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Anatomical Study of *Shorea robusta* Gaertn.

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Abstract

Shorea robusta commonly known as Sal, is one of the multipurpose timber trees in Nepal. This study aims to carry out detailed anatomical investigation of the wood and leaf anatomical traits of Sal. The wood and leaf samples of Sal were collected from Rupandehi district, Nepal. Wood of Sal was found to be diffuse-porous and the vessels were mostly solitary or paired and sometimes in a short radial multiple. Non-septate, vasicentric fibres forming solid tracts connected the vessels and rays; vasicentric parenchyma with lozenge aliform to aliform confluent arrangement; moderately broad, multiseriate rays and the presence of prismatic crystals in rays were the characteristic feature of the wood. Leaves were hypostomatic and the stomata were cyclocytic with four or more subsidiary cells surrounding the guard cells. Stellate, glandular trichomes were present in upper epidermis, while unicellular, simple, glandular, tufted trichomes were present in the lower epidermis. The increase in global trade has resulted in over-exploitation of forest resources, and hence in the present context, the identification and traceability of wood is highly crucial. The outcomes of this study are supposed to help in the identification of wood of Sal through their anatomical study.

Keywords: Cyclocytic stomata, Diffuse-porous wood, Glandular trichomes, Hypostomatic leaves, Sal

Introduction

Shorea robusta Gaertn. locally known as Sal, is a large, multipurpose, tropical tree in the family Dipterocarpaceae. It forms the dominant vegetation type in lowlands, especially Tarai and Siwaliks of Nepal (Chaudhary, 1998), which Stainton (1972) categorized into Tarai Sal forest and Hill Sal forest. It is large, gregarious, extremely light demanding, deciduous tree but seldom seen leafless (Pearson & Brown, 1932/1981; Troup, 1921/1986). It grows best in the lower slopes as well as valleys where the soil is deep, moist and fertile (Troup, 1921/1986). Sal is used as an excellent timber, medicine, fodder and fuel wood (Kumar & Saikia, 2020). Seeds are rich in starch and are edible (Agarwal et al., 2002).

The bark is gray to dark reddish brown that becomes fissured and flaky (Wu et al., 2007), greyish brown and smooth with few longitudinal cracks, in saplings. Whereas, in older trees, the barks are dark brown, thick and rough with longitudinal furrows (Gamble, 1972; Troup, 1921/1986). The bark is rich in tannins which accounts for more than 9% (Agarwal et al., 2002). Heartwood and sapwood have distinct features (Government of Nepal [GoN], 2012; Rao & Juneja, 1971). Heartwood is brown, hard, coarse-grained, strong and durable. Whereas, sapwood is small, pale colored, usually brownish-white and perishable (Pearson & Brown, 1932/1981; Troup, 1921/1986). Wood is hard ranging from moderately heavy to heavy. And, heartwood, in its earlier stage is pale brown and turns to dark reddish-brown with age; tyloses are common; wood is without any characteristic odour and taste (Pearson & Brown, 1932/1981).

Leaves are simple, glaucous. These are about10-25 cm long and broadly ovate at the base and taper into a long point at the apex. New leaves are reddish and soon become delicate green (Soni et al., 2013). Various parts of the plant such as leaves, resin, and bark are rich sources of flavonoids, saponins, steroids, tannins, phenols, etc. And, hence they are used for medicament for the treatment of various conditions (Singh & Kumar, 2018). Apart from this, Sal leaves are extensively collected and used for making leaf-plates, tapari and also used for various religious purposes (Kumar & Saikia, 2020).

Vigorous increase in the demand of Sal wood for construction, as well as for making furniture has resulted in over-exploitation imposing serious threats to its existing populations. Every year many incidents about the illegal smuggling of its timber have become the headlines in the news and articles. Many of those confiscated wood samples of several species including Sal are brought to KATH. Every year 10-15 wood samples (confiscated as well as non-confiscated) are brought to KATH for identification. Therefore, developing accurate anatomical reference for identification of this species through anatomical study is crucial. It is one of the most significant technical prerequisites for the identification and treatments in the laboratory. Hence, the present work aims to carry out the detailed anatomical investigation of the wood and leaf anatomical traits of Sal.

