Microencapsulation of Herbs for Wound Care Textiles

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Among the various antimicrobial agents available today, the natural plant products comprise the major segment. The natural antimicrobial agents derived from herbs could be the promising candidates for medical and healthcare textiles, mainly due to their ecofriendliness. Thousands of herbs, which have antibiotic, therapeutic, and wound healing properties are available in India. In the present work, the antibacterial activites of *Ficus bengalensis*, *Areca* catechu and *Cleome viscosa* were studied *in vitro* on different wound causing pathogens. Both the morphological and biochemical analysis of the wound causing pathogens were carried out before they were isolated, purified, and identified. The wound pathogens were identified to belong to *Staphylococcus, Escherichia, Klebsiella, Serratia, Pseudomonas, Bacillus* and *Proteus* genus. Different combinations, such as 1:1:1, 1:2:1, 1:1:2 of the selected herbs were prepared and assessed for their antibacterial activity. The herbal combination of 1:1:1 exhibited higher zone of inhibition against *Escherichia coli* and *Staphylococcus aureus*. The herbal extract of this combination (1:1:1) was microencapsulated and applied onto the air plasma treated polypropylene nonwoven fabric. The antibacterial activity and the wash durability of the microcapsule-finished fabric were evaluated.

Keywords: Superficial wounds, Bacterial predominants, Polypropylene, Microencapsulation, Ionic gelation, Antibacterial activity.

The use of textiles in medical field has a long tradition. Among the various textile materials used in medical textiles, bandages and wound dressings gained high popularity which find major applications in wound care and prevention of chronic wounds[1]. Despite the fact that traditional textiles fulfilled primary qualities such as biocompatibility, flexibility etc. there is an increasing need for the use of synthetic fibers. As the global demand for traditional textiles especially, for cotton increases day by day, it is high time to replace cotton by synthetic textile materials in wound healing and in prevention of chronic wounds. Textiles are carriers of bacteria and fungi. Control of bacterial growth on the fabric can be achieved (a) by finishing of the textile surface using resins, to fix the antibacterial agents or (b) by grafting the antimicrobial agents on the cellulosic chain, i.e. Cotton, Viscose, Lyocell etc[2].

As consumers are more aware of hygiene and the harmful effects of microorganisms, the demand for antimicrobial textiles is increasing. Although, there are many medicinal plants rich in antimicrobial agents viz. *Aloe vera*, teak, Eucalyptus and Tulsi (*Ocimum basilicum)*, the

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study of their application onto textiles is very limited and not well documented. [1]

When compared to modern synthetic antimicrobial agents, the herbal products have relatively lower incidence of adverse reactions. Due to their reduced cost, the herbal products find themselves as an economic and eco friendly alternative to synthetic antimicrobial agents[3]. It is estimated that there are about 5, 00,000 species of plants on the Earth. Many of these plants possess medicinal properties. Most of them contained secondary metabolites, of which at least 12,000 have been isolated. The wound healing property of many of these plant materials have been used from age old days. Some of these plants also contain compounds such as terpenoids and tannins which were responsible for plants' odor and pigments respectively[4].

Recent developments on plant based bioactive agents have opened up new avenues in this area of research. Many papers reported about the details of applying natural agents, such as neem extracts, natural dyes, chitosan and other herbal products (tulsi, *Aloe vera*, teak tree oil, etc.) on textile substrates [5].

Antibacterial activity of combination of herbs has not been much established and therefore, in this work, an attempt has been made to study the antibacterial activity of combination of some of the medicinal herbs.

As textiles are subjected to washing, the wash durability of the finish is a major issue. Although many herbal extracts applied onto the textile material have shown good antibacterial property, their wash durability is poor. The major limitation of the antimicrobial textiles is the non-durability of the finish. Micro encapsulation is a novel technique used to improve the wash durability of the antimicrobial textile materials. In this technique, the microcapsules were prepared by using the herbal antimicrobial compound as the core material which was encapsulated by any one of the wall (sheath) materials such as modified starch, sodium alginate, gum acacia, etc. These microcapsules were applied onto the textile materials by a suitable method. Uniqueness of micro encapsulation is the smallness of the capsules which provides a means of packaging and storing the core material on a microscopic scale for its slow release under controlled conditions [6].

