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Application of *Terminalia chebula* natural dye on wool fiber—evaluation of color and fastness properties

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Abstract

In the present study, *Terminalia chebula* (Myrobalan/Harda) natural dye extract was used for the development of eco-friendly shades on woolen yarn with different hues and tones. The effect of dye concentration on color strength (*K/S*) of woolen yarn dyed with *T. chebula* was assessed. Increasing the concentration of dye decreased lightness (L^*) values of woolen yarn samples, indicating darker shades. Different metal salts such as alum, ferrous sulfate, and stannous chloride were used to enhance the fastness properties (light, wash, dry and wet rubs) of dyed woolen yarn. Pre-treatment of woolen yarn samples with metal salts has shown encouraging results with better fastness properties and enhanced color strength values. Five percent ferrous sulfate mordanted samples show greater saturation with increasing dye concentration from 0.5 to 15 % (o.w.f.). Fourier-transform infrared (FT-IR) analysis of *T. chebula* dye extract shows presence of carbonyl and hydroxyl functionalities.

Keywords: Natural dye, Harda, Mordants, Color strength, Fastness properties

Background

Natural dyes have been used since time immemorial to color different textile materials. In the nineteenth century, synthetic dyes in view of their low cost, large variety of shades, and high dyeability overtook the use of natural dyes (Samantha and Agarwal 2009; Yusuf et al. 2013). Synthetic dyes and particularly azo-based dyes are recently discovered to pollute the environment. This has motivated textile researchers to re-introduce natural colorants from renewable resources once again into the dyeing industry. Natural dyes produced from plants, animals, insects, and minerals are mostly non-toxic, non-carcinogenic, biodegradable, and environment-friendly in nature with some biological activities (Khan et al. 2010; Shahid et al. 2013). To achieve the sophisticated demand of modern people, a lot of research has been undertaken in the field of natural dyes for obtaining colorful shades on textiles and evaluation of their tolerance to the light, wash, and rub effects (Khan et al. 2015; Islam and Mohammad 2015). Metallic mordants as well as bio-mordants can be used to enhance the color characteristics and fastness properties of natural dyes on a textile substrate (Dalby 1993; Vankar et al. 2008). It is also reported that natural dyes can not only be used to get natural shades but they can also provide functionalities to fabrics such as antibacterial activity (M. I. Khan et al. 2011; S. A. Khan et al. 2012; Shahid et al. 2012), antifungal activity (Yusuf et al. 2015), and ultraviolet protection (Zhou et al. 2015).

Tannin-based dyes are used for textile dyeing for their good substantivity towards them because of more numbers of auxochromic groups in coloring components. *Terminalia chebula* is a medicinal plant grown in India and Southeast Asia (Khan et al. 2005). It is one of the constituents of triphala used in India as medicine. Fruits of *T. chebula* are included in the Indian pharmacopeia under the category astringent. It possesses laxative, diuretic, cardiotonic, and hypoglycemic properties (Naik et al. 2004). The major phytoconstituents present in the fruits are hydrolysable tannins, gallic acid, chebulic acid, chebulic ellagitannins, and gallate esters (Pfundstein et al. 2010).

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The present research paper is aimed to explore the dyeing potential of *T. chebula* on wool fiber. The effect of the dye concentration and different types of metal mordants such as alum, ferrous sulfate, and stannous chloride on color characteristics of developed shades and their fastness properties are also studied.

Materials

Wool yarn (100 % semi-worsted 60 counts) was purchased from MAMB Woollens Ltd. Bhadohi, UP, India. *T. chebula* dye extract in powder form was purchased from Sir Biotech India Ltd. Kanpur, UP, India. Metallic mordants potash alum ($Al_2K_2(SO_4)_4{\cdot}24H_2O$), iron sulfate ($FeSO_4{\cdot}7H_2O$), and stannous chloride ($SnCl_2{\cdot}2H_2O$) were used of laboratory grade.

Instrumentation

Fourier-transform infrared (FT-IR) spectra of *T. chebula* dye powder were obtained on a Perkin Elmer Spectrum RXI FT-IR system in order to investigate and observe auxochromic groups participating in wool-dye interactions (with the resolution of 4 cm^{-1}). Bands in the FT-IR spectra were resolved in accordance with literature data. A Perkin Elmer Lambda-40 double beam UV-visible spectrophotometer was employed for recording absorbance values of dye solutions. A pH/mV meter (BD 1011) from Decibel digital technologies was used for measuring pH of dye solutions.

