Natural Dye Extracted from *Vitex negundo* as a Potential Alternative to Synthetic Dyes for Dyeing of Silk

Venkataramanappa Narayana Swamy¹ · Kurikempanadoddi Ninge Gowda² · Rajagopal Sudhakar²

Received: 15 February 2015 / Accepted: 1 June 2015 / Published online: 26 June 2015
© The Institution of Engineers (India) 2015

Abstract Since the last decade, the application of natural dyes on textile material has been gaining popularity all over the world, possibly because of the increasing awareness of issues concerning the environment, ecology and pollution control. The present paper investigates extraction of natural dye from leaves of the plant *Vitex negundo*, which is an abundant, cheap, and readily available agricultural by-product. Water extracts from *V. negundo* was used to dye silk fabrics. Optimum extraction conditions included pH 9, duration 120 min, and temperature 90 °C. Optimum dyeing conditions included dyeing pH 5 and duration of 60 min. Potash alum, tannic and tartaric acid were used as mordants, all of which are benign to human health and the environment. Color strength and color coordinates in terms of L*, a*, b*, C, and h were examined. A range of shades were obtained when fabrics were dyed with different mordants and mordanting techniques. The extracted dye was tested for some of the eco-parameters using atomic absorption spectrophotometry and GC/MS. The test results were compared with set standards to determine the eco-friendliness of natural dye. Their concentrations were found to be lower than the stipulated limits. Dyed samples were tested for antimicrobial activity against gram-positive and gram-negative bacteria. The dyed silk fabrics showed acceptable fastness properties and

were also found to possess antibacterial activity. It can be concluded that the abundantly available agricultural by-product *V. negundo* has great potential to be effectively utilized as a natural dye for silk.

Keywords Natural dyeing · Heavy metals · Mordants · *Vitex negundo* · Antimicrobial activity

Introduction

Dyeing of textiles using synthetic dyes could have a high negative impact on the environment, as well as on the safety and health of dyers and end-users. Consumers have become more aware of environmental issues; in fact, they are increasingly seeking products with a green or organic label [1]. During the last few decades a growing interest in the use of natural colorants has been evident both in public awareness and scientific activity [2, 3]. This increased interest could be on account of the natural dyes' high compatibility with the environment, relatively low toxicity and low allergic effects. Recent debates in favour of natural dyes consider its positive features such as increased sustainability, renewable resources, environmentally friendly processing, reduced pollution, and green chemistry. The demand for more sustainable processes should be seen as a driving force for identifying new strategies that can bring about a revival of natural dyes in textile dyeing.

Vitex negundo (Linn.) is a large, aromatic shrub belonging to the verbenaceae family and grows in waste lands and is commonly cultivated as a hedge plant throughout India. The upper surface of the leaves is green and the lower surface is silvery in color. It is one of the common plants used in traditional medicine and is reported to have a variety of biological uses [4]. The leaves are

✉ Venkataramanappa Narayana Swamy
gvnnarayan@gmail.com

¹ The Oxford College of Science, Bangalore 560 102, Karnataka, India

² Department of Fashion and Apparel Design, Bangalore University, Bangalore 560 001, Karnataka, India

aromatic, bitter, acid, astringent, anodyne, anti-inflammatory [5], antipyretic or febrifuge, tranquilizer, bronchial smooth muscle relaxant, anti-arthritic, antihelminthic, antibacterial and vermifuge [6, 7]. This plant reportedly showed diverse pharmacological properties such as analgesic, anti-noise receptive, anti-oxidant and anti-hyperglycemic [8]. The major flavonoids present in the leaves are Luteolin-7-glucoside (Fig. 1), casticin (Fig. 2), artemetin (Fig. 3) [9, 10] which make it a possible dye candidate for silk fabric. In the present investigation an attempt has been made to extract natural dye from *V. negundo* leaves, evaluate its dyeing potential, color-fastness properties, heavy metal content, and antimicrobial activity.

