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Wood anatomical features of *Juniperus squamata* Buch.-Ham. ex. D. Don from high mountains of Trans-Himalayan Zone of central Nepal

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Wood anatomical characters were investigated for *Juniperus squamata* Buch.-Ham. ex. D. Don from high mountains of Trans-Himalayan Zone of Manang District of Central Nepal. We studied the anatomical features and the inter-relationship between the anatomical parameters of Himalayan Juniper from 30 different wood samples collected at 4600 m above the mean sea level (msl). Wood samples were boiled at 100ºC in oven, and sectioning was done using the KD-3390 Semi-automatic Microtome. The sections were then dehydrated in alcohol stained with 1% Safranin and fast green solutions, and permanent slides were prepared and observed under microscope. *J. squamata* is a softwood species and is characterized by the presence of distinct narrow annual growth rings with gradual to abrupt transition from earlywood to latewood. Both earlywood and latewood tracheids comprising square to polygonal cells, circular bordered pits and few resin cells arranged in loose tangential bands. The rays were found to be exclusively uniseriate and homogenous; most of the ray cells contained prismatic crystals while cupressoid pits were present in the ray cells. The annual-ring-width showed a positive correlation with both the earlywood and latewood width but a negative correlation with tracheids length. In softwood species like Junipers, tracheid length is an important characteristic, not only for wood and fiber quality, but also for the tree's hydraulic architecture. Furthermore, this is also coupled with the acclimatization of the species in harsh climatic condition of the arid trans-Himalayan region. Dwarf individuals with reduced growth ring dimensions and increased tracheid length ensure effective water transportation towards the shoot system. Therefore, this intra-specific variation in wood anatomical features of *J. squamata* is due to variation in micro-habitat types.

Key words: Annual-rings, cupressoid pits, prismatic crystals, resin cells, tracheids.

Juniperus is the second largest genera of the conifers with 69 species and is native to sub-arctic & temperate Eurasia to tropical African Mountains, North America to Guatemala, and Caribbean regions (POWO, 2024). Due to its adaptability and capacity to thrive in harsh environments, the genus exhibits remarkable variation in its morphology. The members of this genus range from low-growing prostrate mats found above the tree-line to towering trees reaching heights of 50–60m at lower elevations near sea level (Florin, 1963). In Nepal, the genus *Juniperus* is represented by five species and three infra-specific taxa, as documented by Rajbhandari *et al*. (2020) and Shrestha *et al*. (2022). Junipers are known for their medicinal properties, and are used for flavoring, perfumery, and for cosmetic purposes (Rana *et al*., 2022).

J. squamata Buch.-Ham. D. Don, commonly called Himalayan Juniper or Nepalese Juniper is an evergreen coniferous shrub or dwarf trees in

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the family Cupressaceae (Shrestha *et al.*, 2022). *J. squamata* is very close to Drooping Juniper, i.e., *J. recurva* in terms of its uniform foliage and purple-black berries but differs in terms of its broader, shorter, glaucous leaves and ridged seeds (Rajbhandari *et al.*, 2020). It is the second most widely distributed alpine juniper in the world and the most widely distributed juniper in south-temperate Asia (Debreczy & Racz, 2011; Rajbhandari *et al.*, 2020). In Nepal, it commonly occurs on open slopes between 3300–4500 m above the msl (Rajbhandari *et al.*, 2020). Sometimes, it is found extending down to 2440 m (Shrestha *et al.*, 2022). The Himalayan juniper has a rich history of medicinal use. Leaves as well as female cones of Nepali Juniper possess medicinal value (Bean, 1973; Rajbhandari *et al.*, 2020; Shrestha *et al.*, 2022). Studies have shown that the leaf extracts of *J. squamata* possess antibacterial properties, against several pathogenic bacteria responsible for human diseases (Sati & Kumar, 2015). In Nepal, Juniper is extensively used for the preparation of incense and so it is locally known as 'Dhupi'. It is one of the heavily exploited but highly neglected species in terms of its research and conservation. As per the IUCN Red List, its status is 'Least Concern' (IUCN, 2023).

