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Development of antibacterial finish for cotton with *neem* leaves extract and assessment of wash durability

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ABSTRACT : The textiles finished with various chemical substances are hazardous to human body. Due to ill effects of synthetic finishes and increasing awareness about health and hygienic products, the textiles developed with herbal plant extracts are being preferred by the green-minded consumers. In present study, cotton fabric finished with herbal *neem* extract was assessed for antimicrobial efficacy. It was evident that 7 percent *neem* leaves extract showed 70.01 and 50.50 percent inhibition for *Staphylococcus aureus* and *Escherichia coli*, respectively. Aqueous extract of *neem* leaves was found inhibitory agent and showed 18 mm and 16 mm zone of inhibition to growth of *E.coli* and *S.aureus*, respectively. The various optimized conditions i.e. 24 hrs extraction time, 6% concentration of citric acid (cross linking agent), 30 minutes of treatment time and MLR 1:30 for direct method and 1:20 for pad-dry-cure method were used which exhibited highest bacterial resistance. The count, weight and thickness of fabric treated with direct method were towards higher side as compared to pad-dry-cure method. The treated cotton fabric exhibited 87.98 and 89.11 percent inhibition to bacterial attack up to 15 wash cycle in exhaust and pad-dry-cure method, respectively against *E.coli*, similarly, 81.01 and 89.50 percent against, respectively *S.aureus*. Overall the application of herbal extract did not have any adverse effect on the strength and elongation of treated cotton fabrics.

Key words *: Neem* herbal extract, cotton, *S.aureus*, *E.coli*, wash durability

Sustainability is the prime requisite for present scenario in agriculture, environment, textile and clothing industries. An increasing population has created stress on the agricultural land, products, and housing. Such population explosion generated a necessity to utilize more chemically synthesized textiles which adversely affect human health by generating skin allergies and diseases. A lots of textile based materials used in hospitals or hotels are conductive to cross infection or transmission of disease caused by micro-organisms (El-Shafei *et al.,* 2017). So people are moving towards safe clothing having antimicrobial properties. Medical textiles including apron for operation, post-operation task, clothing of patient and medical practitioners are prone towards microbial infection. Antimicrobial agents prevent growth or kills microbes to control their negative effects e.g. odour, deterioration and pungent smell

(Kurshid *et al.,* 2015). Although human skin acts as barrier to the invading microbes but clothing are in direct contact to the skin so hygienic and antimicrobial clothing function as an alternative supportive barrier against infective microbes. Natural fibers possess inherent property which acts as a host for development and growth of micro-organism. Recently many antimicrobial finished textiles are available in market which is synthetic based and hazardous to human health (Sathianarayanan *et al.,* 2010). So there is necessity to develop eco-friendly clothing with antimicrobial finish. The demand of antimicrobial finishes is tremendously increasing due to increasing awareness of people regarding health and hygienic lifestyles (Rosida *et al.,* 2018). Cotton fabric is most widely used for clothing due to its super properties such as higher cellulosic content, comfortable and skin friendly. Cotton finished with herbal finishes are eco-friendly, biodegradable and safe for wearing. Antimicrobial agents prevent growth or kills microbes to control their negative effects e.g. odour, deterioration and pungent smell (Kurshid *et al.,* 2015)

India has vast diversity of medicinal plants which are widely used for medicine and preparation of other formulations. The commonly grown plants like *neem*, aloe vera, *tuls*i, clove, cardamom, eucalyptus and curry leaves posses antimicrobial activity but out of these *neem* is very effective against wide range of microbes and hold retention of antimicrobial activity after repeated washing. It is easily available and cheaper source contributed largely to human health for its curative properties. *Neem* which is scientifically known as *Azadiracta indica* belongs to family *Meliaceae* and is one of the most prominent tropical plant due to its immense pharmaceutical property. Extracts from leaves, bark, fruit, flowers and fruit seed of *neem* plants exhibit antibacterial properties. Since ancient times, every part of neem plant is used in ayurvedic and homeopathic medicine (Reshma *et al.*, 2018).

