



Antimicrobial Finishing on Cotton and Khadi Fabric Using Neem Leaves (*Azadirachta indica*) Extract

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Abstract

The present study was conducted in January, 2021 at NITRA, Ghaziabad, Uttar Pradesh, India, to evaluate the antimicrobial properties of cotton and khadi fabrics treated with neem leaf extract. Neem (*Azadirachta indica*) is a well-known medicinal plant belonging to the family Meliaceae and has been widely used in traditional practices for its healing, skin-protective, immunity-boosting and antimicrobial properties. Owing to its natural bioactive compounds, neem offers a promising eco-friendly alternative for developing functional textile finishes. In this study, neem leaves were extracted using methanol through the Soxhlet extraction method. The extracted neem finish was applied to bleached cotton and khadi fabrics using two techniques: direct application and microencapsulation. The untreated and treated fabric samples were tested against Gram-positive bacteria, *Staphylococcus aureus*, and Gram-negative bacteria, *Klebsiella pneumoniae*, to assess their antimicrobial performance. Further characterization was carried out using Scanning Electron Microscopy (SEM), Fourier Transform Infrared Spectroscopy (FTIR) and wash durability testing. The results showed that neem-treated fabrics possessed noticeable antimicrobial activity. Among the two application methods, microencapsulation proved more effective, as it provided better retention of the neem extract on the fabric surface and maintained antimicrobial performance even after repeated washing. The microencapsulated cotton and khadi fabrics showed durable antimicrobial activity up to 25 washes, making this method more suitable than direct application for developing long-lasting herbal antimicrobial textiles.

Keywords: Medicinal plants, soxhlet method, antimicrobial activity, SEM, FTIR

1. Introduction

Neem, commonly known as Nim or margosa, is a fast-growing tree from the mahogany family (Meliaceae) that has been valued for centuries because of its medicinal and protective properties. Different parts of the neem tree are widely used in traditional medicine for treating skin problems, wounds, infections, acne, and early symptoms of malaria due to their strong antimicrobial and healing effects (Singh et al., 2023; Das and Das, 2022). In recent years, neem has attracted growing attention in the textile and biomedical industries because it offers a natural and eco-friendly alternative to synthetic chemical finishes. Researchers are increasingly exploring plant-based extracts to improve fabric qualities such as appearance, softness, durability, and drape while also providing additional functional properties like antimicrobial protection (Saha et al., 2024).

The increasing use of herbal finishes in textiles is mainly driven

by concerns regarding the harmful environmental impact of synthetic chemicals. Conventional antimicrobial finishes can release toxic substances into the environment and may even contribute to microbial resistance over time. In contrast, natural materials such as *Azadirachta indica* are biodegradable, renewable, and safer for both humans and the environment (Hasan et al., 2022; Hossain et al., 2024). Because of these advantages, textile manufacturers are gradually adopting plant-based treatments as part of sustainable production practices. The use of herbal antimicrobial agents also supports the principles of green manufacturing and circular economy systems by encouraging the use of renewable and non-toxic resources (Johan et al., 2025). As consumers become more conscious about sustainability and health, the demand for environmentally friendly textile products continues to grow.

Modern advancements in textile finishing techniques have further improved the effectiveness of botanical antimicrobial



treatments. Technologies such as microencapsulation and nanoencapsulation help retain herbal extracts on fabrics for a longer time and protect the active compounds from washing and environmental damage. These techniques enhance the durability and long-term antimicrobial performance of treated textiles (Podgornik et al., 2021; Verma and Singh, 2021). Studies have shown that neem-treated cotton fabrics exhibit strong antibacterial activity against harmful microorganisms such as *Staphylococcus aureus* and *Escherichia coli*, even after several washing cycles (Ganesan and Vardhini, 2015; Ibrahim et al., 2020). Similarly, fabrics treated with neem and Aloe vera extracts have demonstrated excellent potential for use in healthcare and hygienic textile applications because of their natural antimicrobial effectiveness (Khurshid et al., 2015). These developments have increased the commercial potential of herbal antimicrobial textiles by improving their durability, stability, and practical usability.

