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Optimization of dyeing process parameters for bioactive cotton and silk fabrics with *Racinus communis* leaf extract

A Musinguzi[1, 2,a], I J Mwasiagi[1], I Nibikora[2] & C Nzila[1]

[1]Department of Manufacturing, Industrial and Textile Engineering, Moi University, P.O. Box 3900-30100, Eldoret, Kenya

[2]Department of Polymer, Textile and Industrialisation Engineering, Busitema University, P.O. Box 236, Tororo, Uganda

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Woven cotton and silk fabric samples have been dyed with *Racinus communis* extract at optimized conditions of extract concentration, temperature, material - to - liquor ratio (MLR), alum concentration and treatment time, and then evaluated for their fastness properties following ISO standards, antimicrobial activity quantitatively against *Staphylococcus aureus* and *Pseudomonas aeruginosa* bacterial strains and wash durability. Central composite design and single-factor design have been used in the optimization process, and based on the lowest number of colony-forming units per milliliter, optimized values are selected. The results indicate that optimized extract concentration of 39%, dyeing temperature of 70ºC, alum concentration of 3g/L, MLR of 1:30 (silk) and 1:40 (cotton), and treatment time of 30min (silk) and 40min (cotton) lead to 99.84% and 99.78% bacterial count reduction against *Staphylococcus aureus* and *Pseudomonas aeruginosa* respectively for dyed cotton fabrics. However, for dyed silk fabrics, a more improved percentage reduction in the bacterial count of 99.88% and 99.83% respectively is realized against both bacterial strains. Retention of the antimicrobial activity of dyed fabrics is found to be more significant even after 5 washes. The fastness properties of rubbing, washing, light, and perspiration for both fabrics range from moderate to excellent.

Keywords: Bioactive fabrics, Central composite design, Cotton, Dyeing process, Fastness properties, *Racinus communis* extract, Silk

1 Introduction

Natural dyes have been a section of human life that acted as a source of color and pigment since prehistoric times until when the first synthetic dye was invented. The introduced synthetic dyes were found to have improved antimicrobial and anti-fungal activities, and reliable shades which are durable and sustainable [1]. However, the side effects reported for synthetic dyes are detrimental to the environment, cause allergy effects to human skin, and diseases [2,3]. In response to this, more interest has been focused on using natural dyes for various applications, among which it includes imparting antimicrobial and infection-resistant properties onto textile materials since they are highly susceptible to microbial attacks. This has been facilitated by active agents found in these natural dyes being non-toxic, non-allergic, and eco-friendly, thus meeting the consumer requirements in regards to hygiene, comfort, and sustainability in general [4]. Natural dyes are not only capable of imparting several functions to textile materials, but can as well be good coloring agents when applied with a mordant since they are adjective [5]. Many plant extracts have already proved to be possessing several medicinal values as well as capable of imparting different color shades depending on mordants used [6,7]. *Racinus communis* is one of the castor oil plants which is grown annually in the Euphorbiaceae family. It is also known for being a softwood tree found in the tropics and temperate regions. The plant leaves have long petiole and palm-like lobed blades, the flowers are categorized as male or female depending on their arrangement at the top of the axis in panicles form. The fruits of the plant are in a three-chambered format, globose capsule with soft spines such that after the capsules mature, they split open into three cavities, thus expelling out the seeds [8]. The presence of the amino acids, fatty acids, flavonoids, phenolic compounds, phytosterol, terpenoids, alkaloids, saponins, tannins, insecticidal, ovicidal among others prevents it from being attacked by microorganisms [9]. Traditionally, *Racinus communis* plant extract is used to treat illnesses like abdominal disorders, aching feet, arthritis, backache, muscle aches, bilharziasis, boils, sores, swellings, chronic headaches, constipation, the expulsion of the placenta, gallbladder pain, and muscular distortion [8]. This present study, therefore,

[a]Corresponding author.
E-mail: musinguzialexb1@gmail.com

was focused on optimizing different dyeing process parameters to achieve the highest antimicrobial activity and fastness properties on dyed cotton and silk fabrics using *Racinus communis* leaf extract.