Wound is defined as the disruption of the cellular and anatomic continuity of a tissue. A wound could be produced by the physical, chemical, thermal, microbial or immunological insult to the tissue[7]. The wound healing process is an integration of cellular and biochemical events. Wound healing results in the re establishment of structural and functional integrity with regain of strength of the injured tissue. Clinically, one often encounters non healing or under healing[8]. Therefore, the aim of treating a wound is to shorten the time required for healing and to minimize the undesired consequences like prolonged hospital stay of the patient which increased the hospital charges, due to the infection in wounds. These wound infections were mainly caused by either the bacterial pathogens present in the wound itself or due to nosocomial infectons[9]

Polypropylene nonwoven is a thermo plastic polymer that could find many uses in technical textiles. The advantages of these polypropylene nonwovens such as excellent strength, breathability, barrier properties, their relatively low cost, resistance to tear, soil, chemical and puncture make them an ideal candidate for medical applications[10]. Though the global annual production of polypropylene is over one million tons, its total hydrophobic nature restrains it to find more applications in Textile arena[11].

Many plant extracts possessing antibacterial properties can be used as textile finishing agents either directly or as microcapsules [5]. Based on their antibacterial efficacy, the herbal extracts with higher antibacterial activity were selected and used for the preparation of microcapsules. The prepared microcapsules were finished onto the air plasma treated polypropylene non-woven fabric by pad-dry-cure method. Using standard test methods, the antibacterial efficiency of the finished fabric was tested against the wound infecting pathogens.

MATERIAL AND METHODS

Collection of medicinal herbs

Based on the phytochemistry and wound healing activity of the medicinal plants[12-14] live and healthy herbal parts of leaves of *Ficus bengalensis* (Banyan), nuts of *Areca catechu* (Betelnut palm) and whole plant of *Cleome viscosa* (Spider flower) were collected from different regions in and around Coimbatore district which were authenticated by the Botanical survey of India, Coimbatore. The plant parts were washed twice in distilled water to remove the epiphytes and other extraneous matter from the plants. These herbal materials were dried under shade and powdered using an electric grinder. The herbal powders were stored in dry containers for further studies.

Fabric & its Surface Modification by Plasma[15]

In this research work, the fabric used was a spun bonded polypropylene nonwoven fabric (Ecotech Products, India) of aerial density of 50 $gm^{-2.}$ Polypropylene is a thermo plastic polymer and it is totally hydrophobic. In order to impart hydrophilicity, a surface modification process using plasma was carried out using atmospheric pressure glow discharge plasma system. The plasma reactor was supplied by M/s Hydro PneoVac, Bangalore. It is a physicochemical method which modifies the surface both physically and chemically without altering the bulk properties[16].

The distance between the electrodes was set as 2 mm and the time of treatment was 20 sec. Air was used as working gas and the air flow rate was 10cc/min. The pressure was maintained at 200 mtorr. The hydrophilicity of the Surface modified substrate was estimated by measuring its wettability through capillary rise method. The capillary rise of the air plasma treated fabric was estimated by the method based on INDA Standard test method 10.3.70R82.

Methanolic extraction of medicinal herbs[17]

12 grams of the herbal plant powder was added to 100 ml of methanol in an airtight conical flask and kept at room temperature overnight. Methanolic extraction of the herbs was obtained by maceration process at room temperature for 7 days in conical flask with occasional stirring and shaking. The methanolic extract was filtered and is ready for finishing onto the air plasma treated polypropylene fabric. Its antibacterial activity was assessed by disc diffusion method.

Selection of Best herbal combinations

Herbal combinations (10 different combinations) were prepared and their antibacterial activity was assessed by disc diffusion method. Out of these, the herbal combination having highest antibacterial activity was selected and analyzed for its antibacterial activity against the wound pathogens.

Processing of postoperative wound samples[9]

The pus samples were collected from different infected sites of patients' wounds using sterile cotton swabs. The collected swabs were stored in airtight sterile containers and then processed to isolate the wound pathogens. These samples were inoculated aerobically on sterile glucose broth, nutrient agar, blood agar, Eosin methylene blue, Mannitol salt agar, Mac Conkey agar, and Chocolate agar and the plates were incubated at 37ºC for 24 hours to 48 hours to obtain the bacterial predominants.

