Cotton Dyeing with Flavonoids using Differential Mordanting Techniques: Colorimetric Investigation and Antibacterial Efficiency

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Abstract

Pure cotton fabric colored by exhaust method using natural dyes taken out from the greeneries of Neem (*Azadirachta indica*) and Tulsi (*Ocimum sanctum*) and core-wood of Khoyer (*Acacia catechu*). Various mordanting methods (not at all mordanting, pre, post, and synchronized mordanting) are applied to keep greater concentration for the three natural colors. Mordant used here was Fitkiri/Potash alum. Spectrophotometric investigation was done by means of a commerce ranked spectrophotometer titled "Datacolor 650" origin USA. The dye fastness to washing was analyzed with ISO (105:CO3), rubbing fastness analyzed with ISO (105/12) ,and fastness to light of the dyed trials were analyzed with BS1006:1990 UKTN test methods respectively. AATCC test system 147-2004 was used to examine the effectiveness of dyed and undyed trials in contrast to 4 microorganisms: *Bacillus cereus* by ATCC (11778), *Escherichia coli* by ATCC (25922), *Pseudomonas aeruginosa* by ATCC (27853), *Staphylococcus aureus* by ATCC (25923). Outcome displays here is noteworthy color deviation among the trials which are un-mordanted-dyed or mordanted for all 3-classes of dyes & mordanting methods change the shades also. Colour strength values for tulsi are slightly better in un-mordanted samples (0.077) where catechu (0.160) and neem (0.055) have better color strength for simultaneously mordanted and dyed samples. The a*- b* plots of the tulsi-dyed samples placed themselves in the green-yellow quadrant and in case of neem and catechu, all dyed samples fell into the yellow-red quadrant. The samples mordanted and dyed simultaneously show minor colorfastness to washing in contrast with pre as well as post-mordanted-coloured trials. The natural coloured fabric was resulted to be inactive counter to any of the bacterial experienced of 24-hr culture in nutrient broth. It may be demonstrated by phenolic complex (plant) solubility in the liquid state as stated in available research works.

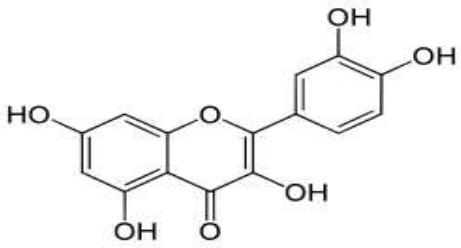
Keywords: Colorimetric Analysis, Minimum Inhibitory Concentration (MIC), Mordanting, Natural colors, Antibacterial efficiency.

1. Introduction

Natural colorants have the past of several hundred years. For instance, indigo dye is acknowledged for quite five thousand years and catechu is being practiced as brownish color in The Republic of India for more than two Thousand years [1]. The world abounds with several natural springs of dyes like trees, fungi, lichens, pests, shellfish, and numerous muds. A

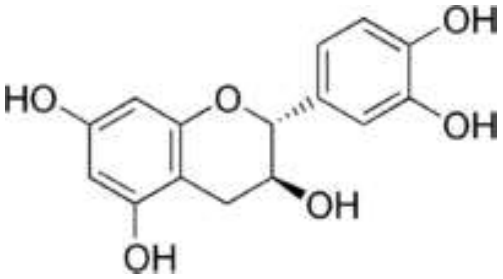
table of forty-seven blossoming plants is used in coloring fabrics for history stated in literature [2]. Several portions of the Plants together with greeneries, floras, pods, pits, howls as well as roots hold complexion resources that give diverse ranges of color on cloths. Though walnut or Allium cepa membranes are some rare natural colors that are functional (non-mordant) direct-color but chemicals needed for the mainstream of them to encourage their bonding with fiber or filaments. They are communally recognized as mordant colors moreover the serving compounds are acknowledged as mordant. Ties amongst dye particles and polymers of fiber resulted from mordanting. Urine, wood ash, plants galls, tannins, and crab-apple juice along with some pure metal compounds are examples of ordinary mordants. Aluminium, chromium, iron, tin & copper salts are examples of metal ions used as mordant. In the process of coloring wearable clothing, mordant may be applied beforehand, during, or afterward the coloring progression, so the mordanting methods are acknowledged as pre-mordanting, synchronized mordanting, and post-mordanting correspondingly.

