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Extraction of Natural Dyes from *Glycyrrhiza glabra* and *Lagerstroemia speciosa* for Wool Dyeing and Testing of its Functional Properties

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A B S T R A C T

In order to sustain the natural dyes, there is increased need to identify sources of natural dyes having high yield, good colour fastness and multifunctional properties. In this study, the dyeing potential of natural dyes extracted from locally available plants namely *Glycyrrhiza glabra* and *Lagerstoermia speciosa* will be evaluated on the basis of their antimicrobial activity and ultraviolet protection factor. The solutions of both the natural dyes were subjected to antibacterial testing against common human pathogens *S. aureus* and *E. coli*. These dyes were applied on wool fabric using standard conditions. The antibacterial activity, colour strength (k/s), UPF and fastness properties of both the dyed samples were assessed. The characterization of dye was done on the basis of FTIR and phytochemical analysis. The results demonstrate that both the natural dyes possess good antimicrobial activity against human pathogens and exhibits good UPF, colour fastness properties as well as colour strength. This study envisages the development of sustainable dyeing technique for wool fabric and also a potential alternative for providing antimicrobial finishing to the fabric.

Introduction

Value addition of textile material by using natural dyes is gaining popularity all over the world. Natural dyes are not an innovation but a revival of rich and prudent tradition and were used in the past until the discovery of synthetic dyes in the middle of 19th century. Most of the natural dyes have inherent antimicrobial properties and could consequently possess high medicinal activity. The antimicrobial activity of some of these dyes is due to the presence of phenol, tannin and quinone in their extracts. When these dyes were applied to textiles, the antimicrobial properties of these plant dyes contribute to the

longer life of the textile materials (Calis *et al.,* 2009)

The main requirements for a basic commercial setup of natural dye stuff production and natural dyeing are agricultural demands and meeting the requirements of technical dye houses. Agricultural demands include low cost production and harvesting of plant material, easy preparation of raw material/ dye powder and easy storage of raw materials. The technical dye houses requirements are simple and rapid dyeing process, acceptable fastness properties, no use of mordants specifically metallic mordants, non toxic properties of dye and its biodegradability in wastewater treatment plant (Bechtold *et al.,* 2003). The study undertaken deals with the sustainable extraction of natural dyes from two plant species namely *Glycyrrhiza glabra* and *Lagerstoermia speciosa*, its application on pure wool fabric and assessment of its functional properties. The findings of this study will be helpful to increase the functionality of textile material such as UPF and antimicrobial activity as well as give acceptable fastness properties. Such multipurpose wool materials can be utilized for making value added products and also reduces the cost of additional finishing treatments. The residual plant material left after dyeing process can be further used as manure, fertilizer or soil conditioner because no chemicals and no organic solvents were used in the study during the entire process including extraction of dyes and dyeing of wool fabric.

Experimental

Materials and Methods

Plant materials

The plant materials i.e. roots of *Glycyrrhiza glabra* commonly known as Mulethi or Liquorice (Family: Fabaceae) and waste leaves of *Lagerstoermia speciosa* or pride of India (Family: Lythraceae) were collected from the nearby areas of Pantnagar, Uttarakhand.

The collected material was washed using distilled water, shade dried, broken into smaller chips and grinded to powder form. Aqueous extraction method was used for dye extraction to avoid use of harmful chemicals as they pollute the nearby water resources.

Textile Materials

Wool fabric purchased from Gandhi Ashram, Haldwani Uttarakhand was used for dyeing with natural dyes. The fabric was washed using 0.5 ml of liquid detergent in 100 ml of water.

The physical properties of fabric such as fabric weight (ASTM D3775), tensile strength and elongation (IS: 1969-1968) were also assessed which are mentioned in Table 1.

Test inoculums for antibacterial testing

Staphylococcus aureus and *Escherichia coli* were used as reference microorganisms for testing antibacterial activity of the dyed and undyed samples. These strains have been used for study, as they are human pathogens and representative of gram positive and gram negative bacteria class.

Chemicals used

In this study, no chemicals were used for extraction of dyes. The chemicals were only used for phytochemical profiling of extracted dyes which are sulphuric acid (H_2SO_4), ferric chloride ($FeCl_3$), sodium hydroxide (NaOH), ether $(C_2H_5)_2O$, hydrochloric acid (HCL), glacial acetic acid (CH_3COOH) and zinc dust (Zn).

