ANALYSIS

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Discovery

The evaluation of antimicrobial activity of cotton fabric dyed with eco-friendly natural colourant extract (*Trianthema pentendra*) against bacteria

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ABSTRACT

Dyeing and testing antimicrobial effect on cotton fabric were achieved using eco-friendly natural extract (Trianthema pentendra). Partitions of extracts were done using ethanol, ethyl acetate, chloroform and hexane solvents. Combinations of mordants (Copper Sulphate: Ferrous Sulphate) in three different ratios (3:1, 1:3, & 1:1) were also used in the order of pre-mordanting, simultaneous-mordanting and post-mordanting. Varieties of colours were achieved, light, washing, rubbing, and pressing fastness properties were also studied. The fabric showed significant washings durability after repeated washings with standard soap. All the fractions were tested for antimicrobial activity against Gram negative (Escherichia coli, and Pseudomonas aeruginosa) and Gram positive

(Streptococcus pyogenes, Staphylococcus aureus) Bacteria. Four different concentrations of plant extracts were used (10, 25, 50 and 100 µl). Methanolic extract was found to be the most active fractions with maximum zone of inhibition (19mm) against Staphylococcus pyogenes.

Keywords; Antimicrobial, Dyeing, Eco-friendly, Mordants

1. INTRODUCTION

The comprehensive focus on plant based natural dyes and other bioactive natural extract in textile coating as antimicrobial textile finish has gained significant momentum. The antibacterial materials such as fabrics, cloths are became important to avoid cross infection by pathogenic microorganisms, especially bacteria such as *Staphylococcus aureus, Escherichia coli* and *Klebsiella pneumoniae,* to control the infestation by microbes, and to arrest metabolism in microbes in order to reduce the formation odour. The major antimicrobial agents for textile coating are chemical agents which have toxic and environmental issues. Textiles for medical and hygienic use have become important areas in the textile industry. Therefore, to reduce/prevent infections, various antibacterial compounds have been used for all types of textiles. Several natural dyes obtained from plants exhibit strong antimicrobial properties. Therefore, coating of antimicrobial plant natural dyes and bioactive plant extract on to cotton fabrics is an emerging technology in the production of medical cloths. This research is aimed at replacing the synthetic dyes (which a times are dangerous or carcinogenic) with abundant natural dyes, which at the same time can act both as dyes and antimicrobial agents.

2. MATERIAL AND METHOD

Cotton Fabric

Cotton fabric was purchased from nearby Factory, Kano and was identified in (Colour/Polymer section Chemistry) department, Bayero University Kano, Nigeria. Mercerization and Scouring of the fabric were carried out according to the method of [1,2].

Plants material

Source; The leaves of Trianthema pentendra were collected at random from Government Day Secondary School Garki town, Jigawa State of Nigeria. on 11th January, 2013. The plant was air dried under the shade for two weeks at room temperature.

Extraction process

The collected leaves was washed, thoroughly and chopped into smaller pieces, shade dried at room temperature and later grinded. After this coarse powder of the plants (500g) *Trianthema pentendra*, was mixed with ethanol at room temperature in a ratio of 500g: 250l of plant: solvent in a beaker. The beaker was left closed for 14 days with random hand shake so that the solvent can take the active component of the leaves. After 14 days the solvent was separated by filtration using a Whatmann filter paper and then solvent was removed under reduced pressure using a rotary evaporator. The collected concentrate was kept at room temperature for further used.

Table 1 Conditions for Dyeing and Mordanting

Dye	Mordant	Mordant: volume of dye bath	Temperature	Time of dyeing
4% owf	2% owf	1:40	80^{o}c	60mins

Fractionation Procedure: Maceration with solvents

Methanol (30ml) was added to the residual ethanol extract obtained from the first maceration process and stirred to dissolve those parts of the ethanol extract which are soluble in methanol. The resulting methanolic solution was collected in an empty beaker. This process was repeated two to three times with (20ml) methanol until the methanol no longer dissolves any part of the ethanol extract. The extract was kept safe for further use. The same process was repeated for the ethyl acetate, chloroform and n-hexane.

