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Antibacterial Activity of Cationised Cotton Dyed with Some Natural Dyes

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Abstract

The present study was taken up as an exploratory study to test if some natural dyes have inherent antimicrobial activity with a view to develop protective clothing using these natural dyes. For enhancing the dyeability of cotton fabric with natural dyes, three cationising agents namely; Monochloro-S-Triazine, Quat 188 and Solfix E were used. Four natural dyes namely; Madder, Logwood, Cutch and Chelidonium majus were tested against common pathogens *Escherichia Coli, Staphylococcus aureus, Aspergillus favus* and *Candida albicans*. Chelidonium majus dye was most effective and showed maximum zone of inhibition there by indicating best antimicrobial activity against all the microbes tested. The color yield of dyed samples was evaluated using K/S measurements. The total difference ΔE CIE (L*, a*, b*) was also measured. The dyed samples were tested to washing, acid and alkaline perspiration, dry and wet rubbing and light fastness. The durability of the antibacterial effect of the dyed cotton fabric was measured after 5, 10 and 20 washing cycles.

Keywords: Cotton; Natural dyes; Antimicrobial; Cationisation; Antibacterial; Macromolecules

Introduction

Increasing the global competition in textile industries created a several challenges for textile researchers. There has been upsurge interest in apparel technology all over the world for much demanding functionality of the products like wrinkle resistance, water repelling, fade resistance and resistance to microbial invasion. Among these, development of antimicrobial textile finish is highly indispensable and relevant since garments are in direct contact with human body [1]. Antimicrobial textiles with improved functionality find a variety of applications such as health and hygiene products, specially the garments worn in contact to the skin and many medical applications such as wound dressings, inflectional control and barrier materials. Cotton fabrics provide ideal environment for microbial growth [2]. Several challenges have been created for apparel researchers due to increasing global demand in textile. Therefore, textile finishes with added value particularly for medical cloths are greatly appreciated and there is an increasing demand on global scale. The consumers are aware of hygienic life style and there is a necessity of textile product with antimicrobial properties. Several antimicrobial agents viz., triclosan, quarternay ammonium compounds and recently nano silver are available for textile finishing [3-11]. However, due to their cost and synthetic in nature which creates environmental problems, natural dyes in textile coloration are gaining significant momentum [12]. This new line of interest is due to stringent environmental standards imposed by many countries due to the usage of synthetic dyes which causes allergic reaction and toxicity. The use of natural products such as chitosan [13] and natural dyes [14-16] for antimicrobial finishing of textiles has been widely reported. Other natural herbal products, such as Aloe Vera, tea, tree oil, Eucalyptus oil and leaf extracts, can also be used for this purpose. Greater interest has emerged in the field of apparel technology using natural colorants, on account of their compatibility with deodorizing properties [12]. Comprehensive literature is available on natural dyes obtained from plants [17].

The aim of the present work is to study the effect of natural dyes (Madder, Logwood, Cutch and *Chelidonium majus*) on the antibacterial properties of cotton fabrics in presence of different cationising agents.

Experimental

Materials

Natural colouring matter: Colouring substance used in this work was extracted from Madder, Logwood, Cutch and *Chelidonium* plants.

Fabrics: Cotton fabrics, mill-scoured and bleached (130 g/m^2) were kindly supplied by Misr El-Beida Dyers Company, Kafr El-Dawar, Alexandria, Egypt.

Methods

Extraction of natural coloring matter: Madder (Table 1a):, Logwood (Table 1b): Cutch (Table 1c): and *Chelidonium majus* (Table 1d): were crushed to the powder form, and then the coloring matter was extracted using (20 g of the powder in 100 ml water) at the boil for one hour. At the end, the solution was filtered off and left to cool down.

Dyeing method:

Dyeing of cotton fabrics: Cotton fabric samples (8.5 g each) were dyed with the natural coloring matter extracted from Madder, Logwood, Cutch and *Chelidonium majus* at liquor ratio 1:40. Dyeing was carried out at pH 5.5 using sodium chloride 5 g/L for 60 minutes at 100°C. The fabric samples were entered to the dyeing solution in a water bath at 70°C then raised to 100°C. The fabrics were dyed for 60 minutes and the dyed samples were rinsed with cold water and washed for 30 minutes in a bath containing 3 g/L of non-ionic detergent at 45°C. Finally, the fabrics were rinsed and air dried.