Materials and Methods

Study area

For anatomical studies, the wood as well as leaf samples were collected from Charpala Community Forest, Rupandehi district, Lumbini province, Central Nepal (27.7022° N, 83.3849° E) during October, 2021 (Figure 1). Collected samples were brought to the National Herbarium and Plant Laboratories, Godawari and anatomical study was carried out (Figure 2A).

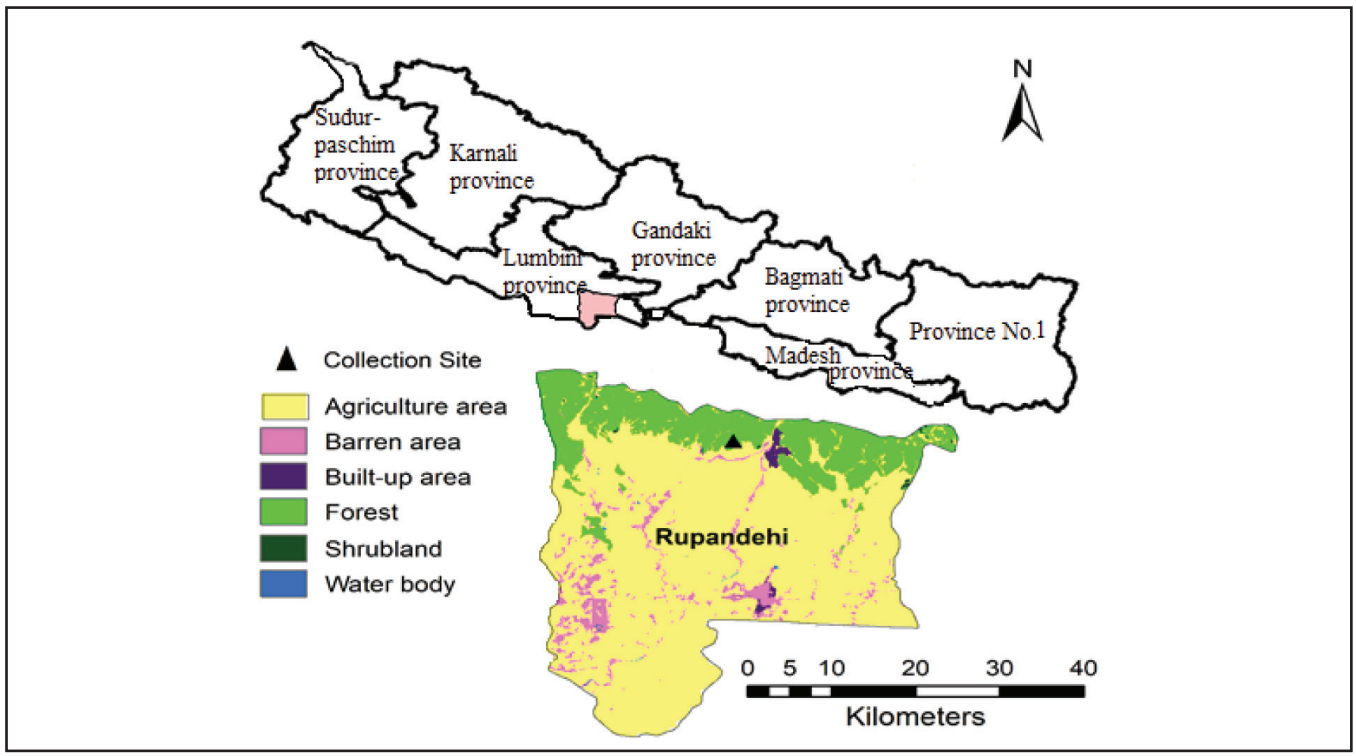

Figure 1: Map of study area showing the collection site in Charpala Community Forest, Rupandehi district