Extraction of natural dyes

The dye solutions were prepared by extracting 2g of each dyestuff in 100 ml of distilled water at 80 °C for 60 minutes and the solutions were filtered using nylon mesh.

Determination of λ-max

The λ-max of the dye solutions were determined using "PC Based Double Beam UV-VIS spectrophotometer. The dye solutions were diluted upto 50 times to record the λ-max.

Assessment of antibacterial activity

The extracted solutions were subjected for antibacterial testing against gram positive *S.aureus* and gram negative *E.coli* bacterias using disc diffusion method (AATCC-30).

The test bacterias were grown overnight in 10 ml nutrient broth i.e. potato dextrose agar and incubated at 37°C. The test bacteria *S.aureus* and *E. coli* were streaked on sterile nutrient agar plates. Each dye solution (10 ml) was impregnated onto a small disc of filter paper with diameter 5.0 mm and placed on top of prepared agar plates. The plates were incubated overnight at 37°C and zone of inhibition was measured.

Characterization of natural dyes

The colouring components present in dyes were identified on the basis of spectroscopic and chemical investigations. The FTIR analysis of both the dyes was done using bruker alpha model spectrophotometer to identify the different kind of materials, molecular composition and structure present in the selected dyes. Phytochemical analysis of both the dyes was also done using standard protocols given by CSWRI, Avikanagar as mentioned below:

Tannins

Five ml of dye extract was placed in a test tube and 2ml of ferric chloride (5%) solution was added. Formation of greenish-black precipitate indicates the presence of tannins.

Anthraquinones

The dye extract was further extracted using ether and filtered. The extract was subjected to caustic soda. Formation of violet colour indicates the presence of anthraquinones.

Anthocyanins and flavanones

1ml of dye extract was mixed with few drops of sodium hydroxide (10%) followed by addition of sulphuric acid. Formation of blue colour followed by yellowish orange colour after addition of acid indicates the presence of anthocyanins. Formation of yellow to orange colour and crimson red after an addition of acid indicates the presence of flavanones.

Coumarins

Dye extract (1ml) was mixed with sodium hydroxide (10%). The solution turns yellow in colour which indicates the presence of coumarins.

Quinones

One ml of dye extract was mixed with 1ml of concentrated sulphuric acid. Sodium hydroxide was also added for confirmation of the test. Red colour formation indicates the presence of quinones. Blue/green colour after addition of alkali confirms the presence of quinones.

Glycosides

In 0.2 ml dye extract, 0.1 ml of glacial acetic acid and few drops of 5% ferric chloride

solution was added. In this mixture, few ml of sulphuric acid was added. The formation of brown ring at interface having greenish blue colour solution indicates the presence of glycoside.

Flavonoid

Dye extract (2ml) was mixed with 2ml zinc dust and HCL was added gradually dropwise. The red colour of the solution indicates the presence of flavonoids in dye.

Phenol

Two ml of dye extract was mixed in 2ml of 2% ferric chloride. Formation of blue green colour indicates the presence of phenol.

Saponin

Two ml of dye extract was shaken vigorously in a test tube. Stable foam formation indicates the presence of saponnin.

Terpenoids

Dye extract (2ml) was mixed with 2ml chloroform and sulphuric acid. The formation of reddish brown colour at the interface indicates the presence of terpenoids in dye.

Natural dyeing of wool fabric with extracted natural dyes

Presoaked samples of wool fabric (2 g) were dyed using the prepared dye solutions at 80°C for 60 minutes. The samples were then cooled, rinsed, washed with water and dried in shade.

Testing of functional properties of prepared fabric

The wool fabrics dyed separately with mulethi and pride of India were further evaluated for its antibacterial activity (AATCC-147), colour strength (k/s), ultraviolet protection factor (UPF) using AATCC-183 method and colour fastness properties.

Evaluation of antibacterial activity (AATCC-147)

The test specimens of size 2.5x5cm were cut and 15-20 ml of nutrient agar medium was poured in respective petri dishes, cool and left to set. The 10 ml nutrient broth containing test organisms was kept overnight in incubator at 37°C and 120 rpm. The 4 mm inoculating loop full of test inoculums was loaded and streaked aseptically to the surface of prepared agar plates. The specimens were gently placed over the agar plates using sterilized forceps. Incubation of plates were done for 24 hours at 37°C.