Isolation, purification and identification of bacterial predominants[18]

The colonies of each representative isolates were then identified by standard bacteriological methods (morphologically and biochemically), subcultured and maintained in sterile nutrient agar slants. The identified bacterial isolates were subjected to an *in vitro* antibacterial assessment with herbal extracts.

Assessment of antibacterial activity of herbal extracts by disc diffusion method [19]

Sterile nutrient agar plates were prepared by pouring 15 ml of nutrient agar media into sterile Petri plates. The plates were allowed to solidify for 5 minutes and 0.1% inoculum suspension of the isolated wound predominants were swabbed uniformly. The inoculum was allowed to dry for 5minutes.The plasma treated nonwoven polypropylene fabric samples (20 mm in diameter) were impregnated in 20 µl of the herbal extract combination and dried aseptically. The discs were placed on the bacterial lawn of agar plates and incubated at 37ºC for 24 h. At the end of incubation, the zone of inhibition formed around the fabric was measured in millimeters and recorded.

Microencapsulation of the herbal extracts [20]

Microcapsules of the best herbal combination were prepared by using the herbal extract as the core material and sodium alginate as the wall material. Microencapsulation was carried out by the ionic-gelation method. In this method, 3% sodium alginate was added to the herbal extract and this was sprayed into the gelation medium of calcium chloride solution, by means of a sprayer. The droplets were retained in calcium chloride for 15 minutes. The microcapsules were obtained by decantation and repeated washing with iso-propyl alcohol followed by drying at 45°C for 12 hours. The microcapsules were characterized for their size using Scanning Electron Microscope (SEM). The microcapsules were then applied onto the air plasma treated polypropylene fabric.

Finishing of the fabric with microencapsulated herbal particles[6]

The fabric was immersed in the microcapsule solution with a liquor ratio of 20:1 using pneumatic padding mangle. Then the fabric was dried at 80 – 85 ºC for 5 min in a drying oven and cured at 120 ºC for 2 min. The finished fabric was assessed for its antibacterial activity by disc diffusion method.

Assessment of antibacterial activity by qualitative method[19]

The antibacterial activity of the finished fabric was estimated by disc diffusion method using Bacteriostasis agar obtained from Himedia (Mumbai). The agar plates were prepared by pouring 15 ml of agar media into sterile Petri plates. The plates were allowed to solidify for 5 minutes. 0.1% inoculum suspension of the isolated wound predominants were swabbed uniformly and the inoculum was allowed to dry for 5 minutes. The microcapsule finished polypropylene fabric of 20 mm diameter was placed on the surface of medium and the plates were incubated at 37ºC for 24 hours. At the end of incubation, the zone of inhibition formed around the fabric was measured in millimeters and recorded. To minimize the error, the test was repeated in triplicates and the arithmetic mean of the zone of inhibition was noted. The antibacterial activity of the microcapsule finished fabric was tested for its wash durability by subjecting to machine laundering, following AATCC 124 test method. The antibacterial activity of the fabric was assessed after every 5 washes.

RESULTS AND DISCUSSION

Modifiction of the fabric surface

Surface modification of the polypropylene nonwoven fabric by air plasma treatment resulted in a hydrophilic surface which would easily absorb the finishing ingredients that would be applied onto it. The wettability of the air plasma treated polypropylene fabric was tested by capillary rise method. Five samples were tested each along the machine direction and five samples in cross machine direction whose arithmetic mean were calculated.

The mean wicking height was estimated as 6.6 cm along the machine direction and 6.1 cm in cross machine direction. The polypropylene nonwoven fabric which was not given any plasma treatment (untreated) could not raise any water level through capillary rise, which reveals that the fabric is totally hydrophobic. So, the wicking height of the untreated fabric was marked as zero, in fig 1.

Isolation, purification and identification of the bacterial isolates from wound samples

The wound samples collected from the patients were subcultured and purified. Morphological and biochemical analysis was carried out. The results of the analysis were presented in table 1.

