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Influence of Enzyme and Mordant Treatments on the Antimicrobial Efficacy of Natural Dyes on Wool Materials

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ABSTRACT

Some of the natural dyes containing tannin as their major chemical constituent can be used to impart antimicrobial efficacy to wool fabrics in addition to dyeing. The treatment with mordant is inevitable during natural dyeing process in order to improve the fastness properties as well as to produce different shades on wool fabrics. Similarly, wool fabrics are subjected to protease enzyme treatment to increase the uptake of dye. In this study, it was attempted to study the effect of such enzyme and mordant treatments on the antimicrobial efficacy of the wool fabrics dyed with selected natural dyes. Wool fabrics were treated with four natural dyes having *in vitro* antimicrobial efficacy to both gram positive and gram negative bacteria with and without the use of enzyme and mordants. The test results showed that the antimicrobial efficacy of dyed wool samples was significantly influenced by enzyme and mordant treatments. The control dyed fabrics showed antimicrobial efficacy only against gram positive *S. aureus* bacteria whereas the enzyme treated fabrics had antimicrobial efficacy against both *S. aureus* and gram negative *E. coli* bacteria. This may be due to 17% higher dye uptake in the enzyme treated materials. The mordant treated wool fabrics generally showed less antimicrobial efficacy against *S. aureus* compared to control dyed fabrics. None of the mordanted materials had shown antimicrobial efficacy against *E. coli* even with enzyme pretreatment. The complex formation between the mordants and active constituents of tannins present in the dyes may be the reason for less or nil antimicrobial efficacies in those wool fabrics.

Key words: Antimicrobial efficacy, enzyme, mordant, natural dyes, tannin

INTRODUCTION

The application of antimicrobial agents to textiles is important in order to obtain aesthetic, hygienic and medical functions (Elshafei and El-Zanfaly, 2011; Elayarajah *et al*., 2011). Natural dye based antimicrobial finishing treatments for textiles are considered as safe, environment friendly and cost effective with additional benefit of colouring in a single stage. The natural dyes are extracted from the different parts of the plants such as bark, leaf, root and flower containing colouring materials like tannin, flavonoids, quinonoids etc. The tannins present in the colouring material impart antimicrobial efficacy to the textile materials during dyeing. Tannins present in such plant leaves are a group of water-soluble polyphenols in the molecular weight range of 300 to 5000 Da. They can be divided into two groups, namely, hydrolysable tannins and condensed tannins. The hydrolysable tannins are esters of polyhydric alcohols with gallic acid or hexahydroxydiphenic acid. The condensed tannins are polymer of flavans. They are also called as proanthocyanidin. It is well documented that the 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* studies (Calis *et al.*, 2009; Chang *et al.*, 1998; Min *et al.*, 2008). Tannins have been traditionally used for curing skin diseases, healing of wounds and burns (Sonibare *et al.*, 2011).

Wool is subjected to alkaline protease enzyme process to improve shrink resistance, handle and dye uptake. During such processes, the cuticle surface of wool is partially or completely damaged (Raja and Thilagavathi, 2010). The damage to cuticle which is the protective outer surface of wool facilitates more uptake of natural dye molecules (Tsatsaroni and Liakopoulou-Kyriakides, 1995). In general, the natural dyes provide only moderate washing and light fastness properties to the dyed materials. In order to improve the fastness properties, the natural dyed materials are treated with metal salts like aluminium sulphate, stannous chloride, potassium dichromate, copper sulphate, ferrous sulphate etc. The metal salt treatment is commonly known as mordanting in textile industry. The metals present in the salt forms coordinate complex with the dye and textile substrate and increase the fastness properties (Raja and Thilagavathi, 2011). There are only limited studies in the literature about the effect of these enzymes and mordant treatments on antimicrobial efficacy of natural dyed materials. Hence, the present study was undertaken to find out the effect of enzymes and mordants on the antimicrobial efficacy of wool textile materials dyed with natural dyes.

MATERIALS AND METHODS

This study was undertaken during the year 2009-2010. A wool fabric of 220 g/sq.m aerial density was used as textile material for this study. The fabric was scoured and bleached by conventional methods using sodium carbonate, non ionic detergent and hydrogen peroxide. The bleached fabric was then treated with alkaline protease enzyme (Perizym AFW) supplied by M/S Textilchemie DR. Petry GMBH, Germany using following recipe:

- Fabric weight: xg
- Wetting agent: 0.5%
- Enzyme: 2% (owm)
- pH: 9 (using buffer solution)
- Temperature: 70°C
- Time: 60 min

The control as well as enzyme treated fabrics were then taken for dyeing experiment.

