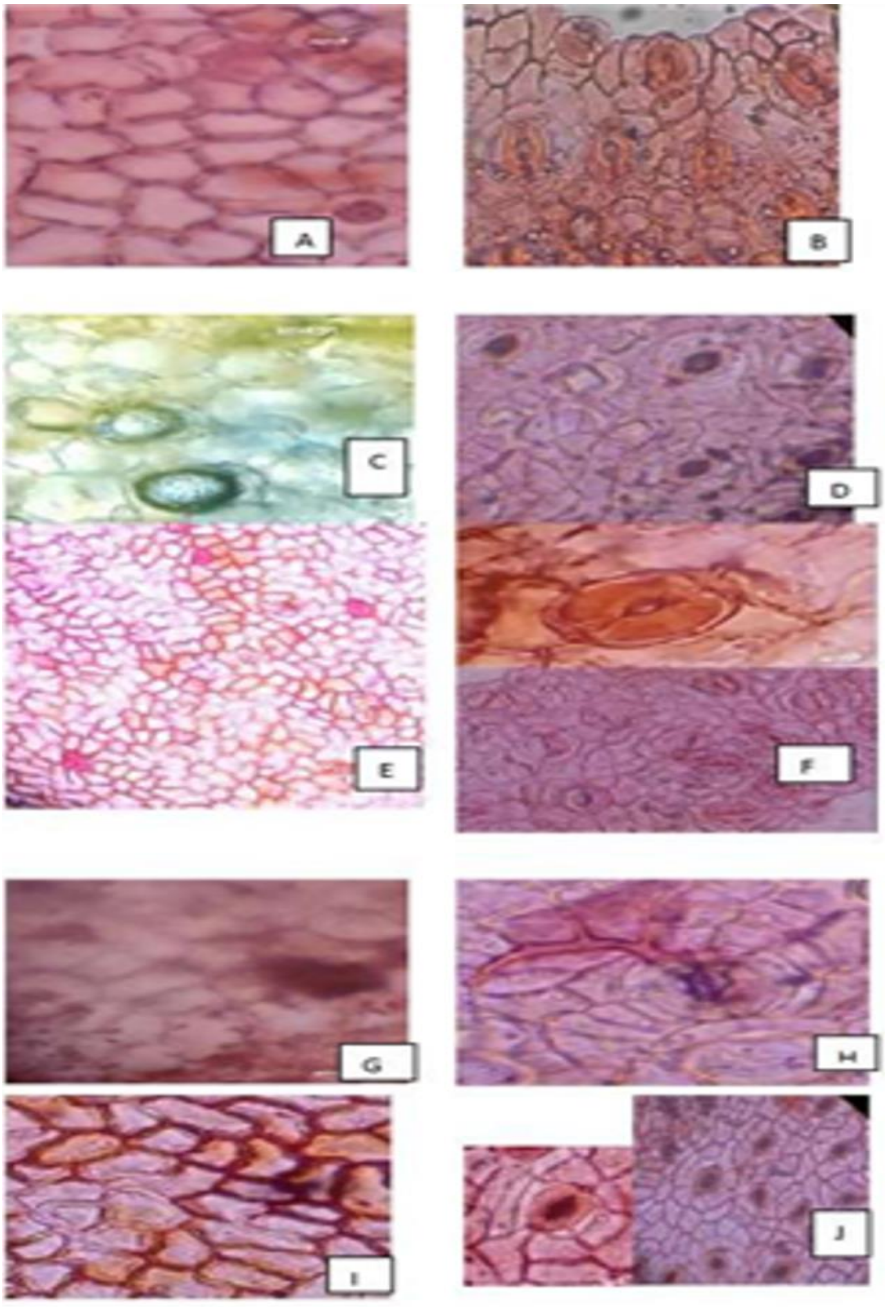


Fig 3. Upper epidermis (left) and Lower epidermis (right) of Citrus species. A-B.. *Citrus aurantifolia* Key lime, C-D. *Citrus sinensis*, Malta, E-F. *Citrus maxima*, pomelo, G-H. *Citrus limonia*, Aroma King Lemon, I-J *Citrus limetta* Risso, sweet lime.