Preparation of cotton fabric for coating

The cotton fabrics were purchased nearby Factory Kano, Nigeria. This was pretreated by caustic boil method. The fabric was washed on hot water with 500mls Na_2CO_3 g/l. Cotton fabric 5.05 g and 2.2 inch length was washed in dissolved 0.3155g /l of Na_2CO_3. The beaker was placed in water bath heated to boil, with continued stirring for 15 minutes. The fabric was removed and dried. The

scoured fabric was bleached by treating with 2gm of ascorbic acid and 3ml of sodium hypochlorite in 200ml of water and maintained at 90°C for 10 min. The fabric was dried and washed in sterile water. It was then dried ready for other processes [3]

Fabric Coating

The exhaust method was used for coating the fabric. The fabric was mordanted with Acetic acid prior to dyeing. The mordant was 5% on weight of the fabric (250 µl of acetic acid for 5.05gm cotton fabric) and the liquor ratio was 40:1. The treated fabric was introduced in to the plant extract i.e 5%, *Trianthema pentendra* and dyed at 50 - 55°C for 45 minutes [3].

Quantitative test (AATCC -100)

The antibacterial property of the dyed fabrics or extracts was carried out according to standard test method reported by *American Association of Textile Chemist and Colorist 100* (AATCC -100). The cotton fabric of 2.2 inch was treated in 5% of *Trianthema pentendra* extracts. The fresh culture of *Streptococcus pyogenes, Staphylococcus aureus, Escherichia coli, and Pseudomonas aeruginosa* were inoculated to sterilized broth agar followed by the transfer of treated fabric sample. These were incubated at 30°C for 24 hours in shaker at 120 rpm. The absorbency was checked at 600nm with sterilized nutrient agar medium as blank. The fabrics were analyzed for their antibacterial durability after repeated wash with standard soap. The fabric sample was placed in soap solution in a beaker with continuous stirring maintained at 40°C for 10min. These were then washed with distilled water and dried. The sample was again tested for antibacterial lproperty. The same procedure was followed for 5 washing [3].

Table 2 Results of Zone of inhibition of plant extract of *Trianthema pentendra*

Plant	Fractions	Conc.	Diameter of zone of inhibitions (mm)			
			1	2	3	4
Trianthema pentendra	Methanol	100	+++	++	+++	+++
		50	+++	+	+	++
		25	++	+	-	+
		10	+	-	-	-
	Ethyl acetate	100	+++	+++	+++	+++
		50	+++	+	+++	+
		25	+	+	+	-
		10	+	-	+	-
	Chloroform	100	+	+	+	++
		50	+	+	+	+
		25	-	-	-	-
		10	-	-	-	-
	Hexane	100	+	+	+++	++
		50	-	-	+	+
		25	-	-	+	-
		10	-	-	-	-

[a]Zones of inhibition (mm); 0-6 = -, 7-9 = +, 10-12 = ++, 12-25 = +++
Microorganisms (1) *Streptococcus pyogenes,* (2) *Staphylococcus aureus,* (3) *Escherichia coli,*(4) *Pseudomonas aeruginosa*

Qualitative test American association of textile chemist and colorist 147 (AATCC-147)

In qualitative test, disc diffusion method was followed. Approximately 25 mg of plant extract was dissolved in 200µl sterile water. Samples of 10, 25 50 and 100 µl were slowly impregnated drop wise on 5mm sterile Whatmann paper disc and also on cotton fabric. The treated samples were placed in intimate contact with agar, which was previously inoculated with overnight culture of *Streptococcus pyogenes, Staphylococcus aureus,* and gram negative organisms, *Escherichia coli, and Pseudomonas aeruginosa* and incubated at 37°C for 18-24 hours. The zone of inhibition was evaluated [3]

Dyeing and Mordanting

Accurately weighed cotton fabric was treated with different metal salts mordants used (cupper sulphate and ferrous sulphate). Three processes of mordanting were used i.e pre mordanting, simultaneous mordanting and post mordanting using 1% weight of the fabric. The dyed material was washed with cold water and dried at room temperature [4].

