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Antimicrobial activity of wool yarn dyed with *Rheum emodi* L. (Indian Rhubarb)

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ABSTRACT

This work is an attempt to examine the effect of *Rheum emodi* L. as dye and its dyed wool yarns against two bacterial (*Escherichia coli* and *Staphylococcus aureus*) and two fungal (*Candida albicans* and *Candida tropicalis*) species. The dyeing was carried out using 5% and 10% o.w.f. dye concentration in presence and absence of ferrous sulphate, stannous chloride and alum mordants. The colour strength, CIELab values and fastness properties of dyed samples were also assessed. FTIR spectra of untreated, mordanted and dyed wool yarn were investigated to study the interaction between fibre, mordant and dye. The structural morphology of wool yarn was investigated by Scanning Electron Microscopy (SEM). The susceptibility tests for *R. emodi* L. were carried out in terms of disc diffusion, growth curve and viability assays against all the tested microorganisms. Dyed samples showed very effective antimicrobial properties showing more than 90% microbial reduction in both bacterial as well as fungal population.

1. Introduction

In present scenario of the era of eco-friendliness, it has become very important for human being to live in the atmosphere of eco-friendly world. The major hindrance that comes in their way is microorganisms which are causative of deterioration, staining, odour and dermal infections [1]. Apart from these effects, microbes cause harm to human beings by transmitting infections and diseases [2–4]. So, it becomes very important to finish all garments with antimicrobial treatment to check the microbial growth on textiles without destroying desirable characteristics of textiles. Antimicrobial finished textiles are used in medical garments, sanitary, napkins, socks, disposable wipers, carpets. A variety of synthetic antimicrobial textile agents have been reported such as organometallics, phenols, quaternary ammonium salts and organo silicones [5]. They are more complex and it will take a long time to complete their natural cycles and return to nature; causing environmental pollution [6,7]. Due to this, eco-friendly natural dyes which inhibit the growth of microorganism as well as dye the textile material can be used for antimicrobial finishings of textiles without causing environmental deterioration. Most of the plants used for extraction of dye are classified as medicinal plants and some of these have shown to possess significant and effective antimicrobial activity [8,9]. The antimicrobial activities of some natural dyes are reported as potent owing to the existence of phenols, tannins, and quinines in their extracts. Natural dyes are believed to be safe because of their near to non-toxic, non-allergic and biodegradable nature [10,11], and are being used extensively in colouration of textile materials [12–14].

Indian Rhubarb/Revandchini (*Rheum emodi* L.), a stout herb of 1.5–3 m in height, distributed in Himalayas from Kashmir to Sikkim at an altitude of 3300–5200 m, is an important medicinal plant, which finds an extensive use in Ayurvedic and Unani systems of medicine [15–17]. It is chiefly used in medicine as a purgative astringent tonic. Roots of the plant are used for cleaning teeth. They are believed to exhibit antimicrobial properties [18], used in colouring of food stuffs [19] and textiles [20,21]. Ultraviolet (UV) protective properties of Rhubarb dyed textiles are also reported [22]. Rhubarb roots contain a large number of anthraquinone derivatives based on chrysophanol, aloe-emodin, rhein, emodin and physcion, the structure of which is given Fig. 1 [23,24].

In present investigation an attempt has been made to evaluate colour measurement, fastness properties and antimicrobial activity of wool yarns dyed with *R. emodi* L. Comparative results of dye exhaustion, colour fastness (light, washing and crocking), Committee International d'Eclairage (CIE) Lab values and colour strength were reported to assess the effect of mordants on dyeing. Fourier Transform Infrared (FTIR) spectra were studied for

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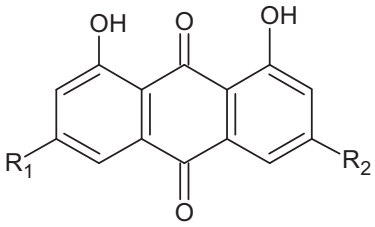

R_1=H,	R_2=CH_3;	Chrysophanol
R_1=H,	R_2=CH_2OH;	Aloe-emodin
R_1=H,	R_2=COOH;	Rhein
R_1=CH_3,	R_2=OH;	Emodin
R_1=CH_3,	R_2=OCH_3;	Physcion

Fig. 1. Active component of *Rheum emodi* L.

interaction between wool fibre-mordant-dye. Scanning Electron Microscopy (SEM) was used to evaluate the change in surface morphology of yarns after mordanting and dyeing.

2. Materials and methods

2.1. Dye and wool yarn

100% semi worsted 60 count wool yarn was purchased from MAMB Woollens Ltd. Bhadohi, India. Powdered *R. emodi* L. dye was obtained from Sir Biotech India Ltd. Kanpur, India. The composition of the *R. emodi* L. extract used in this study, was 85–87% dye component along with 5–6% moisture and 8–9% ash content. All chemicals used were of Laboratory grade.

2.2. Strains and media

Stock cultures of *Escherichia coli* Microbial Type Culture Collection (MTCC) 443 and *Staphylococcus aureus* MTCC 902 were cultured in MacConky agar (HiMedia, India) and stock cultures of *Candida albicans* American Type Culture Collection (ATCC) 10,261 and *Candida tropicalis* ATCC 750 were maintained on slants of nutrient agar at 4 °C. To initiate growth for experimental purposes, one loop full of cells from an agar culture was inoculated into 25 mL of respective nutrient media and incubated at 30–37 °C for 24 h i.e., up to stationary phase (primary culture). The cells from primary culture (10^8 cells mL^{-1}) were re-inoculated into 100 mL fresh Yeast Extract Peptone Dextrose (YEPD or YPD) medium and grown for 8–10 h i.e., upto mid-log phase (10^6 cells mL^{-1}).