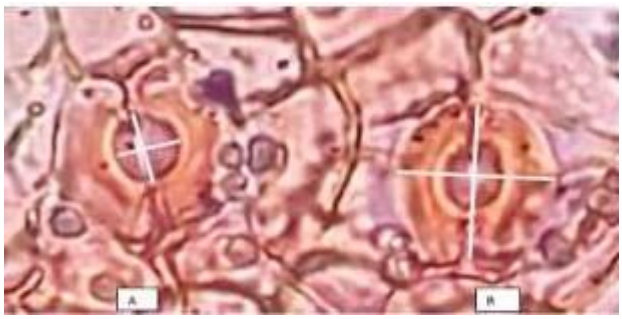


Fig. 4. A. Pore length and pore width B. Stomata length, stomata width of *Citrus*

Table 3: Stomatal characteristics of Citrus

Cultivar	Stomatal index (%)	Stomatal frequency (mm^2)	Stomatal length(µm)	Stomatal Breadth(µm)
Citrus aurantifolia	7.89±1.2	2.54±1.3	6.91±1.2	3.5±1.2
Citrus sinensis,	20±1.6	15.2±2.1	7.1±1.2	5.2±.99
Citrus maxima,	13.8±1.5	8.47±2.2	8.2±1.1	4.5±1.3
Citrus limonia,	15±2.5	12.7±2.3	9.5±1.3	4.6±1.1
Citrus limetta	7.93±1.9	4.23±1.1	7.5±1.3	4.2±1.3

The comparative stomatal analysis among the *Citrus* cultivars revealed significant variation in epidermal and stomatal traits, which are crucial for understanding physiological adaptability and taxonomic differentiation [23](Table 3, 4). *C. sinensis* exhibited the highest stomatal frequency (15.2 mm²), indicating greater gas exchange potential, which may correlate with its larger leaf area and high photosynthetic activity [21]. In contrast, *C. aurantifolia* showed the lowest stomatal index (7.89%) and frequency (2.54 mm²), suggesting reduced transpiration and a more conservative water-use strategy, typical of drought-tolerant species [24]. Stomatal size varied among species, with *C. sinensis* having the longest stomata (15.2 µm), potentially enabling greater pore aperture control in response to environmental cues [25]. These differences in stomatal morphology and distribution emphasize ecological and evolutionary adaptations within the genus *Citrus* and support their taxonomic distinction.

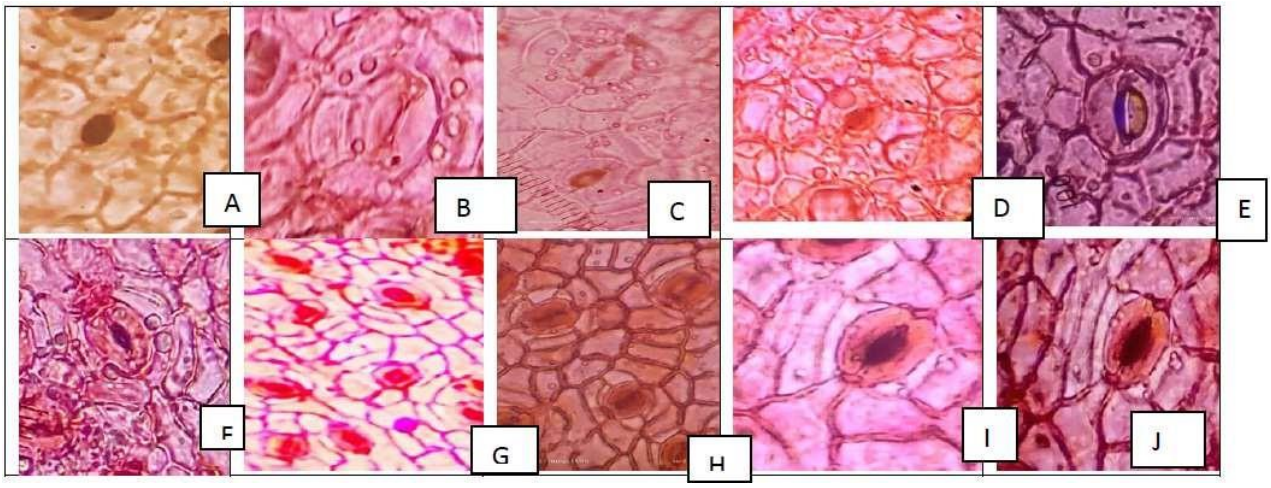
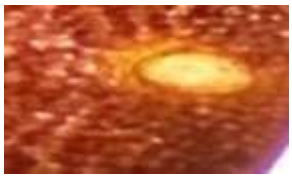
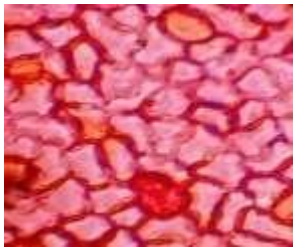
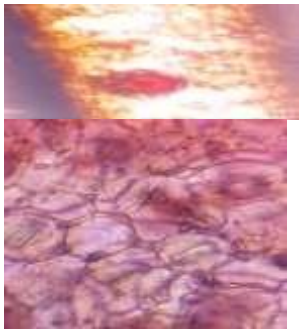
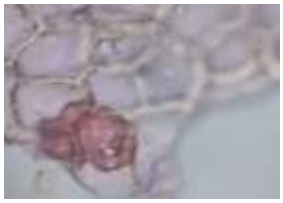