Figure 2: Field study and laboratory study, **A.** Collected wood and leaf samples, **B.** Sectioning with semi-automatic microtome KDEE3390, **C.** Maceration of leaves, **D.** Preparation of temporary and permanent slides

Methods

Wood samples were brought to the KATH Xylarium, kept in normal tap water and placed in hot air oven at 100ºC for softening. Sectioning was done using semi-automatic microtome KDEE 3390 (Figure 2B), and micro-sections of Transverse section (TS), Tangential Longitudinal section (TLS) and Radial Longitudinal section (RLS) of 30 µm were cut. The sections were then dehydrated in alcohol series, stained with safranin and fast green and permanent slides were prepared (Figure 2D). Stomatal study was done by peeling the epidermal layer which was done by boiling small pieces of leaves in Jefferey's solution at 60ºC (Figure 2C). Epidermal peels were washed thoroughly and stained in aqueous safranin. Temporary slides were prepared by mounting in aqueous glycerin and then sealed by DPX mountant. The permanent slides were then studied under compound microscopes at different magnification, and photographs of stem were taken under Olympus CX43 microscope via LC30 camera and that of leaves were taken under Humascope microscope.

Stomatal Index (SI) and Stomatal Frequency (SF) were calculated using the formulas as given below (Rajbhandary, 2015) -

Stomatal Index (SI) = S X 100 / (E+S)

Where, S = Average number of stomata in microscopic field

E = Average number of epidermal cells in microscopic field

Stomatal Frequency (SF) = S/A per mm^2

Where, S = Average number of stomata in microscopic field

A = Area of microscopic field

Results and Discussion

Anatomy of stem

The transverse section of the stem is ribbed due to the presence of fissured bark. The epidermis layer consists of a single row of rectangular cells that is covered with cuticles. In the young stem, some of the epidermal cells give rise to multicellular hairs. Epidermis is followed by collenchymatous hypodermis which consists of three to four layers of cells. Below hypodermis, a multilayered cortex is present that consists of thin walled, parenchymatous cells. Gum canals are common in the cortex. Endodermis is single layered and made up of elongated, barrel shaped cells. The endodermal region is followed by a pericycle that consists of sclerenchymatous cells. The stele consists of conjoint, collateral and open vascular bundles arranged in a ring. The bundles are relatively different in size and number. There are six large bundles located opposite ridges. There is also a large pith in the stem center and consists of polygonal parenchymatous cells which tend to decrease in size towards the periphery. Gum canals are present in pith. However, in matured stem, due to the presence of periderm, the epidermal cells as well as hypodermal region seem to be rudimentary.

Wood structure and composition

Wood is very hard, heavy, close grained and durable. Heartwood is light brown to reddish brown and gradually turns brown with age. Whereas sap wood is pale yellowish to light brown, narrow, distinct from the heartwood. Growth rings are not very distinct or absent. Pores are usually moderately large to medium; visible to the eyes, distinct under the lens; evenly distributed. The mean values of the wood anatomical features are presented in Table 1.

Table 1: Mean values of the wood anatomical features

Vessels			Tracheids			Fibres	Axial Parenchyma		Rays					
									Overall		Procumbent Cells		Upright Cells	
Radial diameter (in µm)	Tangential diameter (in µm)	Length (in µm)	Length(in µm)	Width (in µm)	Wall thickness (in µm)	Length (in µm)	Radial diameter (in µm)	Tangential diameter (in µm)	Length (in µm)	Width (in µm)	Length (in µm)	Height (in µm)	Height (in µm)	Width (in µm)
285	455	280	58	19	2	1250	31	90	920	80	70	20	26	24