2 Materials and Methods

2.1 Materials

A hundred per cent pre-treated plain woven cotton fabric (GSM 126, EPI 54, PPI 27, warp count Nm 34/1 and weft count Nm 34/1) and silk fabric (GSM 83, EPI 39, PPI 36, warp count Nm 22/1 and weft count Nm 22/1) were bought from Rivatex East African Limited, Eldoret-Kenya, and Kawanda Silk Research Center, Kampala-Uganda respectively. Mature leaves of the *Racinus communis* plant were sourced from the wild in Biharwe, Mbarara District – Uganda. From INDO Kenya Enterprises, Eldoret-Kenya, other preparation requirements like sodium hydroxide, distilled water, pestal, and a motor, digital measuring balance, and Whatman No. 1 filter papers all were purchased.

2.2 Methods

2.2.1 Extraction of Medicinal Dyes

The mature plant leaves of *Racinus communis* were grounded into a coarse powder, weighed, and subjected to an aqueous extraction process, keeping the material-to- liquor ratio at 15:250 (w/v) [10]. The extract was filtered and stored at 4ºC in an airtight container after 8 h on a shaker.

2.2.2 Microorganisms and Culture Condition

Following the standard operating procedures, the ATCC, viz *Staphylococcus aureus* and *Pseudomonas aeruginosa* pathogenic bacterial strains were recovered from the storage media and maintained on Muller Hinton (MH) medium.

2.2.3 Optimization of Dyeing Conditions

After preparation of the extract, dyeing parameters, such as extract concentration (% owf), dyeing temperature (ºC), material-to-liquor ratio (MLR), alum concentration (g/L), and treatment time (min), were subjected to the optimization process, whereby central composite design (CCD) was used on extract concentration and dyeing temperature with their selected experimental variables ranging from 11% to 39% (owf) and 56ºC to 84ºC respectively, while maintaining other factors constant as further explained in sub-section 2.2.4. Using a single factor design, the optimization of MLR was done for 1:10, 1:20, 1:30, 1:40 and 1:50 at 39% (owf) extract concentration, 3g/L alum concentration, 70ºC temperature, and 40 min treatment time. The alum concentration was tested for 1, 3, and 5g/L at 39% (owf) extract conc., 1:30 M: L ratio, 70ºC temperature and 40 min treatment time. Then optimum treatment time was studied using 20, 30 40 min, whereby other factors such as 39% extract concentration, 3g/L alum concentration, 1:30 M:L ratio, and 70ºC temperature were kept constant. Thereafter, the dyed fabrics using all the different parameters were tested for bacterial resistance, and based on the lowest number of colony-forming units observed against selected bacterial strains, the optimum conditions were selected.

2.2.4 Central Composite Design

On the basis of the demonstrated bacterial resistance properties by dyed cotton and silk fabric samples, extract concentration and dyeing temperature were optimized using central composite design (CCD) of response surface methodology[11]. The experimental variables were, extract concentration and dyeing temperature. Their coded and actual levels are given in Table 1.

The obtained bacterial count values from testing dyed cotton and silk fabric samples against selected bacterial strains were considered as a response. Then Design Expert 7.0 software package was used in the design and analysis of experiments [12].

2.2.5 Statistical Analysis

With the help of ANOVA, the statistical significance of the regression coefficients and adequacy of the developed model were checked. Response surface plots were drawn to analyze the interaction among the different independent process factors and their effect on the bacterial count.

2.2.6 Dyeing of Fabrics with Optimized Conditions

Considering conventional dyeing technique [13], dyeing was done using optimized dyeing parameters, such as 39% (owf) extract concentration, 70ºC dyeing temperature, 3g/L alum concentration, 1:20 MLR for silk, 1:30 for cotton fabrics, and 30min treatment time for silk, and 40min for cotton. Then the dyed fabric samples were removed, rinsed to remove excess dyes, and dried at 37ºC.