Preparation of Monochloro-S-Triazine (Cationic agent) (Table 2a): Monochloro-S-triazine cationic agent were prepared using a similar method described previously [18]. N-ethyel 2-anilino

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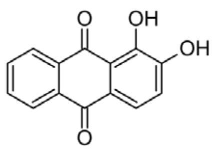
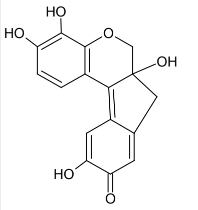
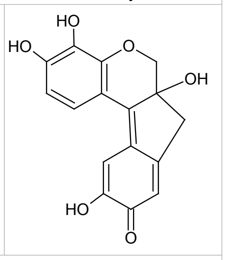
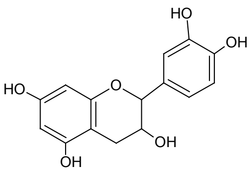
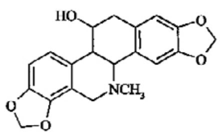
(a) Madder (dye 1)	(b) Logwood (dye 2)	
	Haematein	Haematoxylin
		
(C) Cutch (dye 3)	(C) *Chelidonium majus* (dye 4)	
		

Table 1: Structure of colouring groups of different plants used in this work.

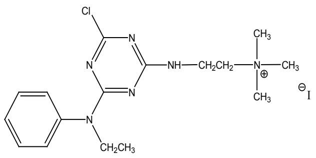
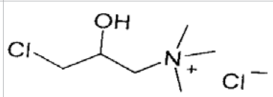
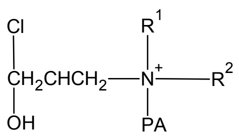
(a): Monochloro-S-Triazine (Cationic Agent)	(b): 3-chloro-2-hydroxy propyl trimethyl ammonium chloride (69%) solution in water (Quat 188)
	
(c): Polyaminochlorohydrin quaternary ammonium (Solfix E)	
 Where R^1, R^2=alkyl, and PA=polyamine	

Table 2: Structure of cationising agents.

4,6dichloro-1, 3, 5-triazine (0.01 mol) was dissolved in hot acetone (80ml) and N, N dimethyl ethylendiamine (.01 mol) in acetone (30 ml) was carefully added, after the initial reaction has subsided the mixture was refluxed for 1h, cooled and intermediate filtered off and washed with acetone. The product was dissolved in hot water, the solution carefully neutralized with sodium hydroxide solution and the precipitate filtered off and dried in vacuum below 40°C, and recrystallized from acetone. The free base was dissolved in acetone and a two molar excess of the appropriate alkyl halide (CH3I) the solution was refluxed for up to 30 h, after which the colour less precipitate was filtered off from the hot solution, washed well with acetone, and dried in a vacuum below 40°C to provide the desired product [18] shown in Table 2a.

Cationisation of cotton fabric:

Cationisation of cotton using the synthesized macromolecule cationising agent (CA): Mercerized cotton fabrics were treated with (CA) at concentration of 10% (owf) in a bath with liquor ratio of 1:50 at 40°C/1h. Then Na_2CO_3, 20 g/l was added and the temperature is raised to 80°C for 30 min.

The reaction was continued for further 2 hours at 80°C. Then the treated fabrics were neutralized using 2% acetic acid at 40°C/5 min. The treated fabrics were washed using 3 g/l nonionic detergent at 60°C/30 min, followed by washing with water and finally drying at room temp.

Cationisation of cotton using (Quat 188) (Table 2b): Solution of Quat (50 g/L, 69% solution) and sodium hydroxide 15 g/L was pad applied to the cotton fabric at 100% wet pick-up. To minimize reactant hydrolysis, the alkali was added to the padding bath just prior to application. The padded samples were then dried at 80°C for 5 minutes and cured at 150°C for 5 minutes, then samples were rinsed with water and neutralized with dilute acetic acid (2 g/L), rinsed with water followed by soaping with 3 g/L nonionic detergent (Hostapal® CV, Clariant) at the boil for 30 min, rinsed with water and air dried [19].

Cationisation of cotton using Solfix E (Table 2c): Cotton fabrics were treated with Solfix E at a concentration of 30% owf at 50°C in a bath with a liquor-to-goods ratio of 30:1 for 1 hour. Then 40 ml of sodium carbonate solution (12% w/v) was added to the treatment bath portion wise at the same temperature and the reaction was continued for further 2 hours. The bath was then drained, the fabric thoroughly rinsed with water followed by soaping with 3 g/l of nonionic detergent (Hostapal® CV. Clarient) at the boil for 30 min, rinsing with water and air dried.

