Application of *Justicia adhatoda* L. leaf extract as antibacterial finish on cotton fabric

SHAZIA MEHTAB, MANISHA GAHLOT and ANITA RANI

Department of Clothing and Textiles, College of Home Science, G.B. Pant University of Agriculture and Technology, Pantnagar-263145 (U.S. Nagar, Uttarakhand)

ABSTRACT: Number of synthetic antimicrobial finishes are available commercially which adversely affect the environment. The present study considers the possibilities of anti-bacterial finish by using plant extracts. The methanolic extract of *Justicia adhatoda* L. leaf was prepared through soxhlet apparatus. Earlier *Justicia adhatoda* L. was named as *Adhatoda vasica* L. Prepared extract was used as finish with citric acid to increase the absorbency of fabric. Finish was applied on organic cotton fabric by pad-dry-cure method in different concentrations through padding mangle. All treated and control samples were subjected to check the antibacterial activity against most representative class of human pathogens. Lyophilized bacterial culture (*Staphylococcus aureus* and *Escherichia coli*) were procured from MTCC IMTECH (Chandigarh). The results indicated that *Justicia adhatoda* L. had the good antibacterial activity against gram positive bacterial strain as compare to gram negative bacteria.

Key words: Antibacterial activity, bacterial reduction, colony forming unit, Methanolic extract, organic Cotton

The inherent properties of textile material provides room for growth of microbes because it is worn next to skin which contains the right combination of organic matter, moisture and give favorable temperature for bacterial growth (Veni and Mani, 2012; Patel and Desai, 2014). Microbial infestation poses danger to both living and non-living things. Apparent smell from the inner garments such as socks, spread of diseases, staining and degradation of textiles are caused due to pathogenic infection (Bajpai and Vankar, 2007).

The term antimicrobial refers to a wide range of technologies that can provide varying degrees of protection against bad effect of microorganisms. Antimicrobials are used to control undesirable growth of bacteria, fungi, mold, mildew and algae. Their control reduces or eliminates the problems that are caused due to microorganisms. Antimicrobial agents vary in their chemical nature, mode of action, cost, substrate to which they are applied, durability and their impact on people and the environment (White and Monticello, 2002).

Plants have continued to play an important role for the maintenance of human health from ancient time (Natarajan and Dhas, 2014). Although synthetic antimicrobial agents effectively inhibit the growth of microbes, but most of them are toxic and can cause adverse effects on human health as well as on environment. Most of plant extracts played an effective role against both gram-positive and gram-negative bacteria depending on the type of components present in the plant extract. Hence, the researches on eco-friendly antimicrobial agents and their application on various textile products gain worldwide importance (Babu and Ravindra, 2014).

MATERIALS AND METHODS

Justicia adhatoda L. leaves have been collected from "Medicinal Plant Research and Development Centre", Pantnagar (Uttarakhand, India). Botanical identification of the samples was confirmed taxonomically. GOTS (Global Organic Textile Standard) certified organic cotton fabric of plain weave with 80/72 thread count, 108.50 g/m^2 weight per unit area and 0.20 mm thickness was procured from "J. C. Overseas INC", Jaipur (Rajasthan). All the bacterial cultures, viz. *Staphylococcus aurous* (MTCC 902)*, Escherichia coli* (MTCC 443) were obtained from the Microbial Type Culture Collection (MTCC), Institute of Microbial Technology (IMTECH), Chandigarh, India.

Extraction process

The leaves were cleaned with water and dried in shade for approximately 8-10 days. When leaves were completely dried, they were ground to fine powder and sieved. Dried powder (20 g) was extracted with methanol

(100 ml) by using soxhlet apparatus at 65°C for 70-72 hours. The obtained extracts were filtered by using filter paper (Whatmann no. 1) and the remaining solvent was evaporated under reduced pressure at 40ºC with the help of rotary evaporator. The dry fractions were stored in refrigerator at 4ºC in airtight glass bottles (Wang and Weller, 2006; Rathinamoorthy and Thilagavathi, 2012).

