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Article

Elucidating the Anatomical Features, Adaptive and Ecological Significance of *Kopsia fruticosa* Roxb. (Apocynaceae)

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Abstract: Anatomical characteristics play a pivotal role in understanding the evolutionary relationship among various plant taxa and identifying species of pharmacological importance. Though the anatomical features of the family Apocynaceae have been widely recognized, there is limited research on the stem wood anatomy of *Kopsia fruticosa*, whereas nothing was previously known about its root wood anatomy. The present work describes and analyses its anatomy and correlates the anatomical features with the habitat and ecology of this plant. The oval shape of the young stem and the presence of unicellular trichomes, stone cells in the pith region, laticiferous canals, calcium oxalate crystals, and vascular bundles of two different sizes, viz., smaller in the broad, flattened region and more prominent on the two narrow sides, are remarkable features of the plant, which collectively may often be helpful in distinguishing *K. fruticosa* from other species of this genus. Apart from the previously known qualitative characteristics of the family Apocynaceae, the coalescence of pit aperture and storied pattern of vessels of *K. fruticosa* are newly observed features of the subfamily Rauvofiòideae. On the other hand, in the root wood, vessels are wider (33–64 µm), less frequent (about 53% more in the stem), and shorter, and the rays are larger (21–46 cells in height) and more frequent than those of stem wood; these are the valuable findings which strongly support the non-climbing nature of the studied plant. The vulnerability and mesomorphy indices for stem wood are 0.914 and 349, respectively, indicating plants' adaptation toward a mesic habitat. The correlation of the anatomical traits of plants with the habitat and ecology represents their survivability in different situations. Consequently, anatomical features such as intraxylary phloem, vessel grouping, the storied pattern of vessels, the simple perforation plate, and intervascular vestured pits suggest that plants can tolerate drought. We firmly believe that the present study's outcome can fulfil the research gaps of this hardy plant.

Keywords: *Kopsia fruticosa*; wood anatomy; ecological significance; vulnerability index; mesomorphy index

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1. Introduction

Biodiversity refers to the range and diversity of life, including all the plants, animals, microorganisms, habitats, and ecosystem functions on planet Earth. Angiosperms (flowering plants) are the most prominent clades of existing seed plants [1]. They have tremendous evolutionary plasticity and evolved to inhabit a wide range of ecological niches and have a variety of growth forms, from minute aquatic plants, herbs, shrubs, and climbers to gigantic trees. This clade is a highly diversified and abundant group of embryophytes, with around

260,000 extant species classified into 416 families [2]. They occupy every habitat on earth except the highest mountain tops.

Plants develop anatomical features and adaptive mechanisms, enabling them to function and thrive in various environmental circumstances. The anatomical characteristics play a significant role in identifying species of pharmacological importance since the commercialized material is scratched and challenging to locate. Moreover, these characteristics can provide essential clues to understanding the various mechanisms and adaptations in plants of different growth forms. In addition, the plant roots' structure, function, and interactions with soil are fascinating breakthroughs that will reveal a great deal about our natural ecosystems and global water, mineral, and carbon cycles and contribute to the long-term security of food supplies [3]. Furthermore, the roots of several perennial plants can pose additional hurdles to biologists owing to their size, depth, and complexity in manipulations. Consequently, it is crucial to study the roots' anatomical structure to understand plants' physiological processes. However, most of our current knowledge of roots is based on research from annual, short-lived plants, primarily focusing on the roots of young plants. Therefore, scientific work on the perennial root is unquestionably at the cutting edge of root biology and function. It will surely be a promising area of research in the future.

Kopsia fruticosa Roxb. is an important species of the Apocynaceae (frequently referred to Dogbane or Oleander family) with an enormous evergreen shrub and spreading growth habit. It is classified as a member of the subtribe Kopsiinae (Vinceae tribe) of the Rauvofiideae subfamily in the most recent Apocynaceae taxonomy [4]. This species is widely distributed in tropical and sub-tropical regions of the world, native to Myanmar, and has been domesticated in India, Malaysia, Indonesia, Thailand, and the Philippines [5]. Some genera of this family are essential timber, while many are grown as ornamentals; non-medicinal uses include food, fodder, wood, poisons, dye, and perfume. The production of milky sap by nearly all species is one of the Apocynaceae family's most notable distinguishing traits. In traditional medicine, they are frequently used to cure malaria, fever, diabetes, gastrointestinal illness, and pain in the Asia-Pacific region [6] and were used as a source of arrow poison, while in cases of tertiary syphilis, ulcerated nostrils are poulticed with the root and other *Kopsia* species [7]. Most of the indigenous species of this plant have indole alkaloids that exhibit a variety of pharmacological actions. Several alkaloids, viz., kopsine, fruticosine, fruticosamine, venacarpine A and B, kopsorinine, and kopsifolines A–F, have been identified from the leaves of *K. fruticosa* [8,9]; among them, kopsine was the first isolated alkaloid. However, kopsorinine was extracted from the stem bark, and eleven additional identified alkaloids were found. Leaf and stem bark extracts were subjected to further isolation work, resulting in novel alkaloids of kopsofinone, kopsimalines A–E, kopsinicine, and kopsiloscines H–J [10].