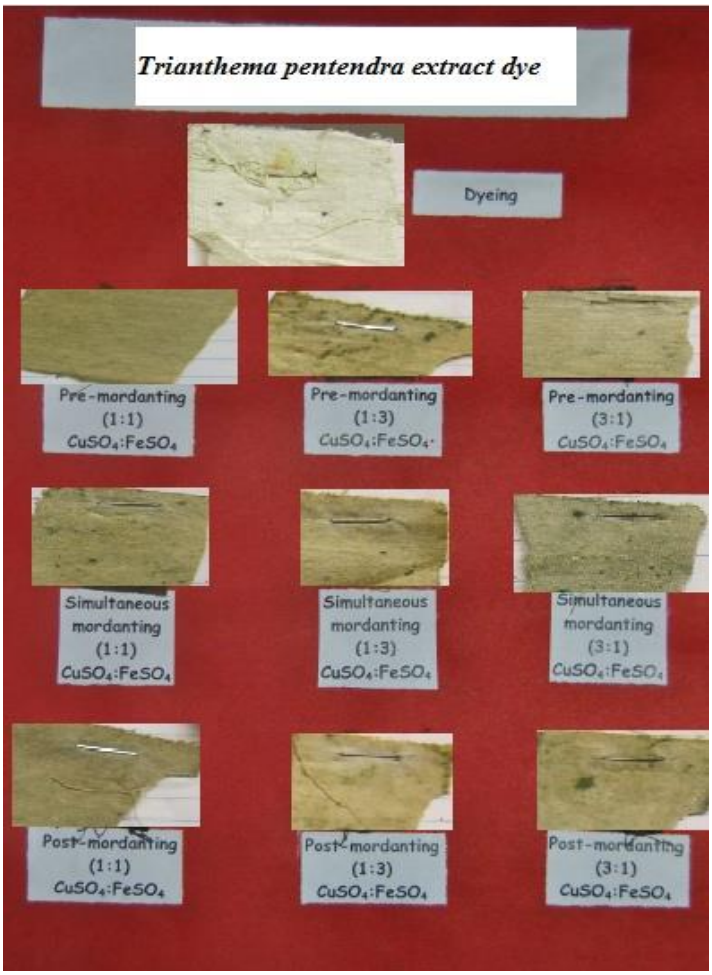
3. RESULT AND DISCUSSION

Durability of Antimicrobial Plant Extract for Plant Trianthema Pentendra

The antimicrobial activity of *Trianthema pentendra* was analyzed against four bacteria viz; Streptococcus *pyogenes*, S. *aureus*, E. *coli* and *Pseudomonas aeruginosa*.

Table 2 showed the result of antimicrobial activity of *Trianthema pentendra,* maximum activity was observed in methanolic extract at different concentrations of the different pathogens (*Streptococcus pyogenes*, S. *aureus*, E. *coli* and *Pseudomonas aeruginosa*) against (19.0, 11.5, 14.8, 113.9) respectively at 100µl/disc concentration. Little or no activity was observed at lower

concentrations at 10µl/disc in S. *aureus* and E. *coli.* Ethyl acetate exhibited remarkable activity at 100µl/disc concentration (17.0, 14.1, 15.0, 12.9) against (*Streptococcus pyogenes*, S. *aureus*, E. *coli* and *Pseudomonas aeruginosa*) respectively. Chloroform extracts of *Trianthema pentendra* possessed a moderate activity to pathogens at 100µl/disc concentration (8.8, 9.3, 8.4, 10.8) against (*Streptococcus pyogenes*, S. *aureus*, E. *coli* and Pseudomonas *aeruginosa*) respectively. Poor or no activity was observed in lower concentrations. Hexane extracts of *Trianthema pentendra* plant showed a magnificent activity, especially in E. *coli* 100µl/disc concentration (13.8) zone of inhibition (ZOI), it showed a very clear ZOI which exceed over 24hours. Hexane extract at 100µl/disc concentration showed activity as (8.8,7.7, 13.8, 9.6) against (Streptococcus *pyogenes*, S. *aureus*, E. *coli* and Pseudomonas *aeruginosa*) respectively. Little or no activity was also observed in lower concentrations this result agreed with the findings of *Adoum, who reported that* In their studies reveled that *Trianthema pentendra* showed strong activity against *Streptococcus pyogene, Proteuos vulgaris* and S. *aureus*[6].



Fastness properties of cotton fabric dyed with plant Trianthema pentendra extract

Four different fastness properties were evaluated. viz light fastness, washing fastness, rubbing fastness and pressing fastness. The fastness rating of cotton fabric dyed with or without mordant 1% owf is presented in Table 3.