The biochemical characteristics of the bacterial isolates were also studied and the results were given in table 2. From the results of the morphological and biochemical analysis, the predominant bacterial pathogens were identified to belong to the following genera and the results were given in table 3.

From table.1, it could be seen from the microscopic morphological analysis and the biochemical analysis, that the wounds carried both Gram positive and Gram negative bacteria. They might be both aerobic and anaerobic. Table 3 shows that the predominant wound pathogens belong to the genus of *Staphylococcus, Escherichia, Klebsiella, Serratia, Pseudomonas and Proteus.*

From the results obtained from our work, it could be inferred that most wounds yielded both aerobes and anaerobes. These results were similar to the outcome of the work done by Sanderson, P. J. *et al* in which they observed that 36 out of 65 septic post-appendicectomy wounds yielded both aerobes and anaerobes. The relative importance of the two types of organisms in the initiation of sepsis in a wound was not known. In their findings, it was also stated that the wounds heal after treatment with antibiotics and sepsis was increased by the presence of both aerobes and anaerobes. The occurrence of wound infection varied with the types of organisms that were grown at the time of wound swab collection. From the wound where both aerobes and anaerobes were recovered, 71 % of wounds later became infected; when anaerobes

Table 1. Morphological identification of wound causing bacterial isolates

Isolate No.	Morphological identification: Colony Morphology	Microscopic observations: Gram Staining	Microscopic observations: Motility
1.	Colonies are circular, convex with entire margin	positive, cocci	-
2.	Medium sized colony with regular margin and convex elevation	negative, short rods	+
3.	Slightly gummy circular, convex with entire margin	negative, short rods	-
4.	Red in appearance, circular with entire margin	negative, short rods	+
5.	Circular convex with entire margin	negative, rods	+
6.	Colonies are large, undulated, circular with flat elevation	positive, rods	+
7.	Medium to large sized colony, shiny cream color colony, swarming growth on agar surface	negative, rods	+
8.	Medium sized colony with regular margin and convex elevation	negative, short rods	+

Table 2. Biochemical identifications of wound causing bacterial predominants

Isolate	Biochemical characteristics of Wound causing bacterial isolates									
No.	I	MR	VP	Citrate	Catalase	Oxidase	Starch	Glucose	Lactose	Urease
1	-	+	+	-	+	-	-	A+/G+	A+/G+	+
2	+	+	-	-	+	-	-	A+/G+	A+/G+	-
3	-	-	+	+	-	+	-	A+/G+	A+/G+	+
4	-	-	+	+	+	-	-	A+/G+	A-/G-	-
5	-	-	-	+	+	+	-	A-/G-	A-/G-	+
6	-	-	-	-	+	-	+	A-/G-	A-/G-	-
7	-	+	-	-	+	-	-	A-/G-	A-/G-	+
8	+	+	-	-	+	-	-	A+/G+	A+/G+	-

Table 3. Identification of bacterial flora from wound samples

S.No	Isolate No.	Genus identified
1	1	*Staphylococcus*
2	2, 8	*Escherichia*
3	3	*Klebsiella*
4	4	*Serratia*
5	5	*Pseudomonas*
6	6.	*Bacillus*
7	7.	*Proteus*

or aerobes alone were recovered, only13 % and 22% respectively became infected [9]

Antibacterial assessment of the herbal extracts and selection of the best herbal combination

The antibacterial activity of the herbal extract was studied against the isolated bacterial cultures and the results were presented in table 4.

The results showed that the three herbal extracts exhibited antibacterial activity against most of the microbial isolates. The next step was to find the effective herbal combination for

Table 4. Antibacterial activity of herbal finished fabric against wound causing bacterial isolate

S. No	Name of the Herbs	Zone of Bacteriostasis (mm) against wound isolates: *Staph.*	*Serratia*	*Klebsiella*	*E. Coli*	*Pseudomonas*	*Bacillus*	*Proteus*
1.	*Ficus bengalensis*	25	24	24	24	24	25	0
2.	*Cleome viscosa*	23	30	28	0	0	30	25
3.	*Areca catechu*	36	0	31	0	0	29	0

preparing wound care textile products. The results were tabulated in the following table 5.

