# Wood anatomy of Holmskioldia sanguinea Retz. and its adaptive and ecological significance 

Manju SAHNEY* ${ }^{*}$, Shakti Nath TRIPATHI and Vibhasa SHUKLA<br>Department of Botany, University of Allahabad, Allahabad-211002, U.P., India.<br>*Corresponding author's email: msahney.au@gmail.com

(Manuscript received 8 November 2016; accepted 22 March 2017; online published 7 April 2017)


#### Abstract

Wood anatomy of Holmskioldia sanguinea Retz. has been done to study the structural variations in stem and root wood and to correlate them with growth habit and ecology of the plant. Vessels are wider and more abundant while rays (uni- to triserate) are taller in stem wood than in root wood, which possesses broad multiseriate rays. Simple perforation plate, sparse vasicentric paratracheal parenchyma and perforated ray cells are the features common in both stem and root woods, while helical thickenings have been recorded only in the stem wood. Lower value of vulnerability, presence of wider vessels with simple perforation plate, and presence of helical thickening are indicators of wood xeromorphy in Holmskioldia sanguinea. Features like wider vessels and extensive height of rays in stem wood are in consonance with the climbing nature of the stem axis.


KEY WORDS: Holmskioldia sanguinea, Rays, Vessels, Wood anatomy.

## INTRODUCTION

Holmskioldia sanguinea Retz. (formerly of Verbenaceae) is a monotypic genus of the family Lamiaceae. It is native of sub-tropical Himalayan regions of India and Pakistan but also occurs widely in South Asia, Mauritius, Indonesia and West Indies (Sayeeduddin and Moinuddin, 1939; Ingole, 2011).

General anatomical features of the family Lamiaceae were described by Solereder (1908), Metcalfe and chalk (1950). Carlquist (1992) presented the quantitative and qualitative data of wood anatomy of 17 genera ( 42 species) belonging to Lamiaceae. Sayeeduddin and Moinuddin (1939) made a preliminary anatomical study of young stem and root of H. sanguinea. However, to the best of our knowledge no information is available on its wood anatomy.

The plant is a perennial straggling evergreen shrub, up to 3-5 meter in height, with drooping branches (Sayeeduddin and Moinuddin, 1939; Bose and Chowdhury, 1991; Gamble, 1972; Ingole, 2011). It has been also described as a scandent/ clambering shrub (Acevedo-Rodríguez, 2005).

It is well known that plants develop anatomical strategies and adaptations for successful ascent of sap, depending on their form and environment. Scandent plants, although high in conductive capability, have a more vulnerable hydrosystem (Carlquist, 1985).

The present study was undertaken to investigate the anatomical features of $H$. sanguinea Retz., which has a climbing stem and drooping branches, in order to ascertain whether the climbing tendency of the shrub also reflects in the anatomical features of stem and root wood. This is the first comprehensive report on the wood anatomy of $H$. sanguinea, exploring the correlation between the habit and ecology of the plant.