The major need of herbal coated clothing is to protect human body from infective microbes. Microbes are present everywhere, some of them are useful and some are harmful to human beings. Out of million microbes, most common pathogenic bacteria effecting human are *Staphylococcus aureus* (Gram+ve) and *Escherichia coli* (Gram-ve) which cause intestinal infection and invade human body through air, water when come in contact with contaminated surface. Microbes also deteriorate the functional and inherent property of fabric by feeding on cellulosic content and cause staining and foul smelling of fabrics. Clothing infested from microbes which are in direct contact with skin instigates several skin disorders, respiratory and other diseases. Natural herbal extract based finishes has therapeutic potential and do not impose side effect to the human beings (Vastrad and Byadgi, 2018). The aim of present investigation was to develop an eco-friendly natural antibacterial cotton fabric using *neem* leaves extract and determine the antibacterial efficacy against *E.coli* and *S.aureus*.

MATERIALS AND METHODS

1. Preparation of cotton fabric: For the preparation of cotton enzymatic desizing and scouring were done as per protocol of Waran *et al.* (2013) and Rajendran *et al.* (2011)

respectively. The scoured fabric was further used for application with herbal finish.

2. Preparation of extract: Aqueous extract of shade dried *neem* leaves was prepared by percolation method i.e. the powdered plant material was soaked in distilled water for 24 hours at a temperature of 60ÚC with occasional stirring. After 24 hours, the liquid was filtered using Whatmann No. 1 filter paper to obtain a clear filtrate. Herbal extract of 1-10% (1-10 g of plant source in 100 ml water) was prepared. The extract was used for determination of minimum inhibitory concentration showing antimicrobial activity.

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

4. Assessment of antimicrobial activity of herbal extract: The susceptibility of micro-organisms to antimicrobial agents was determined by minimum inhibitory concentration. Test bacteria were revived by adding 1 ml of inoculums into 4 ml of NB (Nutrient broth) at 37ºC for 24 hours in incubator. Serial dilution of 24 hours grown test organisms (*S. aureus* and *E. coli*) was done and $1x10^{-7}$ was selected for 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 spreaded on agar plate incubates. The CFU/ml and reduction percentage of bacterial growth was calculated. The minimum zone of inhibition was determined using disc diffusion method. The inoculum was properly spread on sterile nutrient agar plate and the sterile paper discs dipped in different concentration of herbal extract were placed on it and incubated at 37ºC for 24 hours and evaluated the susceptibility of test organism.

5. Application of herbal extract on fabric: Exhaust and pad-dry-cure methods were used for finishing of cotton fabric with herbal extract, citric acid was used as a cross linking agent.

i. Direct (Exhaust) method: The scoured fabric was immersed in antibacterial solution of 7 percent concentration of herbal extract using 1:30 material to liquor ratio (MLR) and 6 percent (owf) cross- linking agent for one hour maintaining temperature of 60^0 C with occasional stirring in water bath. The herbal treated fabric was shade dried.

ii. Pad-dry-cure method: The fabric was immersed in the antimicrobial solution consisting of 7 percent *neem* extract, 6 percent (owf) cross linking agent with MLR 1:20 for 10 minutes maintaining temperature of 60^0 C with occasional stirring. The extract impregnated fabric was passed between the rollers of the pneumatic padding mangle at a speed of 3m/min. at pneumatic pressure of 1 kg/cm^2. The finished fabric was shade dried and cured at 140ºC for 3 minutes in hot air oven.

6. Determination of preliminary data of treated and untreated cotton fabric: 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^{0}C.Three parameters i.e. fabric count (ASTM–D123), weight (ASTM D3776-90) and thickness (BS 2544: 1967) were determined using standard test methods.

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

Colony Forming Units (CFU)=No. of colonies X Dilution factor X Inoculum of bacterial culture (ml)=Counts/ml

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

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

8. Assessment of durability of finish to washing: Durability of herbal finish to washing was evaluated using 'Launder-o-Meter'. The treated fabric was dipped in 2g/l neutral detergent and washed for 30 minutes at 50±2°C 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. The herbal treated samples were subjected to 5, 10 and 15 wash cycles and further preceded for antimicrobial efficiency.