Wood is generally classified based on cellular structure as hardwood (in angiosperms) and softwood (in gymnosperms), based on the presence and absence of vessels. Xylem forms the main portion of wood which is complex tissues composed of tracheids, vessels, xylem parenchyma and xylem fibers (Wimmer, 2002). Among those, tracheids and vessels are chief conducting elements. However, in gymnosperms, vessels are usually absent such that xylem in gymnosperms consist of earlywood and latewood tracheids, and those of angiosperms are more complex due to the presence of vessels as conducting tissues. The structure of wood in gymnosperms is relatively simple, consisting of tracheids and rays where the tracheids are mainly responsible for water transportation and mechanical support (Zhang, 2017). The ray cells, also known as xylem or phloem rays, play crucial roles in plant's vascular system, ensuring efficient resource distribution, storage, structural support, and response to stress or damage due to environmental changes (Evert & Eichhorn, 2006). In most of the Junipers, the wood is pycnoxylic with absence of resin canals but presence of resin cells & homogenous rays and absence of spiral thickenings in tracheids (Rajbhandari *et al*., 2020). Wood of *J. squamata* is similar to other Junipers but is characterized by the presence of crystals in rays and resin cells (Rajbhandari *et al.*, 2020). Woods of *Juniperus* have fragrant or cedar-like odor, and their heart woods are dull red, rose-red to purplish, reddish brown or dull brown (Panshin & de Zeeuw, 1980). Wood anatomical features such as earlywood and latewood width, average length and width of tracheids, parenchymal ray types, and height of rays vary within different species of Junipers (Jowary & Sharefy, 2021).

Studies have shown that wood anatomical features are of adaptive significance. Hydraulic safety and efficiency are influenced by the anatomy of xylem (Lachenbruch & Mcculloh, 2014; Schuldt *et al*., 2016). Xylem anatomy is an important driver to determine growth performance of trees, their survival and capacity to fix carbon (Sperry & Love, 2015; Pandey *et al*., 2020). It is affected by both genetic and environmental factors (Downes *et al*., 2009; Downes & Drew, 2008). However, the environmental conditions can also influence these anatomical features within and among species. Some wood traits are closely associated with both the survival as well as mortality rate, reproductive time, (Swenson & Enquist, 2007; Wright *et al*., 2003) and life span (Sterck *et al.*, 2001) of trees as well as the growth rate of stem diameter and canopy (King *et al.*, 2005). Wood traits are also connected with resource competition among species (Baker *et al.*, 2004), community dynamics and ecosystem functions (Chave *et al.*, 2006; Zhang *et al.*, 2011). Variation in wood anatomical features have been analyzed in angiosperms with several ecological factors, such as macroclimatic divisions, moisture availability, habit, and phenology (Baas, 1973; Oever *et al.*, 1981; Baas *et al.*, 1988; Zhang *et al.*, 1992).

At higher elevations, shrubs adapt to harsh conditions (low temperatures, strong winds, and intense UV radiation) by developing smaller leaves, thicker cuticles, fewer sunken stomata, compact growth forms, and denser wood with narrower vessels, which are crucial for their survival in extreme environments with short growing seasons (Körner, 2003). Unlike in case of many Juniperus species, detailed studies on the wood anatomical features of *J. squamata* are lacking (Phillips, 1968; Herbst, 1978; Panshin & deZeeuw, 1980; ter Welle & Adams, 1998; Bauch *et al*., 2004; Adamopoulos & Kosh, 2011; Vasić *et al*., 2014; Lehejček *et al*., 2017; Jowary & Sharefy, 2021). Pandey *et al*. (2020) in central and eastern Himalayas reported elevation driven variation in wood characteristics of *Rhododendron lepidotum* although they did not find any distinct pattern with elevation. The quantitative changes related to age of trees in wood characters in central Nepal was explained for *Pinus roxburghii* (Joshi & Chalise, 2022). In fact, very few studies have been carried out on wood anatomical features in relation to ecological factors and microclimatic conditions in Nepal. Therefore, the present study was carried out to: (i) study the general anatomy of wood, and (ii) to analyze the variation in wood anatomical parameters of *J. squamata* on its uppermost range of distribution in the high altitude Trans-Himalayan Manang Valley of central Nepal, and (iii) highlight whether there are any differences in qualitative and quantitative wood characters within the intraspecific population.

