Received: 11 March 2020 | Revised: 17 October 2020 | Accepted: 9 November 2020

DOI: 10.1002/jemt.23650

RESEARCH ARTICLE

MICROSCOPY RESEARCH & TECHNIQUE WILEY

Evaluation of natural dyes *Curcuma longa* and *Nyctanthes arbor-tristis* with different mordants on plant tissues under fluorescence microscopy

Nikita G. Shet Verenkar | Krishnan Sellappan

Department of Botany, Goa University, Goa, India

Correspondence
Krishnan Sellappan, Department of Botany, Goa University, Goa, India.
Email: skrish@unigoa.ac.in

Funding information
Department of Science, Technology and Environment, Goa, Grant/Award Number: 8-318-2016/STE-DIR/Accts/470; University Grants Commission, Grant/Award Number: F.3-9/2015/DRS-III

Review Editor: Paolo Bianchini

Abstract

The study reveals the effect of natural dyes *Curcuma longa* (rhizome) and *Nyctanthes arbor-tristis* (corolla tube) on various plant tissues under fluorescence microscopy. The results indicated that the auto-fluorescence was seen in some of the tissues of unstained monocot and dicot stem sections. The use of *Curcuma longa* dye along with different chemical mordants leads to various colors and intensity of fluorescence under different excitation filters (violet: 400–450; blue: 450–500; green: 500–570; yellow: 570–610 and red: 610–750 nm) examined. The extract of *Nyctanthes arbor-tristis* along with the mordants lead to bluish-orange fluorescence of vascular tissues under violet excitation filter. Among the dyes evaluated, *Nyctanthes arbor-tristis* showed better fluorescence than the *Curcuma longa* dye. Hence, the dye extracted from the corolla tube of *Nyctanthes arbor-tristis* is a potential natural dye and could be used as biological stain for staining plant tissues.

KEYWORDS

Curcuma longa, fluorescence microscopy, mordants, natural dyes, *Nyctanthes arbor-tristis*

1 | INTRODUCTION

The microscopic preparations of any biological specimen involves: fixation of the material, followed by dehydration, clearing, embedding and sectioning of the tissue. Free-hand sectioning provides a rapid and inexpensive microscopic observation of the internal structures of living plant tissues (Lux, Shigenori, Jun, & Kaori, 2005). Staining is a crucial technique for differentiating and highlighting the important features of the cells and tissues in biological samples. Some of the biological structures are transparent due to the presence of little or no color pigment in their cells and hence there is a need to employ the stains or dyes that can create a contrast between the cellular structures. The aqueous or alcoholic solutions of the dye are considered as simple stains (Avwioro, 2002). Stains are generally used to add color to the plant and animal tissues, microbes and spores that makes them optically distinct (Korade, Lalita, & Deepika, 2014). Some of the dyes require addition of mordants (chemical salt) which act as a bridge between the dye and the tissues (Avwioro, 2002).

One of the commonly used stains is safranin (Ma, Sawhney, & Steeves, 1993) which stains lignin, chromosomes, nucleoli, cutin, resins and gums and cork (Horobin & Kiernan, 2002; Johansen, 1940; Kasten, 1989; Ruzin, 1999). Some cellular components respond differentially to certain dyes/stains for example eosin dye stains the cytoplasm pink/red whereas the Feulgen's stain imparts red color to the chromosome. The Leishman's stain gives red-pink color to the blood cells while safranin stains the nuclei red. The plant cell walls are composed of cellulose, hemicelluloses and pectins and lignins. The Fast green stain is used to stain cellulose whereas phloroglucinol stain is used for staining lignins.

Fluorescence is an important phenomenon in which a substance absorbs shorter wave length of the light of some color and almost instantaneously re-emits as light of another color in longer wavelength and with lower energy, hence fluorescence occur and the same is observed under fluorescence microscopy. In general, dyes have specific excitation and emission range of wavelengths, but pH may influence the fluorescence emission. However, these changes in fluorescence may also occur due to the increase in dye–dye or dye-substrate interactions

(Lakowicz, 2006). But some cell wall components, such as lignin requires no dye as it auto-fluoresce with maximum absorbance in the UV range (Albinsson, Li, Lundquist, & Stomberg, 1999; Donaldson & Bond, 2005).

Synthetic dyes with azo bonds nitro or amino groups are carcinogenic in nature (Aguoru, Okelionwu, & Olasan, 2016; Braide, Akobundu, Nwaoguikpe, & Njiribaeko, 2011; Suryawanshi, Naik, Kumar, & Gupta, 2017). Also the cationic dye Safranin-O is one of the harmful contaminant which has negative effects on skin, digestive system and respiratory system of humans (Bayazit, 2014). These environmental threats evoked the desire of a researcher to find a substitute for these harmful synthetic dyes. Thus, there was a growing interest in the use of harmless, cheaper, nontoxic and ecofriendly natural dyes.

Plants are considered to be the potential source of natural dyes as many researchers have extracted various colors from local plants (Abubakar, Usman, Etim, Nnadi, & Alaku, 2012; Bhuyan & Saikia, 2003; Egbujo, Adisa, & Yahaya, 2008; Kamel & Najmaddin, 2016; Raheem, Ibnouf, Shingeray, & Farah, 2015; Siva, 2007; Verenkar & Krishnan, 2017; Yan, Pan, & Ji, 2018). Several natural dyes are used in histology, histochemistry and histopathology. One of the most important and commonly used histological dye is haematoxylin (Avwioro, 2002) which is a natural dye produced from *Haematoxylon campechianum* Linn. It is used in the combination with eosin while staining to understand general tissue structures (Avwioro, 2002). Several other natural dyes are used in anatomical field. The ethanolic extract from the leaves of *Lawsonia inermis* Linn. (henna) contains lawsone dye which was used in staining chromosome of *Allium cepa* (Aguoru & Okoli, 2004). The aqueous extract of henna was used for staining plant epidermal cells of *Allium cepa* (Aguoru, Okpe, & Olasan, 2015).