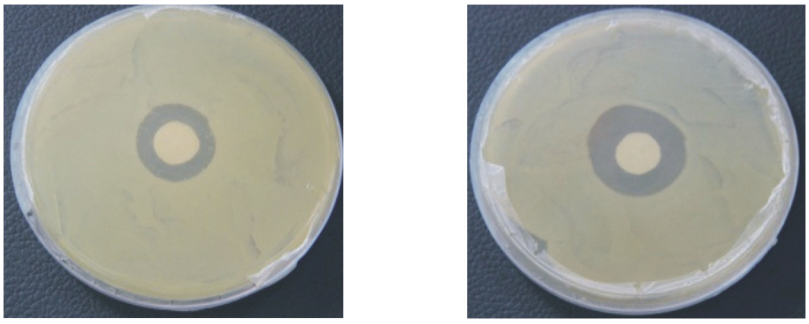
RESULTS AND DISCUSSION

The *neem* leaves extract exhibited 167.24 mg GAE/g total phenolic contents (TPC) and 16 and 18 mm zone on inhibition (ZoI) to test bacteria i.e. *S. aureus* and *E.coli,* respectively. Hence, it was selected for application of antibacterial herbal finish on cotton fabric (Table1 and Fig.1).

Table 1. Plant source selected for finishing of cotton

Local Name	Neem
Common name	Indian lilac
Botanical name	*Azadirachta indica*
Family	Meliaceae
Part used	Leaves

The data presented in Figure2 and 3 indicate that 7 percent concentration of *neem* leaves extract showed maximum turbidity against *S. aureus* (70.07%) and *E.coli* (50.50%). Hence these concentrations were selected for application of finish on cotton fabric. From the Figure1 and 2, it can be inferred that with the increase of concentration above 7 percent, the percent inhibition lowered and showed moderate range (50-62%) of inhibition. The good antimicrobial activity was obtained at 7 percent



S.aureus ***E.coli***

Fig. 1. Maximum zone of inhibition showed by herbal extract against *E.coli* and *S. aureus*

for both the test organisms. So present study provided MIC value of *neem* leves extract against specified test organism. The leaves extract of *neem* showed antimicrobial activity against gram+ve and gram-ve bacteria. It may be due to presence of various biactive components which are in line with study done by Parashar *et. al.* (2018). The variation in the antimicrobial activity at different concentrations of hebal extracts (1 g/100 ml to 10g/ml) may be due to variation in concentration of nutrient broth, high concentration of bacterial inoculum, culture condition and incubation time.

The data presented in Table 2 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 0.29mm thickness. It was observed that all the parameters i.e. count, weight and thickness of the fabric increased after treatment with herbal extract when compared with scoured fabric. It might be due to the absorption of herbal extract 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 got extracted during processing.

With the perusal of results presented in Table 3, it was depicted that tensile strength of scoured fabric was 25.72 kg and the percent elongation was 22.87 percent. The tensile strength of fabric treated with *neem* leaves extract (without cross linking agent) using direct method was 25.70 kg whereas the percent elongation was observed as 23.00. When cross linking agent i.e. citric acid was used with herbal finish, the same trend was observed as strength and elongation

Table 2. Effect of herbal finishing on preliminary properties of treated fabric

Treated Fabrics	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)	62x57	142	0.29	62x57	142	0.29
Without cross linking agent	62x60	172	0.37	63x60	161	0.36
With cross linking agent	63x60	170	0.36	62x60	160	0.34

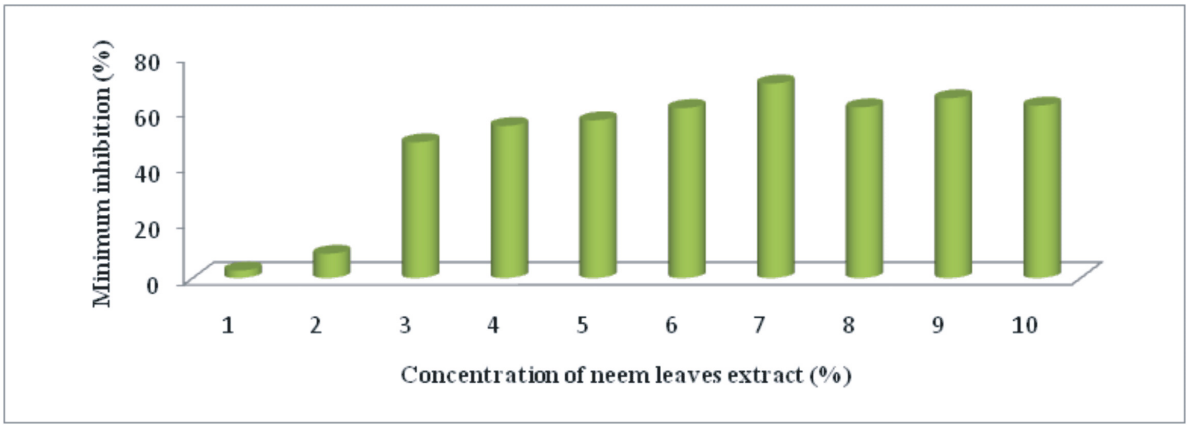


Fig. 1. Minimum inhibitory concentration of *neem* leaves extracts against *S. aureus*

