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Effect of plasma treated wool fabrics using natural fungal pigment for healthcare applications

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ABSTRACT

The present study aims to improve the dyeability and antimicrobial activity of natural extract on wool fabric by using low temperature plasma treatment. The fungal pigments were extracted from the species of *Thermomyces*, purified and characterized using UV-Vis and FTIR spectra and used for dyeing process. An experiment has been designed using Box- Benkhen with three levels and three variables using pH, temperature and time as independent variables with wash, rubbing, light fastness and bacterial reduction (%) as dependent variables and the conditions were optimized. Regression equations have been obtained to analyze the fastness properties and bacterial reduction and the optimum process parameters were identified. The method is also to modify the conventional dyeing process using plasma pretreatment on wool fabric before dyeing with natural fungal extract and to analyze the parameters like wash fastness, rubbing fastness, light fastness and bacterial reduction (%). The results showed that optimum concentration of the fungal pigment was 2% on weight of the fabric and the optimum condition for dyeing was 60 deg C, 30 min at a pH of 3 and the plasma pretreated samples brings better fixation levels, improvement in fastness properties and imparts good antibacterial activity for the dyed wool fabric at optimum condition. It was also inferred that the plasma treated samples does not showed any influence with increase in the treatment time.

KEYWORDS

Antibacterial activity;
Bacterial reduction;
Fungal pigment;
Plasma treatment;
Wool.

INTRODUCTION

The worldwide demand for dyes of natural origin, especially yellow or red pigments, is rapidly increasing in the food, cosmetic and textile sectors[1,2]. Several research projects have so far been carried out to evaluate the techno-economical feasibility of today's dyes with alternative dye crops. Among the species examined, common madder, woad proved to be quite interesting sources of red (alizarin) and yellow (luteolin) dyes respectively, either for their agronomic characteristics or for their dyeing properties[3,4]. The main disadvantages of these natural dyes lies in the order of magnitude of their extraction yield factors (a few grams of pigment per kg of dried raw material). This makes their current market price about 1 US $/g, thus limiting their

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application to high-value-added natural-dyed garments only. To overcome this limitation, it was suggested to exploit the potentiality of other biological sources such as fungi (both moulds and yeasts), bacteria, algae and plant cultures, since appropriate selection, mutation or genetic engineering techniques are likely to improve significantly the pigment production yields with respect to wild organism[5,6]. Among the several pigment-producing micro-organism described in the literature, the fungus Thermomyces has been thoroughly studied[7]. It has been traditionally used for manufacturing food colorants and fermented foods and beverages in southern and far eastern Asia, the latter being also used in medical therapy to promote blood circulation and proper cholesterol levels, prevent gastric and intestinal disorders, stimulate digestion, etc[8]. The several pigments produced by Thermomyces are oligoketides and have been subdivided into three groups; rubropunctain and monascorubrin are orange pigments, presenting different side chains on the ozolactone ring[9]. Their two azoto analogues are the red pigments rubropunctamine and monascorubramine, where as their reduced forms are the yellow pigments monascin and ankaflavin[10]. There are but few natural dye-stuffs that have any direct affinity for wool. Turmeric, saffron, anotta, are about the only representatives, and these are not of much importance in wool dyeing by themselves, although they are sometimes used in conjunction with other natural dyestuffs, when they are applied by a process which is adapted more especially for the other dye-stuff which is used. Plasma treatment of wool top does not damage the fibres; the fibre/fibre friction increases but the differential frictional effect decreases. Other physical properties of wool remain unchanged with the exception of a slight decrease in the loop breaking force. The tenacity of yarns spun from plasma–treated wool top is higher by about 25% and elongation at break point is also higher compared with standard yarns. Plasma treatment considerably reduces the felting potential for any product obtained from modified wool. Good hand washability is usually achievable in a 'plasma only' process; an environmentally acceptable plasma/polymer process is also available. This research attempted to study the influence of plasma treatment on the dyeing potential and microbe resistant characteristics of the natural fungal extract pigmented samples.