Materials and methods

Study area

The study was conducted within the Neshyang Rural Municipality of Manang District which lies within the Annapurna Conservation Area of central Nepal (see Figure 1). The huge Annapurna massif forms the trans-Himalayan range as a semi-arid zone characterized by less than 400 mm annual precipitation (Miehe *et al*., 2001), which forms a typical mountain ecosystem with the diversity of wild flora and fauna (Mayewski &

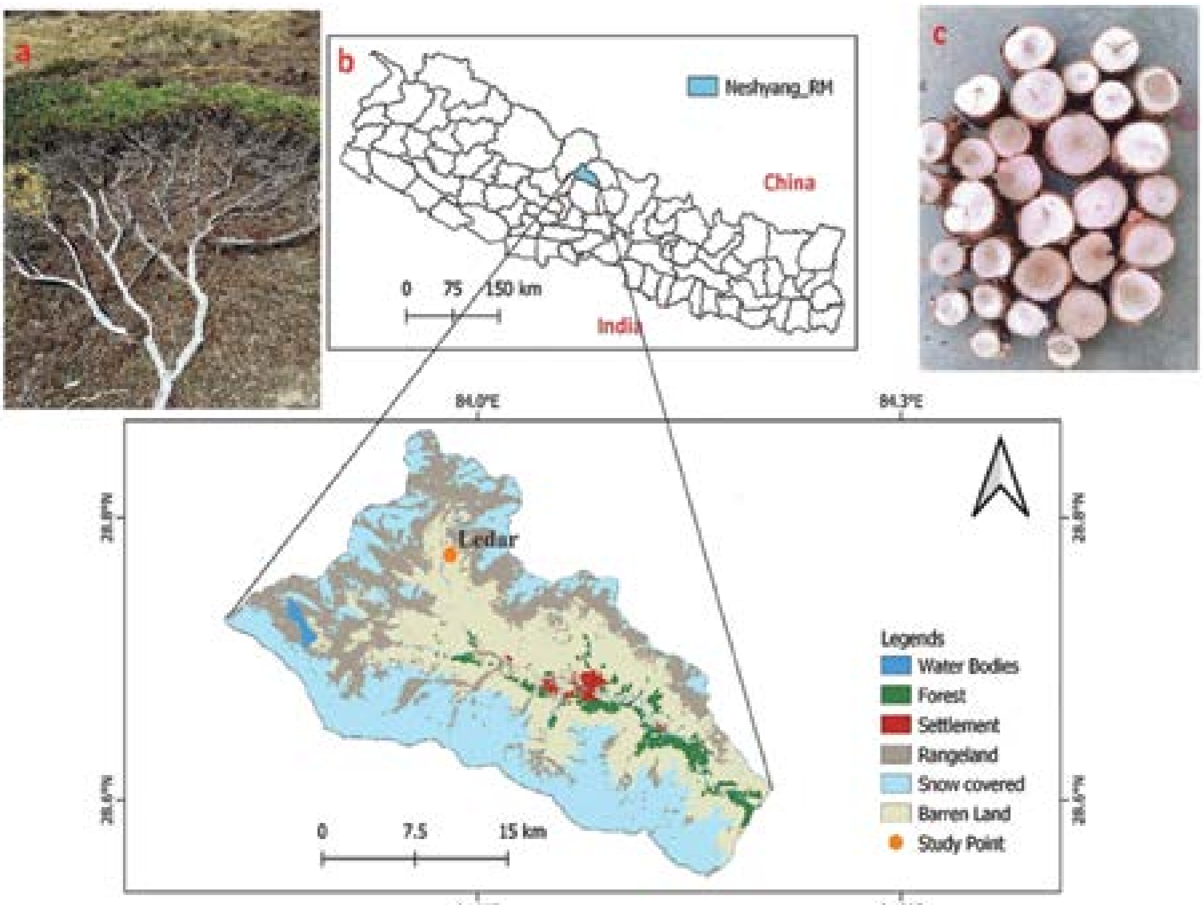

Figure 1: (a) *J. squamata* growing in its natural habitat; (b) location of the study area and the sample collection site in the map of Nepal; and (c) stem discs collected for anatomical study.

Jeschke, 1979). We collected 30 stem discs from 30 individuals of *J. squamata* from the Ledar Area (situated within 28.73^0–28.74^0 N latitudes and 83.97^0–83.98^0 E longitudes at 4600–4690 m altitude) in Manang district, central Nepal during the month of June 2021. The Ledar Area forms the upper part of Manang along the Thorang Khola (stream) in the northwest of Marsyangdi Valley. All the individuals of *J. squamata* were in creeping conditions due to harsh environment at this elevation. For anatomical study, we collected wood samples from one of the largest branches from the main stem of the individual shrubs (see Figure 1c).