Table 1 — Experimental variables and their levels

Variables	Levels				
	-alpha	-1	0	+1	+alpha
Conc., %	10.86	15	25	35	39.14
Temp., ^{0}C	55.86	60	70	80	84.4

2.2.7 Assessment of Antibacterial Resistance, Wash Durability and Fastness Properties of Dyed Fabrics with Optimized Values

The dyed and undyed (control) cotton and silk fabrics were assessed for their antimicrobial properties against *S.aureus* and *P.aeruginosa* bacterial strains following the AATCC Test Method-100:2019. With respect to untreated control samples, microbial inhibition was calculated as a percentage reduction in the number of colony forming units (CFU) using the following formula;

$$R = [(B-A)/B]*100$$

where R is the percentage reduction in microbial colonies; *A*, the CFU/mL for the treated fabric samples after 24h incubation; and *B*, the CFU/mL for the untreated fabric samples after 24h incubation under the same conditions.

For wash durability, the dyed cotton and silk fabrics were investigated after one and five washes following the AATCC-100 test method [5] and thereafter analyzed for fastness properties following the ISO standard methods, such as ISO 105-X12:2016, ISO 105-E04:2013, ISO 105-B02:2014, and ISO 105-C10:2006 for rubbing, perspiration, light, and wash fastness respectively.

3 Results and Discussion

3.1 Effect of Extract Concentration and Dyeing Temperature on Bacterial Count

3.1.1 Central Composite Design of RSM

Table 2 illustrates the bacterial count values of cotton and silk dyed fabric samples with *Racinus communis* dye extract at varying dyeing conditions. As the extract concentration and dyeing temperature increase, bacterial count reduces, thus providing better antimicrobial resistance of the dyed fabrics. However, the bacterial count reduction is majorly a function of the bacterial strain used as well as the extract concentration applied. This may be due to the difference in the bacterial structure compositions [14].

3.1.2 Analysis of Variance (ANOVA) Study

To statistically analyze the results, analysis of variance (ANOVA) has been performed and results are illustrated in Table 3 for bacterial count values of dyed cotton and silk fabrics respectively against *Staphylococcus aureus* (*S. aureus*) and *Pseudomonas aeruginosa* (*P. aeruginosa*) bacterial strains. It can be seen that for cotton and silk fabrics, both models (‘Y_{cs}’ & ‘Y_{cp}’) and (‘Y_{ss}’ & ‘Y_{sp}’) are significant having p-values = 0.000. The extract concentration effect on bacterial count is also significant with a p-value of 0.000 on all the models, thus confirming that a change in extract concentration has a significant effect on dyeing cotton and silk fabrics for microbial resistance, while dyeing temperature has a minor influence.

The second-order regression equations are derived to formulate the relationship between bacterial count (Response ‘Y_{cs}’ & ‘Y_{cp}’) and (Response ‘Y_{ss}’ & ‘Y_{sp}’) and dyeing parameters, such as extract concentration (A) and temperature (B) for cotton and silk fabric samples. For cotton fabric samples, R^2 and adjusted R^2 are 99.06% and 98.40% respectively for the Y_{cs} model, and 96.73% and 94.56% respectively for the Y_{cp} model. This clearly shows that 99.06% and