E. coli, a gram-negative bacterium, was selected due to its popularity of being selected as a test organism and its resistance to common antimicrobial agents [5]. *S. aureus*, a pathogenic gram-positive bacterium, was used because it is the major cause of cross-infection in hospitals and it is the most frequently evaluated species. *C. albicans* and *C. tropicalis* were selected, since these are the common opportunistic pathogens of the immuno-compromised patients [25].

2.3. Mordanting and dyeing

Dye and mordants were used at different % o.w.f (on weight of fibre). Mordanting is done by pre-mordanting method using ferrous sulphate 5%, stannous chloride 1% and alum 10% as mordants. The dyeing was carried out using 5% and 10% dye concentration, at 1:40 ML ratio (material to liquor ratio), maintaining at neutral pH (pH ≈7), in the view of reported significant hydrolytic degradation of protein fibres at highly acidic or basic pH of around or less than 2.5 and above 7 at elevated temperature of dyeing for prolong time [26]. Temperature was raised to 91–93 °C (simmering point) and maintained at that level for 1 h. Dyed samples were washed with the non-ionic detergent safe wash (5 g/L) and rinsed with tap water and dried in shade.

2.4. Determination of exhaustion of dye

Dye uptake was determined by measuring the absorbance of the diluted dyebath samples at wavelength of maximum absorbance (λ_{max} 420 nm) of dye. The percentage of dyebath exhaustion was calculated as follows

$$\%\ \text{Dye Exhaustion} = [(A_0 - A_1)/A_0] \times 100$$

where, A_0 and A_1 are absorbance at wavelength of maximum absorption (λ_{max}) of dyebath before and after dyeing.

2.5. Colour measurement

Dyed samples were prepared for colour measurements, which were carried out by following a standard procedure. Cardboard strips were used for preparing samples for colour measurement. Dyed wool yarns were wounded closely in parallel pattern on a card to a sufficient thickness to prevent show-through. The CIELab and K/S values of dyed samples were obtained on Gretag Macbeth Colour-Eye 7000A Spectrophotometer. By using Kubelka–Munk equation (1)

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

where (K) is absorption coefficient, (R) is reflectance of dyed sample and (S) is scattering coefficient.

2.6. Fastness testing

The dyed samples were tested for colour fastness properties towards light, washing and rubbing (dry and wet) according to standard methods. The light fastness of dyed woolen yarn samples were conducted on Digi light Nx™ having water cooled Mercury Blended Tungsten lamp, according as per test method American Association of Textile Chemists and Colorists (AATCC) 16e-1993 (2004) similar to ISO 105-B02:1994 (Amd.2:2000). The wash fastness of the dyed woolen yarn samples were measured in Digi wash SS™ (Launder-o-meter) as per the ISO 105-C06:1994 (2010) specifications. The samples were also assessed for staining on white adjacent fabrics (cotton and wool). Dry and wet rub fastness of dyed woolen yarn samples were tested using a Digi crock™ (Crockmeter) as per Indian standard IS 766:1988 (Reaffirmed 2004) based on ISO 105-X12:2001 by mounting the fabric on panel and giving ten strokes for both dry and wet rub fastness tests.

2.7. FTIR spectral analysis

FTIR spectra of wool yarn before and after application of iron mordant and dye were obtained on "Perkin Elmer Spectrum RXI-

Table 1
% Dye exhaustion.

Dye (*R. emodi L.*)	% Dye exhaustion			
	Un-mordanted	5% iron	1% tin	10% alum
5%	54	69	67	64
10%	49	64	59	56

Table 2
CIELab values of the dyed wool yarn.

Dye (*R. emodi L.*)	Mordant	L*	a*	b*	c*	h°
5 %	Unmordanted	53.25	14.43	20.27	24.88	54.54
10 %		52.07	16.13	25.67	30.32	57.85
5 %	5% $FeSO_4$	50.25	12.89	17.12	21.43	53.04
10 %		49.94	15.92	25.26	29.86	57.79
5 %	1% $SnCl_2$	56.16	19.24	24.71	31.31	52.09
10 %		53.3	21.33	29.38	36.31	54.01
5 %	10% Alum	58.79	12.46	37.06	39.09	71.41
10 %		54.7	16.16	40.71	43.80	68.34

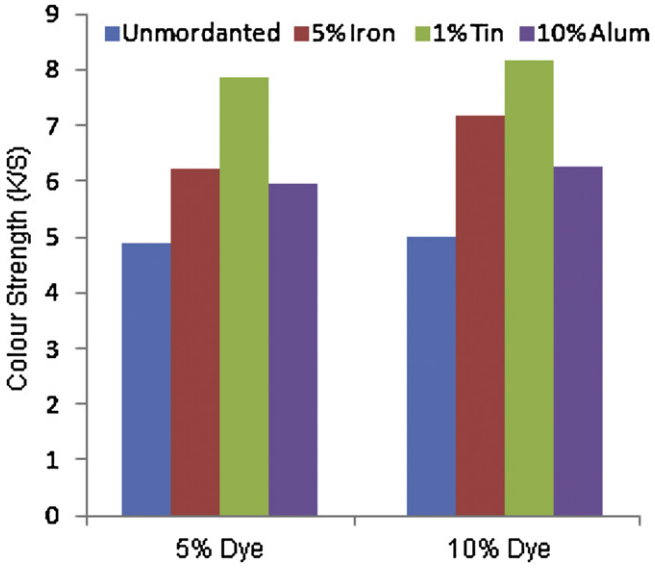

Fig. 2. *K/S* graph of dyed wool yarn.

