Extraction of Natural Dye from *Peepal* Bark (*F.Religiosa*) and its Application on Textile Substrates

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Abstract

The present study attempts to extract natural dye from the bark of F. religiosa to dye cotton, wool and silk. The fabrics were premordanted with alum, myrobalam, eucalyptus leaves, beetal nut, pomegranate peel and ferrous sulfate. Assessment of the dyed samples was done in terms of colour strength (K/S), colour coordinates (L a*, b* values) and fastness properties. Effect of pH on dye uptake was also assessed. A range of colours i.e. from pinks to purples to browns could be obtained on unmordanted samples. The colour was found to further intensify on mordanting and the maximum dye uptake was observed at neutral pH. Wool fabric showed maximum dye uptake followed by silk and cotton. Excellent colour fastness properties of most of the dyed samples were recorded. The dye extracted from Peepal bark showed excellent pharmacological properties. Almost 100% antioxidant activity was obtained when concentration of the dye increased from 10 mg/ml to 100 mg/ml. The dye and the dyed samples showed good antimicrobial activity as almost negligible growth of bacteria was observed for pre mordanted dyed samples thus making the dyed fabrics as bioactive textiles. Bioactive textiles dyed with the bark of F. religiosa can find suitable applications in the health and hygiene industry and in the field of medical textiles.*

Keywords: *Antimicrobial, Cotton, F. religiosa, Natural dye, Peepal, Silk, Wool*

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1. Introduction

The emergent concerns about the eco-system, pollution, ecology, environment and increasing cognizance of problems related to toxicity and health hazards associated with synthesis, processing and use of synthetic dyes have resulted in the substitution of synthetic dyes by more eco-friendly products for colouration [1]. Dyes derived from natural sources have emerged as an important alternative to synthetic dyes. In the recent years, the increased availability of abundant new sources of natural colours has helped to reduce the cost of natural colorants. The use of natural products for imparting antimicrobial properties on textile materials has also been widely researched [2].

One such natural source is *Peepal* tree (*Ficus religiosa Linn*), the oldest portrayed tree in India and a native of the sub-Himalayan tract, Bengal and Central India. Since antiquity, *F. religiosa* has got mythological, religious and medicinal importance in Indian culture. *Ficus Linn* being the largest genus of the family Moraceae comprises about 755 fig tree species worldwide. *F. religiosa* is a Bo Tree, which sheltered the Buddha when he was "enlightened" (Bodhi) as he divined the "truths" [3]. 'Religiosa' refers to 'religion' because the tree is sacred in both Hinduism and Buddhism [3].

The potential therapeutic benefits of leaf juice and fruits of *F. religiosa* have been documented in folk medicine. *F. religiosa* shows diverse range of pharmacological activities like, anticonvulsant, antidiabetic, anti-inflammatory, antimicrobial, analgesic, wound-healing, antioxidant, proteolytic, and anti-amnesic [4]. The leaf juice has been used for the treatment of asthma, cough, sexual disorders, diarrhoea, haematuria, ear-ache, toothache, migraine, eye troubles, gastric problems and scabies [3]; leaf decoction as an analgesic for toothache; stem bark in gonorrhea, bleeding, paralysis, diabetes, as antiseptic, astringent, anti-inflammatory and analgesic [5]. The leaves have been reported to have hypoglycemic, wound healing [6, 6], antimicrobial [8] properties and

methanolic fig extracts of *Ficus Religiosa* are anticonvulsant [6]. Traditionally, the bark is used for its antibacterial, antiprotozoal, antiviral, astringent and antidiarrhoeal properties [8] and also in the treatment of ulcers [9, 10]. The bark of *Peepal* tree constitutes phytosterols like lanosterol, β-sitosterol-D glucoside and stigmasterol [11, 12], because of which they have a well-established and wide spectrum of pharmacological activities. Bark of *F. religiosa* also shows antidiabetic activity and exhibits fall of the blood sugar level [13].

The fruit of *F. religiosa* contains appreciable amounts of total phenolic and total flavonoid content [14] which qualifies them to be used as a tonic and the fruit powder is used to treat asthma.