From the results in table 5, it was found that the combination of herbs were effective against the most common organisms which cause infections, viz. *Staphylococcus aureus* and *Escherichia coli*. The best herbal combination was found to be 1:1:1. The best herbal combination was then microencapsulated by ionic gelation method. These microcapsules were applied onto the air plasma treated non woven polypropylene fabric. Then the antibacterial activity of the microcapsule finished fabric was assessed. The surface morphology of the microcapsule finished fabric was characterized by Scanning Electron Micrograph (SEM) analysis which was presented in Fig 2.

The finished fabric was tested for its antibacterial activity against *Escherichia coli* and *Staphylococcus aureus*. The results of the antibacterial tests were presented in table 6 and Fig 3.

The microcapsule-finished fabric exhibited its antibacterial activity with a zone of inhibition of 29 mm against *Escherichia coli* and 31 mm zone of inhibition against *Staphylococcus aureus*. The antibacterial activity of the microcapsule-finished fabric was tested for its wash durability and the results were presented in table 7.

Table 5. Assessment of Antibacterial activity of Different herbal combinations by Disc diffusion method

S. No	Combinations of Herbal extracts (*Areca catechu* + *Ficus bengalensis* + *Cleome viscosa*) Ratio	Antibacterial activity (Zone of Bacterial inhibition – mm) Disc diffusion method	
		Escherichia coli	*Staphylococcus aureus*
1.	1:1:1	28	17
2.	1:2:1	15	17
3.	2:1:1	16	14
4.	1:1:2	15	14
5.	2:2:1	16	0
6.	1:2:2	15	16
7.	2:1:2	14	13
8.	3:1:2	13	12
9.	1:3:2	18	14
10.	1:2:3	16	14

Table 6. Antibacterial activity of the microcapsule finished fabric

S. No	Fabric sample	Antibacterial activity (Zone of Bacterial inhibition – mm)	
		Escherichia coli	*Staphylococcus aureus*
1.	Control fabric	0	0
2.	Herbal microcapsule finished fabric	29	31

Table 7. Antibacterial activity of the microcapsule finished fabric after washing – AATCC -124 test method

S. No	Wash cycle (Industrial washes)	Antibacterial activity(Zone of Bacterial inhibition – mm)	
		Escherichia coli	*Staphylococcus aureus*
1.	After 5 washes	28	30
2.	After 10 washes	27	28
3.	After 15 washes	26	27
4.	After 20 washes	0	24

From table 7, it is obvious that the antibacterial activity of the microcapsule finished fabric was found to decrease with the increase in the number of washes and the antibacterial activity was found to be minimum even after 20 washes. The herbal extracts exhibited a good potential for antimicrobial activity against *Staphylococcus aureus* and *Escherichia coli*. This result was found to have good correlation with that of the observations of Thilagavathi et al which revealed that the microencapsulated herbal extracts possessed a very good resistance for microbes even after 15 washes. Their results showed that the microencapsulated geranium extract based antimicrobial finish is durable up to 15 washes whereas the direct application of geranium extract onto the fabric, showed activity only up to 5 washes[6].Also the results of the study conducted by Sathiyanarayanan, et al revealed that the herbal extracts encapsulated with acacia gum and cross-linked with a resin gave good antibacterial property and the wash durability of up to 15 wash cycles [4].

The results showed that the microcapsule finished polypropylene fabric retained its antibacterial activity for about 20 washes. The microencapsulation of combination of herbal extracts leads to the slow release of active core materials and preserve the durability for 20 washes.

