

Herbal Antimicrobial finishing of Cotton and Khadi fabric using Moringa (*Moringa oleifera*) leaves extract

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Abstract: Microbes can be found almost everywhere in the environment including in textile materials. Natural fibers are especially prone to attack by microorganisms that cause staining and bad odor along with deterioration of strength and other mechanical properties. Consumers are now increasingly aware of healthy and hygienic lifestyles, and there is an expectation for a wide range of textile products with antimicrobial properties. In the present study, the antimicrobial finish was imparted to cotton and khadi fabrics using Moringa leaf (*Moringa oleifera*) extract through direct and microencapsulation methods to improve the antimicrobial properties of the fabric. Microcapsules were prepared by using Moringa oleifera extract as core material and chitosan as wall material and these were applied on fabric using pad dry cure method. Both treated and untreated samples were subjected to various tests including SEM, FTIR, and antimicrobial efficacy carried out by quantitative and qualitative methods in terms of bacterial reduction and durability of antimicrobial activity against washing. The treated samples showed satisfactory antimicrobial activity against gram positive (*Staphylococcus aureus*) and gram negative (*Klebsiella pneumoniae*) bacteria. The deposition of microcapsules was observed in the SEM analysis, and the active compounds of Moringa leaves (*Moringa oleifera*) extract were also confirmed by FTIR spectroscopy. Microencapsulated herbal samples showed higher resistance to microbes even after 10 wash cycles than directly treated samples.

Key words: Antimicrobial finish, chitosan, FTIR, Moringa, Microencapsulation, SEM

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1. Introduction

Textile materials especially cotton and khadi, are the most preferred fabrics for garment production due to their comfort property and absorbency, however, these fabrics offers an ideal environment for the growth of microbes that causes an obnoxious odor causes staining and also spreading skin diseases. In recent years there has been an increasing awareness of consumers towards health and hygiene coupled with the harmful effects of synthetic-based finishing treatments on fabrics for antimicrobial activity. Consumers are looking for safer alternatives to synthetic chemicals and environmentally friendly processes. Thus, herbal extracts are the best alternatives. Herbal extracts from stem, leaves, flowers fruits and seeds of diverse species of plants exhibits antimicrobial properties and can be used as textile finishing agents either in the crude form or as microcapsules to enhance the durability of finishing treatment. Microencapsulation is a rapidly expanding technology with greater applicability in textiles in recent years.

Microencapsulation may be defined as a micro packaging technique, wherein an active core material is encapsulated in a polymer shell of limited permeability. It is a physiochemical technique that provides textiles with resistance to microorganisms and insects.

Moringa belongs Moringaceae family, *Moringa oleifera* tree has approximately 5 to 10 m height and found in all over the world, as every part of Moringa is used for certain nutritional and medicinal purpose. Besides being a good source of protein, vitamins, oils, fatty acids, micro-macro minerals elements and various phenolics, it is also reported for its anti-inflammatory, antimicrobial, antioxidant, anticancer, cardiovascular, hepatoprotective, anti-ulcer, diuretic, antiurolithiatic, and antihelmintic properties. Its multiple pharmaceutical effects are capitalized as therapeutic remedy for various diseases in traditional medicinal system.^[1]

In the present study we attempted to apply moringa leaf extract to cotton and khadi fabric to impart antimicrobial properties. Two

methods that is direct and microencapsulation were used for this purpose, and both treated and untreated fabrics were subjected to various tests to analyze the efficacy of antimicrobial finishing.

2. Materials and Methods

The aim of this study was to utilize moringa leaf extract for antimicrobial finishing of cotton and khadi fabrics by direct and microencapsulation techniques and to evaluate the treated and untreated samples. The research methodology adopted to carry out the present research has been bifurcated in the following heads and sub-heads.

Raw material used

Fabrics:

Cotton and khadi fabrics were used for the application of antimicrobial finish as these fabrics are cellulosic in nature and offer an ideal condition for microbial growth. The EPI of cotton fabric was 104 and PPI 77 and for khadi fabric EPI was 42 and 35 PPI.

