

Optimization of the Dyeing Conditions of Natural Dye from African Locust Bean (*Parkia biglobosa*) Pod on Cotton Fabrics

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Abstract

This study examines the dyeing performance, color fastness, surface morphology, and antimicrobial properties of cotton fabrics dyed with natural dye extract, both with and without mordant treatment. The dye extract was applied to treated and untreated cotton fabrics, while CuSO_4 , FeSO_4 , and $\text{KAl}(\text{SO}_4)_2$ were used as mordants to produce different color shades. The optimum dyeing conditions for treated and untreated cotton fabrics were identified at dye concentrations of 2% and 1.5%, temperatures of 90°C and 80°C, and dyeing times of 60 and 50 minutes, respectively. Color fastness to washing and sunlight was evaluated, and the surface morphology of dyed and undyed fabrics was investigated using SEM. The dyed cotton fabrics demonstrated good color fastness, with ratings ranging from 3 to 5 under washing and sunlight exposure. Antimicrobial analysis showed that all dyed fabrics, with and without mordants, produced no inhibition zone against the bacteria *Bacillus subtilis* and *Salmonella typhi*, with values of 0.00 ± 0.00 . However, the dyed fabrics exhibited varying inhibition zones against the

fungus *Penicillium notatum*. Treated cotton dyed with copper sulphate mordant showed the highest inhibition zone of 1.1 ± 0.00 mm, whereas untreated cotton dyed with potassium aluminium sulphate mordant showed the lowest inhibition zone of 0.4 ± 0.00 mm. The study concludes that mordant-assisted dyeing can improve shade variation and maintain acceptable color fastness in cotton fabrics, while the antifungal activity of the dyed fabrics suggests potential functional value for naturally dyed textile applications.

Keywords: Natural Dye; Mordant-Assisted Dyeing; Cotton Fabric; Color Fastness; Antifungal Activity

INTRODUCTION

A colored material with an affinity for the surface it is applied on, is called a dye (Kumar *et al.*, 2021). Natural dyes are substances from plants and animals that impart color to foods, cosmetics, drugs, hair, fiber, fur and polymers. Natural dyes may be obtained from several plant parts like flowers, roots, leaves, bark, insects' secretion and minerals (Nwoye and Ezema, 2017). In light of growing environmental consciousness, the significance of natural dyes has gained global attention. The usage of natural dyes has become more popular due to the negative consequences of synthetic dyes and dyeing agents. This is mostly because many nations have rigorous environmental regulations in place to prevent the health risks linked with synthetic textile dyes (Nwadiokwu *et al.*, 2019). Because natural dye extracts contain strong, highly active agents including tannins, flavonoids, quinines, carotenoids, and alkaloids, the utilization of natural colorants holds promise for creating antimicrobial fabrics for aesthetic, hygienic, and medical uses (Verma *et al.*, 2017).

Nigeria has abundant resources in terms of plants which contain dyes, they are found in leaves, bark, roots, seeds, fruits and flowers (Nwoye and Ezema, 2017). African locust bean also known as *Parkia biglobosa*, has long been acknowledged as a vital native multipurpose fruit tree with a variety of domestic uses, including manure, medicine, food, shade, windbreaks, bee food, stabilizing the environment, fuel, livestock feeds, fiber, fish poison, and many more (Sadiku, 2010).

Andema *et al.*, 2026 studied natural dye from African locust bean pod. It was observed that the dye extract was dark brown, with a pH of 5.77, a melting point of 44 °C,

a density of 0.54 g/mL, partial solubility in water, and complete solubility in methanol. The UV-Vis spectrum showed distinct peaks, with the highest absorbance recorded at 287 nm (4.1000), likely associated with carbonyl (C=O) groups characteristic of hydrolysable tannins. Phytochemical screening revealed the presence of tannins, flavonoids, alkaloids, glycosides, phlobatannins, anthraquinones, quinones, terpenoids, steroids, carbohydrate, starch, proteins, and anthocyanins, while saponin was absent. The dye extract demonstrated antibacterial activity against *B. subtilis*, *E. coli*, *S. aureus*, *P. aeruginosa*, and *S. typhi*, and antifungal activity against *P. notatum*, but no antifungal activity was observed against *C. albicans* and *A. niger* at all concentrations (Andema *et al.*, 2026).

Dass *et al.*, (2023) reported that, for both scoured fabric with and without a mordant, the optimal concentration for dyeing with turmeric root dye extract on cotton fabric is 2.0 g/dm³. However, for scoured fabric with and without mordant, the amount of dye absorbed are 21% and 25%, respectively. The optimal dyeing time is 60 minutes for scoured fabric, both without and with a mordant, when utilizing turmeric root dye extract on cotton fabric. However, during the optimum dyeing time, 30% and 33% of the dye are absorbed by scoured fabric with and without mordant, respectively. The optimal dyeing temperature is 90°C for scoured fabric, both with and without a mordant, when utilizing turmeric root dye extract on cotton fabric. At optimum dyeing temperatures, scoured cotton absorbs 48% and 53% of the dye, respectively, with and without a mordant.