Table 2 — Bacterial count at varying dyeing parameters

Factors		Response: Bacterial count, CFU/mL			
		Cotton		Silk	
Extract conc, %	Temp., ^{0}C	*S.aureus*	*P. Aeruginosa*	*S.aureus*	*P. Aeruginosa*
25	70	7.84×10^4	1.70×10^5	2.48×10^4	8.72×10^4
25	70	7.68×10^4	1.72×10^5	2.56×10^4	8.88×10^4
15	60	2.76×10^5	3.32×10^5	4.32×10^4	2.09×10^5
35	80	00	6.72×10^4	4.80×10^3	1.04×10^4
11	70	3.43×10^5	4.40×10^5	9.12×10^4	2.44×10^5
39	70	00	00	00	00
25	84	2.96×10^4	1.43×10^5	1.20×10^4	7.28×10^4
25	70	8.08×10^4	1.69×10^5	2.40×10^4	8.56×10^4
25	56	1.22×10^5	2.14×10^5	3.04×10^4	1.18×10^5
25	70	7.76×10^4	1.68×10^5	2.16×10^4	8.48×10^4
25	70	7.60×10^4	1.71×10^5	2.00×10^4	8.64×10^4
15	80	2.42×10^5	3.17×10^5	4.00×10^4	1.86×10^5
35	60	00	8.64×10^4	8.0×10^3	3.36×10^4

96.73% variations in the data sets can be explained by the models and for the unseen data sets, the adjusted R^2 is 98.40% and 94.56%. The effects of the interaction of the dyeing variables are presented in Fig. 1. Second-order regression equations for cotton are given below:

$$Y_{cs} = 1009839 - 42677\ A - 4836\ B + 482.4\ A^2 + 4.4\ B^2 + 86.0\ AB \quad \ldots (1)$$

$$Y_{cp} = 992298 - 26968\ A - 7123\ B + 482.4\ A^2 + 40.6\ B^2 + 10.0AB \quad \ldots (2)$$

where Y_{cs} & Y_{cp} are the bacterial counts of dyed cotton fabrics against *Staphyloccocus aureus* and *Pseudomonas aeruginosa* bacterial strains respectively (Table 3).

In the case of silk fabric samples, R^2 and adjusted R^2 are 89.55% and 82.09% respectively for the Y_{ss} model, and 99.91% and 99.84% respectively for model Y_{sp}. This implies that 89.55% and 99.91% variation in the data sets can be explained by the models. For the unseen data sets, the adjusted R^2 is 82.09% and 99.84%. The interaction effects of the dyeing parameters are represented on the three-dimensional graphs (Fig. 2). Therefore, the presence of these high R^2 values for all the models in the present study is an indication that the models adequately fit the data sets.

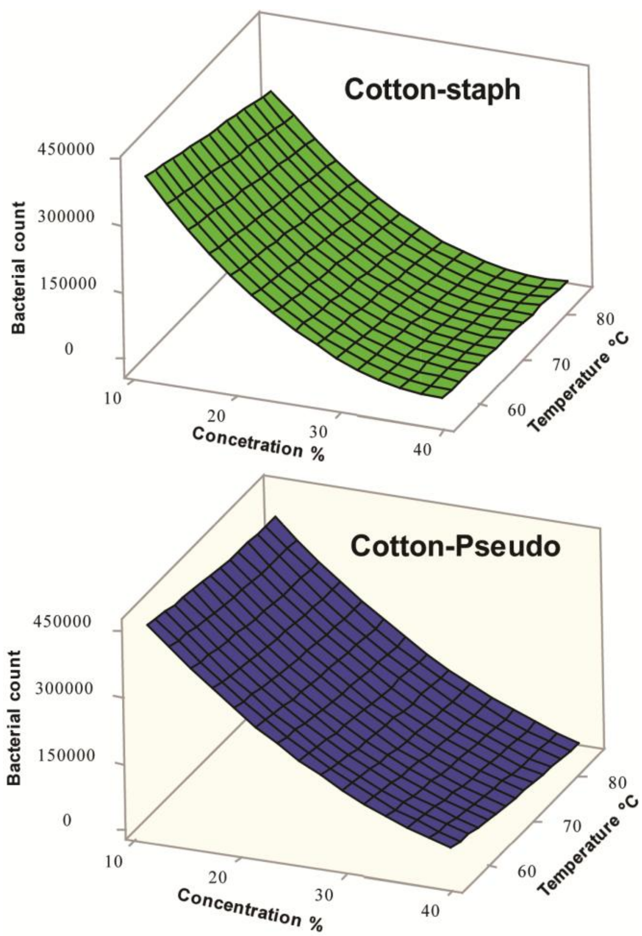

Fig. 1 — Effect of extract concentration and dyeing temperature on bacterial count for dyed cotton

