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Eco-friendly antimicrobial finishing of cotton fabrics using bioactive agents from novel *melia azedarachayan* berries extract and their performance after subsequent washings

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Abstract

Fabrics with antibacterial assets have become essential to organize and manage the infestation by microbes and to reduce the formation of odor. The fabrics with antimicrobial finishes are subjected to subsequent washings and their effectiveness after each wash is of prime concern when consumed by human beings. Cotton fabric has large advantages such as compatibility, absorbency, good dimensional stability, and lower shrinkage. One of their major draw backs is that their degradation occurs due to the bacteria on them. In order to evaluate antimicrobial activity, we have prepared natural and organic extracts from peels of wild tree *Melia azedarachayan* (common name Bakaayan) plant berry. The Bakaayan berries were kept in the ethyl acetate for two weeks and a pitch of berry was obtained through the distillation process and applied to the fabric by padding (drying /curring process) and anti-microbial activities were analyzed after 24 hours by studying bacteria growth on the fabric. After analyzing bacterial growth on fabric by microscope the extracted pitch was found as antimicrobial. In a study, the fabric samples were tested for antimicrobial activity against bacterial strains like Staphylococcus, *E. coli*, Bacillus, *C. albicans* under qualitative and quantitative analysis. The results of the study have indicated that the cotton fabric shows a better microbial resistance against the above-mentioned strains except for *C. albicans*. As per quantitative analysis, the fabric treated with anti-microbial extract showed the best reduction against Staphylococcus. For analyzing the effect of washing on antimicrobial activity; the finished fabrics were washed according to the standard ISO 6330. The washing result shows a gradual decrease in antimicrobial activity after each wash.

Keywords: Antimicrobial finishing, cotton fabrics, bioactive agents, *Melia azedarachayan,* berries

1. Introduction

Antibacterial fabrics are important not only in medical applications but also in terms of daily life usage [1,2]. The application of antimicrobial finishes to textiles can prevent bacteria growth and it has become increasingly prominent for hygienic and medical applications. Habitually, microbial attacks the textile fibers on the textile products especially medical textiles such as surgical gowns, composites, and recovery items and other undergarments and socks etc [3–5]. These items are relatively affected by many kinds of microbes and cause the infection in a sector where it is used like a hospital, medical institutes & dental clinics [6,7]. A microbe generates the bacteria, fungi, and mildew which nourishes the infection by odor, staining & deterioration in this sector and affect the environment rapidly [8]. There ought to be a role of antimicrobial activity to enhance

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textiles ability along with to make textile finishing eco-friendly. Antimicrobial finishes were extracted from many sources like natural, synthetic & animals, but for final aspects choose those which have results in all environmental aspects [9]. Natural antimicrobial finishes are mostly extracted from plants and fruits, among which aloe vera is an important source from plants and a pioneer in antimicrobial finishes [10].

In fruits, pomegranate is traditional for antimicrobials activity but the chemical composition of lemon and orange is also the contest for the control of the microbes attacks [11]. Most of the natural finishes, dyes & pigments are human-friendly and contain the groups called phenols, quinone's, flavonoids, lectines, and polyacetylenes which showed good antimicrobial activity [12]. Some researchers start working on *Melia azedarach* (Bakaayan) as the natural antimicrobial source. This tree wildly exists in the Asian region mainly found in the south Asian countries like India, Pakistan and Nepal, etc [13]. It is also found wildly in the African forests. *Melia azedarach* is antiseptic also because it is capable of preventing infection by stopping the growth of infections agents [14] . *M. azedarach* antibacterial and antioxidant efficacy is based on the solvent for use as a medicinal plant (leaf extracts) with mostly methanol as a solvent [15, 31]. Steroids, alkaloids, phenolic, flavonoids, polyphenols, and glycosides were found in the methanolic leaf extract [16]. M. azedarach was investigated for antibacterial activity against 6 pathogenic strains: Staphylococcus aureus, Escherichia coli, pseudomonas aeruginosa, klebsiella pneumoniae, and proteus vulgaris [17, 18].