The aim of this research is to extract dye from African locust bean pod, optimize the dyeing conditions on the cotton fabric, apply the dye with and without mordants, carry out color fastness test and antimicrobial study of the dyed cotton fabrics.



Plate 1: Dried Pod of the African Locust Bean (*Parkia biglobosa*)

METHODOLOGY

Extraction and Purification of the Dye

A container containing 200 g of the powdered stuff was filled with 500 ml of methanol. For efficient extraction, the mixture was left to stand for a week. Following that, it was filtered, the filtrate was dried off, and the solute was gathered and weighed (Leonard *et al.*, 2022).

Scouring of the Cotton Fabrics

3M sodium hydroxide was used during the scouring procedure. For every cotton fabric, the scouring procedure was conducted for one hour at 60°C using 50 mls of the sodium hydroxide (Otutu *et al.*, 2012).

Bleaching of the Cotton Fabrics

Using a measuring cylinder, 3M of sodium hypochlorite was prepared. The bleaching process of every fabric was then carried out for an hour at 100°C using 100 ml of the prepared solution (D'Cruz, 2020).

Pre-mordanting, Meta and Post Mordanting

In order to perform the mordanting, 3% of the mordant was prepared. After being scoured, the cloth was submerged in 50 ml of the mordant solution for approximately half an hour at 60 °C. It was then removed and manually squeezed before being submerged in the dye bath (Khin and Yee, 2017).

Optimization of dyeing concentration

Several dye concentrations (0.5%, 1.0%, 1.5%, 2.0%, and 2.5%) were made in order to find the optimum dyeing concentration. A constant temperature and time were used to dye the cotton fabric. At the optimum wavelength, the absorbance of dye solutions was measured both before and after dyeing (Mahilrajan *et al.*, 2014).

Optimization of dyeing time

Dyeing was done for 20, 30, 40, 50, 60, and 60 minutes at the constant concentration and temperature in order to maximize dying time. At the optimum wavelength, the absorbance of dye solutions was measured both before and after dyeing. The optimum time for dyeing was determined when the most dye was absorbed (Geetam *et al.*, 2017).

Optimization of dyeing temperature

The dyeing process was run at 50⁰C, 60⁰C, 70⁰C, 80⁰C, 90⁰C, and 100⁰C to maximize the dyeing temperature at constant time and concentration. At the optimum wavelength, the absorbance of dye solutions was measured both before and after dyeing. The optimum dyeing temperature was determined to be the one at which the most dye could be absorbed (Onyesm, 2017).

Dyeing Process of the Fabrics

Using a fixed amount of liquor ratio (1:100), the fabrics was added to the dye solution in the bath and dyed at optimum concentrations, temperatures, times and pH values (10-11 for cotton). To ensure that the dye molecules are well absorbed into the fabric, the fabric was agitated throughout this process. After dyeing, the dyed sample was carefully rinsed with cold water to remove the dye particles that are not absorbed and squeezed by hand then dried at room temperature and stored dried for further analysis (Wanyama *et al.*, 2011).

Color Fastness Test of the Dyed Fabrics

Fastness to washing

All of the dyed fabrics were cleaned and rinsed with water after soaking in laundry soap for an hour. And the fabrics was allowed to air dry at room temperature (Ezeokonkwo *et al.*, 2018).

Fastness to sunlight

For two days, the dyed materials were exposed to sunlight for ten hours every day (Ezeokonkwo *et al.*, 2018).

Antimicrobial Activity of the Dyed Fabrics

Determination of Zone of Inhibition

The dye extract's antimicrobial properties were tested using the hole-in-plate disc diffusion technique. After boring wells in the media with a 6 mm cork borer, 0.2 mL aliquots of the extracts at various concentrations were put within. The agar plate was then incubated at 37 °C for 24 hours. Following incubation, a transparent meter rule was used to measure the diameters of each extract's zone of inhibition in millimeters. Every extract was examined three times (Usman *et al.*, 2018).

Scanning Electron Microscope Analysis of the Dye and Dyed Fabrics

A Quanta 250, FEI, Netherlands Scanning Electron Microscope was used to examine the dimensions and form of sample at various stages. The sample was placed on aluminum stubs using conductive carbon tape, and then it was sputtered with gold using a plasma sputtering equipment under vacuum at 20mA for two minutes. The sample was then inspected and captured on cameras (Cheran *et al.*, 2022).

RESULTS AND DISCUSSION

Extraction and Purification of the Dye Extract

Table 1 showed the extraction of dye from African locust bean (*Parkia biglobosa*) pod via maceration method of extraction. 200 g each of the sample was use to carry out this extraction. The yield of the dye extract is 47.52g and percentage yield is 23.76%. Leonard *et al.*, (2022) reported that the percentage yield of 6.3% (12.93 yield) from the ethanol dye extract of *parkia biglobosa* tree bark after a week extraction.

Table1: Extraction and Purification of the Dye Extract

Locust Bean Pod Powder (g)	Weight after Extraction and Purification (g)	Actual yield (g)	Percentage Yield (%)
200	152.48	47.52	23.76

Scouring of the Cotton fabrics

Table 2 shows the results of scouring the cotton fabrics. It was observed that, the fabrics lost weight after scouring. This may be due to removal of wax and other impurities as reported by Joshua *et al.*, (2023).