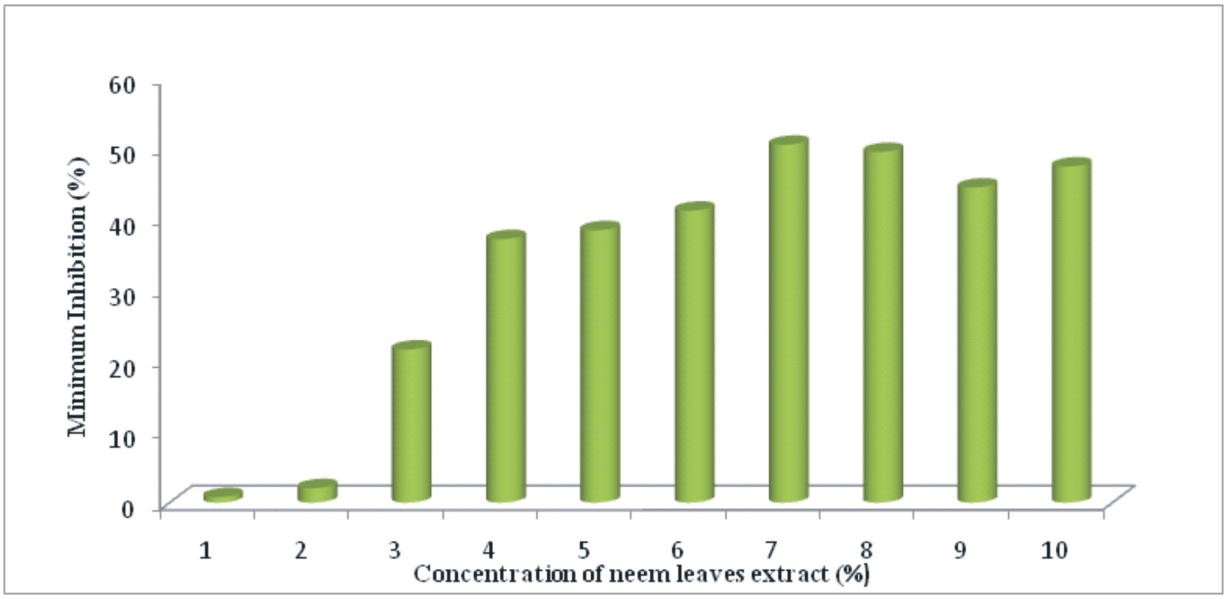


Fig. 2. Minimum inhibitory concentration of *neem* leaves extracts against *E.coli*

Table 3. Effect of herbal finishing on physical properties of treated fabric

Treated Fabrics	Exhaust (Direct) method		Pad-dry-cure method	
	Strength (Kg)	Elongation (%)	Strength (Kg)	Elongation (%)
Scoured (Control)	25.72	22.87	25.72	22.87
Without cross linking agent	25.70	23.00	24.80	24.40
With cross linking agent	25.70	22.00	25.20	24.70

was found almost same and only very minor decrease was observed in percent elongation. When herbal extract was applied with and without cross linking agent by pad-dry-cure method the tensile strength ranged between 24.80 kg to 25.20 kg and the elongation of treated fabric was found 24.40 and 24.70 percent.