MATERIALS AND METHODS

Materials

Bleached 100% wool fabric plain weave, yarn count 14.76 tex with warp 56 ends/cm, weft 27 picks/cm and 120 g/m^2 was used.

Methods

Extraction and estimation of pigment (air-drying process)

Extracellular pigment producing fungi *Thermomyces* species was isolated from soil. The fungal cultures were inoculated on to potato dextrose broth and incubated at 35ºC for 5-7 days; supernatant was filtered through the filter paper. Broth having the pigment was taken in a clean glass Petri plates and placed under hot air in a dust free chamber 40ºC. The plate was covered with a thin muslin cloth to avoid contamination due to dust. After 8 hours of drying, the volume of the broth was reduced to one third. The condensed broth was lyophilized and the powered pigment was stored at 4ºC and is shown in Figure 1a and Figure 1b.

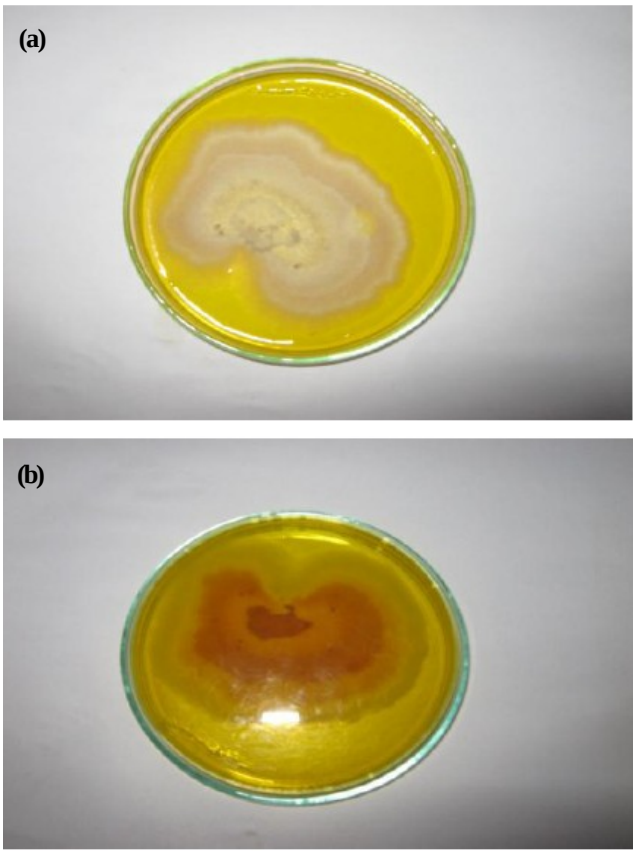

Figure 1 : Samples of extracted pigment

Determination of minimum inhibitory concentration (MIC)

The MIC was performed to test the antimicrobial activity of the methanolic extract of *P.purpuroscens*, *thermomyces* sp and *chatomium sp*. using tube dilution method (claeys et al., 1988) the MIC (minimum inhibitory concentration) was defined as the lowest concentration of antibiotics or extracts that did not show any growth of tested pathogens at a minimum concentration. This test was performed at four concentration of the plant extract (10 mg/ml,1mg/ml,0.1mg/ml and 0.01mg/ml). Twenty-four hours old culture of each organism was used for the study.4/10 dilution of each organism was prepared by serial dilution technique. A four number of sterilized eppendorf tubes were taken and to this 900ul of 4/10 diluted test organism were added. To the first tube 0.1 ml of prepared culture extract was added and serially diluted to the last tube. The four tubes corresponding to four Concentrations of the culture extract was obtained (10mg/ml, 1mg/ml,0.1mg/ml,0.01mg/ml).likewise, and a set of eppendorf tubes was prepared for each organism for each test samples. Simultaneously; controls were also kept for the experiment. For the second set of eppendorf tubes (4 numbers),0.1 ml of the negative control (100 % ethanol) was added to first tube and serially diluted to the last tube. For the third set of eppendorf tubes (4 numbers), 0.1ml of the positive control, ketaconazole for fungi and chleorampheicol for bacteria (10 mg/ml) was added to the first tube and serially dilute to the last tube. For the fourth set of eppendorf tubes (2 numbers), nothing was added so that the tubes contained only the microbial cells. Similar to the ager well diffusion method, the Petri plates were divided into 4 equal quadrants. After incubation of the eppendorf tubes for an hour, 50ul from each of the tubes were spotted on the Petri plates. The plates were then covered and incubated for 24 hours. The growth of the organism for each dilution was observed and thus the minimum inhibitory concentration of the fungal extract was calculated as shown in TABLE 1.