Juniperus is the second largest genus of conifers having about 70 species and 30 varieties under Cupressaceae Family. Among them, *J. squamata* usually grows in poor rocky and sandy soil along the trails of Manang Valley. *J. squamata* is a dwarf spreading, prostrate shrub up to 1m tall with flaky brown to red-brown bark, exfoliating in thin strips or plates, with densely arranged straight or recurved branchlets; and needle-like incurved leaves (Bhattarai *et al.*, 2006; Rajbhandari *et al.*, 2020). *J. squamata* is an important medicinal plant in the highlands of Nepal (Ghimire *et al.*, 2008; Miehe *et al*., 2001).

Anatomical study

All the samples were brought to the National Herbarium and Plant Laboratories (KATH), Godawari, Lalitpur for anatomical study. Wood samples were boiled at 100ºC in oven for softening, and sectioning was done using Semi-automatic Microtome KDEE-3390. The sections were then dehydrated in Ethanol series, stained with 1 % Safranin, and permanent slides were prepared. The permanent slides were then observed under Olympus CX43 Microscope under different magnifications of the objective lens, and photomicrography was done using fitted Olympus LC30 Camera. After photomicrography, the measurement of anatomical parameters was accomplished using Image J Software (Schneider *et al*., 2012). The anatomical parameters were tabulated in Microsoft Excel; data analysis was performed using IBM SPSS version 21 (IBM, 2012).

Altogether, 12 anatomical parameters were considered during this study; the parameters included Growth Ring Width (GRW), Earlywood Tracheid Width (EWTW), Earlywood Tracheid Tangential Diameter (EWTD), Earlywood Tracheid Radial Diameter (EWRD), Latewood Tracheid Width (LWTW), Latewood Tracheid Tangential Diameter (LWTD), Latewood Tracheid Radial Diameter (LWRD), Tracheid Length (TL), Uniseriate Ray Height (URH), number of cells in each ray (NURC), Ray Parenchyma Tangential Diameter (RPCTD), and Ray Parenchyma Vertical Diameter (RPCVD). For each of these anatomical parameters, 20 measurements were taken for each of the samples. The mean values of these anatomical parameters were compared between the collected samples. The data were checked for their normality, and since the data were not normal in distribution, the Spearman's Rank Correlation test was performed to study the correlation between the anatomical parameters.

Results

General anatomy of wood

Growth rings

The growth rings of variable widths were visible in the cross-sections of the sampled *J. squamata* wood; the growth rings were narrow with distinct boundaries between the concentric rings. The width of the growth rings of the sampled *J. squamata* wood in our study ranged from 149.16 µm to 636.69 µm (Table 1). Gradual transition was observed from the earlywood to latewood (Figures 2a & 2b). Wood of *J. squamata* is characterized by the presence of tracheids, resin cells, uniseriate ray parenchyma, and narrow growth rings. The wood is soft, vessel-less, and light-brown to yellowish-brown in color. The heartwood is distinct from the sapwood. Two different sizes of rings in terms of width were noticed in our sampled *J. squamata* wood (Figure 2a). The narrower rings (indicated by blue arrow) reflect less rainfall while the wider rings (indicated by black arrow) reflect abundant rainfall in the respective years.

Tracheids

The tracheids of the sampled *J. squamata* wood comprised square to polygonal cells (see Figure 2b). The widths of the earlywood tracheids were found to be between 8.58–35.68 µm and 8.90–38.40 µm in terms of their radial diameter (RD) and tangential diameter (TD), respectively (Table 1). Similarly, the widths of the latewood tracheids were found to be between 3.40–18.11 µm and 3.11–27.87µm, respectively. The tracheid length ranged from 601.79 µm to 1224.59 µm. The bordered pits were circular, ca. 8 µm in diameter, arranged in uniseriate rows and with fine torus (see Figure 2f). Apart from this, clear signs of some extreme weather events (frost ring) were noticed in some of the collected samples (see Figure 2b).

Resin cells

J. squamata wood was characterized by the presence of resin cells. Resin cells usually diffuse, sometimes in loose tangential bands with 1–2 cells. The walls of resin cells are smooth, and crystals are present inside (Figure 2a, 2b). The dimensions of these resin cells are usually 9–25 µm and 9–24 µm in terms of radial and tangential diameters, respectively, mostly square shaped (as noticed in Figure 2b) and rarely polygonal.