Several researchers have provided the general anatomical features of the family Apocynaceae [11–13] and the wood anatomy of the subfamily Rauvofiòideae [14,15]; however, the literature lacks detailed information on the wood anatomy of *K. fruticosa*. Moreover, nothing has been reported pertaining to its root wood anatomy. Consequently, the present study aimed to investigate the following: (i) the novel anatomical features of the subfamily Rauvofiòideae and (ii) the clear stem and root anatomy of *K. fruticosa* to understand its ecological adaptation in the xeric habitat.

2. Materials and Methods

Plant materials were collected for the present investigation from the district Prayagraj, Uttar Pradesh, India (25.28° N latitude, 81.54° E longitude, and an altitude of 103 m), and Almora, Uttarakhand, India (29.30° N latitude and 79.2° E longitude and an altitude of 1668 m). In order to eliminate air bubbles from the xylem of the samples for better preservation, they were taken to the lab and evacuated using a vacuum pump. Following a 24 h fixing period, materials were placed in formaldehyde alcohol acetic acid for additional processing and preservation. The sections were cut from the sub-apical toward the lower portion at distinct levels. Three types of sections were cut to study the woods, viz., transverse sections (TS), radial longitudinal sections (RLS), and tangential longitudinal sections (TLS). Nevertheless, the TS of the stem were cut at different levels, from the sub-apical to the lower portion. Hand sections as well as microtome sections were prepared. Subsequently, the samples were dehydrated for microtomy using the series of tertiary butyl alcohol (TBA). The dry materials were steadily unfiltered through paraffin wax (58 °C) for three days and immersed in paraffin wax. The sections were stained in safranin (2.0% in 50% ethanol) for 5 min, then rinsed with distilled water, dehydrated twice in a series of alcohols for 3 min each, in 50%, 70%, 95%, and 100%, and were stained with fast green (1.0% in absolute ethanol) for 2 min then entered into xylene and absolute alcohol solution series (25:75, 50:50, and 75:25) and finally in xylene. Then, sections were mounted in Canada balsam. Individual elements of the xylem were isolated by macerating suitable pieces of root and stem wood in Jeffrey's fluid containing equal quantities of 10% nitric acid and 10% chromic acid [16]. All the chemicals and dyes used in the present work were from Sigma-Aldrich®, Saint Louis, MO, USA, Chemicals Private Limited. Macerated slices were thoroughly washed with water and stained with 1.0% aqueous safranin to quantify the dimensional features, viz., vessel element length and xylem fibres. However, tangential dimensions of the vessel lumen were measured on transverse sections. An Olympus binocular compound microscope (Model: CH2i) and a Leica binocular compound microscope (Model: DM2500) were used for examining the anatomical sections and for photography, respectively.

The numerical values given in the descriptions are the mean of thirty measurements. Maximum and minimum values are the single highest or lowest measurements recorded within a species. Eventually, ocular micrometre measurements were converted into µm (Table 1).

Table 1. Conversion of ocular lens measurements.

Lens Objective	
(1 Ocular Unit)	**(µm)**
4×	25
10×	10
40×	2.5
100×	1

The cell counts, measures, and anatomical descriptions follow the recommendations of the IAWA [17]. According to IAWA Committee, fibre length and vessel size (diameter) have been classified into three distinct groups (Table 2).

Table 2. Grouping of fibre lengths and vessel size.

Fibre Lengths			**Vessel Size**		
Class	**Subclass**	**Lengths (µm)**	**Class**	**Subclass**	**Tangential Diameter (µm)**
Short	Extremely short	Less than 500	Small	Extremely small	Less than 25
	Very short	500–700		Very small	25–50
	Moderately short	700–900		Moderately small	50–100
Medium	Medium	900–1600	Medium	Medium	100–200
Long	Moderately long	1600–2200	Large	Moderately large	200–300
	Very long	2200–3000		Very large	300–400
	Extremely long	Over 3000		Extremely large	More than 400

Following Carlquist [18], vessel diameter was divided by vessel frequency to determine wood vulnerability (v), while mesomorphy (m) was computed by multiplying the vulnerability with the average length of the vessel element. Low v and m values (<1 and 50, respectively) imply xeric adaptation, while their high values (>1 and 800, respectively) point to the transformation towards mesic conditions. The average libriform fibre length is divided by the average vessel element length to determine the F/V ratio. The sequences of three numeric values given in the text represent the minimum, mean, and maximum values, respectively.