Natural dyes were extracted from the leaves of silver oak [Grevillea robusta (SOL)], flame of forest [Spathodea campanulata (FOF)], tanner's senna [Cassia auriculata (AL)] and wattle [Acacia decurrens (WL)] were used. The leaf extract of tanner's senna is traditionally used as medicine for skin disease (Dhanasekaran and Ganapathy, 2011). The silver oak and wattle are the rich source of tannin (Bele *et al.*, 2010). The colouring matters from the plants were extracted by aqueous method at boiling condition. The extracts were then converted in to powders using laboratory model spray dryer. The natural dye powders were used for dyeing the fabric using standard procedure. In brief, the wool fabric was treated with 5% concentration of selected natural dyes on the weight of fabric in a dyeing bath at boiling condition for 1 h. Mordants such as aluminum sulphate

[$Al_2(SO_4)_3$], stannous chloride [$SnCl_2$] and ferrous sulphate [$FeSO_4$], of analytical grade were used for mordanting the fabric. The dyed materials were treated with 3% concentration (owm) above metal salts at boiling condition for 1 h in a dye bath itself. Finally, the fabrics were thoroughly washed to remove the surface adhered dyes and metal salts.

The light and washing fastness of the dyed samples were done as per the standard methods, namely, ISO 105 (CO_3) and BS 1006 (BO_2), respectively. The spectral values of the dyed samples were compared with white sample (standard). ΔL, Δa, Δb and ΔE (CIE-2000 based) values determined using a Minolta 508 spectrophotometer with Macbath Match View software in D65 day light. K/S value is linearly related to the concentration of dyes in the material. The colour difference (ΔE) was calculated according to Eq. 1:

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

where, ΔL, Δa and Δb are the difference between sample and standard.

The antimicrobial efficacy of the treated wool fabrics was determined using qualitative Agar Diffusion Method [SN 195920-1992 (Swiss Norm)] as per the literature (Thilagavathi *et al.*, 2005) against Staphylococcus aureus and Escherichia coli. For this test, AATCC bacteriostatic agar medium was used as a growth medium for evaluation. The bacteriostatic agar was sterilized in an autoclave. 15-20 mL of the sterilized agar was then added on a petri dish and allowed to harden for 30 min. The required bacterial culture (24 h incubated) of the test organism was then evenly spread on the agar using a cotton swab. In this experiment, a gram-positive *Staphylococcus aureus* and a gram-negative *Escherichia coli* were taken as representative bacteria for evaluation. The control and enzyme treated fabrics were placed on the solid agar inoculated with bacterial culture. The petri dish was then incubated at 37°C for 18-24 h. At the end of the incubation time, the test dish was observed. The evaluation was made based on 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 of the treated sample. If the test specimen did not show any zone of inhibition, the growth of microorganisms beneath the test specimen in the petri dish was observed. If there was no growth of bacteria beneath the test specimen in the petri dish, it was also inferred that such specimen had 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.

RESULTS AND DISCUSSION

Effect of enzyme and mordant on dye uptake: Our earlier study showed that leaf extracts of deciduous plants, namely, silver oak, wattle, flame of forest, tanner's senna can be used for dyeing wool with and without the use of mordants (Raja and Thilagavathi, 2008). The ΔE, K/S, Washing fastness, light fastness values of control and enzyme treated fabrics were given in Table 1. The fabrics dyed without mordant showed lighter shades. All the three mordants influenced the depth of the shades. Stannous chloride and ferrous sulphate-mordanted fabrics showed a maximum colour depth more than did the aluminium sulphate mordant. The stannous chloride mordants increased the depth of the original shades by about 235% and ferrous sulphate by 536%, whereas the aluminium sulphate mordant increased the depth by about 44%.

Table 1: The effect of enzyme and mordants on the dye uptake and fastness properties

	ΔE		K/S		Washing fastness		Light fastness	
Source/Mordant	Control	Enzyme treated	Control	Enzyme treated	Control	Enzyme treated	Control	Enzyme treated
FOF	39.05	45.42	12.05	13.29	3	3	4-5	4
FOF+AS	37.94	46.38	15.28	16.68	3-4	3-4	6	6-7
FOF+SC	45.74	54.87	42.15	52.99	4	4	6	6-7
FOF+FS	59.84	63.13	77.57	78.32	4	3-4	6	6
AL	32.30	40.95	10.14	11.72	3-4	3	5	5
AL+AS	36.41	46.56	15.45	18.26	4-5	4-5	5-6	6-7
AL+SC	40.84	51.14	30.80	40.41	4	4	5-6	5-6
AL+FS	57.87	61.95	70.39	73.91	3-4	4	6-7	6
WL	29.84	36.54	9.31	10.20	2-3	2-3	5-6	5
WL+AS	34.78	41.90	14.78	15.98	3-4	4	5-6	6
WL+SC	43.36	51.63	37.95	46.22	3-4	3-4	5-6	5-6
WL+FS	56.28	61.82	64.69	73.55	3-4	3-4	6-7	6
SOL	35.46	39.07	10.79	10.54	2-3	2-3	5-6	5-6
SOL+AS	34.91	41.79	15.45	18.26	3	3-4	6	6
SOL+SC	40.63	51.68	30.90	51.37	3-4	3-4	5-6	5-6
SOL+FS	53.59	59.40	56.12	64.38	4	4	6-7	6