## MATERIAL AND METHODS

Fresh materials of both stem and root were collected from plants growing in the Roxburgh Botanical Garden, University of Allahabad ( $25^{\circ} 28^{\prime} \mathrm{N}$, $81^{\circ} 54^{\prime} \mathrm{E}$ ) and the National Botanical Research Institute, Lucknow ( $26^{\circ} 55^{\prime} \mathrm{N}, 80^{\circ} 59^{\prime} \mathrm{E}$ ). The stem and root woods were cut into small pieces and fixed in FAA (Berlyn and Miksche, 1976). Transverse and longitudinal (both T.L.S and R.L.S) sections of $15-20 \mu \mathrm{~m}$ in thickness were cut, stained with safranin-fastgreen combination, and mounted in Canada balsam. Small pieces of stem and root woods were macerated using Jaffery's fluid (Johansen, 1940) and thirty random measurements of the macerated cells were taken, using an ocular micrometer scale, to obtain the mean measurements for each cell type. An Olympus binocular compound microscope (Model No.CH2i) and a Leica binocular compound microscope (Model: DM2500) were used for examining the anatomical sections and for photography, respectively. SEM photographs were taken with Electron Probe Micro Analyser model "JEOL JXA 8100 " operated at 15 KV and $1 \times 10^{-8}$ Amp. at National Centre of Experimental Mineralogy and Petrology (NCEMP), University of Allahabad. Before SEM imaging samples were coated with 30 nm thick carbon using Jeol JEE-420 vacuum evaporator. F/V ratio is calculated by dividing mean libriform fibre length with mean vessel element length. Vulnerability (v) of wood was calculated by dividing mean vessel diameter with vessel frequency, while mesomorphy (m) was obtained by multiplying the vulnerability with mean vessel-element length, following Carlquist (1977). The cell counts, measurements and anatomical description follow the rules of the IAWA Committee, 1989.

## RESULTS

## Stem wood

Vessels- Arranged in semi-ring porous pattern; occur mostly as solitary vessels; occasionally in radial multiple of 3-6 (Fig. 1a); frequency of vessels 145 (140 to $150 / \mathrm{mm}^{2}$ ); individual vessel element oval to circular in cross section; $30-60-115 \mu \mathrm{~m}$ in diameter; vessel elements moderately to extremely large, showing variation in length ( $200-400-600 \mu \mathrm{~m}$ ); perforation plate simple on transverse to slightly oblique end wall (Fig. 1 g ); vessel elements frequently tailed at one or both the ends; helical thickening in vessels (Fig. 1h); intervascular pitting alternate, minute and boarderd (Fig. 1 j ). Vulnerability index (v) and mesomorphy index (m) 0.413 and 165 respectively.

Fibres and Fibre-tracheids- Libriform fibres septate as well as non-septate (Fig. 1e); short in length (350-590-900 $\mu \mathrm{m}$ ) and F/V ratio- 1.47. Starch grains present in septate fibres. Length of fibre-tracheids- $105-250 \mu \mathrm{~m}$.

Parenchyma- Axial parenchyma sparse, vasicentric paratracheal (Fig. 1b). Rays predominantly 2-3 cells wide, 6-27 cells in height, occasionally uniseriate (8-18 cells high); heterocellular with upright and square cells (Figs. 1c, 1d); ray cells frequently perforated (Fig. 1f), starch grains present in ray cells, rhomboidal crystal seen in pith region (Fig. 1i).

## Root wood

Vessels- Arranged in semi-ring porous pattern; occur mostly as solitary vessels, sometimes in radial multiple of 3-6 (Fig. 2a); frequency of vessels 113 (110 to $115 / \mathrm{mm}^{2}$ ); individual vessel elements oval to circular in cross section; $25-55-105 \mu \mathrm{~m}$ in diameter, showing variation in length; normally large in size ( $250-385-500 \mu \mathrm{~m}$ ); perforation plate simple on transverse to oblique end wall (Fig. 2g); intervascular pitting alternate, minute and bordered (Fig. 2i). Vulnerability index (v) and mesomorphy index (m) are 0.486 and 187 respectively.

Fibres and Fibre-tracheids- Libriform fibres septate as well as non-septate (Fig. 2e); medium to short in length ( $350-600-1000 \mu \mathrm{~m}$ ) and F/V ratio- 1.55. Starch grains present in septate fibres. Length of fibre-tracheids- $105-250 \mu \mathrm{~m}$.

Parenchyma- Axial parenchyma sparse, vasicentric paratracheal (Fig. 2b); rays predominantly tetraseriate (sometime upto 6 cells wide); 10-14 cells in height; rarely uniseriate, 10-16 cells in height; heterocellular with upright and square cells (Figs. 2c, 2f); ray cells frequently perforated (Fig. 2d); rhomboidal crystals present (Fig. 2h); starch grains present in ray cells (Fig. 2i).