3. Results

3.1. Stem

As observed in a transverse section, the *K. fruticosa* young stem appears to be approximately oval. The single-layered epidermis is composed of compactly arranged oval cells and covered by a thin layer of cuticle. Furthermore, trichomes are unicellular and vary in length from 92 to 412 µm. The cortex region is formed from a 2–5-celled collenchymatous hypodermis, which surrounds the 4–7-celled parenchymatous cortex that bears starch grains. The collenchymatous portion of the cortex contains solitary stone cells, while the pericycle region has isolated large patches of fibres. Numerous vascular bundles form a continuous oval ring traversed by narrow rays. In the broad, flattened region, the vascular bundles are smaller, while the two narrow sides' vascular bundles are larger in size. Vascular bundles are conjoint collateral, endarch, and open. A short distance away from the vascular bundles on the inner side adjacent to the pith are strands of intraxylary phloem. Larger vascular bundles of the two narrow sides are opposed by larger patches of the intraxylary phloem, while smaller patches of the intraxylary phloem oppose smaller bundles. The presence of calcium oxalate crystals (in the form of prismatic crystals), and laticiferous canals can be observed in the cortex, pericycle, and pith regions. The pith region is made up of parenchymatous cells without intercellular space and has enormous clusters of stone cells. In the stone cells, prismatic crystals are occasionally observed (Figure 1B–H).

In the nodal as well as inter-nodal portions of the young stem, the shape of the vascular cylinder transforms; a section through the foliar apex is oval and exhibits two unilacunar trace nodes. As the leaves are opposite one another, two leaf gaps are apparent. In the region just below the node, there is a continuous vascular cylinder having two raised notches (at the position of the leaf gaps), which steadily depresses and develops two new notches at a right angle to the first notches at the narrower portion of the stem (Figure 1A–C).

