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DEVELOPMENT OF ANTIMICROBIAL COTTON FABRIC THROUGH APPLICATION OF DYE EXTRACTS FROM GALINSOGA PARVIFLORA PLANT LEAVES.

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Abstract: *The present study involves the aqueous extraction of dyes, optimization of dyeing conditions (Extract concentration and dyeing temperature), application of extracted dyes onto cotton fabrics using optimized conditions, SEM analysis of treated fabrics, assessment of their antibacterial activity, and wash durability. Using Central Composite Design (CCD), the optimized dyeing conditions were selected as 39.14 percent and 70^{0}C based on the lowest bacterial count demonstrated by the dyed cotton fabric samples. Results of Analysis of Variance (ANOVA) confirmed that extract concentration has a more significant statistical influence compared to dyeing temperature. Treated cotton fabrics at optimized conditions together with alum as a cross-linking agent exhibited a 98.54 and 97.96 percent reduction in the bacterial count against Staphylococcus Aureus and Pseudomonas Aeruginosa bacterial strains respectively. The retention of the antimicrobial activity of treated fabrics was found to be more significant even after 5 washes thus confirming that Galinsoga Parviflora extract is a potential source of finishes capable of improving antibacterial resistance of cotton fabrics for a longer period.*

Keywords: *Antibacterial activity, Central Composite Design, Cotton fabrics, Galinsoga Parviflora, Optimization.*

1. INTRODUCTION

Cotton materials owning desired characteristics and their proximity to the human body act as a potential medium for adherence, transfer, and propagation of several microbes that cause diseases and infections among people in hospitals and other healthcare-related environments. More so, these microbes lead to detrimental effects like bad odors, color deterioration, and loss of fabric aesthetic properties [1]. As a way of rectifying these shortcomings, natural plant antimicrobial finishes are increasingly being preferred since they are non-toxic, non-allergic, and biodegradable compared to synthetic ones [2]. The application of these natural finishes requires a mordant to improve their bonding capabilities thus enhancing their wash durability [3]. Galinsoga Parviflora is a traditionally known plant that is capable of treating beetle bites and wounds. It belongs to the Asteraceae family and contains many Pharmacological as well as Phytochemical properties [4]. To efficiently benefit from these natural medicinal products, their application parameters are normally optimized using different techniques among which include Central Composite Design of Response Surface Methodology (RSM) and Single-factor design [5]. The current study, therefore, was focused on extracting dyes from Galinsoga Parviflora plant leaves, optimizing the dyeing conditions, analyzing the surface morphology of the treated fabrics, assess their antimicrobial resistance and wash durability.

2. MATERIALS AND METHODS

2.1 Materials

A mercerized, scoured, and bleached 100 percent woven cotton fabric was acquired from Rivertex Limited, Kenya. Galinsoga Parviflora plant leaves were sourced from the wild in the Western part of Uganda. All other requirements were bought from INDO Kenya Enterprises limited.

2.2Methods

2.2.1 Preparation of the dye extract

The collected and dried Galinsoga Parviflora plant leaves were coarsely grounded and weighed. Maintaining the Material to liquor ratio of 15:250w/v were subjected to aqueous extraction by maceration process [6]. Then, the extract was filtered, concentrated, and stored at 4^0C.

2.2.2 Microorganisms and culture condition

Following the SOPs, the Gram-positive and Gram-negative bacterial strains viz. *Staphylococcus aureus* and *Pseudomonas aeruginosa* of ATCC grade were recovered from the storage media and maintained on Muller Hinton medium.

2.2.3 Dyeing of cotton fabric samples and testing for their antibacterial efficacy

Using the material to liquor ratio of 1:40, 3g/l alum concentration at 70^0C for 40min, cotton fabric samples were dyed with extracted dyes, excess dyes removed by padding mangles, and dried under the shade. In optimizing the dyeing process, concentration and temperature varied from 15 to 35% and 60 to 80^0C respectively. Antimicrobial tests were done on dyed fabric samples against selected bacterial strains. Based on the combination that demonstrated the lowest bacterial count was noted as the optimum condition using Central Composite Design.