Wood is a diffuse porous type. Vessels are few, 5-10 vessels per mm^2, mostly solitary or paired, rarely in short radial multiples of 2-3. Solitary vessel oval to round in outline; 230-400 (285) µm and 345-525 (455) µm in radial and tangential diameters respectively (Figure 3A and 3B). Vessel elements 170-340 (280) µm long; perforation plate simple; intervessel pits alternate, bordered (Figure 3H). But, Pearson & Brown (1932/1981) reported that vessel elements are 160-440 µm long and 230-260 µm wide. However, Chalk (1989) considers the size as well as number of vessels are susceptible to environmental influence. Tyloses are common in heartwood (Figure 3I and 3J).

Tracheids vasicentric; 45-80 (58) µm long, 17-25 (19) µm wide; thick walled, wall thickness 1.5-3 (2) µm; simple to minutely bordered pits (Figure 3B, 3G). Fibres vasicentric, non-septate, 750-1800 µm long, 14-25 µm in diameter; thick walled; forming broad, nearly solid tracts connecting the vessels and rays (Figure 3B and 3D), 50-130 (78) µm wide tracts; simple to minutely bordered pits. Inter-fiber pits are simple and minute. Fibre lumen is usually filled with reddish-brown gummy deposits. Fibres influence both strength as well as shrinkage of wood (Anoop et al., 2019) due to which the wood of *Shorea robusta* is very hard and is very difficult to cut.

Wood parenchyma apotracheal as well as paratracheal; apotracheal parenchyma diffuse; paratracheal parenchyma vasicentric, exist in narrow tangential bands surrounding the pores, lozenge aliform to aliform confluent and thin walled. Individual cells in axial parenchyma 23-45 (31) µm and 50-125 (90) µm in radial and tangential diameters respectively. Prismatic crystals present in axial parenchyma cells (Figure 3C and 3D).

Rays heterogeneous, moderately broad; made up of parenchymatous tissue; multiseriate, usually 3-4 cells wide, rarely uniseriate; large rays 4-10 seriate; 550-1335 (920) µm long and 60-110 (80) µm wide; distinct under the lens; 4-12 per mm (Figure 3E and 3F). Rays comprise of procumbent cells, upright cells and sometimes square marginal cells. Procumbent cells in radial view, 49-95 (70) µm long and 14-29 (20) µm high. Similarly, upright cells in radial view, 21-28 (26) µm high and 18-33 (24) µm wide. Square cells as well as upright cells restricted to marginal rows (Figure 3F).

Anatomy of leaf

Leaves in *Shorea robusta* are dorsoventrally flattened. The epidermal region is uniseriate with rectangular shaped cells, covered with thick cuticles and is followed by mesophyll tissue. Upper epidermis/ adaxial surface (UE) is dark green in color compared to lower epidermis/ abaxial surface (LE). Leaves are hypostomatic due to the presence of stomata on the lower epidermis (Figure 4A-4D). The mesophyll is dorsiventral, heterogeneous and is differentiated into palisade parenchyma and spongy parenchyma. Palisade layer is 2-3 layered and is made up of elongated cells that are vertically arranged and parallel to each other. Spongy parenchyma lies below the palisade parenchyma and is made up of round to oval cells that are loosely arranged. The mean values of the leaf anatomical features are presented in Table 2.

Both the upper epidermal as well as lower epidermal cells have straight anticlinal walls; epidermal cells are polygonal in shape (Figure 4A-4D). Bulliform cells are usually absent in the epidermis. Guard cells typically kidney-shaped and ostiole are located on the same level relative to the epidermal cells.