Neem-based textile finishes are now finding applications in healthcare textiles, sportswear, home furnishing products, and protective clothing. Fabrics treated with neem help reduce microbial growth and unpleasant odour, making them more hygienic and comfortable for everyday use. Such textiles are particularly useful in medical products and personal care items where cleanliness and antimicrobial protection are essential. In addition, researchers are developing plant-based nano emulsions and herbal finishes for denim and nonwoven fabrics to provide safer and eco-friendly antimicrobial protection without relying on harsh synthetic chemicals (Venkatraman et al., 2026). Several studies also suggest that sustainable textile finishing using natural plant extracts can improve fabric functionality while ensuring environmental safety and user comfort (Karthik et al., 2022). Therefore, neem-based antimicrobial finishing has emerged as a promising solution for developing sustainable, functional, and environmentally responsible textile products.

2. Materials and Methods

2.1. Materials

The experiment was conducted during January, 2021 at NITRA, Ghaziabad, Uttar Pradesh, India. Cotton (EPI 104 and PPI 77) and khadi fabrics (EPI 42 and PPI 37) were selected for the application of antimicrobial finish using extract of Neem leaves.

Neem leaves extract was used as core material for antimicrobial finish on selected cotton and khadi fabrics. Leaves were collected from the campus of SHUATS, Prayagraj, Uttar Pradesh, India.

Various chemicals, such as chitosan, silicon oil, citric acid, amylase, hydrogen peroxide, NaOH, etc. were used for specific purposes. Chitosan was used as wall material for microcapsules, citric acid as a cross linking agent and silicon oil for preparation of emulsion. Amylase served as desizing agent, H₂O₂ as bleaching agent and NaOH as scouring agent (Plate 1).



Plate 1: *Azadirachta indica*

2.2. Methodology

2.2.1. Pre-treatment of fabrics

Before applying antimicrobial finish on cotton and khadi fabrics, the entire length of fabric was desized, scoured and bleached for which ten grams of amylase enzyme solution per litre of water was prepared maintaining the material: liquor ratio 1:50. The solution was heated to 50–60°C. Cotton and khadi fabrics were dipped into the solution separately and stirred gently till fabric turns slightly yellowish in colour. Then fabrics were kneaded and squeezed and finally rinsed under tap water and then dried in sunlight.

For scouring and bleaching, a detergent solution containing 3 g of NaOH, 5 g of Na₂CO₃, 1 g of wetting agent (soap) and 3 g of H₂O₂ were added in 1000 ml of water. The solution was prepared, maintaining the material: liquor ratio 1:50. It was heated to 100 oC and cotton and khadi fabrics were dipped into this solution separately and stirred gently for about 60 min. After pre-treatment, the fabrics were thoroughly washed with hot water and finally rinsed with cold water. Then samples were dried in sunlight.

2.3. Application of antimicrobial finish

2.3.1. Extraction of plant material

Neem leaves were collected and thoroughly washed with distilled water to remove dust and other unwanted particles. The leaves were then shade-dried at room temperature and crushed into small pieces, which were stored in airtight containers for further extraction. The overall methodology followed in the present study is illustrated in Figure 1a, whereas the detailed procedure for neem leaf extraction is shown in Figure 1b. Cotton and khadi fabrics were selected as the textile substrates, neem leaves were used as the plant material, and methanol served as the extraction solvent. The antimicrobial activity of the treated fabrics was tested against *Staphylococcus aureus* and *Klebsiella pneumoniae*.

