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ASSESSMENT OF WASH DURABILITY OF EUCALYPTUS HERBAL EXTRACT DERIVED ANTIMICROBIAL FINISH

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Abstract

The living standards of people are increasing tremendously due to increase in wealth, fame and material goods. Textile market is at boom to fulfill the necessity and clothing desires of people. So lot of chemicals treatments are given to make best clothing for consumers which are hazardous to skin and cause various skin allergies. Herbal clothing becomes the centre of choice for solving health issue for which different plants may be an intelligent choice. Eucalyptus is widely known for its medicinal property from ancient time. Every part of Eucalyptus posses large amount of flavonoids, propanoids, alkaloids and tannins which constitutes the major medicinal property like antitumor, antiviral, anticancer, anti-inflammatory, antibacterial, antiseptic, anti-diabetic effect. So cotton fabric treated with eucalyptus herbal extract was tested for antimicrobial activity and further retention of treatment after washing. It was observed that herbal finish with 9% eucalyptus leaves extract resulted in 57.32% and 48.89% inhibition for *S. aureus* and *E. coli* respectively. The zone of inhibition was obtained 15 mm and 18 mm with *E. coli* and *S. aureus* respectively. The highly optimized condition like extraction time of 24 Hrs (Single merceration), 6% concentration of citric acid, treatment time (30 minutes), MLR (1:30) and 1:20 for exhaust and pad dry cure methods. Out of these two application methods pad dry cure method showed highest percent inhibition of microbes instead of exhaust method. The positive increment was observed with exhaust method for fabric comparison to pad-dry-cure method, weight and thickness of treated fabric increased as compared to control sample. Whereas strength and elongation remained constant after treatments. The higher inhibition of 23.09% and 28.29 % was observed after 15 wash cycle respectively in exhaust and pad-dry-cure method for *E. coli*. Similarly, 12.35 and 13.82 percent inhibition was recorded against *S.aureus* in exhaust and pad dry cure method with citric acid respectively.

Key words: Eucalyptus, cotton, aqueous herbal extract, *S.aureus*, *E.coli*, wash durability, wash cycle

Introduction

Eucalyptus globules which is commonly known as 'Safeda' in India belongs to Myrtaceae family which is native to Australia and Tasmania and introduced in India in 1843 as a fuel tree (Kesharwani *et al.,* 2018). It is evergreen, tall tree and widely known for its medicinal property from ancient time. Every part of eucalyptus posses large amount of flavonoids, propanoids, alkaloids and tannins which constitutes the major medicinal property like antitumor, antiviral, anticancer, anti-inflammatory, antibacterial, antiseptic, anti-diabetic effect. The essential oil extracted from fresh leaves consist of high content of Cineol (70%) which is very useful for medicinal purpose (Shiferaw *et al.,* 2019). Such essential oil acts as a antimicrobial agent (Winska *et al.,* 2019). In recent decades, people are moving toward organic therapeutic treatment instead of chemical, so use of eucalyptus in different area has been increased tremendously. Dramatic increase in population created shortage of food , cloth and land which is prime requirement of living being (Dhoka, 2018). To fulfill clothing demand of people within limited time, textile industries doing various chemical treatments which are very hazardous to the skin. Even people now prefer herbal clothing instead of chemically treated fabrics. Medical textiles are also more susceptible toward microbial infection. Herbal clothing is greatly in demanded for medical, regular and sanitary purpose. Cotton which is considered as most suitable fabric for clothing because of high cellulose content, biodegrable and skin friendly was selected for herbal clothing preparation. The present investigation is an attempt of preparing herbal cotton cloth using Eucalyptus extract.

Two most pathogenic bacteria *Staphylococcus aureus* (Gram positive) and *Escherichia coli* (Gram negative) were taken for present investigation. These organisms commonly present in environment and cause severe skin and gastrointestinal infection. These microbes also propagate rapidly in clothing because of cellulosic content of fabric which lead to discoloration and foul smell of fabric. Clothing finished with herbal extract will have therapeutic potential against such microbes. So aim of present investigation was to develop herbal clothing with antimicrobial activity and assess the retention of effect of finish after different wash cycles.

Materials and Methods

Preparation of cotton fabric: As per protocol of Rajendran *et al.* (2011) fabric was enzymatically desized and scoured. This treated fabric was further used for herbal finish.