TABLE 1 : Minimum inhibitory concentration of *thermomyces* sp, *P.purpuroscens and Chaetomium sp*. against pathogens

Pathogens	Dilution 1 (10 mg/ml)			Dilution 2 (1 mg/ml)			Dilution 3 (0.1 mg/ml)			Dilution 4 (0.01 mg/ml)		
	1	2	3	1	2	3	1	2	3	1	2	3
Gram positive bacteria												
Enterococcus	I	I	I	I	I	I	I	I	NI	NI	NI	NI
Bacillus subtilis	I	NI	NI	I	NI	NI	I	NI	NI	NI	NI	NI
B.cereus	I	I	NI	I	I	NI	I	I	NI	I	NI	NI
Gram negative bacteria												
Escherichia Coli	I	I	NI	I	NI	NI	I	NI	NI	NI	NI	NI
Vibrio Cholerae	I	I	NI	I	NI	NI	I	NI	NI	NI	NI	NI
Salmonella typhi	I	NI	NI	I	NI	NI	I	NI	NI	NI	NI	NI
Fungi												
C.albicans	I	I	NI	I	I	NI	I	NI	NI	I	NI	NI
C.neoformans	I	I	NI	I	I	NI	I	NI	NI	NI	NI	NI
Control												
Solvent control		NI			NI			NI			NI	
Chloramphenicol		I			I			I			NI	
Ketoconazole		I			I			I			I	

Selection of mordant

The natural mordant myrobolan was chosen for the dyeing process. The fabric specimen was initially premordanted before treating with the natural fungal extract pigment for the dyeing process.

Plasma pretreatment on wool fabric

Low temperature treatment of wool fabric is done using RF and DC sputtering unit. The temperature and pressure used for the treatment of wool fabric is room temperature and atmospheric pressure respectively. According to frame size wool fabric is cut and treated for

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three different time periods of 15sec, 45sec and 80sec.

Dyeing of plasma pretreated wool fabric by natural fungal extract

Plasma treated samples for 15 sec, 45 sec, 80 sec and untreated sample were steeped using natural dye by the following procedure. Samples were steeped in the mordant bath prepared with 5 % (owm) on weight of material of myrobalan. The bath ratio was 1:20. Mordanting was done at a temperature of 30 deg C for 20 min. Samples were rinsed with tap water and squeezed. The mordanted samples were steeped in the dye bath with Liquor to ratio of 1:20 that prepared by 5% solution (owm) of extracted dye at pH 4.5-5.5 in presence of acetic acid. Dyeing of sample was done at 30 deg C for 20 min. The sample were rinsed with tap water and dried at 60 deg C for 20 min.

TESTING

The untreated and treated samples were tested for various measurements by standard test procedures. Color fastness properties of the samples were assessed using AATCC standards -Fastness to washing (AATCC Test Method 61-2009), Fastness to rubbing/crocking (AATCC Test Method 8-2007) and Fastness to Light (AATCC Test Method 16-2004). The dyed samples were analysed for the spectral values K/S determined using a Minolta 508 spectrophotometer with Macbeth Match View software (X-Rite, USA) in D65 daylight.