Experimental Procedure

Materials

The degummed mulberry silk fabric (purchased from a trader in Bangalore) weighing 40 g/m² with a yarn density of 132 ends/inch and 116 picks/inch was used. The dry leaves of *V. negundo* were collected from the Jnanabharathi campus, Bangalore University. Potassium aluminum sulphate

[KAl(SO₄)₂·12H₂O], tannic acid [C₇₆H₅₂O₄₆], tartaric acid [C₄H₆O₆] of analytical grade were used as mordants. Distilled water was used for extraction of dye and preparation of chemical solutions.

Dye Extraction

Fresh *Vitex negundo* leaves were dried at room temperature till completely dry and then crushed using a mixer grinder and then used as the raw material for dye extraction. Optimization of extraction was performed at different temperatures (30, 50, 80, 90 and 100 °C) for different pH (5, 7, 8, 9 and 10) and for different durations (45, 60, 75, 90 and 120 min). The extracted dye was filtered and cooled to room temperature. The dye extracted at optimum conditions was filtered and evaporated using a roto-evaporator and the obtained dye powder was used for dyeing experiments.

Method of Mordanting

Aluminum potassium sulphate, tannic acid and tartaric acid were used for mordanting silk. Pre- and post-mordanting techniques were used for this study. Pre-mordanting included pre-treatment in a bath containing 5 and 10 % (owf) mordant at an MLR of 1:40 at 60 °C for 30 min and dried at room temperature and for post-mordanting, the dyed samples were treated with 5 and 10 % (owf) mordants at a MLR ratio of 1:40 at 60 °C for 30 min.

Dyeing

All dyeings were carried out in a dye bath maintained at pH 5 containing 10 % dye (owf) at MLR 1:40 in a beaker dyeing machine. The dyeing was started at 40 °C then the temperature was raised to 90 °C and dyeing was continued for 60 min. The dyed samples were subsequently rinsed and soaped in 1 gpl nonionic detergent at 70 °C for 15 min and dried.

Determination of Exhaustion of Dye

Dye uptake was determined by measuring the absorbance of the diluted dye bath samples at a wavelength of maximum absorbance (λ_{max} 420 nm) of dye. The percentage of dye bath exhaustion was calculated as follows:

$$\% \text{ Dye exhaustion} = [(A_0 - A_1)/A_0] \times 100,$$

where A_0 and A_1 are absorbance at a wavelength of maximum absorption (λ_{max}) of dye bath before and after dyeing.

Fig. 1 Luteolin-7-glucoside

Fig. 2 Casticin

Fig. 3 Artemetin

Determination of Color Strength and Color Parameters

Color was evaluated by means of K/S and CIELAB color coordinates with illuminant D65/10° observer on Gretag Macbeth Color Eye 7000 A Spectrophotometer. Four measurements were made for each sample and the reflectance values over a range of 350–750 nm were recorded. The K/S values were assessed using the Kubelka–Munk equation.

$$K/S = (1 - R)^2 / 2R,$$

where R is the observed reflectance at $\lambda_{\max}$, K is the absorption coefficient, and S is the light scattering coefficient.

The colors are given in CIE Lab co-ordinates, L corresponding to the lightness (100 = White, 0 = Black), a^* to the red-green co-ordinate (positive sign = red, negative sign = green) and b^* to the yellow-blue co-ordinate (positive sign = yellow, negative sign = blue). C is a measure of chroma (saturation) and represents the distance from the neutral axis. h is a measure of hue and is represented as an angle ranging from 0° to 360°. Angles that range from 0° to 90° are reds, oranges and yellows. 90°–180° are yellows, yellow-greens and greens. 180°–270° are greens, cyan and blue. 270°–360° are blue, purples, magentas and return to reds.

Evaluation of Fastness Properties

The dyed fabrics were assessed for light-fastness according to ISO 105 B02 using Xenotest light-fastness apparatus. Color changes were assessed using the blue wool standards. Wash-fastness was evaluated according to ISO 105 C02 using launderometer. Rubbing-fastness evaluation was performed according to ISO-X12 using crock meter. The change in the color of dyed fabrics and the staining of the adjacent white fabric were evaluated by grey scale standards.