Finishing of the fabrics using extracts

Prior to finish fabric was washed with dilute soap solution to remove the impurities so that they do not interrupt in further finishing process. The fabric was washed in a bath containing distilled water (1:10 M:L ratio) and 2 g/l non-ionic detergent at 60°C for 30 min. After washing the fabric was thoroughly rinsed with water to remove the soap solution completely and dried at room temperature (Banupriya and Maheshwari, 2014).

Three different concentrations (5%, 10%, and 15%) of *Justicia adhatoda* leaf methanolic extract was mixed with 2% citric acid as crosslinking agent, subsequently it was added to distilled water at 1:20 M:L ratio. For finishing of fabric pad dry cure method was used. Samples were dipped in prepared solution for 30 min at 40°C subsequently padding was carried out in padding mangle by maintaining the temperature with 3.5-4 kg/cm^2 pressure. Curing of finish were carried out at 100°C for 15-20 min in hot air oven and dried at room temperature (Saranya and Bagyalakshmi, 2016).

SEM analysis

Fabric morphology was characterized by SEM (Scanning Electron Microscope, Model LEO 435 VP).

Assessment of the antibacterial activity by AATCC Test Method 100-2004

Bacterial cultures were revived for assessment of antibacterial activity. For the preparation of inoculum single loopful of isolated colonies was inoculated into 4 ml sterilized nutrient broth and incubated at 37±2 ºC (99 ± 3 °F) for 24 hours.

For quantitative test treated and control swatches were inoculated with the incubated test organisms. After incubation, 100 ± 1 ml sterile distilled water was added and serial dilutions of water and plate were prepared. Different dilutions were prepared and results were reported at 10^6 for control and treated fabric. After 48 hours incubation at 37 ± 2°C bacterial count was recorded by direct counting from agar plate and percentage reduction was calculated using following equation:

$$\% \text{ Reduction } (R) = 100 (C-A) / C$$

A= the no of bacteria recovered from the treated specimen over desired contact period time and C= the number of bacteria recovered from the untreated control specimen immediately after inoculation.

Assessment of wash durability

The washing fastness of antimicrobial treatment was evaluated after repetitive washing by using Launder-O-Meter according to AATCC test method 61(2a)-2011. The washing duration was 45 min and a solution of liquid detergent (0.56±0.1 %), total liquor 150 ml was used at 49 ± 3°C. The fabric used for the test was 50 X 150 mm. Subsequently, the treated cotton fabric was rinsed three times in distilled water at 40 ± 2°C and air dried. One wash of Launder-O-Meter is considered as five home washings as mentioned in to AATCC Test Method 61-2009.

Statistical analysis

All experiments were performed in triplicates. Data were expressed as mean value. For the estimation of significant difference in antibacterial activity between before and after washing paired t-test has been used. It was considered statistically significant if value of $p < 0.05$ at 5% level of significance.

RESULTS AND DISCUSSION

SEM *analysis*

In the SEM picture (Figure 1(b)) fibre texture was altered as compared to control fibre (Figure 1(a)). A layering of plant extract can be observed on treated fabric but these changes did not significantly alter the fabric properties like thermal insulation, air permeability and moisture regain etc. Heliopoulos. *et al*. (2013).

Antibacterial analysis

Significant bacterial reduction was observed with 15% concentration of plant extract treated fabric samples. More than 85% and 70% bacterial reduction was observed against gram positive bacteria and gram negative bacteria respectively as shown in Table 1. Control and blank samples did not show any resistance against both the bacteria.

Durability of antibacterial activity

The bacterial reduction after each laundering cycle is listed in Tables 2 and 3 for *Staphylococcus aureus* and *Escherichia coli* respectively. Difference in antibacterial activity was considered highly significant as all the p-values were less than 0.05.

