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WOOD ANATOMICAL CHARACTERISTICS OF SOME *MAGNOLIA* SPECIES FROM SIKKIM, INDIA

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Abstract: **The present study was carried out in four *Magnolia* species namely*M. cathcartii, M. champaca, M. doltsopa, M. lanuginosa*. The wood samples were collected fromstraight trees of selected*Magnolia* speciesfrom forests of North Sikkim, NE India.The aim of the study was to provide detailed account of qualitative and quantitative anatomical characteristics of these species. The common anatomical characteristics among species were diffuse porous wood with distinct growth rings marked by marginal parenchyma(except in *M. doltsopa*), scalariform perforation plate in vessels, presence of oil cells in rays, intervessel pits scalariform (except in *M. doltsopa*).The features like demarcation of growth ring boundary by radially flattened thick fibres, presence of pith flecks, biseriate rays, scanty paratracheal parenchyma in *M. doltsopa* and distended rays in *M. lanuginosa* were the distinct features of these species. The quantitative anatomical characteristics also exhibited significant variation among species. In conclusion, both qualitative and quantitative anatomical features are important for identification of *Magnolia* species.**

***Index terms*: Anatomical characteristics, Marginal parenchyma, Magnolia species, Oil cells, Scalariform perforation**

I.INTRODUCTION

Magnoliaceae is one of the primitive families of angiosperms. It comprises of evergreen and deciduous trees and shrubs with approximate 240 species which are widely distributed in tropical, sub-tropical and temperate zones of Southern and Eastern Asia (Shi et al,2000). A number of genera like *Aromadendron, Michelia, Angelitia, Paramichelia, Sampacca* and *Taulama* had been placed in sub family Magnoliodeae. But, based on the DNA work (Azuma et al, 2000, 2001; Kim et al, 2001), morphological consideration (Figlar,2000) and nomenclatural changes (Shankar,2020) have merged these genera into a single genus *Magnolia.* The name of this genus was given in honour of French botanist Pierre Magnol by famous Carl Linnaeus in 1737. It is represented by 327 taxain the world. Of which, 10% oftaxa occur in NE India, Central and East Himalaya, Nepal and Bhutan. Only one species *M. nilagirica* is present in Western Ghats- Sri Lanka global hotspot of biodiversity and other species are endemic to NE India.

The wood of *Magnolia* is distinct in sapwood and heartwood. Sapwood is creamy white to greyish colour and heartwood is medium to dark brown sometimes with green purple or black streaks, moderately heavy, straight grained with medium to fine uniform texture, easy to work with both hand and machine tools and finishes well. Because of its unique characteristics like resistance to split, better gluability and dimensionally stable after seasoning, the wood is used for making

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furniture, toys, plywood, interior work and turnery articles(Chowdhury & Ghosh,1958).

Most of the workers have paid attention towards the taxonomic research regarding distribution, taxonomic diversity and endemism (Kundu et al, 2009; Shankar,2020), molecular systematics (Azuma et al, 2000, 2001; Kim et al, 2001) and traditional analysis of morphological characters (Figlar,2000;Figlar&Noteboom,2004). Currently, *Michelia*is considered as a part of species rich genus *Magnolia* (APG IV, 2016). The available literature reveals that wood anatomy of family Magnoliaceae is homogeneous (Chauhan &Dayal, 1992; Chen et al, 1993). Chen et al (1993) reported distinguishable wood anatomical features between *Magnolia* and *Mangelitia* despite of various overlapping characters between them. However, the wood anatomy of evergreen species of *Magnolia* and *Michelia* were similar. Wroblewska (2015) reported new aspects of phylogenetic relationship between *Magnolia* species based on vessel characteristics and also compared the obtained data with phylogenetic trees, based on fossil records and plastid gene expression. They also revealed a link between the type of perforation plate and degree of evolutionary specialization with *Magnolia* genus. In India, Chauhan and Dayal (1992) examined eight*Michelia* species available in xylarium of FRI Dehradun and considered fibre shape, vessel, and ray characteristics for separation of *Michelia* species. However, there is limited report on wood anatomy of *Magnolia* species of Sikkim. Hence, the aim of present study is to provide detailed account of qualitative and quantitative anatomical characteristics of four *Magnolia* species.