These compounds in plants seem to play a major role in defence function against pathogens such as bacteria, fungus, and viruses that invade them [19]. The sugar moiety has a significant role in influencing the bioavailability of flavonoids, which are often found in glycosylated modification in plants [20]. Drying fresh green leaves at temperatures between 21 and 30 degrees Celsius for 30 days was researched by Sabira Sultana and colleagues [21]. The antibacterial properties of the peel extract are directly related to the constituents that they contain. Propine and corydaline alkaloids were shown to be beneficial in a variety of bacteria in the tests [22, 29, 30]. Lactone and polyacetylene compounds were also found to be effective in a number of bacteria. Peels include phenolic and flavonoid inhibitors, which were shown by phytochemical investigation to be powerful components [23, 27, 28]. Traditional work of natural antimicrobial is mostly based on and moved around Aloe Vera plant and pomegranate fruit extracts. Pakistan is an agricultural country and increased growth rate of seasonal life lot of peel fruit which used in daily life or some are wild ones [24]. Today a lot of chemicals are banned due to environment safe regulation by ECOTEC, so natural fruits peel follow this regulation by their chemical composition and have the ability to resist microbial effect [25] This research analyzes the best natural fruit extract which is eco-friendly and locally available in abundance. During laundry washing, the repeated loading and aggression caused the removal of antimicrobial finishes from the surface of the fabric. In this research, we have discussed the durability of an anti-microbial finish with multiple items of washing and discussed some of the ways to improve it. The wash durability of treated fabrics was analyzed as per the ISO test method, after being subjected to several repeated wash cycles.

The objective of this study was to evaluate the antibacterial activity of (100% Cotton fabric) treated with the natural extract M. azedarachayan (Bakaayan) against several pathogenic microorganisms. It is essential to enhance the antibacterial activity of textiles in use; hence, the antimicrobial property of unwashed and washed fabrics was studied in comparison. The overall research methodology is shown at Fig. 1.

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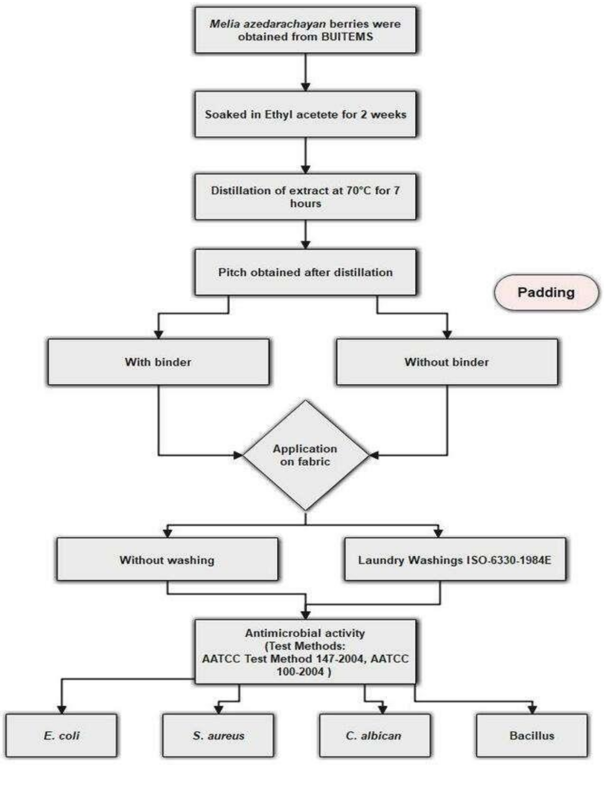

Fig. 1 Flow chart of research methodology

2. **Materials and Methods**

2.1 Chemicals and samples

100% cotton fabrics were obtained from Gul Ahmad textiles, Pakistan. The berries of "*Melia azedarach*ayan" (Bakaayan) tree extract was obtained from BUITEMS, Pakistan. All other reagents and chemicals of analytical grades or higher purity were purchased from Merck (Darmstadt, Germany). The hydraulic padder has used for padding application for agent and fabric. The BUITEMS processing lab accessories like padder and dry heat chamber has used for it.

2.2 Preparation of Fabrics

This study used 100% pure bleach treated cotton cloth received the supplier (Gul Ahmed textile Karachi). After the cloth was tumble-dried, it was rinsed in a washing machine at 40 °C for 45-50 minutes with a wetting agent. Fabric and water specifications employed in this research are presented in detail in Tables 1 and 2.