Measurement of the antimicrobial activity using the agar diffusion disc method: A filter paper sterilized disc saturated with measured quantity of the sample is placed on plate containing solid bacteria medium (nutrient agar broth) or fungal medium (Dox S medium) which has been heavily seeded with the spore suspension of the tested organism. After inoculation, the diameter of the clean zone of inhibition surrounding the sample is taken as a measure of the inhibitory power of the sample against the particular test organism [20-22].

Testing

Color measurements of dyed fabrics: The color yield of both dyed and mordanted samples was evaluated by light reflectance technique using the Perkin-Elmer, UV/V Spectrophotometer (Model, Lambda 3B). The color strength (K/S value) was assessed using Kubelka-Munk equation.

Colour-difference formula ΔE CIE (L*, a*, b*):

The total difference ΔE CIE (L*, a*, b*) was measured using the Hunter-Lab spectrophotometer (model: Hunter Lab DP-9000), Reston, Virginia.

The total difference ΔE CIE (L*, a*, b*) between two colors each given in terms of L*, a*, b* is calculated from:

$$\Delta E^*=[(\Delta L^*)2 + (\Delta a^*)2+ (\Delta b^*)2]1/2$$

Where:

ΔE* value: is a measure of the perceived colour size of the colour difference between standard and sample and cannot indicate the nature of that difference.

ΔL*value: indicates any difference in lightness, (+) if sample is lighter than standard, (-) if darker.

Δa* and Δb* values: indicate the relative positions in CIELAB space of the sample and the standard, from which some indication of the nature of the difference can be seen.

Fastness properties: The dyed samples were tested to washing, acid and alkaline perspiration, dry and wet rubbing and light fastness according to AATCC standard methods.

Durability of antibacterial properties: The durability of the antibacterial effect of the dyed cotton fabric was measured after 5, 10 and 20 washing cycles.

Results and Discussion

Cationisation of cotton fabric using different macromolecules cationising agents

Cationisation of cotton fabric using Quat (Table 2b): Similar to the case of reactive dyeing of cellulosic fibers, the reaction of Quat with cotton fabrics would lead to the formation of ether linkage between Quat and cellulose. However, this desirable reaction will be affected with the side reaction that takes place between Quat and water, thus lowering the efficiency of the treatment process [23,24]. Pad-dry curing technique was used in this work for the pretreatment. The concentration of Quat 50 g/l were used for cotton treatment.

Cationisation of cotton fabric using Solfix E (Table 2c): Solfix E is a polyaminochlorohydrin quaternary ammonium polymer with epoxide functionality that can react with cellulose via ether formation in the presence of alkali. Therefore the reactivity of the reagent in aqueous alkaline medium during the pretreatment process is similar to that of reactive dyes during the dyeing process of cellulosic fibers. Especially regarding the hydrolytic effect of water that competes with ether formation.

Based on the well-documented cationisation information in the literature [25,26] the best pretreatment can be obtained by avoiding as much as possible the competing hydrolytic side reaction. To achieve this, the cotton fabric was impregnated with Solfix E in the pretreatment bath at 50°C for 1 h before performing the alkali fixation process.

The effect of cationic reagent concentration on the nitrogen content of the cationised cotton fabrics

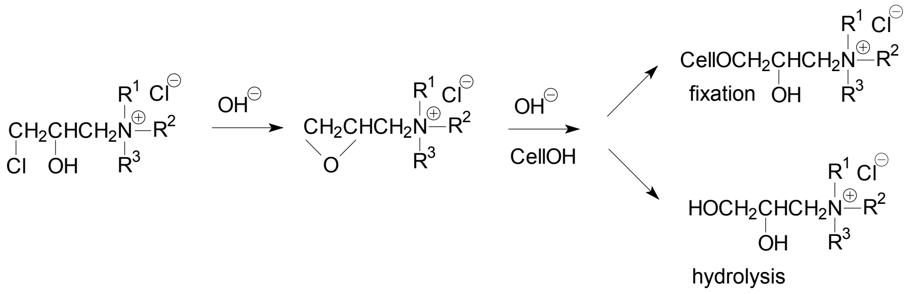
In a previous report 0.16% nitrogen content was obtained using more than 60 g cationic reagent per 100 g of cotton fabric, however, in this work 0.13% nitrogen content was achieved using less than 40 g of cationic reagent per 100 g of cotton fabric. The effect of cationic reagent concentration on the nitrogen content of the cationised cotton fabrics can be clear that as the nitrogen content of the cotton fabric increases as the concentration of cationic reagent increases. Leveling off at a reagent concentration of 30% owf. This result can be analyzed by the fact that the pretreatment process is a solid-liquid phase reaction, which proceeds by the movement of the cationic molecule from the liquid phase to the solid surface of the fiber by virtue of its ionic interaction with the surface negative charge of cotton fabric. The cationic reagent diffuses within the fabric and becomes covalently fixed to the available primary hydroxyl groups on the fibers, There for, it is expected that the process is diffusion-controlled and competes with hydrolytic effect of water. Similar behavior has been reported for cationisation using other cationic agents [27] (Scheme 1).