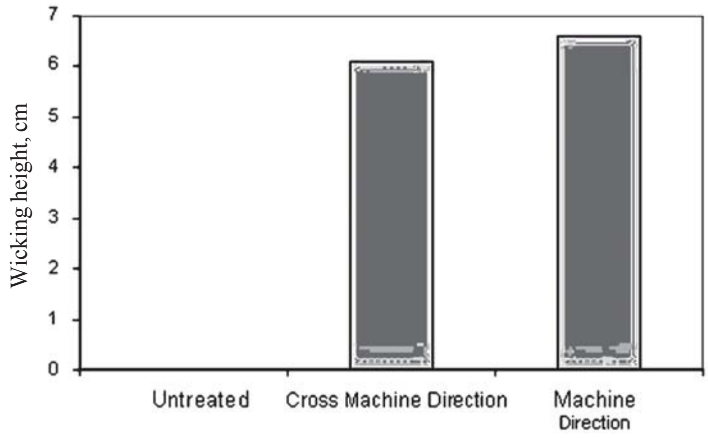

Fig. 1. Wettability of plasma treated polypropylene Nonwoven Fabric

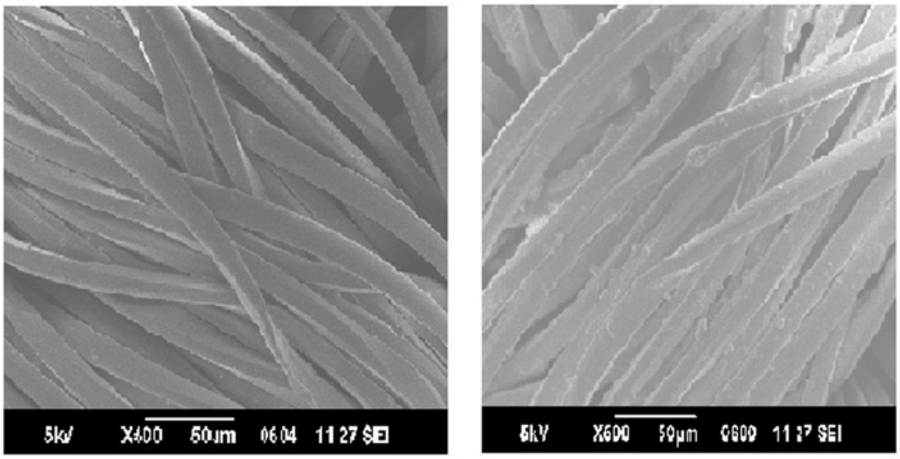

Fig. 2. SEM Micrograph of (a) Air plasma treated and (b) Polypropylene fabric finished with Herbal microcapsules