2.2.4 Central Composite Design (CCD) and Statistical analysis with ANOVA

Using Central Composite Design of Response Surface Methodology [5], dyeing conditions for the plant extract from Galinsoga Parviflora were optimized. Experimental variables, their coded and actual levels are illustrated in Table 1.

Table 1: *Experimental variables and their levels*

Variables	Levels				
	-α	-1	0	1	α
Conc (%)	10.86	15	25	35	39.14
Temp (^{0}C)	55.86	60	70	80	84.4

After testing dyed cotton samples against selected bacterial strains, bacterial count values obtained were considered as the response variables. Using the Design Expert 7.0 software package [7], all the design and analysis of experiments were done. Then the adequacy of the developed model and its statistical significance were checked using ANOVA and Response surface plots drawn to analyze the interaction among several independent process factors and their effect on the bacterial count

2.2.5 SEM analysis, Antimicrobial resistance assessment and wash durability tests of dyed cotton fabrics with optimized values.

Using Tescan Vegas 3 control software at 3kV and a scale of 20µm, SEM analysis was done for the treated and untreated (control) fabrics. The antimicrobial activity of cotton fabrics was tested against selected bacterial strains based on AATCC Test Method-100:2019 and the microbial inhibition was calculated as a percentage reduction in the number of Colony Forming Units (CFU) with respect to untreated control samples after inoculation and incubation as per the stated formula; R = [(B-A)/B]*100. Where; R- Percentage reduction in Microbial colonies, A – CFU/ml for the treated fabric samples after 24hrs incubation, B – CFU/ml for the untreated fabric samples after 24hrs incubation under the same conditions. For wash durability, the dyed cotton fabrics were investigated after one and five washes following the AATCC-100 test method [3].

3. RESULTS AND DISCUSSION

3.1. Central Composite Design and Statistical analysis

From Table 2, bacterial count values reduce as extract concentration and temperature increase although the reduction in bacterial count varied which may have been influenced by the different bacterial strains used since they are known to have different bacterial structures [8]. Then Figure 1 clearly indictated that there was a reduction in bacterial count values for treated cotton fabric at optmum conditions in relation to untreated one.

Table 2: *Bacterial counts at varying dyeing parameters*

No.of Runs	Conc. Of Extract (%)	Temp. (^{O}C)	Bacterial Count (CFU/ml)	
			Cotton fabric	
			Staphylococcus aureus	*Pseudomonas Aeruginosa*
1	25	70	2.0×10^5	2.92×10^5
2	25	70	1.94×10^5	2.94×10^5
3	15	60	4.23×10^5	4.98×10^5
4	35	80	6.64×10^4	1.43×10^5
5	10.86	70	5.06×10^5	5.62×10^5
6	39.14	70	**2.56×10^4**	**6.96×10^4**
7	25	84.14	1.61×10^5	2.48×10^5
8	25	70	1.98×10^5	2.94×10^5
9	25	55.86	2.08×10^5	3.19×10^5
10	25	70	1.98×10^5	2.91×10^5
11	25	70	1.96×10^5	2.90×10^5
12	15	80	4.01×10^5	4.61×10^5
13	35	60	9.12×10^4	1.70×10^5

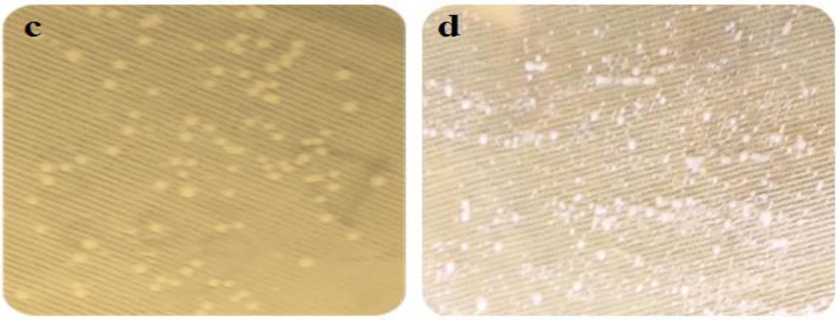

Fig. 1. *Shows the reduction in bacterial count of the treated cotton fabric **(c)** at optimum conditions based on the number of colony forming units (CFU/ml) as compared to the untreated cotton fabric **(d)**.*

Table 3 illustrates the ANOVA results for the bacterial count values against Staphylococcus aureus and Pseudomonas Aeruginosa whereby both models (‘Y_{cs}’ and ‘Y_{cp}’) are significant with a p-value = 0.000. Also, the linear and square effects of extract concentration for both models are significant while only the linear effect of temperature is significant for all models thus confirming that a change in extract concentration has a more significant effect on cotton dyeing for antimicrobial resistance as compared to dyeing temperature.