II.STUDY SITE AND EXTRACTION OF WOOD SAMPLES

Five straight trees with uniform crown and no visible defects of *Magnolia* species namely*Magnolia cathcartii, Magnolia champacaMagnoliadoltsopaandMagnolialanuginosa* wererandomly selected from upper Mallam Phamtam, North Sikkim. Thegeographicalcoordinates of the site are 27°26'21.2"N 88°35'51.4"E. Wood samples of 5cm×5cm×3cm size were collected at breast-height of each tree with the help of a hammer and a chisel. The wood samples were packed inperforated polythene bags, properlylabelledand broughttothelaboratoryfor further investigations.

A. PROCESSING OF WOOD SAMPLES AND PREPARATION OF PERMANENT SLIDES

Collected samples were cut into small blocks of $2cm^3$size. These blocks werefixedinFAA(Formalin-aceto-alcohol)for24-48hrs.andthenpreservedin70%alcohol.The preserved blocks were cut in 3 planes namely Cross Section (C. S.),Tangential Longitudinal Section (T. L. S.) and Radial Longitudinal Section (R. L. S.)withthehelpofaslidingmicrotome(LeicaSM2000 R). The sections were stained by following standard method and permanent slides were prepared (Johansen,1940).

B. Maceration

Thin shavings of wood taken from the radial side of each specieswereteatedwithFranklin'ssolutionat60˚Cfor 24hourstilltheybecomesoftandwhiteincolour.Themaceratedmaterialwaswashedwithdistilledwater2-3times and gently shaken toobtain fluffy mass of fibres.It was stained by adding 2-3 drops of safranine and temporary slideswerepreparedbyusing50% glycerol. The fibre length and vessel length were measured from these slides with an ocular micrometer at 40x magnification.

C. Tissue proportion and measurement of cell dimensions

Fibre, vessel, parenchyma, and ray proportion were determined on cross section at 100x by selecting random 10 fields from each replicate of selected species. Length of fibres and vessels were measured randomly from temporary slides of each sample of every species. The measurements were taken with the help of an ocular micrometer at 40x magnification. Number of vessels per mm^2 were counted in cross section by using graph eyepiece at 100x magnification and ray per mm were also taken in cross section with the help of an ocular micrometer at 100x magnification. 10 random fields per sample were selected randomly for each replicate of a species. Other vessel, fibre and ray dimensions like vessel diameter, ray height (at 40x magnification),fibre diameter and fibre lumen diameter (at 400x magnification) were measured with the help of Scope image 9.0 software. Ray

width was measured in terms of number of cells. The counts/ number of these parameters were taken according to Wheeler et al (1989)

D. Photography

The photomicrographs of selected species were taken with the help of Leicaimageanalysissystem atdifferentmagnificationsfortheiranatomicalfeatures .

E. Statisticalanalysis

One-way ANOVA followed by Tukey's testwasperformedusingSPSS16software.

III.RESULTS

The qualitative and quantitative features of *Magnolia* species were presented inTables 1-2and the tissue percentage of selected species was given in Figure3.The anatomical descriptions of *Magnolia*species are givenbelow:

A.Magnolia cathcartii(hook f. &Thomson)Noot (Fig. 1: A-C)

Vernacularname:Titlichamp (Nepali)

Anatomicalfeatures

Growthrings: Distinct,markedbybandsof marginalparenchyma.

Vessels:Diffuse-porous,mostly solitary, in radial multiples of 2-8, oval in outline, oblong shaped,564.08 -1401.65 µm (Mean 815.69 ± 165.97 µm) in length, 41.83 – 81.10 µm (Mean59.51±82.88 µm) in diameter, vessel frequency 26-75 (Mean 45.22±12.65) per mm^2,scalariform perforation plate, intervessel pits scalariform, vessel- ray pits with muchreduced border to apparently simple, pits horizontal (scalariform / gash like), vesselpercentage19.27.

Fibres: Thin to thick walled, 1384.56- 1957.19 µm (Mean 1656.34±150.57 µm) long,21.38-324.77µm(Mean59.51±82.88µm)and16.32-246.27µm(Mean45.27±62.49µm)indiameterandlumendiameter,wallthickness3.58-78.50µm(Mean14.25±20.62µm), septatefibrespresent,fibrepercentage 36.55.