After 24 hours of incubation the plates were examined for growth interruption along the streaks of inoculums and beneath the specimen. The clear zone of inhibition beyond the edges of specimen was determined. The average width of inhibition zone for test specimen was calculated beyond the edges of specimens was determined using following formula:

$W = T - D/2$ Eq. (A.1)

Where, W= width of clear zone of inhibition in mm.

T= total diameter of fabric specimen and clear zone in mm

D= diameter of the fabric specimen in mm

Measurement of colour strength and UPF of dyed samples

The colour strength of dyed fabric samples were measured using “Premier Colourscan SS5100A” spectrophotometer. The samples

were folded twice and placed at the eye of the instrument and the light was projected onto it. The samples orientation was changed to five times and readings were recorded.

$k/s = [\{(1-R)^2 / 2R\}]$ **Eq. (A.2)**

Where R is the reflectance, K is absorbance and S is the scattering

Ultraviolet protection factor of dyed and undyed specimens were determined using AATCC-183:2004 test method at NITRA Ghaziabad. The instrument used for measuring UPF of samples was Compsec M 350 UV-visible spectrophotometer. The specimen of size 10 x 10 cm was mounted on the port of instrument under standard atmospheric conditions. The UPF of the specimen was firstly taken in one direction, second measurement was taken at 45° to the first orientation and third measurement was taken at 45° to the direction of the second measurement. The average of three measurements of spectral transmittance was calculated.

The formula used for determining UPF of each specimen was:

$$UPF = \frac{\Sigma E\lambda \times S\lambda \times \Delta\lambda}{\Sigma E\lambda \times S\lambda \times T\lambda \times \Delta\lambda} \quad \text{.....Eq. (A.3)}$$

Where, E_λ is relative erythemal spectral effectiveness, S_λ is solar spectral irradiance, T_λ is average spectral transmittance of the specimen (measured) and Δ_λ is measured wavelength interval (nm)

Assessment of colour fastness

The wool fabric dyed using mulethi and pride of India dyes were tested for various fastness properties such as washing fastness (IS: 687-1979), rubbing fastness (IS: 766-1956) and light fastness (IS: 2454:1985).

Results and Discussion

Wool fabric specifications

The physical properties of wool fabric including fabric weight, tensile strength and elongation were assessed. The results are shown in Table 1.

Determination of λ-max

The λ-max of both the dyes were recorded by diluting the solution upto 50 times and was observed to be 390 nm in case of *Glycyrrhiza glabra* and 400 nm in case of *Lagerstoermia speciosa* dye.

Assessment of antibacterial activity of extracted dyes

The extracted dyes were subjected to test against antibacterial activity using disc diffusion method and results are shown in Table 2. The zone of inhibition was measured against both the bacteria i.e. *S.aureus* and *E.coli*.

It was found that *Glycyrrhiza glabra* showed excellent inhibition properties against *S.aureus* (10 mm) as compared to *E.coli* (6mm). Snafi, (2018) also reported that alcoholic extract of roots of *Glycyrrhiza glabra* showed strong antibacterial activity against *S.aureus* with an inhibition zone of 22mm and *E.coli* with inhibition zone of 15 mm.

The lowest zone of inhibition was obtained in case of *Lagerstoermia speciosa* against *S.aureus* (3mm) whereas the dye possesses good antibacterial activity against *E.coli* (6mm). The findings of Diab *et al.*, 2012 reported that methanolic extracts of Lagerstoermia leaves had bactericidal activity against *S.aureus* with inhibition zone of 30 mm and *E.coli* (26mm).

Characterization of *Glycyrrhiza glabra* and *Lagerstoermia speciosa* dye

FTIR analysis of *Glycyrrhiza glabra* and *Lagerstoermia speciosa* dye

The spectral ranges obtained from FTIR have been used in identifying the components present in the dye extract. The FTIR peak values and functional groups of *Glycyrrhiza glabra* (GA) and *Lagerstoermia speciosa* (LS) are shown in Figure 1. The dye extract of *Glycyrrhiza glabra* (GA) showed characteristic absorption bands at 1063 cm^{-1}, 1682 cm^{-1} and at 3310 cm^{-1}. The peak at 1063 cm-1 represents the presence of aliphatic amines (Theivandran *et al.,* 2015).

The peaks 1682 cm^{-1} to 1670 cm^{-1} are representing –C=C- stretching vibrations which indicates the presence of alkanes. A small peak at 1281 cm^{-1} indicates the presence of ester group. The frequency range of 3800-3200 cm^{-1} present in O-H stretching vibration confirms the presence of alcohols/phenols.