The second-order regression equations for silk fabric is given below:

$$Y_{ss} = 5036 - 6917\ A + 4285\ B + 88.5\ A^2 - 33.5\ B^2 \quad \ldots (3)$$

$$Y_{sp} = 743013 - 17548\ A - 8004\ B + 179.7\ A^2 + 47.7\ B^2 - 2.0\ AB \quad \ldots (4)$$

where Y_{ss} & Y_{sp} are the bacterial counts of dyed silk fabrics against *Staphyloccocus aureus* and *Pseudomonas aeruginosa* bacterial strains respectively.

Extract concentration demonstrated a superior effect on bacterial count as opposed to dyeing temperature for both cotton and silk dyed fabrics (Figs 1 and 2). The optimized values are found to be at 39% and 70ºC. The study also demonstrate that at lower extract concentration and temperature, a slight decrease in the bacterial count is observed. This could have been attributed to the low concentration gradient of the extract and the inability of the fibers in the fabric's composition to swell[15]. At very high concentrations and temperatures, the bacterial count values increase due to dyed fabric saturation and decrease in dye molecule stability[12].

3.2 Effect of MLR on Bacterial Count

To study the effect of MLR on the bacterial count, dyeing of cotton and silk fabric samples has been done at 1:10, 1:20, 1:30, 1:40, and 1:50 with 39% extract concentration at 70ºC.

From Fig. 3(a), it is evident that the bacterial count of *Staphylococcus aureus* and *Pseudomonas aeruginosa* is lowest at MLR 1:30 and 1:20 on cotton and silk fabrics respectively, and hence these values are selected as the optimum conditions. Material-to-liquor ratio less than that may not result in uniform absorption of the contents from the water bath thus, leading to the increase in bacterial count. However, a further increase in material-to-liquor ratio results in less deposition of the medicinal plant extracts onto the fabrics, thereby rising the bacterial count values [16].

Also, the study reveals that silk fabric samples demonstrate the lowest bacterial count at MLR 1:20

Table 3 — ANOVA results for bacterial count at different dyeing variables on Cotton fabric and silk fabrics

Source	DF	*Staphylococcus aureus*		*Pseudomonas aeruginosa*	
		F-Value	P-Value	F-Value	P-Value
Cotton fabric					
Model	5	148.18	0.000	77.16	0.000
A	1	638.53	0.000	368.77	0.000
B	1	17.42	0.004	5.26	0.056
A^2	1	82.21	0.000	11.75	0.011
B^2	1	0.01	0.936	0.26	0.623
A*B	1	1.50	0.260	0.926	0.926
Error	7				
Lack-of-fit	3				
Pure error	4				
Total	12				
Silk fabric					
Model	5	12.00	0.003	1483.74	0.000
A	1	51.49	0.000	6980.58	0.000
B	1	1.36	0.281	174.51	0.000
A^2	1	5.65	0.049	258.77	0.000
B^2	1	0.81	0.398	18.23	0.004
A*B	1	0.00	1.000	0.02	0.896
Error	7				
Lack-of-fit	3				
Pure error	4				
Total	12				