FOF: Flame of forest; AL: Tanner's senna; WL: Wattle; SOL: Silver oak; AS: Aluminium sulphate; SC: Stannous chloride; FS: Ferrous sulphate

The ΔE and K/S values of the enzyme treated fabrics were 17% higher over the control wool fabrics. The ANOVA test also confirmed that there was a statistically significant difference existing between the colour difference values of control and enzyme treated wool fabrics. This result was attributed to the removal of surface cuticle of the wool during protease treatments which facilitated the higher dye uptake.

The dyed fabrics without mordanting showed poor to moderate washing fastness ratings of 2-3 and 3. The addition of mordants increased the washing fastness to good with the rating of 4. The light fastness of dyed fabrics was also moderate (4-5) and good (6-7) without and with mordant, respectively. The mordants in the order of ferrous sulphate>aluminium sulphate>stannous chloride increased the light and washing fastnesses of the fabrics. However, there was no significant change in washing and light fastness ratings between the control and enzyme treated fabrics.

Antimicrobial character of selected natural dyes: The *in vitro* study using all the four natural dye extracts loaded filter papers showed that all the natural dye extracts had antimicrobial efficacy against both *S. aureus* and *E. coli* (Raja and Thilagavathi, 2011). From the results, it was confirmed that all the selected natural dyes in powder form had antimicrobial efficacy against both gram positive and gram negative bacteria. The presence of 5-10% tannins in the selected dye sources was the reason for their antimicrobial nature. The antimicrobial mechanisms of tannins can be summarized as follows (1) Tannin binds the proteins and enzymes present in the cell wall of microorganisms and inhibit their growth (2) Tannins also have the ability to bind vital metal ions used by the microorganisms for their growth (Biradar *et al.*, 2008). (3) The gallic acid released from tannins is able to inhibit the growth of microorganisms by reacting with their cell wall and inhibiting their metabolism (Han *et al.*, 2007). Hence, the ester linkage between gallic acid and glucose in tannin is important to the antimicrobial potential of these compounds.

Table 2: The effect of enzyme and mordants on the antimicrobial efficacy of dyed wool fabrics against *S. aureus*

	Zone of inhibition (mm)*							
	Dyed sample		After 5 washes		After 10 washes		After 15 washes	
Source/Mordant	Control	Enzyme treated	Control	Enzyme treated	Control	Enzyme treated	Control	Enzyme treated
FOF	24	26	22	22	**	**	-	-
FOF+AS	20	22	20	22	18	20	**	**
FOF+SC	20	20	**	**	-	-	-	-
FOF+FS	18	18	-	-	-	-	-	-
AL	20	24	**	22	**	**	-	-
AL+AS	20	24	**	**	**	**	-	-
AL+SC	18	18	-	-	-	-	-	-
AL+FS	-	-	-	-	-	-	-	-
WL	29	32	26	30	20	20	**	**
WL+AS	25	27	22	22	**	20	**	**
WL+SC	25	25	20	22	**	**	-	-
WL+FS	21	22	20	20	-	-	-	-
SOL	21	23	20	21	-	-	-	-
SOL+AS	20	23	18	20	-	**	-	-
SOL+SC	18	20	-	**	-	-	-	-
SOL+FS	-	-	-	-	-	-	-	-