Table 2: Mean values of the leaf anatomical features

Stomata		Stomatal Pore	Stomatal Frequency	Stomatal Index	Trichomes			
					In Upper Epidermis	In Lower Epidermis	Basal glands	
Length (in µm)	Width (in µm)	Length (in µm)	SF (per mm^2)	SI	Type	Type	Diameter (in µm)	Frequency (per mm^2)
19.71	13.2	9.78	533.81	28.889	stellate, glandular	simple, unicellular, tufted, glandular	26.62	22

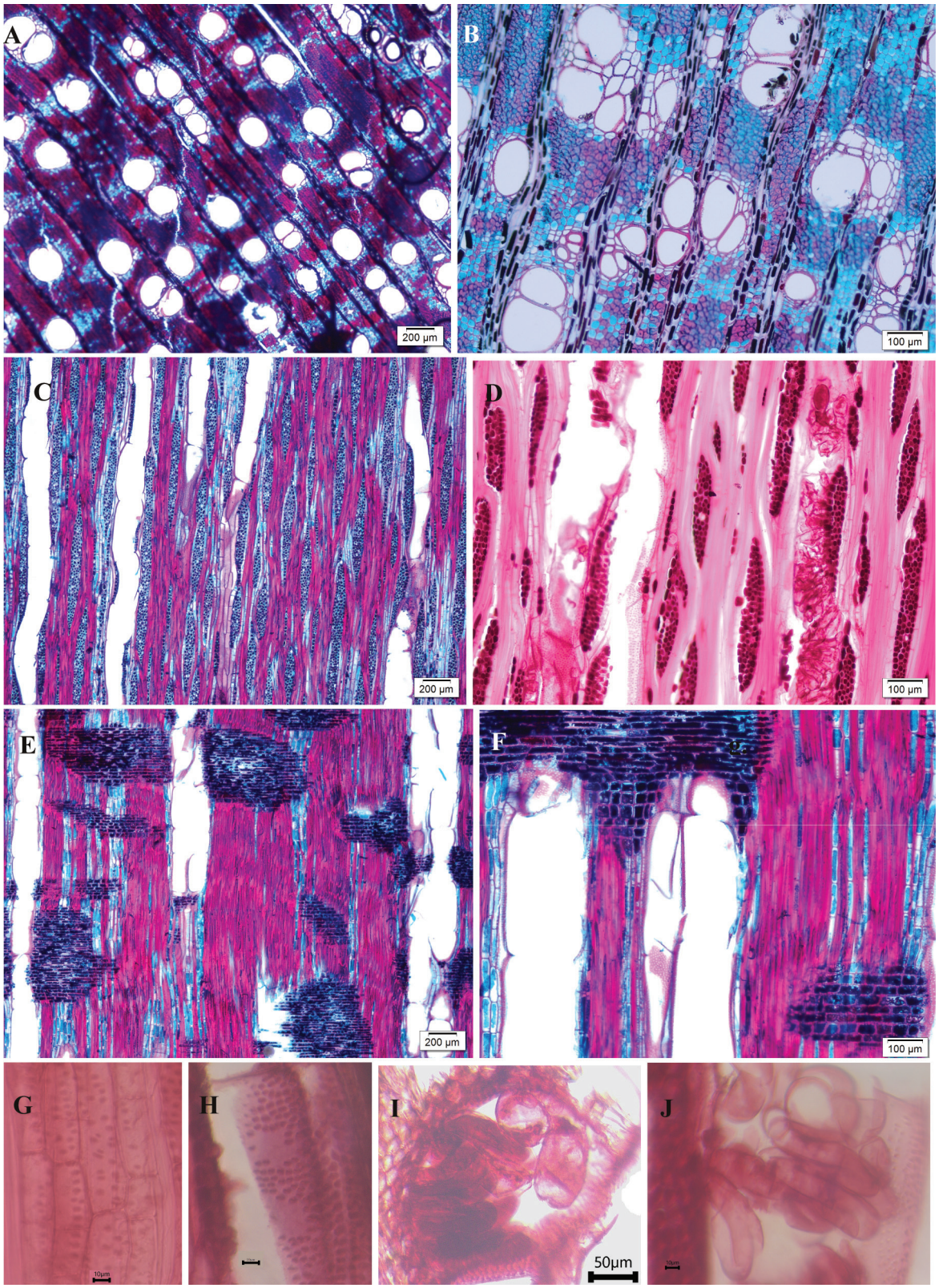

Figure 3: Wood anatomy of *Shorea robusta*, **A & B.** Cross-section of wood (TS) showing solitary vessels as well as paired vessels in **A** and **B**, radial multiple of 3 vessels in **(B)**, **C & D.** TLS of wood showing multiseriate rays with few ray cells having prismatic crystals, **E & F.** RLS of wood showing heterogeneous rays with procumbent cells, upright cells and square marginal cells, **G.** Tracheids in TLS, **H.** Vessels in TLS, **I & J.** Tyloses in vessels, in TS **(B)** and in TLS **(D)**. Magnification: **(A, C, E)** (4x+2x), **(B, D, F)** (10x+2x), **(G, H, J)** (40x+0.5x), **(I)** (10x+0.5x)

Stomatal length and width range from 15-26 (19.71) µm and 9.87-14.41 (13.2) µm respectively (Figure 4C and 4D). Stomatal pore varied from 7.81-11.26 (9.78) µm in length. Stomatal frequency was found to be 533.81 per mm^2 and Stomatal index was found to be 28.889. Stomata are cyclocytic (Figure 4C and 4D) where each stoma is surrounded by four or more subsidiary cells that form a narrow ring around the guard cells (Cotthem, 1970).

Trichomes are present in both upper as well as lower epidermis, in veins and veinlets, more frequent in lower epidermis (Figure 4A and 4B). Stellate, glandular trichomes in upper epidermis (Figure 4E and 4G) whereas simple, glandular, unicellular and tufted trichomes were found in lower epidermis (Figure 4F). Basal glands in 18.28-34.38 (26.62) µm in diameter, 20-26 (22) per mm^2 (Figure 4H). Noraini & Cutler (2009) also reported the presence of simple, unicellular and tufted trichomes in the genus *Parashorea* of family Dipterocarpaceae.