Parenchyma: Marginal or in seemingly marginal bands, 4-8 cells per parenchymastrand,parenchymapercentage 21.63.

Rays: Mostly multiseriate and biseriate rarely uniseriate, mean ray height and raywidth 306.21-613.83 µm (Mean 461.44±62.78 µm) and 34.89 - 80.85 µm (Mean52.78±11µm).Bothhomocellularandheterocellularrays.Homocellularraysofuprightand/orsquarecells,mainbodyofprocumbentcellswith1-3marginalrowsofsquareand/or upright cells in heterocellular rays.Rays 4-8 (Mean 5.74± 0.98) per mm, raypercentage61.69.

Secretary elements: Oil cells associated with square ray cells and present amongfibres.

B. Magnolia champaca (L.) Baill. Ex Pierre (Fig. 1: D-G)

Vernacular name: Phulchampa (Nepali)

Anatomicalfeatures

Growthrings:Distinct, markedbybandsofmarginalparenchyma.

Vessels: Diffuse porous,mostly solitary, in radial multiples of 2 - 3, oval in outline, barrel to oblongshaped 470.07-914.49 µm (Mean 681.51±103.28 µm) in length, 7.44-117.97 µm(Mean55.74±39.50 µm) in diameter, vessel frequency 14 – 31 (Mean 20.26±4.01)per mm^2, scalariform perforation plates, intervessel pits scalariform, opposite in 2-3rows, vessel - ray pits with much reduced border to apparently simple, pits horizontal(scalariform,gashlike)andpresentthrough outtheray,tylosespresent,vesselpercentage32.18.

Fibres: Thin walled, 1282.00-1888.81 µm (Mean 1546.78±150.72 µm) long, 17.27-29.43 µm (Mean 21.95±2.77 µm) and 11.88-22.93 µm (Mean 15.63±2.38 µm) indiameterandlumendiameter,wallthickness3.83-8.57µm(Mean6.33±1.05µm),fibrepercentage32.36.

Parenchyma: Marginal or in seemingly marginal bands, scanty paratracheal, 5-8cellsper parenchymastrand,parenchymapercentage 11.64.

Rays: Mostly multiseriate, mean ray height and ray width 147.62-568.01 µm (Mean 372.55±78.18 µm) and 36.31- 91.28 µm (Mean 62.92±12.65 µm),

both homocellular and heterocellular rays, homocellular rays of either procumbent cells or upright and/or square cells, main body of procumbent ray cells with marginal rows of upright and / or square cells in heterocellular rays. Rays 3 - 9 (Mean 5.92±1.12) per mm, ray percentage 23.82.

Secretaryelements-Oil cells present in rayandparenchyma.

C.Magnolia doltsopa(Buch. - Ham ex DC) Figlar (Fig.1: H-K)

Vernacular name: Rani champ, Safed champ (Nepali)

Anatomicalfeatures

Growthrings:Distinct andmarkedbyradiallyflattenedthickwalledfibres.

Vessels: Diffuse porous,mostly solitary and in radial multiples of 2-3, oval in outline, barrel shaped,230.76 - 615.36 µm (Mean 435.54±78.06 µm) in length, 42.36 - 106.64 µm (Mean68.16±13.92 µm) in diameter, vessel frequency 13-36 (Mean 22.70±5.36) per mm^2,scalariform perforation plate, intervessel pits alternate, vessel- ray pits with muchreduced border to apparently simple, pits rounded or angular, vessel percentage16.91.

Fibres: Thintothickwalled,786.29-1187.99µm(Mean985.09±94.26µm)long,14.59-227.20µm(Mean58.75±74.23µm)and8.70-190.20µm(Mean46.49±61.44µm) indiameterandlumendiameter,wallthickness3.14-45.23µm(Mean12.03±12.98µm),septatefibrespresent,fibrepercentage43.09.

Parenchyma: Scantyparatracheal,diffuse,3-6cellsperparenchymastrand,parenchymapercentage 25.82.