Table 2: Scouring of the Cotton Fabrics

Weight of fabric before scouring (g)	Weight of fabric after scouring (g)	Weight Loss (g)
0.475	0.454	0.021

Bleaching of the Cotton fabrics

Table 3 shows the results of bleaching the cotton fabrics. It was observed that, the fabrics lost weight after bleaching. This also may likely be due to removal of wax and other impurities which causes the fabric to become whiter and brighter as reported by Joshua *et al.*, (2023).

Table 3: Bleaching of the Cotton Fabrics

Weight of fabric before bleaching (g)	Weight of fabric after bleaching (g)	Weight Loss (g)
0.454	0.449	0.005

Optimization of the Dyeing Concentration

The results for the optimization of the dyeing concentration of treated and untreated cotton fabrics is presented in Figure 1. The optimum concentration of treated cotton fabric was 1.5% and the amount of dye absorbed was 30.30%. While the optimum concentration of the untreated cotton was 2.0% and the amount of dye absorbed was 26.35%. It was observed from the graph that as the concentration increases, the amount of dye absorbed also increased. However, after 1.5% concentration, the amount of dye absorbed by the treated cotton begins to decrease. While after 2% concentration, the amount of dye absorbed by the untreated cotton also decreases. Dass *et al.*, (2023) similarly reported that, as the concentration of dye increased, more dye was absorbed until the cotton fabric reached equilibrium.

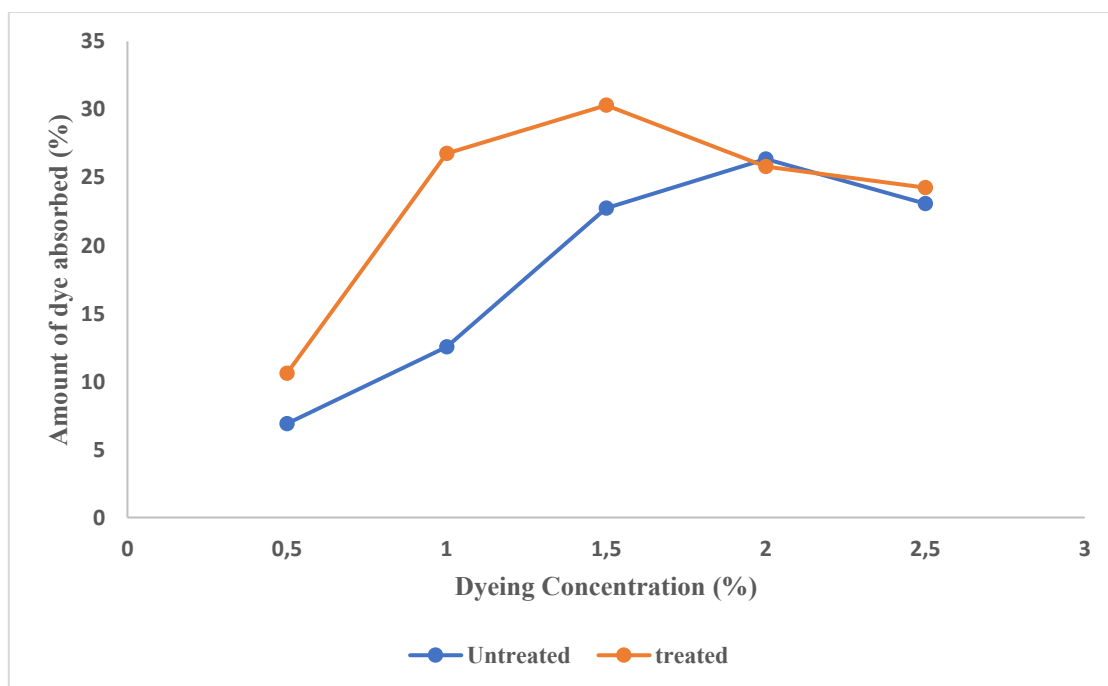


Figure 1: Optimization of the Dyeing Concentration of Treated and Untreated Cotton Fabric

Optimization of the Dyeing Time

The results for the optimization of the dyeing time of treated and untreated cotton fabrics is presented in Figure 2. The optimum dyeing time of treated cotton fabric was 50

minutes and the amount of dye absorbed was 20%. While the optimum dyeing time of the untreated cotton was 60 minutes and the amount of dye absorbed was 18.24%. It was observed from the graph that as the dyeing time increases, the amount of dye absorbed increases too. However, after 50 minutes, the amount of dye absorbed by the treated cotton begins to decrease. While after 60 minutes, the amount of dye absorbed by the untreated cotton also decreases. According to Wanyama *et al.*, (2014), the amount of dye absorbed increases as dying time increases. Higher absorbance is produced by longer dyeing times until dye exhaustion reaches equilibrium.