It is evident from Table 4 that the

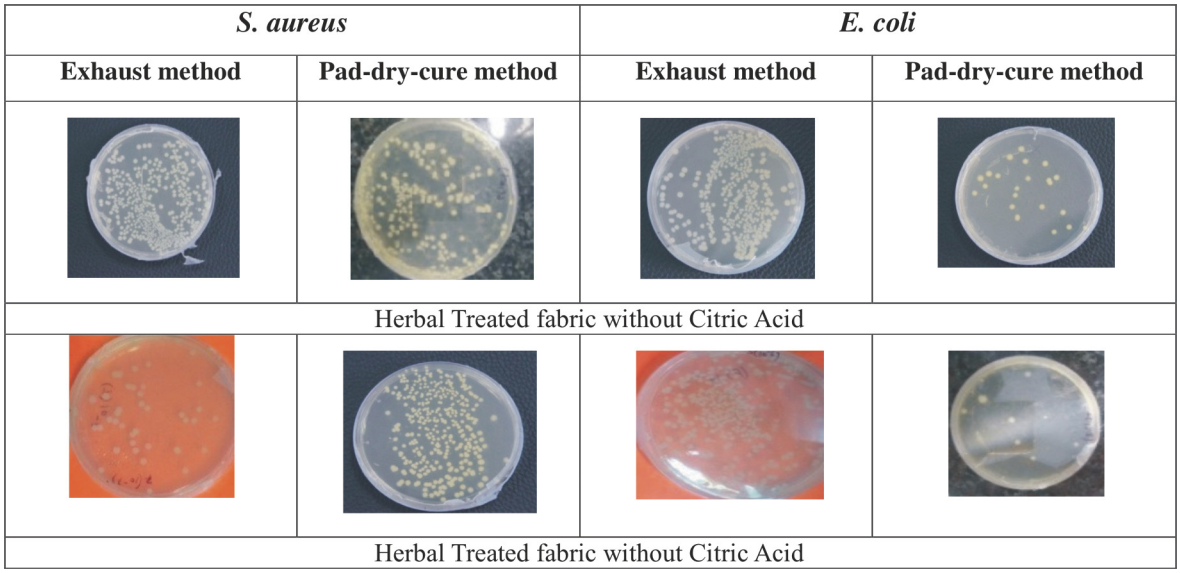


Fig. 4. Antibacterial activity of herbal extract treated fabrics

bacterial count of *neem* leaves extract treated cotton (scoured) fabric was 6.80 x10^8 and 5.42 x10^8 for *S. aureus* with exhaust and pad-dry-cure method, respectively. With the application of herbal extract without cross linking agent, the bacterial count observed was 6.65x10^8 and 5.05x10^8 for *S. aureus* with exhaust and pad-dry-cure method, respectively. When herbal extract was applied with cross linking agent, the bacterial count was observed 4.95x10^8 and 4.60x10^8 in direct and pad-dry-cure method, respectively. The bacterial count of treated (without cross linking agent) fabric was 3.45x10^8 and 2.90x10^8 for *E.coli* with direct and pad-dry-cure method, respectively. On treated sample (with cross linking agent) the observed bacterial count was 3.50x10^9 and 3.75x10^9 in direct and pad-dry-cure method, respectively. Decrease in bacterial count of fabric treated using *neem* leaves extract and *neem* leaves extract along with cross-linking agent indicated the increase in bacterial resistance. However, *neem* leaves extract with cross linking agent provided more resistance against the growth of both the test bacteria.

Figure 4 indicates that in exhaust and pad-dry-cure application method there was visible difference in growth of both the bacteria. The more decrease in bacterial count of both the bacteria was observed in pad- dry-cure method, the reason for the same might be that

Table 4. Antibacterial activity of herbal extract treated fabrics

Treated Fabrics	*S. aureus*		*E. coli*	
	Exhaust method	Pad-dry-cure method	Exhaust method	Pad-dry-cure method
Scoured	6.80 x10^8	—	5.42 x10^8	—
Without cross linking agent	6.65x10^8	5.05x10^8	3.45x10^8	2.90x10^8
With cross linking agent	4.95x10^8	4.60x10^8	3.50x10^9	3.75x10^9

the more antibacterial constituents got attached on fabric surface from the concentrated padding bath which subsequently inhibited the growth of bacteria.

The effectiveness of herbal extract treatment was assessed after 5, 10 and 15 wash cycles. It is evident from the data in Tables 5 and 6 that fabrics treated with herbal extract, with or without citric acid, exhibited very good resistance to bacterial attack up to 15 wash cycles. It was inferred that further the resistance to bacterial attack was higher when herbal treatment was given using pad-dry-cure method as compared to exhaust method. The cotton fabric coated with herbal extract and citric acid 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 which might be the reason that after 15 wash cycles there was higher percent reduction of bacteria than that of control which is without citric acid. The results of the study are in consonance with Fadhel *et. al.* (2012) who also found that with the increase in washing cycles the bacterial resistance also reduced.