Preparation of extract: Fresh leaves of eucalyptus were collected from CCSHAU campus and dried in shade. The dried leaves were ground to fine powder. To extract the plant material percolation method was used i.e. the powdered plant

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material was soaked in distilled water for 24 hours at a temperature of 60˚C and stirred occasionally. After 24 hours, the liquid was filtered using Whatmann No. 1 filter paper to obtain a clear filtrate. Herbal extract of 1-10% (10 g of plant source in 100 ml water) was prepared. The extract was used for determination of minimum inhibitory concentration showing antimicrobial activity.

Selection of microbial test culture: Two viable strains of *S. aureus* and *E. coli* bacteria were used for present investigation which was procured from Department of Animal Biotechnology, Lala Lajpat Rai university of Veterinary Sciences, Hisar.

Assessment of antimicrobial activity of herbal extract: The minimum inhibitory concentration of aqueous extract of eucalyptus leaves was determined. Test organisms were revived by adding 1 ml of inoculums in 4 ml of NB (Nutrient broth) at 37ºC for 24 hours in incubator. Serial dilution of 24 hours grown test organism (*S. aureus* and *E. coli*) was done and $1x10^{-7}$ was selected for detecting MIC (minimum inhibitory concentration) study. Various serial dilutions of broth, herbal extract and bacterial inoculums were prepared and incubated at 150 rpm at 37ºC for 24 hours. The MIC was determined in UV-visible spectrophotometer provided OD (optical density) using broth without inoculum as a test control and from each sample 50µl of solution was spread on agar plate incubates. The CFU/ml and reduction percentage of bacterial growth was calculated. The minimum zone of inhibition was determined by using parallel streak method (AATCC 143 1993). Similar procedure was used and five parallel streaks approximately 60 mm in length with 10 mm apart were made. The sterilized and herbal extract treated fabric of specific concentration was placed on streak and gently pressed to assure proper attachment with agar plate. Incubated the plate at 35+2ºC for 24 hour in incubator. Next day examined the incubated plates for interruption of growth along and beneath the specimen. The average width of a zone of inhibition along the streak on either side of the test specimen was calculated using following formula.

$$W = (T-D)/2$$

Where, W= Width of clear zone of inhibition in mm,

T= total diameter of test specimen and clear zone in mm,

D= diameter of the specimen in mm.

Application of herbal extract on fabric: Exhaust and Pad-Dry-Cure methods were used for finishing of cotton fabric with herbal extract, citric acid (6%) was used as a cross linking agent.

i. **Direct (Exhaust) method:** The scoured fabric was impregnated in the antibacterial solution in the ML Ratio: 1:30, concentration of antimicrobial stock solution: 7%, cross linking agent (Citric acid): 6% owf for one hour maintaining the temperature of 60^0 C with occasional stirring. The herbal treated cotton fabric was dried in shade.

ii. **Pad-dry-cure method**: For application of herbal finish through pad-dry-cure method the treatment solution with 9% concentration of stock solution , 6% cross linking agent (citric acid) owf was prepared and fabric was impregnated in the solution for 10 minutes maintaining the temperature of 60ºC with occasion stirring. After that the fabric was passed between the rollers of the pneumatic padding mangle at a speed of 3mt/min. at pneumatic pressure of 1 kg/cm^2. The fabric was shade dried and cured for 3 minutes at 140ºC.

Determination of preliminary data of treated and untreated cotton fabric: Three parameters i.e. fabric count, weight and thickness of fabric was determined. The fabric was conditioned prior to determination of fabric dimensions under standard test conditions i.e. relative humidity of 65 ± 2 percent and temperature of 27 ± 2^0C.

I. **Fabric count:** Pick glass with pointer was used to determine the fabric count of the woven cotton fabric using ASTM–D123 test method.

ii. **Fabric weight:** Samples were cut at random from the fabric with the help of round cutter of GSM and weight of fabric was determined using ASTM D3776-90 test method.

iii. **Fabric thickness:** Fabric Thickness Gauge was used to determine thickness of the samples using BS 2544: 1967 test method.