Methods

Mordanting

Pre-mordanting method was opted for which three eco-friendly mordants alum ($Al_2K_2(SO_4)_4{\cdot}24H_2O$), iron sulfate ($FeSO_4{\cdot}7H_2O$), and tin chloride ($SnCl_2.2H_2O$) were selected. Woolen yarn samples were soaked in water before mordanting. The mordants were dissolved in water, and soaked woolen yarns were immersed into mordant solution at about 30 °C. Temperature of mordant solution was raised at a constant rate up to 91–93 °C and kept at this temperature for 60 min with constant stirring. Unused mordants on woolen yarn were removed by rinsing with tap water.

Optimization of mordants

Three different mordants such as alum ($Al_2K_2(SO_4)_4{\cdot}24H_2O$), iron sulfate ($FeSO_4{\cdot}7H_2O$), and tin chloride ($SnCl_2{\cdot}2H_2O$) used in this study were optimized for their better performance on wool in terms of achieving higher color strength and better fastness properties without sacrificing much of the mordant. Alum, iron sulfate, and tin chloride were optimized in the concentration range of 1.0–10, 1.0–5, and 0.1–1 % o.w.f (on weight of fiber). In view of the toxic nature of some metal salts, higher concentrations were not selected in this study.

Optimization was done on the basis of absorbance values (percentage exhaustion), recorded before and after dyeing with a UV-vis spectrophotometer, and percentage exhaustion was calculated by using the following equation.

$$\text{Percentage exhaustion} = \frac{\text{initial absorbance} - \text{final absorbance}}{\text{initial absorbance}} \times 100 \quad (1)$$

Based on the absorption and visual appearance, each mordant was selected in a particular optimized concentration (Prabhavathi et al. 2014) and was evaluated for colorimetric and fastness characteristics.

Dyeing

Un-mordanted and mordanted woolen yarns were immersed into dyebaths of varying concentrations (15, 8, 3, 1, and 0.5 % o.w.f.) maintained at M:L (material to liquor) ratio of 1:40 at neutral pH conditions. Temperature of dyebath was raised at a constant rate up to 91–93 °C and kept at that temperature for 60 min with constant stirring to achieve uniform dyeing. Acid (HCl) (pH = 4.0) and alkali (Na_2CO_3) (pH = 9.0) after-treatment was performed to dyed samples to demonstrate the effect of pH on dyeing processes. Dyed and after-treated woolen yarn samples were washed with non-ionic detergent Safewash, Wipro (5 ml/L), and rinsed with tap water. The fibers were dried in shade at room temperature.

Color measurements

Color measurements of dyed woolen yarn were carried out by following standard procedures. Estimation of color parameter values in terms of *K*/*S* and CIE-$L^*a^*b^*$ values were recorded on Gretag Macbeth color-eye 7000A spectrophotometer connected to a computer with installed software of MiniScan XE Plus. Color strength (*K*/*S*) value was calculated by using Kubelka-Munk equation.

$$\frac{K}{S} = \frac{(1-R)^2}{2R} \quad (2)$$

where *K* is the absorption coefficient, *S* is the scattering coefficient, and *R* is the reflectance of dyed samples.

Chroma (c^*) and hue angles ($h°$) were calculated using the following equations:

$$\text{Chroma } (c^*) = \sqrt{a^2 + b^2} \quad (3)$$

$$\text{Hue angle } (h^{o}) = \tan^{-1}\left({}^{b}/_{a}\right) \quad (4)$$

Fastness determination

Light fastness of the dyed samples was conducted on Digi light Nx™, having water cooled by Mercury Blended

Tungsten lamp, according to test method ISO 105-B02:1994 (Amd.2:2000). Wash fastness was measured in Digi wash SS™ (Laundrometer) as per the ISO 105-C06:1994 (2010) specifications. Dry and wet rub fastness of dyed samples were tested using Digi crock™ (Crockmeter) as per Indian standard IS 766:1988 (reaffirmed 2004) based on ISO 105-X12:2001 by mounting the fabric on a panel and giving ten strokes for both dry and wet rub fastness tests. The samples were assessed for staining on white adjacent fabric (cotton and wool).

Results and discussion

FT-IR spectra of *Terminalia chebula* dye

FT-IR analysis was used to identify the possible auxochromes responsible for the substantivity of dye. FT-IR spectra (Fig. 1) of extract powder of *T. chebula* were recorded which showed bands at 3240 and 2980 cm^{-1} due to O–H and aromatic C–H stretching vibrations, respectively; presence of bands at 1710 and 1595 cm^{-1} is due to C=O and C=C stretching vibrations, respectively. Stretching vibrations at 1201 and 1040 cm^{-1} are for C–O stretching peaks.

