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STUDY ON THE APPLICATION OF VITEX AGNUS-CASTUS NATURAL DYE FOR WOOL

Dr. V. Narayana Swamy*, Dr. K. N. Ninge Gowda, Dr. R. Sudhakar
Department of fashion and apparel design, the oxford college of science, Bangalore 560 102. India.
affiliation : Bangalore University
Head, department of fashion and apparel design, Bangalore University, India
Department of fashion and apparel design Bangalore University, India

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ABSTRACT

The present paper investigates extraction of natural dye from leaves of the plant *Vitex agnus-castus,* which is an abundant, cheap, and readily available agriculture by -product. Leaf extracts can be readily sorbed by wool fabrics directly with mordants all of which are benign to human health and the environment. Color values and color coordinates in terms of L*, a*, b*, C, and h were examined. Dyed wool fabrics demonstrated acceptable fastness properties. The extracted dye was tested for some of the eco-parameters using AAS and GC/MS. 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.

KEY WORDS: antimicrobial properties, dyeing, heavy metals, mordants, *Vitex agnus-castus*, wool

INTRODUCTION

Consumers have become more aware of environmental issues; in fact, they are increasingly seeking products with a green or organic label. During the last few decades a growing interest in the use of natural colorants has been evident both in public awareness and scientific activity [1-4]. This increased interest could be on account of the natural dye's high compatibility with the environment, relatively low toxicity and low allergic effects [5-8]. 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 agnus-castus (Linn.) belongs to family Verbenaceae, is an aromatic, ornamental and deciduous shrub native to the arid and semi arid Mediterranean and Western Asia and widely cultivated in the warm temperate regions and subtropics. It is a medicinal plant [9] traditionally used for the treatment of several health problems and symptoms, such as premenstrual ones and spasmodic dysmenorrheal, certain menopausal conditions, insufficient lactation and acne exhibited stronger antioxidant activity [10]. The major flavonoids present in the leaves are casticin (Fig 1), orientin (Fig 2), vitexin (Fig 3) [12-15] which makes it a possible dye candidate for wool fabric. In the present investigation an attempt has been made to extract natural dye from *Vitex agnus-castus* leaves, evaluate its color measurement, color-fastness properties, heavy metal content, and antimicrobial activity.

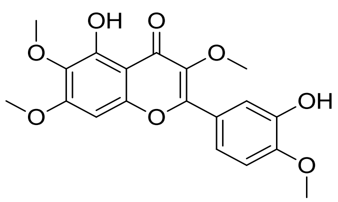

Fig. 1 Casticin

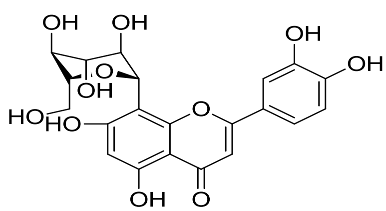

Fig. 2 Orientin

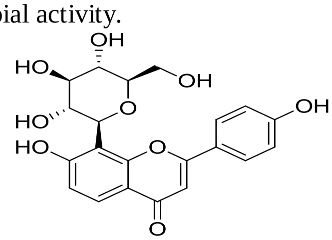

Fig. 3 Vitexin

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EXPERIMENTAL MATERIALS

The degummed, scoured *Bombyx mori* wool fabric described previously [16] was used. The dry leaves of *Vitex agnus-castus* were collected from Hosur, Tamilnadu. Potassium aluminum sulphate [$KAl(SO_4)_2.12H_2O$], tannic acid [$C_{76}H_{52}O_{46}$], tartaric acid [$C_4H_6O_6$] were used as mordants, all of analytical grade and purchased from Merck. Distilled water was used for extraction of dye and preparation of chemical solutions.

DYE EXTRACTION

Fresh *Vitex agnus-castus* leaves were dried at room temperature till complete 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 resulting extract was distilled enough to obtain a pasty mass through this process of distillation. This pasty extract was then washed with a non-polar solvent such as petroleum ether and freeze dried to yield solid coloring materials for dyeing.