Rays

The rays of *J. squamata* wood were exclusively uniseriate and homogenous (as seen in Figure 2e, 2f). The ray height (of the parenchyma cells alone) of our sampled wood ranged from low to medium (46.14 µm to 101.24 µm). Generally, the ray height had of 1–5 cells (occasionally 6–7 cells), but few larger rays with up to 10 cells in height. The rays of the parenchyma cells of the studied samples were found to be 10.87−19.42 µm and 17.32−29.15 µm in terms of TD and vertical diameter (VD), respectively (Table 1). The parenchyma cells of this species were smooth-walled with thick vertical walls. Prismatic crystals were present in the ray cells of our sampled wood (Figure 2c, 2d, 2e, 2f). Besides, cupressoid pits were present in the ray cells, and were more numerous in the marginal ray cells as compared to the other ray cells (see Figure 2f). The mean values of the anatomical parameters of the studied samples are presented in Table 1.

Table 1 : Mean values of the anatomical features in the studied samples of *J. squamata*

Sample code	Growth ring width (µm)	Earlywood tracheid (µm)			Latewood tracheid (µm)			Tracheid length (µm)	Uniseriate ray		Ray parenchyma cells (µm)	
		Width	TD	RD	Width	TD	RD		Height (µm)	No. of cells	TD	VD
MJ1	231.59	291.20	13.50	15.42	65.18	5.49	8.12	1093.69	84.69	4	14.33	20.18
MJ2	262.32	228.80	8.90	8.58	41.73	3.11	3.40	1089.83	76.64	3	17.11	27.19
MJ3	210.11	284.63	23.12	25.04	60.10	10.36	18.11	1038.28	101.24	3	17.46	29.15
MJ4	156.69	342.50	23.09	20.89	65.62	9.46	14.95	789.44	90.41	3	11.82	17.75
MJ5	636.69	342.76	13.32	16.48	33.42	6.05	12.78	804.98	56.22	3	12.88	19.77
MJ6	972.27	815.10	18.97	16.36	94.27	10.27	6.09	745.29	65.11	4	11.41	17.32
MJ7	557.23	362.89	21.30	19.86	82.07	12.23	7.09	714.36	64.63	3	13.23	17.87
MJ8	254.85	218.51	15.70	16.37	55.10	10.64	7.02	885.62	49.14	2	10.87	22.76

Sample code	Growth ring width (µm)	Earlywood tracheid (µm)			Latewood tracheid (µm)			Tracheid length (µm)	Uniseriate ray		Ray parenchyma cells (µm)	
		Width	TD	RD	Width	TD	RD		Height (µm)	No. of cells	TD	VD
MJ9	352.55	426.20	18.26	18.56	73.40	11.34	7.10	601.79	66.08	3	12.12	20.24
MJ10	576.09	276.98	15.99	16.52	55.56	11.31	6.28	844.11	65.37	3	12.89	22.49
MJ11	357.09	215.95	16.09	16.29	45.34	12.13	7.46	881.21	62.69	3	15.84	18.95
MJ12	481.21	292.50	17.17	17.59	39.55	13.42	5.99	965.13	62.36	3	16.69	19.75
MJ13	459.01	487.25	23.46	28.28	56.52	16.92	10.10	834.12	59.99	3	11.71	18.48
MJ14	315.64	276.70	19.28	16.23	49.37	14.79	7.61	971.18	66.20	3	14.03	19.86
MJ15	340.37	349.29	19.21	17.59	37.44	13.53	6.73	758.66	58.72	3	15.54	20.04
MJ16	291.25	251.26	23.25	15.97	31.85	15.04	6.18	903.34	60.50	3	12.77	19.70
MJ17	332.59	255.47	16.80	16.16	39.48	12.35	7.13	882.88	57.05	3	14.89	19.68
MJ18	401.16	329.58	33.84	27.13	42.25	21.85	11.71	1053.35	77.60	4	16.20	23.74
MJ19	628.70	542.78	18.02	16.77	53.21	11.89	6.95	782.49	55.10	3	13.97	17.39
MJ20	523.15	191.14	20.84	15.83	43.00	14.48	7.52	819.41	60.89	3	16.57	19.53
MJ21	532.35	450.13	18.19	21.71	59.88	11.36	7.41	1224.59	60.55	3	19.42	23.20
MJ22	383.87	436.98	38.40	35.68	54.73	27.87	11.75	1104.72	70.28	3	16.06	23.83
MJ23	465.89	299.93	16.29	18.21	71.58	10.15	6.67	798.68	79.58	4	11.85	19.23
MJ24	525.96	427.16	21.84	20.94	63.59	14.68	5.91	735.46	46.14	3	11.63	19.56
MJ25	231.94	297.21	28.41	27.30	47.58	19.36	8.57	802.65	50.18	3	12.19	19.03
MJ26	374.67	310.63	18.18	14.47	50.67	12.65	5.56	845.99	85.69	3	14.88	24.75
MJ27	149.16	74.49	20.53	16.35	17.86	11.64	7.73	618.09	59.13	3	12.38	21.37
MJ28	374.25	300.25	17.56	16.32	50.91	13.66	6.54	978.64	56.76	3	14.45	19.74
MJ29	351.40	265.04	18.41	20.46	33.85	14.11	8.13	913.24	62.41	3	14.44	19.68
MJ30	286.68	220.46	16.89	16.62	39.65	13.88	6.14	943.55	75.28	4	14.20	22.79
Overall mean	400.56	328.79	19.83	18.99	51.82	12.86	8.09	880.82	66.22	3.18	14.13	20.83
SD	200.42	156.43	6.31	5.97	22.10	5.20	3.33	205.39	26.67	1.33	2.82	4.47