CONCLUSION

Efforts were done for developing an eco-friendly natural antibacterial finish from *neem* leaves extract for application on cotton fabric. The findings of this study suggested that *neem* leaves extract treated herbal finished fabric possessed high antimicrobial activity even after

Table 5. Wash durability of herbal finishing against *S. aureus*

Treated fabrics	Percent Reduction in Bacterial Count					
	Wash cycles duration(Exhaust method)			Wash cycles duration (Pad-dry-cure method)		
	5th	10th	15th	5th	10th	15th
Without cross linking agent	96.12	87.32	78.98	98.25	90.65	87.31
With cross linking agent	97.24	85.32	81.01	99.35	92.36	89.50

Table 6. Wash durability of herbal treated fabrics against *E. coli*

Treated fabrics	Percent Reduction in Bacterial Count					
	Wash cycles duration(Exhaust method)			Wash cycles duration (Pad-dry-cure method)		
	5th	10th	15th	5th	10th	15th
Without cross linking agent	97.55	92.77	83.98	98.66	93.58	86.24
With cross linking agent	98.26	93.54	87.98	99.38	94.68	89.11

15 wash cycle which can be used for preparing herbal clothing with multiple time use. These herbal finished fabrics can be further used for preparation of 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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REFERENCES

El-Shafei, A., El-Bisi, K.M., Zaghloul, S. and Refai, R. 2017. Herbal textile finishes-Natural antibacterial finishes for cotton fabric. *Egypt Jour. Chem.* **60** :161-80.

Fadhel, B.B., Aissi, A., Ladhari, N., Deghrigue, M., Chemli, R. and Joly, JP. 2012. Antibacterial effects of two Tunisian Eucalyptus leaf extracts on wool and cotton fabrics. *Jour. Tex. Ins.* **103** :1197-04.

Khurshid, M.F., Ayyoob, M., Asad, M. and Shah, S.N.H. 2015. Assessment of eco-friendly natural antimicrobial textile finish extracted from aloe vera and neem plants. *Fib. Text. East. Europe.* **6** : 120-23.

Parashar, G. Sutar, N. and Sanap, S. 2018. Antibacterial activity of mixture of leaf extracts of Neem (*Azadirachta indica L.*) and Tantani (*Lantana camara*). *Internat. Jour. Pharma. Sci. Res.* **9** : 2545-49.

Rajendran, R., Sundaram, S.K., Radhai, R. and Rajapriya, P. 2011. Bioscouring of cotton fabric using pectinase enzyme its optimization and comparison with conventional scouring process. *Pakistan Jour. Bio. Sci.* **14** : 519-2011.

Rajendran, R., Radhai, R., Savitha, R., Sundaram, S.K., Kongarasi, K., Geethadevi, C. and Rajalakshmi, V. 2017. A study on the antimicrobial property of the cotton fabric imparted with *Michaelia champaca* leaf extract loaded nanoparticles. *Internat. Jour. Pharm. Sci. Res.* **8** : 1235-44.

Reshma, A., Priyadarisini, V.B. and Amutha, K. 2018. Sustainable antimicrobial finishing of fabrics using natural bioactive agents- A review. *Internat. Jour. Life Sci. Pharma Res.* **8 :** 10-20.

Rosida. A., Sharifah, S.S., Asdren, Z. and Nizam. 2018. Antibacterial and wash durability activity of cotton fabric treated with *Punica granatum* and *Citrus limon* peel extracts against skin pathogens. *Malaysian App. Bio. Jour.* **47** : 129-35.

Sathianarayanan, P.M., Bhat, V.M., Kokate, S.S. and Walunj, E.V. 2010. Antibacterial finish for cotton fabric from herbal products. *Indian Jour. Fibre Text. Res.* **35** : 50-58.

Vastrad, J.V. and Byadgi, S.A. 2018. Eco-friendly antimicrobial finishing of cotton fabric using plant extracts. *Internat. Jour. Curr. Micro. App. Sci.* **7 :** 284-92.

Vigneswaran, C., Ananthasubramanian, M., Anbumani, N. and Kandhavadivu, P. 2013. Ecofriendly approach to improve pectinolytic reaction and process optimization of bioscouring of organic cotton textiles. *Jour. Eng. Fibers Fabrics.* **8** : 121-33.

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