Activity retention

To find out the retention of washed samples on the basis of antibacterial activity after each laundering cycle, percent retention was calculated by comparing before and after laundering bacterial reduction. Activity retention of laundered samples was considerably decreased due to less durability of finish.

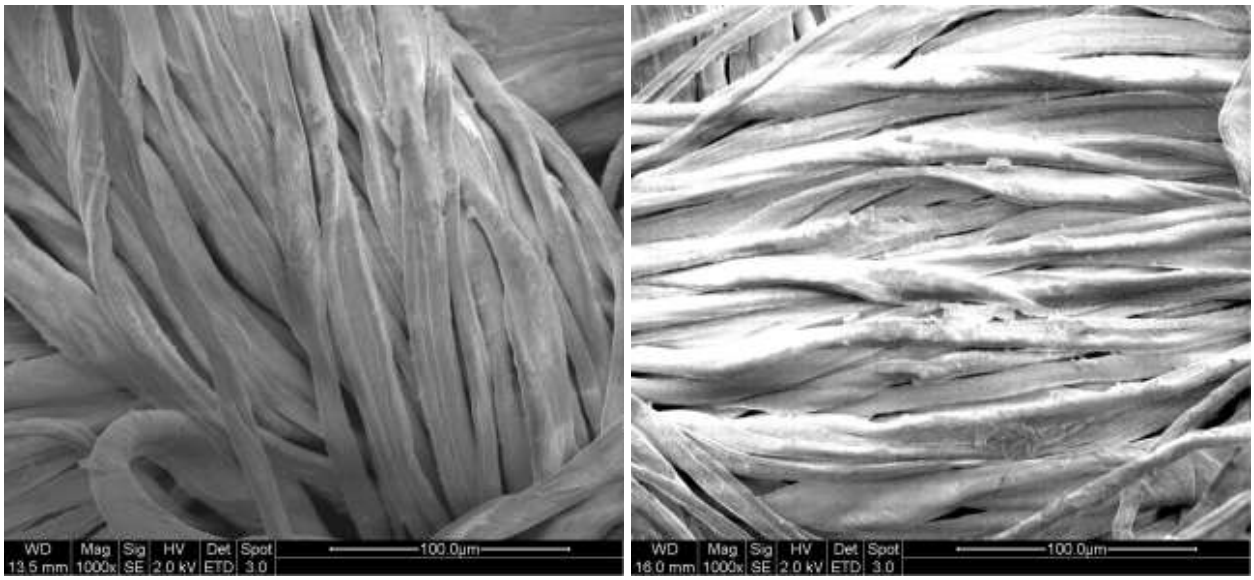

Fig. 1: SEM of (a) organic cotton fabric (control) and (b) *Justicia adhatoda* extract treated fabrics using the direct method of application

Table 1: Quantitative assessment of antibacterial activity

Samples	*Staphylococcus aureus* (gram +ve)				*Escherichia coli* (gram-ve)			
	CFU on fabric at zero contact time	R% at zero contact time	CFU on fabric at 24 h contact time	R% at 24 h contact time	CFU on fabric at zero contact time	R% at zero contact time	CFU on fabric at 24 h contact time	R% at 24 h contact time
Control	$6.25x10^6$	-	$5.87x10^5$	-	$1.627x10^7$	-	$8.68x10^6$	-
Blank	$7.62x10^6$	-21.92	$6.21x10^5$	-5.79	$1.918x10^7$	-17.88	$9.71x10^6$	-11.86
I	$2.08x10^6$	66.72	$1.24x10^5$	78.87	$6.127x10^6$	62.34	$2.11x10^6$	75.69
II	$1.57x10^6$	74.88	$1.01x10^5$	82.79	$5.04x10^6$	69.02	$1.930x10^6$	77.76
III	$0.08x10^6$	87.20*	$4.84x10^4$	91.75*	$2.125x10^6$	86.93*	$1.006x10^5$	88.41*

I: 5% concentration of plant extract, II: 10% concentration of plant extract, III: 15% concentration of plant extract, Blank: Treated with methanol, Control: No treatment given, *highest bacterial reduction and R%: bacterial reduction.