The FTIR analysis of *Lagerstoermia speciosa* dye has absorption bands at 575 cm^{-1}, 1040 cm^{-1}, 1169 cm^{-1}, 1466 cm^{-1}, 1654 cm^{-1}, 1736 cm^{-1}, 2878 cm^{-1}, 2927 cm^{-1}, 3298 cm^{-1} and 3763 cm^{-1}. The peak at frequency of 1040 cm^{-1}, 2927 cm^{-1} and 3298 cm^{-1} were strong while other peaks were medium to weak. The strong peaks at 1040 cm^{-1} represents the presence of aliphatic amines, 2927 cm^{-1} indicates the presence of alkanes and smooth peak at 3298 cm^{-1} strongly indicates the presence of alcohols/phenols.

Phytochemical profiling

The phytochemical analysis of *Glycyrrhiza glabra* and *Lagerstoermia speciosa* dye extract indicated the presence of various bioactive compounds as shown in Table 3. The Glycyrrhiza dye extract indicates the presence of tannins, quinones, glycosides, phenol, saponins and terpenoids. Sharma *et al.,* (2013) also reported the presence of tannins, terpenoids, glycosides and saponins in the methanolic root extract of *Glycyrrhiza glabra*.

The qualitative phytochemical analysis of *Lagerstoermia speciosa* dye extract confirms the presence of tannins, anthocyanins, flavanones, glycosides, phenol and saponins. Azad *et al.,* (2019) also confirms the presence of glycosides, saponins and phenols in the ethanolic leaf extract of *Lagertoermia speciosa*.

Dyeing of wool fabric with natural dyes and testing of its functional properties

The filtered solutions of both the dyes were used for dyeing of wool fabric at 80°C for 60 minutes. The colours obtained on wool fabric using *Glycyrrhiza glabra* and *Lagerstoermia speciosa* dyes are shown in Figure 2a and b.

These dyed fabrics were tested for antibacterial activity against *S.aureus* and *E.coli* using AATCC-147 method. The UPF and colour strength of both the dyed samples were also tested. The results obtained are shown in Table 4.

It is evident from Table 4 that the *Lagerstoermia speciosa* dyed samples showed good antibacterial activity against *E.coli* (2cm) but very less antibacterial activity against *S.aureus* (0.2cm). In case of *Glycyrrhiza glabra* dyed samples, the inhibition zone (Fig. 3) was more against *S.aureus* (2cm) but less against *E.coli* (1cm). When compared to the antibacterial activity of dye solutions shown in Table 2, the zone of inhibition reduces in case of dyed fabric.

This may be due to the fact that the dye concentration was not sufficient enough for antibacterial activity on dyed fabric.

Table.1 Physical properties of wool fabric

Properties		Wool
Fabric Weight (g/cm^{-1})		2.321
Tensile Strength (KgF)	Warp	34.15
	Weft	36.9
Elongation (%)	Warp	38.5
	Weft	38

Table.2 Antibacterial activity of extracted natural dyes

Natural Dye	Zone of Inhibition (mm)	
	S. aureus	*E.coli*
Glycyrrhiza glabra	10	6
Lagerstoermia speciosa	3	6

Table.3 Phytochemical analysis of extracted dyes

Phytochemicals	*Glycyrrhiza glabra*	*Lagerstoermia speciosa*
Tannins	+	+
Anthraquinones	-	-
Anthocyanin and Flavanones	-	+
Coumarins	-	-
Quinones	+	-
Glycosides	+	+
Flavonoids	-	-
Phenol	+	+
Saponins	+	+
Terpenoids	+	-

Key: + Present - Absent

Table.4 Functional properties of fabric dyed using glycyrrhiza & lagerstoermia dyes

Name of the plant	Colour strength (k/s)	Zone of Inhibition (cm)		UPF	UV protective class
		S.aureus	*E.coli*		
Blank wool	0.844	0	0	60.6	Excellent
Lagerstoermia speciosa	16.595	0.5	2	146	Excellent
Glycyrrhiza glabra	15.523	2	1	107	Excellent

Table.5 Colour fastness properties of dyed samples

Name of the plant	Washing fastness		Light fastness	Crocking fastness			
				Dry		Wet	
	CC	CS		CC	CS	CC	CS
Lagerstoermia speciosa	3	4-5	5	5	4-5	4-5	4-5
Glycyrrhiza glabra	4	4	5	5	4-5	5	4