METHOD OF MORDANTING

Mordanting process with aluminum potassium sulphate, tannic acid and tartaric acid was performed. 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 a MLR ratio of 1:40 at 60°C for 30 min. After that the pre-treated sample was rinsed with tap water and dried at room temperature. 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

Wool fabric was added to a bath containing 10% of dye solution at a MLR ratio of 1:50 at 90°C. The dyeing was maintained for 60 min at acidic pH value (adjusted using acetic acid). The dyed sample also underwent sequential treatments: rinsing with tap water, washing in a bath containing 1 g/l nonionic detergent at a material and liquor ratio of 1:30 at 50°C for 10 min, a second rinsing with tap water and drying at room temperature.

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:
% Dye exhaustion = $[(A_0-A_1)/A_0]\times100$,
where A_0 and A_1 are absorbance at a wavelength of maximum absorption (λ_{max}) of dye bath before and after dyeing.

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 λ_{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 degrees to 360 degrees. Angles that range from 0 to 90 degrees are reds, oranges and yellows. 90 to 180 degrees are yellows, yellow-greens and greens. 180 to 270 degrees are greens, cyan and blue. 270 to 360 degrees 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 properties were evaluated according to ISO 105 C02 using launder meter. 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.

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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 [17].

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 text 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 [18-19]. 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.

$$R = [(A-B)/A]\times100,$$

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: % Retention of antimicrobial activity = $\frac{\mathrm{Rn}}{R0}\times 100$, where R_0 = per cent of microbial reduction before washing and R_n= 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 solutions [20]. The extracted dye was also analyzed for the presence of banned aryl amines after reductive cleavage and isolation using GC/MS.

STATISTICAL ANALYSIS

Data pertaining to K/S values were expressed as mean ± S.E. effect of mordant, dosage and different techniques and analyzed by two-factor analysis of variance [21]. When a significant F ratio was found, least significant differences (LSD) were used to assess the difference between group means. Probability values $p<0.05$ were considered significant.

RESULTS AND DISCUSSION

OPTIMIZATION OF DYE EXTRACTION

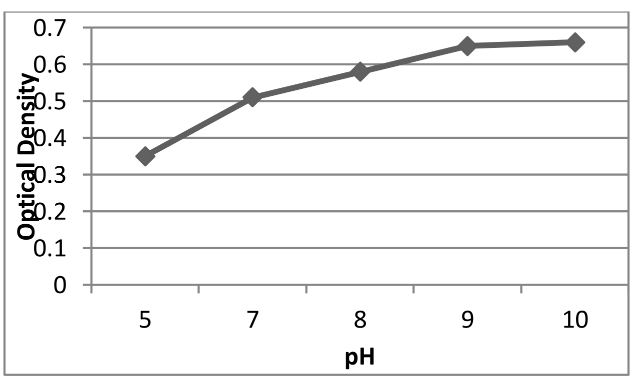

Fig. 4 Effect of pH on dye extraction

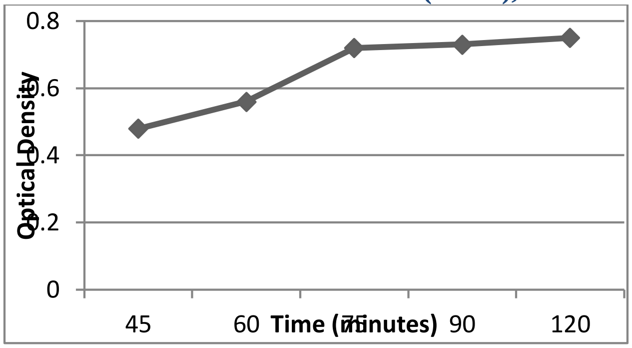

Fig. 5 Effect of duration on dye extraction

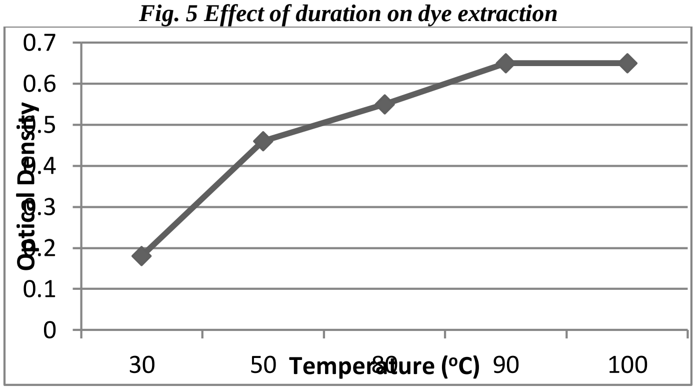

Fig. 6 Effect of temperature on dye extraction

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 in pH 9 (Fig 4), duration 90 (Fig 5) minutes at 90°C (Fig 6) temperature. Figure 5 shows that absorbance increases with increasing duration but, after 90 minutes the sediment in the extracts may be attributed.