For the extraction process, glass wool was placed inside the Soxhlet tube to facilitate filtration. Approximately 20 g of dried

neem leaf powder was transferred into the Soxhlet apparatus attached to a round-bottom flask. About 300 mL of methanol was added as the extraction solvent, and the extraction was carried out at 60°C for 3 h. After extraction, the obtained solution was filtered into a beaker and dried in an oven at 40°C until the desired consistency was obtained. The concentrated extract was then stored in a refrigerator at 4°C for further use

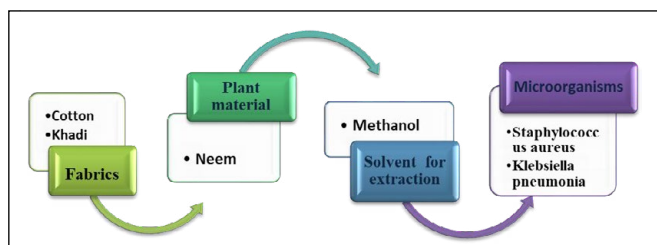


Figure 1a: Schematic diagram of methodology

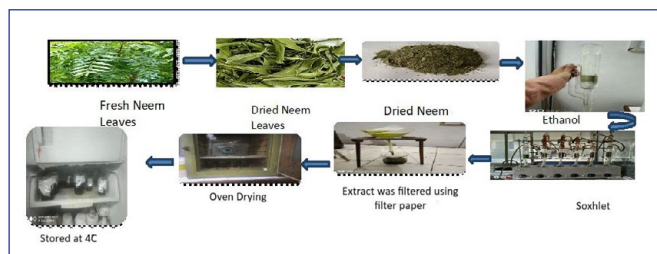


Figure 1b: Process of extraction of plant extract

in microcapsule preparation and textile finishing applications. The complete extraction procedure, including drying of leaves, powder preparation, Soxhlet extraction, filtration, oven drying, and refrigerated storage, is presented in Figure 1b (adapted from Ibrahim et al., 2020).

2.3.2. Application on fabric

The extract has been applied on the fabrics using the following two methods namely direct application and microencapsulation

2.3.2.1. Direct application

The above methanol extract of Neem leaves was directly applied on cotton fabric by pad-dry –cure method. Methanol extract (500 ml) and citric acid (6%) as cross-linking agents were taken and stirred completely. Samples were immersed in stirred extract of Neem leaves for 30 min. 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 a hot air oven which was used for drying, condensation and fixation. The samples were dried and cured at 102°C for 3 min.

2.3.2.2. Microencapsulation

To prepare the microcapsules, 2.5 g chitosan (as a wall material in microencapsulation) was dissolved in 100 mL distilled water with 1% acetic acid by mechanical stirring; kept overnight till a clear solution was obtained. Silicon oil (0.5 ml) and 2 g of core material (extract of Neem leaves) were slowly added

with continuous stirring conditions for an emulsion. Stirring continued for another 15 min. Coactivation of chitosan was done by addition of sodium hydroxide (1 g in 100 ml). Stirring was stopped and the mixture was allowed to rest for a period of 30 min at 0–5°C in the refrigerator to develop microcapsules. After that, solution was kept at 5–10°C for 24 h, filtered, and then washed thrice with distilled water.

For the application of microcapsules, a solution of filtered microcapsules and distilled water was prepared and samples were immersed in microcapsule solution for 30 min then padded through padding mangle at a pressure of 2.5 psi. Treated samples were dried at 80°C for 5 min in a 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*) microorganisms.

2.4. Functional analysis of finish

2.4.1. Quantitative analysis

Assessment of quantitative bacterial reduction was carried out by AATCC test method 100:2014 for both treated and control samples. Those showing activity were evaluated quantitatively. Treatment 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 amounts 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 sample⁻¹ (swatches in jar) not as the number of bacteria ml⁻¹ 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 was 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).

2.4.2. Qualitative method

Qualitative assessment of antimicrobial activity was carried out under 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 petri dish. 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 ensured that no surface breakage should occur while making the streaks.