Rays: Mostlybiseriate,multiseriateraysalsopresent,meanrayheightandraywidth165.69-334.72µm(Mean248.85±40.13µm)and20.62-233.33µm(Mean37.13±33.00 µm), rays heterocellular, main body of procumbent ray cells with 1-3marginal rows of upright and/or square cells. Rays 2-10 (Mean 6.28±1.55) per mm,pithflecks present,raypercentage 14.18.

Secretaryelements:Oilcells presentin rays,parenchymaandamongfibres.

D.Magnolialanuginosa(Wall.)Figlar&Noot. (Fig.2: A-E)

Vernacularname:Phursechamp/Gogaychamp(Nepali)

Anatomicalfeatures

Growthrings: Distinctandmarkedbybandsof marginalparenchyma.

Vessels: Diffuse porous,mostly solitary, in radial multiples of 2 - 4, oval in outline, oblong shaped,495.71-965.77µm(Mean706.41±95.34µm)inlength,44.42-75.09µm(Mean56.21±6.98 µm) in diameter, vessel frequency 33 - 88 (Mean 56.21±6.98) per mm^2,scalariform perforation plates, intervessel pits scalariform, vessel- ray pits with muchreduced border to apparently simple, pits horizontal (scalariform, gash like), vesselpercentage16.91.

Fibres: Thin walled, 1333.28-1837.53 µm (Mean 1670.79±110.11 µm) long, 20.62-38.08 µm (Mean 27.99±3.34 µm) and 14.75 - 31.19 µm (Mean 20.51±3.77 µm) indiameter and lumen diameter, wall thickness 9.26-11.98 µm (Mean 8.09±2.98 µm),septatefibrespresent,fibrepercentage43.23.

Parenchyma: Marginal or in seemingly marginal bands, 4-8 cells per parenchymastrand,parenchymapercentage15.27.

Rays - Mostly multiseriate, mean ray height and ray width 310.78-710.30 µm (Mean447.92±75.27 µm) and 39.19- 81.50 µm (Mean 59.95±9.91 µm), rays heterocellular,mainbodyofprocumbentraycellswith1-2rowsofuprightand/orsquaremarginal cells,raysdistendednearmarginalparenchyma.Rays4-9(Mean6.14±1.06)permm,raypercentage 24.54.

Secretaryelements-Oil cellspresentin rays.

The results given in Table 1 showed highly significantvariation in anatomical parameters among species. However, vessel diameterand ray frequency exhibited non-significant variation. Vessel length was significantlylonger in *Magnolia cathcartii*whereas vessel diameter was significantly greater in*Magnolia doltsopa*. Vessel frequency was higher in *Magnolia lanuginosa*.The fibres of *M.*

*lanuginosa*were longer than other species whereas fibre diameterand fibre wall thickness were significantly higher in *M. cathcartii*than other species.A significant variation in ray height and ray width and non-significant variationinrayfrequencywere recorded amongspecies.