RESULTS AND DISCUSSION

Effect of process parameters and plasma treated samples on K/S value

A positive value of the coefficient for X2 (surfactant loading) indicates a favorable effect on K/S value. Optimized K/S value was observed at temperature (30°C), pH (3.0) and time (40 min) from the regression coefficient. The reason might be due to the influence of the diffusion characteristics and depth of penetration of the natural fungal pigment on the fabric specimen. The K/S value of Low temperature plasma treated samples shows positive significant difference comparatively to the untreated sample. From TABLE 2, it is inferred that from the various plasma treated samples, 15s treated sample shows greater K/S value compared to 45 and 60 seconds treated samples as the color strength reduces with respect to the increase in treatment time. It proves that the plasma treated samples increases the depth of absorption of the natural fungal extracted pigments on the wool fabric specimen. Hence, the relative color strength of the natural fungal dyed sample is greatly influenced by the plasma treatment.

Effect of process parameters and plasma treated samples on color fastness ratings

From the regression analysis, optimized wash and rubbing fastness rating were observed at temperature (30°C), pH (3.0) and time (40 min). The reason might be due to the reactivity and fixation levels of the natural fungal pigment on the fabric specimen. From TABLE 3, the color fastness values of low temperature plasma treated samples for wash and rubbing exhibits better fastness ratings comparatively to the untreated sample. It is inferred that from the various plasma treated samples, 15s treated sample shows positive significant difference in fastness ratings compared to 45 and 60 seconds treated samples as increase in treatment time reduces the depth of shade. It is inferred that the plasma treated samples influence the degree of fixation and depth of penetration of the natural fungal extracted pigments on the wool fabric specimen. From the regression analysis, optimized Light fastness rating was observed at temperature (30°C), pH (3.0) and time (40 min). The increase in plasma treatment time affects Light fastness ratings hardly than wash and rubbing fastness. The results infer that the surface adsorption characteristic of the natural fungal pigment influences the fabric properties. Hence, the durability of the natural fungal extracted dyed wool fabric samples can be increased by subjecting to plasma treatment.

TABLE 2 : K/S value of plasma pretreated dyed samples

Sample		K/S value
Untreated sample		0.337
Plasma	15 sec	0.531
treated	45 sec	0.478
sample	80 sec	0.411

Effect of plasma treated samples on antibacterial activity

From the regression analysis, optimized values were

observed at temperature (60°C), pH (3.0) and time (40 min).Here, the antibacterial reduction % decreases with increase in temperature and pH but increases with increased time. A high value of antibacterial efficacy can be obtained up to a certain Level of all 3 independent variables, but above this an increase in the Level of independent variables Leads to a decrease in the antibacterial efficacy. The antibacterial activity of Low temperature plasma treated samples exhibits positive significant difference in bacterial reduction % comparatively to the untreated sample. From TABLE 4, it is inferred that from the various plasma treated samples, 15s treated sample shows overall good bacterial reduction % with respect to *E.coli* Gram (-) bacteria and *S.aureus* Gram (+) bacteria compared to 45 and 60 seconds treated samples as the bacterial protection is not influenced with respect to the increase in plasma treatment. It is inferred that the plasma treated samples influence the antibacterial activity of the natural fungal extracted pigment on the wool fabric specimen. As plasma treatment exhibits good bacterial reduction% for both +ve and -ve bacteria, it can be a better solution for the medical application. The results infer that a part of the plasma treatment, natural mordant and pigment also influence the pathogenic bacterial reduction to a greater extent. The same result was also reported by textile and leather samples dyed with five different fungal pigments.

