NC STATE UNIVERSITY

College of Textiles, NC State University

JTATM Journal of Textile and Apparel, Technology and Management

Volume 7, Issue 2, Fall 2011

Dyeability and Antimicrobial Properties of Cotton Fabrics Finished with *Punica Granatum* Extracts

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ABSTRACT

The dry rind of the pomegranate (Punica granatum) has been used as dye stuff since earlier times. In the present study the dye extracts were obtained from the rinds of the P. granatum and used for dyeing the cotton fabrics. The fabrics were assessed for antimicrobial, dyeability and fastness properties. The color strength (K/S) of dyed fabric was assessed through dyeability test. The antibacterial assessment is performed qualitatively by disc diffusion method and parallel streak method (AATCC 147) and quantitatively by percentage reduction test (AATCC 100) against the test organisms Escherichia coli and Staphylococcus aureus. The fabrics were also assessed for fastness properties such as wash fastness, light fastness and rubbing fastness as per the AATCC standards. The fabrics were finally subjected to wash durability tests. The dyeability results showed that the fabrics dyed with the P. granatum rind extracts have high K/S value when compared with the untreated control fabric. The natural extract dyed fabric has prominent antimicrobial activity which was evidenced by clear zone of bacterial inhibition in qualitative tests and bacterial reduction in quantitative tests whereas the control fabric has no antibacterial activity. Wash durability test showed that the antimicrobial activity of the fabrics was durable up to 10 wash cycles.

Keywords: natural dye, cotton fabrics, antimicrobial, dyeability

1. INTRODUCTION

Up to the end of the 19th century natural dyes were the main colorants for textiles. The introduction of synthetic dyes led to an almost complete replacement of natural dyes, due to the favorable application properties of synthetic dyes. Besides a wide range of available colors, higher reproducibility and improved quality of dyeing could be achieved at lower specific cost (Thomas Bechtold, 2006). It has become increasingly important for antibacterial agents to meet environmental and low toxicity criteria, while retaining their functionality. Therefore, it is vital to research and develop ecofriendly

antibacterial agents extracted from plants/animals for textile applications. The effect of various plants on bacteria has been studied by a number of researchers (Liolios, *et al.*, 2007; Pereira, 2007; Jasso de Rodrigiez, *et al.*, 2007). However, despite the fact that there are many natural antibacterial agents, few studies in the open literature have explored their antibacterial activity on textile materials.

The use of natural products such as natural dyes for antimicrobial finishing of textile materials has been widely reported (Gupta, *et al.*, 2004 & 2005). Many natural dyes obtained from various plants are known to have antimicrobial properties. Recently there has been a revival of interest in the use of natural dyes in textile coloration. This is a result of the stringent environmental standards imposed by many countries in response to the toxic and allergic reactions associated with the use of synthetic dyes. A widespread interest has emerged in the dyeing of textile fibers using natural colorants, on account of their high compatibility with environment, softer color shades, naturalness, lower toxicity and antibacterial/anti-allergic/deodorizing/anti-cancer properties, harmonizing natural shades or just the novelty (Hill, 1997; Bechtold, 2003; Kenneth, 1973; Montazer, 2007; Popoola, 2000; Angelini, 1997). It is well known that problems in dyeing with natural dyes are the low exhaustion of natural colorants and the poor fastness of dyed fabrics. Attempts to overcome these problems have been mainly focused on the use of metallic salts as mordants, which are traditionally used to improve fastness properties or exhaustion and to develop different shades with the same dye (Hwang, *et al.*, 1998; Shin, *et al.*, 1999; Cho, 1999; Cristea, 2006; Lee, *et al.*, 2000; Lee, 2004). There were studies that attempted to use different natural sources for coloring cotton fabrics but there are no literature concerning the utilization of rind of the fruit *P. granatum* for dual functionalization of the fabrics for antimicrobial and dyeability properties.

This study investigates the antibacterial functionality of cotton fabric dyed using natural aqueous dyeing solutions obtained by extraction from pomegranate (*Punica granatum*). *Staphylococcus aureus* and *Escherichia coli*, the microorganisms typically known to grow on textiles, were used as test organisms for the antibacterial study. The antibacterial activity of the treated fabrics were examined as per standard AATCC methods and found to have remarkable antibacterial activity. In addition to the antimicrobial functionality the natural extract dyed fabrics were also assessed for the fastness and durability properties.