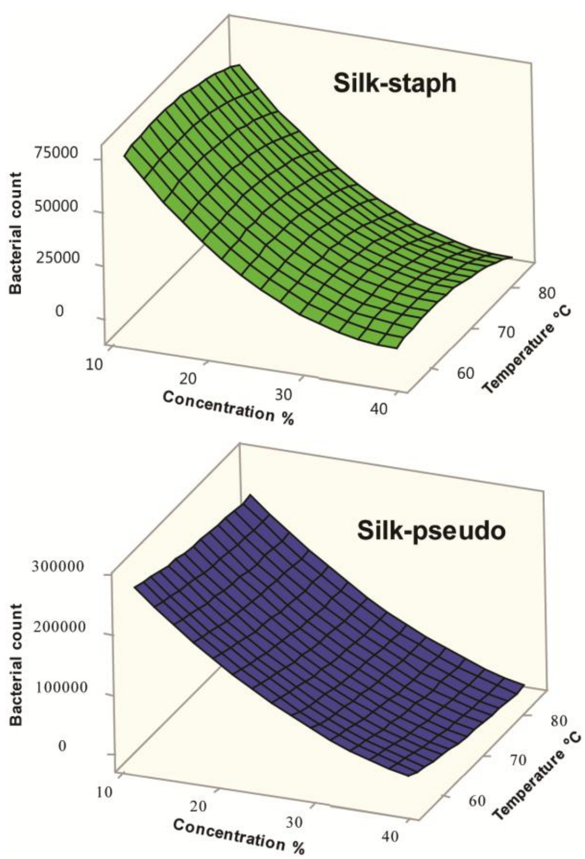

Fig. 2 — Effect of extract concentration and dyeing temperature on bacterial count for dyed silk

as compared to cotton fabric samples with the lowest bacterial count at MLR 1:30. This slight difference may have been attributed to the presence of many active sites on polypeptide chains of silk yarns/fabrics thus, its ability to bind better with natural dyes [17].

3.3 Effect of Alum Concentration on Bacterial Count

The concentration of alum in the dye bath is varied at 1, 3, and 5 g/L to determine the optimum value. As indicated in Fig. 3(b), the bacterial count of both test bacterial strains (*S. aureus* and *P. aeruginosa*) is observed to be lowest at 3g/L concentration of alum on dyed cotton and silk fabrics and thus considered as the optimum value. At low concentrations of the mordant, the affinity of the dye to the fabrics is less, leading to poor antimicrobial activity and high bacterial count. On the other hand, high mordant concentrations result in uneven dyeing due to dye agglomeration, leading to a reduction of dye affinity to the fabrics [18].

3.4 Effect of Treatment Time on Bacterial Count

The influence of treatment time from 20min to 40min on bacterial count has been examined and the results are presented in Fig. 3(c). It is evident that the lowest bacterial count for cotton and silk fabrics against *S. aureus* and *P. aeruginosa* bacterial strains was observed after 40min and 30min of the dying