Fig. 5: Stomatal diversity of five Citrus species. A- Actinocytic stomata, B- Cyclocytic Stomata, C- Laterocytic Stomata, D- Stephanocytic Stomata, E- Amphilaterocytic Stomata, F- Actinocytic Stomata, G- Actinocytic Stomata, H- Amphilaterocytic Stomata, I- Incomplete Stephanocytic Stomata, J- Amphianisocytic Stomata

Table 4: Epidermal characters of five *Citrus* species

Cultivar	Epidermal cell length	Epidermal cell breadth	Inclusions and secretory structure	Sheath cells	Contiguous stomata	Megastomata
Citrus aurantifolia Key lime	14.75±1.26	6.26±1.0		+	+	Present
Citrus sinensis, Malta	16.65±1.23	7.5±1.22		_	+	Present
Citrus maxima, pomelo	13.75±1.41	8.22±1.3		_	+	Absent
Citrus limonia, Aroma King Lemon	14.35±1.11	7.8±1.5		+	_	Present
Citrus limetta Risso, sweet lime	20±2.5	6.25±1.23		+	+	Present

The epidermal anatomical characteristics of the five *Citrus* cultivars exhibited notable interspecific variation, particularly in epidermal cell size, presence of sheath cells, contiguous stomata, and megastomata traits that aid in taxonomic identification and ecological adaptation [17] (Table 4). *C. limetta* displayed the widest range in epidermal cell length (12.5–27.5 µm), suggesting high phenotypic plasticity, while *C. limonia* had the highest number of upper epidermal cells. indicating dense epidermal layering potentially linked to stress tolerance [20]. Sheath cells were present in all cultivars except *C. sinensis* and *C. maxima*, a feature considered important for structural support and vascular protection [12]. Contiguous stomata were observed

in most cultivars, though absent in *C. limonia*, implying species-specific stomatal development patterns [21]. The presence of megastomata in all cultivars except *C. maxima* underscores its taxonomic distinctiveness and possibly a different ecological strategy regarding gas exchange and water regulation [19]