Optimization of mordant concentrations

Metal salt mordants have different interactions with wool and thereby may darken, brighten, or drastically alter the final color of the dyed wool samples. In this study, wool yarns were pre-treated with different types of metal salts (alum, ferrous sulfate, stannous chloride).

Figure 2a–c shows the effect of alum, iron sulfate, and tin chloride concentrations on the percentage exhaustion values of *T. chebula*-dyed woolen yarns. On increasing the concentration of alum (1–10 % o.w.f.), iron sulfate (1–5 % o.w.f.), and tin chloride (0.1–1.0 % o.w.f.) mordants, respectively, percentage exhaustion values increase. This increase may be attributed to the increasing interaction of respective metal ions or their higher coordinating ability with wool fiber and dye molecules.

From the experimental results, 10.0 % (o.w.f.) alum, 5.0 % (o.w.f.) iron sulfate, and 1.0 % (o.w.f.) tin chloride concentrations were found to give maximum exhaustion results and were taken as optimized mordant concentrations for subsequent dyeing experiments with *T. chebula* natural dye.

Color characteristics

Colorimetric characteristics (*K*/*S* and CIE-$L^*a^*b^*$ values) of woolen yarn dyed with different concentrations of *T. chebula* (15.0, 8.0, 3.0, 1.0 and 0.5 % o.w.f.) with optimized mordant concentrations were analyzed (Fig. 3). Negatively charged dye molecules (anions) and metal (cations) have strong affinity for positively charged amino and negatively charged carboxyl groups (isoelectric nature of wool fiber), respectively. Electrostatic forces of attraction (ionic bonding) between coloring components and wool functional groups are responsible for the uptake of dye by wool in dyeing bath in addition to hydrophobic forces of attraction (van der Waals forces). From the dye-metal coordination complexes, the vacant sites are used to form coordinate bonds with the uncharged amino ($–NH^-$) and carbonyl (C=O) groups of the amide group of wool (Fig. 4).

Effect of concentration of dye on color strength of dyed woolen yarn

With the increase in the concentration of dye from 0.5 to 15.0 % (o.w.f.), an increase in color strength (*K*/*S*) is observed resulting in deeper shades with increase in the