Fig.1 FTIR spectra of *Glycyrrhiza glabra* and *Lagerstoermia speciosa* dye

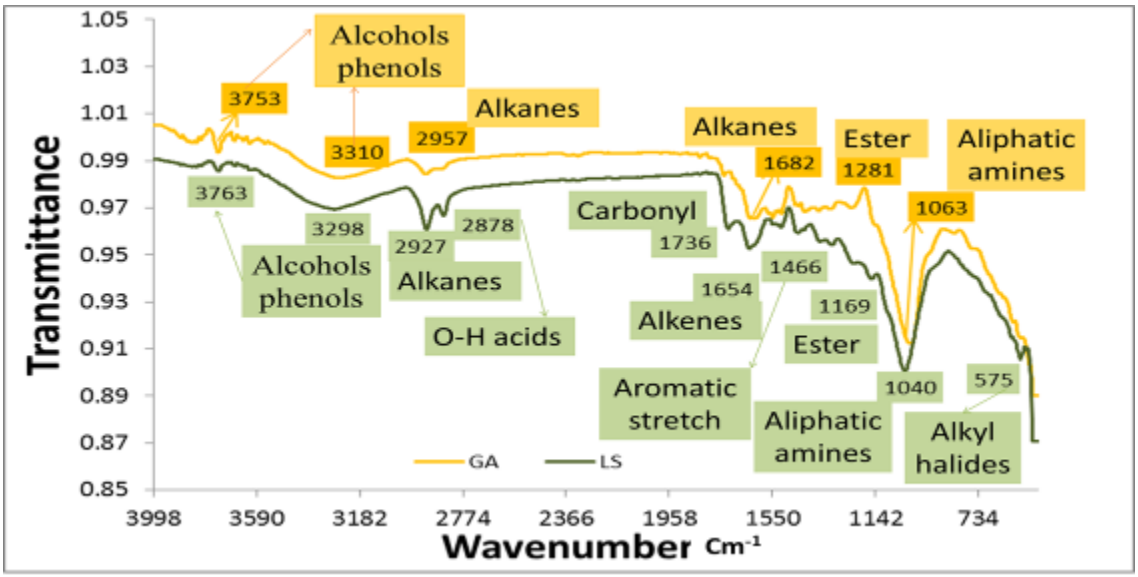
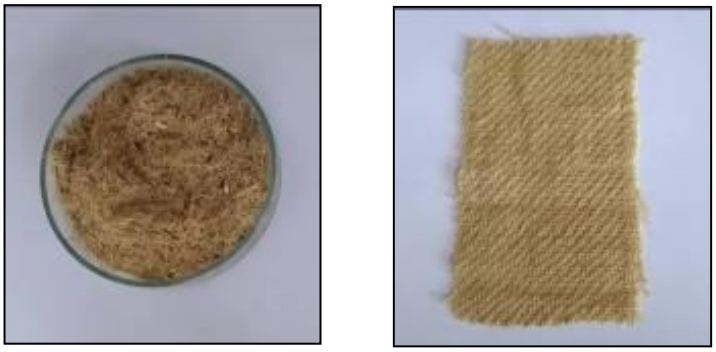


Fig.2 Dye powders and colours obtained on wool fabric

a) Glycyrrhiza glabra



b) Lagerstoermia speciosa

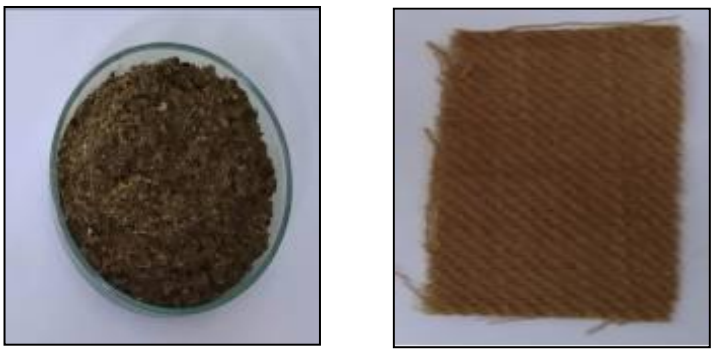
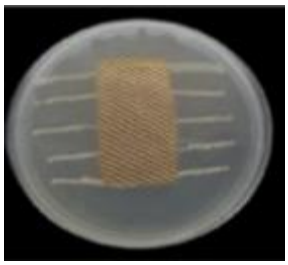
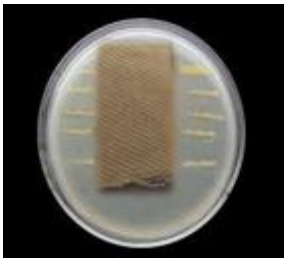