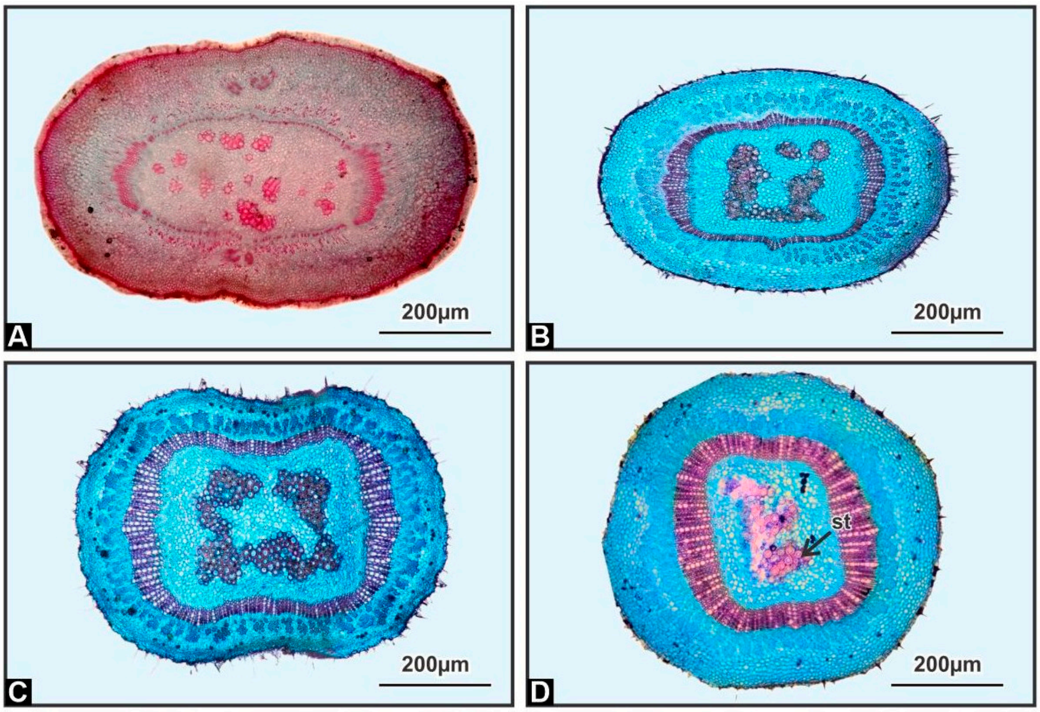

Figure 1. *Cont.*

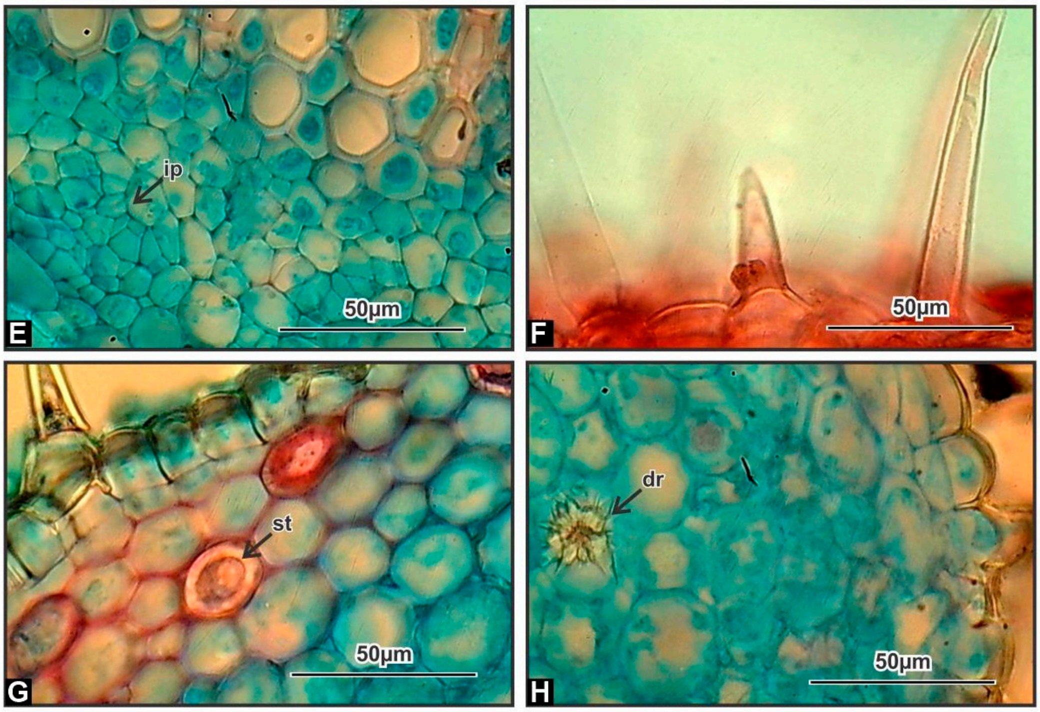

Figure 1. Anatomy of *Kopsia fruticosa* Roxb. stem: (**A**) TS of the stem through the node (foliar apex); (**B**–**D**) TS of the young stem from sub-apical toward the lower portion at distinct levels showing the development of secondary growth; (**E**) magnified portion exhibits intraxylary phloem; (**F**) magnified image exhibits unicellular hairs; (**G**) magnified image exhibits the stone cells in collenchymatous region; (**H**) magnified image exhibits the druses crystal (a type of calcium oxalate crystal) in pith region; ip—intraxylary phloem; dr—druses; st—stone cell.

3.2. Stem Wood

The vessel arrangement is a diffuse porous pattern with a frequency of 44–47–49/mm^2. Individual vessel elements varied from very small to moderately small (31–43–56 µm) in diameter and 202–382–552 µm in length (Table 3). They are angular in the cross-section and are often radial multiples of 2–11. Vessel perforation plates are always simple and present either terminal or sub-terminal in position. Intervascular pits are vestured, alternate, and small to minute, but the arrangement pattern is occasionally coalescent. Vessels are sometimes arranged in a storied pattern (Figure 2A,C–E).

Table 3. Dimensions of wood elements (stem and root) of *Kopsia fructicosa;* values are the mean ± standard error (n = 30).

Wood Elements	Stem Wood	Root Wood
Vessel length (µm)	382 ± 3.95	302 ± 1.48
Vessel diameter (µm)	43 ± 0.30	39 ± 0.27
Vessel Frequency (Number/mm^2)	47 ± 0.06	31 ± 0.05
Fibre tracheid length (µm)	196 ± 2.19	195 ± 2.15
Libriform fibre length (µm)	752 ± 6.44	611 ± 5.81

The values of the vulnerability (v) and mesomorphy (m) indices are 0.914 and 349, respectively. Both septate as well as non-septate libriform fibres are present, which range from extremely short to medium (402–752–1105 µm) in length, and fibre tracheids are 152–196–500 µm in size, while tracheids are totally absent (Table 3, Figure 2G). The ratio of F/V is 1.97. The parenchyma is axial, mostly apotracheal, diffuse, and occasionally scanty paratracheal. Rays are typically uniseriate (5–9 cells tall), although they can sometimes also be compound (5–9 cells in height), and are heterocellular with a higher frequency of upright cells than square cells. The axial parenchyma and ray cells exhibit rhombic crystals (Figure 2B,D–F).