Plant material

Fresh leaves of Moringa (*Moringa oleifera*) were collected and its extract was used as core material for antimicrobial finish on selected Cotton and khadi fabrics.

Chemicals used for the study and their purposes

Pre-treatment of fabrics: Before applying antimicrobial finish on cotton and khadi fabrics, the entire length of the fabric was desized, scoured and bleached using standardized recipes

Desizing

Desizing is generally done to remove starch or sizing material from the gray fabric to make the fabric more absorbent without undergoing any chemical or physical damage. The recipe given by **Parmar (2016)** was used for desizing of cotton and khadi fabric.

An amylase enzyme solution per 1000 ml of water was prepared maintaining the material: liquor ratio 1:50. It was heated to 50-60°C. Cotton and khadi fabrics were dipped into the solution separately and stirred gently. Then

fabrics were kneaded and squeezed and finally rinsed under tap water and then dried in sunlight.

Bleaching and Scouring

Scouring and bleaching were carried out simultaneously by using the recipe given by **Parmar (2016)**. A detergent solution containing 3gm of NaOH, 5gm of Na₂CO₃, 1gm of wetting agent (soap) and 3 gm of H₂O₂ added in 1000 ml of water. Separate solutions were prepared for both cotton and khadi fabrics maintaining the material: liquor ratio 1:50. Solutions were heated to 100°C and cotton and khadi fabrics were dipped into prepared solution separately and stirred gently to about 60 minutes. After pretreatment the fabrics were thoroughly washed with hot water and finally rinsed with cold water. Then samples were allowed for drying in sunlight and after that kept in dry place.^[9]

Application of antimicrobial finish

Method of extraction of plant material

Moringa leaves were plucked, sorted and washed with water to remove dust particles and were dried under shade at room temperature. After that crushed into small pieces and stored in air tight containers for extraction. The Soxhlet apparatus was used for extraction process. In this sequence dried and crushed leaves were weighted 20 gm by electronic weighing balance; some glass wool was added into Soxhlet tube as filtering material then weighted crushed leaves were added into Soxhlet tube and tube was fixed with round bottom glass. Finally 300 ml of methanol was added as solvent and fixed in the Soxhlet extractor for 3 hours at 60° temperature. After 3 hours, extract was filter into a beaker incubated at 40°C till required consistency was achieved and finally stored in refrigerator at 4°C for formation of microcapsule as core material.

Fabric treatment with plant extract

Direct application method:

Methanol extract of the Moringa leaves was directly applied on cotton fabric by pad-dry – cure method. 500ml of methanol extract and 6% of citric acid as cross linking agent were taken and stirred completely. Samples were immersed in stirred extract of Moringa leaves for 30 minutes. After that samples were taken out and padded on padding mangle at a

pressure of 2.5 psi with 2 dips and nips to give a wet pick up of 85%. Drying and curing was carried out in hot air oven which was used for drying, condensation and fixation. The samples were dried and cured at 102°C for 3 minutes.

Microencapsulation method:

Formation of microcapsules

2.5 g of chitosan (as a wall material in microencapsulation) was dissolved in 100 ml of distilled water with 1% acetic acid by mechanical stirring and kept overnight till a clear solution was obtained. Silicon oil (0.5 ml) and 2g of core material (extract of Moringa leaves) were slowly added in this solution with continuous stirring for making an emulsion. Stirring was further continued for another 15 minutes. Coacervation of chitosan was done by addition of sodium hydroxide (1g in 100 ml). Stirring was stopped and the mixture was allowed to rest for a period of 30 minutes at a temperature of 0°-5°C in refrigerator for the formation of microcapsules. After that solution was kept at 5°C-10°C for 24 hours, filtered and washed thrice with distilled water.

Application of microcapsules on fabric

For the application of microcapsules, a solution of filtered microcapsules and distilled water was prepared and samples were immersed in microcapsule solution for 30 minutes then padded through padding mangle at a pressure of 2.5 psi. Treated samples were dried at 80°C for 5 minutes in hot air oven to cure the samples. The cured samples were evaluated for antimicrobial efficacy against gram positive (*Staphylococcus aureus*) and gram negative (*Klebsiella pneumonia*) microorganism.