Table 3: *ANOVA results for the bacterial count values at different dyeing variables on treated cotton*

		Staphylococcus aureus		*Pseudomonas Aeruginosa*	
Source	DF	F-Value	P-Value	F-value	P-value
Model	5	370.00	0.000	276.79	0.000
Conc.	1	1751.59	0.000	1352.46	0.000
Temp.	1	12.55	0.009	20.19	0.003
Conc*Conc	1	83.52	0.000	10.96	0.013
Temp*Temp	1	0.10	0.757	0.00	0.970
CONC*Temp	1	0.01	0.919	0.16	0.699
Error	7				
Lack-of-Fit	3				
Pure Error	4				
Total	12				

The second-order regression equations (1 & 2) were generated to model the relationship between bacterial count (Response ‘Y_{cs}’ & ‘Y_{cp}’) and dyeing variables of extract concentration (A) and Temperature (B) for cotton fabric samples. The R^2 and adjusted R^2 were 99.62% and 99.35% respectively for the Y_{cs} model and then for the Y_{cp} model, R^2 and adjusted R^2 were 99.50% and 99.14% respectively. This implies that 99.62% and 99.50% variations in the data sets can be explained by the models. For the unseen data sets, the adjusted R^2 for each model is 99.35% and 99.14% respectively. The interaction effects of the dyeing variables are represented on the three-dimensional graphs, response surface plots in Fig. 1

$$Y_{cs} = 885425 - 36114\,A + 672\,B + 394.1\,A^2 - 13.9\,B^2 - 6.0\,AB \quad \textbf{(1)}$$

$$Y_{cp} = 1010908 - 26682\,A - 26682\,B + 161.9\,A^2 + 1.9\,B^2 + 26.0\,AB \quad \textbf{(2)}$$

Whereby;

Y_{cs} & Y_{cp} are the bacterial counts of the dyed cotton fabric against *Staphylococcus aureus* and *Pseudomonas Aeruginosa* bacterial strains respectively

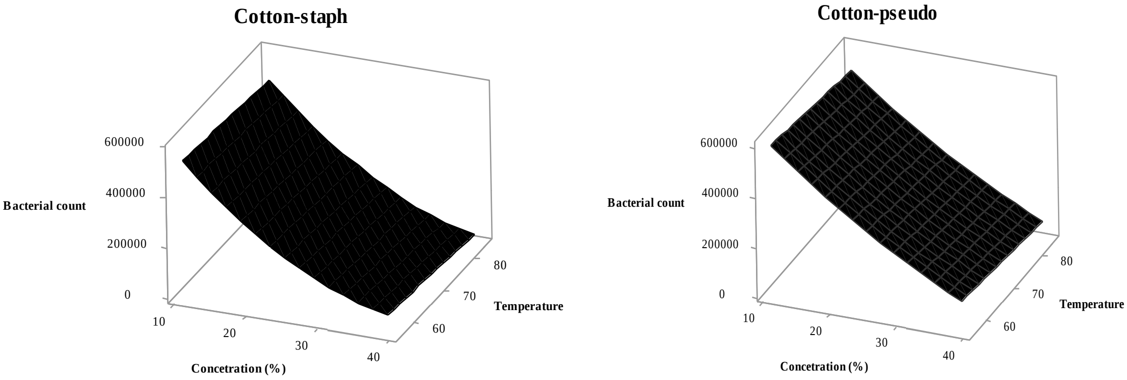

Fig.2. *Effect of extract Concentration and dyeing temperature on Bacterial count for dyed cotton.*

As presented in Figure 2, extract concentration has proved to be a key factor for dye application onto cotton fabric for improved bacterial resistance as opposed to dyeing temperature. The optimized values were found to be at 39.14% and 70^{0}C. Also, it was observed that at lower extract concentrations and temperatures, there was a slight decrease in bacterial count values. This could have been attributed to the low concentration gradient of the extract and the inability of the fibers in the structure of the fabrics to swell [9]. Then at very high concentrations and temperatures, the bacterial count values rise more due to dyed fabric saturation and a decrease in dye molecule stability [10].