The amount of dye uptake by wool fabrics dyed with *Vitex agnus-castus* leaves was expressed as percentage of exhaustion in Table 1. The maximum exhaustion was observed in the case of post- mordanted tartaric acid and alum-mordanted samples

EFFECT OF DYEING CONDITIONS

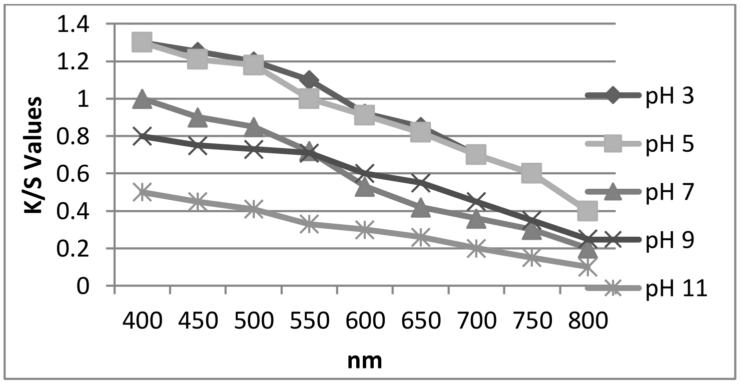

Fig. 7 Effect of dye bath pH on color strength of dyed wool fabrics

Figure 7 show that the pH of the dye bath has a considerable effect on the dye- ability of wool fabric. As the pH increases, the dye-ability decreases; the effect of the dye bath pH can be attributed to the correlation between dye and wool fibers. Since the dye used is water soluble containing anionic groups, it would interact ionically with the protonated terminal amino groups of wool fibers at acidic pH via ion-exchange reaction [22]. This ionic attraction would increase the dye-ability of the fiber as clearly observed. At pH > 5, the dyeing qualities of wool decreased markedly, in this part, all the protonated terminal amino groups fixed on wool fabric interact with the dye, thus no additional dye is adsorbed.

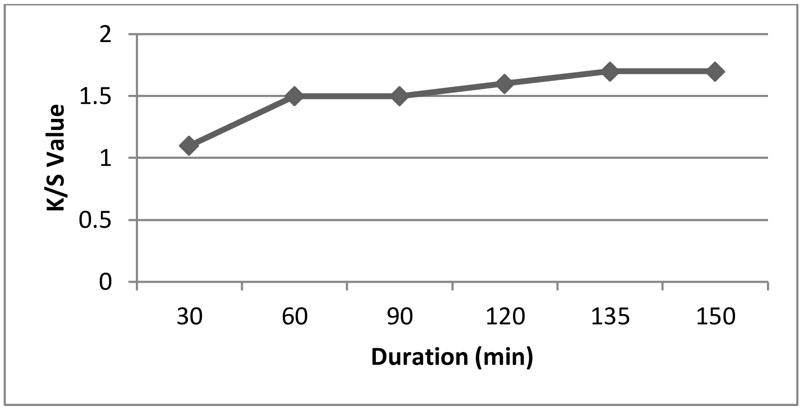

Fig. 8 Effect of dyeing time on the color strength of wool fabrics

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 wool fabrics reach saturation and do not absorb any more natural dyes.

DETERMINATION OF COLOR COORDINATES OF DYED SAMPLES

Table 1 *Colorimetric data of wool samples dyed with Vitex agnus-castus leaf extract*

Mordant	Method	% Dye exhaustion	Mordant %	K/S	L*	a*	b*	C	h
Undyed	-		-	-	90.42	2.25	0.11	2.19	365.715
Control	-	52	-	1.15	55.21	14.12	09.12	42.18	46.55
Alum	Pre	82	5	3.01	53.68	3.03	15.62	16.16	78.60
		85	10	5.61	44.98	1.55	12.42	16.85	83.18
	Post	87	5	4.12	51.58	3.51	13.68	14.15	75.36
		90	10	6.55	45.87	1.42	10.18	10.16	82.42
Tannic	Pre	66	5	2.62	53.54	3.75	16.25	16.69	76.69
		82	10	3.00	52.12	3.56	15.54	16.35	76.84
	Post	81	5	3.61	53.77	3.62	14.66	14.48	75.25
		83	10	4.02	51.28	2.85	13.45	13.78	78.26
Tartaric	Pre	84	5	4.05	51.45	2.89	13.65	13.62	78.66
		88	10	5.68	48.71	1.42	12.59	12.91	83.47
	Post	86	5	4.01	49.21	1.19	12.35	12.78	75.78
		90	10	6.09	44.22	1.42	10.15	10.15	83.59

Note: pre: premordanting, post: postmordanting. Control: wool dyed without mordant; undyed: fabric without mordant and dye.

