

〈Research Paper〉

A Pre-treatment Process for Natural Dyeing of Wool to Impart Durable Antimicrobial Efficacy

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Abstract— A pre-treatment process has been developed for natural dyeing of wool by which the dyed materials have been imparted antimicrobial efficacy against both gram-positive and gram-negative bacteria durable up to 20 washes. In this process, wool fabrics were treated with citric acid under oxidizing condition prior to dyeing. The treated fabrics were then dyed with four different types of natural dye powders obtained from leaves of silver oak, wattle, tanner's senna and flame of forest. All the natural dyes produced yellowish brown colour on wool fabrics. The washing and light fastness properties of the fabrics subjected to pre-treatment were one grade higher compared to those of the dyed fabrics without pre-treatment. The pre-treated wool fabrics showed antimicrobial efficacy against both gram-positive bacteria (*Staphylococcus aureus*) and gram-negative bacteria (*Escherichia coli*). The fabrics dyed without pre-treatment showed antimicrobial efficacy against gram-positive bacteria (*Staphylococcus aureus*) only. The durability of antimicrobial efficacy was higher in pre-treated and dyed wool fabrics compared to the dyed fabrics without pre-treatment.

Keywords: *antimicrobial, citric acid, natural dyes, tannin, wool*

1. Introduction

The textile materials are good media for generation and spreading of microorganisms. Keratin and cellulose can be as nutrients for the growth of microorganisms¹⁾. The growth of microorganisms in the textile materials causes unpleasant smell, staining, loss of mechanical strength etc., and it can create health-related problems to the wearer. Hence, antimicrobial finishing of textile materials is necessary to avoid infections of the wearer from the harmful microorganisms. Wool materials have been treated with various synthetic antimicrobial agents like quaternary ammonium compounds (QAc), polyhexamethylene biguanide (PHMB), nano silver, etc. to impart antimicrobial efficacy. Such antimicrobial agents of high cost limit their use for common wool materials like shawl, sweater etc. They are also considered to have negative

impact on environment.

There are several studies in literature on natural dyes extracted from *Quercus infectoria*, curcumin, etc.^{2,3)} which have been used to give both dyeing and antimicrobial finishing to the textile materials. Earlier, we have developed a natural dyeing process for wool using dyes extracted from the leaves of deciduous plants, namely, silver oak, wattle, flame of forest, tanner's senna⁴⁾. The tannins present in such plant leaves are a group of water-soluble polyphenols in the molecular weight range of 300 to 5000 Da. The tannins can be divided into two groups, namely, hydrolysable tannins and condensed tannins. The hydrolysable tannins are usually compounds containing a central core of glucose or other polyhydric alcohols esterified with gallic acid (gallotannins) or hexahydroxydiphenic acid (ellagitannins). The condensed tannins are polymer of flavan-3-ol (catechin) units. They are also called as proanthocyanidin. It is well documented that the

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tannins present in the different parts of the plants like bark, leaf, fruit etc., have antimicrobial property to several strains of bacteria through *in vitro* studies⁵⁻⁷. The antimicrobial mechanisms of tannins can be summarized as follows: (i) Tannin binds the proteins and enzymes present in the cell wall of microorganisms and inhibit their growth, (ii) Tannins also have the ability to bind vital metal ions used by the microorganisms for their growth⁸, (iii) The gallic acid released from tannins is able to inhibit the growth of microorganisms by reacting with their cell wall and inhibiting their metabolism.

The antimicrobial efficacy of natural dye extracts depends on the source of tannins and their concentration on the substrate. Generally, the natural dyed materials exhibit good antimicrobial efficacy against gram positive bacteria rather than gram negative bacteria. In order to produce antimicrobial efficacy against gram negative bacteria, higher concentration of natural dyes on the substrate is required. However, the absorption of natural dye on textile material cannot be increased after particular saturation point. The organic acids like citric acid has antimicrobial efficacy against several strains of bacteria and widely used in the food industry and for treatment of chronic wounds^{9,10}. Based on the above facts, in this research a pre-treatment is proposed, where citric acid was incorporated on wool prior to dyeing. The pre-treated wool was then dyed with the natural dyes extracted from tannin-containing leaves. Finally, the antimicrobial efficacy of the dyed materials with and without pre-treatment was determined and compared.