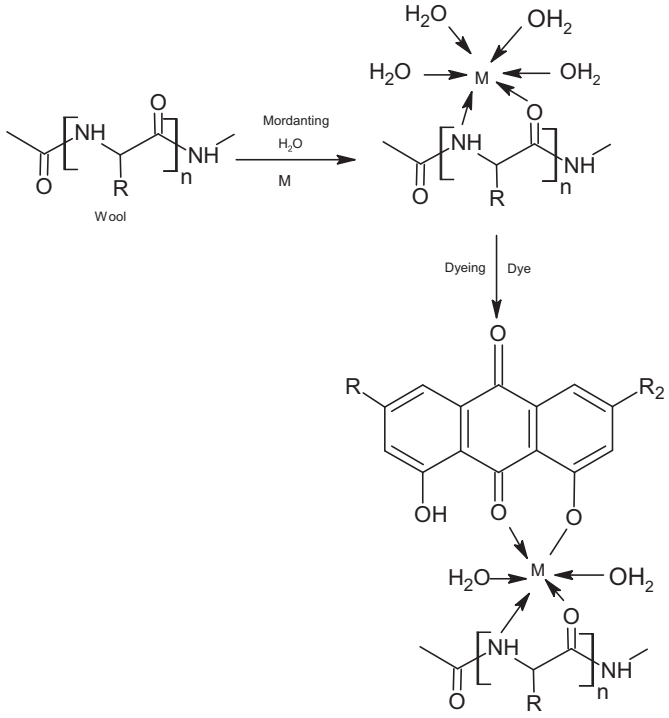

Fig. 3. wool-mordant-dye interaction.

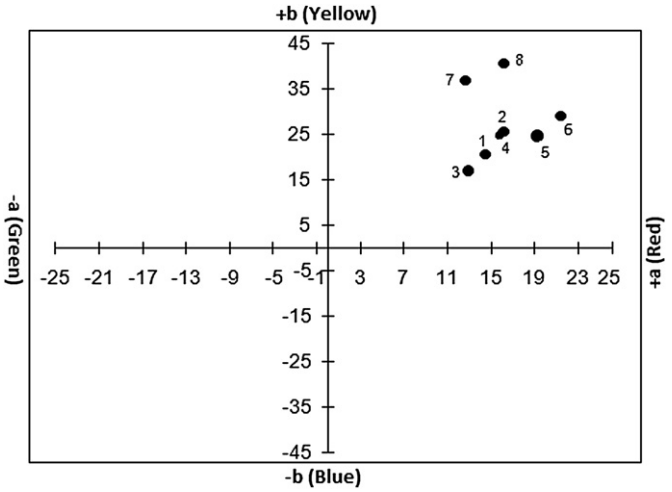

Fig. 4. a*–b* plot of dyed wool yarn. (1) 5% *R. emodi* L., (2) 10% *R. emodi* L., (3) 5% ferrous sulphate + 5% *R. emodi* L., (4) 5% ferrous sulphate + 10% *R. emodi* L., (5) 1% stannous chloride + 5% *R. emodi* L., (6) 1% stannous chloride + 10% *R. emodi* L., (7) 10% alum + 5% *R. emodi* L., (8) 10% alum + 10% *R. emodi* L.

FTIR System" to investigate and observe the type of interaction between fibre-mordant-dye.

2.8. SEM studies of dyed woolen yarns

Surface morphology of wool samples (untreated, mordanted and dyed) were investigated by SEM. Samples were glued to aluminium stubs with colloidal silver paint for conductivity and coated with gold by sputter coating method for 3 min in an argon atmosphere. Gold coated samples were observed and imaged digitally on a LEO 435VP Scanning Electron Microscope at 10 kV accelerating voltage.

2.9. Growth studies

Growth studies of tested microorganisms were done as described earlier [27] with slight modifications. Prior to testing, test microorganisms were sub-cultured at least twice and grown for 24 h at 35 °C

Table 3
Fastness properties of the dyed wool yarn.

Dye (*R. emodi* L.)	Mordant	Light fastness	Wash fastness			Rub fastness	
			c.c.	c.s.	c.w.	Dry	Wet
5 %	Un-mordanted	4	4	4–5	4	4–5	4
10 %		3	3	4	3	3–4	3
5 %	5% $FeSO_4$	4–5	3	5	4	3	3–2
10 %		4	3	4	3	3	2
5 %	1% $SnCl_2$	4–5	4	5	4	4–5	3–4
10 %		4	3–4	3–4	2–3	4	2
5 %	10% Alum	4	4	5	4	4	3–4
10 %		4	3–4	3–4	2–3	4	2

c.c. = colour change c.s. = colour staining of cotton c.w. = colour staining of wool
Wt % of mordant and dye raw material is taken with respect to o.w.f. (i.e., 50 g).