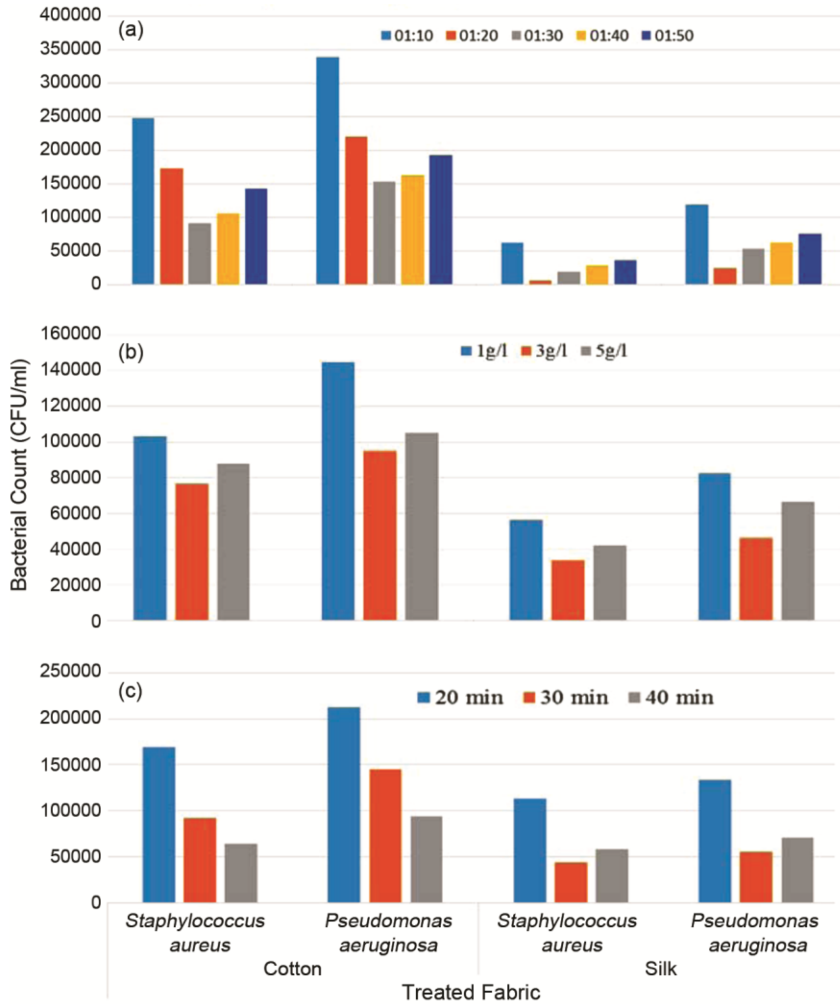

Fig. 3 — Effects of dyeing parameters on bacterial count (a) MLR, (b) alum conc. and (c) treatment time

process. This is true due to the adsorption saturation of the extracted dyes thus no further increase in uptake of the dye is observed after that duration [19,20].

3.5 Assessment of Antibacterial Resistance, Wash Durability and Fastness Properties

On comparing the bacterial count of the treated fabrics with those of untreated controls (Fig. 4), it is found that the reduction in the bacterial count is enumerated into percentages, as can be evident by the % reduction in bacterial count (cotton: 99.84% for *S. aureus* and 99.78% for *P. aeruginosa*; and silk: 99.88% for *S. aureus* and 99.83% for *P. Aeruginosa*). Both dyed fabrics demonstrate a high percentage bacterial count reduction against selected bacterial strains (*S. aureus* and *P. aeruginosa*); although with silk fabrics, the percentage reduction is slightly higher. This variation may have been as a result of the existence of more active sites on silk fabric structure, thus more medicinal dyes forming covalent bonds

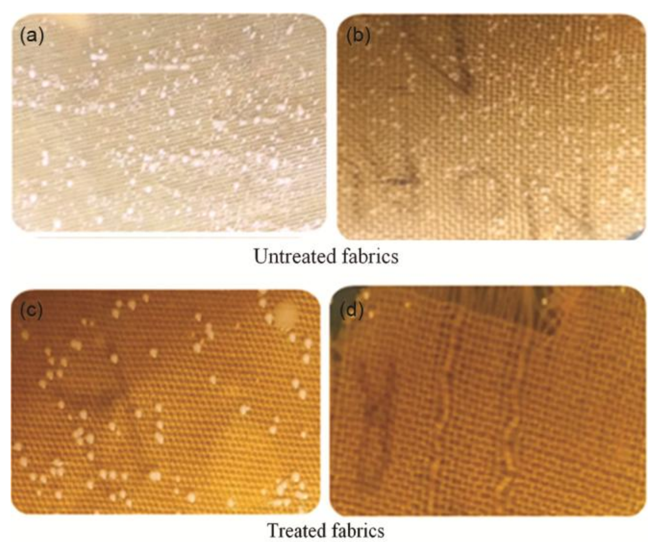

Fig. 4 — Antimicrobial properties exhibited by cotton (a) and silk (b) untreated (Control) fabrics compared with the treated cotton (c) and Silk (d) fabrics

Table 4 — Dyed cotton and silk fabrics retention of antimicrobial activity

Bacteria	Fabric	Dyeing	After 1 wash		After 5 washes	
			R %	A %	R %	A %
S.aureus	Cotton	Control	Nil	Nil	Nil	Nil
		Dyed	97.2	97.4	89.0	91.8
P. aeruginosa	Cotton	Control	Nil	Nil	Nil	Nil
		Dyed	96.9	97.2	87.7	90.7
S.aureus	Silk	Control	Nil	Nil	Nil	Nil
		Dyed	98.7	98.8	93.3	94.6
P. aeruginosa	Silk	Control	Nil	Nil	Nil	Nil
		Dyed	98.3	98.5	91.0	92.7

R – Reduction in CFU, and **A** – Activity retention.