2. Materials and Methods

2.1 Pre-treatment Process

Analytical grade hydrogen peroxide, citric acid and aluminium sulphate were used as such. A pure wool fabric was used for this study. The wool fabrics were pre-treated with citric acid by two methods viz direct method and under oxidizing condition. In the direct method, wool fabric was treated with 3% (owm) citric acid in acidic pH for

90 min at boiling condition. In the second method, wool fabric was treated with 3% (owm) citric acid under oxidising condition by using 3% (owm) hydrogen peroxide at pH 5. The treatment was given for 90 min under boiling condition.

2.2 Dyeing of Wool with Natural Dyes

Four plant leaves, namely, silver oak [*Grevillea robusta* (SOL)], flame of forest [*Spathodea campanulata* (FOF)], tanner's senna [*Cassia auriculata* (AL)] and wattle [*Acacia decurrens* (WL)], were collected from Western Ghats of India and were used to extract dye.

2.3 Extraction of Colouring Materials and Conversion to Powder

All the plant leaves were dried in shadow to a moisture content of 10-20% and then ground into powder. 40g of each crude powder was then soaked in 200ml of water for 24h followed by boiling in water bath for 2h for extraction of water soluble colorants. The crude extract was then filtered and spray dried using Labultima LU 222 spray dryer into powder. The purified natural dye powders were used for dyeing experiments.

2.4 Dyeing and Mordanting

All the dyeing experiments were carried out by exhaustion method in a water bath, keeping material to liquor ratio to 1:50. The wool fabrics (5g) were introduced into dye bath containing 5% dye (owm) at room temperature and the temperature was increased to 85°C with gentle stirring. The dyeing was then continued for one hour. The simultaneous mordanting with aluminium sulphate was carried out in the same bath after exhaustion of the dye. The temperature of the bath was allowed to cool below 50°C and the mordant solution of 3% concentration (owm) was added. The temperature was then raised to 85°C and maintained for another one hour. After mordanting, the fabrics were taken out of the bath and thoroughly washed with water, followed by washing with detergent.

2.5 Testing Methods

The light and washing fastness of the dyed fabrics were done as per the standard methods, namely, ISO 105(CO3) and BS 1006(BO2), respectively. The spectral values of the dyed samples were compared with white sample (standard). ΔL , Δa , Δb , K/S and ΔE (CIE 2000) values were determined using a Minolta 508 spectrophotometer with Macbeth Match View software in D65 day light. The colour difference (ΔE) was calculated according to Eqn 1:

$$\Delta E = (\Delta L^2 + \Delta a^2 + \Delta b^2)^{1/2} \quad (1)$$

Where, ΔL , Δa , Δb are the difference between sample and standard. If, $\Delta L < 0$, the sample is darker than the standard; $\Delta L > 0$, the sample is lighter than the standard; $\Delta a < 0$, the sample is greener than the standard; $\Delta a > 0$, the sample is redder than the standard; $\Delta b < 0$, the sample is bluer than the standard; $\Delta b > 0$, the sample is more yellow than the standard. K/S value is linearly related to the concentration of dyes in the material.

2.6 Testing of Antimicrobial Efficacy

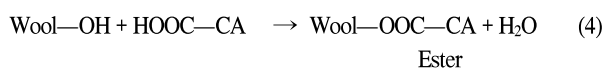
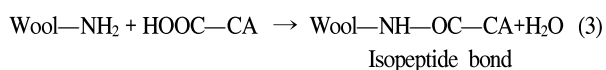
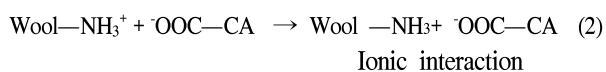
The antimicrobial efficacy of the treated wool fabrics along with their different washed fabrics was determined using qualitative Agar Diffusion Method (SN 195920) against gram positive and gram negative bacteria viz. *Staphylococcus aureus* (*S.aureus*) and *Escherichia coli* (*E.coli*) respectively. In this method, the evaluation was made on the basis of absence or presence of an effect of bacteria in the contact zone under the specimen and the possible formation of a zone of inhibition around the test specimen. The area of inhibition zone in mm was a measure of antimicrobial effectiveness. If the test specimen did not show any zone of inhibition, the growth of microorganisms beneath the test specimen was observed. If there was no growth of bacteria beneath the test specimen in the petri dish, it was also inferred that such specimen has low level (Below 70%) of antimicrobial efficacy i.e. bacteriostatic in nature. Initially an *in vitro* study was made with pure natural dye extracts (5%

owm) by coating them on a sterilized filter paper and tested against the above bacteria using Agar Diffusion Method in order to confirm their antimicrobial efficacy.