Table 3 Fastness properties Trianthema pentendra

PLANT	MORDANTING	RATIO	LIGHT	WASHING	RUBBING	PRESSING
Tranthema pentendra	Without	-	4-5	2	3	3
	Solvent + mordant	1:1	3-4	2	3	3
	Pre-mordanting	3:1	6-7	3	3	2
		1:3	4-5	2	4	3
		1:1	5-6	3	3	4
	simultaneous	3-1	5-6	3	3	4
		1-3	4-5	3	3	4
		1-1	5-6	3	3	5
	Post mordanting	3:1	6-7	4	3	4
		1:3	5-6	4	3	4
		1:1	6	4	3	4

Key

Light fastness rating:1 – poor,2 – fair,3 – moderate,4 – good,5 – better,6 – very good,7 –best and 8 – excellent

Wash, rubbing and pressing fastness rating: 1 – poor, 2 – fair, 3 – good, 4 – very good and 5 –excellent.

Assessment of colour fastness properties

Fastness to wash, light pressing and rubbing of the dyed samples were assessed using standard procedures; [5].

Light Fastness

Fastness to light of the cotton fabric dyed with *Trianthema pentendra* extract showed a very good rating. Dyeing without mordant gave fair to fair results (Table 3). Solvent dyeing gave fair to moderate results of (2-3). Pre-mordanting dyeing gave very good to excellent results (6-7) at 3:1 $CuSo_4/FeSo_4$ and 1:1 $CuSo_4/FeSo_4$ ratios gave good to better (4-5) and better to very good (5-6) respectively, (Table 3). Simultaneous mordanting dyeing of a cotton fabric with *Trianthema pentendra* gave moderate result at all combinations of mordant ranging from good to very good results. Post-mordanting dyeing gave best results out of all (6-7, 5-6, and 6) in a (3:1, 1:3 and 1:1) $CuSo_4/FeSo_4$ ratios respectively (Table 3).

Wash Fastness

Cotton fabric dyed with extract from Trianthema pentendra, showed the fastness to washing results as follows. Without mordant the dyeing gave fair result with fastness rating of 3 (Table 3) The tannins present in T. pentendra seedpod extract having a phenolic structure, can form metal chetalate with different mordants, hence, after mordanting, these tannins are insoluble in water, ultimate improving washing, water, and perspiration fastness[**7**]. Solvent dyeing gave poor to fair results with the value (2) while the pre-mordanting dyeing gave moderate results (3, 2, 3) in (3:1, 1:3 and 1;1) $CuSo_4/FeSo_4$ ratios of mordanting combinations respectively. While Simultaneous and post mordanted dyed fabrics gave good to very good results at all combinations of mordants.

Rubbing fastness

Fastness to rubbing was observed vary between averagely good to very good at all combinations except in 1:1 ratio of $CuSO_4/FeSO_4$ simultaneous mordanted technique (Table 3).

Pressing fastness

The pre mordanted, simultaneous mordanted and post mordanted cotton fabric exhibited good to better results except in 1:1 ratio of simultaneous dyeing process where an excellent result was obtained (Table 3). The major problem encounter with this dye extract is that it's not suitable for aqueous dyeing. It is clear that ferrous sulfate and copper sulfate mordants exhibited greater ability to form coordinate complexes with the dye. As the coordination numbers of ferrous sulfate and copper sulfate are 6 and 4 respectively, some co-ordination sites remained unoccupied when they interact with the fiber. Functional groups such as amino and carboxylic acid groups on the T. *pentendra* extracts can occupy these sides. Thus this metal can form a ternary complex on one site with the fiber and on the other side with the dye [8].

Quantitative analysis of microbes and washing durability of cotton fabric treated with Trianthema pentendra

The Result below of standard test method Quantitative test (AATCC-100) Fig. 2 shows the growth of pathogens after several washings with standard soap.