Functional analysis of finish:

Quantitative bacterial reduction test for antimicrobial reduction

Assessment of quantitative bacterial reduction was carried out by AATCC test method 100:2014 for both treated and control samples. samples showing antimicrobial activity were evaluated quantitatively. Treated and control samples were inoculated with test organisms (*staphylococcus aureus* and *Klebsiella pneumonia*). After incubation, the bacteria were eluted from the swatches by shaking in known amount of neutralizing solution. The number of bacteria present in this liquid was determined and the percentage reduction by the treated specimen was calculated. The bacterial counts were reported as the number of bacteria per sample

(swatches in jar) not as the number of bacteria per ml of neutralizing solution. '0' counts at 10⁰ dilution was reported as "less than 100". The percentage reduction (*R*) of bacteria by the specimen treatments was calculated using the following formula:

$$R = 100 (B \times A) / B$$

where, *A* is the number of bacteria recovered from the inoculated treated test specimen swatches in the jar incubated over the desired contact period; and *B*, the number of bacteria recovered from the inoculated treated test specimen swatches in the jar immediately after inoculation (at '0' contact time).

Qualitative method for testing presence or absence of antimicrobial activity

Qualitative assessment of antimicrobial activity was carried out by AATCC 147-2004 test method. Sterilized nutrient agar [cooled to 47±2°C (117±4°C)] was dispensed by pouring 15±2 ml into each standard (15×100 mm) flat bottomed Petridis. Agar was allowed to get firm before inoculating. Inoculum was prepared by transferring 1.0±0.1 ml of a 24 h broth culture into 9.0±0.1 ml of sterile distilled water contained in a small flask. It was mixed well using appropriate agitation. Using a 4 mm inoculating loop, one loopful of the diluted inoculum was loaded and transferred to the surface of the sterile agar plate by making five streaks approximately 60 mm in length, spaced 10 mm apart covering the central area of standard petri dish without refilling the loop. It was taken care that no surface breakage should take place while making the streaks.

FTIR

The FTIR spectra of cotton, khadi and extract of Moringa were measured using Perkin Elmer FT-IR Spectrometer (SPECTRUM TWO). The wave number range was 450-4000 cm⁻¹ and the resolution was 1 cm⁻¹

Surface morphology of treated, untreated and washed samples was observed under "Carl Zeiss EVO 18 Special" at a magnification of 50X to 2500, 000X on and at acceleration voltage of 20 KV. Before the assessment, all the samples were sputtered under vacuum with gold.

Wash durability of treated fabric:

After the evaluation of functional analysis of treated samples, washing fastness was done for treated samples to evaluate the durability of

antimicrobial property against washing using AATCC 100 test method up to 10 cycles wash cycles.

3. Results And Discussion

The results obtained from the study as well as relevant discussion have been summarized under following heads:

Result of quantitative bacterial reduction

Assessment of quantitative bacterial reduction was carried out by AATCC 100:2004 test method and the results are reported in Table 2. In AATCC 100:2004 test method the treated sample show the bacterial reduction of samples in percentage.

Results clearly reveal that both the cotton and khadi fabric samples treated with Moringa extract showed good antimicrobial properties against gram positive (*Staphylococcus aureus*) and gram negative (*Klebsiella pneumonia*) microorganism. In case of direct application methods both the samples (cotton and khadi) showed higher percentage of antimicrobial reduction against both the organisms (95.77 to 96.91%) as extract was applied directly on the fabric surface. On the other hand percentage of antimicrobial efficacies against both the organisms in microencapsulation application method found less compared to direct application method.

Result of qualitative method for assessing presence or absence of antimicrobial activity

Qualitative assessment of antimicrobial activity was carried out under AATCC 147-2004 test method and the results are reported in Table 3.

It was observed that both untreated samples (cotton and khadi) did not show antimicrobial properties hence microbial growth was observed on these samples. However, treated samples showed presence of antimicrobial property thereby no microbial growth was found on these samples.