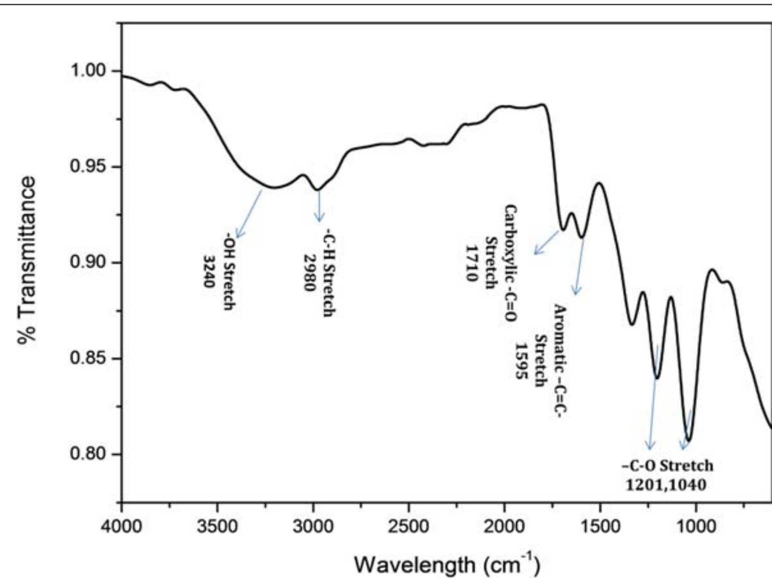

Fig. 1 FT-IR spectrum of *Terminalia chebula* dye

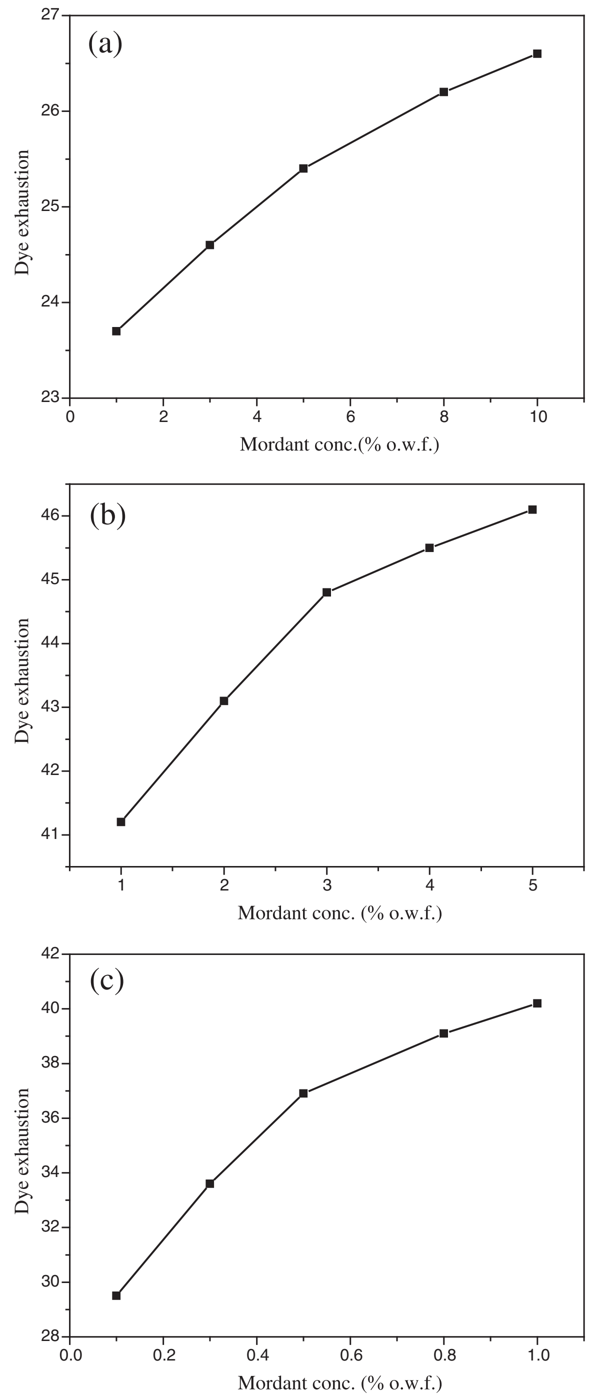

Fig. 2 Optimization of mordants concentration. **a** Alum. **b** Iron. **c** Tin

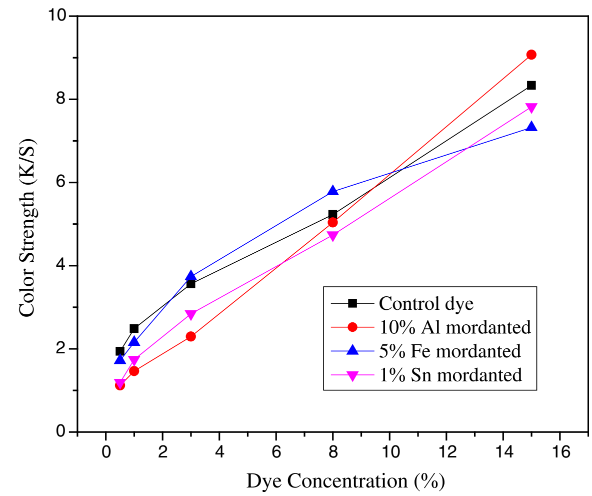

Fig. 3 Effect of dye concentration on color strength (*K/S*) of dyed woolen fiber

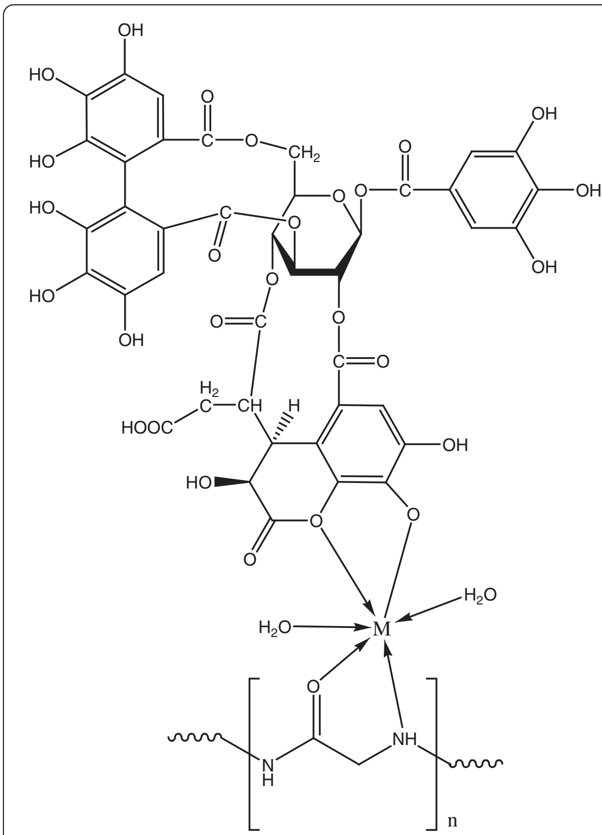

Fig. 4 Complex formation between chebulagic acid, metal ion, and functional groups of wool fiber

dye adsorption. Due to increase in concentration of dye in dyebath, concentration gradient increases, which eventually results in an increase in the rate of diffusion of dye onto the surface of woolen yarn from the dyebath solution. A lower concentration of dye produces shades of gray-colored tones while a higher concentration of dye shifts color to the yellow region.