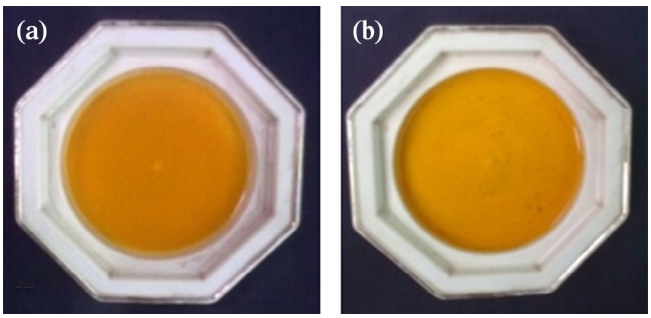

FIGURE 1 Dye extracts. (a) *Curcuma longa* (rhizome); (b) *Nyctanthes arbor-tristis* (corolla tube) [Color figure can be viewed at wileyonlinelibrary.com]

Curcuma longa Linn. is a perennial herb belonging to family Zingiberaceae which is well known traditional Indian spice and used in Ayurvedic and Chinese systems of medicine. It is native to tropical South Asia. The curcumin is the bio-active component of turmeric which is used as a natural dye (Priyadarsini, 2014) source of coloring agent for foods, textiles and cosmetics. It contains medicinal properties like anti-diabetic, anti-ulcer, anti-coagulant, anti-fertility (Yadav & Tarun, 2017) anti-viral, anti-microbial, anti-fungal (Moghadamtousi et al., 2014) anti-oxidant, anti-inflammatory, antiseptic, and anticancer. Researchers have attempted to use the curcumin for staining collagen and muscle fibers (Kumar, Singh, Singh, Singh, & Singh, 2014). On the other hand, Abraham et al. (2017) found that the turmeric can excellently replace the eosin stain.

Nyctanthes arbor-tristis Linn. is a well-known plant widely distributed in India, including sub-Himalayan regions and southwards to Godavari which belongs to family Oleaceae. The coloring matter nyctanthin is present in the corolla tube of the plant (Gokhale, Tatiya, Bakliwal, & Fursule, 2004). Flowers also contain other chemicals like glucose, tannin, carotenoid and glycosides (Hiremath, Hiremath, Mohapatra, & Das, 2016).

The present study was undertaken to evaluate the staining potential of dye extracts of *Curcuma longa* (rhizome) and *Nyctanthes arbor-tristis* (corolla tube) on dicot and monocot stem sections and to study and understand the fluorescence in different plant tissues under various excitation filters using fluorescence microscopy.

TABLE 1 Effect of natural dyes *Curcuma longa* and *Nyctanthes arbor-tristis* on monocot and dicot stem sections

	Curcuma longa		*Nyctanthes arbor-tristis*	
Staining duration (min)	**Dicot stem**	**Monocot stem**	**Dicot stem**	**Monocot stem**
5	Good	Fair	Fair	Fair
15	Good	Fair	Good	Good
20	Excellent	Excellent	Excellent	Excellent
30	Over-stained	Over-stained	Over-stained	Over-stained

TABLE 2 Auto-fluorescence of dicot and monocot stem sections under different excitation filters in fluorescence microscopy

			Different excitation filters				
Unstained section	**Major plant tissues examined**	**Bright field microscopy**	**Violet 400–450 nm**	**Blue 450–500 nm**	**Green 500–570 nm**	**Yellow 570–610 nm**	**Red 610–750 nm**
Dicot stem	Vascular tissues	No color	Light blue	Light green	Red	Yellow	Light blue
	Epidermis	No color	Light blue	Light green	Red	Yellow	Light blue
Monocot stem	Vascular tissues	No color	Blue	Green	Red	Orange	Bluish-pink
	Epidermis	No color	Blue	Green	Red	Orange	Bluish-pink
	Ground tissue	No color	No color	No color	No color	No color	No color

TABLE 3 Effect of *Curcuma longa* dye and chemical mordants on dicot and monocot stem sections under bright-field microscopy and under different excitation filters in fluorescence microscopy

				Different excitation filters				
Dyes and mordants used	**Stems**	**Major plant tissues examined**	**Bright field**	**Violet 400–450 nm**	**Blue 450–500 nm**	**Green 500–570 nm**	**Yellow 570–610 nm**	**Red 610–750 nm**
Curcuma longa	Dicot	Vascular tissues Epidermis	Yellow Yellow	Green Green	No fluorescence	No fluorescence	No fluorescence	Over-fluoresced
	Monocot	Vascular tissues Epidermis Ground tissue	Yellow Yellow No color	Green Green Green	No fluorescence	No fluorescence	Green Green Green	Over-fluoresced
Curcuma longa + $CuSO_4$	Dicot	Vascular tissues Epidermis	Yellow Yellow	Green Green	No fluorescence	No fluorescence	No fluorescence	Over-fluoresced
	Monocot	Vascular tissues Epidermis Ground tissue	Yellow Yellow No color	Green Green Green	No fluorescence	No fluorescence	Green Green Green	Over-fluoresced
Curcuma longa + $FeSO_4$	Dicot	Vascular tissues Epidermis	Yellow Yellow	Green Green	No fluorescence	No fluorescence	No fluorescence	Over-fluoresced
	Monocot	Vascular tissues Epidermis Ground tissue	Yellow Yellow No color	Green Green Green	No fluorescence	No fluorescence	Green Green Green	Over-fluoresced
Curcuma longa + $K_2Cr_2O_7$	Dicot	Vascular tissues Epidermis	Yellow Yellow	Green Green	No fluorescence	No fluorescence	No fluorescence	Over-fluoresced
	Monocot	Vascular tissues Epidermis Ground tissue	Yellow Yellow No color	Green Green Green	No fluorescence	No fluorescence	Green Green Green	Over-fluoresced

2 | MATERIALS AND METHODS

2.1 | Preparation of dye extracts from *Curcuma longa* and *Nyctanthes arbor-tristis*

The rhizomes of *Curcuma longa* were collected fresh from Valpoi, Sattari Goa, India. The rhizome was cut 0.5 cm thick and dried at normal room temperature for 5 days. They were milled and 1 g of powder was weighed using digital balance and dissolved in 20 ml of distilled water and boiled at 60°C for 12 hr in water bath to get maximum dye. The dark yellowish dye obtained was filtered using Whatman filter paper (Grade-A) and used for staining (Figure 1a). The corolla tube of *Nyctanthes arbor-tristis* was collected from Quepem, Goa, India. The collected materials were shade dried at room temperature and extracted in (1 g/20 ml) distilled water for 12 hr in water bath at 60°C to get the maximum dye extract. The extract obtained was filtered using Whatman filter paper (Grade-A) (Figure 1b).