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 samples. Post-mordanted samples of both alum and tartaric acid were found to have more a prominent effect on colour strength than others. From a* and b* the color gamut of wool samples dyed with *Vitex agnus-castus* 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.

C* value shows alum and tannic acid mordanting results in brighter shades whereas tannic acid increases the dull shade.

FASTNESS TESTING

Table 2 *Fastness properties for wool fabric dyed with Vitex agnus-castus leaf 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	5
		10	4	4	5	5	5
	Post	5	3	3	5	5	5
		10	4	4	5	5	5
Tannic 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
Tartaric acid	Pre	5	3	4	5	5	5
		10	4	4	5	5	5
	Post	5	3	4	5	5	5
		10	4	4	5	5	5

Note**: cc color change, cs color staining, control: wool dyed without mordants**

Light-fastness was observed from the colour fastness data (Table 2) that all the samples dyed with *Vitex agnus-castus* exhibit moderate to good light-fastness i.e., 3-5. The dyed samples show moderate to good wash-fastness ratings ranging from 3-4 on grey scale 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 on the grey scale.

ANTIBACTERIAL PROPERTIES

Table 3 *Antibacterial activity against E. coli of wool samples (10%) dyed with Vitex agnus-castus leaf extract with and without mordants after dyeing and laundering*

Mordants	Method	Mordant %	Bacterial Reduction (%)	
			After dyeing	After laundering
Control	--	--	70.6	70.0
Alum	Pre	5	80.0	79.5
		10	81.1	79.5
	Post	5	78.2	76.0
		10	80.1	78.9
Tannic acid	Pre	5	87.6	86.4
		10	91.5	92.0
	Post	5	88.2	85.5
		10	94.5	93.0
Tartaric acid	Pre	5	80.6	79.1
		10	81.5	80.1
	Post	5	76.5	75.1
		10	81.2	80.5

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***Table 4** Antibacterial activity against Staphylococcus aureus of wool samples (10%) dyed with Vitex agnus-castus leaf extract with and without mordants after dyeing and laundering*

Mordants	Method	Mordant %	Bacterial Reduction (%)	
			After dyeing	After laundering
Control	--	--	65.6	64.6
Alum	Pre	5	75.8	75.4
		10	77.5	77.0
	Post	5	79.0	78.1
		10	80.4	80.0
Tannic acid	Pre	5	84.0	83.0
		10	85.1	85.5
	Post	5	86.0	83.0
		10	86.1	85.5
Tartaric acid	Pre	5	80.5	78.1
		10	81.0	76.0
	Post	5	79.2	78.0
		10	85.8	83.0

The antibacterial activity of mordanted and unmordanted dyed wool fabrics against *E. coli* and *S. aureus* was assessed. Tables 3 and 4 show the antibacterial results of samples dyed using *Vitex agnus-castus* 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 high dye uptake. Therefore, this study was also carried out on the mordanted wool samples. The antibacterial activity of dyed samples has dramatically increased after mordanting. Regardless of the nature of the mordant used in dyeing, samples with different mordant treatments showed different levels of reduction 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. Among the three mordants, tannic acid had almost the 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 [23]. Post-mordanted samples with 10 % mordant show a higher color strength and fastness properties, but there is significant reduction in their antibacterial properties after mordanting. They claim that it is due to the complex formation between active function groups of the dye and the metal salt mordants[24-25].