Phenolic compounds are mostly found in tree-associated natural dyes. They are recognized for remedial and herbal applications [3]. Grounded up on their various structural parts, they can be clustered as quinones, flavonoids, tannins, lignanes, curcuminoids, coumarins, stilbenes, and other subcategories [4,5] Except them, Flavonoids are one of the utmost plentiful dyes originate in floras, greeneries, pods and pits. They are nearly fifty percent of natural dyes applied in fabric and recorded in color index [3]. Flavones and Flavonols; and the principal Available Flavanols' like Quercetin (Figure 1) &Kaemferol are the main dye molecules in the class of flavonoids [3]. Flavonoids have been recognized as a bearer of antioxidants, anti-inflammatory, anti-allergic, anti-carcinogenic, and antimicrobial functions [6].



Quercetin

Figure 1 (a). Example of flavonoid.



Catechin

Figure 1 (b). Example of flavonoid.

Neem (Azadirachta indica), a usually recognized medicinal plant, contains Quercetin in its leaves [7, 8]. Another remedial plant Tulsi (Ocimum sanctum/ tenuliflorum), acknowledged as Holy Basil, is also a foundation of Flavonoid. Seven diverse flavonoids specifically Rutin, Luteolin glucoside, Naringin, Coumarin, Apigenin, Kaempherol, and Silymarin were noticed in leaf excerpt of holy basil [9]. Nine flavonoids [(+)-catechin, (−)-epicatechin, (+)-afzelechin, (−)-epiafzelechin, (+)-mesquitol, kaempferol, quercetin, quercetin 3-methyl ether, and caryatin] were secluded from the ethanolic excerpt of Acacia catechu leaf [10]. Core wood of A. catechu contains 66.9% catechin and 23.1% epicatechin [11,12]. Though numerous flavonoids have antimicrobial properties, they functionally be subject to their Abstraction techniques, the kind of solvents utilized for extraction, and the minimum inhibitory concentration (MIC). Methanolic excerpt of neem leaves unusually inhibited the development of Staphylococcus aureus

nevertheless Streptococcus pyogenes and Pseudomonas aeruginosa [13]. The uppermost flavonoid is present in methanol extraction of neem leaves but not any finding in water extraction [14]. They also found no distinguished antibacterial efficacy of aqueous extraction neem leaves equated to Gram-positive bacterium *S. aureus* and Gram-negative bacteria *E. coli,P. aeruginosa* and *P. vulgaris*. Here 1.03%, 5.33%, and 1.83% of total phenol, flavonoid, and tannin present respectively in 50% ethanolic excerpt of neem leaves, which in a concentration 0.5~1.50 mg/ml displayed noteworthy antibacterial action in contradiction of *E. coli* and *S. aureus* [15]. Antibacterial action of *A. indica* (Neem) leaves and barks extraction in aqueous solvent using centrifuge method (@4000rpm) in contradiction of Gram-negative pathogenic microorganisms (*Escherichia coli, Salmonella typhi* and *Vibrio cholerae*) and Gram-positive bacteria (*Bacillus subtilis*) [16].

Antimicrobial action of wool fabric colored with 4 natural colorants Acacia catechu, Kerria lacca, Quercus infectoria, Rubia cordifolia, and Rumex maritimus, in contradiction of *Escherichia coli, Bacillus subtilis, Klebsiella pneumoniae, Proteus vulgaris,* and *Pseudomonas aeruginosa* resulted as the dyed fabrics exhibited very limited antibacterial activity. This is due to textile material having low contraction of these dyes which is below MIC [17]. Dyeing of wool fabric with aqueous and ethanolic extracts of *Camelia Sinensis*, *Rubia tinctorum*, *Curcuma longa, Crocus sativus*, and *Lowsoniainermis*are found some level of antibacterial activity in contrast to *Staphylococcus aureus, Escherichia coli,* and *Pseudomonas aeruginosa* bacteria [18]. Eleven natural dyes and found only a few selected dye solutions showed efficacy against Gram-negative bacteria *E. coli, K. pneumonoiae* and *P. vulgaris*; and cotton fabric dyed with only three on them exhibited some degree of antibacterial actions [19]. They also found that antimicrobial activity of natural dyes while on textiles decreased when mordants ($CuSO_4$ and $FeSO_4$) were used, which could be due to metallic salt chelation of the color.