Evaluation of the Antimicrobial Activity

Escherichia coli (*E. coli*) a gram-negative bacterium, was selected due to its popularity of being selected as a test organism and its resistance to common antimicrobial agents. *Staphylococcus aureus* (*S. aureus*), a pathogenic gram-positive bacterium was used as it causes major cross-infection in hospitals and is the most frequently evaluated species [11].

Circular fabric swatches were tested using 1 ± 0.1 mL of bacterial inoculums in a 200 mL container. The inoculums were a nutrient broth culture containing 1.0×10^5

colony forming units (CFU)/mL of bacteria. For test swatches to be in contact with the bacteria for 24 h, 100 mL of sterilized distilled water was poured into the vessel and was shaken in a bacterial suspension and the reduction in bacterial activity in standard time was measured [11, 12]. The efficiency of the antimicrobial treatment was determined by comparing the reduction in bacterial concentration of the treated sample with that of the control sample expressed as a percentage reduction in standard time.

$$R = [(A - B)/A] \times 100,$$

where R is the percentage reduction in microbial population; A represents the number of bacteria colonies in the control and B represents the number of bacteria colonies in the treated fabrics.

Determination of Durability of Antimicrobial Activity to Washing

Antimicrobial activity of dyed samples was evaluated after 1, 5 and 10 washing cycles and the durability of antimicrobial property was calculated in terms of per cent retention of antimicrobial activity using the formula given below:

$$\% \text{ Retention of antimicrobial activity} = \frac{R_n}{R_0} \times 100,$$

where R_0 is the per cent of microbial reduction before washing and R_n is the per cent of microbial reduction after n wash cycles.

Elemental Analysis

Heavy metal concentrations in the digested samples were determined with A6300 Shimadzu flame atomic absorption spectrophotometer with Shimadzu auto sampler (Asc-600). The calibration curves were prepared separately for all the metals by running different concentrations of standard

Fig. 4 Effect of pH on dye extraction

Fig. 5 Effect of duration on dye extraction

Fig. 6 Effect of temperature on dye extraction

solutions [13, 14]. The extracted dye was also analyzed for the presence of banned aryl amines after reductive cleavage and isolation using GC/MS.

Results and Discussion

Dye Extraction Conditions

Extracts obtained under various conditions were estimated by measuring their absorbance at 420 nm. From several sets of experiments, it was observed that the yield of the dye extract was better at pH 9 (Fig. 4), duration 120 min (Fig. 5) at 90 °C temperature (Fig. 6). The amount of dye uptake by silk fabrics dyed with *V. negundo* leaves was expressed as percentage of dye exhaustion and results are shown in Table 1. The maximum exhaustion was observed in the case of tartaric acid samples followed by alum and tannic acid mordanted samples.

Effect of Dyeing Conditions

Figure 7 shows that the pH of the dye bath has a considerable effect on the colour strength of silk fabric. With the increase in pH the colour strength of the dyed fabrics showed a decrease in colour strength indicating acidic pH to be the ideal for better dye uptake.

Figure 8 shows the effect of dyeing time on the color yield obtained for the dyed fabrics. This figure indicates that the color yield increases as the time increases up to 60 min, and then a plateau is attained after 90 min up to 150 min. A longer dyeing time means higher color strength until dye exhaustion attains equilibrium and there is no significant increase after further increase in dyeing time. This seems to indicate that silk fabrics reach saturation and do not absorb any more dye.