2. MATERIALS AND METHODS

2.1. Materials

The fruits of pomegranate (*Punica granatum*) were collected from the agricultural farms situated in and around the Coimbatore district, Tamil Nadu, India. The rind part alone was separated and used for the extraction process. The reagent grade chemicals copper sulphate and aluminum potassium sulfate (alum) were procured from Hi-media, Mumbai, India. Fabrics with the standard specifications were purchased from National Textile Corporation Limited (Coimbatore, Tamil nadu) as listed in Table-1.

Table 1. Specifications of the fabric used for treatment

Type	Pre Finishing	Warp Count	Weft Count	Ends per Inch	Picks	Width
100% Woven Cotton Fabric	Bleached	20^{s}K	20^{s}K	54	40	122 cm

2.2. Bacterial Strains

In this study, to investigate the antibacterial activity of cotton fabrics dyed with natural colorant from pomegranate, evaluation was carried out with *Staphylococcus aureus* MTCC 6538, a Gram-positive bacterium and *Escherichia coli* MTCC 6539, a Gram negative bacterium. These two organisms are reference strains used for antimicrobial susceptibility testing according to AATCC standard method. The strains were cultured on nutrient agar (Himedia, Mumbai, India) and incubated aerobically at 37^0C overnight.

2.3. Preparation of sample

The fruit rinds of pomegranate were dried under shade to remove the moisture content. After it has completely dried the fruit rinds were ground to a fine powder and sieved to remove any large residues. The dry powder obtained was used for the process of extraction.

2.4. Extraction of Dye

2.4.1. Aqueous Extraction

Aqueous dye solution was prepared, by adding 10g of the *P. granatum* rind powder to 100 ml of distilled water in a beaker. The extraction was done at 100^0C for 1 hour. The hot solution was filtered through a Whatmann No 1 filter paper to remove any plant residue and obtain a clear filtrate. The filtrate was stored at 4^0C and used for dyeing of cotton fabric.

2.4.2. Ethanolic Extraction

To prepare ethanolic extract, 100 ml of ethanol was added to 20g of powder in a sealed conical flask and kept at room temperature for 48 hours. The solution was filtered to obtain a clear filtrate. To remove ethanol, the filtrate was condensed through a rotary vacuum evaporator at 60^0C for 30 mins. The ethanol free filtrate was used for the preliminary assessment of antimicrobial activity.

2.5. Preliminary Assessment of antimicrobial activity of P. granatum rind extracts:

The antimicrobial activity of the crude extracts of *P. granatum* rind was assessed preliminarily by disc diffusion method. Filter paper disc (diameter 2 cm) was prepared by treating with 100 µl of both aqueous and ethanolic extracts and allowed to air dry. The discs were placed in intimate contact with AATCC bacteriostasis agar, which has been previously inoculated (mat culture) with the test organisms. Two test organisms namely, *Staphylococcus aureus* and *Escherichia coli* were used in the study. The plates were incubated at 37^0C for 18-24 hrs. The presence of a clear zone after incubation indicates the antimicrobial effectiveness of the extracts.

2.6. Mordanting and Dyeing Process of Cotton Fabrics

Alum and copper sulphate were used as mordant according to previous literature. Alum was used as pre-mordant, in which the fabric was treated with 3% alum 60^0C for 20 mins. The mordanted fabrics were dyed with the extracts. Copper sulphate was used as a mordant along with the dyeing bath. To the boiling water 20% of the powder of *P. granatum* rinds were added and stirred well for 30 minutes. Then 5% copper sulphate was dissolved in water and added to the bath, stirred well and boiled for another 5 minutes. The solution was filtered and used for further dyeing process.

Cotton fabric was dyed by the standard method as prescribed by Gulrajani and Gupta, 2001 for natural dyes. The dyeing of fabric with extracts was carried out at 10% of (on weight of fabric), at 1:30 (material liquor ratio) for 30 mins at 80^0 C at neutral pH. Dyed samples were rinsed in cold water and dried under shade. After dyeing the fabric were washed with 5% soap solution (neutral soap), rinsed and dried under shade.

2.7. Dyeability of Dyed Cotton Fabric

The color strength (K/S) of dyed fabric was assessed using Kubeka-Munk equation. K/S of the dyed samples was measured using a spectrophotometer (x4000).

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

where R is the reflectance value of the dyed fabric at λ max, K is the absorption co-efficient, S is scattering co-efficient.