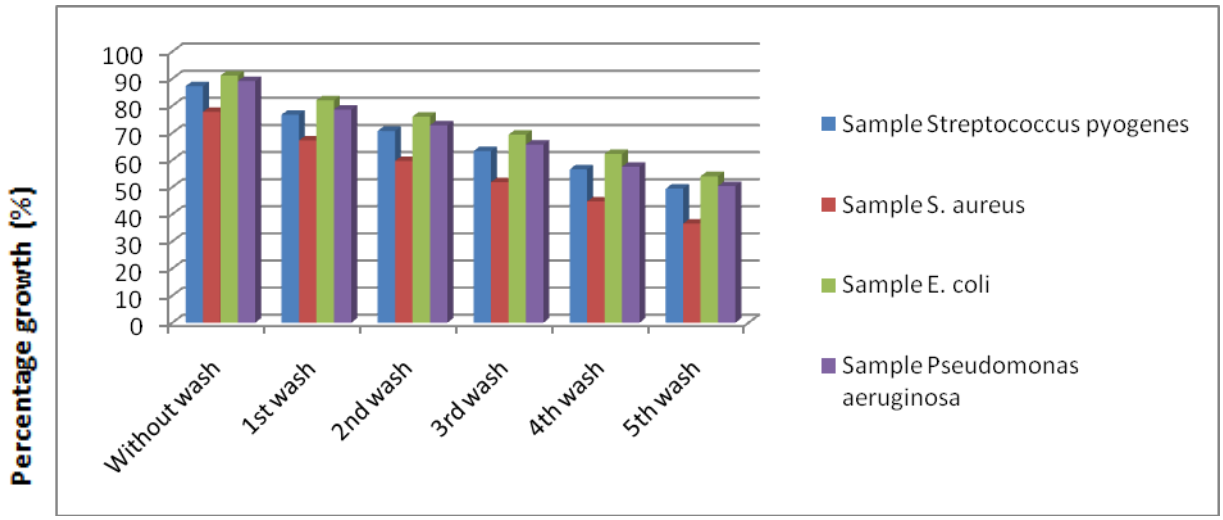


Figure 2 Result of Quantitative test (AATCC-100) for *Trianthema pentendra*

Basic idea came from the inception of end used of treated material, the durability to washing of functional treatment for disposable material; it only requires temporary functional properties for all other applications. The functional properties should be reasonable resistant to wash. To test the durability to home laundering. The antimicrobial activities of mordanted and non mordanted fabrics were tested after 1st-5th wash against Streptococcus pyogenes, S. aureus, E. coli and Pseudomonas aeruginosa bacteria. The findings are shown in Fig 1.

From Fig 1, it can be observed that the antimicrobial activity of treated (0 wash) cotton fabric with Trianthema *pentendra* extract as dye and 1% $CuSO_4$/$FeSO_4$ as mordants showed 87%, 77%, 91% and 90% reductions in growth against Streptococcus pyogenes, S. aureus, E. coli and Pseudomonas aeruginosa bacteria. After 1st washings cycle, it shows further decreasing drastically as 77%, 67%, 82%, and 78% respectively. Further to 3rd washings cycle of cotton fabric shows 63%, 52%, 70%, and 66%. The cotton fabric was found to exhibits good antimicrobial activity up to fifth washings. However, the cotton fabric sample treated dyed with *Trianthema pentendra* extract in combinations with 1% $CuSO_4$/$FeSO_4$ retained almost 75% antimicrobial activity of some bacteria even after 5 washing. This perhaps this may be due to presence of cupper ion which form insoluble copper complex "lake" with tannins from Trianthema *pentendra* extract and further form a dye molecules and retain the antimicrobial activity after repeated washing. Fig. 2 showed the clear growth of microorganism after repeated washing. T. pentendra extract are very active against gram (-) bacteria i.e E. coli and *Pseudomonas aeruginosa*. Moderate results were found in S. *aureus* while the least went to Streptococcus *pyogenes*.

4. CONCLUSION

Natural colourant extract from *Trianthema pentendra* was be successfully used for dyeing cotton fabric to a wide range of colours from light soft to dark colour by using combination of mordants. Test samples exhibits excellent fastness to rubbing and pressing, (*Trianthema pentendra* plants), good for light fastness and good to excellent for washing (except pre-mordanting in *Trianthema pentendra*). It has further confirmed that the plants used in this study could be used for fabric finishing against microbes, skin transmission infection and clinical used clothes. Due to availability of natural extracts can served as noteworthy source of raw material in the future.

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