2.2 | pH of natural dyes

The pH of natural dyes was measured using pH meter and all the readings were taken in replicates of three.

2.3 | Sectioning

Free-hand thin transverse section (t.s.) from fresh samples of both dicot stem (*Chromolaena odorata*) and monocot stem (*Cyanodon dactylon*) were taken. Several free-hand sections were obtained and transferred into clean distilled water for further staining procedure.

2.4 | Preparation of mordant solutions

Three chemical mordants were prepared in distilled water (0.1 g/20 ml) viz. potassium dichromate ($K_2Cr_2O_7$), copper sulfate ($CuSO_4$), and ferrous sulfate ($FeSO_4$).

2.5 | Staining procedure with natural dyes

Thin sections of about (10 μm) thickness of both dicot and monocot stem were chosen and transferred to staining solution, that is, dye extracts of *Curcuma longa* and *Nyctanthes arbor-tristis*. The sections were allowed to stain for 5, 15, 20, and 30 min and then transferred in distilled water to remove excess stain. This was carried out to see

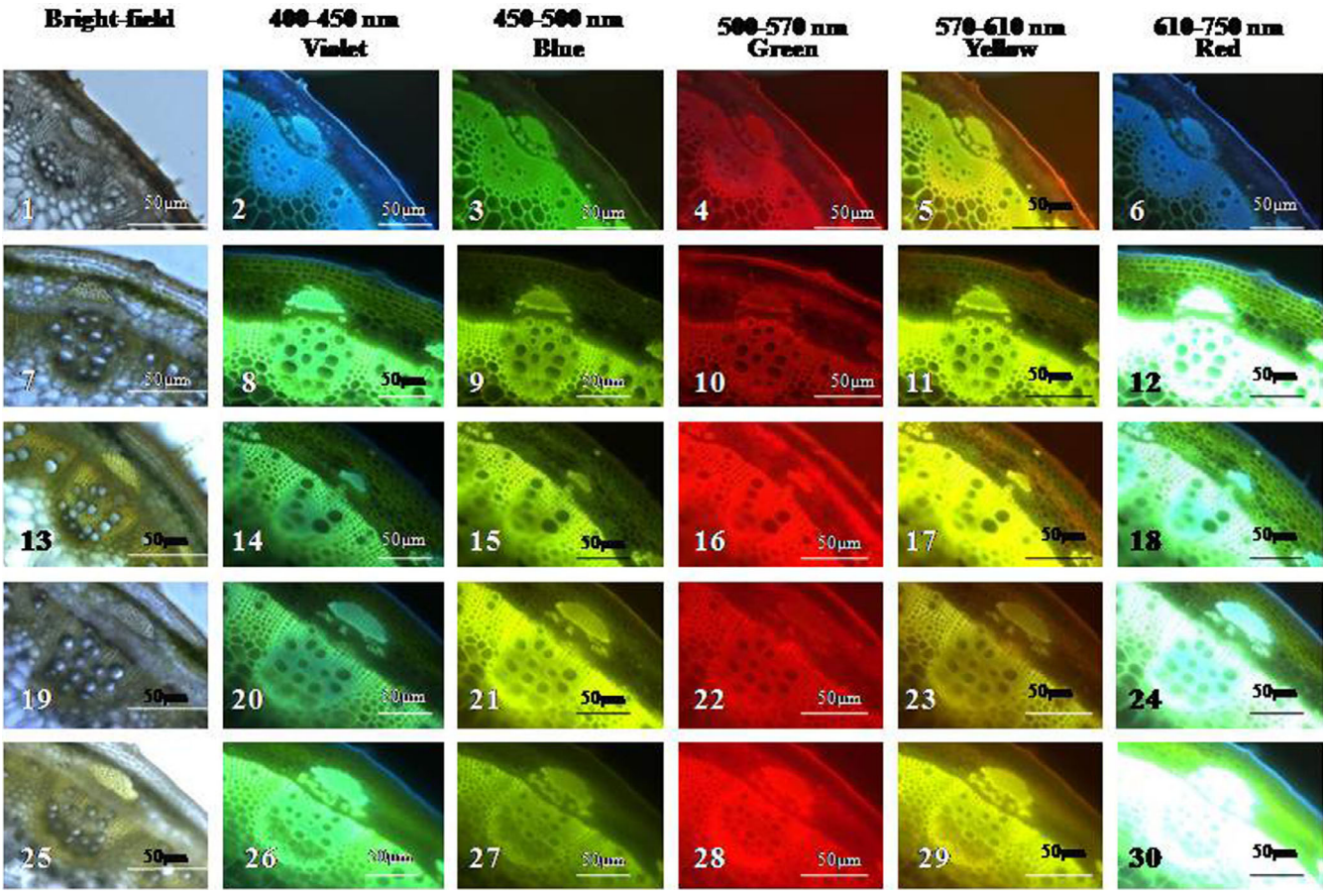

FIGURE 2 Fluorescence images of transverse section of dicot stem stained with *Curcuma longa* dye and along with different mordants (×200). 1. Unstained section; 2–6. Auto-fluorescence; 7–12. Stained with *C. longa* dye; 13–18. *C. longa* dye and $CuSO_4$ mordant; 19–24. *C. longa* dye and $FeSO_4$ mordant; 25–30. *C. longa* dye and $K_2Cr_2O_7$ mordant [Color figure can be viewed at wileyonlinelibrary.com]