Table 1. Tested Fabric Specification

Fabric composition	Weave type	Area weight (g/m²)	Warp yarn count (tex)	Weft yarn count (tex)	CIE Whiteness index (WI)	Absorbency (Sec)
Bleached 100% cotton	Plain	115.6	30	30	66.5	0.7

Table 2. Tested water specifications

H	'otal hardness ɔpm)	'otal dissolve solids ɔpm)
.9	4.6	13

2.3 *Melia azedarach*ayan (Bakaayan) tree extract

*Melia azedarach*ayan berries were obtained from wild trees, in the first stage (Full green form) (Fig. 2). The leaves were washed with distilled water and cleaned. Berries were put in ethyl acetate in a 2:1 ratio for 2 weeks in a closed flask. After two weeks the mixture turned brown (Fig. 2) and was filtered for distillation at 70° C for 7 hours. The resultant was referred to as "pitch" as shown in Fig. 2 ©.

2.4 Application of pitch on fabric

The solutions of the pitch were prepared according to Table 3. Test specimens were cut in 25.0 ± 0.1cm rectangular and dipped in the respective solution with the help of a hydraulic padder see Fig. 3(a). After the padding process, the next process of application is fixation. Fixation at various temperatures was performed and after fix 10 washings for each temperature sample, the antibacterial activity was checked to find out the optimal temperature for fixation. From Fig. 3 © the temperature of 50℃ was found to be optimal. After optimizing the curing temperature fixation was performed at 50℃. The antimicrobial fabric samples were prepared according to Table 4. After curing the samples were weighed again to calculate the pickup dye of the samples, Fig. 3 shows the application and curing parameters of applied antimicrobial material. Further analysis has been revealed in surface microscopy.

Table 3. Solutions composition prepared from the pitch

Solution	Extract (mL)	Water (mL)
A	25	75
B	50	50
C	75	25

2.5 Domestic Washing Test ISO: 6330–2012E

ISO: 6330 assessed the completed materials' antibacterial activity after numerous wash cycles. Type A (Annex B & Annex D) is the origin of the machine in 2012E, where we used processes Nos. 1A & 5B. Repeating the domestic cleaning processes increases the frequency of times they are washed by. Standard detergent was used to clean the final products. According to a reference detergent, ECE detergent was applied with sodium perborate excluding any cleaning enhancers like optical brightener and enzymes (3% owf).

A) Select the appropriate wash programme for the type of washer being used. Set the domestic washing number, water levels, and pull out the right knobs on a washing machine (FOM 71S Wascator).

B) Start the machine. Put the dissolved powder into the machine by raising the cover on the top of the machine when the water level is above the base level in the view tube. Rinse the beaker with a fresh supply of warm water to flush off any remaining residue.

C) Discard the wash load after the programme is complete. Swatches of the same cloth with no antibacterial coating were utilized as a negative control in all studies. They were the standard.

Table. 4. Application parameters for *Melia azedarachayan* extract

Source extract	***Finishing Concentration/%***	***Pickup/%***	***Finishing temp& time***	
			Drying	**Curing**
***Melia azedarach*ayan**	1g, 5g, 10g /100 ml	80	50℃, 10 min	150℃, 3min

2.6 AATCC 100-2004 Test Method (Assessment of anti-bacterial finishes on textile material)

A steel die was used to cut test specimens to a diameter of 5.0 ± 0.1 cm. Test specimens were individually injected with 150 µL of working culture on sterile Petri plates. Each specimen was inoculated and then put in a 100 mL screw cap jar with neutralizing agent (3 percent Tween-80 and 0.3 percent lecithin in sterile distill water). There was no toxicity seen in neutralizing agents against tested pathogens. For one minute, the jars were shaken forcefully, and repeated dilutions were performed. Transferring 0.1ml of each appropriate dilution to TSA was done for each of the three dilutions. Fig. 4 shows an E. coli agar plate that has grown effectively. After 48 hours of incubation at 37°C, a colony counter device was used to count the number of colonies as CFU/ml (Acolyte Super colony Counter, Symbiosis). For the microscopic views of the staining examinations of E. coli and S. aureus, please see