2.8. Antimicrobial Activity Assessment of Dyed Cotton Fabric

Antimicrobial activity was evaluated by both qualitative and quantitative test methods. The following are the descriptions of test methods employed for this study.

2.8.1. Qualitative Assessment by Agar Diffusion Method (SN 195920-1992)

Fabric dyed with natural colorants and undyed control fabric samples were placed in intimate contact with AATCC bacteriostasis agar, which has been previously inoculated (Mat culture) with an inoculum of test organisms in petridishes. Two test organisms namely; *Staphylococcus aureus* and *Escherichia coli* were used for the study. The plates were incubated at 37^0C for 18-24 hrs. After incubation, a clear area of uninterrupted growth underneath and along the side of the test material indicates antibacterial effectiveness of the fabric.

2.8.2. Qualitative Assessment by Parallel Streak Method (AATCC 147-2004)

Sterile bacteriostasis agar was dispensed in petriplates. 24 hours broth cultures of the test organisms (*E. coli* and *S. aureus)* were used as inoculums. Using 2 mm inoculation loop, 1 loop full of culture was loaded and transferred to the surface of the agar plate by making 7.5cm long parallel streaks 1cm apart in the center of the plate, without refilling the loop. The test specimen was gently pressed transversely, across the five inoculums of streaks to ensure intimate contact with the agar surface. The plates were incubated at 37°C for 18-24 hours. After incubation, a streak of interrupted growth underneath and along the side of the test material indicates antibacterial effectiveness of the fabric.

2.8.3. Quantitative Assessment by Percentage Reduction Test (AATCC 100-2004)

Specimens of the test material were shaken in a known concentration of bacterial suspension and the reduction in bacterial activity in standard time was measured. The efficiency of the antimicrobial treatment was determined by comparing the reduction in bacterial concentration of the treated sample with that of control sample expressed as a percentage reduction in standard time.

$$\% \text{ Reduction} = [(A-B)/B] \times 100 \quad (2)$$

where A and B are the surviving cells (CFU/ml) for the flasks containing the control (blank cotton fabric) and test samples (natural dye treated cotton fabric), respectively, after 18 hrs of contact time.

2.10. Wash Durability Test (Sarkar *et al., 2003*)

The wash durability of antimicrobial activity of the dyed sample was evaluated after different wash cycles. The sample was washed with 5% neutral soap solution for 20 mins. Washed sample was tested for the retention of antimicrobial activity after 2, 4, 6, 8 and 10 launderings by AATCC-100 test method as described in 2.8.3.

2.11. Fastness Properties

The fastness properties of the dyed fabric such as the acidic perspiration, alkaline perspiration, wash fastness, light fastness and rubbing fastness were assessed with guidelines from standard AATCC testing methods.

2.11.1. Wash fastness using Launder O Meter (AATCC- 110106)

Fabric sample of size 6''x 2'' was taken. Staple multi fiber test fabric along one edge of technical face of sample.

Sample was set aside. 150 ml of water and 0.225g of detergent (0.15% wt of liquor) were added to each canister. 50 steel balls were added into canister. Blank gasket was placed into canister lid. Sample was pressed into lid and lid was closed. Canister was clamped. Then rotor was started and run for 2 minutes at 140^0C to pre heat the canister and solution. Now the cover of one canister was unclamped. The samples were added to each canister in the row. After finishing the row was re-clamped again. Rotor was manually turned to the next row. The process was repeated until all samples were loaded. Then canisters were removed and each sample contents were added to separate beaker. Each sample were rinsed for 3 times and in 1 minute with de-ionized water, excess water was removed. Sample was dried in oven (106^0F or 71^0C) for 1 hour before evaluation.

2.11.2. Rubbing Fastness (IS: 766-1956)

Two sample pieces of not less than 14x5 cm, one piece having the long direction parallel to the warp yarn and the other parallel to the weft yarns were cut. These two pieces were used for dry rubbing test and two other similar pieces were cut and used for wet rubbing test. A Crock meter was used as the rubbing device. The untreated fabric was used as the control.

2.11.2.1. Dry Rubbing

One test piece was taken and fixed to the rubbing device. A piece of the dry treated fabric was fixed in place over the end of the finger of the running device and rubbed to and fro in a straight line along a 10 cm long track on the (dry) test piece ten times in ten seconds with a downward force of 900g on the finger. The second test piece of untreated cloth was treated in a similar manner.