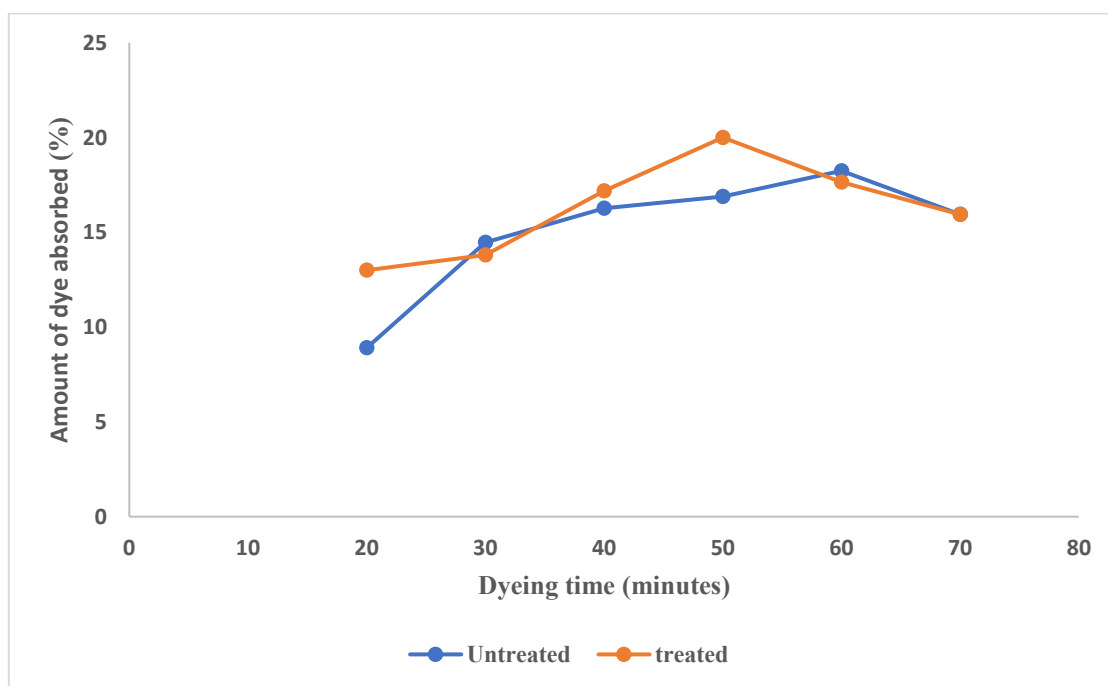


Figure 2: Optimization of the Dyeing Time of Treated and Untreated Cotton Fabric

Optimization of the Dyeing Temperature

The results for the optimization of the dyeing temperature of treated and untreated cotton fabrics is presented in Figure 3. The optimum dyeing temperature of treated cotton fabric was 80°C and the amount of dye absorbed was 19.11%. While the optimum dyeing temperature of the untreated cotton was 90°C and the amount of dye absorbed was 18.56%. It was observed from the result that as the dyeing temperature increases, the amount of dye absorbed also increases. However, after 80°C, the amount of dye absorbed by the treated cotton begins to decrease. While after 90°C, the amount of dye absorbed by the untreated cotton also decreases. According to Wanyama *et al.*, (2014), the amount of

dye absorbed increases as the dyeing temperature rises. Higher absorbance is produced by longer dyeing temperatures until equilibrium is reached by the dye.

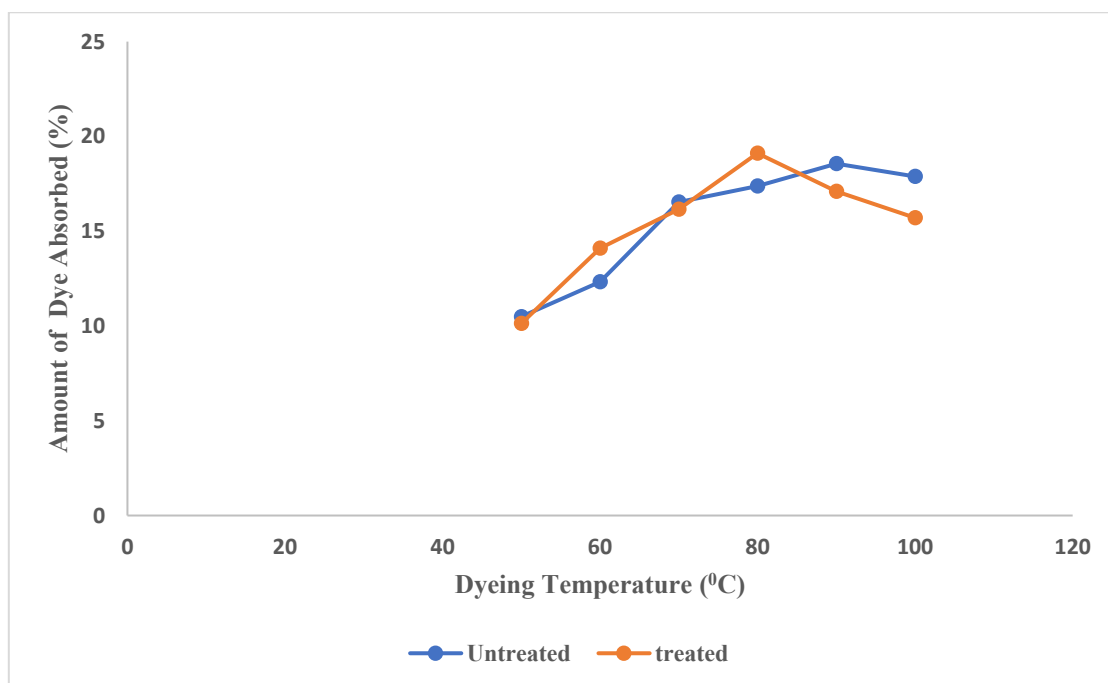


Figure 3: Optimization of the Dyeing Temperature of Treated and Untreated Cotton Fabric