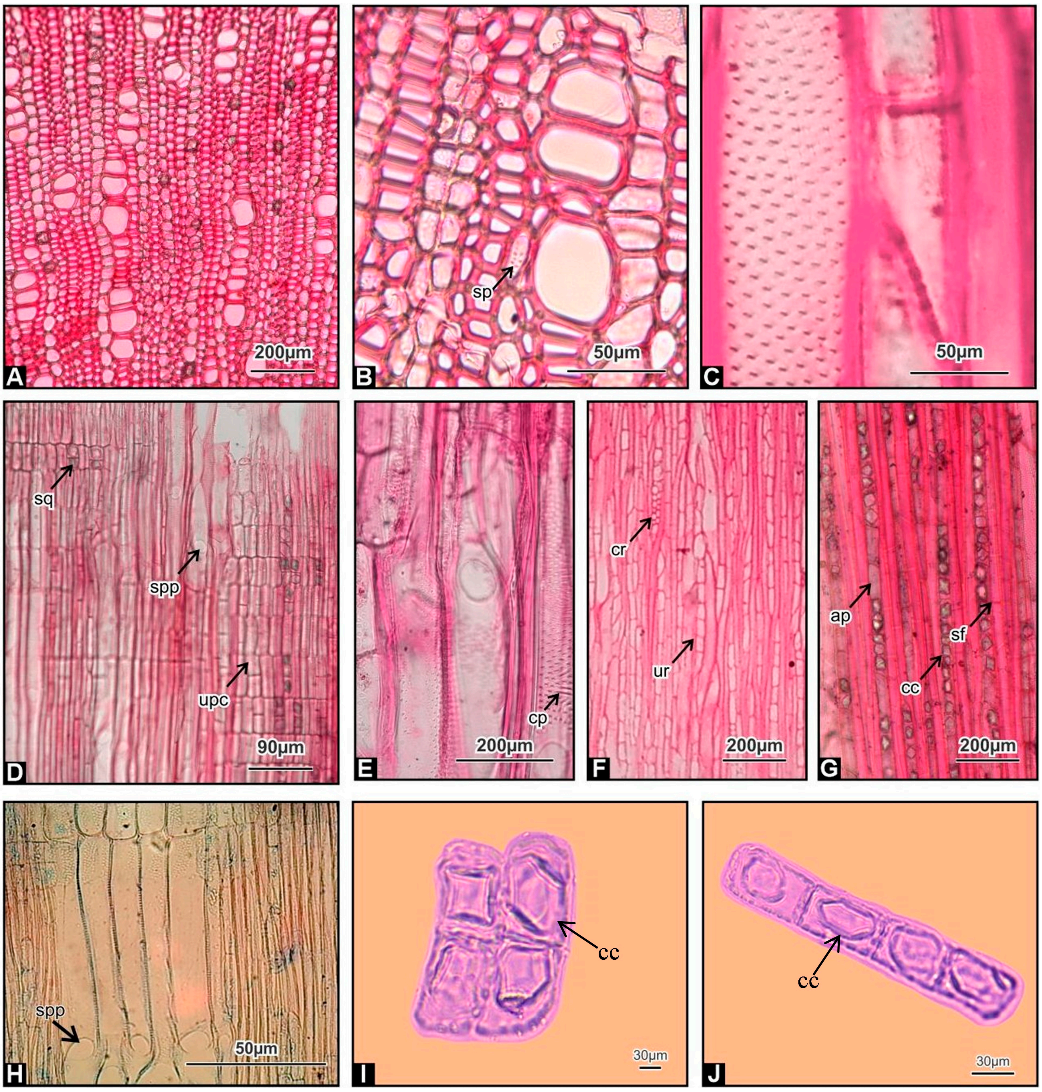

Figure 2. Wood anatomy of *Kopsia fruticosa* Roxb. stem: (**A**) TS revealing a radial arrangement of vessels; (**B**) magnified image revealing scanty paratracheal parenchyma; (**C**) TLS revealing vestured pits (alternate); (**D**) RLS revealing simple perforation plate, upright and square cells; (**E**) TLS image of simple perforation plate and coalescent pit; (**F**) image of uniseriate and compound rays; (**G**) image of rhomboidal shape calcium oxalate crystals in axial parenchyma; (**H**) RLS revealing storied arrangement of vessels; (**I**) square parenchyma cells with chambered calcium oxalate crystals; (**J**) upright parenchyma cells with chambered calcium oxalate crystals; sp—scanty paratracheal parenchyma; spp—simple perforation plate; cc—calcium oxalate crystal; ap—axial parenchyma; upc—upright cell; sq—square cell; sf—septate fibre; cp—coalescent pit; cr—compound rays; ur—uniseriate rays.