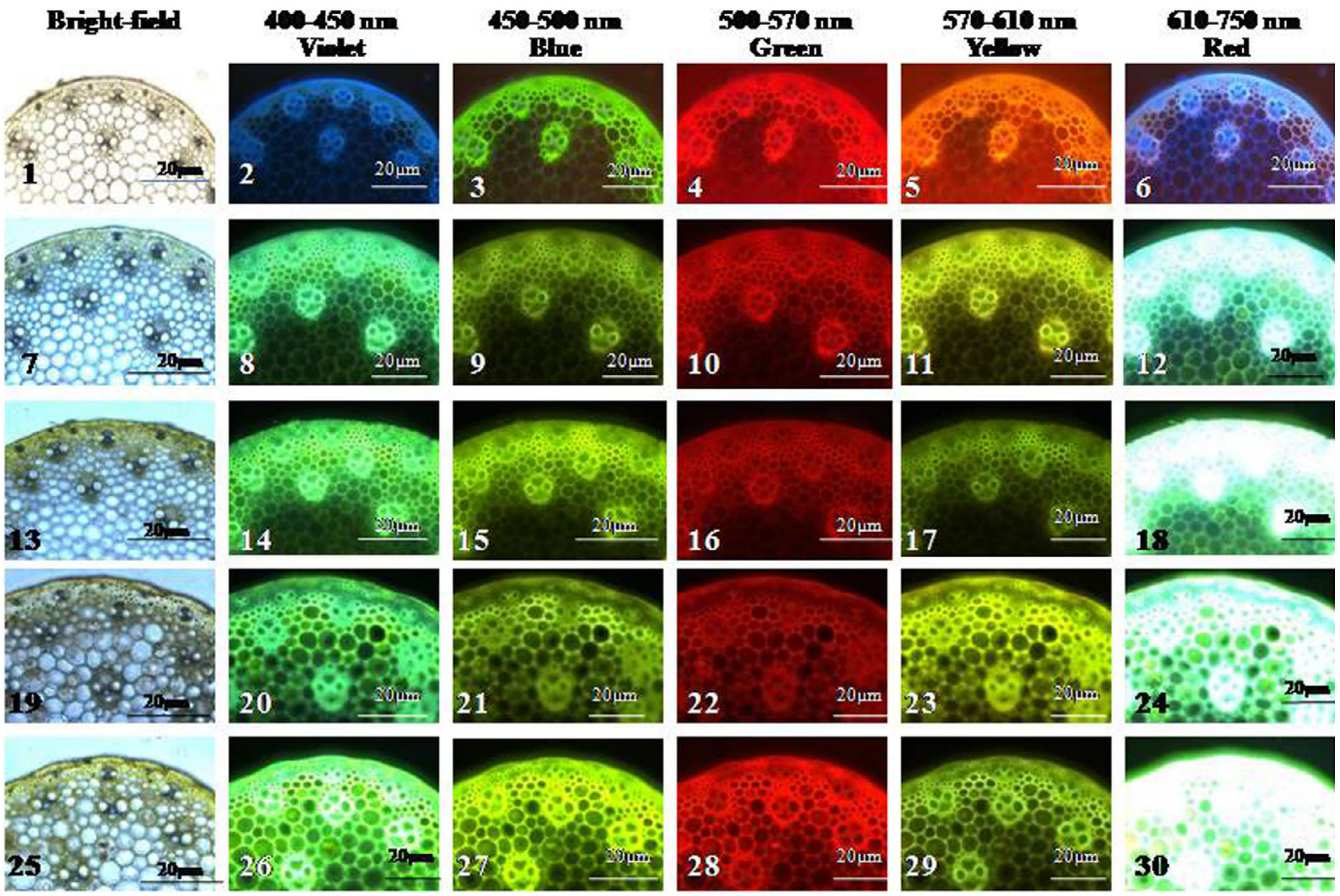

FIGURE 3 Fluorescence images of transverse section of monocot stem stained with *Curcuma longa* dye and along with different mordants (×400). 1. Unstained section; 2–6. Auto-fluorescence; 7–12. Stained with *C. longa* dye; 13–18. *C. longa* dye and $CuSO_4$ mordant; 19–24. *C. longa* dye and $FeSO_4$ mordant; 25–30. *C. longa* dye and $K_2Cr_2O_7$ mordant [Color figure can be viewed at wileyonlinelibrary.com]

the optimal staining period for both the dyes. The stained sections were then mounted with glycerin on a clean glass slide and observed under bright-field and fluorescence microscopy.

2.6 | Mordant staining

The sections were treated with each mordant separately, prior to staining in natural dye extract. The sections were individually stained in mordant solution of $K_2Cr_2O_7$, $CuSO_4$, and $FeSO_4$ for 10 min to enhance the staining intensity and then transferred to respective natural dyes viz. *Curcuma longa* and *Nyctanthes arbor-tristis* for 20 min. The sections were later transferred to distilled water to remove the excess stain and mounted on clean glass slide using glycerin. The stained sections were then observed under bright-field and fluorescence microscopy.

2.7 | Photography

The stained sections were observed under bright-field and fluorescence microscopy attached with digital camera and image analyzing system (OLYMPUS BX 53 microscope). The microphotographs were taken using ×20 objectives (for dicot) and ×40 objectives (for monocot). The slides were observed under five different excitation (fluorescence) filters with the wavelength 400–450 nm (violet), 450–500 nm (blue), 500–570 nm (green), 570–610 nm (yellow), and 610–750 nm (red). The staining intensity of each section was studied.

3 | RESULTS

The use of dyes to improve the contrast in plant tissues has been substantiated in this work. In both the natural dyes, staining period of 5–15 min gave fair to good staining in the dicot and monocot stem sections. However, 20 min staining period lead to excellent staining of vascular bundles in dicot and monocot stem with clear differentiation of tissues. But at 30 min, the stain was too intense which completely overshadowed in distinguishing the plant tissues as shown in (Table 1). Therefore, 20 min was considered as an optimal staining time for both the natural dyes. Both dyes are acidic in nature with pH (6.10 ± 0.01) of *Curcuma longa* dye and pH (4.32 ± 0.01) of *Nyctanthes arbor-tristis* dye.

TABLE 4 Effect of *Nyctanthes arbor-tristis* dye and chemical mordants on dicot and monocot stem sections under bright-field microscopy and under different excitation filters in fluorescence microscopy