## DISCUSSION

Among the various anatomical features reported in the present work, semi-ring porous wood, simple perforation plate, alternate and minute intervascular
pitting, sparse vasicentric paratracheal parenchyma, heterogeneous rays (upto 4-12 cells wide) and septate fibres are the features of Lamiaceae wood (Metcalfe and Chalk, 1950). However, vessels of Lamiaceae are reported to be small and minute, while in $H$. sanguinea wide to narrow vessels have been observed. In the present work, perforated ray cells have been recorded. Carlquist (1992) also reported such cells in some members of Lamiaceae viz. Phyllostegia lantanoides and Tinnea rhodesiana.

## Comparative wood anatomy of stem and root wood

Some qualitative features, viz. wide and narrow vessels, simple perforation plate, sparse vasicentric paratracheal parenchyma, fibre-tracheids, septate fibres, heterogeneous rays, starch grain in ray parenchyma and perforated ray cells are common to both stem and root woods. Stem wood differs from root wood in having helical thickening bands in the vessels. Quantitatively, vessels are wider and more abundant in stem wood than in root wood. Rays (uni- to multiseriate) are taller in stem wood and broader (up to 6 cells) in root wood.

Usually within a species root wood tends to have wider vessels than stem wood but woody climbers are known to have stem wood with wider vessels than in root wood (Fisher and Ewers, 1995; Ewers et al., 1997). H. sanguinea also shows presence of wider vessels in the stem wood than in the root wood, which can be correlated to the climbing nature of the plant.

In addition, vessel elements in both stem and root of $H$. sanguinea have simple perforation plates. It is generally believed that vessel elements with simple perforation plate are more efficient in conduction of water due to having less friction. Our observations on the presence of helical thickening bands in the stem wood of $H$. sanguinea substantiate the earlier report of Carlquist (1992) in several other genera of the family Lamiaceae. The presence of helical thickening in vessels of stem wood has been correlated with climates that are cool or dry or both (Carlquist, 1975, 1983). It may be mentioned here that the climatic conditions at Allahabad and Lucknow, from where the present plant material of $H$. sanguinea was collected, are neither extremely dry nor cold.

The plant shows a normal secondary growth. In the stem wood, the frequency of tall uni- to triseriate rays between the lignified tissue (tracheary elements and fibres) is high. Presence of softer tissue in the hard lignified tissue may provide flexibility to the drooping and the climbing branches. Axial parenchyma is sparse vasicentric; species with sparse axial parenchyma often have septate fibres containing starch that may have a role in facilitating water transport under draught condition when embolism is likely to occur (Harrar, 1946). It may also be mentioned here that F/V ratio in the stem wood of $H$. sanguinea is 1.47 which is indicative of optimal mechanical strength of the plant axis (Carlquist, 1988).


Fig. 1. Stem wood anatomy of Holmskioldia sanguinea. (a) T.S. showing semi-ring porous wood. (b) T.S showing sparse paratracheal parenchyma (c) T.L.S. showing uniseriate to triseriate rays. (d) R.L.S. showing upright and square ray cells. (e) T.L.S. showing libriform fibres, septate fibres and starch grains. (f) Magnified portion showing perforated ray cells. (g) T.L.S. showing simple perforation plate. (h) T.L.S. showing helical thickenings in vessels. (i) Magnified portion of crystals in pith. (j) SEM image of bordered pits. Labels: $\mathrm{sp}=$ sparse paratracheal parenchyma, ur $=$ uniseriate ray, $\mathrm{tr}=$ triseriate ray, $\mathrm{pc}=$ perforated ray cell, $\mathrm{sf}=$ septate fibre, If $=$ libriform fibre, $\mathrm{pp}=$ perforation plate, $\mathrm{cr}=\mathrm{crystal}, \mathrm{bp}=$ bordered pit, $\mathrm{sg}=$ starch grain. Scale bars: (a), (c), (d), (e) = $200 \mu \mathrm{~m}$; (b), (f), (g), (h), (i) $=50 \mu \mathrm{~m}$; (j) $=10 \mu \mathrm{~m}$.