3.3. Root Wood

The vessel arrangement is a diffuse porous pattern with a frequency of 29–31–33/mm^2. Individual vessel elements ranged from very small to moderately small (33–39–64 μm) in diameter and 262–302–452 μm in length (Table 3). They are angular in the cross-section and are often radial multiples of 2–5 while occasionally solitary or in the cluster. Vessel perforation plates are always simple and present either terminal or sub-terminal in position. Intervascular pits are vestured, alternate, and small to minute but occasionally the arrangement pattern is coalescent (Figure 3A,C–E,K).

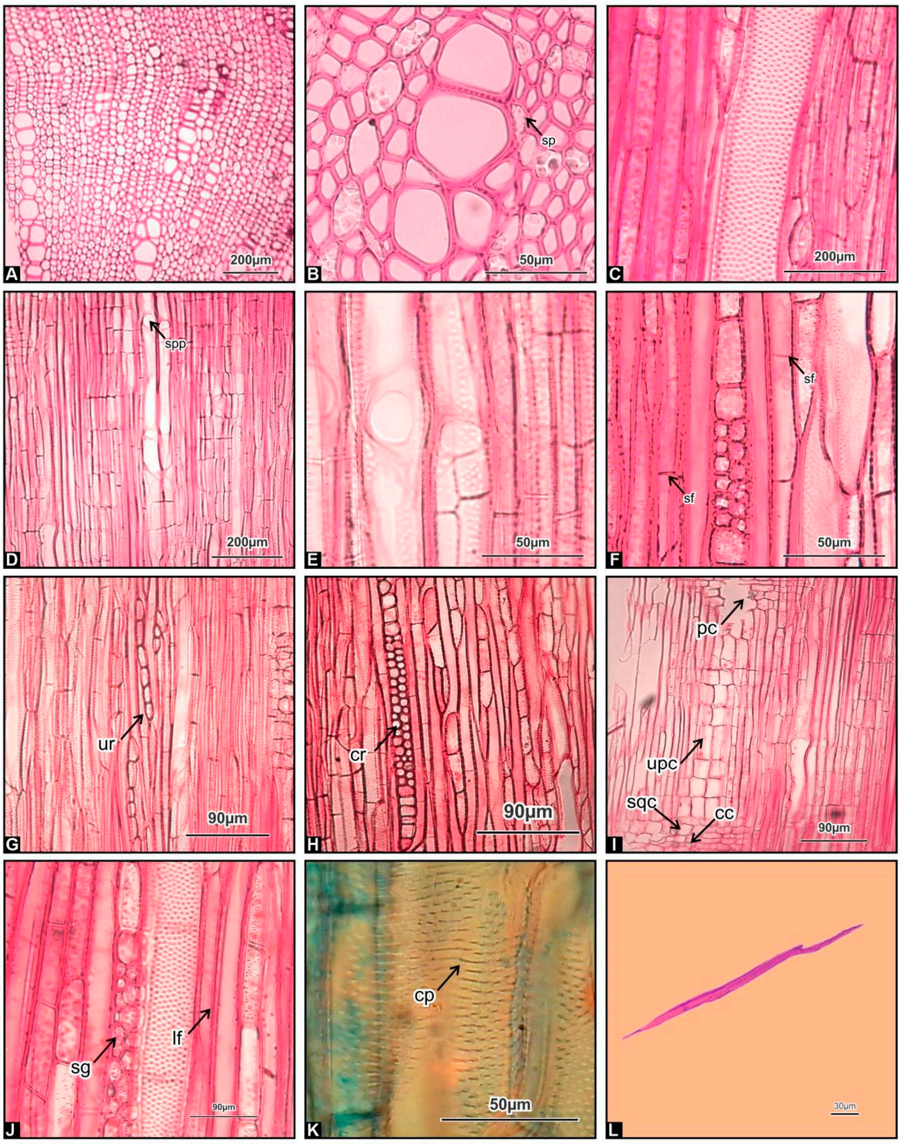

Figure 3. Wood anatomy of *Kopsia fruticosa* Roxb. root: (**A**) TS revealing a radial arrangement of vessels; (**B**) magnified portion showing scanty paratracheal parenchyma; (**C**) image showing alternate vestured pits; (**D**) RLS revealing simple perforation plate; (**E**) magnified portion of simple perforation plate; (**F**) image showing septate fibres; (**G**) TLS image of uniseriate rays; (**H**) section showing compound rays; (**I**) section showing upright, square, and procumbent cells with rhomboidal calcium oxalate crystals; (**J**) section showing rays with starch grains and libriform fibres; (**K**) magnified portion showing coalescent pits in vessels; (**L**) a libriform fibre; sp—scanty paratracheal parenchyma; spp—simple perforation plate; sf—septate fibre; ur—uniseriate rays; cr—compound rays; upc—upright cell; sqc—square cell; pc—procumbent cell; sg—starch grain; lf—libriform fibre; cc—chambered crystal; cp—coalescent pits.