				Different excitation filters				
Dyes and mordants used	**Stems**	**Major plant tissues examined**	**Bright field**	**Violet 400–450 nm**	**Blue 450–500 nm**	**Green 500–570 nm**	**Yellow 570–610 nm**	**Red 610–750 nm**
Nyctanthes arbor-tristis	Dicot	Vascular tissues	Yellow	Bluish-orange	Orange	No fluorescence	Red	Bluish-orange
		Epidermis	Yellow	Bluish-orange	Orange		Red	Bluish-orange
	Monocot	Vascular tissues	Yellow	Bluish-orange	Orange	No fluorescence	No fluorescence	Bluish-orange
		Epidermis	Yellow	Bluish-orange	Orange			Bluish-orange
		Ground tissue	Yellow	Bluish-orange	Orange			
Nyctanthes arbor-tristis + $CuSO_4$	Dicot	Vascular tissues	Yellow	Bluish-orange	Orange	No fluorescence	Red	Red
		Epidermis	Yellow	Bluish-orange	Orange		Red	Red
	Monocot	Vascular tissues	Yellow	Bluish-orange	Orange	No fluorescence	No fluorescence	Bluish-orange
		Epidermis	Yellow	Bluish-orange	Orange			Bluish-orange
		Ground tissue	Yellow	Bluish-orange	Orange			
Nyctanthes arbor-tristis + $FeSO_4$	Dicot	Vascular tissues	Yellow	Bluish-orange	Orange	No fluorescence	Red	Bluish-orange
		Epidermis	Yellow	Bluish-orange	Orange		Red	Bluish-orange
	Monocot	Vascular tissues	Yellow	Bluish-orange	Orange	No fluorescence	No fluorescence	No fluorescence
		Epidermis	Yellow	Bluish-orange	Orange			
		Ground tissue	Yellow	Bluish-orange	Orange			
Nyctanthes arbor-tristis + $K_2Cr_2O_7$	Dicot	Vascular tissues	Yellow	Bluish-orange	Orange	No fluorescence	Red	Bluish-orange
		Epidermis	Yellow	Bluish-orange	Orange		Red	Bluish-orange
	Monocot	Vascular tissues	Yellow	Bluish-orange	Orange	No fluorescence	No fluorescence	Bluish-orange
		Epidermis	Yellow	Bluish-orange	Orange			Bluish-orange
		Ground tissue	Yellow	Bluish-orange	Orange			

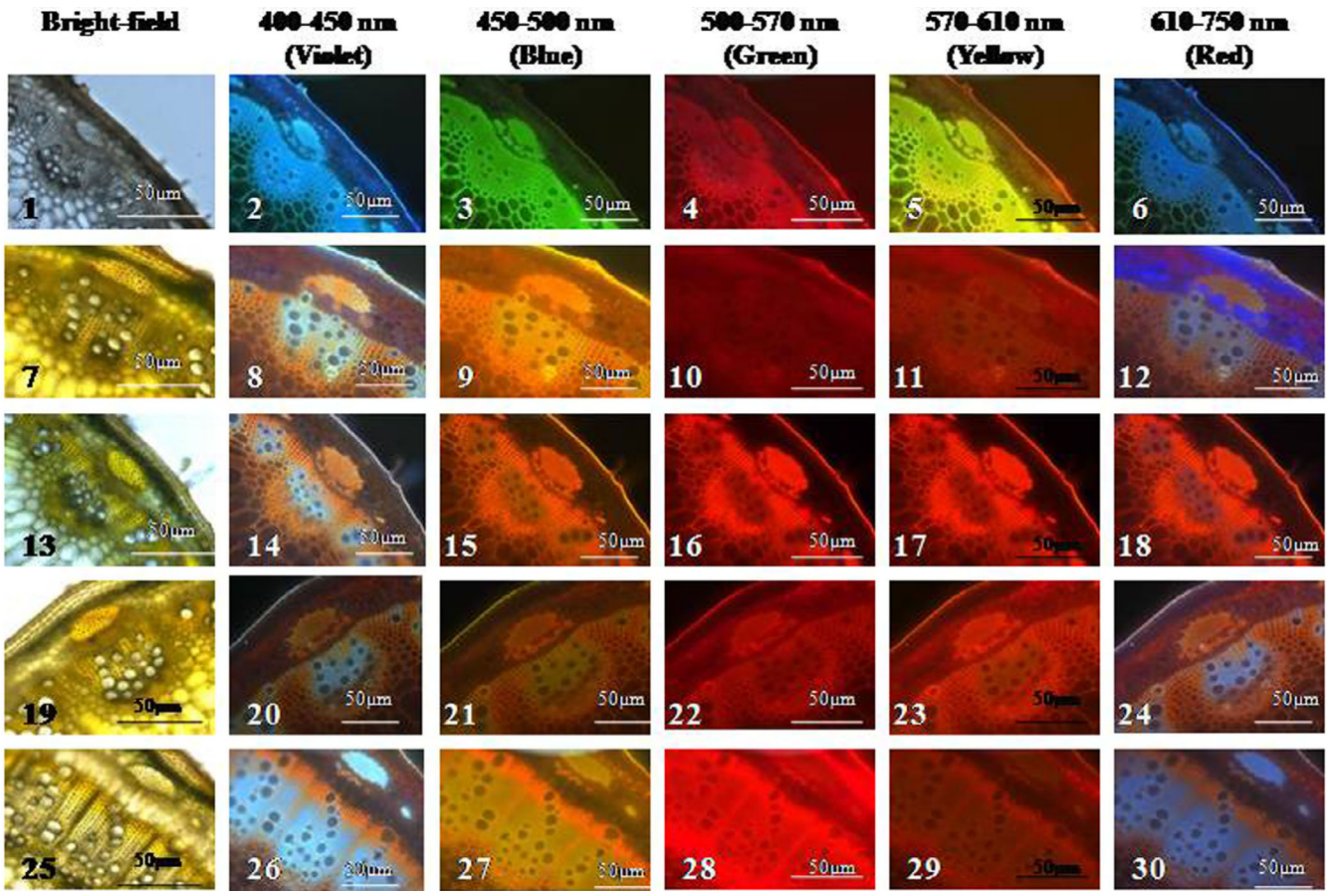

FIGURE 4 Fluorescence images of transverse section of dicot stem stained with *Nyctanthes arbor-tristis* dye and along with different mordants (×200). 1. Unstained section; 2–6. Auto-fluorescence; 7–12. Stained with dye *Nyctanthes arbor-tristis*; 13–18. *Nyctanthes arbor-tristis* dye and $CuSO_4$ mordant; 19–24. *Nyctanthes arbor-tristis* dye and $FeSO_4$ mordant; 25–30. *Nyctanthes arbor-tristis* dye and $K_2Cr_2O_7$ mordant [Color figure can be viewed at wileyonlinelibrary.com]

3.1 | Fluorescence study of dicot and monocot stems under different excitation filters

The unstained section of dicot stem when observed under different fluorescence (excitation) filters lead to auto-fluorescence of the sections. The vascular tissues showed different colors of fluorescence with different excitation filters: light blue with violet and red filters, light green with blue filter, red with green filter and yellow with yellow filter.