3.2 SEM analysis, Antimicrobial resistance assessment, and wash durability tests of dyed cotton fabric with optimized values.

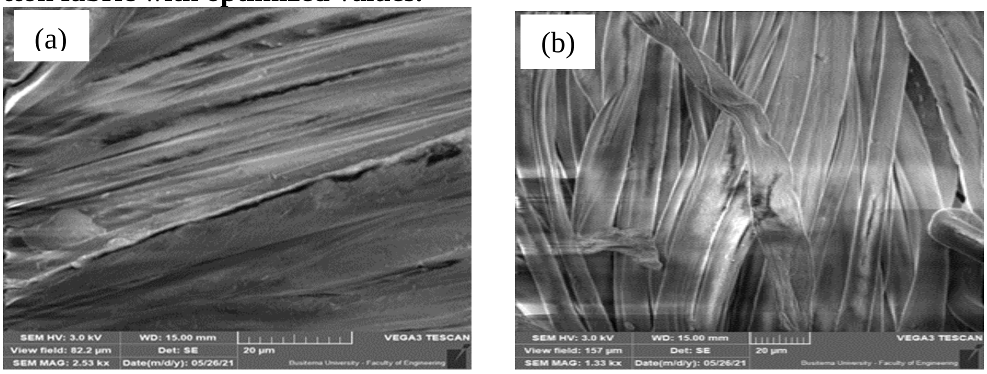

Fig 3: *SEM pictures indicating the growth of bacteria on (a) undyed cotton and (b) dyed cotton fabric at optimized conditions.*

Table 4: *Antibacterial efficacy of dyed cotton.* **Table 5***: Dyed cotton fabric retention of antimicrobial Activity*

Plant extract	**Reduction in Bacterial Count (%)**	
	Cotton fabric	
	Staphylococcus aureus	*Pseudomonas Aeruginosa*
GP	98.54	97.96

Bacteria	**Cotton fabric**	**After 1 wash**		**After 5 washes**	
		R (%)	A (%)	R (%)	A (%)
S.aureus	Control	Nil	Nil	Nil	Nil
	Dyed	96.3	97.76	85	88.7
P. aeruginosa	Control	Nil	Nil	Nil	Nil
	Dyed	92.7	94.8	78.56	85.8

GP - Galinsoga Parviflora, R - Reduction in CFU, A - Activity retention.

As shown in Figure 3 and Table 4, dyed cotton fabrics exhibited a bacterial count reduction percentage of 98.54 and 97.96 against Staphylococcus aureus and Pseudomonas Aeruginosa bacterial strains respectively. This result demonstrated a significant antibacterial effect against the selected bacterial strains which may have been attributed to the higher level of medicinal dye uptake [11]. The study further confirmed that due to differences in chemical compositions of their cell walls, the percentage reduction was much higher in the case of Staphylococcus compared to Pseudomonas bacterial strain. The results of wash durability as illustrated in Table 5 showed that even after 5 washes, the dyed fabric was able to retain over 85% activity which may have been a result of a mordant used that improved the dye fixation into the fabric structure [12].

4. CONCLUSIONS

The optimized dyeing parameters were 39.14% and 70^0C for extract concentration and temperature respectively. However, extract concentration was found to have more effect on the antimicrobial activity of the finished cotton fabrics. At optimized conditions, a 97.96 to 98.54% reduction in the bacterial count against both bacterial strains was realized. Durability studies showed that even after 5 washes, 86-98% activity was retained thus confirming that it is possible to develop cotton fabric materials with durable antimicrobial properties using Galinsoga Parviflora plant extracts.

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