3. Results and Discussion

3.1 Bond Formation between Wool and Citric Acid

During pre-treatment, the following bond formation is likely to form between wool and citric acid. Citric acid ($\text{HOOC-CH}_2\text{-C(OH)(COOH)-CH}_2\text{-COOH}$) has three carboxylic groups and one hydroxyl group in its structure capable of forming ionic, isopeptide or ester bonds with amino and hydroxyl groups present in the wool polymer side chains. The wool polymer contains basic amine ($-\text{NH}_2$) groups due to the side chain of histidine, arginine and lysine amino acids and polar hydroxyl ($-\text{OH}$) groups due to the side chain of serine, threonine and tyrosine amino acids¹¹. Under oxidising condition and at acidic pH, wool is more reactive due to swelling of its hydrophobic cuticle cells which hinders the reaction with citric acid. The acidic pH during oxidization of wool enhances the availability of polar side chain groups of wool due to ionisation. Under these conditions, the citric acid can react with the basic amine groups and hydroxyl groups of wool and form ionic, amide and ester linkages as depicted below



The previous studies in this subject also indicated that the reaction between wool and citric acid was possible only under oxidising conditions^{12,13}. This may be due to the fact that the side chain amino and hydroxyl groups of wool fabric were already involved in the isopeptide, ionic, hydrogen bond

Table 1. ΔL , Δa and Δb value of dyed fabrics

Source		ΔL	Δa	Δb	ΔE	K/S	W.F	L.F
FOF	Pre-treated	-41.4	11.2	8.7	43.71	17.31	4	6-7
	Control	-32.5	11.3	16	37.94	15.28	3-4	6
AL	Pre-treated	-38.1	6.1	8.5	39.55	16.23	4-5	6-7
	Control	-30.5	7.8	18.3	36.41	15.45	3-4	5-6
WL	Pre-treated	-24.8	4.5	18.2	31.57	12.10	4	6
	Control	-28.5	6.4	18.5	34.78	14.78	3-4	5-6
SOL	Pre-treated	-29	5.7	14.7	32.99	16.23	4	6
	Control	-31.4	5.4	14.3	34.91	15.45	3	6

with the acidic side chain groups of other wool amino acids like aspartic acid, glutamic acid etc. and if treated directly with citric acid, it may not be able to form bond formation with citric acid. From the above, it was inferred that the citric acid under oxidizing condition reacted with the wool and formed different types of bonds like ester, isopeptide etc.

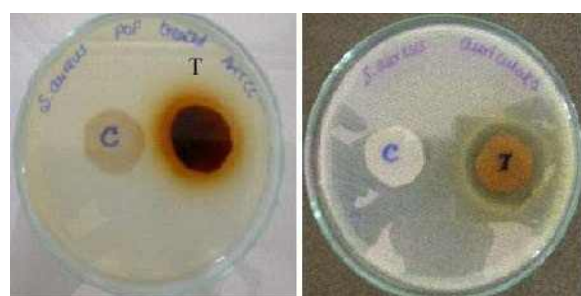
3.2 Dyeing

All the four aqueous leaf extracts dyed both the pre-treated and control wool fabrics satisfactorily. For each group, four dyeing operations were carried out using all the four leaf extracts. The dyeability of leaf extracts resulted from the presence 6-10% of tannins in their chemical constitution. The ΔL , Δa , Δb , K/S , ΔE , washing fastness and light fastness values of dyed samples are given in Table 1. In brief, fabrics subjected to pre-treatment were 4% darker, 3% less redder and 25% less yellow in colour than control fabrics. The K/S and ΔE values of dyed fabrics showed that all the pre-treated fabrics except WL dyed fabrics absorbed more dye compared to the control fabrics. The presence of citric acid in pre-treated wool fabric influenced the dye uptake. Citric acid with its three carboxylic and a hydroxyl group was able to form crosslinkages with the wool and natural dye molecules. Thus, citric acid acted as a mordant and increased the uptake of natural dyes on wool as well as fixation. The washing and light fastness of fabrics subjected to pre-treatment was higher (4 & 4-5) compared to control fabrics (3 & 3-4) due to the reasons aforementioned.