2.5. FTIR analysis

Fourier transform infrared spectra of the extract of Neem on cotton and khadi were recorded using the Perkin Elmer FT-IR Spectrometer (SPECTRUM TWO). The wave number range was kept 450–4000 cm^{-1} and the resolution was 1 cm^{-1} .

2.6. SEM analysis

Scanning electron microscopy (SEM) was used to analyse the presence, binding and availability of microcapsules on the fabric surface. All the treated, untreated and washed samples were observed under instrument 'Carl Zeiss EVO 18' at a magnification of X 50 to 2500, 000 and acceleration voltage of 20 KV. Before the assessment, all the samples were sputtered under vacuum with gold.

2.7. Wash durability

After the functional analysis of treated samples, wash fastness was performed on treated samples to evaluate the fastness against treated samples up to 10 cycles wash cycles using AATCC 100 test method.

3. Results and Discussion

The results obtained from the study as well as relevant discussion are summarized here under.

3.1. Quantitative bacterial reduction

Assessment of quantitative bacterial reduction has been carried out and the results were shown in Table 1.

The result clearly revealed that all the treated fabric samples (cotton and khadi both) showed good antimicrobial properties against Gram positive (*S. aureus*) and Gram negative (*K. pneumonia*) microorganisms. It might be due to

the reason that treated fabrics did not allow the growth of microorganisms.

In the case of cotton samples, a higher percentage of antimicrobial efficacy was obtained against *S. aureus* (99.23 direct and 98.01% microencapsulation) in both the application methods. On the other hand, the percentage of antimicrobial efficacies against *S. aureus* was found to be slightly less as compared to *K. pneumonia* reduction efficacy (Singh et al., 2023).

Similar results were obtained on khadi samples. Both direct and microencapsulation methods showed higher efficacy of reduction against *Klebsiella pneumonia* as compared to *Staphylococcus aureus*. However, no bacterial reduction was observed in case of untreated samples for cotton and khadi.

3.2. Qualitative method for assessing antimicrobial activity

Qualitative assessment of antimicrobial activity has been carried out and the results are reported in Table 2.

It was observed that both untreated samples (cotton and khadi) did not show antimicrobial properties hence microbial growth was observed on these samples (Plate 1). However treated samples show presence of antimicrobial property, thereby no microbial growth on these samples was observed (Plate 2 to 6).

The qualitative test proved effective for assessing the primary antibacterial agent or treated fabrics, provided the agent could leach out. As shown in Table 2, all treated fabrics exhibited significant antibacterial activity against both Gram-positive (*Staphylococcus aureus*) and Gram-negative (*Klebsiella pneumonia*) bacteria. In contrast, the untreated fabrics allowed microbial growth, displaying no antibacterial properties.

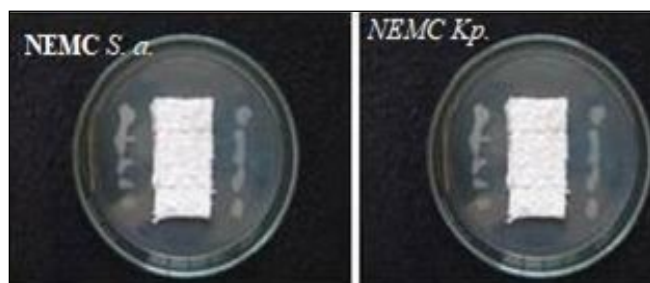
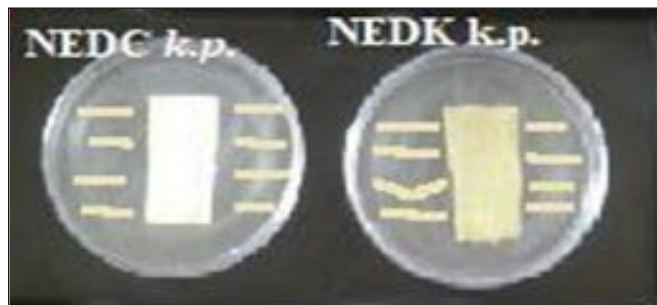
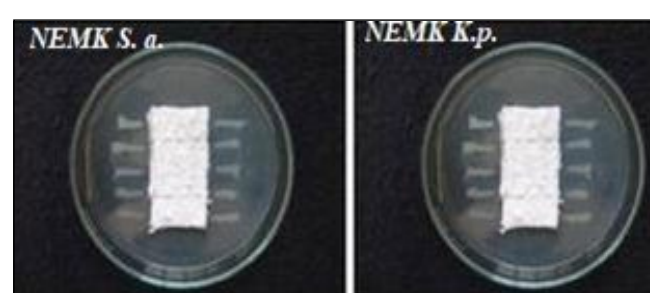
Table 1: Assessment of bacterial reduction