Dye extracted from *F.religiosa* has been reported to be applied on Chitosan treated cotton fabric [15]. Similar work has also been reported on silk [16, 17]. However, not many studies have been reported on the use of natural mordants for dyeing with the colour extracted from *Peepal* bark and the measurement of antimicrobial and antioxidant properties of the dyed substrate.

The present study investigates the extraction of dye from *Peepal* bark and its application on cotton, wool and silk. The effect of various natural and synthetic mordants have also been studied on the colour uptake using a pre-mordanting method and the fastness properties in terms wash, rub, light and perspiration were determined. The biological (antimicrobial and antioxidant) properties of the dye and dyed samples were also studied.

2.Materials and Methods

2.1 Material

Cotton, wool and silk fabrics were procured and scoured to prepare them for dyeing. The fabric specifications are given in Table 1.

Table 1: Specifications of the fabrics used

Fabric	GSM	Ends per inch (epi)	Picks per inch (ppi)
Cotton	660	106	62
Wool	1169	56	53
Silk	260	76	64

Analytical grade sodium carbonate and glacial acetic acid were used to maintain pH.
Natural mordants such as *Harda* (Myrobalam), Betel nut (*Supari*), Pomegranate peel and Eucalyptus leaves and chemical mordants such as alum (Merck) and ferrous sulphate (Merck) were also used for the study.
Peepal bark was collected from the local gardens of Delhi.

2.2. Methods

2.2.1 Characterisation of the raw material

Raw material was characterized in terms of the moisture content and ash content.

2.2.1.1 Moisture content

Moisture content of the raw material was determined according to ASTM D 629-99.
The percentage moisture content was calculated as given in equation 1:

$$\text{Moisture content } (\%) = \frac{W_m - W_d}{W_m} \text{ X } 100 \ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots (1)$$

Where W_m is the weight of dye in moisture equilibrium state at 20°C and 65% relative humidity, and W_d is weight of dye dried at 105°C for 60 min.

2.2.1.2 Ash content

Ash content of the raw material was determined according to AOAC method (AOAC, 2000). The percentage ash content was calculated as in equation 2:

$$\text{Ash content (\%)} = \frac{\text{Weight of ash (grams)}}{\text{Weight of sample (grams)}} \text{ X } 100 \ldots\ldots\ldots\ldots\ldots\ldots\ldots (2)$$

2.2.2. Extraction of dye

20 g of *Peepal* bark was extracted in 1000 ml of distilled water at boil for 60 mins. The extracted mixture (pH 7) was cooled to room temperature and filtered using Whatman No. 1 filter paper. The dye was extracted at three pH levels (pH 5, 7 and 9).

The extract was dried and colourant. Yield percentage was estimated on the basis of dry weight as given in equation 3:

$$\text{Yield (\%)} = \frac{\text{Weight of extract (grams)}}{\text{Weight of dried bark (grams)}} \text{ X } 100 \ldots\ldots\ldots\ldots\ldots (3)$$

2.2.3 Visible spectrum of the extracted dye

The visible spectra of the extracted dye were recorded in the wavelength range from 400 – 700 nm, on D-2750 UV-Visible spectrophotometer.

2.2.4 Application of dye on different textile substrates

2.2.4.1 Pre-mordanting of textile substrates: Substrates (cotton, wool and silk) were pre-mordanted with 5% owf alum, *myrobalam*, eucalyptus leaves, beetal nut, pomegranate peel and ferrous sulfate at 80°C for 30 minutes followed by drying at room temperature.

2.2.4.2 Dyeing of textile substrates

Pre-mordanted cotton, wool and silk fabrics were dyed with the aqueous dye extract of *Peepal* bark obtained at varying pH (acidic, neutral and alkaline). The dyeing was carried out for 60 mins at boil. M.L.R was maintained at 1:30 for cotton and wool and 1:80 for silk. Dyeing was carried out on volume basis, therefore, 50% of the extracted dye liquor was taken to make the required M.L.R. To study the effect of pH on dye uptake, fabrics were dyed at pH 5, 7 and 9. The dyed samples were cold rinsed, soaped with 0.5 g/l Lissapol N at 50ºC for 20 mins followed by drying at room temperature.