Anatomical Study

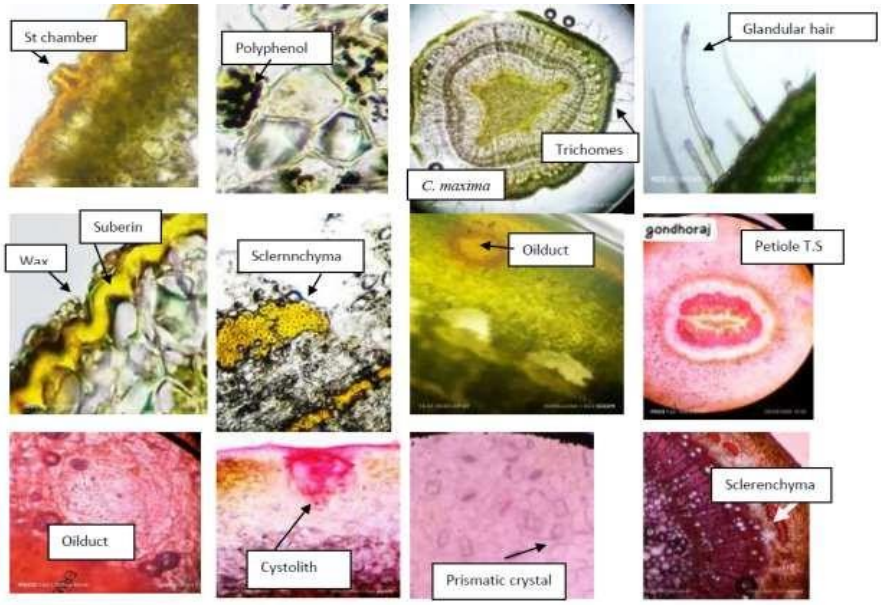


Fig. 6: A.T.S of leaf and stem of five *Citrus* species.B.T.S. of stem of *C. sinensis*

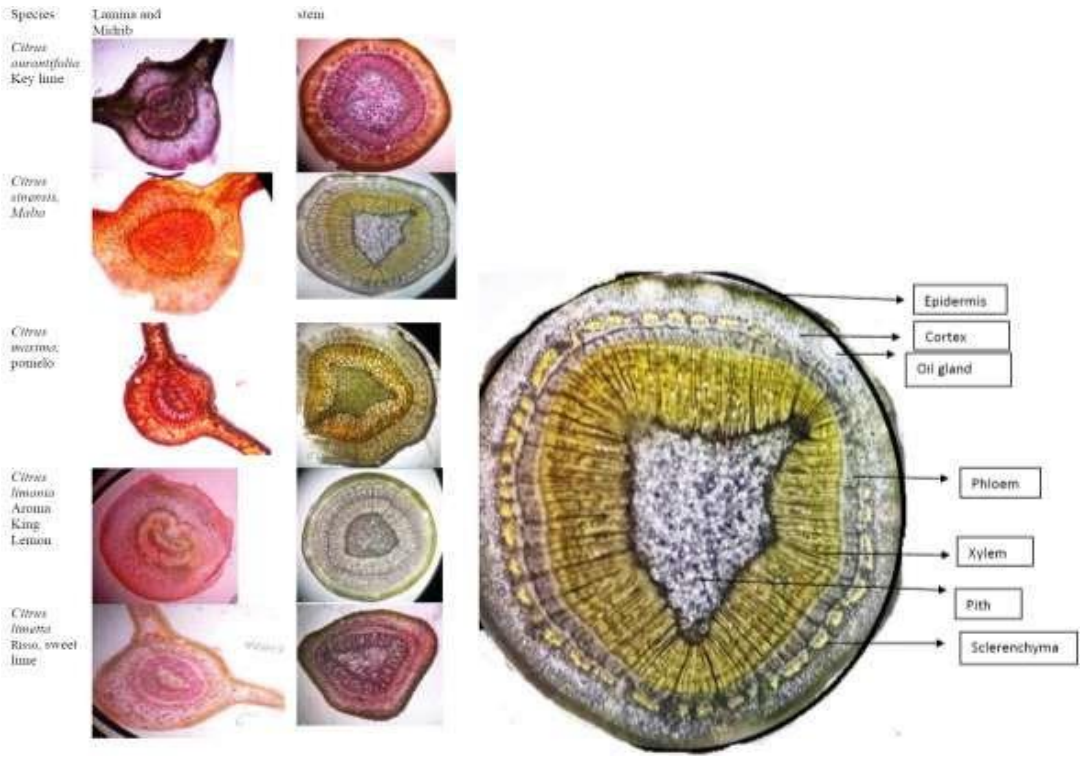


Fig. 7: Anatomy of leaf and stem of *Citrus* species showing diverse characters

Microscopy of Leaf Transverse Section (T.S.)