2.11.2.2. Wet Rubbing

One test piece (dry) was taken and fixed to the rubbing device. The test piece was soaked in distilled water and squeezed so that it contained its own weight of water. The wet piece of the test piece was placed over the end of the finger of the testing device and rubbed it to and fro in a straight line along a 10 cm long track on the dry test piece ten times in the seconds with a downward force of 900g on the finger. The piece was dried at room temperature. The second piece of the untreated fabric was treated in a similar manner. The degrees of the staining of the treated and untreated fabrics were evaluated with the help of geometric grey scales (staining) and the numerical ratings were assigned.

2.11. 3. Light Fastness (AATCC test method 16-1993)

Light fastness was evaluated according to the AATCC test method 16-1993. Each treated and untreated fabric samples were exposed to xenon arc lamp for 20 hours. After that the change in color of the test fabric samples were evaluated with the help of geometric grey scales and the numerical ratings were assigned for both treated and untreated fabrics.

2.11.4. Acidic and Alkaline Perspiration (IS: 971-1956)

The acidic test liquor was prepared by dissolving 2.65g of sodium chloride and 0.75g of urea per liter and the pH was adjusted to 5.6 with the addition of acetic acid. The alkaline test liquor was prepared by dissolving 3g of sodium chloride per liter and the pH was adjusted to 7.2 with the addition of sodium bicarbonate. One of the composite specimen was wetted thoroughly in the acidic test liquor using liquor to specimen ratio of 50:1 (mg) and allowed to retain in the liquor for 30 minutes at room temperature. Care was taken specially while wetting the specimen to see that it was uniformly saturated. The liquor was poured off and the specimen was placed in between two glass plates under a force of 4.5 kg. The glass plates were kept with the specimens in the perspirometer and placed in the hot air oven for 4 hours at 37^0 C. At the end of this period, the specimen was removed and the test piece was separated from the two pieces of the untreated fabric and dried in air at a temperature not exceeding 60^0 C. The

second composite specimen was also treated similarly using alkaline test liquor. The change in color of the test pieces (treated in both acidic and alkaline broth) and the degree of staining of the corresponding two pieces of untreated cloth were evaluated with the help of geometric grey and the numerical ratings were assigned for both treated and untreated fabrics.

3. RESULTS AND DISCUSSION

3.1. Preliminary Assessment of Antimicrobial Activity of Extracts of P. granatum Rind

Natural extracts from *P. granatum* was screened for their antimicrobial activity against *E. coli* and *S. aureus* by disc diffusion method and the zone of inhibition was measured. The results of the screening test were reported in table 2. Even though the antibacterial activity was observed the same with slight variation in both the extracts, based on the ease of process and cost effectiveness the aqueous extract was use for further studies.

Table 2. Preliminary assessment of antimicrobial activity of the natural dye

S. No	Type of extract of *P. granatum*	Antibacterial activity (Zone of Inhibition in mm)	
		E. coli	*S. aureus*
1	Aqueous	8	14
2	Ethanolic	10	17

3.2. Color Probabilities of Cotton Fabric treated with Natural Dyes

Cotton fabric was dyed with aqueous extracts of *P. granatum* along with mordant compound and different shades of color were obtained. Even though both the mordants are suitable for textile application, alum was preferred for further studies as alum was used as a pre-mordant and the process of pre mordanting is easier when compared to the post mordanting process. The main problem associated with the simultaneous mordanting was that the modant as such is not completely soluble in the dye solution. Gupta and Laha (2007) showed that the use of alum as mordant enhanced the antimicrobial activity and also made the treatment fast to multiple washes.

3.3. Dyeability Test

The cotton fabric dyed with natural dye was evaluated for dyeability using spectrophotometer. Surface depth of the color, K/S value of the dyed samples was determined on a spectrophotometer. The K/S value of dyed material was directly proportional to the amount dye present in the material. The K/S value of the dyed sample was found to be 2.70, whereas the K/S value of the control fabric was very low. Manonmani *et al.,* 2009 has showed that dyeing the cotton knitted fabric with natural dye extracted from *Acacia catechu* has good depth of dyeing (K/S value = 3.1) when dyed along with mordant whereas the control fabric dyed with the extract alone showed less K/S value (K/S value = 1.9).

3.4. Antimicrobial Activity Assessment of the Dyed Cotton Fabric

3.4.1. Qualitative assessment by Agar Diffusion Method (SN 195920-1992)

The results of agar diffusion method against the test organisms *S. aureus* and *E. coli* are given in Fig 1.