Sample code		Bacteria	No. of bacteria sample ⁻¹ (CFU)		Bacteria reduction %
			'0' h (B)	'24' h(A)	
Control (untreated)	Cotton	<i>S. aureus</i>	1.35×10 ⁵	1.44×10 ⁷	NS*
		<i>K. pneumonia</i>	1.41×10 ⁵	1.27×10 ⁷	NS
	Khadi	<i>S. aureus</i>	1.38×10 ⁵	1.55×10 ⁷	NS
		<i>K. pneumonia</i>	1.47×10 ⁵	1.26×10 ⁷	NS
Treated with Neem extract	Cotton	Direct	<i>S. aureus</i>	0.010×10 ⁵	99.23
			<i>K. pneumonia</i>	0.016×10 ⁵	98.93
		Microcapsulation	<i>S. aureus</i>	0.040×10 ⁵	96.36
			<i>K. pneumonia</i>	0.036×10 ⁵	98.01
	Khadi	Direct	<i>S. aureus</i>	0.021×10 ⁵	98.12
			<i>K. pneumonia</i>	0.026×10 ⁵	98.07
		Microcapsulation	<i>S. aureus</i>	0.040×10 ⁵	96.36
			<i>K. pneumonia</i>	0.036×10 ⁵	98.01



Table 2: Qualitative assessment of antimicrobial activity on cotton and Khadi fabrics treated with Neem Extract

Sample codes			Zone of inhibition (mm)		Result of antimicrobial activity
			<i>S. aureus</i>	<i>K. pneumonia</i>	
Control	Cotton		0	0	-
	Khadi		0	0	-
Neemextract treated samples	Cotton	Direct Application	25±0.6	23±0.5	+
		Microencapsulated	24±0.5	21±0.7	+
	Khadi	Direct Application	24±0.2	22±0.8	+
		Microencapsulated	23±0.6	20±0.4	+

Plate 2: Antimicrobial activity of control cotton and khadi fabric against *Staphylococcus aureus* and *Klebsiella pneumonia*Plate 3: Neem direct treated cotton and Khadi samples against *Staphylococcus aureus*Plate 5: Microencapsulated Neem treated cotton samples against *Staphylococcus aureus* and *Klebsiella pneumonia*Plate 4: Neem direct treated cotton and Khadi samples against *Klebsiella pneumonia*Plate 6: Microencapsulated Neem treated Khadi samples against *Staphylococcus aureus* and *Klebsiella pneumonia*

For both treated fabrics (cotton and khadi), no zone of inhibition was observed for either microorganism. This suggested that neem extract not only prevents microbial growth on the fabric surface but also facilitated the elimination of bacteria by leaching them out (Karthik et al., 2022).

3.3. Fourier transform -infra-red (ftir) spectra of neem leaves extract

The FTIR spectrum of Neem leaves extract, manifested in Figure 2 reveals that strong stretching band appeared around 3390.46 cm⁻¹ showed the presence of N-H stretching and

bending vibration of amine group NH_2 and OH the overlapping of the stretching vibration of attributed for phenolic group of Neem leaf extract molecules. Decrease in intensity of 3433.25 cm^{-1} simply involves the involvement of phenolic groups in neem leaves extract. The FTIR spectra shows an absorption peak at 1633 cm^{-1} which could be attributed to amid $\text{C}=\text{O}$ stretching indicating the presence of $-\text{COOH}$ group in Neem leaf extract. Hence, the presence of these functional groups validates that flavones and terpenoids molecules were present in the extract exhibiting antimicrobial activity. (Sarbudeen et al., 2022).