The purpose of this paper is to investigate the result of mordanting techniques on colourimetric characteristics, fastness properties, and antimicrobial efficacy of cotton textiles colored with the natural colorants taken out from the greeneries of Neem and Tulsi & Khoyer succeeding ancient coloring in aqueous medium by exhaust method. A high absorption of color was upheld for coloring all '3' samples with the variance of mordanting systems (not at all mordanting, pre, synchronized, and post-mordanting). In case of mordanting, a high percentage of mordant potassium alum or fitkiri was applied. The rationale of maintaining a high solution of dye and mordant in coloring is to ensure the high presence of Flavonoids in dyed samples for potential antibacterial activity.

2. Materials and Methods

2.1. Materials

a) Fabric: 100% cotton knitted fabric (weight 160 g/m^2) in single jersey construction was received in scoured or bleached from Dulal Brother Ltd. located in Gazipur, Bangladesh.
b) Natural dyes: Neem (*Azadirachta indica*) and Holy Basil, locally known as Tulsi (*Ocimumtenuliflorum/sanctum*) plant leaves were collected from a garden located at Kabirpur, near the Savar area of Bangladesh. Wood catechu (*Acacia catechu*) in solid form originated from the Gaibandha district was collected from a local supplier.
c) Chemical: Laboratory grade Potassium alum or fitkiri was bought from the local marketplace for use as mordant.
d) Anti-bacterial test: '3' gram-positive bacteria: Bacillus cereus, Pseudomonas aeruginosa, Staphylococcus aureus; and one gram-negative bacterium

Escherichia coli used for the anti-bacterial efficacy test. A 24-hr culture in nutrient broth was used for the analysis.

2.2. Methods

2.2.1. Extraction of dyes from leaves

Collected Neem and Tulsi leaves were washed with distilled water and dried under the sun for several days until they were fully dry. Dried leaves were crushed into small flakes by a grinder. A 5% aqueous stock solution was made for each type of leaves after boiling the flakes for 1 hour maintaining a liquor ratio of 1:20; and cooling down to room temperature and filtering to get a clean dye solution.

2.2.2. Dye extraction from wood catechu

Cubes of catechu were mechanically crushed into powder. A 5% solution was prepared by mixing 50 gm of catechu powder into 1 liter of water. This dye solution was boiled for 1 hour, cooled down to room temperature before filtering to grow a clean colour solution.

2.2.3. Mordanting Procedure

a) Pre-mordanting: Earlier the application of the mordant, knitted fabric was drenched in water at room temperature. 15% (on the weight of fabric, owf) of Potassium alum was liquified in water maintaining a liquor ratio of 1:40. This cotton fabric was deep in the prepared mordant resolution. Then increased 80^0 C temperature and maintained it for 30 minutes. After mordanting, the fabric was directly transferred to the dye bath described in the next section (2.2.4).

b) Post-mordanting: After dyeing an un-mordanted piece of fabric following the procedure mentioned in section 2.2.4, the dyed piece was preserved with mordant in an isolated bath maintaining 15% Potash alum in 1:40 liquor at 80^0 C for 30 minutes.

c) Simultaneous mordanting-dyeing: In this technique, 15% (owf) mordant was added to the dye bath (see section 2.2.4) while dyeing an un-mordanted piece of fabric.