Table 1 Colorimetric data of silk samples dyed with *Vitex negundo* leaves extract

Mordant	Method	% Dye exhaustion	Mordant %	K/S	L*	a*	b*	C	h
Undyed	–	51	–	–	90.53	2.25	0.15	2.18	365.75
Control	–	–	–	1.25	55.33	14.22	09.45	42.08	46.65
Alum	Pre	80	5	3.34	53.81	3.13	15.82	16.13	78.80
		88	10	5.70	44.71	1.51	12.50	16.93	83.01
	Post	51	5	4.01	41.68	3.52	13.61	14.08	75.49
		51	10	6.97	44.57	1.32	10.13	10.22	82.53
Tannic	Pre	65	5	2.83	53.43	3.85	16.21	16.66	76.62
		81	10	3.05	52.98	3.69	15.88	16.30	76.91
	Post	51	5	3.68	43.87	3.70	14.12	14.60	75.31
		51	10	4.16	41.38	2.81	13.39	13.68	78.14
Tartaric	Pre	86	5	4.16	51.61	2.83	13.60	13.80	78.21
		89	10	5.70	48.71	1.51	12.50	12.93	83.31
	Post	51	5	4.58	49.28	1.29	12.41	12.66	75.25
		51	10	6.99	44.01	1.32	10.13	10.22	83.52

pre Premordanting, post postmordanting, Control silk dyed without mordant, undyed fabric without mordant and dye

Fig. 7 Effect of dye bath pH on color strength of dyed silk fabrics

Fig. 8 Effect of dyeing time on the color strength of silk fabrics

Determination of Color Coordinates of Dyed Samples

The colorimetric data (Table 1) indicates the depth and natural tone of dyed and mordanted samples. K/S value of the dyed samples shows that use of mordants considerably increased dye absorption leading to higher K/S values in the case of mordanted samples than in un-mordanted

Table 3 Antibacterial activity against *E. coli* of silk samples (10 %) dyed with *Vitex negundo* leaves extract with and without mordants after dyeing and laundering

Mordants	Method	Mordant (%)	Bacterial reduction (%)	
			After dyeing	After laundering
Control	–	–	70.8	70.2
Alum	Pre	5	80.3	80.1
		10	82.5	80.3
	Post	5	80.1	78.4
		10	82.6	80.9
Tannic acid	Pre	5	88.5	87.1
		10	95.5	94.0
	Post	5	89.6	87.9
		10	96.3	95.2
Tartaric acid	Pre	5	81.0	78.6
		10	82.8	81.0
	Post	5	80.5	78.9
		10	81.5	80.0

Table 2 Fastness properties for silk fabric dyed with *Vitex negundo* leaves extract

Mordant	Method	Mordant %	Light	Wash fastness		Rubbing fastness	
				CC	CS	Dry	Wet
Control	–	–	2	3	4	5	4
Alum	Pre	5	3	3	5	5	4
		10	4	3	5	5	5
	Post	5	4	4	5	5	5
		10	4	4	5	5	5
Tannic acid	Pre	5	3	3	5	5	4
		10	3	4	5	5	5
	Post	5	4	3	5	5	5
		10	4	4	5	5	5
Tartaric acid	Pre	5	3	3	5	5	5
		10	3	4	5	5	5
	Post	5	4	3	5	5	5
		10	4	4	5	5	5

cc Color change, cs color staining, control silk dyed without mordants

samples. It can be attributed to the complex formation between fabric, mordant and dye. Post-mordanted samples of both alum and tartaric acid were found to have more prominent effect on colour strength than others. From a^* and b^* the color gamut of silk samples dyed with *V. negundo* indicates a red yellow zone. The L^* values were lower in the case of all post-mordanted samples corresponding to darker shades, whereas higher in the case of 5 % pre-mordanted samples.