Table 4
Sensitivity index defined by a ratio of diameter of Inhibition Zone (in mm) to the concentration (in mg/mL) for the *R. emodi* L. dye in all the tested isolates.

Isolates	*R. emodi* L. dye
	Sensitivity index
Escherichia coli	2.51 ± 0.034
Staphylococcus aureus	2.875 ± 0.047
Candida albicans	2.625 ± 0.056
Candida tropicalis	2.938 ± 0.072

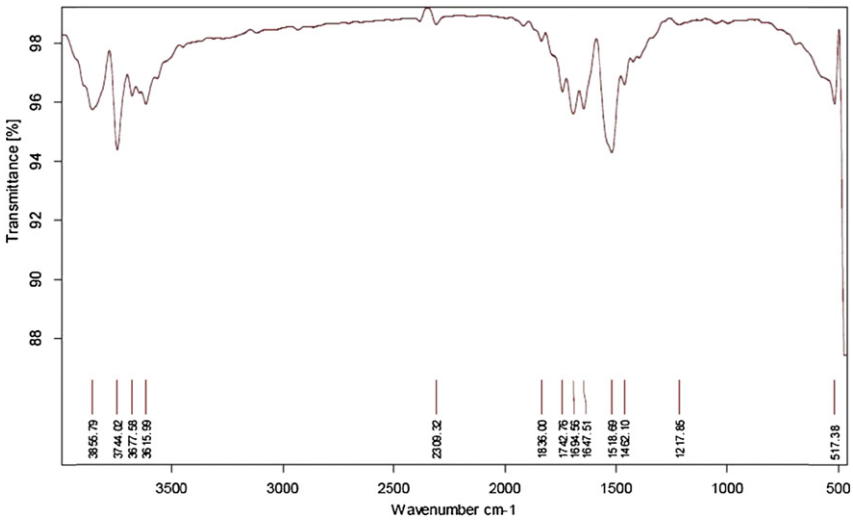

Fig. 5. FTIR spectra of untreated wool yarn.

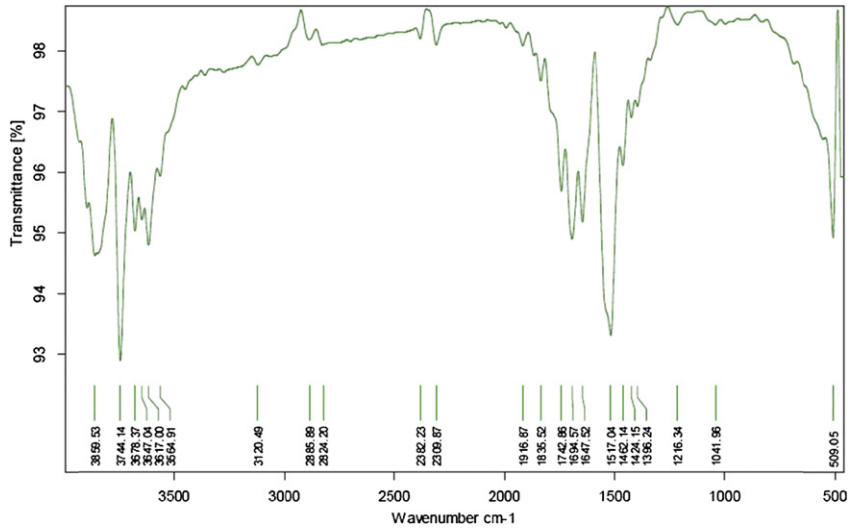

Fig. 6. FTIR spectra of mordanted wool yarn.

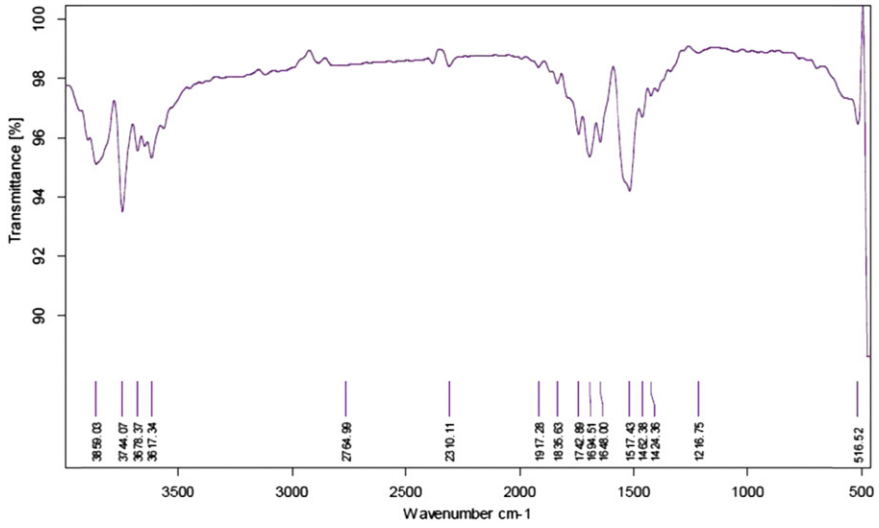

Fig. 7. FTIR spectra of *R. emodi* L. dyed wool yarn.

on Sabouraud Dextrose Agar (SDA) plates. For growth studies, 10^6 cells (optical density $A_{600} = 0.1$) of test strains were grown aerobically in 50 mL media on automated shaker set at 35 °C with agitation of 200 rpm. *R. emodi* L. dye in concentrations of 5% and 10% along with negative (distilled water) and positive controls (1% w/v of ampicillin for bacteria and 1% w/v of fluconazole for yeasts) for each test isolate were also added to the cultures. At pre-determined time points (after every 2 h) for 24 h, aliquots were removed and growth was followed turbidometrically at λ_{max} 595 nm using LABOMED Spectrophotometer (Culver City, California, USA). Optical density was recorded for each concentration against time (hours).