Taiwania


Fig. 2. Root wood anatomy of Holmskioldia sanguinea. (a) T.S showing semi-ring porous wood. (b) T.S showing sparse paratracheal parenchyma. (c) T.L.S. showing uniseriate and multiseriate rays. (d) Magnified portion showing perforated ray cells. (e) T.L.S. showing libriform fibres and septate fibres. (f) R.L.S. showing upright and square ray cells. (g) T.L.S. showing simple perforation plate. (h) Magnified portion of crystals. (i) SEM image of starch grains and bordered pits. Labels: sp =sparse paratracheal parenchyma, ur = uniseriate ray, trr = tetraseriate ray, $\mathrm{pc}=$ perforated ray cell, sf = septate fibre, If = libriform fibre, pp = perforation plate, $\mathrm{cr}=$ crystal, $\mathrm{bp}=$ bordered pit, $\mathrm{sg}=$ starch grain. Scale bars: (a), (c), (f) $=200 \mu \mathrm{~m}$; (b), (d), (e), (g), (h) $=50 \mu \mathrm{~m}$; (j) $=10 \mu \mathrm{~m}$.

Presence of perforated ray cells in the uniseriate to multiseriate rays in the wood of $H$. sanguinea is important. Such cells function as conducting elements and usually connect vessels located at opposite sides of the ray. These cells are known to occur in lianas (Angyalossy et al., 2009, 2012; Pace and Angyalossy, 2013).

Rhomboidal crystals are common in the wood of Lamiaceae (Carlquist, 1992). Sayeeduddin and Moinuddin (1939) reported the occurrence of oxalate of lime in the form of prismatic, rod like simple or aggregate crystals in all parts of $H$. sanguinea plant, but quite meagre in roots. However, in the present study calcium oxalate crystals have been observed only in root wood; in the case of stem these are seen in the pith region only.

Carlquist (1992) recognized vulnerability (v) and mesomorphy (m) of wood as indicators of ecological tendencies of the plant. Low values of $v$ and $m$ (less than 1 and 50, respectively) indicate adaptation to xeric conditions, whereas their high values (more than 1 and 800, respectively) point towards adaptation to mesic conditions. These indices undergo a decline in plants grown under environmental stress such as atmospheric pollution (which often leads to water stress), thus showing a shift towards xeromorphy due to stressful habitat condition (Iqbal et al., 2010). However, in $H$. sanguinea, vulnerability index for stem wood is lower than 1 ( 0.41 ), indicating its adaptation to xeric conditions, while mesomorphy index (165) represents an intermediate condition between xeric and mesic plants.

Anatomical features of the stem wood of $H$. sanguinea like wider vessels and extensive height of rays support the climbing nature of its stem axis. Presence of perforated ray cells is also a liana feature, while lower value of vulnerability, presence of wider vessels with simple perforation plate, presence of helical thickening are indicators of wood xeromorphy in the species studied.

## ACKNOWLEDGEMENTS

We are thankful to Professor D.K. Chauhan, Department of Botany, University of Allahabad, and Ms. Arpita Tripathi, CIMAP, Lucknow, for their help in collection of the materials. We also thank Dr. M.M. Dwivedi, National Centre of Experimental Mineralogy and Petrology (NCEMP), University of Allahabad for help with electron microscopy, and the reviewers of the MS for useful suggestions.

## LITERATURE CITED

Acevedo-Rodríguez, P. 2005. Vines and Climbing Plants of Puerto Rico and the Virgin Islands.The United States National Herbarium Vol. 51.Dept. Bot., National Museum of Natural History, Washington, DC. 483pp.
Angyalossy, V., G. Angeles and C. Madero-Vega. 2009. The mirror effect on xylem and phloem radial conduction. Proceedings of the Sixth Plant Biomechanics Conference, Cayenne, 156-162.