It was observed that the treated cotton fabrics absorbed more dye than the untreated cotton fabrics at optimum concentration, time and temperature. This may likely be due to, the effects of fiber swelling that improve dye diffusion. Secondly, it can be explained by the relationship between treated cotton fibers and dye structure (Mansour and Ali, 2019).

Dyeing of the Cotton fabric with and without mordant

The results of dyeing treated and untreated cotton are presented in Plate 2 to 9. It was observed that all the treated and untreated cotton fabrics dyed with and without mordants, showed varying degree of shades. The treated and untreated fabrics dyed with mordants using copper sulphate, ferrous sulphate and aluminum sulphate produced darker shades than those without mordants. This may likely be as a result of the interaction between the dye's chromophore and the various metals in the mordants, which may be responsible for these different shades, which also promote better dye absorption and retention on the fabric fibers (Islam *et al.*, 2024). According to Kulkarni *et al.*, (2011), both copper sulfate and ferrous sulfate can form coordination complexes; their respective coordination numbers are 4 and 6. When interacting with the fiber, functional groups like

amino and carboxylic acids can occupy the vacant positions. As a result, the metal salt forms a ternary complex with one site containing the fiber and the other containing the dye. Islam *et al.*, (2024) reported that when mahogany sawdust dye was applied to cotton, dyed samples had an overall increase in color due to the use of mordants throughout the dyeing process. Coordination bonds between cellulose and metal ions and their functional groups are possible. As a result, the metallic salts enhanced the fastness characteristics and dye uptake capacity. Locust bean pod dye is naturally a brown color. However, when reacted with CuSO_4 the color changes to bronze brown, FeSO_4 changes the color to blue black or black while $\text{KAl}(\text{SO}_4)_2$ changes the color to tan or pale brown. The treated cotton fabrics produced deeper and brighter shades than the untreated cotton fabrics because they are likely to absorb more dye than the untreated ones. According to Mansour and Ali (2019), treated cotton dyed with *Hibiscus sabdariffa* shown superior dye absorption compared to cotton that were not treated with chitosan.