WASH DURABILITY OF ANTIMICROBIAL ACTIVITY

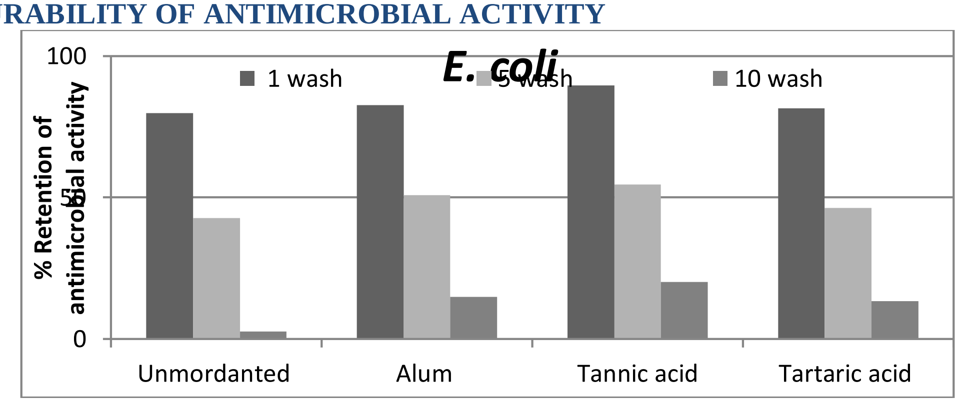

***Fig. 9** % Retention of antimicrobial activity to washing against E. coli*

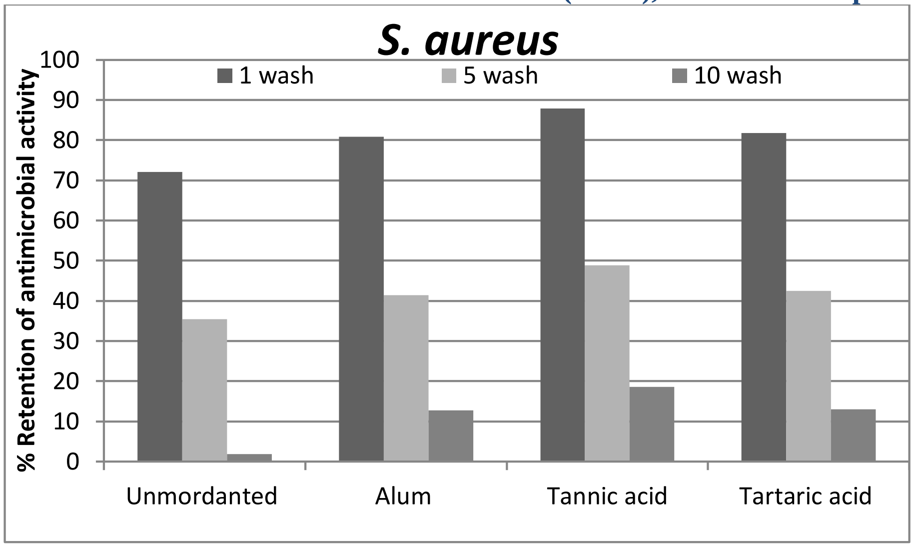

Fig. 10 % Retention of antimicrobial activity to washing against S. aureus

Figures 9 and 10 represent the durability of antimicrobial activity in terms of % retention of microbial activity after washing. Microbial % reduction of dyed wool fabric samples were examined over three different launderings 1, 5 and 10. Samples dyed with tannic acid mordants were found to be most effective against both tested microbes also durable washing. After 5 washing cycles microbial activity was reduced to less than 45% of the initial stage against both 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.

ANALYSIS OF ELEMENTS IN DYED SAMPLES

Table 5 *Concentration of red listed chemicals in the extracted dye*

Parameters	Permissible range (ppm)	Dye (ppm)
Heavy metals		
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

***Notes*: NT: not traceable; Ab: Absent**

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

CONCLUSION

The aim of this study is to use industrial agriculture waste as a natural coloring agent in the textile industry. For this purpose, commercially available *Vitex agnus-castus* leaves were used to obtain natural dye pigments by the extraction method. The extraction of natural color from plants is a sustainable process towards waste utilization. All the dyed wool samples were found to have good color- fastness to washing, rubbing and acceptable color-fastness to light; therefore, the colorant can be an alternative to synthetic dyes. Also, antibacterial activity was tested 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 because on testing, the red listed chemicals are either absent or their concentration is much below the stipulated limits. The data generated from the present study could 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 enable modern science and technology to serve further the global demand for dyed textiles using safe and eco-friendly dyes from natural sources. It can be concluded that the abundantly available agricultural by-product *Vitex agnus-castus* has great potential to be effectively utilized as a natural dye for wool textiles.

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