The vulnerability and mesomorphy indices are 1.26 and 380, respectively. Both septate and non-septate libriform fibres are present. They are extremely short to medium in length (302–611–1000 µm). Fibre tracheids are 105–195–410 µm in size. Bifurcate fibres are also present, but tracheids are totally absent (Table 3, Figure 3J,L). The ratio of F/V is 2.02. The parenchyma is axial, predominantly apotracheal, and diffuse; however, sometimes scanty paratracheal parenchyma is also present. Rays are frequently compound (21–46 cells in height) and heterocellular with square, upright, and procumbent cells. Rhomboidal-shaped calcium oxalate crystals and starch grains are visible in the parenchymatous cells (Figure 3B,G–J).

4. Discussion

Plant anatomy is a critical component for species identification as well as being essential for understanding growth processes and nutrient and water mobilization in trees [19]. Therefore, it is crucial to clarify the wood anatomy, including the different cell types that form it and their function, organization, and structural characteristics. The present study evaluates, compares, and explains the anatomy of the wood (stem and root) of *K. fruticosa* from two different altitudes in India. Previously reported qualitative features of the family Apocynaceae, such as stone cells, internal phloem, laticiferous canals, and calcium oxalates (prismatic and druses), have also been noted in the current study [11]. Intraxylary or internal phloem is a significant feature that describes the sieve components that distinguish between the pith and the proto-xylem [20–22]. Internal phloem was first discovered in the family Cucurbitaceae and later in Solanaceae and Apocynaceae. Subsequently, its existence is confirmed in several other species. The secondary xylem has a functional and evolutionary significance closely linked to plant architecture and changing environmental circumstances. Ecological factors can impact all dimensions of xylem structure, either qualitatively or quantitatively. Further, because of the complexity of the dicotyledonous wood system, several characteristics may be used in their identification, i.e., the presence or absence of vessels, distribution of vessels in tissues, types of rays, distribution of axial parenchyma, and types of perforation plate and pits and their arrangements. The presence of distinct intraxylary phloem strands that occur from the starting of primary growth in the young stem of *K. fruticosa* may be associated with an increased translocation of photosynthates and also be an adaptive functionality to protect the sieve elements, which become nonfunctional during an unconducive climate or in hot summers [23]. Furthermore, crystals of calcium oxalate and stone cells are seen in the stem. Stone cells serve as special repositories of plant secretory products and metabolic waste [24], whereas calcium oxalate crystals are recognized for their participation in various essential functions, viz., tissue calcium regulation, metal detoxification, and herbivore defence [25]. In addition, the presence of collenchymatous hypodermis and sclerenchymatous pericycles supply tensile strength to the young stem.

Among the anatomical features seen in this study, radial multiple of vessels, the diffuse porous structure of wood, the simple perforation plate, apotracheal and scanty paratracheal axial parenchyma, and heterocellular rays are the previously identified traits of the stem wood of the family Apocynaceae [11]. However, intervascular pitting of Apocynaceae is reported to be vestured, alternate, and small to minute, while in vessels of *K. fruticosa*, including the above characteristics, coalescence of pit aperture and the storied pattern of vessels are newly observed features of the subfamily Rauvofiòideae. In intervascular pitting, the coalescence of apertures gives an impression of scalariform pitting. Sidiyasa and Baas [26] also reported such pits in the *Alstonia* of the subfamily Apocynoideae.

Like stem wood, root wood exhibits these qualitative features except for the storied pattern of vessels, which occurs only in stem wood. It is important to mention that while the plants were collected from two different altitudes, no significant morphological variations were observed. However, an assessment of the quantitative comparison of stem and root wood confirms significant differences in the length, frequency, and diameter of vessels as well as rays' distribution. The rays are broader and more frequent in the root wood than stem wood, while the vessels are shorter, more comprehensive, and less frequent. The stem wood has a mean vessel frequency of approximately 53% higher than root wood. In stem woods' axial and ray parenchyma, rhomboidal-shaped chambered calcium oxalate crystals are consistently seen but are quite meagre and infrequent in root wood.