Effect of using different cationising agents on the color strength

Table 3 illustrated the results of using different cationising agent and its effects on the color strength and color data. For cotton samples dyed with natural coloring matter extracted from Madder, Logwood, Cutch and *Chelidonium majus*.

From Table 3, it can be observed that for dyed cotton samples, by using Monochloro-S-Triazine as cationising agent with madder and cutch plants, the highest color strength was achieved. Contrary, using of Solfix E and Quat gave the highest color strength with Logwood and *Chelidonium majus* plants, respectively.

L*, a* and b* are presented the lightness, red/green ratio and yellow/blue ratio, respectively. Table 3 showed that, L*, a* and b* data are in agreement with color strength results. Values of a* and b* located in the positive region for all dyed samples, indicating that the dyed samples had a yellowish-reddish color. Samples dyed with madder and logwood exhibited more reddish color while the others (Cutch and *Chelidonium majus*) had more yellowish color.



Where:

For CHTAC $R^1, R^2, R^3 = CH_3$

and for PAQAC R^1, R^2=alkyl and R^3= polyamine

Plants	Wave length	Different types of Cationising agents	(K/S) Color strength	Color data (CIE Lab)			
				L*	a*	b*	(ΔE)
Madder	500	Blank	0.76	67.69	12.45	7.04	0
		Quat*	0.75	68.01	12.97	7.14	0.62
		Solfix E**	1.85	55.18	10.19	14.89	14.94
		Cationising agent***	2.05	53.27	14.25	8.28	14.58
Logwood	400	Blank	2.93	55.62	6.28	19.53	0
		Quat*	3.22	52.72	5.92	15.96	4.61
		Solfix E**	6.77	42.12	6.72	15.81	14.01
		Cationising agent***	4.00	48.79	5.62	16.10	7.65
Cutch	400	Blank	1.75	63.66	11.59	19.18	0
		Quat*	2.17	58.41	12.12	18.19	5.37
		Solfix E**	2.68	57.28	11.46	17.60	6.57
		Cationising agent***	2.83	52.66	12.50	16.84	11.33
Chelidonium	400	Blank	3.24	58	3.25	21.45	61.94
		Quat*	3.24	57.4	4.05	19.59	60.81
		Solfix E**	1.28	69.62	2.03	17.06	71.71
		Cationising agent***	2.28	61.95	3.03	19.36	64.97

Quat*----- 3-chloro-2-hydroxy propyl trimethyl ammonium chloride (69% solution in water)

Solfix E**---Polyaminochlorohydrin quaternary ammonium

Cationising agent***------------ Monofunctional cationic agent of monochlorotriazine type

Table 3: Effect of using different Cationising agent on the color strength and color data for cotton fabric dyed with natural coloring matter extracted from Madder, Logwood, Cutch and *Chelidonium majus*.

Plants	Different types of Cationizing agents	Crocking		Acidic Perspiration			Alkaline Perspiration			Washing fastness			Light fastness	
		Dry	Wet	St.*	St.**	Alt.	St.*	St.**	Alt.	St.*	St.**	Alt.	40h	ΔE
Madder	Blank	4	3	3-4	4-5	4	4-5	4-5	4	4-5	4-5	4	5-6	2.62
	Quat*	3-4	2-3	3-4	4	4	4-5	4-5	4	4-5	4-5	4	4-5	4.96
	Sulfix E**	4	2-3	4	4	4	4-5	4-5	4	4-5	4-5	3-4	4	5.29
	Cationizing agent***	3-4	2	4	4-5	4	3-4	4	4	4-5	4-5	4	5-6	2.45
Logwood	Blank	4	2-3	3	4	3-4	3-4	4	4	4-5	4-5	4	4-5	4.68
	Quat*	3-4	2	3-4	4	4	4	4-5	4	4-5	4-5	4	5-6	2.94
	Sulfix E**	3-4	2	3	4	4	3-4	4	4	4-5	4-5	4	5	3.22
	Cationizing agent***	3-4	1-2	3	4	4	3	3-4	3	4-5	4-5	4	6	1.57
Cutch	Blank	3-4	3-4	4	4	4	4	4-5	4	3-4	4	3-4	6	1.28
	Quat*	3-4	3	4	4	4	3-4	4	4	3-4	4	3-4	6	1.79
	Sulfix E**	4-5	3-4	4	4-5	4	4	4-5	4	3	4	3-4	5	3.52
	Cationizing agent***	3-4	2	4	4-5	4	3-4	4-5	3	4	4	4	5-6	2.90
Chelidonium	Blank	4	2-3	2-3	2	3	2	2	3	2	2	3	5-6	2.19
	Quat*	3	2	2-3	2-3	3	2-3	2	3	3-4	3	3-4	6	1.43
	Sulfix E**	3-4	3-4	3	3	3	3-4	3	3-4	3-4	3	3-4	5-6	2.67
	Cationizing agent***	3-4	3	2-3	2	3	2-3	2	3	3	3	3	5-6	2.69

