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Antiseptic treatment for human foot wounds using piper betel extract finished bamboo/cotton fabrics

K Ramya[a] & V Maheshwari

Department of Costume Design & Fashion, PSG College of Arts & Science, Coimbatore 641 014, India

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In this study, the extracts of *Piper betel* leaves have been obtained and finished on to the natural and regenerated bamboo/cotton knitted fabrics. The finished fabrics are assessed for the antibacterial activity against the selected pathogens *Staphylococcus sp., Bacillus sp., Klebsiella sp., Pseudomonas sp.,* and *Proteus sp.,* which are commonly present in the human foot wounds. The obtained results are compared with the untreated fabrics and also with the fabric samples treated with two antibiotics, viz Cefixime and Levofloxacin which are commonly used for the treatment of the foot wounds. The herbal extract treated fabrics show good antibacterial activity against the pathogens, which makes them to have an antiseptic property. To enhance the wash durability of the finished samples the herbal extracts are microencapsulated and their wash durability is assessed using the standard methods.

Keywords: Antiseptic finishing, Bamboo/cotton fabrics, *Bacillus sp., Klebsiella sp.,* Microencapsulation, *Piper betel*, *Pseudomonas sp., Proteus sp., Staphylococcus sp.,* Wash durability

Recently there has been upsurge interest in apparel technology all over the world for much demanding functionality of the products like wrinkle resistance, water repellence, fade resistance and resistance to the microbial invasion[1]. Today, with the increasing awareness of environmental concerns, an important legislation on eco-toxicological considerations has been introduced. Among various finishes, development of antimicrobial textile finish is highly indispensable and relevant since garments are in direct contact with the human body. The antimicrobial (antiviral, antibacterial and antifungal compounds) property plays a vital role, as they are directly in contact with the human skin[2]. The carbohydrates in the cellulosic fabrics serves as the nutrients and the energy sources for the growth of micro organisms, which leads to unpleasant odour, stain, discoloration and reduction in the strength properties[3]. The products like innerwear and socks are in contact with the human skin, which are highly prone to sweat for a long period of time that may lead to the skin infections and discomfort to the wearer[4]. For these reasons, it has been the essential property for a textile to resist or minimize the bacterial growth during use and storage. The textile finishes with added values particularly for medical cloths are greatly appreciated and the rapid growth in the field of medical textiles and their end uses has generated many opportunities for the application of antimicrobial finishes.

Recognising the importance of plant materials as antimicrobial agents, research has been initiated in the areas of producing bioactive textiles for the protection of wearer from common microbes causing cross infections. Natural antimicrobial agents are non-toxic and non-allergenic and do not cause the problems of microbial resistance[5]. There is a growing interest in plants with antimicrobial activity. Scientists are increasingly becoming involved in the screening of such plants with the aim of establishing their potential antimicrobial effects and identifying the compounds responsible for the antimicrobial properties[6]. Hence, there is an indispensible need for more investigation in the development of ecofriendly antibacterial agents extracted from plants for textile applications. Although certain natural antibacterial agents are available at present, only few studies have been explored for their antibacterial activity on textile materials and also there is need to generate progressive and consolidated data on antimicrobial finished product of textiles particularly in the preparation of medical cloths[7].

The present study aims at developing an ecofriendly and natural antimicrobial finish on textile fabrics using the extracts of *Piper betel* (Linn). It belongs to family *Piperaceae* and is commonly known as '*Paan*'. It is extensively grown in Srilanka, India, Thailand, Taiwan and other Southeast Asian countries. The parts of *Piper betel* utilized are leaves, roots, stems, stalks and fruits. Betel leaf has broad antiseptic properties and to date there have been no reports in the literature on the occurrence of cases of

[a]Corresponding author.
E-mail: ramyakathiresan@yahoo.com

allergy to the plant. The use of betel leaf as an antiseptic is increasing. The active substances in betel leaf are phenol and its derivatives. The phenolic derivatives contained in betel leaves have a five-fold greater antibacterial potency than phenol itself[8-10]. As garments are subjected to washing, the wash durability of finishes is a major issue. Even though many of the herbal extracts like tulsi leaves, pomegranate, cassia senna, etc have shown good antimicrobial property after applying on textile fabrics, their wash durability is poor[2,11]**.** Hence, in this study microencapsulation technique has been used to fix the herbal extracts on the fabrics. The extracts were applied to the bamboo/cotton knitted fabric by direct application method and microencapsulation method. An extensive study was conducted to assess the antiseptic efficacy against the selected human foot wounds and their wash durability by employing standard test methods [12].