The FTIR analysis of untreated and treated samples were presented in Figure 3 (a and b) The phytochemicals responsible for the antimicrobial properties were identified and quantified and the frequencies were documented.

As evidenced by Figure 3 the FT-IR results, the absorbance of the control samples was in the ranges 3200 to 3600 cm^{-1} and 1000 to 1500 cm^{-1} , and Presence of $-\text{OH}$ functional groups. Absorption range from 1600 to 1900 cm^{-1} , confirmed the

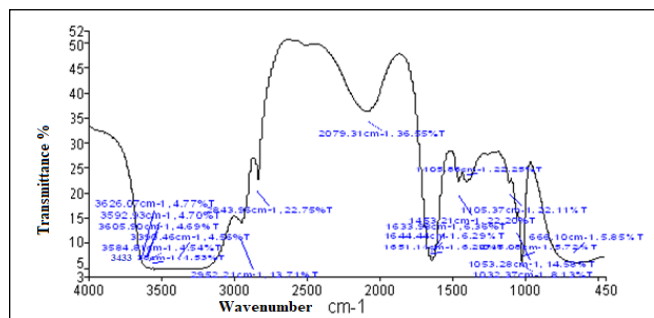


Figure 2: Fourier transform infra-red spectra (FTIR) study of *Neem* leaves extract

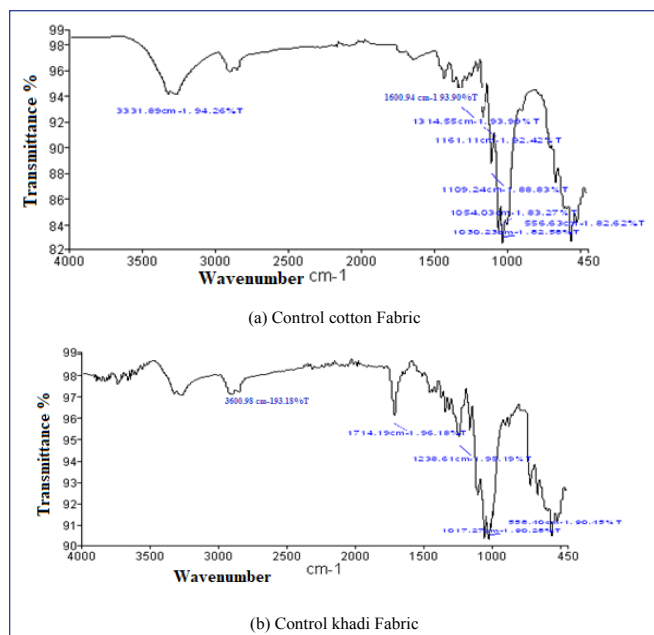
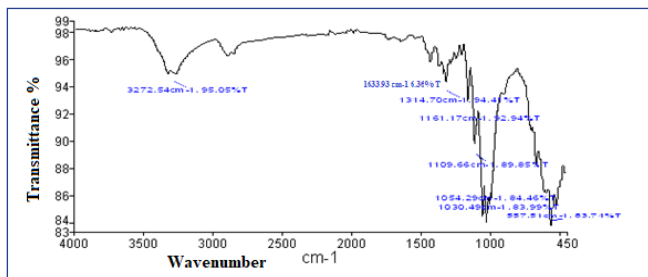
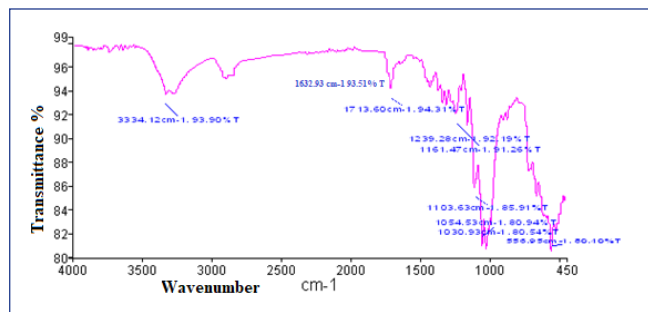