TABLE 3 : Color fastness values of plasma pretreated dyed samples

Sample		Wash fastness	Rubbing fastness	Light fastness
Untreated sample		2-3	3-4	2-3
Plasma	15 sec	4-5	4- 5	7-8
treated	45 sec	3-4	3-4	5-6
sample	80 sec	2-3	3-4	4-5

Characterization of pigment from *Thermomyces*

From Figure 2 a, the optical density of the pigment extract was determined in a wide range of spectra using a UV-Visible spectrometer and the absorbance was recorded. The spectrum shows that the maximum absorbance of the specimen between 250-300 nm which confirms the presence of protein and carbohydrate groups in the fungal pigment. From Figure 2 b, the absorption in the region 3000-3500 cm^{-1} confirms the presence of N-H (str) group. The absorption in the region 1500-1750 cm^{-1} confirms the presence of C=O (str) group. Hence, from the observation the affinity of sample towards protein fibres was confirmed.

TABLE 4 : Antibacterial activity of plasma pretreated dyed samples

Sample		*Bacterial reduction (%)*	
		Escherichia coli	*Staphylococcus aureus*
Untreated sample		45%	48%
Plasma	15 sec	47%	50%
treated	45 sec	43%	41%
sample	80 sec	44%	47%

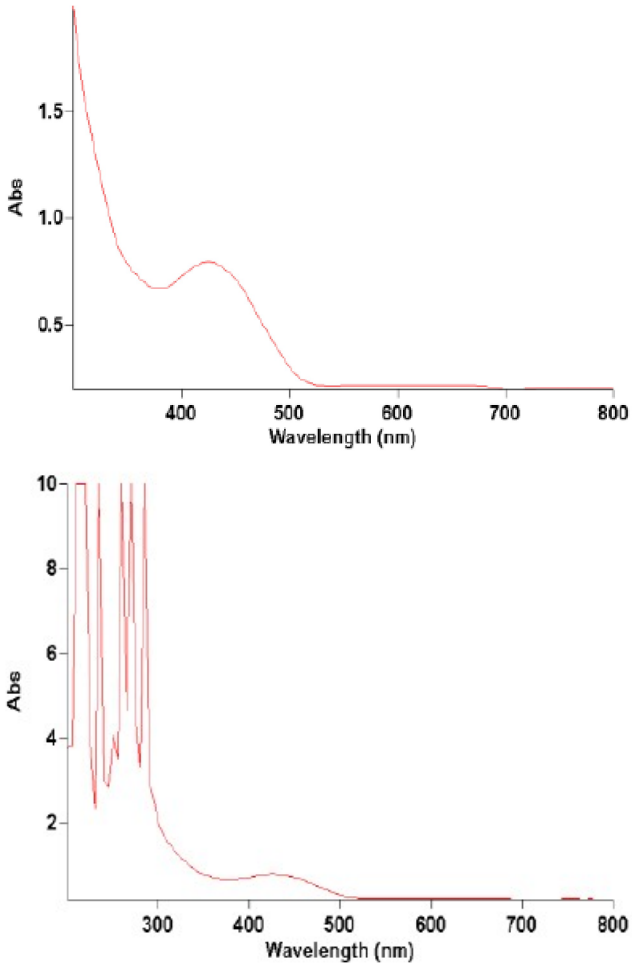

Figure 2a : UV-Vis spectra of extracted fungaL pigment

Air permeability

The ASTM D 737-04 standard test method was followed. The sample was tested at R.H.65%+/-2% and temperature 21deg C +/-1degC. The air permeability value of wool fabric was found to be 4.86 cm^3/