2.2.4. Dyeing procedure

The exhaust dyeing method was followed for this research. In every case of dyeing the un-mordanted or pre-mordanted fabric pieces, a material: liquor -1:40 was maintained and dyeing was carried out at 80°C in 45 minutes. The concentration of dye was 30% (owf) maintained in the day-bath prepared separately from each of the previously prepared stock solutions of Neem, Tulsi, and Catechu. Dye amount (D) was calculated using the formula 1:

D = (Fabric Weight X Required % of Dye) / % of Stock Solution … (1)

In case of simultaneous mordanting, dye bath was prepared by adding mordant with the dye solution and maintaining an M:L of 1:40. After dyeing, in all cases, the fabric pieces were air-dried for 10 to 12 hours. No rinsing and washing were carried out after dyeing to ensure the maximum presence of Flavonoids in the dyed samples to assist their antibacterial actions.

2.2.5. Determination of color strength

Reflectance percentage of completely dyed trials (not at all mordanting, pre, post, and simultaneous mordanting) without any after wash was measured by means of a quality manufacturing marked spectrophotometer "Datacolor 650" [20].

Strength of colour (K/S values) of the coloured cloths was demonstrated by Kubelka-Munk equation (eq. 2).

$$K/S = (1 - R_{\lambda max})^2 / 2R_{\lambda max} \quad \ldots (2)$$

Where the absorption coefficient is K, the scattering coefficient is S, and the reflection factor of the cloths is $R_{\lambda max}$ at the wavelengths of extreme absorbance.

2.2.6. Determination of color parameters

The colorimetric constraints (*L, a, b*) for all colored examples (un-mordanted, pre-mordanted, post-mordanted, and simultaneous mordanted-dyed) were achieved by means of the same spectrophotometer having light source D65, with a standard observer of 10°. According to the theory of CIELAB colour space, *L** corresponds to the lightness and darkness (where, hundred = white, zero = black), positive *a** refers to the red and negative *a** is green coordinate, and positive *b** is related to the yellow and negative *b** blue coordinate. The L*, a*, b* values of each sample were determined by comparing the colour differences against a white reference tile having spectra values of L* = 97.06, a* = 0.41, and b* = 0.57 and using equation 3.

$$\Delta L^* = L^*_{sample} - L^*_{reference} \text{ (X-rite, 2016)} \quad \ldots (3)$$

For example, when measured against the white reference tile, the color differences of the un-mordanted tulsi-dyed sample was found ΔL* -26.70, Δa* -2.22 and Δb* 23.25, Therefore, the L*, a*, b* values of this sample were calculated as:

$$L^*_{sample} = 97.06 + (-26.70) = 70.36$$
$$a^*_{sample} = 0.41 + (-2.22) = -1.81$$
$$b^*_{sample} = 0.57 + 23.25 = 23.82$$

All the calculated values are presented in Table 1. The colour difference of the all mordanted-dyed samples was compared against the un-mordanted sample of each dye group (Neem, Tulsi and Catechu) by using the CMC tolerancing formula (eq. 4), established by CMC (Colour Measurement Committee) of the Society of Dyers and Colourists in Great Britain in 1988. [20,21].

The CMC formula is based on CIE lightness (ΔL*), chroma (ΔC*), and hue (Δh*) differences. The system calculated the CMC tolerance using equation 4 mentioned below.

$$\Delta E^*_{CMC} = \sqrt{\left(\frac{\Delta L^*}{lS_L}\right)^2 + \left(\frac{\Delta C^*}{cS_C}\right)^2 + \left(\frac{\Delta h^*}{S_H}\right)^2} \quad \ldots (4)$$

Where, ΔE^*_{CMC} is the CMC tolerance or the difference, and the S_L, S_C, and S_H are the CMC allowance aspects of lightness, Chroma and Hue that adjust the CIE differences (ΔL*, ΔC*, ΔH*) depending upon the location of the standard in CIE 1976 color space [20,21]. The two aspects l and c are persistent, are well-defined by the handler, and importance of lightness and Chroma relation to the hue of resulting colors.

2.2.7. Color-fastness properties of colored samples

The wash fastness of the colored examples was tested at 60°C for 30 minutes according to ISO (105:CO3) method. The color fastness against dry and wet rubbing of dyed samples was evaluated according to ISO 105/12 method. Fastness ratings were determined by comparing the variations in dye shades of the colored samples under D65 light (artificial day light) in a color matching cabinet from Verivide, USA, and with the help of the ISO Grey scale standards.