As we know, plants' internal structure (young stem and wood) is a key feature of their successful survival in different habitats. Yang et al. [27] correlated the relationship between wood structure and environmental conditions as a survival strategy. Variations in the hydraulic architecture of the xylem, particularly quantitative features such as vessel frequency, vessel grouping, type of perforation plates, vessel element length, and width, are correlated with ecological parameters such as water availability, temperature, and geographical variables (e.g., latitude and altitude). A vessel, also known as the trachea, is built of a series of axially superimposed cells of which intervening end walls have perforations. Vessel diameter is an important factor influencing the conduction of water. It is well established that maximal conductivity and safety from embolism are strongly related to vessel frequency and diameter. A higher frequency of vessels in the stem might be helpful in balancing water transport ability [28,29]. One of the fundamental organizing principles of hydraulic architecture has been noted as the tendency of the roots of trees and shrubs to have wider vessels than the stem [30]. However, the reduced lumen of vessels in the stem aids the mechanical strength of the stem. In the root wood of *K. fruticosa,* vessels are wider than stem wood. It has been reported that due to the presence of wide vessels, the roots of diffuse porous plants are potentially more vulnerable to drought-induced embolism than the stem [31]. In that condition, the presence of starch grains in the parenchyma cells of root wood of *K. fruticosa* is helpful in the removal of embolism, thus increasing the conduction of water in vessels. Simultaneously, it is observed that a storied pattern of vessels is present in the stem wood of *K. fruticosa*. Carlquist [32] mentioned that the arrangement of a storied pattern of vessels indicates the tendency of the plant to survive in desert areas.

In addition, the arrangement of vessels is a characteristic feature of the secondary xylem, which helps in the identification of angiospermous wood. In the stem and root wood of *K. fruticosa,* vessels are arranged radial, multiple, and in a diffuse porous pattern. It was reported that the species in which growth begins early during the growing season are ring porous, whereas species in which growth begins late are diffuse porous [33]. Similar relationships between evergreen and deciduous habits have been discovered. Diffuse porous dicotyledons are generally evergreen, but ring porous dicotyledons are typically deciduous [34].

Perforation plates and inter-vessel pits are particularly crucial for water transport, making up the link across vessel elements inside individual vessels and adjoining vessel walls. Pits in the walls of vessels vary in their shape, size, and arrangement, especially in relation to the type of adjoining cell. Intervascular pits between contiguous vessels are the most conspicuous and taxonomically significant. Vessels in the stem and root wood of *K. fruticosa* possess simple perforation plates and vestured pits. It is supposed that vessel elements with simple perforation plates are more potent in water conduction due to low friction. Vestured pits show protuberances from the secondary cell wall [35]. These are the bordered pits with pit cavities wholly or partially lined with projections from the secondary wall [17]. Vestured pits are hypothesized to serve an essential role in the prevention of cavitations and in improving vessel performance by helping to repair embolisms during drought conditions [36–38]; they are also a sound taxonomic characteristic.

5. Conclusions

This work, for the first time, reported insights into featuring the young stem and wood anatomy of *K. fruticosa.* Apart from the previously known characteristics of the family Apocynaceae, the coalescence of pit aperture in vessels and the storied pattern of *K. fruticosa* are newly observed features of the subfamily Rauvofiòideae. It has a dual therapeutic and poisonous characteristic. It is common to observe that misidentified plants lead to mortality; therefore, specific anatomical features may be helpful in identifying plants. Several anatomical features of the young stem and wood of the *K. fruticosa*, such as the oval shape of the young stem, the presence of unicellular trichomes, the shape of the vascular cylinder in the nodal as well as inter-nodal portions, vascular bundles of two different sizes (smaller in the broad, flattened region and more prominent on the two narrow sides), and the cluster of stone cells in the pith region, may be collectively helpful in distinguishing it from other species of this genus. The correlation of the anatomical features with the habitat and ecology represents the plant's survivability in different conditions. Moreover, it has been documented that low vulnerability index and mesomorphy index values (<1 and 50, respectively) represent an adaptation to xeric conditions, while their high values (>1 and 800, respectively) show transformation to mesic conditions. Accordingly, the presence of v and m values of 0.914 and 349 in the present investigation for stem wood indicate an adaptation of the plant towards mesic conditions. Meanwhile, anatomical features such as vessel grouping, simple perforation plate, intervascular vestured pits, and intraxylary phloem suggest that plants can tolerate drought. Hence, the observed anatomical characteristics provide the framework for research on interspecific and intraspecific variations in woody species, which help us to understand the structural adaptations of the plants with respect to adverse environmental conditions. This framework may also be employed for insight into the plants' physiological processes and for solving taxonomic problems and may be crucial for pharmacology as well as for elucidating phylogenetic relationships.

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