2.10. Disc diffusion assay

Strains were inoculated into liquid YPD medium and grown overnight at 35 °C and then cells were pelleted and washed three times with distilled water. Approximately 10^5cells/mL were inoculated in molten agar media at 40 °C and poured into 90-mm-diameter petriplates. Filter discs were kept on solid agar and dye was spotted on disc. Test compound dye dissolved in double distilled water with final concentrations of 1%, 5% and 10% and solvent control (distilled water) was pipetted onto 4-mm-diameter filter disc. The diameters of zones of inhibitions were recorded in millimeters after 48 h and were compared with that of control. The experiments were performed on both, the bacterial and fungal (yeast) strains.

Index of sensitivity defined as

$$\sum \text{Zone diameter(mm)/concentration(mg/mL)} = \text{clearing(mm/mg)}$$

Values were shown in terms of Mean ± standard error of all the experiments.

2.11. Determination of antimicrobial activity of dyed yarn

The antimicrobial activities of dyed wool specimens were tested. The 2.54 cm^2 yarns (undyed and dyed) were introduced in the 10 mL nutrient broths inoculated with a desired microbe and incubated overnight at 37 °C. The reduction of microbial growth by dyed yarn was expressed as follows:

$$R = B - A/A \times 100$$

where R = % reduction in microbial population; B = absorbance (595 nm) of the media inoculated with microbe and undyed yarn; A = absorbance (595 nm) of the media inoculated with microbe and dyed yarn. The greater the growth, higher is the turbidity, and optical density (absorbance) were therefore, directly proportional to the number of microbial cells in the media.

2.12. Statistical analysis

Each experiment was performed twice and in triplicate. Results obtained were expressed in terms of mean ± standard error. Statistical analyses were performed and P value ≤ 0.05 was considered significant.

3. Results and discussion

3.1. Dye exhaustion

The amount of dye uptake by wool yarn samples was expressed as percentage of exhaustion expressed in tabular form in Table 1. The maximum exhaustion was observed in case of iron mordanted samples followed by tin, alum and unmordanted woolen yarn samples. The difference in exhaustion rate was because of difference in interaction between fibre-mordant-dye.

3.2. Colour measurement

CIELab values of dyed woolen yarn samples are given in Table 2. From K/S plot (Fig. 2) it is observed that mordanted samples have shown higher K/S value than unmordanted ones and highest colour strength is observed in case of alum mordanted wool yarn samples followed by ferrous sulphate, stannous chloride and unmordanted samples. It can be attributed to the complex formation between yarn, mordant and dye (Fig. 3). The a*–b* plot (Fig. 4), indicates that all the samples of wool (unmordanted and mordanted) were found in red yellow zone. It is also observed that stannous chloride mordanted samples were shifted towards redder side whereas alum mordanted samples were found to be shifted towards yellow

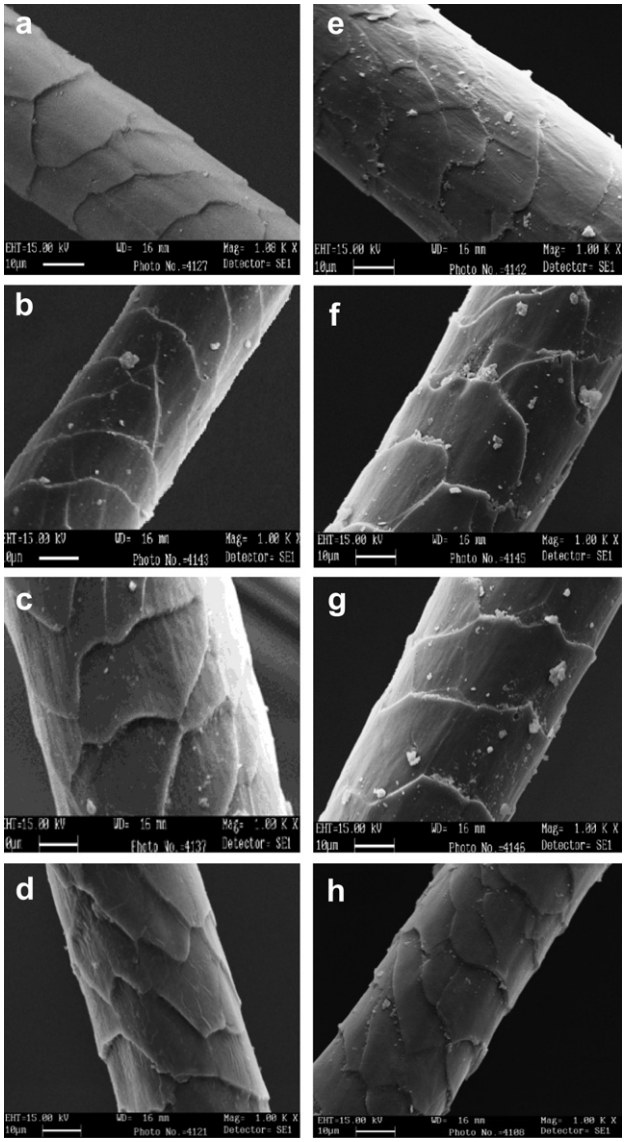

Fig. 8. SEM pictures of the wool yarn showing surface morphology. (a) Untreated wool yarn, (b) 5% ferrous sulphate mordanted, (c) 1% stannous chloride mordanted, (d) 10% alum mordanted, (e) Un-mordanted wool yarn dyed with 10% *R. emodi* L., (f) 5% ferrous sulphate mordanted wool yarn dyed with 10% *R. emodi* L., (g) 1% stannous chloride mordanted wool yarn dyed with 10% *R. emodi* L. (h) 10% alum mordanted wool yarn dyed with 10% *R. emodi* L.