Table 5 — Fastness properties of dyed cotton and silk fabrics

Fabric	Light fastness	Wash fastness		Fastness to perspiration				Rub fastness	
				Acidic		Alkaline			
		CC	CS	CC	CS	CC	CS	Dry	Wet
Cotton	4-5	3-4	4	4	3-4	3-4	3-4	4-5	4
Silk	5	4-5	5	4	4-5	4	4-5	4-5	4

CC–Colour–Change, CS–Colour–Staining, 3– Moderate, 4– Good and 5– Excellent.

leading to improved antimicrobial properties [6]. The study also reveal that the bacterial count reduction percentage for both dyed fabrics is much higher against the *S. aureus* bacterial strain as compared to the *P. aeruginosa* bacterial strain. This slight variation may have been attributed to the differences in the chemical composition of their cell walls [21]

The results of wash durability as illustrated in Table 4 showed that even after 5 washes, the dyed fabrics are able to retain over 90% activity, which may have been as a result of a mordant used that improves the dye fixation into the fabric structure [22]. However, with silk fabric, the activity retention is slightly higher than that of cotton. This could have been due to the presence of many active sites on its structure [6].

Colourfastness being a property of a dye to retain its color when applied on to a textile substrate and subjected to light, rubbing, and perspiration among other conditions, its assessment on dyed cotton and silk fabrics has been carried out using a standard greyscale (Table 5). It can be seen that both cotton and silk dyed fabrics demonstrate commercially acceptable fastness ratings as per international standards of 4 to 5 for light and rubbing. For wash and perspiration fastness, the rating is generally good (3-4 to 4) for cotton fabrics and very good to excellent (4-5 to 5) for silk fabrics[23]. The presence of tannins in the *Racinus communis* plant extract, as well as a mordant used contribute much in the bonding of the dye to the fabric matrix, thus providing better fastness properties[7]. The study also confirms that the fastness properties for dyed silk fabrics are more significant as opposed to dyed cotton fabrics. This variation may have been attributed to the formation of binding linkages between the dye molecules and the carboxyl groups found in silk fibers which are not present in cotton fibers[24].

4 Conclusion

Prior to the dyeing of cotton and silk fabrics with aqueous extracts from *Racinus communis* plant leaves, dyeing parameters are optimized and selected, based on the higher bacterial resistance properties (lower bacterial count). For cotton fabric samples, the optimized values are 39%, 70ºC, 1:30, 3g/L, and 40 min for extract concentration, dyeing temperature, Material-to-Liquor ratio, alum concentration, and treatment time respectively. However with silk fabric samples, the optimized values for material-to-liquor ratio and treatment time are 1:20 and 30min respectively, which are different values from those recorded on cotton fabric. At optimum conditions, dyed cotton and silk fabrics exhibit a 99.78 - 99.88% reduction in the bacterial count of *Staphylococcus aureus* and *Pseudomonas aeruginosa.* However, the percentage reduction is slightly higher for silk fabrics as compared to cotton. Durability studies show that even after 5 washes, 91-99% activity is retained. Also, dyed fabrics demonstrate acceptable fastness properties which range from moderate (3) to excellent (5), thus confirming that natural plant dyes are capable of dyeing textile materials as well as imparting antibacterial properties. However, further research can be done on testing these treated fabrics against other common Gram-positive and Gram-negative bacterial strains, and thereafter, assess their wash durability and other physical and mechanical properties.

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