Leaf Shape:

The leaves were dorsiventral with a prominent midrib. They were foliate to elliptic in shape with an acuminate apex. In transverse view, the midrib was prominently elevated and round or big arc-shaped, 2 lobed (Fig.7).

Epidermis:

The upper epidermal cells were squarish, thick-walled, and covered with a smooth cuticle. In surface view, the cells appeared polygonal with thick, straight walls. The lower epidermal cells were thick walled and hypostomatic

Mesophyll:

The mesophyll was differentiated into two zones:

- The adaxial zone comprises three layers of short palisade cells.
- The abaxial zone consisted of compact layers of spongy parenchyma with large intercellular air chambers. Vascular strands of lateral veins were embedded within the mesophyll.

Wide, circular secretory cavities were present, each surrounded by thick-walled, spindle-shaped epithelial cells. These cavities contained amorphous inclusions.

Prismatic calcium oxalate crystals were abundantly distributed in a characteristic pattern. These were located in the subepidermal layers of the adaxial surface. The cells containing these crystals were wide, circular, filled with mucilage, and termed idioblasts**.**

Vascular System:

The vascular system was large and double-stranded. It consisted of multiple short, compact, and parallel rows of xylem composed of both vessels and fibers. The vessels were angular to circular in outline, thick-walled. Phloem was present as a thick band beneath the abaxial bundle and located outside the xylem of the abaxial strand.

Ground Tissue:

The ground tissue comprised large, thin-walled, compact parenchyma cells (Fig. 7).

Microscopy of Stem Transverse Section (T.S.)

The investigation revealed notable similarities in the anatomical features of *Citrus* species (Fig. 7), including non-occluded vessels, radial multiple pore arrangement, presence of multiseriate and uniseriate rays, and ray widths exceeding half the pore diameter. These shared features suggest a close affinity among the species and support their classification under a single genus. Traumatic intercellular canals were observed in all species This aligns with previous reports identifying *C. medica, C. grandis,* and *C. reticulata* as ancestral and true *Citrus* species [26]. All species, except *C. aurantifolia*, exhibited confluent paratracheal parenchyma, while *C.*

aurantifolia showed vasicentric aliform parenchyma. This suggests a divergent evolutionary path for *C. aurantifolia* and a moderate affinity with the rest. The uniform pore shapes in all species except *C. aurantifolia* further support this. These anatomical distinctions emphasize the taxonomic value of wood anatomy, which has been instrumental in clarifying the phylogenetic relationships within plant groups and in resolving the position of taxa with uncertain affinities.

Histochemical Localization of Phytochemicals in five *Citrus* sp

Phytochemical screening of the leaf, stem, and petiole tissues of Citrus revealed the presence of various bioactive compounds, including alkaloids, starch, tannins, reducing sugars, proteins, flavonoids, amino acids, and lipid. Notably, the concentration and diversity of these phytochemicals were found to be higher in the xylem compared to other tissues. These compounds are known for their significant antimicrobial properties and have been reported to exhibit curative potential against a range of human pathogens, including *Escherichia coli, Pseudomonas aeruginosa, Klebsiella pneumoniae,* and *Staphylococcus aureus*. The findings suggest that *Citrus* tissues, particularly the xylem, may serve as a valuable source of natural compounds for the development of treatments against various microbial infections [27].