Figure 5. Since S. aureus is gram-positive and verified by its spherical form, it keeps its purple hue, but E. coli

looks pink in color because it is gram-negative. Additional jars were also made to gather data on the treatment's bactericidal action during the course of its contact time (60 minutes). *Percent reduction of bacteria by the specimen treatments was calculated using following formula:*

$$R = 100\,(B - A)/B \qquad 1.1$$

Where, **R** is the reduction,
A is the number of bacteria recovered from the inoculated treated test specimen swatches in the jar incubated over desired contact period.
B is the number of bacteria recovered from the inoculated treated test specimen swatches in the jar immediately after inoculation. Swatches of the same fabric construction which containing no antibacterial finish were used as negative control in all experiments.

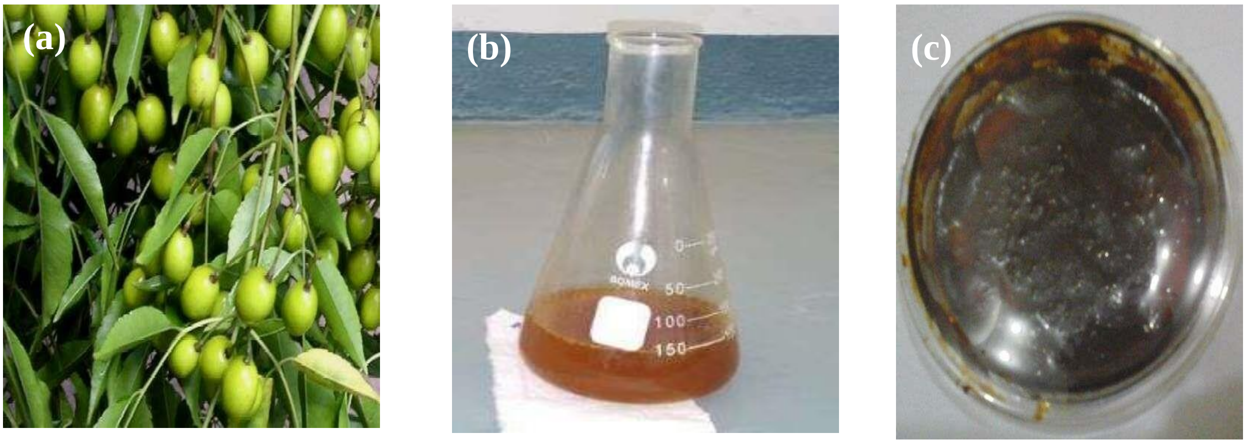

Fig. 2 (a) Berries of the tree *Melia azedarachayan*, (b) Extract from *Melia azedarachayan* berries, (c) Pitch from extract of *Melia azedarachayan* berries