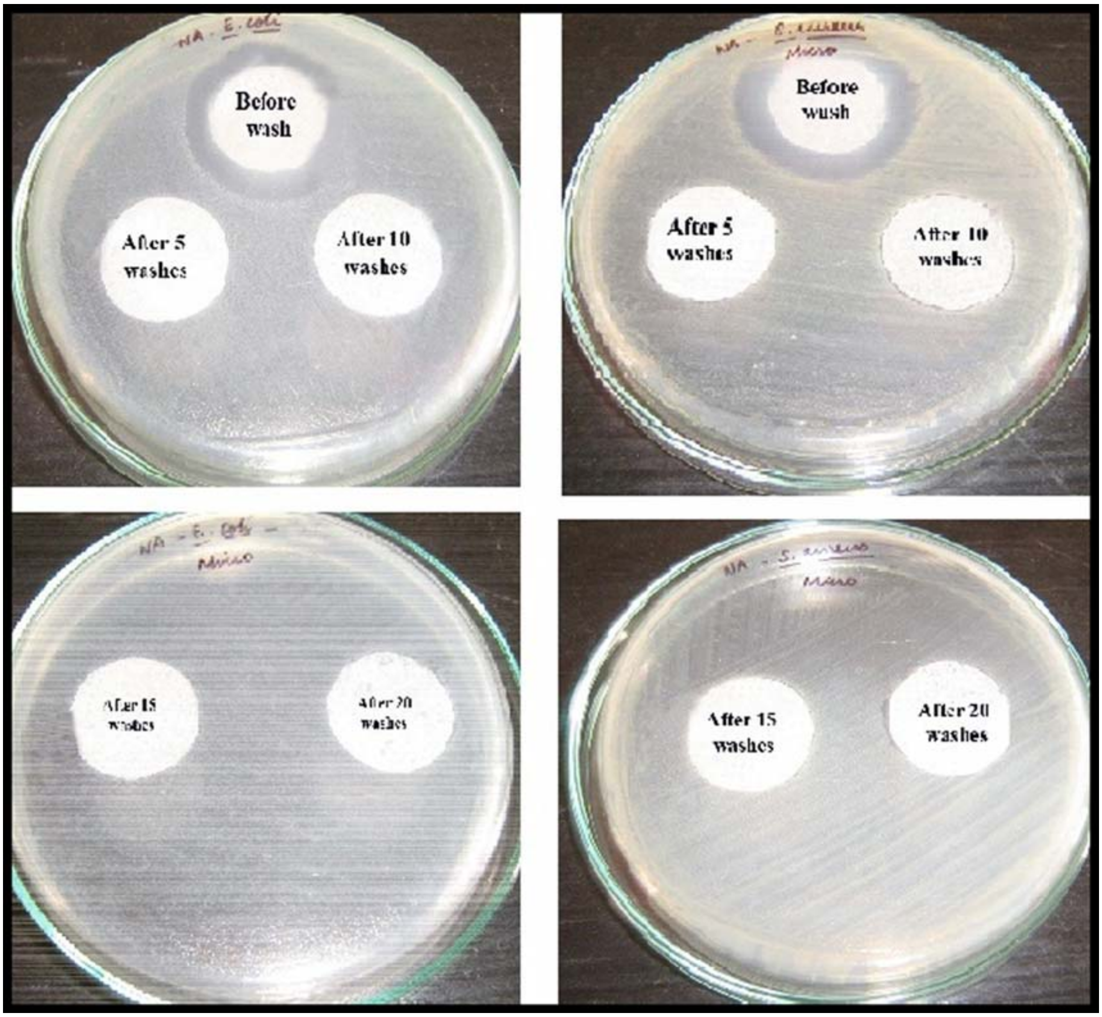

Fig. 3. Antibacterial activity of the microcapsule finished fabric after washing

Escherichia coli *Staphylococcus aureus*

Poly-herbal preparations containing these herbs have been claimed to be useful in treating Gram negative and Gram-positive infections. This property of the herbal combination is effective in causing the acceleration of migration and mitosis of epithelial cells and promotion of contraction of myo-fibroblasts. These myo-fibroblasts are responsible for wound contraction and accelerates wound healing [21].

CONCLUSION

Antimicrobial textiles with improved functionality find a wide range of applications such as the garments worn close to the skin, several medical applications such as infection control and barrier materials, health and hygiene products etc. In this work, the wound samples were collected and the predominants were isolated and identified. Three medicinal herbs, *Ficus bengalensis*, *Areca catechu* and *Cleome viscosa* were collected. The extracts of the collected herbs were obtained by using methanol as solvent. The antibacterial activity of the herbs, both individually and in combination were studied against the isolated wound organisms. Then, to increase the wash durability, the best herbal combination was microencapsulated and applied onto the textile substrate. The substrate used for this work was a totally hydrophobic polypropylene nonwoven fabric. An ecofriendly technique of surface modification was followed using plasma technology to convert the hydrophobic surface of polypropylene to hydrophilic. The air plasma treated polypropylene fabric finished with the herbal microcapsules was tested for their antibacterial activity both before and after 20 washes. The results disclosed that the microcapsule finished fabric had an antibacterial activity of zone of inhibition of 29 mm against *Escherichia coli* and 31 mm against *Staphylococcus aureus* before washing. The microcapsule finished fabric had no zone of inhibition against *Escherichia coli* and had a zone of inhibition of 24 mm against *Staphylococcus aureus*, washed after 20 cycles.

Unlike other textile processes, the technique of surface modification used in this work did not add any pollution load to the environment. The process of surface modification using plasma technology is a water free process and therefore water conservation is an added advantage. The extracts of medicinal herbs have good antibacterial activity which confirms the work of Raina *et al* [21]. Microencapsulation is a rapidly growing technology and finds greater applicability in textiles in recent years. Microcapsules of herbal combination enhance the wash durability of the fabric. The microcapsules also provide a controlled release of the medicinal extracts which accelerates wound healing. The herbal microcapsule-finished fabric could be suitably designed to develop wound care textile products. Plants or chemical entities derived from many other medicinal herbs available abundantly in Asia, especially in India, need to be identified and formulated suitably for the treatment and management of wounds. Our research work has also answered the question of acceptability of a hydrophobic polypropylene nonwoven fabric for wound care textiles after due surface modification process and microencapsulation

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