Table 5: Histochemical Localization of Phytochemicals in five *Citrus* sp

Reagents	Colour	Metabolite	*C.aurantifolia*	*C.sinensis,*	*C.maxima*	*C. limonia*	*C. limetta*
Wagner's	Red brown	Alkaloid	Epidermis, cortex, xylem	Epidermis, Xylem, Sclerenchyma, pith	Xylem, Sclerenchyma, epidermis, pith	Epidermis, cortex, xylem	Epidermis, Xylem, Sclerenchyma, pith
Potassium iodide (KI).	Blue-Black	Starch	Chloroplasts, amyloplasts in pith cells, trichome, ray cells and sclerenchyma	Chloroplasts,amyloplasts in pith cells,trichome, ray cells and sclerenchyma	Chloroplasts,amyloplastsin pith cells,trichome, ray cells and sclerenchyma	Chloroplasts,amyloplasts in pith cells,trichome, ray cells and sclerenchyma	Chloroplasts,amyloplastsin pith cells,trichome, ray cells and sclerenchyma
Lead Acetate	Yellow	Tanin	Epidermis, sclerenchyma, medullary ray, pith cells	medullary ray, pith cells	Epidermis, sclerenchyma, medullary ray, pith cells	medullary ray, pith cells	Epidermis, sclerenchyma, medullary ray, pith cells
Benedict's	Brick red	Reducing sugar	Epidermis, Bark	Epidermis, Bark	Epidermis, Bark	Epidermis, Bark	Epidermis, Bark
Lugol 's 2 %	Dark Brown	Protein	Primary xylem, storage tissues	medullary ray, Primary	Hypodermis, medullary ray,	Primary xylem	Hypodermis, medullary ray,

				xylem	Primary xylem		Primary xylem
10% sodium hydroxide (NaOH)	Yellow	Flavo noid	Hypodermis, periderm, primary xylem	primary xylem	Hypodermis, periderm, primary xylem	primary xylem	Hypodermis, periderm, primary xylem
Ninhydrin	purple derivat ives, which are called Ruhem ann's purple	Amino Acids	Primary xylem, storage tissues	Hypodermis, medullary ray, Primary xylem	Bark, pith , primary xylem	Hypodermis, medullary ray, Primary xylem	Primary xylem, storage tissues
Sudan III	red,	Lipid	Cuticle, Oil glands, mesophyll,	Xylem, Sclerenchyma ,epidermis, pith, Cuticle	Oil glands, mesophyll Xylem, Sclerenchyma,	Xylem, Cuticle Sclerenchyma ,epidermis, pith	Oil glands, mesophyll, Cuticle

The histochemical analysis of five *Citrus* cultivars revealed the localization of key primary and secondary metabolites, which supports their taxonomic differentiation and highlights their pharmacological potential [28]. Alkaloids were widely distributed across tissues, with *C. sinensis* and *C. limetta* showing the most extensive presence—spanning epidermis, xylem, sclerenchyma, and pith—suggesting their potential medicinal value [29]. Starch was consistently detected in chloroplasts, amyloplasts, trichomes, ray cells, and sclerenchyma across all species, confirming a conserved pattern of carbohydrate storage [29]. Flavonoids and proteins exhibited species-specific localization, with flavonoids often concentrated in hypodermis and xylem, while proteins were mostly confined to the medullary ray and primary xylem, indicating tissue specialization for defense and metabolic activity [30]. Lipids were predominantly found in oil glands, mesophyll, and cuticle layers—especially in *C. aurantifolia* and *C. limetta*—which are characteristic features of aromatic and essential oil-producing species in the Rutaceae family [31].

4. CONCLUSION

The study of five *Citrus* cultivars—*C. aurantifolia*, *C. sinensis*, *C. maxima*, *C. limonia*, and *C. limetta*—showed clear differences in their stomata, outer cell structures, and chemical contents. Each species had unique features: *C. maxima* lacked both sheath cells and megastomata, *C. sinensis* had the most stomata and showed alkaloids in many tissues, and *C. limonia* did not have contiguous

stomata. *C. aurantifolia* had the fewest stomata and a special pattern of lipid distribution, while *C. limetta* had average stomatal traits, megastomata, and specific protein placement. These differences help explain how each plant adapts to its environment and can be used to tell the species apart. Combining anatomical and chemical traits help us better understand the relationships among *Citrus* species and their potential medical uses by making it easier to identify, classify, and study their useful compounds and healing properties.

ETHICS APPROVAL AND CONSENT TO PARTICIPATE

Not applicable.

HUMAN AND ANIMAL RIGHTS

No animals or humans were used for the studies that are based on this research.

CONSENT FOR PUBLICATION

Not applicable.

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CONFLICT OF INTEREST

The authors have no conflict of interest

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