side of red yellow zone. The lightness (L*) values were higher in case of dyed samples mordanted with alum and stannous chloride which corresponds to lighter shades. Whereas, the lightness (L*) values were found to be lower in case of dyed samples mordanted with ferrous sulphate which corresponds to deeper shades.

3.3. Fastness testing

Dyed woolen yarns were subjected to test for fastness towards light, wash and rub (dry and wet). It was observed from colour fastness data (Table 3) that wool samples mordanted with ferrous sulphate and stannous chloride have shown improvement in light fastness ratings of 4–5, as compared to unmordanted wool samples rating of 3–4 and alum mordanted samples (rating of 4). All the dyed samples show fairly good to good wash fastness ratings of 3–4 on grey scale and slight staining on adjacent fabric (cotton and wool) in some samples. The dry rub fastness ratings of dyed samples were found to be in between 3 and 5 and wet rub fastness ratings were of 2–4 on grey scale.

3.4. FTIR spectral analysis

The wool fibre is complex in structure and essentially composed of 20 amino acids, which can be divided into four distinct groups: cationic, anionic, polar and non-polar. The main functional groups of amino acids are carboxylic (−COOH) and amino ($-NH_2$). In FTIR spectra (Fig. 5) of untreated wool, the main characteristic peaks are in between 1700 and 1000 cm^{-1}, which include peaks for amide I, amide II and amide III appeared at 1647 cm^{-1}, 1518 cm^{-1} and 1217 cm^{-1} respectively. When spectra of raw wool yarn and mordanted wool yarn (Figs. 5and 6) were compared with each other, one new peak was appeared at 1041.90 cm^{-1}, which could be attributed to the SO_4^{-2} group of ferrous sulphate [28] which has been used for mordanting.

Measuring changes in secondary structure of wool fibres were assessed in three ranges: 3000–2500, 2000–1700 and 1500–1000 cm^{-1}. The wave number shift of amide I, amide II and amide III bands were found to be affected distinctly by the dye. FTIR spectra of mordanted and *R. emodi* L. dyed wool were shown in Figs. 6 and 7. By the comparison of intensities of bands, it is observed that there is slight increase in intensities of bands in case of mordanted wool (1647.52 and 1517.04 cm^{-1}) in comparison to *R. emodi* L. dyed wool (1648.00 and 1517.43 cm^{-1}). This could be as a result of the existence of anthraquinone groups in *R. emodi* L. dye.

3.5. SEM of wool samples showing surface morphology

The surface morphological features of woolen yarn were shown in Fig. 8. SEM photograph for the untreated wool shows a normal morphology (Fig. 8a). Some particles of the mordants have been found (seen) on the yarn surfaces (Fig. 8b–d). The unmordanted wool samples dyed with *R. emodi* L. have shown the deposition of some dye molecule on the surface of wool yarn (Fig. 8e). The mordanted wool samples dyed with *R. emodi* L. again showed the