The colour value was determined by using the Kubelka–Munk equation as given in equation 4:

$$K/S = \frac{(1-R)^2}{2R} \ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots (4)$$

2.2.5 Assessment of fastness properties

For assessing the fastness to light, wash, rub and perspiration, pre-mordanted fabrics were dyed with aqueous extract obtained at neutral, acidic and alkaline pH. Light fastness was assessed in accordance with AATCC 16 – 2004 on Xenotest alpha high energy light fastness tester. Colour fastness to washing was assessed in launder-O-meter in accordance with the method prescribed in IS: 3361 – 1984 (ISO – II); rubbing on a crockmeter as per AATCC 8 – 2007; and perspiration on a perspirometer in accordance with the method prescribed in AATCC 15 – 2007.

2.2.6 Biological Properties

2.2.6.1 Antioxidant Properties

Antioxidant activity of the dye extracted from *Peepal* bark and the dyed samples was measured by DPPH assay. The textile substrates pre mordanted with alum, ferrous sulphate, *harda* and eucalyptus and then dyed with the dye extracted (aqueous medium at pH 7) were analyzed for their antioxidant activity. The undyed and the unmordanted dyed samples were taken as control.

2.2.6.2 Antimicrobial Properties

Test organism: Gram negative bacteria *(Escherichia coli)*

Culture medium: Nutrient agar

Test specimens

(a) Dye solution of aqueous dye extract (neutral pH) prepared at different concentrations viz. 0.5 %, 1%, 5%, 10% w/v

(b) Dyed fabrics: The textile substrates premordanted with alum, ferrous sulphate, *harda* and eucalyptus and then dyed with the dye extracted in aqueous medium at pH 7 were analyzed for their antimicrobial activity.
Qualitative assessment of the dye in solution form was done by AATCC 30 method (Disc diffusion method).
Qualitative assessment of the antimicrobial activity of the fabric samples was done by parallel streak method (AATCC 147).

3. Results and Discussion

3.1 Characterisation of raw material and extracted dye

3.1.1 Yield % and colour
The dye was extracted from *Peepal* bark in an aqueous medium at varying pH (5, 7 and 9). Yield % was determined and the results are presented in Table 2.

Table 2: Yield % and colour of dye residue obtained from *Peepal* bark after extraction

Medium and pH of extraction	Colour of dye obtained	Yield %
Aqueous, 5	Light red	13 %
Aqueous, 7	Red	11 %
Aqueous, 9	Deep red	40 %

Higher amounts of yield, ~40% was obtained when extraction was done in aqueous medium at alkaline pH as compared to acidic and neutral pH.

3.1.2 Moisture and Ash content
The raw material was characterized in terms of moisture and ash content. Powder of *Peepal* bark was found to have a moisture content of 0.7 % and ash content of 5.4 %.

3.1.3 Visible Spectra of extracted dye
Figure 1 shows the visible spectra of 1 % v/v dye extracted in aqueous medium at various pH viz. neutral, acidic and alkaline.

The visible spectra of the dye extracted in aqueous medium at pH 5 and 7 showed the λ_{max} at 495 nm. The dye extracted at alkaline pH showed a bifurcation in the peak with λ_{max} at 440 nm and 520 nm, thus indicating that the colour obtained under these conditions has undergone some structural change. Also, it can be observed that the dye extracted at alkaline pH has maximum absorbance at 1.5% v/v as compared to that at acidic and neutral pH at same concentration.

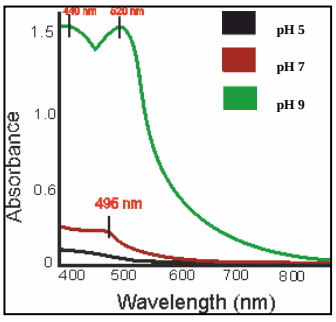


Figure 1- Visible spectra of dye extracted from *Peepal* bark in aqueous medium at varying pH

3.2 Application of extracted dye on premordanted textile substrates
The dye was extracted as well as applied at varying pH values to study the effect of pH on dye uptake (measured in terms of *K/S*) and the results of unmordanted dyed samples are summarised in Table 3.