*Diameter of test specimen is 15 mm. **No growth of bacteria beneath the fabric

Effect of enzymes on antimicrobial efficacy: The results showed that all the four selected natural dyed fabrics exhibited clear zone of inhibition against *S. aureus*. However, the FOF and WL dyed fabrics showed higher zone of inhibition of 24 and 29 mm, respectively compared 20 and 21 mm of AL and SOL dyed fabrics against *S. aureus*. This may be due to the presence of higher tannin content in the above sources (Table 2). However, none of the dyed fabrics showed antimicrobial efficacy against *E. coli*. The antimicrobial efficacy of enzyme treated and dyed materials showed increased zone of inhibition against *S. aureus* irrespective of the dye source. The AL dyed fabric exhibited 20% increase in the antimicrobial efficacy against *S. aureus* due to enzyme treatment. Other sources exhibited about 9% increase in the antimicrobial efficacy. With respect to the antimicrobial efficacy of dyed fabrics against *E. coli* , it was observed that enzyme treated fabrics showed bacteriostatic effect against *E. coli* i.e., there was no growth of *E. coli* in those samples under test condition compared to the nil antimicrobial efficacy exhibited by control dyed fabrics. The result may be due to the fact that the gram positive *S. aureus* is more susceptible to the action of tannins than gram negative *E. coli* bacteria. Possibly because of the presence of outer membrane layer in the cell wall of gram negative species that serve as an effective barrier. However, the literature reveals that some tannin containing natural compounds have shown antimicrobial ability against gram negative bacteria also (Chattopadhyay *et al*., 2007). From the results, it was observed that the antimicrobial efficacy of the dyed fabrics was also dependent on the concentration of the tannins in the dyed materials. There should be sufficient concentration of antimicrobial agents on the fabric to inhibit the growth of microorganisms. Otherwise, even if the particular agent has *in vitro* antimicrobial efficacy, the effect will not be seen on the textile materials. Based on the above, it was inferred that the enzyme treatment on wool fabrics increased the dye uptake above the Minimum Inhibitory Concentration level (MIC) against the bacteria. This may the reason for larger zone of inhibition of enzyme treated fabrics against *S. aureus* compared to control fabrics. The same was the reason for bacteriostatic effect of enzyme treated wool against *E. coli* compared to nil antimicrobial efficacies of control fabrics. The antimicrobial efficacy of the dyed fabrics against *S. aureus* was fast up to 10 washes in the case of FOF and AL sources. The WL source showed durable antimicrobial efficacy up to 15 washes whereas of SOL source produced antimicrobial efficacy durable up to five washes only. There was no significant effect observed in the durability of the finish due to enzyme treatment.

Effect of mordants on antimicrobial efficacy: The results showed that, all the mordanted wool fabrics showed less antimicrobial efficacy against *S. aureus* than their corresponding dyed fabrics without mordant irrespective of the source. The FOF and WL dyed and aluminium sulphate mordanted fabrics showed 16 and 13% reduction in zone of inhibition against *S. aureus* compared to insignificant reduction in other two sources dyed fabrics (Table 2). The stannous chloride mordanted fabrics exhibited on the average 12% reduction in zone of inhibition against *S. aureus* irrespective of the dye source. It was observed that all the dyed fabrics using aluminium sulphate and stannous chloride mordants showed antimicrobial efficacy against *S. aureus*. However, in the case of ferrous sulphate mordant treated fabrics, only the dyed samples of FOF and WL sources exhibited antimicrobial efficacy against *S. aureus*. The AL and SOL source dyed fabrics with ferrous sulphate mordant did not show any antimicrobial efficacy against *S. aureus*. Among the mordants, the aluminium sulphate mordanted fabrics were shown higher antimicrobial efficacy against *S. aureus* and longer durability compared to stannous chloride mordanted fabrics. None of the mordanted fabrics of selected dye sources showed any antimicrobial activity against *E. coli*. During mordanting, the considerable proportion of tannins present in the dye sources combine with the metal ions and form insoluble complexes (Schofield *et al*., 2001). Thus the metal binding capacity of tannins, which is one of the reasons for their toxicity against microorganisms, is reduced. Among the metal salts, tannins have strong affinity for ferrous sulphate. This may the reason for low or nil antimicrobial efficacies of the ferrous sulphate mordant treated fabrics against both types of bacteria. After mordanting, the hydrolysis of ester bond present in tannin between gallic acid and flavane could not be taken place. This process resulted in non release of gallic acid which is the major compound responsible for antimicrobial efficacy of the selected dye sources. The above facts may be the reason for the low and nil antimicrobial efficacies of the mordant treated fabrics against *S. aureus* and *E. coli*.

CONCLUSION

The enzyme and mordant treatments significantly influenced the dye uptake and antimicrobial efficacy of wool fabrics dyed with selected natural dyes. Enzyme treatment increased the uptake of the dye on wool and provided the antimicrobial efficacy against *E. coli* bacteria compared to control dyed materials which showed antimicrobial efficacy only against *S. aureus*. The mordant treatment increased the fastness to washing and light properties of dyed wool fabrics. However, it reduced the antimicrobial efficacy of dyed fabrics due to their complex formation tendency with reactive groups of tannin present in dye molecules responsible for antimicrobial property. Among the mordants, aluminium sulphate treated wool fabrics showed both higher fastness and durable antimicrobial properties compared to stannous chloride and ferrous sulphate.

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