Assessment of antimicrobial property of herbal treated fabrics: The determination of bacterial resistance property was quantitatively determined using AATCC Test Method-100. Both control (untreated) and treated samples were tested against bacterial resistance. The colonies of bacteria were counted manually and total colony forming units were calculated using following formula:

Colony forming Unit (Counts/ml) = No. of colonies x Dilution factor x Inoculum of bacterial culture (ml)

The results were enumerated as percent reduction in bacterial count of the treated fabrics in comparison to the bacterial count of untreated control fabric and was calculated as

$$\text{Reduction in bacterial count (\%)} = \frac{\text{CFU /ml of the untreated control- CFU/ml of the treated fabric}}{\text{CFU/ml of untreated control}} \text{ X } 100$$

Assessment of durability of finish to washing: Herbal extract treated fabric was tested for wash durability using 'Launder-o-Meter'. The fabric was dipped in 2g/l neutral detergent and washed for 30 mints at 50±2ºC temperature which was taken as one cycle. After each cycle fabric was washed with cold water, shade dried and again placed in Launder-o-meter. Fabric was subjected to 5, 10 and 15 wash cycles. Samples were collected after each fixed washing cycle and further proceeded for antimicrobial efficiency.

Results and Discussion

On the basis of total phenolic contents (TPC) and maximum zone on inhibition (ZoI) to test bacteria, eucalyptus leaves extract was selected for application of antibacterial herbal finish on cotton fabric. Figure 1 shows different turbidity in test tube consisting of microbial inoculum, liquid broth and various concentration of eucalyptus leaves extract. The lower turbidity indicated higher inhibition of microbes and vice versa. It provides an estimation of effective concentration of herbal extract. Further parallel streak method was used for calculating inhibition zone. The data presented in Table 2 indicate 9% concentration of eucalyptus leaves extract showed minimum turbidity against *S. aureus* and *E. coli*. Hence these concentrations were selected for application of finish.

From the figure 2 and 3, it was depicted that with the increase of concentration of plant extract above 9 percent, the percent inhibition was lower and showed moderate response of range 46.56 percent inhibition. The good antimicrobial activity 98.89% was confined to 9% for both the test organisms. So present study provide MIC value of eucalyptus

leves extract against specified test organism. The leaves extract of eucalyptus might have possessed the antimicrobial activity against gram+ve and gram-ve bacterial due to presence of various bioactive components (Vecchio *et al.,* 2016). The variation in the antimicrobial activity of different concentration of hebal extract (1 g/100ml to 10g/100ml) may be due to to various concentration of nutrient broth, high concentration of bacterial inoculum, culture condition and incubation time and period.

On the basis of parallel streak method, the zone of inhibition observed with eucalyptus leaves extract was 18 mm and 15 mm for *S.aureus* and *E.coli*, respectively (Figure 4). Sterile fabric swatches of (2.2 inch) dipped in membrane filter (0.45 µM, millex-HV, PVDF) sterilized eucalyptus leaves extract showed a better inhibitory effect.The data presented in Table 4 indicate that fabric count of desized and scoured cotton fabric used for the study was 62x57 ends and picks per square inch, weighing 142 gm/m^2 and having 0.29 mm thickness. The data in Table 5 depict that all the parameters *i.e.* count and thickness of the fabric increased after treatment with herbal extracts when compared with untreated control fabric. It might be due to the absorption of herbal extracts by the fabric.

Further, comparison of fabric treated with exhaust method and pad-dry-cure method exhibited that the count, weight and thickness of fabric treated with direct method was towards higher side. The reason for the same might be that in pad-dry-cure technique, the fabric was under pressure between the rollers and excess liquor gets extracted during processing.

With the perusal of results obtained in Table 6, it was found that tensile strength of control fabric treated with *eucalyptus* leaves extract using direct method was 25.90 kg. The percent elongation was observed as 22.20. When cross linking agent i.e. citric acid was used, the same trend was observed as strength and elongation was found almost same and only very minor difference was observed. With the application of herbal extract with and without cross linking agent in pad-dry-cure method the tensile strength ranged between 25-26 kg The elongation of treated fabric was found 23-25percent.

It is evident from Table 7 that with the treatment of *eucalyptus* leaves extract, the bacterial count of treated cotton (control) fabric was $5.55x10^8$ and $4.15x10^8$ for *S. aureus* with exhaust and pad-dry-cure method, respectively. On treated sample with cross linking agent the bacterial count was observed $4.12x10^8$ and $3.65x10^8$ in direct and pad-dry-cure method, respectively. The bacterial count of treated cotton (control) fabric was $4.64x10^8$ and $4.05x10^8$ for *E. coli* with direct and pad-dry-cure method, respectively. On treated sample (with cross linking agent) the observed bacterial count was $3.85x10^9$ and $3.95x10^9$ in direct and pad-dry-cure method, respectively. Figure 5 indicate visual growth of bacteria under different treatment methods.