Table 1 CIE-*L*a*b** values and fastness properties of un-mordanted dyed wool samples

								Wash fastness	Rub fastness		Light fastness
Dye %	Medium (pH)	*L**	*a**	*b**	*c**	*h*°	*K/S*	c.c.	Dry	Wet	
15	Neutral	47.6	4.8	17.8	18.4	74.8	8.33	3	4/5	4/5	5
	Acidic	49.9	5.5	18.6	19.4	73.5	6.95	4	4/5	3/4	5
	Alkaline	43.4	6.4	19.4	20.4	71.9	15.27	5	4/5	4/5	5
8	Neutral	48.1	4.1	16.1	16.6	75.8	5.23	5	4/5	4/5	5
	Acidic	51.4	5.9	18.6	19.5	72.4	5.71	4	4/5	4/5	5
	Alkaline	49.5	5.6	19.6	20.4	73.9	7.42	2	4/5	4/5	5
3	Neutral	55.4	4.4	17.9	18.4	76.3	3.56	4	4/5	4/5	5
	Acidic	57.0	4.4	18.6	19.2	76.6	3.80	4	4/5	4/5	5
	Alkaline	56.4	4.3	18.5	19.0	76.8	3.39	3	4/5	4/5	5
1	Neutral	57.1	3.9	15.9	16.4	76.2	2.48	4/5	4/5	4/5	5
	Acidic	62.0	4.7	19.7	20.2	76.6	2.56	3	4/5	4/5	5
	Alkaline	59.7	3.6	16.5	16.9	77.6	2.24	3/4	4/5	4/5	5
0.5	Neutral	60.8	2.8	15.3	15.6	79.6	1.94	4	4/5	4/5	5
	Acidic	65.9	4.2	19.9	20.3	78.1	1.99	3/4	4/5	4/5	5
	Alkaline	63.4	2.8	15.4	15.7	79.6	1.59	4/5	4/5	4/5	5

Abbreviations: *c.c.* color change

Effect of pH

Wool yarns were dyed with *T. chebula* in a dyebath maintained at neutral pH. To widen the shade range, dyed wool yarns were after-treated with acidic and alkaline solutions. After treatment of dyed wool yarn samples with acidic and alkaline media, appreciable changes in color parameters and fastness properties were observed. Acidic treatment lightens the shades as supported by low color strength values which may be due to hydrolysis of complexes and wool-dye interaction in acidic medium. Alkaline medium treatment improved the wash fastness (color change), but acidic medium treatment decreases the tolerance to washing. The different colors obtained on wool in neutral, acidic, and alkaline media are shown in Table 5.

Table 2 CIE-*L*a*b** values and fastness properties of alum-mordanted dyed wool samples

								Wash fastness	Rub fastness		Light fastness
Dye %	Medium (pH)	*L**	*a**	*b**	*c**	*h*°	*K/S*	c.c.	Dry	Wet	
15	Neutral	54.2	2.6	22.3	22.5	83.3	9.07	4	4	3	4/5
	Acidic	55.8	5.0	19.5	20.1	75.5	4.63	3	4/5	4	4/5
	Alkaline	51.8	4.7	22.3	22.8	77.9	10.43	4	4	3	4/5
8	Neutral	61.9	2.1	23.4	23.5	84.7	5.03	4	4	4	4/5
	Acidic	56.4	4.6	17.9	18.5	75.5	3.65	3/4	4/5	4/5	4/5
	Alkaline	62.6	3.4	23.3	23.5	81.4	4.04	4	4/5	4/5	4/5
3	Neutral	69.6	1.6	22.5	22.6	85.9	2.29	4	4/5	4/5	4/5
	Acidic	62.6	3.4	17.9	18.2	79.0	2.26	4	4/5	4/5	4/5
	Alkaline	70.7	2.5	21.2	21.3	83.2	1.76	3/4	4/5	4/5	4/5
1	Neutral	73.2	1.3	20.6	20.7	86.1	1.46	4	4/5	4/5	4/5
	Acidic	66.8	3.2	18.7	18.9	80.2	1.95	4	4/5	4/5	4/5
	Alkaline	72.9	2.1	20.7	20.8	84.1	1.35	4	4/5	4/5	4/5
0.5	Neutral	73.8	1.3	18.7	18.8	85.9	1.12	4	4/5	4/5	4/5
	Acidic	75.0	1.9	18.2	18.3	83.8	1.02	2	4/5	4/5	4/5
	Alkaline	72.9	2.3	20.1	20.2	83.3	1.25	4	4/5	4/5	4/5