The unstained monocot stems section also auto-fluoresced under different excitation filters. The vascular tissues showed different colors of fluorescence with different excitation filters: blue with violet filter, green with blue filter, red with green filter and orange with yellow filter and bluish pink with red filter. However, no fluorescence was seen on ground tissue (Table 2).

The vascular tissues and the epidermis of the dicot stem section were stained yellow with *Curcuma longa* dye under bright-field microscopy. With the use of mordants $K_2Cr_2O_7$, $CuSO_4$, and $FeSO_4$ along with the dye the yellow color was profound, than that of individual staining with *Curcuma longa* dye. In violet excitation filter vascular tissues fluoresced green when stained with the dye individually as well as with $K_2Cr_2O_7$, $CuSO_4$, and $FeSO_4$ mordants. The blue, green and yellow filters did not show any fluorescence even on application of all the mordants along with the dye. The red excitation filter over-fluoresced the tissues. No special effects on fluorescence were seen with the application of all the three chemical mordants (Table 3 and Figure 2).

In monocot stem the vascular tissues and epidermis stained yellow in color. However, no staining was seen in the ground tissue under bright-field microscopy. The violet excitation filter fluoresced the vascular tissue, epidermis and ground tissue in green color when stained with *Curcuma* dye individually and along with the mordants. However, no fluorescence was seen in blue and green filter. The yellow excitation filter leads to green fluorescence of vascular tissues whereas the red filter over-fluoresced the cell components (Table 3 and Figure 3).

The dye from *Nyctanthes arbor-tristis* imparted yellow coloration to the vascular tissues and the epidermis in dicot stem. However, the color was more profound on use of chemical mordants $K_2Cr_2O_7$, $CuSO_4$, and $FeSO_4$ under bright-field microscopy. In violet and red excitation filters the vascular tissues fluoresced bluish-orange with the use of all mordants, except $CuSO_4$ mordant which lead to red

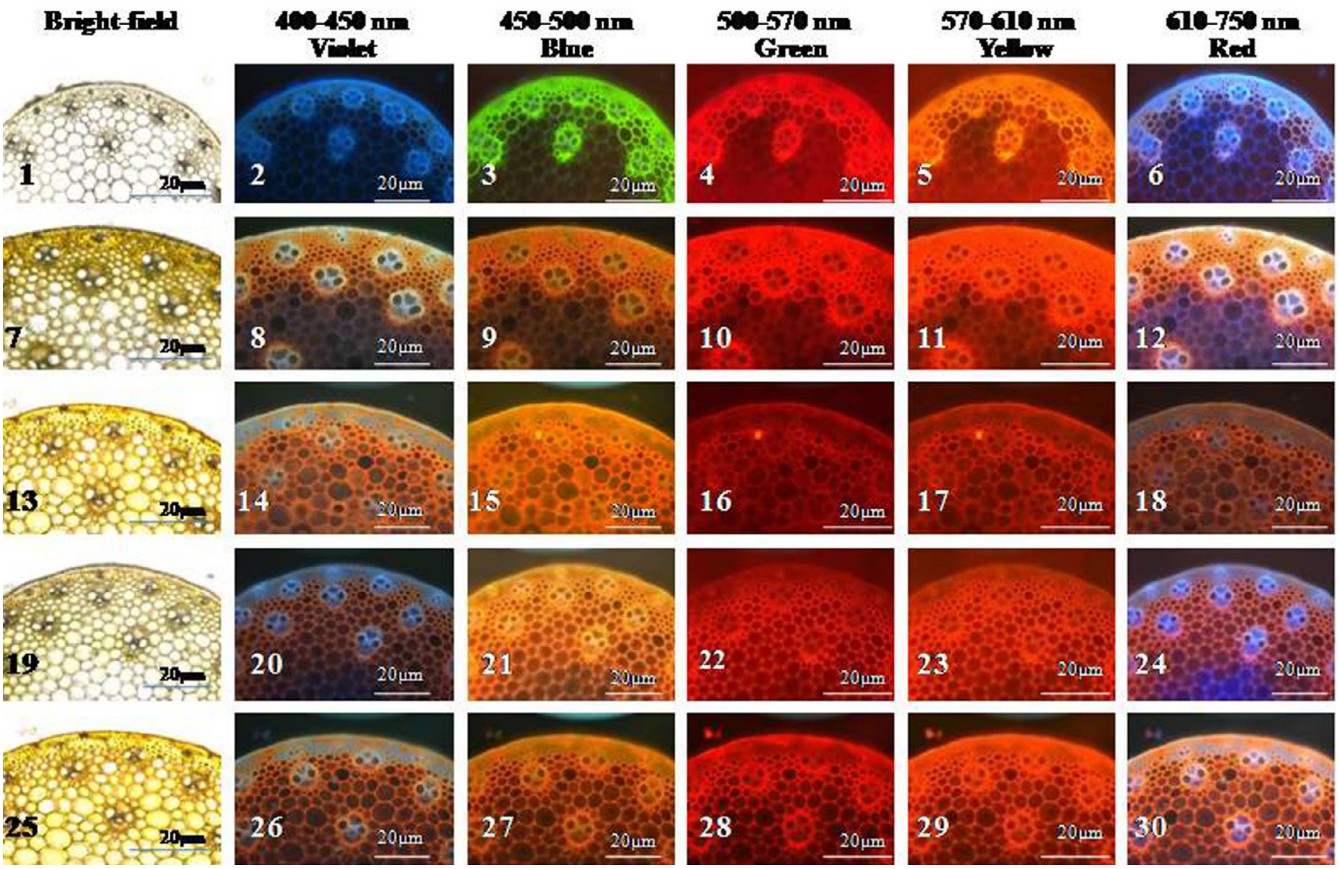

FIGURE 5 Fluorescence images of transverse section of monocot stem stained with *Nyctanthes arbor-tristis* dye and along with different mordants (×400). 1. Unstained section; 2–6. Auto-fluorescence; 7–12. Stained with *Nyctanthes arbor-tristis* dye; 13–18. *Nyctanthes arbor-tristis* dye and $CuSO_4$ mordant; 19–24. *Nyctanthes arbor-tristis* dye and $FeSO_4$ mordant; 25–30. *Nyctanthes arbor-tristis* dye and $K_2Cr_2O_7$ mordant [Color figure can be viewed at wileyonlinelibrary.com]

fluorescence in red excitation filter. The blue filter showed orange fluorescence whereas no fluorescence was seen in green excitation filter. In yellow filter the vascular tissues and epidermis fluoresced bright red in color (Table 4 and Figure 4).