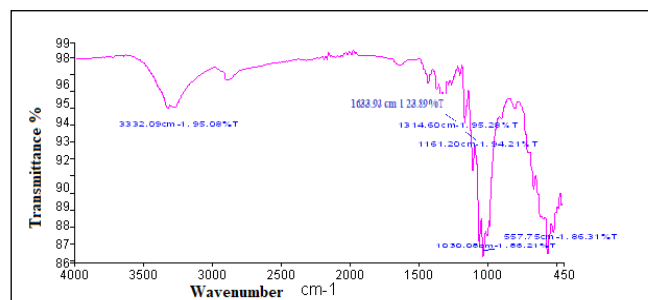
Figure 3 (a and b): FTIR Spectra of control cotton and khadi samples



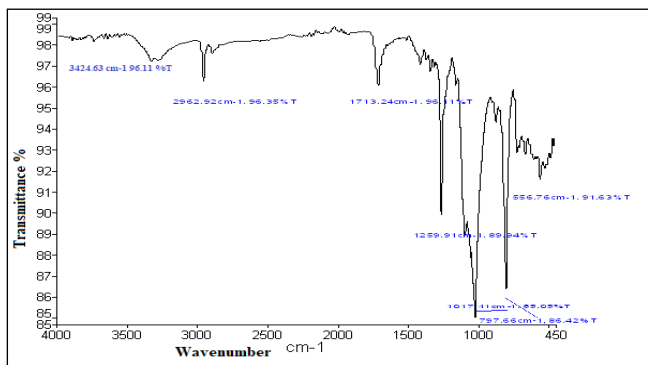
(a) Direct application of *Neem* extract on cotton fabric



(b) Direct application of *Neem* extract on khadi fabric



(c) *Neem* extract microencapsulated cotton fabric



(d) *Neem* extract microencapsulated khadi fabric

Figure 4: (a, b, c and d) FTIR Spectra of *Neem* treated cotton and khadi samples

absence of carbonyl groups ($-\text{C}=\text{O}$) in the incomplete sample, reported by Chen and Chang (2007).

The finished cotton fabric contains a lot of neem extract, groups such as methyl ($\text{C}-\text{H}$) asymmetric/symmetric stretching, ketones, primary or secondary OH bonds, alkyl sulfones and primary amines ($\text{C}-\text{N}$) in the range 3433.41 ; CN stretching of

2970.48, 1712.85, 1327.07, 1157.33, and 1026.16 cm^{-1} Amino groups or microencapsulated neem samples were presence of only one normal polymeric hydroxyl group (OH) stretch of 3433.41 cm^{-1} reported by Shanmugavasan and Ramachandran (2011).

FTIR analysis was performed to identify the presence of flavanones and terpenoids in neem leaf extracts involved in stabilizing chitosan particles. Representative FTIR spectra of pure neem leaf extract and chitosan were shown in Figure 4 in which a strong stretch band appears around 3424.63 cm^{-1} . The figure shows the presence of N-H stretching and bending vibrations of the amine groups NH_2 and OH, the water stretching vibrations and the overlap of the phenolic groups of neem leaf extract molecules (El-Naggar et al., 2020).

3.4. SEM studies

Both treated and untreated cotton and khadi samples were analysed using SEM test and the results are shown in Plate 6 and 7. Plate 6 a and b showed that both untreated samples (cotton and khadi) had smooth and clean surfaces due to the removal of all impurities including starch during the pre-treatment process. However, treated samples (Plate 7 a and b) showed rough and uneven surface due to deposition of microcapsules made by chitosan as wall material.