Fastness Testing

Color fastness properties (light, washing and rubbing) of the *V. negundo* dyed silk fabric samples are given in

Table 4 Antibacterial activity against *Staphylococcus aureus* of silk samples (10 %) dyed with *Vitex negundo* leaves extract with and without mordants after dyeing and laundering

Mordants	Method	Mordant (%)	Bacterial reduction (%)	
			After dyeing	After laundering
Control	–	–	65.1	64.0
Alum	Pre	5	75.6	75.6
		10	77.1	77.0
	Post	5	79.1	78.2
		10	80.8	80.5
Tannic acid	Pre	5	84.6	83.6
		10	85.7	85.1
	Post	5	86.4	86.0
		10	87.9	86.0
Tartaric acid	Pre	5	80.0	78.5
		10	81.5	81.3
	Post	5	80.2	80.0
		10	81.8	81.0

Table 2. All the samples dyed with *V. negundo* exhibited moderate to good light-fastness i.e., 3–4. The dyed samples showed moderate to good wash-fastness ratings ranging from 3 to 4 on gray scale for change in colour and no staining on adjacent fabrics were observed. The wet rub-fastness of the dyed samples was found to be between 4 and 5 and dry rub-fastness ratings were 5.

Antibacterial Properties

The antibacterial activity of mordanted and unmordanted dyed silk fabrics against *E. coli* and *S. aureus* was assessed. Tables 3 and 4 show the antibacterial results of samples dyed using *V. negundo* with 10 % dye concentration after dyeing, after laundering and exposure to light. The majority of natural dyes need a mordant in the form of a metal salt to create an affinity between the fiber and the pigment. These metals form a ternary complex on one side with the fiber and on the other side with the dye. Such a strong coordination tendency enhances the interaction between the dye and the fiber, resulting in higher dye uptake.

The antibacterial activity of dyed samples has increased after mordanting. Regardless of the nature of the mordant used in dyeing, samples with different mordant treatments showed different levels of improvement in antibacterial activity. By applying pre and post-mordant treatment, the antibacterial activity of the dyed samples not only increased, but also remained stable after laundering. The antibacterial results also revealed that dyed fabrics after laundering have almost similar antibacterial activity against gram positive and gram negative bacteria. The bacterial inhibition may be attributed to the active substances present within the coloring matter as also to the well known fact that the metallic salts used as mordants exhibit toxic effects against the pathogens. Among the

Fig. 9 % Retention of antimicrobial activity to washing against *E. coli*

Fig. 10 % Retention of antimicrobial activity to washing against *S. aureus*

Table 5 Concentration of red listed chemicals in the extracted dye

Parameters	Permissible range (ppm)	Dye (ppm)
<i>Heavy metals</i>		
Arsenic	0.02	0.001
Cadmium	0.1	Ab
Chromium	1.0	0.02
Cobalt	1.0	Ab
Copper	10	0.25
Lead	0.08	Ab
Mercury	0.02	Ab
Nickel	1	0.01
Zinc	10	0.02
Pesticides	–	NT
Banned aryl amines	–	NT

NT not traceable, Ab absent

three mordants, tannic acid showed highest antibacterial activity against both bacteria. Many metallic salts are shown to inhibit the growth of microorganisms or destroy them at very low concentrations. Metal may have toxic effects either in its free state or in metallic compounds. The mechanism of metal antibacterial action is suggested to be either through protein binding or formation of reactive oxygen species [15].

Wash Durability of Antimicrobial Activity

Figures 9 and 10 represent the durability of antimicrobial activity in terms of % retention of microbial activity after washing. Microbial % reduction of dyed silk fabric samples were examined over three different launderings viz., 1, 5 and 10. Silk fabric samples mordanted with tannic acid were found to be most effective against both tested

microbes. After 5 washing cycles microbial activity was reduced to <45 % of the initial stage against both bacteria.

Analysis of Elements in Dyed Samples

The metal content in dyed silk samples is shown in Table 5. Results showed the extremely low quantities of heavy metals extracted from the *V. negundo* leaves extract. As the concentrations are much below the stipulated limits the extracted dye can be considered eco-friendly.