(a) **(b)**

Figure 1. Plates showing antibacterial activity by agar diffusion method of (1) *P. granatum* extract treated fabrics (2) Untreated fabric against (a) *E. coli* and (b) *S. aureus*.

There was clear zone of inhibition around the fabric dyed with natural extract from *P. granatum* against both test organisms in contrast with control fabric which allowed the growth of organism. The natural extract treated sample exhibited a zone of 14.2 mm for *S. aureus* and 10.8 mm inhibition for *E. coli*. Gupta and Laha (2007) investigated the antimicrobial activity of cotton fabric treated with *Quercus infectoria* against *E. coli* and *B. subtilis* and they found that 12% *Quercus infectoria* in combination with 5% alum can act as a good antimicrobial agent against Gram negative bacteria *E. coli* and Gram positive bacteria *B. subtilis*.

3.4.2. Qualitative Assessment by Parallel Streak Method (AATCC 147-2004)

While evaluating the antimicrobial activity of natural dye treated cotton fabric as tested by Parallel Streak method a clear zone of inhibition was observed for both the test organisms. The result of parallel streak method is presented in figure 2. In the case of the antimicrobial activity of natural dye treated cotton fabric by parallel streak method, the zone of inhibition was observed to be 12.8 mm for *S. aureus* and 9.4 mm for *E. coli*. The results of the parallel streak method for the fabrics dyed with the extract of rind of *P. granatum* correlate with the results of Satiyanarayanan *et al.*, (2010). In his attempt to finish the cotton fabrics with *P. granatum* extracts, the antibacterial activity of treated fabric against the test organism *S. aureus* was found to be12.6 mm (Zone of inhibition).

(a) **(b)**

Figure 2. Plates showing antibacterial activity by parallel streak method of *P. granatum* extract treated fabrics against (a) *E. Coli* and (b) *S. aureus*

3.4.3. Quantitative Assessment by Percentage Reduction Test (AATCC 100-2004)

In the test against *E. coli* and *S. aureus* in AATCC bacteriostasis broth, inoculated control and inoculated test fabric was evaluated for percentage bacterial reduction by cell counting. The results of the percentage reduction test are shown in table 3. The reduction percentage for *E. coli* and *S. aureus* correspond to the bacterial numbers on the respective control test of $9.5x10^6$ per milliliter. The reduction percentage was found to be 95.7% for *S. aureus* and 89.4% for *E. coli*.

Table 3. Antibacterial activity of the dyed fabric by Percentage Reduction Test

Fabric sample	Test Organism	Survival cells (CFU/ml)		% Bacteria Reduction
		Control fabric	Treated fabric	
Cotton fabric treated with *P. granatum* extract	*S. aureus*	$9.5x10^6$	$0.4x10^6$	95.7
	E. coli	$9.5x10^6$	$1x10^6$	89.4

Han and Yang (2005), observed an inhibition rate of 70% against *S. aureus* when 0.01% of curcumin was applied to the fabric and also 70% inhibition rate against *E. coli* with 0.05% of curcumin. It was attributed that bacterial inhibition is due to the slow release of active substances from the fabric surface.

3.5. Wash Durability Test for Antimicrobial Activity

Wash durability test carried out with the test fabrics showed that the significant antimicrobial activity was actively retained in the fabrics treated with extract up to 5 washes (Fig-3) even after repeated wash cycles. After 5 wash cycles the % bacterial reduction was very low and there was no activity found in the fabrics after 10 washes.

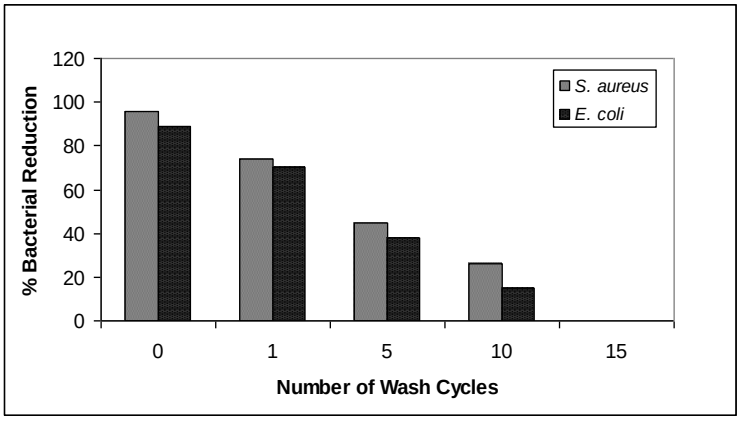

Figure 3. Wash Durability Test

The results of the wash durability test was similar to that of Sathianarayanan *et al.,(*2010), who confirmed that the wash durability of the fabrics treated with *P. granatum* extract alone retained the antimicrobial activity only up to 5 wash cycles, which gradually decreased and became nil after 10^{th} washes.