Table 3 - Colours obtained on unmordanted textile substrates

Fabric	pH of Dyeing		
	5	7	9
Cotton			
Wool			
Silk			

Intersting colours ranging from pinks to purples to browns could be obtained on unmordanted samples. Silk and wool fibers fibres showed significantly less affinity for dyes in neutral or alkaline media. This may be because there are more carboxylate ions (COO^-) than ammonium ions ($–NH_3^+$) on wool and silk in the alkaline medium, due to the formation of $–NH_2$ groups from $– NH_3^+$ ions via their interaction with OH^- ions. Therefore, protein fabric becomes anionic, and due to electrostatic charges, repulsion occurs between the colorants and the protein fibres, ultimately resulting in a lowering of the dye uptake.

To study the effect of mordants, the textile fabrics were pre-mordanted with mordants such as alum, *harda*, eucalyptus leaves, *supari*, pomegranate peel and ferrous sulphate and then dyed. The colours obtained for cotton, wool and silk have been presented in Tables 4, 5 and 6 respectively.

Table 4: Dyeing of Unmordanted and Mordanted Cotton Fabric

Mordants	pH of Dyeing		
	Acidic	Neutral	Alkaline
Unmordanted			
Alum			
Ferrous sulphate			
Harda			
Eucalyptus			
Pomegranate peel			
Supari			

Table 5: Dyeing of Unmordanted and Mordanted Wool Fabric

Mordants	pH of Dyeing		
	Acidic	Neutral	Alkaline
Unmordanted			
Alum			
Ferrous sulphate			
Harda			
Eucalyptus			
Pomegranate peel			
Supari			

Table 6: Dyeing of Unmordanted and Mordanted Silk Fabric

Mordants	pH of Dyeing		
	Acidic	Neutral	Alkaline
Unmordanted			
Alum			
Ferrous sulphate			
Harda			
Eucalyptus			
Pomegranate peel			
Supari			

A range of shades viz. pinks, red, browns and black could be obtained with different mordants. In general, it was observed that brighter and darker shades were obtained on dyeing after mordanting as compared to unmordanted samples. The activity sequence for the pre-mordanting method was: ferrous sulphate > Alum > Supari > Pomegranate peel > Eucalyptus > *Harda*. It was seen that most mordants showed maximum colour value when dyed at neutral pH for cotton and silk samples. Ferrous sulphate yielded very dark brown to almost black colour on all the textile substrates. The wool fabrics in general gave very dark shades in comparison to silk and cotton.

This can be attributed to the ferrous ion showing a secondary valency of six in its coordination complexes instead of its normal valency of two; it forms six coordination bonds with donor atoms (ligands). Silk consists of amide linkages containing nitrogen and oxygen atoms with lone pairs of electrons which also have the ability to act as ligands. Therefore, ferrous atoms simultaneously form coordination bonds with silk and colorant molecules resulting in fixation to the fibre [18]. The appearance of darker shades of colorants with iron and zinc ions agrees with results reporting that these ions form more stable complexes with anthocyanines rather than aluminum ions [18].

3.2 Fastness properties

The unmordanted dyed cotton, wool and silk showed good wash fastness properties. The light fastness of the dyed samples was observed to be very good and ranged between 5-6. The wash fastness rating of the samples ranged between good to excellent under all the dyeing conditions. The ratings for staining of the adjacent fabrics for all the dyed samples ranged from 4.5 to 5 and there was no change in colour observed for the dyed sample under test.

The pre-mordanted samples gave excellent wash fastness properties. Grey scale ratings ranged between 4.5- 5 for staining of the adjacent fabric, without any corresponding change in colour. Perspiration fastness of pre-mordanted cotton, wool and silk was excellent with rating of 5 for both acidic and alkaline perspiration solution. The rub fastness of the pre-mordanted cotton, wool and silk samples was also good. Rub fastness of the dyed cotton, wool and silk was marginally better for dry rub fastness than wet.