Conclusion

In the present study natural dye extracted from the leaves of *V. negundo* was applied on silk fabrics and the performance attributes of the dyed fabrics with respect to colour strength, colour fastness and antibacterial activity was assessed. All the dyed silk samples exhibited good colour fastness to washing, rubbing and acceptable color-fastness to light; therefore, the colorant can be an alternative to synthetic dyes. Also the dyed samples exhibited antibacterial activity against two bacteria gram-positive bacterium, *S. aureus* and gram-negative bacterium *E. coli*. It was found that the extracted dye is safe and eco-friendly as the red listed chemicals are either absent or their concentration is much below the stipulated limits. The data generated from the present study should encourage the economic viability of natural dye production to be considered on a commercial scale. This would help towards utilization of plant waste; to uplift the socio-economic growth in rural areas; and to serve further the global demand for dyed textiles using safe and eco-friendly dyes from natural sources.

Acknowledgments The authors are thankful to the Bangalore University, Bangalore for providing the financial support under interdisciplinary collaborative research fund.

References

1. S. Ratnapandian, S.M. Fergusson, L. Wang, Application of Acacia natural dyes on cotton by pad dyeing. *Fibers Polym.* **13**(2), 206 (2012)
2. M. Mirjalili, K. Nazarpour, L. Karimi, Eco-friendly dyeing of wool using natural dye from weld as co-partner with synthetic dye. *J Clean. Prod.* **19**(2011), 1045 (2011)
3. V. Narayana swamy, K.N. Ninge gowda, R. Sudhakar, Extraction and application of natural dye from byproduct of *Psidium guajava* L. leaves. *J. Natl. Fibers* **10**(2013), 257 (2013)
4. Y. Choia, H. Jeonga, J. Lee, Antioxidant activity of methanolic extracts from some grains consumed in Korea. *Food Chem.* **103**, 130 (2007)
5. The wealth of India: Raw materials publication & Information Directorate, CSIR, New Delhi, vol. **X**, p 520 (1976)
6. M.G. Dharmasiri, J.R. Jayakody, G. Galhena, S.S. Liyanage, W.D. Ratnasooriya, anti-inflammatory and analgesic activities of mature fresh leaves of *Vitex negundo*. *J Ethnopharmacol* **87**(2–3), 199 (2003)
7. R.P. Samy, S. Ignacimuthu, A. Sen, Screening of 34 Indian Medicinal plants for antibacterial property. *J. Ethnopharmacol.* **62**(2), 173 (1998)
8. V.R. Tandon, Medicinal uses and biological activities of *Vitex negundo*. *Natl. Prod. Radiance* **4**(3), 162 (2005)
9. P.L. Ladda, C.S. Magdum, *Vitex negundo* Linn: ethnobotany, phytochemistry and pharmacology—a review. *Int. J. Adv. Pharm. Biol. Chem.* **1**(1), 111 (2012)
10. A.K. Meena, U.S. Niranjana, M.M. Rao, M.M. Padhi, R. Babu, A review of the important chemical constituents and medicinal uses of *Vitex* genus. *Asian J. Tradit. Med.* **6**(2), 54 (2011)
11. R. Singh, A. Jain, S. Panwar, D. Gupta, S.K. Khare, Antimicrobial activity of some natural dyes. *Dyes Pigm.* **66**, 99 (2005)
12. S. Baliarsingh, A.K. Panda, J. Jena, T. Das, N.B. Das, Exploring sustainable technique on natural dye extraction from native plants for textile: identification of colorants, colourimetric analysis of dyed yarns and their antimicrobial evaluation. *J. Clean. Prod.* **37**(2012), 257 (2012)
13. V. Narayana Swamy, K.N. Ninge Gowda, R. Sudhakar, Dyeing properties of natural dye *Syzygium cumini* on silk. *J. Inst. Eng. India Ser. E* **95**(1), 11 (2014)
14. H. Lokeshwari, G.T. Chandrappa, Impact of heavy metal contamination of Bellandur Lake on soil and cultivated vegetation. *Curr. Sci.* **91**(5), 622 (2006)
15. H.F. Mansour, A.A. Haroun, Ultrasonic efficiency on the photofading of Madder dyed silk using egg albumen and aluminium ions chelating. in *The 6th International Conference of Textile Research Division*, Cairo, Egypt: National Research Centre 15–67 (2009)