The effectiveness of herbal extract treatment was assessed after 5, 10 and 15 wash cycles. It is evident from the data in Table 8 and 9 that fabrics treated with the herbal extracts exhibited very good resistance to bacterial attack up to 15 wash cycles. Further the resistance to bacterial attack was higher when herbal treatment was given using pad-dry-cure method as compared to exhaust method. Cotton fabric coated with herbal extract and citric acid (6%) showed better response than that of without citric acid. The citric acid acts as a cross linking agent or intermediate between cotton fabric and herbal extract. Due to its binding nature it helps slow release of bound herbal extract on washing that is the reason that after 15 wash cycles there was higher percent reduction of bacteria than that of control which is without citric acid. Table 8 showed wash durability after 5th, 10th and 15th cycle of washing of herbal cloth.

The retention of antimicrobial effect was decreased after each cycle but very lower reduction observed after 10th cycle to 15th cycle. In exhaust and pad-dry cure method (without citric acid) 82.77% and 86.67% reduction respectively observed after 15th cycle in *S.aureus* bacteria. But good percentage reduction was observed in both methods respectively with citric acid that is 85.73% and 88.20% in *S.aureus*. Similarly table 9 showed 86.50% and 88.25% reduction in control condition in exhaust and pad-dry cure method and 87.15% in exhaust with 88.77% in pad-dry cure with citric acid for *E.coli* bacteria.

Conclusion

The research were done for developing an eco-friendly natural antibacterial finish from eucalyptus leaves extract for application on cotton fabric. The salient findings of this study indicate that *eucalyptus* leaves extract treated herbal finish showed high antimicrobial activity even after 15th wash cycle which can be used for preparing herbal clothing with multi time used. These herbal finishes can be further used for preparation various health and hygiene products like gloves, medical textile, packaging and even storage of things which are vulnerable to bacterial attack. The study also showed the improved long lasting antibacterial activity due to combine effect of citric acid and herbal extract in pad-dry-cure method.

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Table 1: Plant source selected for finishing of cotton

S. No.	Local Name	Common name
1.	English Name	Eucalyptus
2.	Common Name	Safeda
3.	Botanical Name	*Eucalyptus globulus*
4.	Family	Myrtaceae
5.	Part used	Leaves

Table 2: Minimum inhibitory concentration of herbal extract against *E. coli*

Concentration of herbal extract	Minimum inhibitory concentration (%)									
	1	2	3	4	5	6	7	8	9	10
Bacterial reduction (%)	01.59	03.30	05.26	07.72	08.94	11.64	15.56	34.80	48.89	46.56

Table 3: Minimum inhibitory concentration of eucalyptus leaves extract against *S. aureus*

Concentration Of herbal extract	Minimum inhibitory concentration (%)									
	1	2	3	4	5	6	7	8	9	10
Bacterial reduction (%)	00.86	30.37	32.71	34.18	38.85	40.56	43.11	50.01	57.32	52.49

Table 4: Preliminary data of the selected fabric

Fabric	Parameters		
	Fabric count (Ends x Picks per inch)	Fabric weight (gm/m^2)	Thickness (mm)
Cotton	62x57	142	0.29

Table 5: Effect of herbal finishing on preliminary properties of treated fabric

Treated fabric	Exhaust method			Pad-dry-cure method		
	Fabric Count (Ends x picks)	Fabric weight (gm/m^2)	Thickness (mm)	Fabric Count (Ends x picks)	Fabric weight (gm/m^2)	Thickness (mm)
Scoured (Control)	66x60	180	0.35	63x61	165	0.34
With cross linking agents	63x61	175	0.35	62x60	166	0.34
Without cross linking agents	62x60	172	0.37	63x60	161	0.35

Table 6: Effect of herbal finishing on physical properties of treated fabric

S. NO.	Treated Fabric	Exhaust (Direct) method		Pad-dry-cure method	
		Strength (Kg)	Elongation (%)	Strength (Kg)	Elongation (%)
1.	Scoured (Control)	25.90	22.20	26.30	23.50
2.	Without cross linking agents	26.88	21.90	25.50	25.70
3.	With cross linking agents	26.89	21.89	26.23	23.56

Table 7: Antibacterial activity of herbal extract treated fabrics

S. NO.	Cross linking agent	*S. aureus* (CFU/ml)		*E. coli* (CFU/ml)	
		Exhaust method	Pad-dry-cure method	Exhaust method	Pad-dry-cure method
1.	Scoured (Control)	$5.55x10^8$	$4.15x10^8$	$4.64x10^8$	$4.05x10^8$
2.	Without cross linking agents	$5.50x10^8$	$4.23x10^8$	$4.69x10^8$	$4.00x10^8$
3.	With cross linking agents	$4.12x10^8$	$3.65x10^8$	$3.85x10^9$	$3.95x10^9$