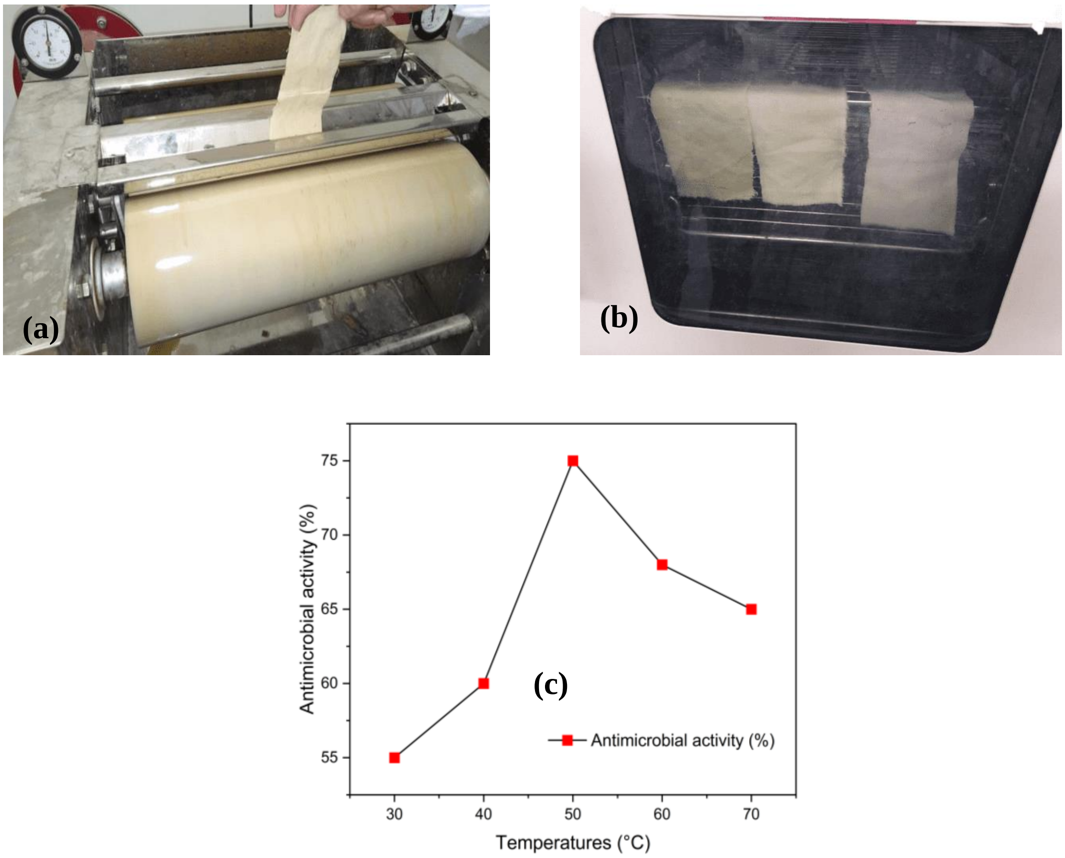

Fig. 3 (a) Applied *Melia azedarachayan* berries on fabric through padding, (b) Curing the treated sample in oven, (c) Optimization of curing temperature

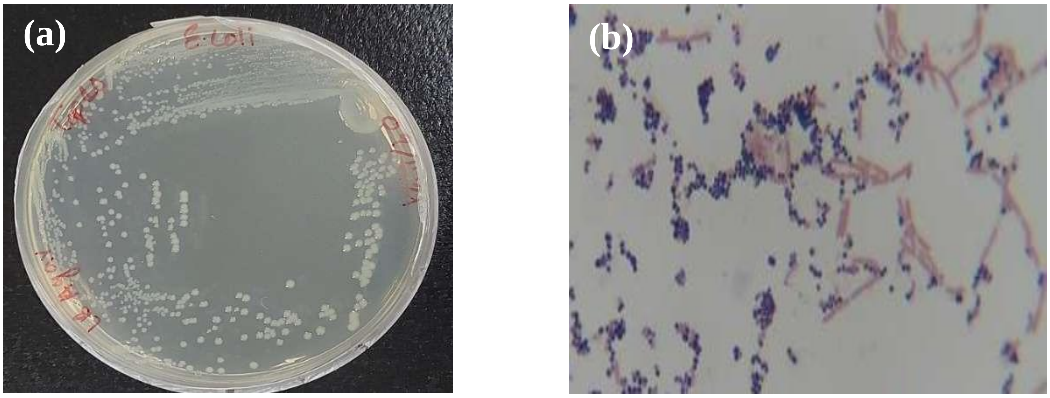

Fig. 4 (a) *E. coli* grown on agar plate for the gram staining, (b) Gram staining of *E. coli* and *S. aureus* for the qualitative analysis of the stocks