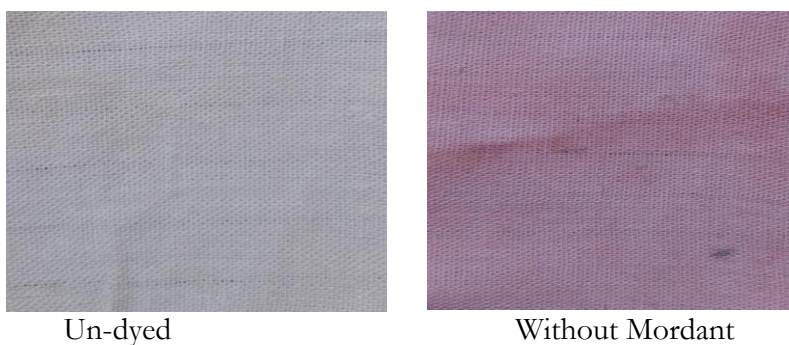


Plate 2: Treated Cotton without mordant and un-dyed Fabrics

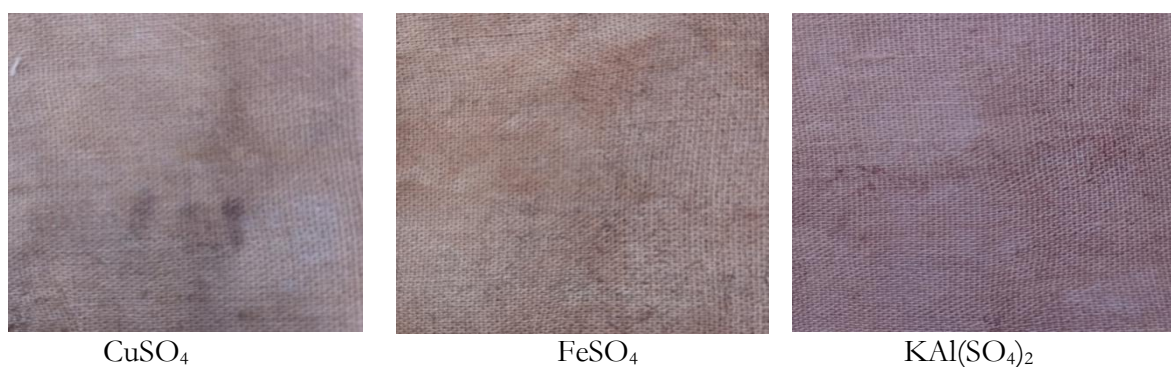


Plate 3: Pre-mordanted Treated Cotton Fabrics

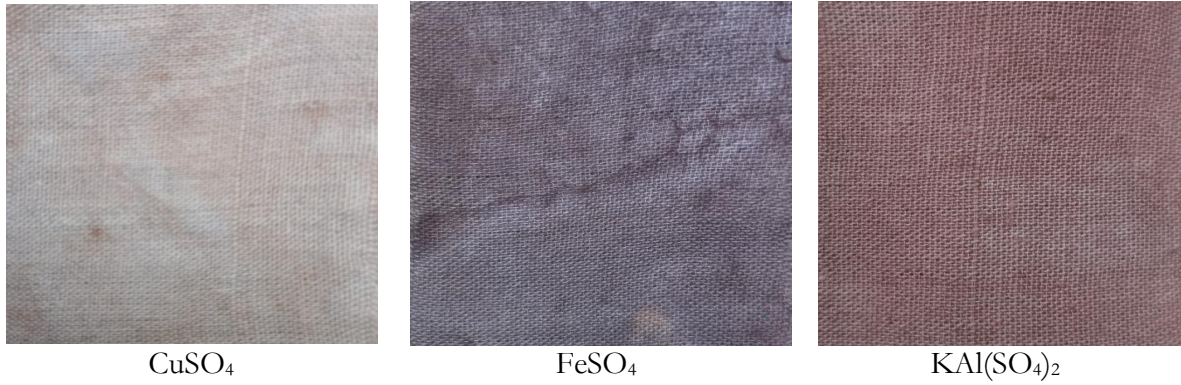


Plate 4: Meta-mordanted Treated Cotton Fabrics



Plate 5: Post-mordanted Treated Cotton Fabrics



Plate 6: Untreated Cotton without mordant and un-dyed Fabrics

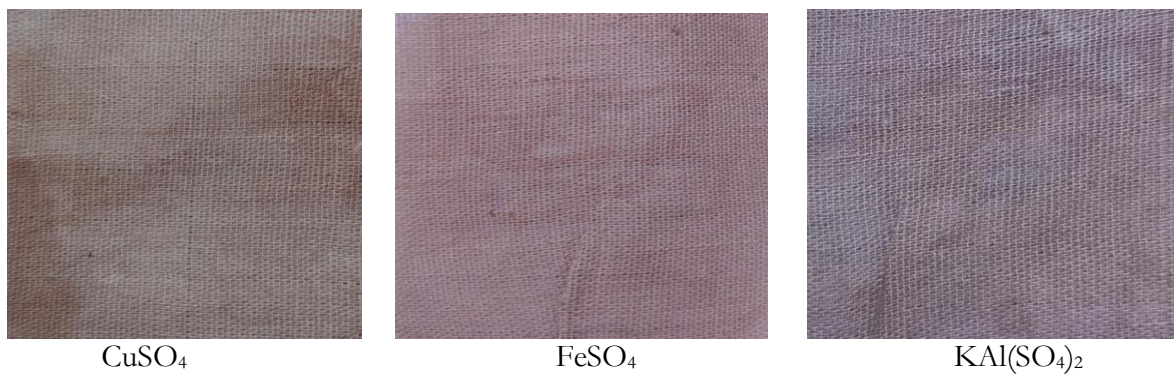


Plate 7: Pre-mordanted Untreated Cotton Fabrics

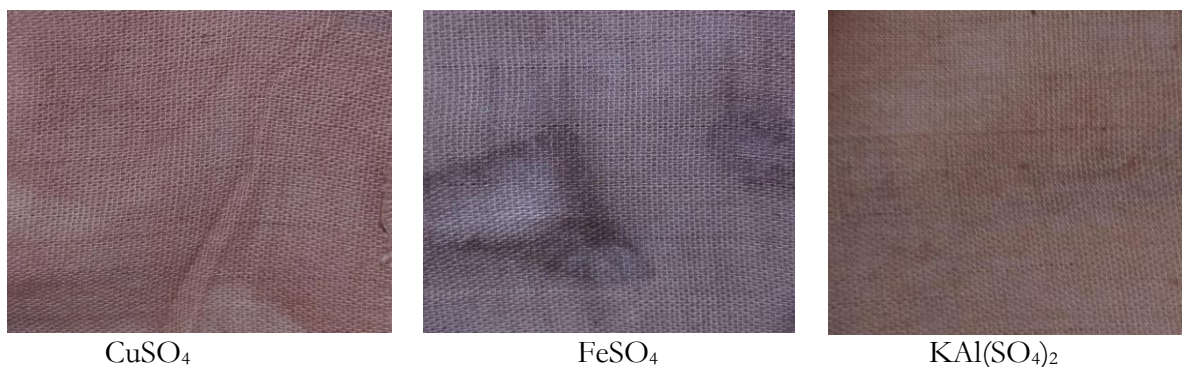


Plate 8: Meta-mordanted Untreated Cotton Fabrics

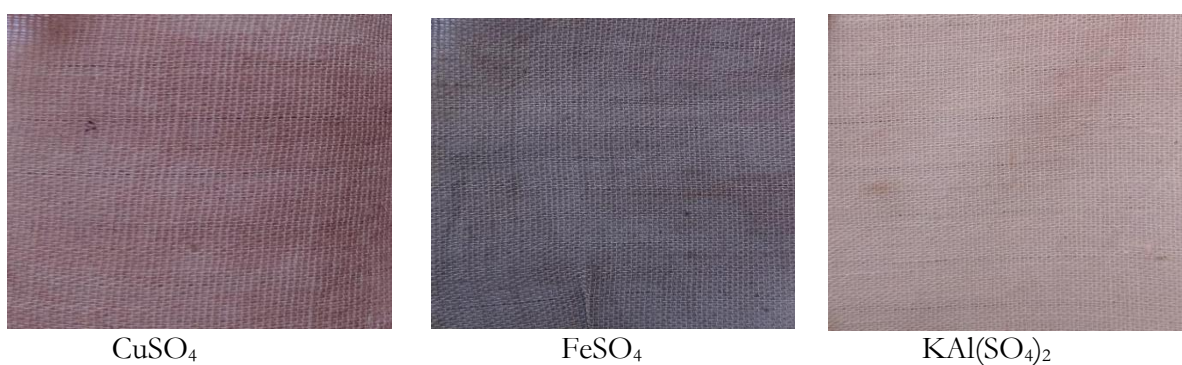


Plate 9: Post-mordanted Untreated Cotton Fabrics

Color fastness test of the dyed fabrics

Sunlight Test

The results of the sunlight test of the treated and untreated cotton dyed with extract from the locust bean pod is presented in Table 4. It was observed that the treated cotton fabrics dyed with mordants showed excellent colorfastness (4-5) than the control (without mordant) which is good. Similarly, the untreated cotton fabrics with mordants showed good colorfastness (3-4) than the control which is fair. The interaction between the dye, mordant, and fiber is responsible for this enhancement, which could lead to improved color stability and retention when exposed to light (Islam *et al.*, 2024). This could also be because a complex with a transitional metal is likely to form, protecting the chromophore from photolytic degradation. The chromophoric group absorbs photons, which then dissipate their energy by resonating within the six-member ring, creating and protecting the dye and enhancing its light fastness. The cause may be the stable configuration of electrons, which is resistant to photodegradation by UV light (Dilshad and Sannapapamma, 2018). It

was also observed all the pre-mordanted and meta-mordanted treated and untreated fabrics showed the best colorfastness results (4-5) than the post-mordanted (3, 4). Interestingly, copper sulphate showed excellent results (4-5). Kanchana *et al.*, (2013) also reported that cotton dyed with marigold using copper CuSO_4 showed excellent light fastness. Abdu and Yusuf, 2015 also reported that during the application of onion skin dye on cotton fabrics, the pre-mordanting using copper sulphate yield the best colour fastness.

Table 4: Sunlight test of Treated and Untreated Cotton

Mordant	Pre		Meta		Post	