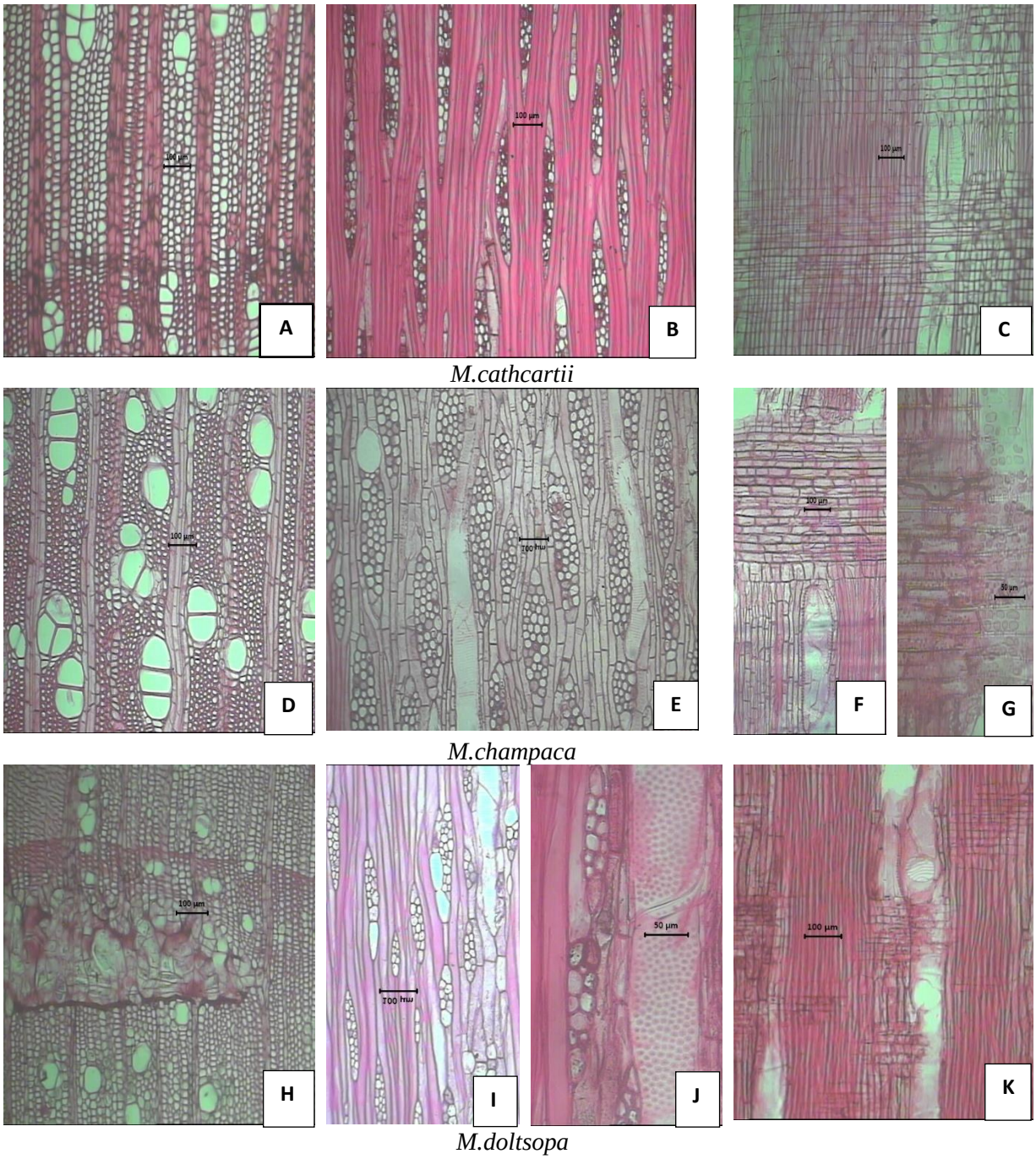