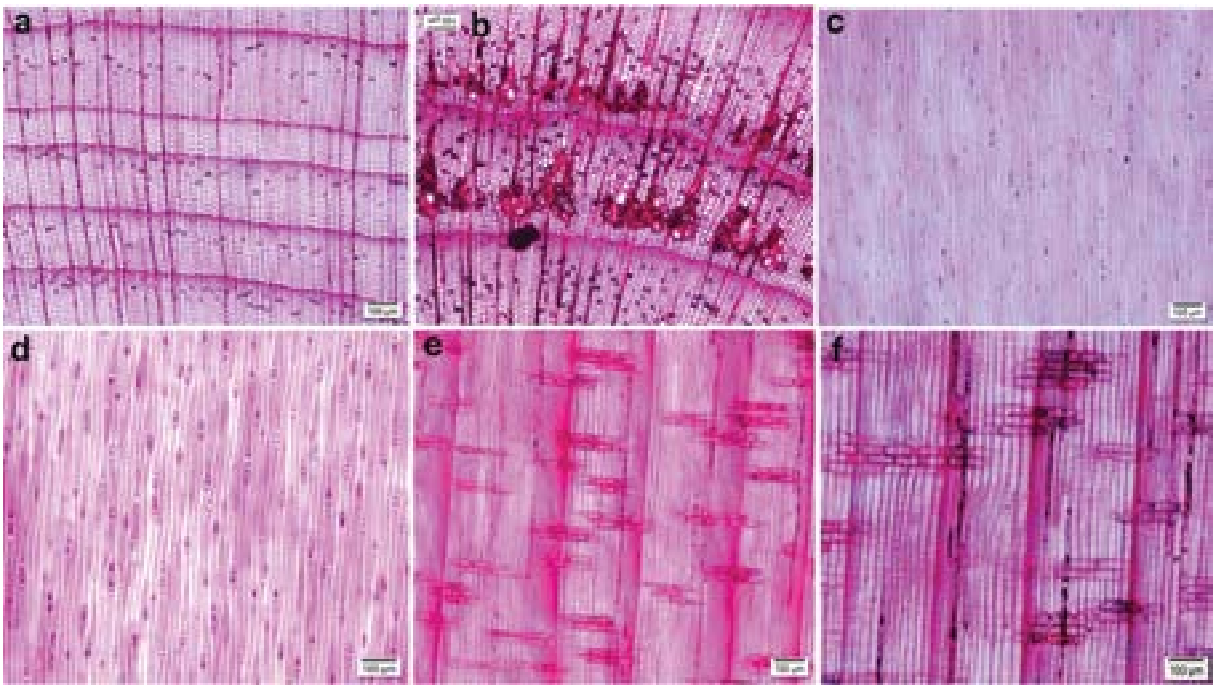

Figure 2: Wood anatomical structure in *J. squamata*: (a) transverse section of wood showing growth rings, tracheids, resin cells, and frost rings; (b) black arrow showing wide rings and blue arrow showing narrow rings; (c, d) tangential longitudinal section of wood showing uniseriate rays, prismatic crystals in ray cells; and (e, f) RLS showing homogenous rays, cupressoid pits in ray cells, and tracheids with circular bordered pits. ***All photomicrographs taken at (10× + 0.5×) magnification.***