Experimental

Bamboo-cotton (50:50) knitted fabric (single jersey) was selected for the application of antiseptic finish. The leaves of the *Piper betel* were collected from Virudhunagar District, Tamilnadu.

Selection of Human Foot Wound Pathogens

The test organisms like *Staphylococcus sp., Bacillus sp., Klebsiella sp., Pseudomonas sp.,* and *Proteus sp.,* which are present in the human foot wounds[13,14] , were procured from a diagnostic centre at Coimbatore, Tamilnadu, India.

Preparation of Plant Extracts

The collected fresh and healthy leaves of *Piper betel* were washed with tap water followed by distilled water and dried in shade. The dried leaves were ground into a fine powder and then used for the study. For extraction, each 3g of dry powder was taken and mixed into 50mL of 80% methanol. The container was closed and kept for overnight. After overnight incubation, the extract was filtered through filter paper and evaporated at room temperature to concentrate the extract. This methanol extract was used for the application on fabrics.

Selection of Controls

For the assessment of the antiseptic property, two controls namely positive control and the negative control (untreated) were selected. Two commercially available antibiotics namely Cefixime and Levofloxacin commonly used for the treatment of the foot wounds were selected.

Finishing of Fabrics

Following two methods were used for the finishing of fabrics.

(i) Direct Application Method—The prepared methanol extract was directly applied on the bamboo/cotton fabric using pad-dry-cure method.

(ii) Microencapsulation Method—Microcapsules containing herbal extracts were prepared employing 3% of sodium alginate. Equal proportion of sodium alginate and extracts was prepared separately and then sprayed into calcium chloride solution by means of a sprayer. The formed droplets were retained in calcium chloride for 15 min to harden the capsules. The microcapsules were obtained by decantation and repeated washing with isopropyl alcohol followed by drying at 45ºC for 12h. The microcapsules were then used for finishing on the bamboo/cotton fabrics by exhaustion method using 8% citric acid as binder. The fabric was kept immersed in the solution for 30 mins at 50ºC. After finishing, the fabric was removed, squeezed, dried at 80ºC for 5 min and finally cured at 120ºC for 2 min.

Assessment of Antibacterial Efficacy

Antibacterial efficacy was assessed using AATCC 147 standards. The bamboo/cotton fabric samples were cut in rectangular shape with 25 × 50 mm for analysis. Sterile bacteriostasis agar was dispensed into petri dishes. Broth cultures (24 h) of the test organisms were used as inoculums. Using sterile inoculation loop, the test organisms were streaked, considering 5 lines with 4 mm width over the surface of the agar plate. Pre-sterilized samples were placed over the culture inoculated agar surface by using sterile forceps. After placing the samples, all the plates were incubated at 37 ºC for 18- 24 h. After incubation, the plates were examined for the zone of bacterial inhibition around the fabric sample. The size of the clear zone was used to evaluate the inhibitory effect of the sample.

Assessment of Wash Durability

The antibacterial activity of the finished bamboo/cotton fabric was tested according to EN ISO 20645 against *Staphylococcus sp., Bacillus sp., Klebsiella sp., Pseudomonas sp.* and *Proteus sp.* Nutrient agar plates were prepared by pouring 15 mL of media into sterile petri dishes. The agar plates were allowed to solidify for 5 min and 0.1% inoculum was swabbed uniformly and allowed to dry for 5 min. The finished fabric with the diameter of 2.0 ± 0.1 cm was placed on the surface of medium and the plates were kept for incubation at 37 ºC for 24 h. At the end of incubation, the zone of inhibition formed around the fabric was measured in millimeters and recorded.

Results and Discussion

Antiseptic Efficiency of Finished Samples

The antiseptic finishing was performed for the untreated fabric samples, samples treated with the selected antibiotics and the samples treated with the extracts of *Piper betel* and their efficacy was assessed against the selected human foot wound pathogens (Table 1 and Fig.1).

The untreated fabrics show clear growth of the pathogens under them with no inhibition. The treatments with the *Piper betel* inhibit microbial growth as is evident from the absence of growth under the treated samples. The *Piper betel* treated samples show an antisepsis benefit ranging from 53% to 86% of the antisepsis benefit of the antibiotics (Cefixime and Levofloxacin) against *Staphylococcus sp., Klebisiella sp., Pseudomonas sp.,* and *Proteus sp.*, but as in the case against *Bacillus sp.,* the *Piper betel* treated samples show 50% higher antisepsis than the Cefixime antibiotic.