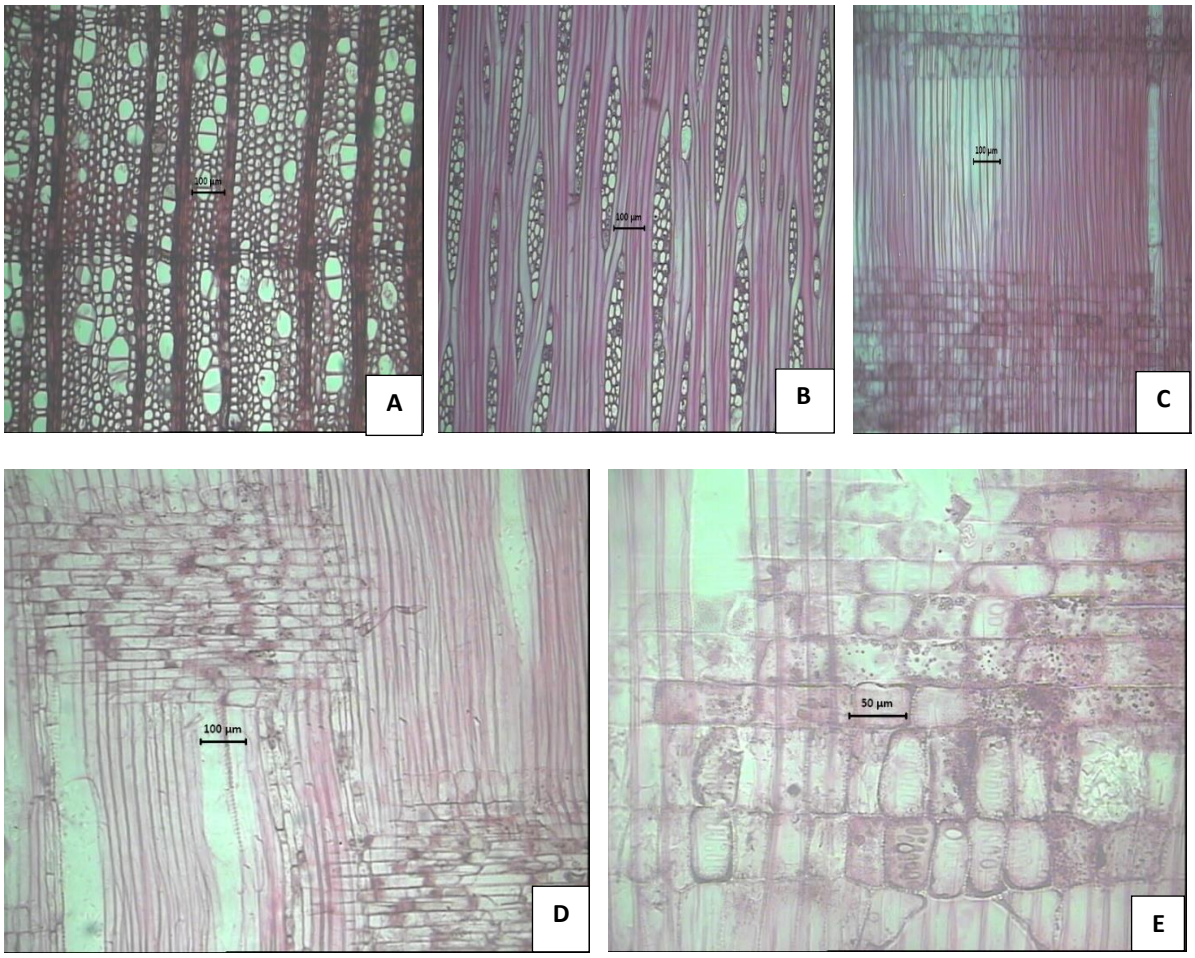