Qualitative test was found to be good for testing the main agent or treated fabrics, providing the antibacterial agent used were capable of leaching out. It is observed from Table 2 that all the treated fabrics showed antimicrobial activity against gram positive (*Staphylococcus aureus*) and gram negative (*Klebsiella pneumonia*) microorganism whereas untreated fabrics showed the growth of microorganisms which means antibacterial activity was not found in treated samples.

In case of both the treated fabrics (i.e. cotton and khadi) there was no zone of inhibition for both the organisms. The zone of inhibition that indicates that Moringa leaves extract not only prevent the growth of microbes on the fabrics but also leaches out the bacteria.

FTIR

FTIR test of Control and treated samples were performed and the results are shown in figure 1 and 2. The overlapping Spectrum of cotton and khadi control sample treated directly and sample treated with microencapsulation method are shown in figure. 1 and 2. Figure 1 reveals the presence of typical group of *Moringa oleifera* on the surface of treated samples. The peaks 3288 cm^{-1} observed in direct application as well as in microencapsulation application which indicates the occurrence of stretch free vibration type alcohol with O-H, 2915.8 cm^{-1} stretch and 1430.6 cm^{-1} peaks represent Alkanes and Alkyls acid with C-H and O-H correspondingly. The presence of C-H stretch organic amine is confirmed with the presence of 1031.4 cm^{-1} band. Similarly figure 2 also reveals the presence of typical group of *Moringa oleifera* on the surface of treated samples. Peaks 3341.8 cm^{-1} indicates the occurrence of stretch free vibration type alcohol with O-H, 2900.8 cm^{-1} and 1710.7 cm^{-1} represents organic aromatic group and carbonyl acid with C=C and O-H correspondingly. The presence of functional group like organic (=C-H bending), carbonyl acid (C-O stretch), organic (alkane; C-H stretch), and carbonyl (amide N-H stretch) are confirmed which indicate the presence of phenolic group on treated samples and indicating be reason for antimicrobial efficacy.

Table 1 Chemicals used with their purposes

S. No.	Chemicals used	Purposes
1.	Chitosan	Wall material of microcapsules
2.	Distilled water	Dissolving the chitosan
3.	Silicon oil	Making emulsion in the formation of microcapsules
4.	Citric acid	Cross linking agent
5.	Amylase	Desizing agent
6.	Na ₂ CO ₃	Scouring agent
7.	H ₂ O ₂	Bleaching agent.

Table 2 Assessment of bacterial reduction

S. No.	Sample	Test organism	No. of bacteria per sample (CFU)		Bacteria reduction in%	
			'0' hrs(B)	'24' hrs(A)		
1.	Control (untreated)	Cotton	<i>Staphylococcus aureus</i>	1.35×10 ⁵	1.44×10 ⁷	Nil
			<i>Klebsiella pneumonia</i>	1.41×10 ⁵	1.27×10 ⁷	Nil
		Khadi	<i>Staphylococcus aureus</i>	1.38×10 ⁵	1.55×10 ⁷	Nil
			<i>Klebsiella pneumonia</i>	1.47×10 ⁵	1.26×10 ⁷	Nil
2.	Samples treated with Moringa extract Using microencapsulaton method	Cotton	<i>Staphylococcus aureus</i>	1.82×10 ⁵	0.105×10 ⁵	94.23
			<i>Klebsiella pneumonia</i>	1.20×10 ⁵	0.086×10 ⁵	92.83
		Khadi	<i>Staphylococcus aureus</i>	1.54×10 ⁵	0.065×10 ⁵	95.77
			<i>Klebsiella pneumonia</i>	1.67×10 ⁵	0.089×10 ⁵	94.67
3.	Samples treated with Moringa extract using direct application method	Cotton	<i>Staphylococcus aureus</i>	1.42×10 ⁵	0.065×10 ⁵	95.42
			<i>Klebsiella pneumonia</i>	1.12×10 ⁵	0.037×10 ⁵	96.91
		Khadi	<i>Staphylococcus aureus</i>	1.32×10 ⁵	0.045×10 ⁵	96.59
			<i>Klebsiella pneumonia</i>	1.56×10 ⁵	0.058×10 ⁵	96.28