Abbreviations: *c.c.* color change

Table 3 CIE-$L^*a^*b^*$ values and fastness properties of iron-mordanted dyed wool samples

								Wash fastness	Rub fastness		Light fastness
Dye %	Medium (pH)	*L**	*a**	*b**	*c**	*h*°	*K/S*	c.c.	Dry	Wet	
15	Neutral	47.3	4.1	16.2	16.7	75.6	7.32	4	4/5	4/5	5
	Acidic	47.4	3.8	16.3	16.7	76.8	7.28	4	4/5	4/5	5
	Alkaline	38.2	6.2	17.7	18.8	70.5	21.15	3/4	4/5	4	5
8	Neutral	51.9	4.5	17.8	18.4	75.6	5.78	3/4	4/5	4/5	5
	Acidic	52.9	4.9	18.8	19.4	75.3	5.48	3	4/5	4/5	5
	Alkaline	42.9	6.1	19.2	20.2	72.3	15.57	4/5	4/5	4/5	5
3	Neutral	54.9	3.6	16.1	16.5	77.3	3.73	4	4/5	4/5	5
	Acidic	56.3	3.3	16.1	16.5	78.3	3.17	4	4/5	4/5	5
	Alkaline	47.6	5.2	19.6	20.3	75.0	9.57	4	4/5	4/5	5
1	Neutral	55.9	2.0	11.5	11.7	79.9	2.15	4	4/5	4/5	5
	Acidic	67.3	3.3	16.8	17.2	78.8	1.42	3/4	4/5	4/5	5
	Alkaline	55.1	3.6	14.9	15.3	76.3	2.67	3/4	4/5	4/5	5
0.5	Neutral	57.5	1.2	10.3	10.4	83.2	1.72	3/4	4/5	4/5	5
	Acidic	67.5	2.4	15.7	15.9	81.3	1.32	2	4/5	4/5	5
	Alkaline	56.3	2.9	12.6	13.0	76.7	2.12	4	4/5	4/5	5

Abbreviations: *c.c.* color change

Effects of mordants on colorimetric characteristics

Some transition metal ions and particularly iron salts in our case strongly bind with natural dye molecules due to their ability to form strong coordination complexes and thus produce deep color on the fabric (Uddin 2014). Better octahedral complexation property of iron leads to higher *K/S* values and the color change to grayish of iron-mordanted dyed woolen yarn than un-mordanted yarn (Mihalick and Donnelly 2006). Metal mordants highly affect the lightness and a^* and b^* values (Tables 1, 2, 3, and 4); as in the case of alum and tin, lightness increases up to 75 or more; and the a^*-b^* plot (Figs. 5 and 6) shifts towards the more yellow region owing to the lightening of shade property of alum and tin mordants. In the case of iron mordants, lightness decreases up to 60 or less, and

Table 4 CIE-$L^*a^*b^*$ values and fastness properties of tin-mordanted dyed wool

								Wash fastness	Rub fastness		Light fastness
Dye %	Medium (pH)	*L**	*a**	*b**	*c**	*h*°	*K/S*	c.c.	Dry	Wet	
15	Neutral	54.8	4.9	20.6	21.2	76.4	7.81	4	4/5	4	5
	Acidic	53.9	5.6	20.3	21.1	74.5	8.20	3/4	4/5	4	5
	Alkaline	46.2	4.2	19.2	19.7	77.5	12.98	4	4/5	4	4/5
8	Neutral	62.4	4.0	21.5	21.8	79.3	4.73	4	4/5	4	5
	Acidic	61.7	4.6	21.2	21.7	77.6	4.52	3/4	4/5	4	4/5
	Alkaline	54.8	4.8	21.6	22.1	77.3	7.30	4	4/5	4	5
3	Neutral	62.5	3.0	18.0	18.2	80.4	2.84	4/5	4/5	4	5
	Acidic	62.9	3.6	19.4	19.7	79.3	3.33	3/4	4/5	4	5
	Alkaline	59.7	3.3	20.2	20.5	80.5	4.13	4	4/5	4	5
1	Neutral	70.4	1.7	18.6	18.7	84.8	1.74	4/5	4/5	4/5	5
	Acidic	69.7	2.6	18.5	18.7	81.8	1.81	3/4	4/5	4/5	5
	Alkaline	68.1	2.3	20.1	20.3	83.3	2.17	4	4/5	4/5	5
0.5	Neutral	75.1	1.1	18.6	18.7	86.7	1.19	4/5	4/5	4/5	5
	Acidic	75.0	1.8	18.6	18.7	84.4	1.22	4	4/5	4/5	5
	Alkaline	73.8	1.4	20.4	20.5	86.0	1.41	4	4/5	4/5	5