3.3 Antimicrobial Efficacy of Dyed Fabrics

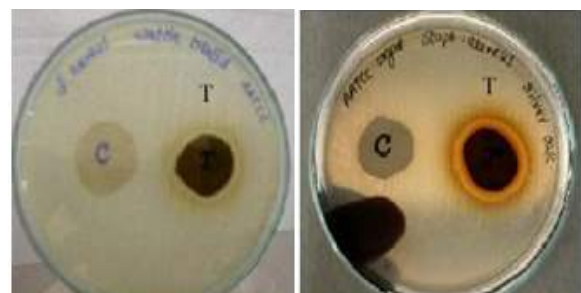
The *in-vitro* study using all the four natural dye extracts loaded filter papers (Figs. 1 & 2) showed that all the four natural dye extracts had antimicrobial efficacy to both *S.aureus* and *E.coli*. From the results, it was inferred that all the selected natural dyes in powder form have antimicrobial efficacy against both gram positive and gram negative bacteria. The antimicrobial efficacy of control and pre-treated wool fabrics along with their differently washed samples against *S.aureus* are shown in Table 2. Both control and pre-treated fabrics showed a clear zone of inhibition against *S.aureus* (Figs. 3 & 4). However, the dyed fabrics subjected to citric acid pre-treatment showed a larger zone of inhibition against *S.aureus*. The antimicrobial efficacy of the pre-treated and dyed fabrics was durable up to 20 washes compared to 5 washes in the case of control fabrics. The undyed fabric but pre-treated with citric acid under oxidising condition did not show any zone of inhibition against *S.aureus*. However, it showed the absence of bacteria growth beneath the test specimen. The result implied that the above pre-treated wool fabric had low level of antimicrobial efficacy due to the presence of crosslinked citric acid.

The antimicrobial efficacy of control and pre-treated wool fabrics along with their differently washed samples against *E.coli* are shown in Table 3. The pre-treated and dyed fabrics showed a clear zone of inhibition against *E.coli*, (Fig. 5) in the case of all dye sources. The antimicrobial efficacy of the pre-treated and dyed fabrics was durable up to 15



(a) Flame of forest

(b) Tanner's Senna

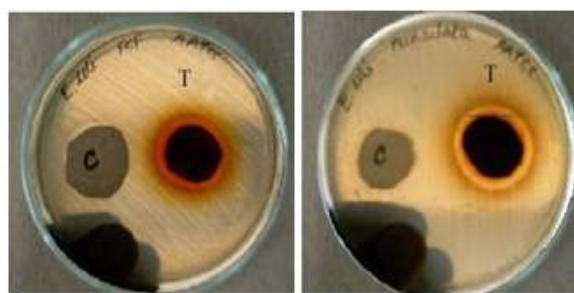


(c) Wattle

(d) Silver oak

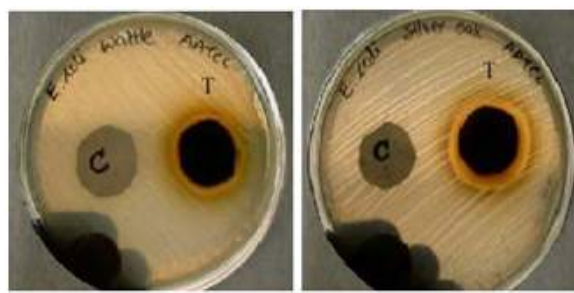
Fig. 1. Zone of inhibition against *S. aureus* – Natural dyes treated filter paper.

washes. However, the fabrics showed low level of antimicrobial efficacy even after 20 washes i.e there was no growth of bacteria beneath the test specimen. The control as well as pre-treated fabrics



(a) Flame of forest

(b) Tanner's Senna



(c) Wattle

(d) Silver oak

Fig. 2. Zone of inhibition against *E. coli* – Natural dyes treated filter paper.

without dyeing did not show any zone of inhibition against *E.coli*. The antimicrobial efficacy of the dyed materials depends upon the nature of dye source, concentration, type of pre-treatment etc.



(a) Flame of forest

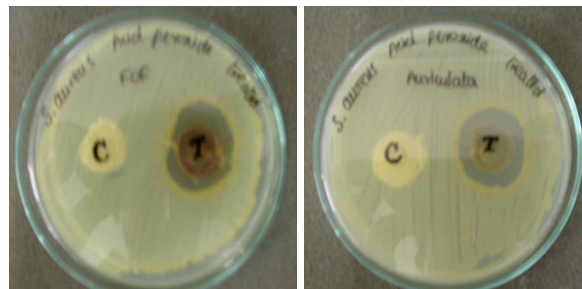
(b) Tanner's Senna



(c) Wattle

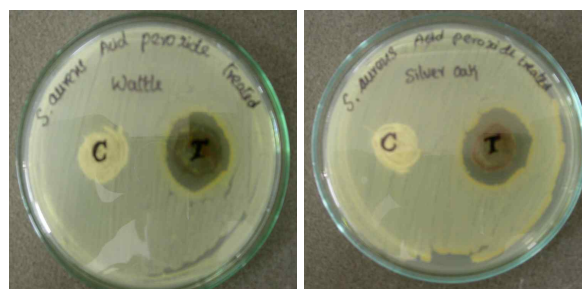
(d) Silver oak

Fig. 3. Zone of inhibition against *S. aureus* – Dyed fabrics without pre-treatment.