Table 3 Assessment of presence or absences of bacteria (Zone of inhibition)

Sample			Test organism	Zone of inhibition (mm)		Result of Anti - microbial activity
				Zone of inhibition	Growth of specimen	
Control (untreated)	Cotton		<i>Staphylococcus aureus</i>	No zone	Present	Antimicrobial activity Absent
			<i>Klebsiella pneumonia</i>	No zone	Present	Antimicrobial activity Absent
	Khadi		<i>Staphylococcus aureus</i>	No zone	Present	Antimicrobial activity Absent
			<i>Staphylococcus aureus</i>	No zone	Present	Antimicrobial activity Absent
Treated with Moringa extract	Cotton	Direct application	<i>Staphylococcus aureus</i>	No zone	Absent	Antimicrobial activity present
			<i>Klebsiella pneumonia</i>	No zone	Absent	
		Microcapsulation	<i>Staphylococcus aureus</i>	No zone	Absent	Antimicrobial activity present
			<i>Klebsiella pneumonia</i>	No zone	Absent	
	Khadi	Direct application	<i>Staphylococcus aureus</i>	No zone	Absent	Antimicrobial activity present
			<i>Klebsiella pneumonia</i>	No zone	Absent	
		microcapsulation	<i>Staphylococcus aureus</i>	No zone	Absent	Antimicrobial activity present
			<i>Klebsiella pneumonia</i>	No zone	Absent	

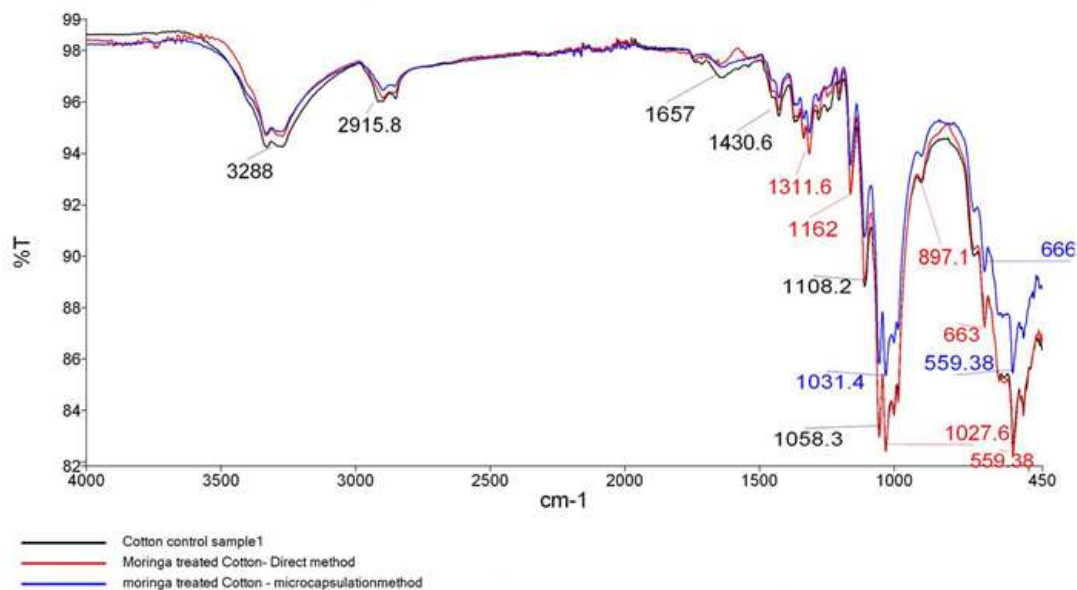


Figure 1 Overlapping of FTIR Spectra of cotton control sample, Moringa treated Cotton – Direct method and Moringa treated Cotton –Microencapsulation method