Table 2: Durability assessment of antibacterial activity against *Staphylococcus aureus*

		Percentage of bacterial growth reduction					
		Number of laundering cycle			Activity retention (%)		
Samples	Before washing	1	2	3	1	2	3
Treated	89.83	58.11	42.28	-	64.68	47.06	-
p-value	-	0.000216	0.00551	-	-	-	-

Table 3: Durability assessment of antibacterial activity against *Escherichia coli*

		Percentage of bacterial growth reduction					
		Number of laundering cycle			Activity retention (%)		
Samples	**Before washing**	**1**	**2**	**3**	**1**	**2**	**3**
Treated	77.10	53.22	-	-	69.02	-	-
p-value	-	0.0035	-	-	-	-	-

CONCLUSION

It can be concluded that organic cotton fabric samples treated with *Justicia adhatoda* significantly reduced the growth of bacteria on textile material. It can be used as an effective antibacterial finish, which is non-toxic and sustainable. The finish can be applied on bed linens, pillows, socks, dresses, gloves, inner garments, air filters, curtains, bath-mates, uniforms etc. the durability of the finish can be increased using microencapsulation and nano-technology.

REFERENCES

Babu, K.M. and Ravindra, K.B. (2014). Bioactive antimicrobial agents for finishing of textiles for health care products. *The journal of The Textile Institute*, 2-10.

Banupriya, J. and Maheshwari, V. (2014). Comparison between herbal and conventional methods in antimicrobial finishes. *International Journal of Fiber and Textile Research,* 4(2): 41-43. Retrieved on March 23, 2015 from http://www.urpjournals.com

Bajpai, D. and Vankar, P.S. (2007). Antifungal textile dyeing with mahonia napaulensis D.C. leaves extract based on its antifungal activity. *Fibers and polymers*. 8(5): 487-494.

Heliopoulos, N.S., Papageorgiou, S.K., Galeou, A., Favvas, E.P., Katsaros, F.K. and Stamatakis, K. (2013). Effect of copper and copper alginate treatment on wool fabric. Study of textile and antibacterial properties. *Surface and Coatings Technology*. 235:24-31.

Natarajan, V. and Dhas, A.S.A.G. (2014). Phytochemical Composition and in vitro Antimicrobial, Antioxidant Activities of Ethanolic Extract of Leptadenia reticulata [W&A] Leaves. *Middle-East Journal of Scientific Research*. 21 (10): 1698-1705.

Patel, M.H. and Desai, P.B. (2014). Nano hernal grafted medical textiles for production of antimicrobial textile. *International Journal of Fiber And Textile Research*. 4 (3): 49-54.

Rathinamoorthy, R. (2012). Optimisation of process conditions of cotton fabric treatment with Terminalia chebula extract for antibacterial application. *Indian Journal of Fibre & Textile Research*. 38: 293-303.

Saranya, N. and Bagyalakshmi, G. (2016). Antimicrobial finishing on cotton fabric with mint stem extract. *Fibre2fashion*. Retrieved from www.fibre2fashion.com.

Veni, K. and Mani, A. (2012). Glabra and a Study of the Anti-Microbial and Thermal Properties on Cotton Fabrics for Eye Syndrome. *Journal of textile and apparel technology and management*. 7 (3): 10-9.

Wang, L. and Weller, C.L. (2006). Recent advances in extraction of nutra- ceuticals from plants. *Trends in food science & technology*, 7: 300-301.

White, W.C. and Monticello, R.A. (2002, Oct. 24-26). *Antimicrobial Performance of Medical Textiles*. Paper presented at IFAI Exposition. Charlotte, U.S. Retrieved July 22, 2016 from http://tetradyn.com/products/bioprotection/Antimicrobial_Performance_of_Medical_Textils.pdf

Received: June 14, 2016
Accepted: July 27, 2016