Abbreviations: *c.c.* color change

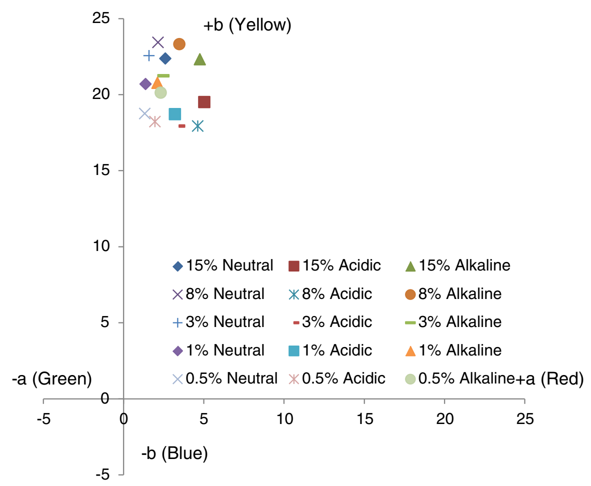

Fig. 5 *a**-*b** plot for alum-mordanted dyed woolen fiber

the *a**-*b** plot (Fig. 7) shifts far away from the yellow co-ordinate in comparison to control-dyed (Fig. 8) woolen yarn owing to the saddening property of iron mordants. The chroma values (*c**) were found minimum in the case of iron-mordanted samples in the range of 11–20 and maximum in alum-mordanted samples in the range of 18–24, whereas the values of tin-mordanted samples were found in the range of 18–22. The hue angles are in the range of 72° to 87°, and all the dyed woolen yarns were found in the yellow-red quadrant. Shade cards of all dyed samples are given in Table 5.

Fastness properties

Light, wash, and rub fastness properties (Tables 1, 2, 3, and 4) were evaluated on a grayscale and observed as average to excellent range.

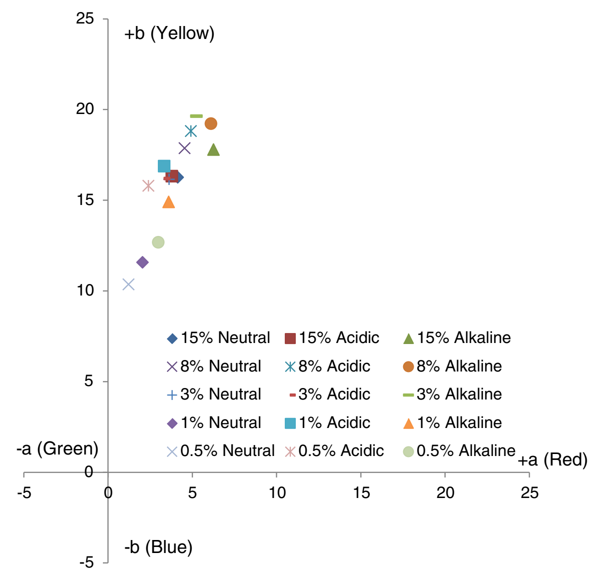

Fig. 7 *a**-*b** plot for iron-mordanted dyed woolen fiber

Light fastness

For the light fastness, it was observed that all the woolen yarns dyed with *T. chebula* showed very good fastness results to light. The alum-mordanted dyed woolen yarns were found comparatively less tolerable towards light in comparison to control-, iron-, or tin-mordanted dyed woolen yarns (Maulik and Agarwal 2014). This is probably due to weak coordination bonding of alum with dye molecule, and the photolytic degradation happens, but in the case of iron, strong coordination bonding prevents photolytic degradation by protecting the chromophore with dissipation of energy of absorbed photons to the chelate structure formed with metal ions (Jothi 2008).