The light fastness of all colored trials was assessed following the BS1006:1990 UKTN (page 89-94) using a Microscale tester (Equiptex, Yorkshire, UK). The trials were uncovered to artificial light for 248 hours under a mercury arc (500w) fading lamp that produces an equivalent color temperature of Xenon arc fading lamp recommended in the ISO 105-BO2:2014 test method. Blue wool standards were used to assess the changes in the color of dyed samples after exposure.

2.2.8. Assessment of the antibacterial activity

(a) Organisms

Antibacterial properties of the undyed and colored cotton fabric examples were tested compared to '3' gram-positive bacteria: *Bacillus cereus* ATCC 11778, *Pseudomonas aeruginosa* ATCC 27853, *Staphylococcus aureus* ATCC 25923; and one gram-negative bacterium

Escherichia coli ATCC 25922. A 24-hr culture in nutrient broth was used for the analysis.

(b) Sample preparation and testing

Each fabric sample was cut into squares of dimension 2x2 cm. Four different dyed fabric samples from each natural dye (i.e. samples dyed without mordanting, with pre-mordanting, with simultaneous mordanting, and with post-mordanting). A piece of the clear undyed cotton fabric, used in the preparation of the dyed samples, was used as control. The AATCC test method 147-2004 was used as the test protocol. Test organisms were immunized into the nutrient broth from stock cultures at 37ºC for 24 hours. 1.0ml of the incubated culture was diluted in 9mL of the ringer's solution. This was used to make five streaks on a Mueller Hilton (MH) agar using a sterile inoculating loop, without refilling the loop. The cut fabric samples were placed on the center of and to overlap the five streaks made on the agar and pressed to ensure sufficient contact was made with the culture and agar surface. The agar plates were nurtured at 37ºC for 24 hours before observation. The experiment was performed twice and in duplicates.

3. Results and Discussion

3.1. Colorimetric Properties

CIELab and K/S values of colored cotton fabrics are presented in Table 1. Figure 2 shows the effect of mordants on color strength while figures 3, 4, and 5 exhibit the a*- b* plots of the samples dyed with tulsi, neem, and catechu respectively. It is clear from figures 3,4 and 5 that tulsi-dyed samples placed themselves in the green-yellow quadrant and in case of neem and catechu, all dyed samples fell into the yellow-red quadrant. It is obvious from table 1 and figure 2 that the mordant (Potassium alum) exhibited some effects in dyeing with neem and catechu; however, it did not positively influence the color strength of tulsi-dyed samples.

When compared against standard 'White Tile' (L*=97.06; a*=0.41; b*=0.57), the lightness (L*) value of the colored samples which are mordanted beforehand was found inferior than the colored samples which are

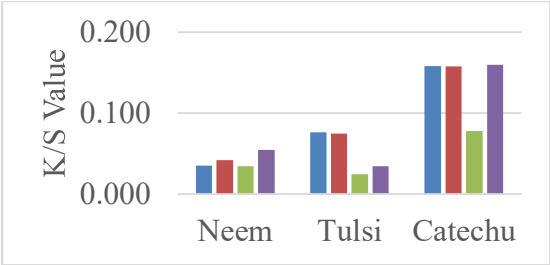


Figure 2. Effect of mordants on color Strength

Table 1. CIE Lab values of colored cotton cloths.

Source of Natural Dye	Dyed Samples	L*	a*	b*	K/S
Tulsi	Un-Mordanted	70.36	-1.81	23.82	0.077
	Pre-mordanted	71.91	-3.16	29.02	0.075
	Post-mordanted	84.09	-1.03	18.34	0.025
	Simultaneous mordanted-dyed	82.71	-1.35	19.04	0.035
Neem	Un-Mordanted	77.65	3.01	21.75	0.035
	Pre-mordanted	74.86	0.69	31.36	0.042
	Post-mordanted	79.58	2.06	20.88	0.035
	Simultaneous mordanted-dyed	73.85	1.31	29.53	0.055
Catechu	Un-Mordanted	52.60	14.87	36.62	0.158
	Pre-mordanted	55.85	15.09	33.34	0.158
	Post-mordanted	65.47	9.16	27.31	0.078
	Simultaneous mordanted-dyed	51.45	14.28	35.59	0.160

post-mordanted. It indicates the post-mordanted-dyed samples were comparatively darker in shade than the pre-mordanted-dyed samples (see table 1). However, simultaneously mordanted and colored examples resulted in lighter shades than all post-mordanted samples of each dye group.