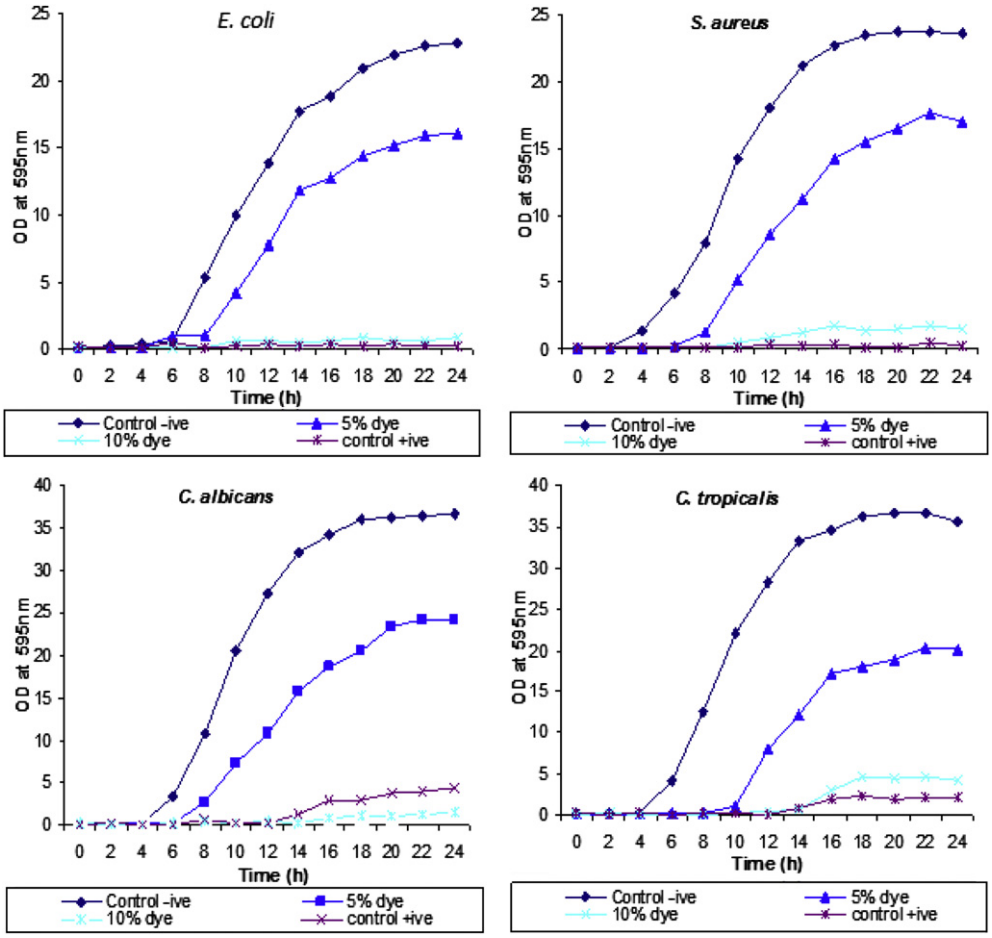

Fig. 9. The effect of various concentrations of the dye on the growth of *E. coli, S. aureus, C. albicans and C. tropicalis*. The cells were grown with 0% dye (Control −ive), 1% w/v of fluconazole for yeasts and 1% w/v of ampicillin for bacteria (Control +ive), 5% dye and 10% dye.

deposition of some dye molecule on yarn surfaces (Fig. 8f–h). From pictures of the dyed samples it is observed that coatings of dye molecules on mordanted wool samples are more than unmordanted sample.

3.6. Antimicrobial activity of R. emodi L. in solution

Antibacterial and antifungal activities of the *R. emodi* L. dye when compared with standard antibacterial and antifungal drugs (ampicillin and fluconazole) showed significant antimicrobial properties.

3.7. Growth studies

Growth studies test showed the effect of increasing concentrations of the dye on the test microbes (Fig. 9), which depicts the growth rates of *E. coli, S. aureus*, *C. albicans* and *C. tropicalis* in presence of *R. emodi* L. dye at 5% and 10%. The absorbance obtained for the growth control (only organism) showed that the test cultures reached the stationary growth phase after 16–18 h showing a normal growth pattern. The curve depicts a lag phase in the initial phase of growth, active log phase and stationary phase. All the test microorganism isolates were found to be susceptible to the test dye. At lower value (5%) of the dye, the test microorganisms show the extension of the lag phase by 2–6 h and growth was suppressed with respect to the control. More than 90% inhibition was observed when cells were treated with 10% of the dye. It was worth to note that at the higher values, the dye showed more inhibitory effect in *C. albicans* than the commercially used antimicrobials.

3.8. Disc diffusion assay

The results summarized in Table 3 give the sensitivity assay, using standard discs of *R. emodi* L. dye, ampicillin and fluconazole. All the isolates *E. coli*, *S. aureus, C. albicans and Candida torpicalis* showed high degree of sensitivity. It is greatest for *C. tropicalis* (2.938 ± 0.072) and least for *E. coli* (2.51 ± 0.034) isolates. The most important thing noticed is, that the dye was slightly more effective against fungus as compared to bacteria. The results showed that, in case of control disc no zone of inhibition was observed, as far as our study is concerned, distilled water is used as a solvent (control), have no effect on the tested organisms. Hence we can effectively conclude here that whole of the antimicrobial effect is because of the dye.

3.9. Antimicrobial activity of R. emodi L. on wool substrate

Having studied the antimicrobial effect of dye in solution, the next step was to access their effectiveness on substrate (woolen yarn). The wool yarn samples dyed with *R. emodi* L. were used as a model system. The majority of natural dyes need a mordant in the form of a metal salt to create an affinity between the fibre and the colour pigment. These metals form a ternary complex with fibre on