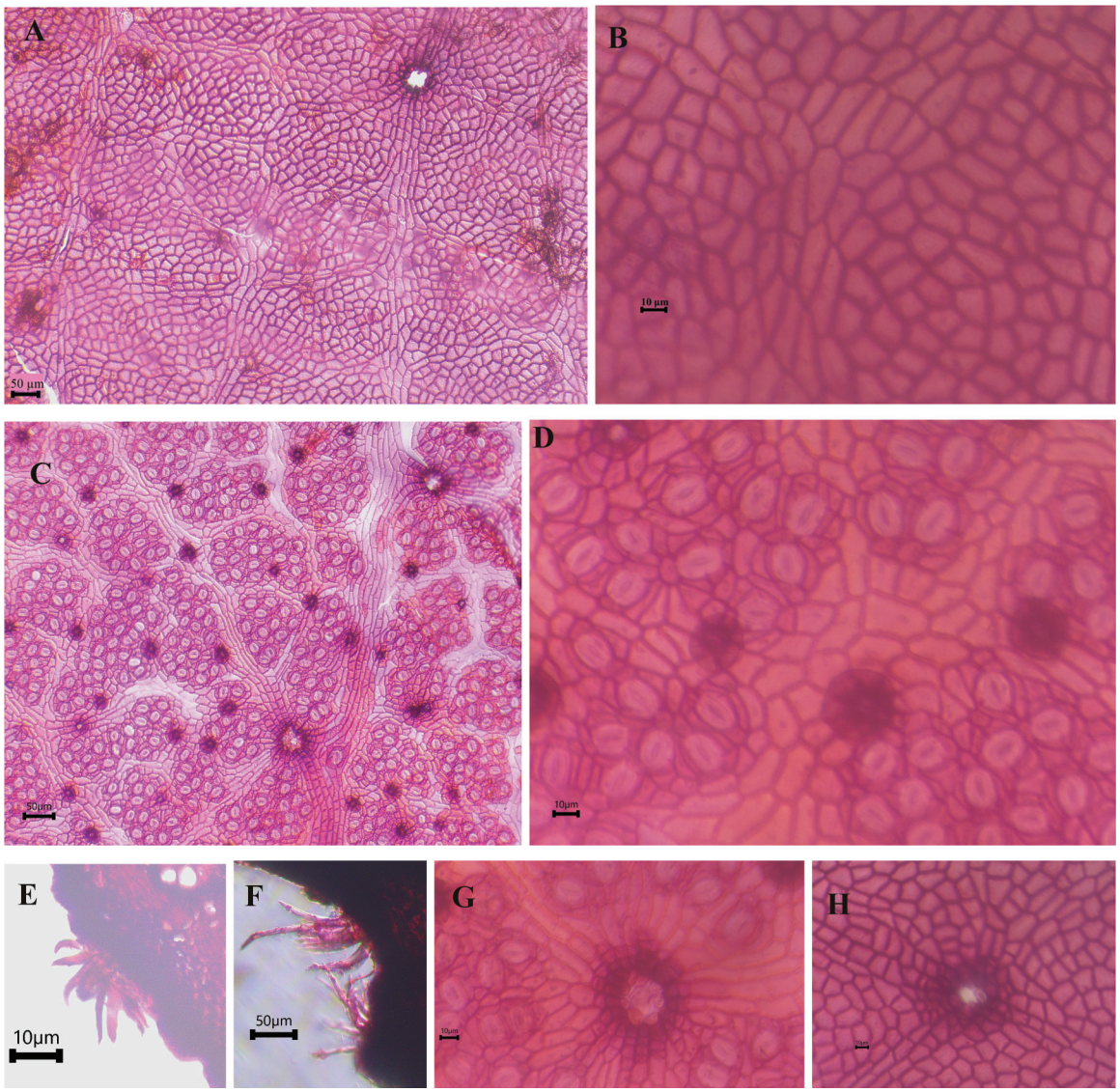

Figure 4: Leaf anatomy of *Shorea robusta*, **A & B.** Upper epidermis showing straight anticlinal walls, **C & D.** Lower epidermis showing stomata and basal glands of trichomes, **E**. Stellate, glandular trichomes in upper epidermis, **F.** Simple, unicellular, glandular, tufted trichomes in lower epidermis, **G.** Basal gland of unicellular, glandular trichomes in lower epidermis, **H.** Basal gland of simple, unicellular, glandular, tufted trichomes in upper epidermis. Magnification: **(A, C, F)** (10x+0.5x), **(B, D, E, G, H)** (40x+0.5x).

Conclusion

Shorea robusta is an important member in Dipterocarpaceae that is characterized by the presence of very hard, durable wood with distinct heartwood and sapwood. Diffuse porous wood; mostly solitary or paired vessels; non-septate fibres forming broad strands connecting vessels and rays; vasicentric parenchyma with aliform and aliform confluent arrangement; broad, multiseriate rays and the presence of prismatic crystals in rays are the characteristic feature of the wood. Similarly, hypostomatic leaves cyclocytic stomata; stellate, glandular trichomes in upper and unicellular, simple, glandular, tufted trichomes in lower epidermis are the characteristic feature of leaves. However, the anatomical features sometimes can be of adaptive value so further studies on the comparative anatomical examination of same plant species existing in different ecological regions would be of great importance.

Author Contributions

All the authors were involved in concept developing and research designing. P. Chalise and Y.R. Paneru carried out field work for sample collection. P. Chalise carried out anatomical examination, photomicrography, prepared and revised the manuscript. Y.R. Paneru accompanied the first author during photomicrography, prepared the map of study area and also revised the manuscript. L. Joshi edited and reviewed the manuscript.

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