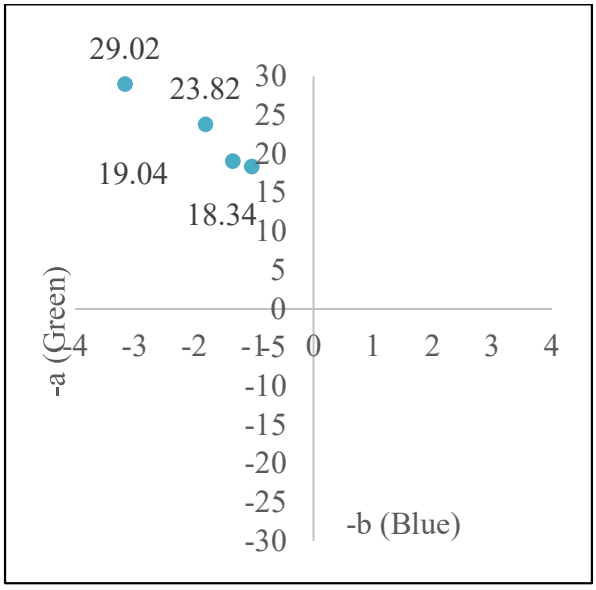


Figure 3. a*- b* plot of samples dyed with *Tulsi* extract.

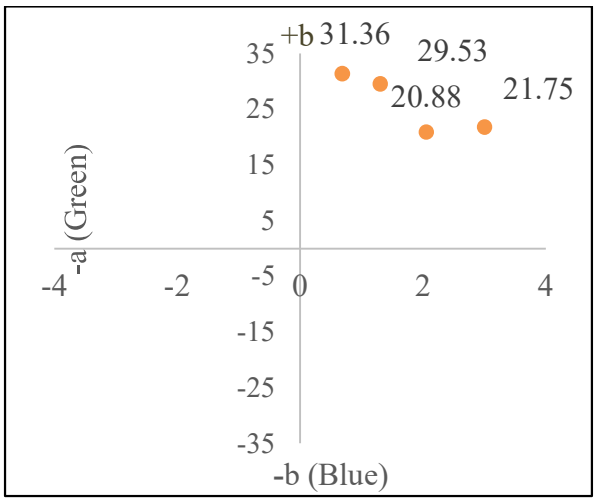


Figure 4. a*- b* plot of samples dyed with *Neem* Extract.

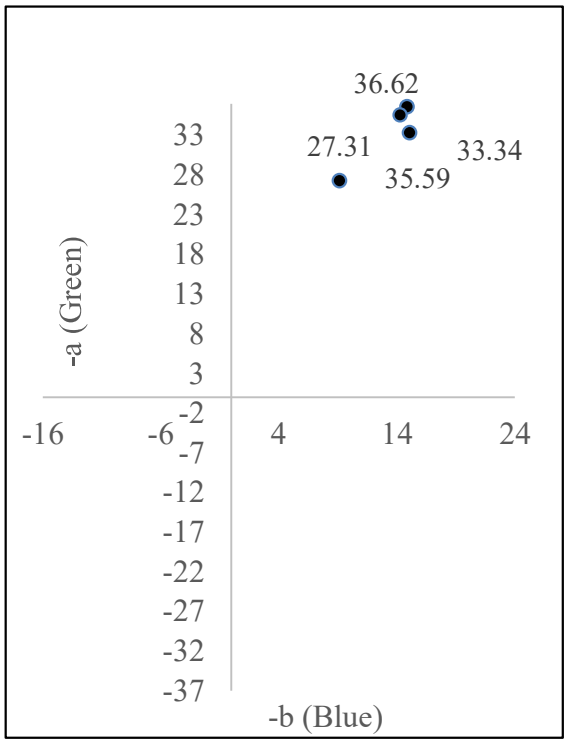


Figure 5. a*- b* plot of samples dyed with *Catechu* extract.