Table 4: Color fastness for cotton fabric dyed with natural coloring matter extracted from Madder, Logwood, Cutch and *Chelidonium*

St. *staining on cotton St. **staining on wool Alt. Alteration

From all colorimetric data, the difference in color data may be attributed to the difference in acidity for each plant used. Also, it depends on the concentration of the three cationic agents used in the Pre-treatment.

Color fastness results

From Table 4 it can be seen that, fastness properties of fabrics dyed with Madder, Logwood, Cutch and *Chelidonium* plants were assessed using blue scale. Comparing the fastness properties of the fabrics dyed with different plants, it is found that for all mordants, the dry crocking fastness is good, while the wet one is fair. Also, the acid and alkaline perspiration are good to very good. While, the washing fastness for the *Chelidonium* plant is the lowest one among the four plants. Finally, the light fastness is very good.

Antibacterial results

Table 5 shows the antibacterial properties of dyed fabrics against both *Staphylococcus aureus* (G+) and *Escherichia coli* (G-) bacteria expressed as inhibition zone. It is clear from Table 5 that all samples exhibit antibacterial properties against both types of bacteria. It is clear that the antibacterial properties against *Staphylococcus aureus* (G +) was found to be greater than that of *Escherichia coli* (G-) which

Sample	Inhibition zone diameter (mm/1 cm sample)					
	Escherichia coli (Gram -ve) bacteria			Staphylococcus aureus (Gram +ve) bacteria		
	5W	10W	20W	5W	10W	20W
Madder + quat	12	11	13	15	15	15
Madder + cationizing agent	12	12	14	17	15	15
Madder + solofix	13	12	15	17	16	17
Logwood + quat	14	12	13	16	16	14
Logwood + cationizing agent	13	12	13	16	16	15
Logwood + solofix	13	11	14	15	15	15
Cutch + quat	12	11	13	15	14	15
Cutch + cationizing agent	12	11	13	15	14	14
Cutch + solofix	11	12	13	15	15	15
Chelidonium + quat	12	12	12	16	16	14
Chelidonium + cationizing agent	13	11	13	16	16	15
Chelidonium + solofix	12	11	12	16	13	15

W: washing cycle

Table 5: Antibacterial activity of the dyed cotton with the four natural dyes after 5, 10 and 20 washing cycles.

can be attributed to the differences in the structure between the two types of bacteria. Table 5 also shows the durability of the antibacterial properties of the treated fabrics with different number of washing cycle (5, 10, and 20 washing cycles). It can be seen from Table 5 that the antibacterial properties are not affected by repeated washing up to 20 washing cycles but in some cases the antibacterial properties increased. It is obvious that the results of antibacterial properties against staph (G+) were found to be greater than that of *E. coli* (G-) as mentioned above due to the same reason of differences in the structure of bacteria. The antibacterial properties of the treated samples can be attributed to the presence of different types of cationising agents in all cases (Quat, Cationising agent and Solfix). The ability of these cationising agents to form true covalent bonds with cotton fabrics leads to the durability of the antibacterial properties against repeated washing.

Conclusion

Cationic groups were successfully formed on the surface of cotton using 3-chloro-2-hydroxy propyltrimethyl ammonium chloride, Polyaminochlorohydrin quaternary ammonium and Monochloro-S-triazine cationic agent, and the cationisation of fabrics strongly increases adsorption of the four natural dyes on the surface of the fibers due to the change of surface charge on the cellulose fibers. Cationised cotton dyed with these dyes exhibited stronger antibacterial activity. Cationic modification can be used for modification of cellulosic fibers to increase the antibacterial activity and increase the stability after repeated washing cycles.

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