The yellow color dye from *Nyctanthes arbor-tristis* (corolla tube) stained the monocot section bright yellow under bright-field microscopy. The violet and red excitation filters lead to bluish-orange fluorescence of the tissues, whereas the blue filter lead to orange fluorescence. No fluorescence was observed in green and yellow filters (Table 4 and Figure 5).

When *Nyctanthes arbor-tristis* dye was used, all the components fluoresced distinctly in both the monocot and dicot stem section under all the excitation filters except green filter.

4 | DISCUSSION

Staining facilitates the observation of cells and tissues under a microscope with the use of dye that has an affinity to the cell organelle (Kumar, Mehul, Das, & Solanki, 2015). A good biological stain should be effective, cheap, eco-friendly and the source must be easily available (Kharbude & Agarwal, 2000).

Curcumin is an active ingredient of turmeric which imparts characteristic yellow color to the plant tissue (Priyadarsini, 2014). The turmeric contains flavonoids, which are typically polyphenolic compounds. Phenols are acidic, due to their ability to release the hydrogen from their hydroxyl group and hence the extract stains the basic parts of the cell, mainly protein part of the cytoplasm (Priyadarsini, 2014). The curcumin was also used for staining sections of skin, liver, intestine, kidney, lung, and spleen (3 mm) of human tissue at post-mortem examination. The staining with curcumin was excellent and hence proved to be an alternative source to eosin stain (Avwioro et al., 2007).

Nyctanthin is the coloring component present in the corolla tube of *Nyctanthes arbor-tristis* flowers which acts as a source of yellow dye for staining plant tissues (Smitha, Sachidananda, Subhas, & Dinesha, 2014). This dye is also used for dyeing and painting of cotton and silk with Kalamkari technique using bamboo stick (Deshmukh & Dongre, 2015).

The fluorescence microscopy gave distinct colors to the vascular tissue and epidermis. The dyeing of tissues is dependent on binding forces to the tissues, or they will simply be rinsed out of the tissue when the section is washed in another reagent (Papawee, Suppaluk, & Natthawut, 2011). The positive ions are released by the dyestuff

which binds covalently to the negative ions present on the cell wall of the tissue this is due to the electrostatic bond reaction between the dye charge and the different cell parts of the cell tissue (Chukwu et al., 2011). Both the dyes used viz. *Curcuma longa* (pH—6.10) and *Nyctanthes arbor-tristis* (pH—4.32) are acidic in nature with low pH values and thus stains the basic part of the cell. Since, the *Nyctanthes arbor-tristis* being more acidic in nature leads to good fluorescence of the components in all five excitation filters. None of the sections were over-fluoresced in any of the filters like the use of *Curcuma* dye which lead to over-fluorescence of both monocot and dicot sections under red filter. Hence, *Nyctanthes arbor-tristis* can be used as an excellent fluorescent dye to distinguish the plant cell components.

ACKNOWLEDGMENTS

This work was financially supported by the Department of Science, Technology and Environment, Goa, India (grant number 8-318-2016/STE-DIR/Accts/470); University Grants Commission, New Delhi, India, at the level of DRS-III (grant number F.3-9/2015/DRS-III).

CONFLICT OF INTEREST

No potential conflict of interest was reported by the authors. Data sharing is not applicable to this article as no new data were created or analyzed in this study.

ORCID

Nikita G. Shet Verenkar https://orcid.org/0000-0003-3279-5306
Krishnan Sellappan https://orcid.org/0000-0001-7367-9542

REFERENCES

Abraham, M., Nambiar, S., Charagannavar, V., Augustine, D., Sowmya, S. V., Babu, A., & Rao, R. S. (2017). Comparison of staining efficacy between turmeric and eosin: A histological study. *Journal of Clinical and Diagnostic Research*, *11*(11), 5–8.

Abubakar, S., Usman, A. B., Etim, V., Nnadi, O., & Alaku, C. (2012). Application of organic dyes from roselle calyx (*Hibiscus sabdariffa* Linn) for mycological staining. *International Journal of Infectious Diseases*, *1*(9), 687–690.

Aguoru, C. U., Okelionwu, S., & Olasan, J. O. (2016). Value of *Hibiscus rosa-sinensis* petal extract as biological stain in plant systematic research. *Journal of Chemical, Biological and Physical Sciences*, *6*(2), 388–395.

Aguoru, C. U., & Okoli, B. E. (2004). Studies on *Baphia nitida* Lood (cam wood) crude extract as chromosome stain. *Annals of Research Nigeria*, *4*, 41–45.

Aguoru, C. U., Okpe, F. O., & Olasan, J. O. (2015). Comparative staining efficacy of safranin and *Lawsonia inermis* L. aqueous ethanolic leaf extract on epidermal cells of *Allium cepa* L. *International Journal of Current Research in Biosciences and Plant Biology*, *2*(4), 108–111.

Albinsson, B., Li, S. M., Lundquist, K., & Stomberg, R. (1999). The origin of lignin fluorescence. *Journal of Molecular Structure*, *508*, 19–27.

Avwioro, O. G. (2002). *Histochemistry and tissue pathology: Principles and techniques*. Nigeria: Claverianun Press.

Avwioro, O. G., Onwuko, S. K., Moody, J. O., Agbedahunsi, J. M., Oduola, T., Ekpo, O. E., & Oladele, A. A. (2007). *Cucuma longa* extract as a histological dye for collagen fibres and red blood cells. *Journal of Anatomy*, *210*(5), 600–603.

Bayazit, S. S. (2014). Investigation of safranin O adsorption on superparamagnetic iron oxide nanoparticles (SPION) and multi-wall carbon nanotube/SPION composites. *Desalination and Water Treatment*, *52* (37–39), 6966–6975.

Bhuyan, R., & Saikia, C. N. (2003). Extraction of natural colourants from roots of *Morinda angustifolia* Roxb.- their identification and studies of dyeing characteristics on wool. *Indian Journal of Chemical Technology*, *10*(2), 131–136.