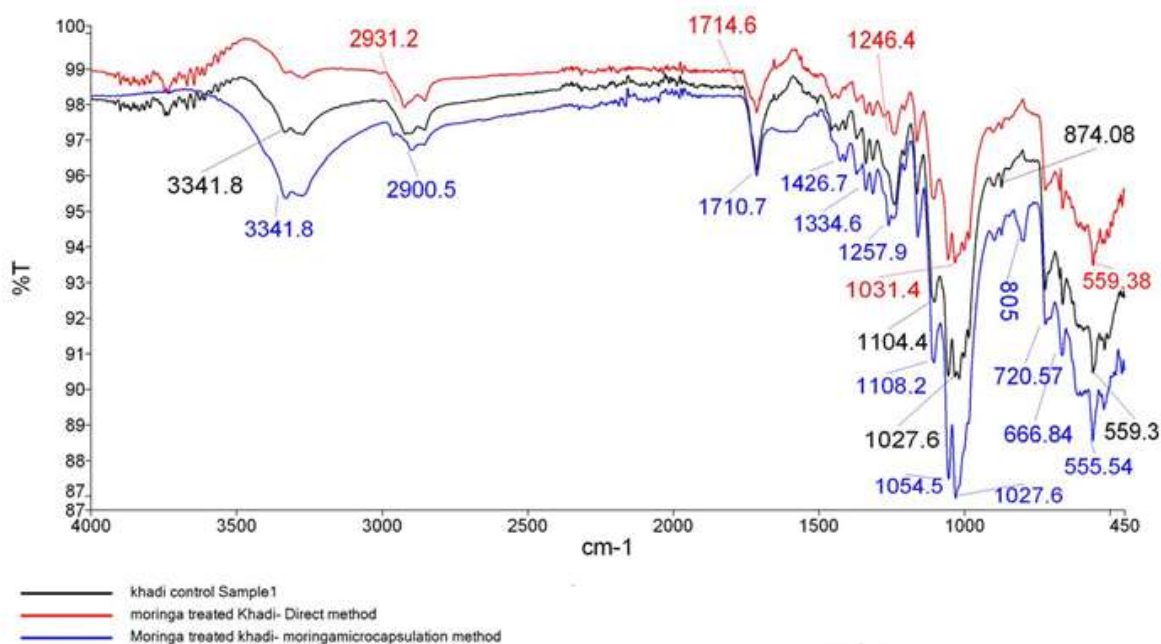
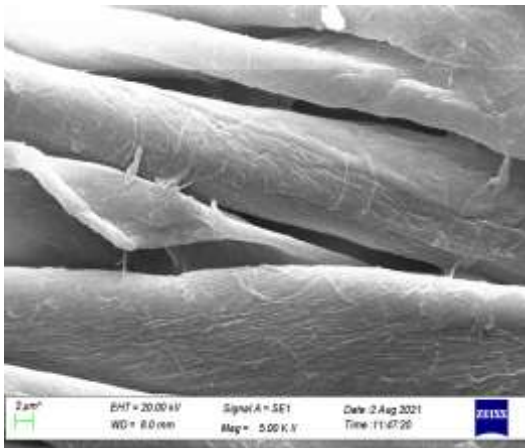


Figure 2 Overlapping of FTIR Spectra of Khadi control sample, Moringa treated Khadi – Direct method and Moringa treated Khadi –Microencapsulation method

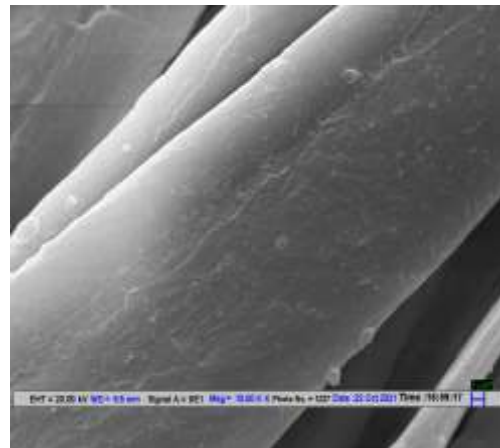
SEM (Scanning Electron Microscope)

Both treated and untreated cotton and khadi samples were analysed using SEM test and the results are shown in Plate no. 1(a) to (d). From the plate no. 1 (a) and(b) it was observed that both untreated samples (cotton and khadi) showed smooth and clean surface because of