Fig.1. *Magnolia* spp. C. S.- Diffuse porous wood with distinct growth rings due to marginal parenchyma (A), vessels in radial multiple of 2-3 (D), pith flecks present (H); T. L.S.- Multiseriate rays, parenchyma strands and oil cells present in rays (B, E, I), intervessel pits scalariform (E) and alternate (J); R.L.S.-Homocellular rays of procumbent cells (C, H), heterocellular rays of procumbent cells with one marginal row of square/upright cells (C, F), vessel ray pits rounded (G) and scalariform perforation (K).



M. lanuginosa

Fig. 2. *M. lanuginosa* Diffuse porous wood with distinct growth rings due to marginal parenchyma, vessels in radial multiple of 2-3 (A); T. L.S.- Multiseriate rays, parenchyma strands and oil cells present in rays (B); R.L.S.-Homocellular rays of procumbent cells (C); heterocellular rays of procumbent cells with one marginal row of square/upright cells (D); vessel ray pits scalariform perforation (E).

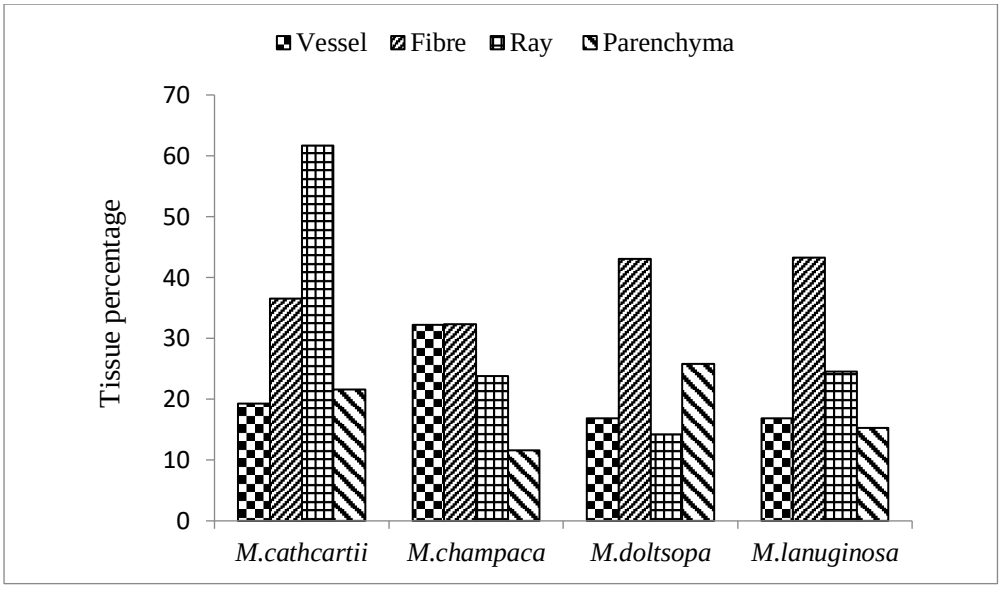


Fig.3.Tissue percentage in *Magnolia* species

Table 1. Qualitative anatomical characteristics of *Magnolia* species

S. No.	Features	*M. cathcartii*	*M. champaca*	*M.doltsopa*	*M. lanuginosa*
1.	Growth rings	Distinct	Distinct	Distinct	Distinct
2.	Porosity	Diffuse porous	Diffuse porous	Diffuse porous	Diffuse porous
3.	Vessel grouping	Solitary, radial multiple of 2-5, cluster	Solitary, radial multiple of 2-3	Solitary, radial multiple of 2-3	Solitary, radial multiple of 2-3
4.	Solitary vessel outline	Oval	Oval	Oval	Oval
5.	Vessel shape	Oblong	Barrel to oblong	Barrel	Oblong
6.	Perforation plate	Scalariform	Scalariform	Scalariform	Scalariform
7.	Intervessel pits	Scalariform	Scalariform	Alternate	Scalariform
8.	Intervessel pits arrangement	Single row	2-3 rows	-	Single row
9.	Vessel ray pitting				
10.	Tyloses	Absent	Present	Absent	Absent
11.	Fibres	Thin to thick walled	Thin walled	Thin to thick walled	Thin walled
12.	Septate fibres	Present	Absent	Present	Present
13.	Axial parenchyma	Marginal	Marginal, scanty paratracheal	Scanty paratracheal, diffuse	Marginal
14.	Ray width	Multiseriate, rarely uniseriate & biseriate	Multiseriate	Biseriate &Multiseriate	Multiseriate
15.	Ray composition	Homocellular&heterocellular	Homocellular&heterocellular	Heterocellular	Heterocellular
16.	Distended ray	Absent	Absent	Absent	Present
17.	Secretory elements (Oil cells)	Present in rays and fibres	Present in rays and axial parenchyma	Present in rays, fibres and axial parenchyma	Present in rays
18.	Pith flecks	Absent	Absent	Present	Absent

Table 2. Quantitative anatomical characteristics of *Magnolia* species

Sl. No.	Parameters	*M.cathcartii*	*M. champaca*	*M. doltsopa*	*M.lanuginosa*
1.	Vessel Length(µm) (Mean±SD)	815.69±165.97^{c}	681.51±103.28^{b}	435.54±78.06^{a}	706.41±95.34^{b}
2.	Vessel Diameter(µm) (Mean±SD)	58.97±10.85^{ab}	55.74±39.50^{a}	68.16±13.92^{b}	56.21±6.98^{a}
3.	Vessel Frequency(/mm^2) (Mean±SD)	45.22±12.65^{b}	20.26±4.01^{a}	22.70±5.36^{a}	56.64±17.40^{c}
4.	Fibre Length (µm) (Mean ±SD)	1656.34±150.57^{c}	1546.78±150.72^{b}	985.09±94.26^{a}	1670.79±110.11^{c}
5.	Fibre Diameter(µm) (Mean±SD)	59.51±82.88^{b}	21.95±2.77^{a}	58.75±74.23^{b}	27.99±3.34^{a}
6.	FibreLumenDiameter (µm) (Mean ±SD)	45.27±62.49^{b}	15.63±2.38^{a}	46.49±61.44^{b}	20.51±3.77^{a}
7.	Fibre WallThickness (µm)(Mean ±SD)	14.25±20.62^{b}	6.33±1.05^{a}	12.03±12.98^{ab}	8.09±2.98^{ab}
8.	Ray Height (µm) (Mean ±SD)	461.44±62.78^{c}	372.55±78.18^{b}	248.85±40.13^{a}	447.92±75.27^{c}
9.	Ray Width (µm) (Mean ±SD)	52.78±11.00^{b}	62.92±12.65^{c}	37.13±33.00^{a}	59.95±9.91^{bc}
10.	No. of rays/mm (Mean ±SD)	5.74±0.98^{a}	5.92±1.12^{a}	6.28±1.55^{a}	6.14±1.06^{a}