	Treated	Untreated	Treated	Untreated	Treated	Untreated
CuSO_4	4-5	4	4-5	3	4	3
FeSO_4	4	4	4-5	3-4	4	3
$\text{KAl}(\text{SO}_4)_2$	4-5	4	4	3-4	4	3
Without (Control)	3-4	2-3	-	-	-	-

Ratings:1=very poor, 1-2= poor, 2= slightly fair, 2-3= fair, 3= moderate, 3-4= good, 4= very good, 4-5= excellent, and 5= outstanding.

Islam *et al.*, 2024 also reported that, cotton treated with mahogany sawdust dye without mordant got a somewhat fair rating (2), indicating a visible fading or color change when exposed to light. The remaining samples, on the other hand, showed a rating of 3, indicating a slight improvement in light fastness. Kanchana *et al.*, (2013) reported that cotton fabrics treated with marigold flower dye extract exhibits good light fastness. This is because the chromatophore is shielded from photolytic destruction by the complex that forms with the metal. The light fastness of dyes appears to be significantly influenced by their pattern of substitution. A particular substituent can either enhance the electron density at the molecule's reaction site, which may promote oxidation, or decrease it, which would result in an increase in the case of reduction. It is clear that the two hydroxyl groups in marigold flowers offer good fastness. The treated cotton fabrics showed excellent color fastness in the presence of sunlight than the untreated cotton fabrics. Finally, it was observed that the dyed cotton fabrics exhibit good colorfastness in sunlight which suggests that, the locust bean pod dye works better on cotton fabrics.

Washing Test

The results of the washing test of the treated and untreated cotton dyed with extract from the locust bean pod is presented in Table 5. It was observed that the treated cotton fabrics dyed with mordants showed good colorfastness (4) during the washing than

the control (without mordant) which is moderate (3). Similarly, the untreated cotton fabrics with mordants showed moderate colorfastness (3) than the control which is fair (2-3). The presence of metals in the mordants may likely affect the interaction with the dye-fiber during washing, which explains the rise in the rate of color change in the mordanted fabrics (Islam *et al.*, 2024). It was also observed all the pre-mordanted and post-mordanted treated fabrics showed the best colorfastness results (4) than the meta-mordanted (3). While all the mordanted untreated fabrics showed moderate color change (3).