Braide, W., Akobundu, C., Nwaoguikpe, R. N., & Njiribaeko, L. C. (2011). The use of extracts from four local Nigerian plants for staining of selected bacteria and moulds. *African Journal of Microbiology Research*, *5*(1), 79–86.

Chukwu, O. O. C., Odu, C. E., Chukwu, D. I., Hafiz, N., Chidozie, V. N., & Onyimba, I. A. (2011). Application of extracts of Henna (*Lawsonia inermis*) leaves as a counter stain. *African Journal of Microbiology Research*, *5*(21), 3351–3356.

Deshmukh, A., & Dongre, S. (2015). Natural yellow colour from corolla of *Nyctanthes arbor-tristis* Linn. for dyeing and painting on cotton and silk for value addition. *Biolife*, *3*(2), 353–357.

Donaldson, L. A., & Bond, J. (2005). *Handbook of textile fibre structure: Fluorescence microscopy of wood*. New Zealand: Elsevier.

Egbujo, E. C., Adisa, O. J., & Yahaya, A. B. (2008). A study of the staining effect of roselle (*Hibiscus sabdariffa*) on the histologic section of the testis. *International Journal of Morphology*, *26*(4), 927–930.

Gokhale, S. B., Tatiya, A. U., Bakliwal, S. R., & Fursule, R. A. (2004). Natural dye yielding plants in India. *Natural Product Radiance*, *3*(4), 228–234.

Hiremath, V., Hiremath, B. S., Mohapatra, S., & Das, A. K. (2016). Literary review of parijata (*Nyctanthes arbor-tristis* Linn.) an herbal medicament with special reference to ayurveda and botanical literatures. *Biomedical and Pharmacology Journal*, *9*(3), 1019–1025.

Horobin, R. W., & Kiernan, J. A. (2002). *Conn's biological stains: A handbook of dyes, stains and fluorochromes for use in biology and medicine*. United Kingdom: BIOS Scientific Publisher.

Johansen, D. A. (1940). *Plant microtechnique: Some principles and new methods*. New York, NY: McGraw-Hill.

Kamel, F. H., & Najmaddin, C. (2016). Use of some plants colour as alternative stain in staining of bacteria. *Kirkuk University Journal-Scientific Studies*, *11*(3), 248–253.

Kasten, F. H. (1989). *The origins of modern fluorescent microscopy and fluorescent probes: Cell structure and function by microspectrofluorometry*. Florida: Elsevier.

Kharbude, B. V., & Agarwal, A. (2000). Different colour stains of different plant extract. *Journal of Chromatography*, *347*, 447–452.

Korade, D., Lalita, S., & Deepika, M. (2014). Application of aqueous plant extracts as biological stains. *International Journal of Scientific Engineering and Research*, *5*(2), 1586–1589.

Kumar, N., Mehul, J., Das, B., & Solanki, J. B. (2015). Staining of Platyhelminthes by herbal dyes: An eco-friendly technique for the taxonomist. *Veterinary World*, *8*(11), 1321–1325.

Kumar, S., Singh, N., Singh, A., Singh, N., & Singh, R. (2014). Use of *Curcuma longa* L. extract to stain various tissue samples for histological studies. *AYU*, *35*(4), 447–451.

Lakowicz, J. R. (2006). *Principles of fluorescence spectroscopy: Protein fluorescence*. New York, NY: Springer.

Lux, A., Shigenori, M., Jun, A., & Kaori, I. (2005). An improved method for clearing and staining free-hand sections and whole-mount samples. *Annals of Botany*, *96*, 989–996.

Ma, Y., Sawhney, V. K., & Steeves, T. A. (1993). Staining of paraffin-embedded plant material in safranine and fast green without prior removal of the paraffin. *Canadian Journal of Botany*, *71*, 996–999.

Moghadamtousi, S., Kadir, H., Hassandarvish, P., Tajik, H., Abubakar, S., & Zandi, K. (2014). A review on antibacterial, antiviral and antifungal activity of curcumin. *BioMed Research International*, *2014*, 1–2.

Papawee, S., Suppaluk, R., & Natthawut, T. (2011). Extraction of natural histological dye from black plum fruit (*Syzygium cumini*). *Journal of the Microscopy Society of Thailand*, *4*(1), 13–15.

Priyadarsini, K. I. (2014). The chemistry of curcumin: From extraction to therapeutic agent. *Molecules*, *19*, 20091–20112.

Raheem, E. M. A., Ibnouf, A. A. O., Shingeray, O. H., & Farah, H. J. (2015). Using of *Hibiscus sabdariffa* extract as a natural histological stain of the skin. *American Journal of Research Communication*, *3*(5), 211–216.

Ruzin, S. E. (1999). *Microtechnique: Plant microtechnique and microscopy*. New York, NY: Oxford University Press.

Siva, R. (2007). Status of natural dyes and dye-yielding plants in India. *Current Science*, *92*(7), 916–925.

Smitha, P. S., Sachidananda, G., Subhas, C. M., & Dinesha, R. (2014). Stability and fluorescent studies of the coloring content of flowers of *Nyctanthes arbor-tristis*. *Global Journal of Biology, Agriculture and Health Sciences*, *3*(4), 80–82.

Suryawanshi, H., Naik, R., Kumar, P., & Gupta, R. (2017). *Curcuma longa* extract-Haldi: A safe, eco-friendly natural cytoplasmic stain. *Journal of Oral and Maxillofacial Pathology*, *21*(3), 340–344.

Verenkar, N. G. S., & Krishnan, S. (2017). Some potential natural dye yielding plants from the state of Goa, India. *Indian Journal of Natural Products and Resources*, *8*(4), 306–315.

Yadav, R., & Tarun, G. (2017). Versatility of turmeric: A review the golden spice of life. *Journal of Pharmacognosy and Phytochemistry*, *6*(1), 41–46.

Yan, S., Pan, S., & Ji, J. (2018). Silk fabric dyed with extract of Sophora flower bud. *Natural Product Research*, *32*(3), 308–315.

How to cite this article: Shet Verenkar NG, Sellappan K. Evaluation of natural dyes *Curcuma longa* and *Nyctanthes arbor-tristis* with different mordants on plant tissues under fluorescence microscopy. *Microsc Res Tech*. 2020;1–10. https://doi.org/10.1002/jemt.23650