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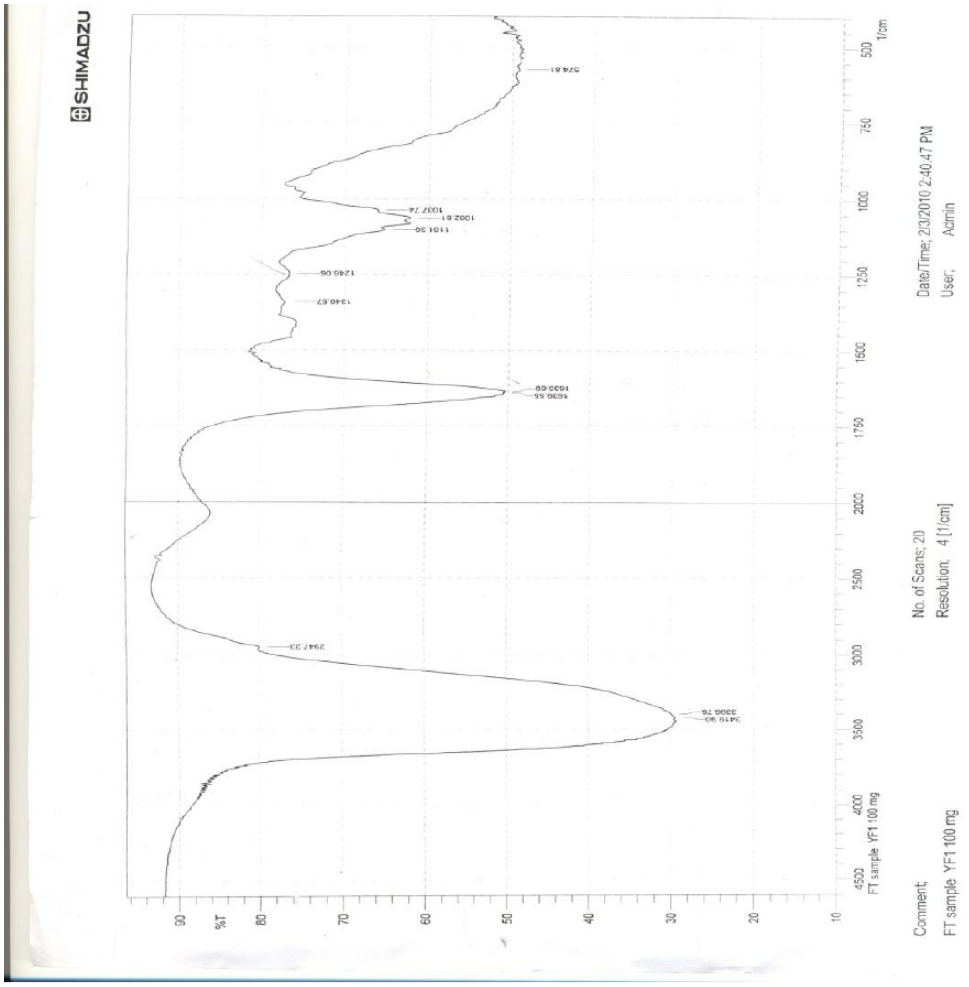

Figure 2b : FTIR absorption spectra

cm.sq./sec.

CONCLUSION

100% wool fabric was given low temperature plasma treatment with different durations and colored using the natural fungal extract *Thermomyces* and the effect of coloring behavior, fastness results and antibacterial activity were analyzed. The process parameters like pH, temp and time duration was varied and the results were optimized. The results were examined for coloring the protein fabric specimens at 2% on weight of the fabric and the optimum condition for coloration and antibacterial finishing was found to be 30°C, 60 min at a pH of 3. The analysis was focused on the surface modification of wool fabric using Low temperature plasma treatment for improvement in dye fixation levels of natural fungal extracted pigment and antibacterial efficacy of wool fabric. The results showed that the Low temperature plasma pretreated wool fabric exhibit better results than untreated wool fabric specimen. Color strength, color fastness ratings and antimicrobial activity were found to be good for 15sec plasma pretreated wool fabric compared to 45 and 80s treated samples. Hence, plasma treated samples did not show any influence with increase in the treatment time and the process proves to be more eco-friendly in nature and it can be used for healthcare application to develop wound dressing, face mask, sutures and surgical drapes.

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