Wood anatomical parameters

Wood anatomical parameters refer to the structural features and characteristics of wood at the microscopic-level. These parameters are important for understanding the physical and mechanical properties of wood as well as its biological and ecological functions (Wheeler, 1983). We had considered Growth Ring Width (GRW), Earlywood Width (EWW), Earlywood Tracheids Tangential Diameter (EWTD), Earlywood Tracheids Radial Diameter (EWRD), Latewood Width (LWW), Latewood Tracheid Tangential Diameter (LWTD), Latewood Tracheid Radial Diameter (LWRD), Tracheid Length (TL), Uniseriate Ray Height (URH), Number of Uniseriate Ray Cells (NURC), Ray Parenchyma Cell Tangential Diameter (RPCTD), and Ray Parenchyma Cell Vertical Diameter (RPCVD) for analyzing the anatomical parameters of the sampled *J. squamata* wood. Spearman's Rank Correlation Test was carried out between the anatomical parameters studied. The results of this correlation test is presented in Table 2. The GRW exhibited strong positive correlation with the EWW and LWW while it had significant negative correlation with the TL, LWRD, URH, and RPCVD (Table 2). Similarly, the EWW showed significant positive correlation with the EWTD, EWRD, and LWW while it had significant negative correlation with the RPCTD and RPCVD. However, the EWTD exhibited a significant positive correlation with the EWRD, LWTD, and LWRD. Similarly, the EWRD also exhibited a significant positive correlation with the LWW, LWTD, and LWRD (Table 2).

Similarly, the LWW exhibited a significant positive correlation with the URH as well as NURC while a significant negative correlation with the LWTD and RPCTD. Likewise, the LWTD showed a significant positive correlation with the LWRD while it had a significant negative correlation with the URH. In contrast, the LWRD showed a significant positive correlation with the URH (see Table 1). However, the TL exhibited a significant positive correlation with the RPCTD and RPCVD. Similarly, the URH exhibited a significant positive correlation with the NURC, RPCTD, and RPCVD. Likewise, the RPCTD exhibited a significant positive correlation with the RPCVD (see Table 1).

Table 2 : Spearman's Rank Correlation Test between the anatomical parameters studied

	GRW	EWW	EWTD	EWRD	LWW	LWTD	LWRD	TL	URH	NURC	RPCTD	RPCVD
GRW	1.000											
EWW	**0.371****	1.000										
EWTD	-0.069	**0.157****	1.000									
EWRD	0.028	**0.264****	**0.499****	1.000								
LWW	**0.176****	**0.357****	0.043	**0.131****	1.000							
LWTD	0.057	0.049	**0.514****	**0.340****	**-0.104***	1.000						
LWRD	**-0.105***	0.079	**0.330****	**0.439****	0.011	**0.130****	1.000					
TL	**-0.083***	-0.051	-0.055	0.061	-0.031	0.029	0.044	1.000				
URH	**-0.130****	-0.063	-0.005	0.026	**0.086***	**-0.103***	**0.087***	0.074	1.000			
NURC	0.028	-0.002	0.019	0.041	**0.108****	-0.002	0.043	-0.041	**0.836****	1.000		
RPCTD	0.011	**-0.093***	-0.016	0.016	**-0.153****	0.077	-0.003	**0.341****	**0.092***	-0.025	1.000	
RPCVD	**-0.193****	**-0.162****	0.022	0.023	-0.075	-0.023	0.020	**0.208****	**0.137****	-0.031	**0.219****	1.000

** Correlation is significant at 0.01 level (2-tailed); and

* Correlation is significant at 0.05 level (2-tailed).

Discussion

Wood is characterized by the presence of tracheids, extremely narrow latewood and comparatively broader earlywood, resin cells, uniseriate ray parenchyma, homogenous rays, narrow growth rings and distinct growth ring boundaries. Inside Wood (2023) and Rajbhandari *et al.* (2020) also reported the presence of similar wood anatomical features in Himalayan Juniper. Anatomical studies conducted on other Juniper species and members of the Cupressaceae family worldwide mentioned homogenous rays with smooth walls and cupressoid pits in other members of Cupressaceae, such as *Tetraclinis* (Esteban *et al.*, 2015). We found the wood anatomical features of *J. squamata* differed from other Juniper species in terms of ray cells, resin cells and their density, and presence of crystals in ray and resin cells. In *J. squamata*, crystals are present in ray cells and resin cells. However, crystals are absent in ray cells and resin cells in *J. recurva*, *J. communis*, and *J. indica*, (Rajbhandari *et al.*, 2020).