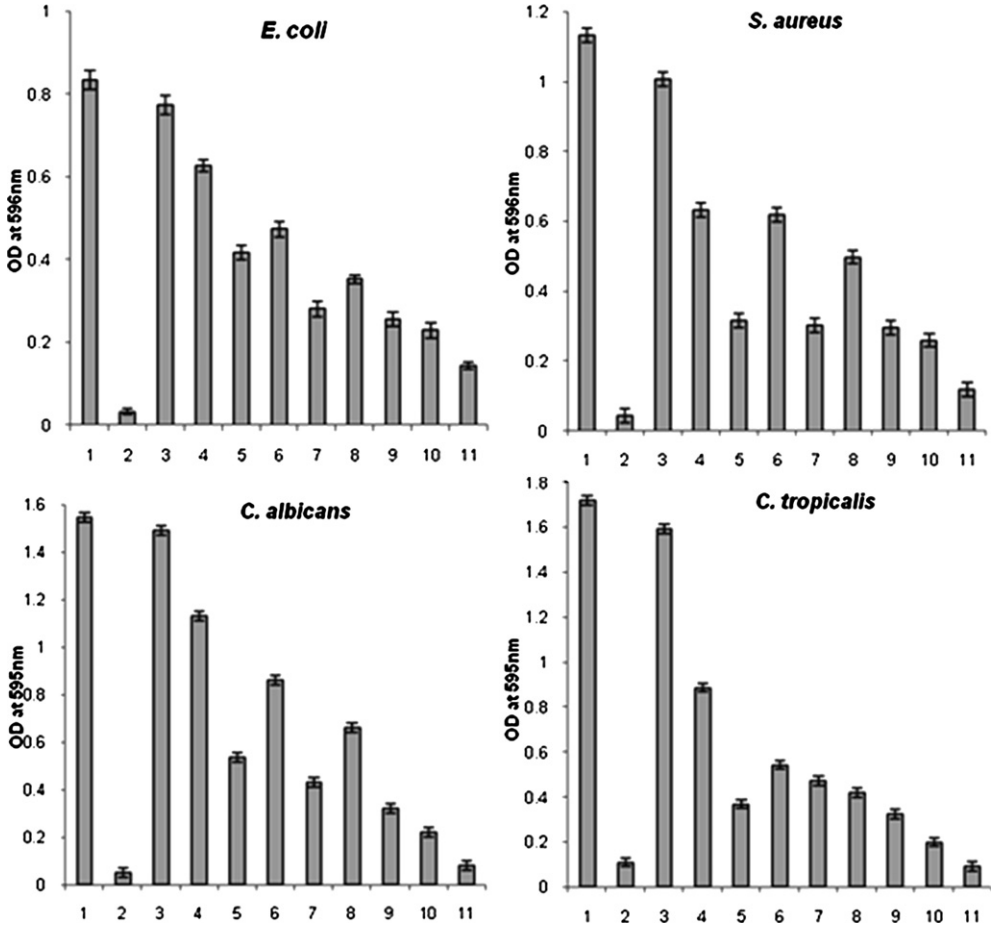

Fig. 10. Antimicrobial activity of the wool yarn treated with *R. emodi* L. dye. Bar 1 represents the control cells without any treatment, 2 represents the treatment of cells with their respective known available antimicrobial, 3 is untreated wool, 4 & 5 represents 5% ferrous sulphate with 5 and 10% dye, 6 & 7 are 1% stannous chloride with 5 and 10% dye, 8 & 9 are 10% alum with 5 and 10% dye, 10 & 11 represents wool yarn treated with 5 & 10% dye, respectively.

Table 5
Antimicrobial activity of *R. emodi* L. dyed wool yarn.

Microbe	% Microbial reduction										
	Blank	Ampicillin/ Fluconazole	Untreated wool	10% *R. emodi* L.				5% *R. emodi* L.			
				Unmordanted	5% iron	1% tin	10% alum	Unmordanted	5% iron	1% tin	10% alum
E. coli	0	95.6	1.3	82.3	49.9	66.3	69.7	72.4	24.8	43.2	57.7
S. aureus	0	98.1	4.2	90.1	72.1	73.3	73.8	77.3	44.2	45.5	56.1
C. albicans	0	97.6	5.5	93.4	65.2	72.2	79.2	85.1	26.8	44.2	57.2
C. tropicalis	0	93.6	4.3	95.3	78.5	72.5	83.1	87.8	48.4	65.3	75.5

one side and on the other side with the dye. Such a strong co-ordination tendency enhances; the interaction between the fibre and the dye, resulting in high dye uptake, therefore, the study was also carried out on the mordanted wool yarn samples dyed with different concentrations of the dye. Antimicrobial activity of commercial antimicrobials (ampicillin for the bacteria and fluconazole for the fungi) were also studied for comparison with *R. emodi* L. dyed woolen yarn samples (Fig. 10). The results were summarized in Table 5. It can be observed from the Table 4 that wool yarn dyed with 5% of the dye shows inhibition upto 72–77% in bacteria and 85–88% in fungi. The significant enhancements in antimicrobial activity (82–90% in bacteria and 93–95% in fungi) were observed when 10% dye was used. The %-age inhibition however, decreases for the dye when mordanted samples were examined. The %-age inhibition by the wool yarn mordanted with 5% ferrous sulphate and dyed with 10% dye ranges from 50 to 78% whereas wool yarn mordanted with 1% stannous chloride dyed with same %-age of dye have shown 66–73% microbial inhibition. 10% Alum mordanted wool yarn have shown highest %-age inhibition (70–83%) of microbial growth.

This is an interesting finding regarding antimicrobial activity of naturally dyed wool substrate and requires further in depth studies, hence, it may be recommended that the *R. emodi* L. dye can be used for dyeing wool textiles as an alternative to very expensive, synthetic and toxic antimicrobial agents.

4. Conclusion

In present study an environment friendly approach was tried to impart colour and antimicrobial properties to woolen yarns with *R. emodi* L. extract. On the basis of experimentation and observation following conclusions were drawn:

- Dyeing with extract of *R. emodi* L. resulted in bright yellowish green shades with subtle change in hue and tone of shade on using metal salt mordants.
- Extract of *R. emodi* L. was found active against all tested microbes in solution as well as after application on wool yarn. Dye was found slightly more effective against fungus as compared to bacteria.
- Mordants have shown positive effect on colour strength as well as fastness properties but lowered the antimicrobial activity to some extent.

This effort has shown that *R. emodi* L. dye can be used suitably for producing value-added environment friendly apparel and other textile products with increased protection against microbial deterioration of dyed fabrics and also as safety measure against pathogenic microbes.

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