3.6. Color Fastness Property to Washing

The color fastness properties of the cotton fabric dyed with natural dye from *P. granatum* was tested according to ISO standard terms. According to the standard grey scale for color change and grey scale for color staining the results are observed and presented in table 4. The color fastness property of *P. granatum* dyed fabric to washing was around 4-5. Thus the results for color fastness for washing were ranging from good to excellent level.

Table 4. Color fastness properties to washing

S. No	Color fastness to washing	Color fastness rating (Dyed sample)
1.	Change in color	4
2.	Staining on wool	4-5
3.	Acrylic	4-5
4.	Polyester	4-5
5.	Nylon	4-5
6.	Cotton	4
7.	Acetate	4-5

1-Very Poor, 2- Poor, 3- Moderate, 4- Good, 5- Excellent

3.7. Color Fastness Property to Light and Rubbing

The color fastness properties of the cotton fabric dyed with natural extract were tested according to ISO standard terms. According to the standard grey scale for color change and grey scale for color staining the results are observed and presented in table 5.

Table 5. Color fastness property to light and rubbing

S. No	Dyed sample	Color fastness rating to lighting	Color fastness rating to rubbing	
			Dry Rubbing	Wet Rubbing
1.	*P. granatum*	4-5	4-5	4

1- Very Poor, 2- Poor, 3- Moderate, 4- Good, 5- Excellent

The color fastness property of *P. granatum* dyed fabric to lighting and rubbing was around 4-5. Thus the results for color fastness for light and rubbing were ranging from good to excellent level.

3.8. Color Fastness Properties to Perspiration

The color fastness properties of the cotton fabric dyed with natural dye from *P. granatum* were tested according to ISO standard terms. According to the standard grey scale for color change and grey scale for color staining the results are observed. The results of color fastness to perspiration are shown in table 6.

Table 6. Color fastness property to perspiration (alkali & acid)

S. No	Color fastness to Perspiration	Color fastness rating of samples dyed with *P. granatum*	
		Alkali	Acid
1.	Change in color	3-4	4
2.	Staining on wool	3-4	3-4
3.	Acrylic	3-4	3-4
4.	Polyester	3-4	3-4
5.	Nylon	3-4	3-4
6.	Cotton	4	4
7.	Acetate	4	4

1- Very Poor, 2- Poor, 3- Moderate, 4- Good, 5- Excellent

The color fastness property of *P. granatum* dyed fabric to perspiration was around 3-4. Thus the results for color fastness for perspiration were in moderate to good level. The fastness properties of the fabrics dyed with natural colorant (*Acacia catechu*) has been previously investigated by Manonmani *et al.*, (2009) and it has been reported that the color fastness to light was in the range of 4(Good) and fastness to dry rubbing and wet rubbing were also satisfactory (in range of 3-4).

4. CONCLUSIONS

The natural dying solutions were obtained by extraction from rind of *P. granatum* and used for dyeing cotton fabrics. Dyeability and fastness properties of the dyed sample were studied. The natural extracts with cotton fabric had more affinity towards fabric and more stability of colorant to light, rubbing, washing and perspiration. The fabric dyed with the natural colorant from *P. granatum* extracts displayed excellent antibacterial activity against both the test organisms used. These results clearly demonstrate that utilizing extracted natural colorants as dyeing materials significantly facilitate obtaining quality fabrics having both dyeability and antibacterial properties. There is no doubt that these natural extract finished fabrics may help in increasing the export to

developed countries where the use of Azo dyes has been banned. Also the present method of extraction and application of dyestuff form natural colorants may help to optimize the technical aspects of natural dyeing process.

5. ACKNOWLEDGEMENTS

The authors wish to thank Dr. R. Vijayaraghavan, Director and Dr. Beer Singh, (Sc 'F'), Head, PD Division, DRDE Gwalior for their interest and encouragement for this project. They are also thankful to Management, Secretary and Principal, PSG College of Arts & Science, Coimbatore, for providing necessary facilities to carry out this work.

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