Table 5: Washing test of Treated and Untreated Cotton

Mordant	Pre		Meta		Post	
	Treated	Untreated	Treated	Untreated	Treated	Untreated
CuSO ₄	4	3-4	3	3	4	3
FeSO ₄	4	3-4	3-4	3	3-4	3
KAl(SO ₄) ₂	4	3	3	3-4	4	3
Without (Control)	2	2-3	-	-	-	-

Ratings:1= very poor, 1-2= poor, 2= slightly fair, 2-3= fair, 3= moderate, 3-4= good, 4= very good, 4-5= excellent, and 5= outstanding

Islam *et al.*, 2024 also reported that, the untreated sample had a color change rating of 3, suggesting sufficient wash fastness, when mahogany sawdust dye was applied to cotton without mordant. In contrast, color change ratings ranged from 3 to 4 for all mordanted samples. The marigold dye has an exceptional rate of washing fastness. When a dye complexes with a mordant, it becomes insoluble and changes color quickly. A certain substituent may raise the electron density surrounding the molecule's reaction site, promoting oxidation, or it may decrease the electron density, leading to an increase in the case of reduction (Kanchana *et al.*, 2013). It was observed that, the treated cotton fabrics exhibit excellent color fastness during washing than the untreated cotton fabrics. Finally, it was observed that all the dyed cotton fabrics showed good colorfastness.

Antimicrobial Study of the Dyed Fabrics

The result of the antimicrobial study of the dyed fabrics is presented in Table 6. From the results, it was observed that all the bacteria (*B. subtilis*, *S. typhi*) showed no zone of inhibition against all the fabrics dyed with and without mordants. This may likely be due to resistance developed by the bacteria against the antimicrobial agent. This ability of microorganisms such as bacteria to develop resistance to antimicrobial agent is called antimicrobial resistance (AMR) This resistance means that the antimicrobial agent is no

longer as effective or entirely ineffective in killing or inhibiting the growth of the microorganisms (Mahizhchi *et al.*, 2024).

Table 6: Zone of Inhibition (mm) of Dyed fabrics

S/N	Fabrics	Mordants	B. subtilis	S.typhi	P.notatum
1.	Treated cotton	CuSO ₄	00.00±0.00 ^a	00.00±0.00 ^a	1.1±0.00 ^a
		FeSO ₄	00.00±0.00 ^a	00.00±0.00 ^a	1.0±0.00 ^a
		KAl(SO ₄) ₂	00.00±0.00 ^a	00.00±0.00 ^a	1.0±0.00 ^a
		Without	00.00±0.00 ^a	00.00±0.00 ^a	1.0±0.00 ^a
2.	Untreated cotton	CuSO ₄	00.00±0.00 ^a	00.00±0.00 ^a	0.8±0.00 ^b
		FeSO ₄	00.00±0.00 ^a	00.00±0.00 ^a	0.5±0.00 ^b
		KAl(SO ₄) ₂	00.00±0.00 ^a	00.00±0.00 ^a	0.4±0.00 ^b
		Without	00.00±0.00 ^a	00.00±0.00 ^a	0.9±0.00 ^b

Values of mean across columns with different alphabetical superscripts are statistically different ($p < 0.05$) $n=3$

It was also observed that all the dyed fabrics inhibited the growth of *P.notatum*. This confirms the antifungal activity of the locust bean pod dye extract. The treated cotton dyed with copper sulphate mordant showed the highest zone of inhibition of 1.1 ± 0.00^a mm while the untreated cotton dyed with potassium aluminum sulphate mordant showed the lowest zone of inhibition of 0.4 ± 0.00^b mm. Interestingly, all the fabrics dyed with copper sulphate mordant showed high zone of inhibitions, this may be primarily because copper ions (Cu^{2+}) ions act as a potent non-specific fungicide that disrupt essential cellular functions, leading to a metabolic failure and death. It is known that copper sulfate damages the cell wall of *penicillium* species, preventing their growth. Copper is a multisite fungicide that mostly inhibits central catabolic and metabolic processes by displacing iron atoms in iron-sulfur clusters of dehydratases and targeting thiolate groups (Farahmand *et al.*, 2023). Also, the treated cotton fabrics showed high zones of inhibition than the untreated. This may likely be as a result of the more dye absorbed by the treated fabrics than the untreated fabrics. Musinguzi *et al.* (2019), reported that the cotton fabrics dyed with FeSO₄ exhibited the highest antibacterial activity on *S. aureus* and *P. aeruginosa*. This is accurate since different mordants employed under appropriate conditions result in varying dye fixation levels, which in turn generate variable inhibition zones.

CONCLUSION

This research reveals the optimum dyeing temperature, concentration and time of the African locust bean pod dye extract on cotton fabrics. The dyed cotton fabrics produced different shades and also exhibit good color fastness during washing and on exposure to sunlight. The dyed cotton fabrics possesses notable antimicrobial activity. The findings of this research demonstrate that dye extract from African locust bean *Parkia biglobosa* pod is an effective natural dye for cotton fabric.

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