(a) Flame of forest

(b) Tanner's Senna



(c) Wattle

(d) Silver oak

Fig. 4. Zone of inhibition against *S. aureus* – Pre-treated and dyed fabrics.

Table 2. Antimicrobial efficacy of pre-treated and control fabrics against *S.aureus*

Source of dye			Zone of Inhibition (mm)* against <i>S.aureus</i>			
			After 5 washes	After 10 washes	After 20 washes	
Undyed	Pre-treated	**	-	-	-	
FOF	Pre-treated	25	24	22	20	
	Control	24	**	-	-	
AL	Pre-treated	26	26	24	21	
	Control	20	**	-	-	
WL	Pre-treated	27	26	22	20	
	Control	29	25	**	-	
SOL	Pre-treated	22	22	20	**	
	Control	21	**	-	-	

* Diameter of test specimen is 15 mm

** No growth of bacteria beneath the fabric

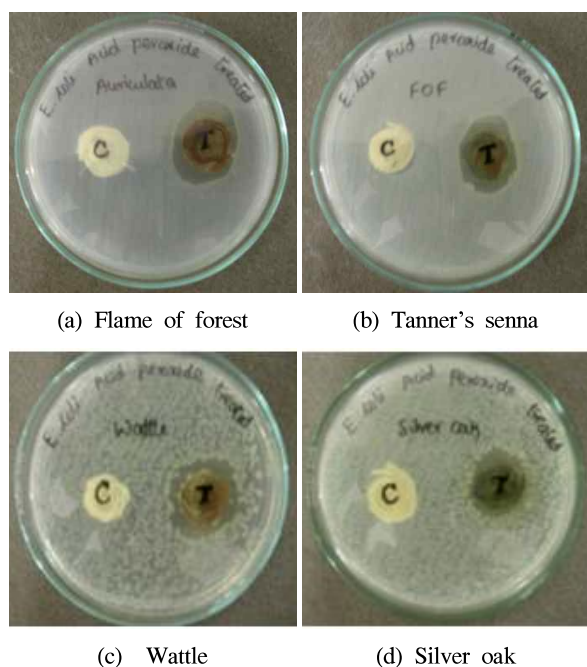


Fig. 5. Zone of inhibition against *E. coli* - Pre-treated and dyed fabrics.

The gram positive *S.aureus* is more susceptible to the action of tannins than gram-negative *E.coli* bacteria. Possibly the presence of outer membrane layer in the cell wall of gram negative species may serve as an effective barrier¹⁴⁾ against the action of antimicrobial agents. The antimicrobial efficacy of the dyed materials depends on the concentration of the tannins. The pre-treated wool fabrics had citric acid, a known antibiotic substance but its concentration was not sufficient to provide 100% antimicrobial efficacy to the fabric. When the pre-treated fabrics were dyed, the combined effect of citric acid and the tannins present in the dyes provided antimicrobial efficacy against *E.coli*. Due to the same reasons, pre-treated and dyed fabrics showed higher antimicrobial efficacy against *S.aureus* compared to control fabrics.

Table 3. Antimicrobial efficacy of pre-treated and control fabrics against *E.coli*

Source			Zone of Inhibition (mm)* against <i>E.coli</i>			
			After 5 washes	After 10 washes	After 20 washes	
Undyed	Pre-treated	-	-	-	-	
FOF	Pre-treated	20	20	18	**	
	Control	-	-	-	-	
AL	Pre-treated	18	18	16	**	
	Control	-	-	-	-	
WL	Pre-treated	22	20	18	**	
	Control	-	-	-	-	
SOL	Pre-treated	23	21	18	**	
	Control	-	-	-	-	

* Diameter of test specimen is 15 mm

** No growth of bacteria beneath the fabric

4. Conclusion

A pre-treatment process incorporating citric acid on wool was developed to enhance the antimicrobial efficacy and durability of natural dyed material. The presence of citric acid in the pre-treated wool improved the dye uptake and fastness. The pre-treated and dyed wool fabrics exhibited antimicrobial efficacy to both gram-positive and gram-negative bacteria durable up to 20 washes. The wool fabrics dyed without pre-treatment showed antimicrobial efficacy only to gram-positive bacteria with durability up to 5 washes.

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