Microencapsulation of Herbal Extract and Wash Durability of Samples

Table 2 shows that the antiseptic activity of the *Piper betel* extracts finished samples is good against all the selected wound pathogens when directly applied onto the fabrics, but it shows poor wash durability. This clearly indicates that the extracts are only coated on the surface of the fabrics without any bonding on to the fabrics, which is removed during repeated washings. In order to overcome this problem and to enhance the wash durability of the finished fabrics, the effective microencapsulation method is followed.

Table 2 also indicates that the fabrics treated with the herbal extracts by microencapsulation show good resistance against the wound pathogens. At the same time, it is found to have good wash durability up to 10 and 20 washes and gradually decreases upto 30 washes. The antiseptic activity in case of microencapsulated method is not affected by the laundering as compared to that of the direct application method, due to the sustained release of the antibacterial compounds from the herbal extracts because of the microencapsulation technique.

The *Piper betel* extracts have found to have good antibacterial activity against the wound pathogens

Table 1—Antiseptic activity of herbal extract treated fabrics against the control samples

Sample	Zone of inhibition, mm				
	Staphylococcus sp.	*Bacillus sp.*	*Klebsiella sp.*	*Pseudomonas sp.*	*Proteus sp.*
Untreated (negative control)	0	0	0	0	0
Samples treated with Antibiotic I – Cefixime (positive control)	43	23	42	33	44
Antibiotic II – Levofloxacin (positive control)	50	62	65	51	63
Piper betel	30	35	35	35	38

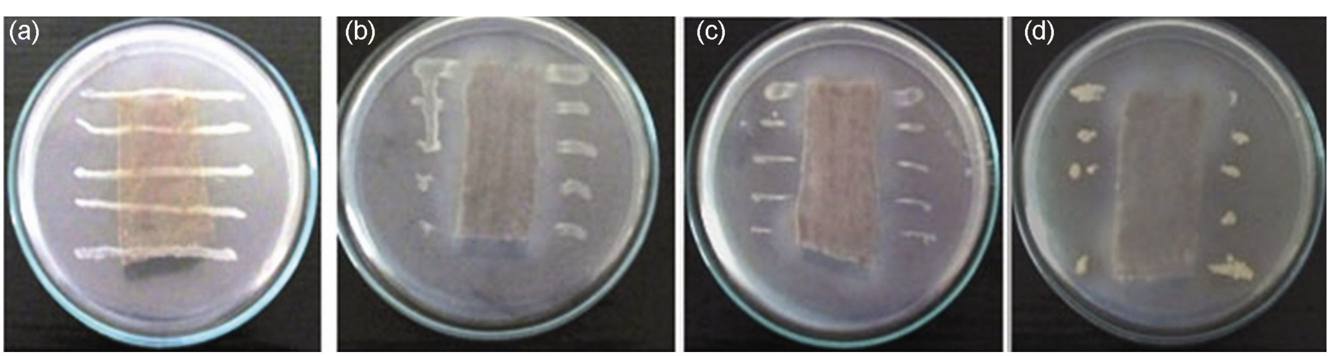

Fig. 1—Zone of inhibition of fabrics (a) untreated sample, (b) *Piper betel* extract treated sample, (c) Cefixime treated sample, and (d) Levofloxacin treated sample.

Table 2—Wash durability of *Piper betel* extract treated fabrics by direct application and microencapsulation method

Piper betel extract treated sample	No. of washes	Zone of inhibition, mm				
		Staphylococcus sp.	*Bacillus sp.*	*Klebsiella sp.*	*Pseudomonas sp.*	*Proteus sp.*
Direct application	0	30	35	35	35	38
	10	22	22	22	22	22
	20	0	0	0	0	0
	30	0	0	0	0	0
Microencapsulation	0	35	38	39	38	45
	10	33	36	25	30	25
	20	30	35	24	28	24
	30	28	35	23	0	0

which acts as an antiseptic. The extracts are directly applied on to the fabric by pad-dry-cure method, but the only disadvantage is that the wash durability of the directly treated fabrics is poor. But the fabrics treated by microencapsulation method show good antiseptic property and wash durability. Hence, the direct application method is suitable for the fabrics being used for single use applications like bandage gauze, surgical cloths, baby diapers, sanitary napkins, and the fabrics that need repeated launderings like garments, bed linens, socks, hospital fabrics, etc. can be microencapsulated. *Piper betel* is easily available all over the world, and there is a good opportunity for the implementation and the commercialization of the herbal extracts finished textile fabrics to act as an antiseptic products. As the extracts are purely from the natural resources, the finished fabric is ecofriendly and satisfies the social and the environmental needs.

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