Table 2 shows the colour difference and CMC tolerance of mordanted samples when compared against un-mordanted sample in each dye group. The CMC colour differences (ΔE^*_{CMC})of all mordanted-dyed samples of dye group tulsi, neem, and catechu were found very high when measured against the colour of the un-mordanted samples, except the simultaneous mordanted-dyed fabric sample of catechu which is ΔE^*_{CMC} of less than 1.

3.2. Colour fastness properties

Table 3 shows that cotton fabrics dyed with tulsi, neem, and catechu in the presence of mordant have greater colour-fastness to wash compared to un-mordanted-dyed samples. This can be explained by the formation of metal complex formed through harmonization of metallic particles with definite assemblies of colors such as '2' hydroxyl assemblies, ora hydroxyl assemblies and a carbonyl assemblies in adjacent locations, which causes the binding

Table 2. Colour differences and CMC tolerance of mordanted samples compared against un-mordanted samples.

Source of Natural Dye	Dyed Samples	Colour Differences in D65 10 Deg					
		$\Delta L^*/Ls_L$	Δa*	Δb*	$\Delta C^*/Cs_C$	$\Delta h^*/S_H$	E^*_{CMC}
Tulsi	Un-Mordanted	0	0	0	0	0	0
	Pre-mordanted	-0.63	-1.69	5.87	4.28	1.92	4.74
	Post-mordanted	3.72	0.87	-4.04	-2.87	-1.46	.92
	Simultaneous mordanted-dyed	3.12	0.32	-1.93	-1.39	-.50	.46
Neem	Un-Mordanted	0	0	0	0	0	0
	Pre-mordanted	-0.41	-3.73	10.98	7.26	10.09	2.43
	Post-mordanted	0.46	-0.92	-0.20	-0.40	1.27	40
	Simultaneous mordanted-dyed	-0.92	-3.11	9.48	6.20	8.84	0.83
Catechu	Un-Mordanted	0	0	0	0	0	0
	Pre-mordanted	-0.25	1.39	-4.09	-1.27	-4.19	4.39
	Post-mordanted	4.53	-5.27	-9.63	-5.29	-0.50	.96
	Simultaneous mordanted-dyed	0.30	-0.63	-1.31	-0.70	-0.15	0.78

of dye molecules with the fibre polymer [22]. Among the mordanted ones, pre- and post-mordanted dyed samples are better in terms of wash resistance when compared against the simultaneously mordanted-dyed samples within each dye group.

Table 3 shows that cotton fabrics dyed with tulsi, neem, and catechu in the presence of mordant have greater fastness to rubbing compared to the un-mordanted dyed samples. Pre-mordanted and post-mordanted samples are better in comparison with mordanted samples on both dry and wet rubbing. As for fastness to light, it can be well-known that, the fabric samples dyed with tulsi, neem, and catechu in general, do not show very good light fastness.

Table 3. Color fastness properties.

Natural dye	Dyed samples	Fastness to wash		Fastness to Rubbing		Fastness to light
		Change in color	staining on cotton	Dry	Wet	
Tulsi	Un-Mordanted	3/4	4/5	3/4	3/4	1
	Pre-mordanted	4/5	4/5	4	3/4	2
	Post-mordanted	4/5	4/5	4	3/4	4
	Simultaneous mordanted-dyed	3/4	4	3/4	3	1
Neem	Un-Mordanted	3/4	4/5	4	3/4	2
	Pre-mordanted	4/5	4/5	4/5	3/4	2
	Post-mordanted	4/5	4/5	4/5	3/4	3
	Simultaneous mordanted-dyed	3/4	4	4	3/4	3
Catechu	Un-Mordanted	2/3	1/2	4/5	2/3	*
	Pre-mordanted	3/4	4/5	4/5	1/2	2
	Post-mordanted	4	4	4/5	4	*
	Simultaneous mordanted-dyed	1/2	3/4	4/5	4/5	3