3.3 Assessment of Antioxidant Activity

Oxygen-centered free radicals and other reactive oxygen species (ROS) can be generated as by-products during oxidative progresses of living organisms. Many human diseases, including accelerated ageing, cancer, cardiovascular disease, neurodegenerative disease and inflammation, are linked to excessive amounts of free radicals [19]. The antioxidants are necessary to cure these diseases [20]. Antioxidant agents, particularly those from natural sources are much in demand since they function as free-radical scavengers and chain breakers.

Antioxidant activity of the dye extracted from *Peepal* bark (aqueous, pH 7) and the samples (cotton, wool and silk) dyed with aqueous extract of the dye at neutral pH was assessed by DPPH method. Different concentrations viz. 10, 20, 40, 60, 80 and 100 mg/ml of the dye were taken and the antioxidant activity was determined as shown in Table 7.

Table 7: Antioxidant Activity of the dye extracted from *Peepal* bark

Concentration (mg/ml)	Absorbance (at 517nm)	Antioxidant activity (%)
0	0.692	0
10	0.15	78.32
20	0.07	89.88
40	0.04	94.21
80	0.04	94.21
100	0.04	94.21

It was observed that almost 90% antioxidant activity was obtained even at the lower concentration of 20 mg/ml. When the concentration was increased to 100mg/ml almost 95% activity was observed. The natural dye extracted from *Peepal* bark contains phytochemicals such as phenols, tannins and serotonin which contains electron donor groups. These donors react with free radicals and convert them to more stable products and terminate the radical chain reaction. Because of this unique property of this dye, it has immense potential in medical textiles. The results of antioxidant activity of the dyed samples are shown in Table 8.

Interestingly, all the mordanted samples showed almost 100 % antioxidant activity. This may be because the mordant in combination with the dye together are able to able to generate enough phytochemicals to react with the free radicals thus giving antioxidant activity in the range of 90-100%. Most of the mordants used also contained tannins.

Table 8: Antioxidant Activity of Fabric Samples

	Cotton		Wool		Silk	
Mordant	**Absorbance (at 517nm)**	**Antioxidant Activity (%)**	**Absorbance (at 517nm)**	**Antioxidant Activity (%)**	**Absorbance (at 517nm)**	**Antioxidant Activity (%)**
Undyed	0.977	0	0.785	0	1.003	0
Unmordanted	0.753	22.92	0.65	17.19	0.77	23.23
Ferrous Sulphate	0.02	97	0.00	100	0.138	86.24
Alum	0.031	98.66	0.072	90.82	0.03	97.00
Harda	0.041	95.80	0.003	99.61	0.007	97.30
Eucalyptus leaves	0.034	96.51	0.079	89.93	0.15	85.04

3.4 Assessment of Antimicrobial Activity

The dye extracted from *Peepal* bark in aqueous medium at neutral pH and the textiles dyed with them were qualitatively assessed for their antimicrobial activity against gram negative bacteria, *E.coli.* The extracted dye samples were assessed by disc diffusion method and the dyed samples were assessed by parallel streak method.

Different concentrations of the extracted dye were taken viz. 0.5 %, 1.0 %, 5 % and 10 % w/v. No clear zone of inhibition was observed in case of dye (Plate I). In addition to this, there was a complete lack of growth underneath the discs at all the concentrations, thus indicating that the dyes are bactericidal in nature and not bacteriostatic.