Angyalossy, V., G. Angeles and M. R. Pace. 2012. An overview on the anatomy, development and evolution of the vascular system of lianas. Plant Eco.and Diversity 5: 167-182.
Berlyn, G. P. and J. P. Miksche. 1976. Botanical Microtechnique and Cytochemistry. Ames: Iowa State University Press, 326 pp.
Bose, T. K. and B. Chowdhury. 1991. Tropical Garden Plants in Colour. Horticulture and Allied Publishers. 452pp.
Carlquist, S. 1975. Ecological Strategies of Xylem Evolution. University of California Press, Berkeley. 259 pp.
Carlquist, S. 1977. Ecological factors in wood evolution: a floristic approach. Am. J. Bot. 64(7): 887-896.
Carlquist, S. 1983. Wood anatomy of Onagraceae: further species; root anatomy; significance of vestured pits and allied structures in dicotyledons. Ann. Missouri Bot. Gard.69(4): 755-769.
Carlquist, S. 1985. Observations on functional wood histology of vines and lianas: Vessels dimorphism, tracheids, vasicentric tracheids, narrow vessels, and parenchyma. Aliso 11(2): 139-157.
Carlquist, S. 1988. Comparative Wood Anatomy. Systematic, Ecological and Evolutionary Aspects of Dicotyledon Wood. Springer-Verlag, Berlin, Heidelberg. 385 pp.
Carlquist, S. 1992. Wood anatomy of Lamiaceae. A survey with comments on vascular and vasicentric tracheids. Aliso 13(2): 309-338.
Ewers, F. W., M. R. Carlton, J. B. Fisher, K. J. Kolb and M. T. Tyree. 1997. Vessel diameters in root versus stem of tropical lianas and other growth forms. IAWA Journal 18(3): 261-279.
Fisher, J. B. and F. W. Ewers. 1995. Vessel dimensions in lianas and tree species of Gnetum (Gnetales). Am. J. Bot. 82(11): 1350-1357.
Gamble, J. S. 1972. A Manual of Indian timbers: an account of the growth, distribution and uses of the trees and shrubs of India and Ceylon with descriptions of their wood-structure. Bishen Singh and Mahendra Pal Singh, Dehra Dun. 868pp.
Harrar, E. S. 1946. Notes on starch grains in septate fiber-tracheids. Tropical Woods, 85: 1-9
IAWA Committee. 1989. List of microscopic features of hard wood identification. IAWA Bull.10(3): 219-332.
Iqbal, M., J. Jura-Morawiec, W. Wloch and Mahmooduzzafar. 2010. Foliar characterstics, cambial activity and wood formation in Azadirachta indica A. Juss. as affected by coal-smoke pollution. Flora 205(1): 61-71.
Ingole, S. N. 2011. Diversity and useful products in some Verbenaceous member of Melghat and Amravati regions, Maharashtra, India.Biodiversitas 12(1): 146-163.
Johansen, A. C. 1940. Plant Microtechnique.McGraw-Hill, New-York, 523pp.
Metcalfe, C. R. and L. Chalk. 1950. The Anatomy of Dicotyledons: Vols 1 and 2., Clarendon Press, Oxford, 1500 pp .
Pace, M. R. and V. Angyalossy. 2013. Wood evolution: a case study in the Bignoniaceae. Int. J. Plant Sci. 174(7): 1014-1048.
Solereder, H. 1908. Systematic Anatomy of the Dicotyledons, Vols1 and 2. Clarendon Press, Oxford, 1175 pp.
Sayeeduddin, M. and M. Moinuddin. 1939. Anatomical study of Holmskioldia sanguinea Retz. (Verbenaceae). J. Indian Bot. Soc. XVIII (2): 31-33