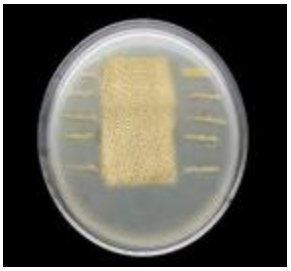
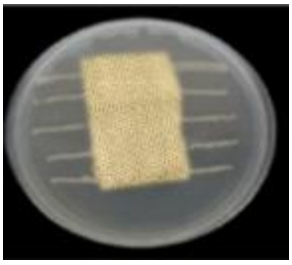
Fig.3 Zone of inhibition produced by dyed wool fabric

Lagerstoermia speciosa

S.aureus ***E.coli***

Glycyrrhiza glabra

The amount of dye absorbed by the fabric may be less which ultimately reduces the antibacterial activity of dyed fabric as compared to solution. The results were also in line with the statement of Singh *et al.*, 2004 that the reduction in antimicrobial activity was observed when extract of dye was applied on wool fabric. This is due to the reason that concentration of dye was not sufficient enough for fabric to impart high antibacterial activity. It is also inferred from the data that the *Lagerstoermia speciosa* dyed wool exhibit higher k/s value i.e. 16.595 as compared to *Glycyrrhiza glabra* (15.523).

In case of ultraviolet protection factor, it was observed from the Table 4 that *Lagerstoermia speciosa* dyed samples exhibits higher ultraviolet protection (146) as compared to *Glycyrrhiza glabra* (107) dyed samples. Both the dyed samples were found to be in excellent category of UPF according to AS/NZS-4399 standards. Herbazest, 2015 also mentioned that licochalcone an active compound in licorice roots provides protection against UV rays.

Kolakul and Sripanidkulchai, 2018 also reported the protective effects of *Lagerstoermia speciosa* extract against UV-A radiation and mentioned it as a potential therapeutic agent to prevent skin photo aging.

Assessment of colour fastness properties

The *Lagerstoermia speciosa* dyed samples as indicated in the Table 5 showed noticeable change in colour in washing fastness and slight to negligible colour staining.

The washing fastness of *Glycyrrhiza glabra* dyed samples showed slight change in colour and slight staining on wool fabric. In case of fastness to light, the *Lagerstoermia speciosa*

and *Glycyrrhiza glabra* dyed samples showed good fastness to light.

In case of *Lagerstoermia speciosa* dyed samples no change in colour (5) was observed during dry crocking but exhibited slight to negligible colour staining on cotton fabric. The dyed samples also showed negligible to slight colour change (4-5) and colour staining (4-5) on cotton fabric during wet crocking.

Table 5 also shows that there is no change in colour (5) during dry crocking *of Glycyrrhiza glabra* dyed fabric but slight to negligible staining was observed on cotton fabric. The wet crocking of *Glycyrrhiza glabra* dyed fabric show no change in colour (5) and slight staining was observed on cotton fabric.

This study envisages the development of new potential natural dye sources and its utilization for imparting functional as well as aesthetic properties through sustainable dyeing of wool fabric. The plants under study utilize waste leaves of *Lagerstoermia speciosa* and the roots of *Glycyrrhiza glabra* which can be obtained through sustainable crop production. The present work showed that *Lagerstoermia speciosa* and *Glycyrrhiza glabra* gives acceptable colours on wool fabric. Both the dyes give excellent antibacterial activity in solution and dyed fabric also showed good antibacterial activity against human pathogens. Both the dyed fabrics provide excellent protection against harmful UV rays. The people who live at higher altitudes are more exposed to UV rays so these dyed wool fabrics will be a potential alternative in developing protective clothing to provide sun protection and also protect wearer's skin from harmful bacterial infections. It is a sustainable technique which may foresee the viability of natural dye production on commercial scale. The study also serves as a potential alternative for upliftment of rural societies in developing countries like India as well as provides alternate employment opportunities to meet their basic needs.

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