Plate I

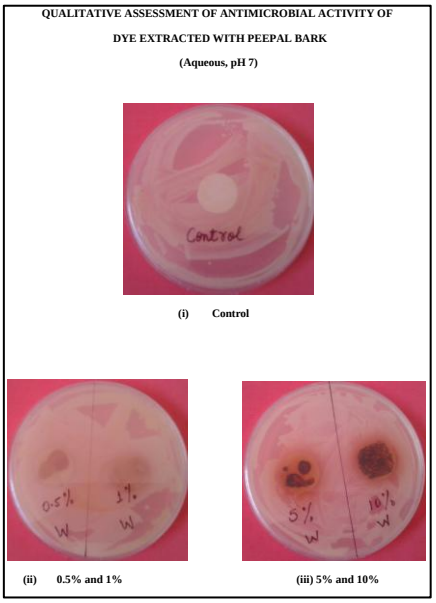
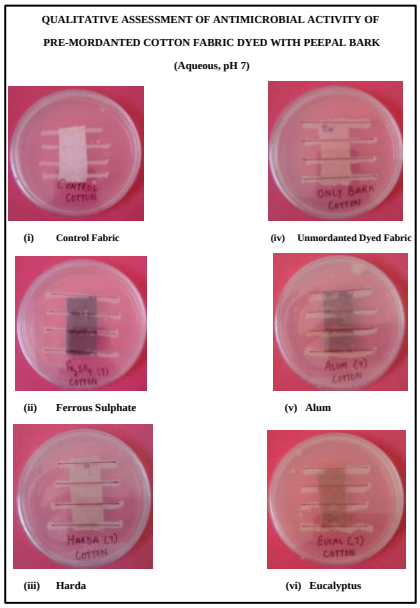


Plate II



The qualitative assessment of the antimicrobial activity of the pre mordanted and dyed samples did not show any growth of bacteria on the fabric surface (Plates II, III, IV). Better results were obtained for silk and cotton as compared to wool. On the contrary, bacterial growth was observed in the case of undyed and unmordanted fabrics.

Plate III

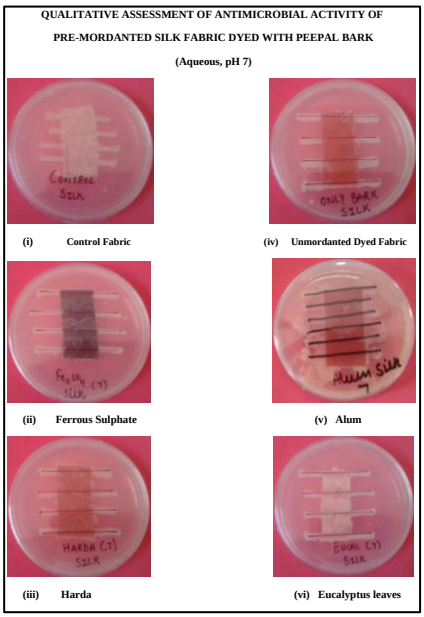
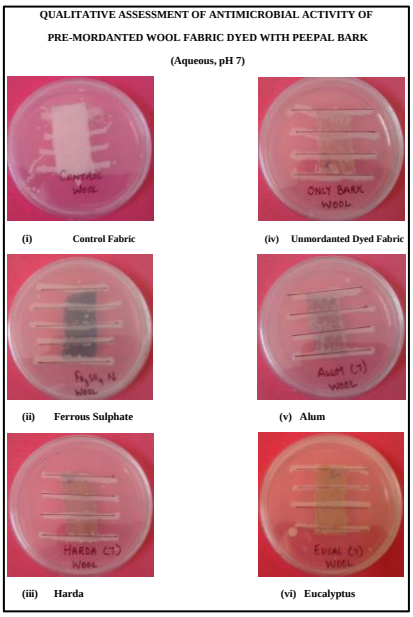


Plate IV



3. Conclusion

The rapid growth in medical textiles and their end uses has generated many opportunities for the application of innovative finishes. Antimicrobial textiles with improved functionality find a variety of applications such as health and hygiene products, especially the garments worn close to the skin and several medical applications such as, infection control and barrier material. Owing to this, there is considerable interest in developing textiles based on ecofriendly agents which can help to reduce effectively the ill effects associated with the microbial growth on textile material.

In the present study, colours with a wide shade range could be obtained on cotton, wool and silk using dye extracted from *Peepal* bark with excellent fastness properties. Various combinations of selected natural and chemical mordants not only resulted in expanding the shade card but also improvement in the fastness properties. Excellent antioxidant and antimicrobial properties could be obtained for the dye as well as dyed samples which can be successfully used in the health and hygiene industry and other medical textile applications.

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