Each annual-ring is made up of concentric rings of earlywood and latewood; thus, the positive correlation between the GRW with the EWW and LWW is justified. However, the negative correlation between the GRW and TL means that when the width of growth ring increases, the length of tracheid decreases. The negative relationship between tracheid length and annual-ring width was reported in conifers including *Pinus sylvestris* (Fabisiak & Fabisiak, 2021) and *Pseudotsuga menziesii* (Douglas Fir) from Netherlands (Kort, 1990). The relative proportions of the three tissues (tracheids, rays, and parenchyma) are generally similar among gymnosperms (Cheng, 1985). However, they markedly vary in angiosperm tree species. Zhang *et al.* (2017) showed smaller coefficient of variation in gymnosperm wood traits than in angiosperm ones. Similarly, great variation in wood trait was observed due to the influence of climate in angiosperms (Cheng, 1985; Carlquist, 2001). Our results showed that annual-ring width exhibited strong negative correlation with uniseriate ray height as well as size of ray parenchyma cells. However, a positive correlation was seen between Tracheid length and uniseriate ray height as well as tangential and vertical diameters of ray parenchyma cells. We also found a positive correlation between ring width and latewood proportion as well. Annual-ring width showed positive and strong correlation to early and latewood width, and negative correlation with tracheid length and latewood proportion in *Thuja occidentalis* (Bouslimi *et al*., 2019). These results coincide with our findings.

We found a considerable intra-specific variation in the wood anatomical features within the sample individuals of *J. squamata*. These anatomical modifications can sometimes be due to acclimatization of the plant to its surrounding environment (Vasić *et al*., 2014) or due to the bioclimatic condition of the surroundings (Castagneri *et al*., 2017). For example, the tracheid size in *Picea abies* in the Italian Alps was influenced by the climate during the growing season and the early-summer temperature influenced cell enlargement at higher elevations while the water availability contributed to the cell enlargement at lower elevations (Castagneri *et al*., 2017). The study site in Manang lies in the trans-Himalayan region where there is very limited availability of soil water, and the climatic condition is also harsh. Here, the bushes of Juniper usually grow in the rocky habitat, where the snow melts earlier such that it can take advantage of the moisture obtained from the melting of snow after receding icecaps. Therefore, the bushes of Juniper can form a distinct micro-habitat within their surroundings. Dwarf individuals with reduced growth ring widths and increased tracheid length insures effective water absorption and transportation towards the shoot system. Parallel to our findings, studies have indicated that the intra-specific variation in wood anatomical features among different growth forms of Juniper is closely linked to the plant's hydraulic architecture (Beikircher & Mayr, 2008). Similarly, elevation-driven variations in the wood characteristics of *Rhododendron lepidotum* were reported in the central and eastern Himalaya, though no distinct pattern with elevation was found (Pandey *et al*., 2020). However, age-related quantitative changes were reported in the wood characteristics of *Pinus roxburghii* trees in central Nepal (Joshi & Chalise, 2022).

Conclusion

The sampled *J. squamata* wood was characterized by the presence of tracheids, resin cells, uniseriate ray parenchyma, and narrow growth rings with abrupt transition from earlywood to latewood. Both the earlywood and latewood tracheids of *J. squamata* comprised square to polygonal cells with circular bordered pits and a few resin cells. Rays were exclusively uniseriate, homogenous, with cupressoid pits. Annual-ring width showed a positive correlation with both the earlywood and latewood width, but a negative correlation with tracheids length. We found that there was a higher variability of structures due to modification in the surrounding environment and micro-climatic condition at the uppermost range of *J. squamata*. Therefore, dwarf individuals with reduced growth ring dimensions and increased tracheid length insure effective water absorption and transportation in harsh conditions. However, wood characters are changed quantitatively but not qualitatively with respect to change in ecological factors. Further studies on the comparative anatomical examination of same plant species existing in different ecological regions as well as in different microhabitats within same ecological regions would ideally reveal the anatomical changes modified by environmental conditions.

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Author's contribution statement

P. Chalise: Development of study tools, sample preparation, anatomical measurement, preliminary data analysis and first draft preparation.

A. Tiwari: Research ideas, sample collection, data analysis, review and final editing of manuscript.

Data availability

The data collected for this study is available from the Figshare repository https://www.doi.org/10.6084/m9.figshare.25225511.v2 (I think this link has to be developed by the editorial from the supplementary data we have submitted)

Conflict of Interest

The authors declare no conflict of interest.

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