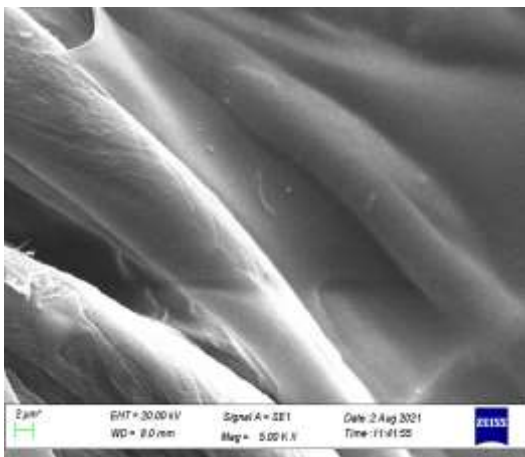
pretreatment process all impurity including starch get washed off. However treated samples plate no. 1 (c) and (d) showed rough and uneven surface due to deposition of microcapsules made by chitosan as wall material.



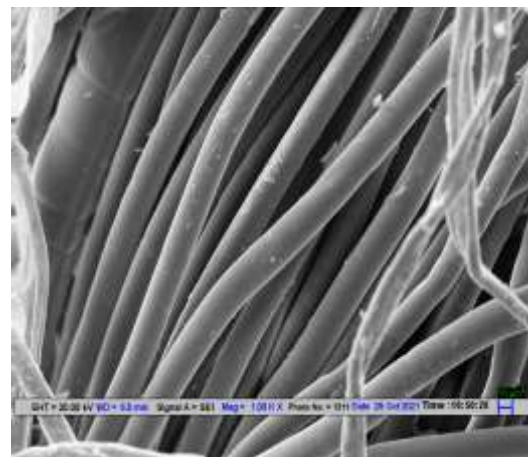
(a)



(b)



(c)



(d)

Plate 1 SEM photographs of (a) Untreated Cotton fabric; (b) Untreated Khadi fabric (c) Microencapsulated Cotton fabric (d) Microencapsulated Khadi fabric

Table 4 Wash durability of treated samples

Washing cycles	Efficacy of bacterial reduction (in Percentage)								
	Direct method				Microencapsulation method				
	Cotton		Khadi		Cotton		Khadi		
	Sa	Kp	Sa	Kp	Sa	Kp	Sa	Kp	
0	98	96.3	96.5	97.2	97	98.3	96	97.2	
1	48	52	19	36	95	93	94	93	
3	26	31.1	21	18	88	89	87	88	
5	14	16.2	13	13	79	82	81	84	
7	11	10.9	6.2	6.9	65	73	73	75	
10	2.3	3.2	3.2	3	52	64	65	63	
Sa- <i>Staphylococcus aureus</i> ,				Kp- <i>Klebsiella pneumonia</i>					

Wash durability of treated samples

Wash durability test was performed on both cotton and khadi fabrics treated with Moringa leaves extract through direct and microencapsulation method as using standard test method AATCC 100 and the results are reported in Table no. 4.

The result of washing durability of finishing was observed and it was found that in both the methods all the treated samples showed higher efficacy of bacterial reduction. However on subsequent washing the efficiency of treated fabrics for bacterial reduction get decreased. It was observed that decrease in efficacy of bacteria reduction was more in directly treated as compared to microencapsulated samples. This may be due to the reason that finish is applied directly on fabric while in microencapsulation process make bonding with fibre that make it more resistant to washing.

4. Conclusion

Microencapsulation of herbal extract (Moringa leaves) has been done successfully by phase coacervation method using herbal extract as core material and wall material chitosan as well followed by its application on to fabric using pad-dry-cure and direct method. It is found that cotton and khadi fabric treated with direct application, sample showed higher percentage of antimicrobial efficacy in both

the application method against *Klebsiella pneumonia* and *Staphylococcus aureus*. The Wash durability test comparing microencapsulated and direct applied herbal extract for both cotton and khadi fabric revealed that microencapsulated samples retained their activity up to 10 wash cycles.

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