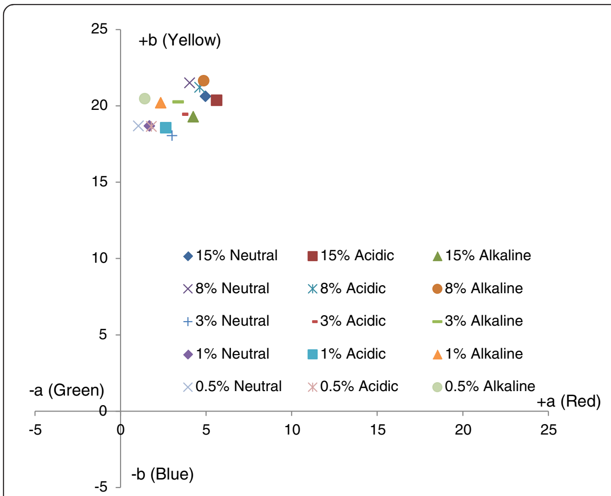

Fig. 6 *a**-*b** plot for tin-mordanted dyed woolen fiber

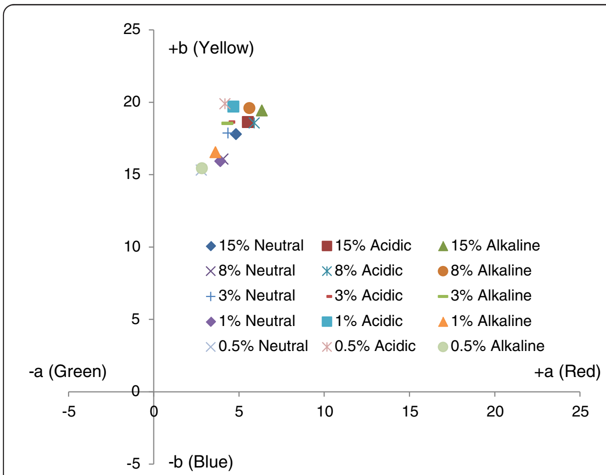

Fig. 8 *a**-*b** plot for control-dyed woolen fiber

Table 5 Shade cards for *Terminalia chebula* natural dye

Dye conc.	Dyeing and treatment medium pH	Un-mordanted	Mordant used		
		Control	Alum	Iron	Tin
15%	(Neutral pH-7)				
	(Acidic pH-4)				
	(Alkaline pH-9)				
8%	(Neutral pH-7)				
	(Acidic pH-4)				
	(Alkaline pH-9)				
3%	(Neutral pH-7)				
	(Acidic pH-4)				
	(Alkaline pH-9)				
1%	(Neutral pH-7)				
	(Acidic pH-4)				
	(Alkaline pH-9)				
0.5%	(Neutral pH-7)				
	(Acidic pH-4)				
	(Alkaline pH-9)				

Wash fastness

From the wash fastness data in Tables 1, 2, 3, and 4, all the *T. chebula*-dyed woolen yarns (un-mordanted as well as mordanted) showed a good to excellent wash fastness rating of 3–5, and negligible staining on adjacent fabrics (cotton and wool) was observed. Relatively good fastness to washing for un-mordanted dyed woolen yarn is attributed to the affinity of coloring components to the yarn in the form of H bonding, ionic bonding, and van der Waals forces (Khan et al., 2015; Yusuf et al., 2015).

Rub fastness

Un-mordanted and mordanted dyed woolen yarns were tested for dry and wet rub fastness properties and were found more or less the same (4–5 on the grayscale) for both un-mordanted and mordanted dyed samples. Rub fastness of alum-mordanted samples were relatively found somewhat less than that of un-mordanted and mordanted with iron or tin which was due to weak coordination complexation of aluminum ions.

Conclusions

This study evaluated the color parameters (K/S, L^*, a^*, b^*) and fastness properties of woolen yarns dyed with *T. chebula* natural dye. The use of different metal salt mordants produced a wide range of beautiful shades with good color and wash fastness (light, wash, and rub fastness) properties. It was found that the K/S values of dyed woolen yarn were found higher in the case of iron mordants and lightness was increased with alum and tin mordants. Furthermore, it was found that alum mordants affected negatively the light fastness. In the end, it can be said that *T. chebula* natural dye can provide bright hues with good color fastness properties with different types of mordants.

Competing interests
The authors declare that they have no competing interests.

Authors' contributions
MS and FM designed the experiment. MS conducted most of the experiments. SI interpreted the experimental data and drafted the manuscript. MNB helped MS in performing experiments on dyeing studies of the Harda plant. LJ helped to analyze the experiment. MAK provided technical help. All authors read and approved the final manuscript.

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