* *Negative effect, fabric became darker after exposure*

All the fastness ratings were found to be under 4 (see Table 3). However, the pre- and post-mordanted-dyed samples of tulsi and neem exhibited higher light fastness ratings than that of un-mordanted samples. This is because the formation of the metal complex decreases the electron mass on the chromophore and improves the confrontation to photo-oxidation [23]. Fabric dyed with catechu without mordant and post mordanting method showed negative result due to photochromic.

3.3. Antibacterial properties

Antibacterial properties of all of dyed cotton fabric samples were tested in contrast to‘3’ gram-positive bacteria: Bacillus cereus

ATCC 11778, Pseudomonas aeruginosa ATCC 27853, Staphylococcus aureus ATCC 25923; and one gram-negative bacterium *Escherichia coli* ATCC 25922 . One undyed piece, of fabric was used as a control in each case. No colored example was initiated to be active in contradiction of bacteria experienced which can be described according to the solubility of phenolic complexes of plant in liquid as stated in available works [17-19,24]. Figure 5 shows the examples of some disc diffusion assays done in this work, where no zone of inhibition was seen. In one hand, the update of these natural dyes into textiles in aqueous medium is below MIC and on the other hand, the chelation of the color by the metallic salt is known for reducing the antimicrobial activity of natural dyes [17, 19].

This work demonstrates that the traditional water-based dyeing method is not effective, even if with a high concentration of dyes, to ensure any anti-bacterial action of cotton textiles dyed with natural Flavonoids, which in their pure form are known to have antibacterial power.

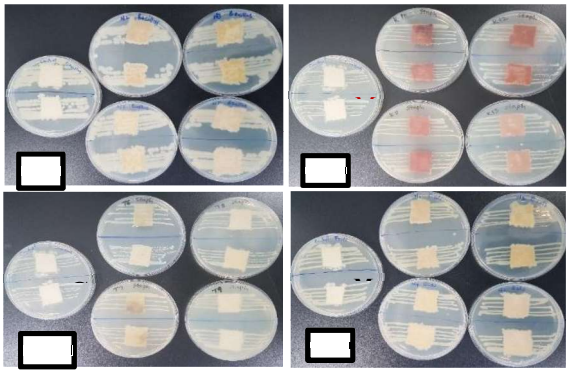


Figure 6. Disc diffusion assay of mordanted-dyed samples against A: *Bacillus cereus* with Neem; B: *Staphylococcus aureus* with Catechu; C: *Staphylococcus aureus* with Tulsi;D: *Escherichia coli* with Neem.

4. Conclusion

Neem (Azadirachta indica) and Tulsi (Ocimum sanctum/ tenuliflorum) are two commonly found medicinal plants and Catechu (*Acacia catechu*) is a commonly used spice in south Asian countries. The paper investigated the effect of mordanting techniques on Colorimetric characteristics, fastness performance, and antimicrobial effectiveness of cotton textiles colored with the extract of Neem, Tulsi, and Catechu. Though these three specific plants are renowned for anti-bacterial properties, color variation resulted for different samples and fabric shade varied with different mordanting techniques, color strength slightly better for catechu and neem after mordanting. Although the mordanting techniques have been found to have some positive effect on the dye strength and performance of cotton fabrics colored with these three natural colors, surprisingly the antibacterial action cannot be achieved on cotton fabric using the traditional water-based. Dyeing method even if high concentration of dyes was used. All of these three plants are known to be proven sources of Flavonoids [7-12]. However, these functional phenolic compounds can be efficiently extracted using an alcoholic (ethanol and methanol) media. In textile dyeing, non-aqueous solvent dyeing is a not-traditional approach to coloration but not unknown and complicated. Therefore, a possible way of achieving anti-bacterial properties onto cotton textiles would be to dye them with Flavonoids in alcoholic medium. Our ongoing research is going to address this issue.

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