Valueswithsameletterinthesamerowarenotsignificantlydifferentat0.05probabilitylevel.

IV.DISCUSSION

Most of the qualitative anatomical characteristics were uniform in *Magnolia*species.Alltheselectedspecieswerediffusep orouswithdistinctringsduetomarginal bands of parenchyma except *Magnolia doltsopa*. Scalariform perforation plates werepresent in all species. Intervessel pits were scalariform in three species namely *Magnoliacathcartii*,*MagnoliachampacaandMagnol ialanuginosa*.Pitswerearrangedinsinglerowin*M.cath cartiiandM.lanuginosa*whereasthesewerearrangedin 2-3rowsin*M.champaca.M.doltsopa*hadalternate intervessel pits.

Chenetal(1993)dividedMagnoliasintotwogroupsb ased onanatomicalcharacters.Theevergreenspecieshaves calariformperforationplatesanddeciduousspecieshav esimpleperforationplates.Sinceallthesselectedspecies havescalariformperforation plates which show that all selected species are evergreen. The vessel raypits were with much reduced border to apparently simple, pits scalariform (gash like)as intervessel pits. However, *M. doltsopa*had rounded pits. The present study is inconfirmation with the findings of Chen et al (1993). Septate fibres were occasionallyseen in selected species except *M. champaca*which may be due to less percentageofparenchyma.Pith flecks were observed in *M. doltsopa*which are the patches of irregularlyarranged mass of parenchyma cells within the wood. Carlquist (1988) reported injury to the cambium by insectinfestation and also cold and drought conditions are responsible for the formation of pith flecks. Inthepresentstudy,thepithflecksin*M.doltsopa*maybe duetocoldconditionofSikkim.Axial parenchyma was marginal or in seemingly marginal bands in selectedspecies except *M. doltsopa*. However, scanty paratracheal and diffuse parenchymawere also observed in the species and corroborates the findings of Chen et al (1993) andMertzetal(2014).Oilcellsarecharacteristicsfeatur eofthefamilyMagnoliaceaeandare associated with rays,parenchyma oramongfibres.

Fibres,vessel,rayandparenchyma are the main xylem elements of hardwoods.Thepercentageoftheseelementsvaryfrom speciestospecies.Inthepresentstudy,*M.doltsopa*had maximum fibre percentagewith minimum percentage of parenchyma.Also, the fibres were thin to thick walled in *M. doltsopa*which shows that its wood isharderthanother species.Allthequantitativeanatomicalcharacteristics ofvessels,raysandfibresexhibitedhighlysignificantva

riationwithinandamongspecies.Thesignificantvariation within species may be due to extraction of wood samples from trees ofunknown age. The present study is in agreement with the findings of other workers (Singh etal,2019;Wangkhemetal,2020).

V. CONCLUSIONS

The results of present study showed homogeneous structure among *Magnolia* species. There were some distinct anatomical features like both multiseriate and biseriate rays in *M. cathcartii*, intervessel pits scalariform arranged in 2-3 rows in *M. champaca*, biseriate rays, scanty paratracheal parenchyma, intervessel pits rounded, alternate, biseriate rays in *M. doltsopa* and distended rays near the marginal parenchyma in *M. lanuginosa* which can be used to identify individual species of *Magnolia*. There was also significant variation in quantitative anatomical characteristics among species. Hence, the present study shows that both qualitative and quantitative anatomical characteristics are important for identification of *Magnolia* species.

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