Plate 7 showed the surface morphology of control cotton (a) and control khadi (b) fabric. Both control samples showed a smooth surface of fibres which was obtained by the pre-treatment (at 500X) of fabrics, as all added finishes and impurities got washed out from the surface of the fabric. (Ahmed et al., 2022)

3.5. Wash durability of the finished fabric

All the treated samples were washed for up to 25 cycles to check durability of antimicrobial finish and the results are reported in Table 3.

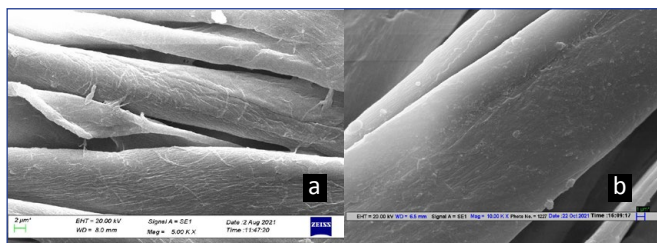


Plate 7: SEM view of (a) Control Cotton fabric, (b) Control Khadi fabric

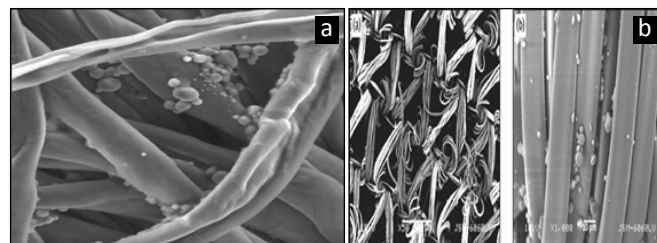


Plate 8: SEM view of microencapsulated *Neem* extract treated samples (a) Cotton fabric (b) Khadi fabric

Table 3 showed the reduction in bacterial activity after each wash cycle. Subsequent reduction in antibacterial activity was observed with increase in wash cycle. It was also clear from the Table that reduction in bacterial activity was higher in case of direct application method as compared to microencapsulated cotton samples (Khan and Malik, 2024).

Neem extract treated samples showed very good antibacterial activity up to 25 washes in microencapsulation method but it was significantly reduced in case of direct application method (Plate 8 a and b)..

Table 3: Wash durability of treated Cotton fabric against microbial property

Samples	Number of washes	Antibacterial activity reduction (%)			
		Direct application method		Microencapsulation method	
		<i>Sa</i>	<i>Kp</i>	<i>Sa</i>	<i>Kp</i>
Neemtreated cotton sample	0 wash	98	96	98	98.3
	1 wash	90	88	97.6	97.1
	3 washes	81	79	96	95.4
	5 washes	72	67	95.3	95.0
	7 washes	50	54	94.9	93.8
	10 washes	34	41	86	88
	15 washes	29	26	72	69
	20 washes	13	12	46	42
	25 washes	3	1	28	24
Neemtreated khadi sample	0 wash	97.3	99	96.8	98
	1 wash	90	91	94.2	92
	3 washes	83	82	93.4	87
	5 washes	66	69	91.3	88.2
	7 washes	59	39	86	84.5
	10 washes	36	21	72.3	71
	15 washes	20	13	46	52
	20 washes	10.60	6	33.49	30
	25 washes	1	0	20	19.51

4. Conclusion

Neem extract effectively imparted antimicrobial properties to cotton and khadi fabrics, whether applied directly or through microencapsulation. While the direct application method demonstrated notable antimicrobial activity, the microencapsulation method excelled in wash durability, maintaining antimicrobial properties for up to 25 wash cycles, unlike the negligible retention seen in the direct application. Therefore, neem extract could be used in both methods to enhance fabric hygiene, making it a valuable approach for health-conscious textile treatments.

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