Table 8: Wash durability of herbal finishing against *S. aureus*

S.NO.	Treated Fabric	Percent Reduction in Bacterial Count					
		Wash cycles duration (Exhaust method)			Wash cycles duration (Pad-dry-cure method)		
		5^{th}	10^{th}	15^{th}	5^{th}	10^{th}	15^{th}
1.	Without cross linking agents	98.55	86.25	82.77	97.85	94.76	86.67
2.	With cross linking agents	99.48	88.12	85.73	98.64	96.88	88.20

Table 9: Wash durability of herbal treated fabrics against *E. coli*

S.NO.	Treated fabrics	Percent Reduction in Bacterial Count					
		Wash cycles duration (Exhaust method)			Washcycles duration (Pad-dry-cure method)		
		5^{th}	10^{th}	15^{th}	5^{th}	10^{th}	15^{th}
1.	Without cross linking agents	98.66	93.88	86.50	98.46	94.19	88.24
2.	With cross linking agents	99.01	94.10	87.15	99.28	96.53	88.77

Fig 1: Different test tube consisting different concentration of herbal extract, liquid broth and microbial inoculum showing different turbidity

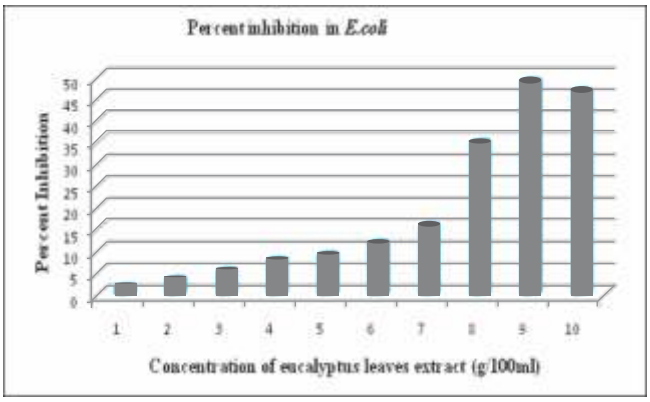

Fig 2: Minimum inhibitory concentration of eucalyptus leaves extracts against *E.coli*

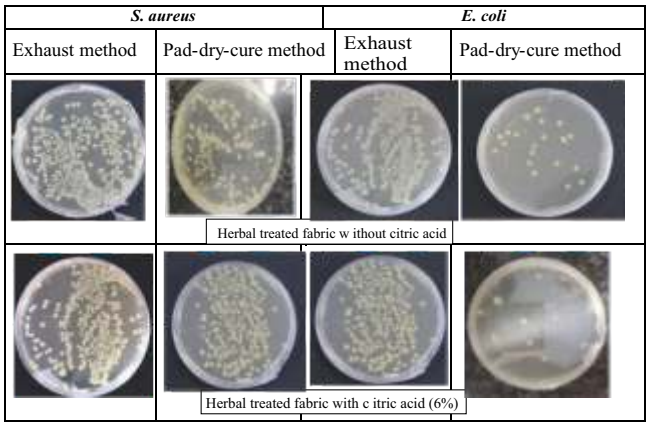

Fig 5: Antibacterial activity of herbal extract treated fabrics

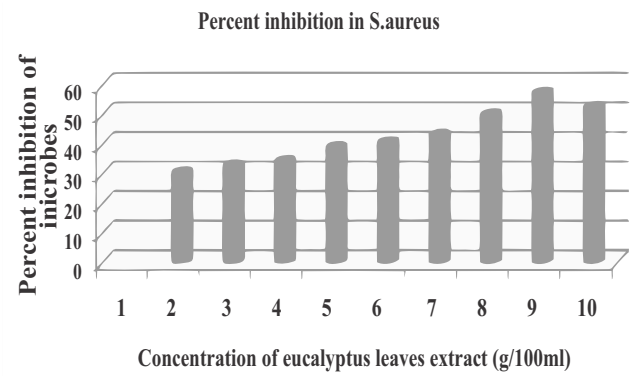

Figure 3: Minimum inhibitory concentration of eucalyptus leaves extracts against *S. aureus*

(A) *S. aureus* **(B) *E. coli***

Fig 4. Maximum zone of inhibition showed by eucalyptus leaves extract against *E. coli* and *S. aureus*