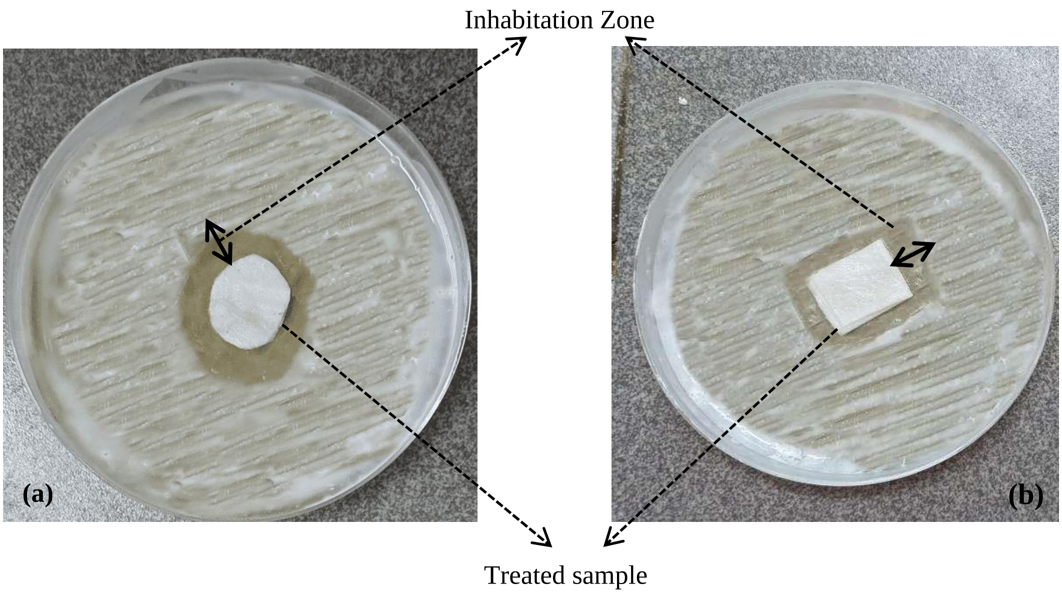

Fig. 5 Antimicrobial activity test at cotton samples, (a) bacillus subtilis, (b) candida albicans

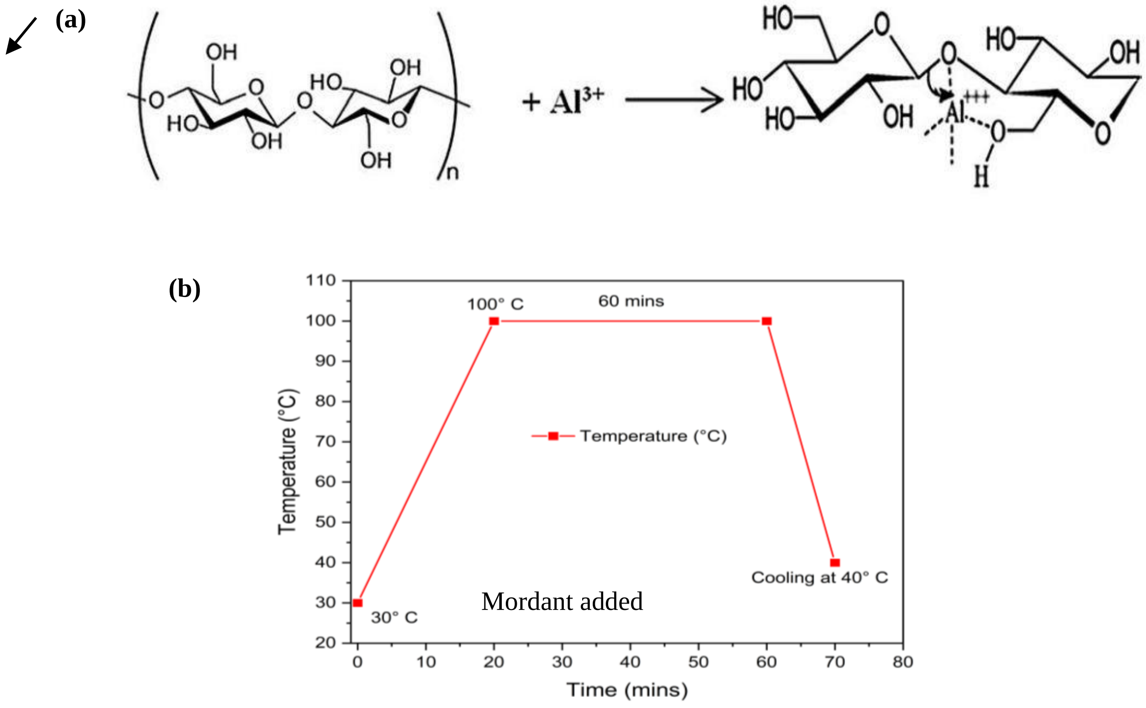

Fig. 6. (a) Co-ordination bond formation between 100% cotton fabric and alum [26], (b) Fabric mordanting process curve

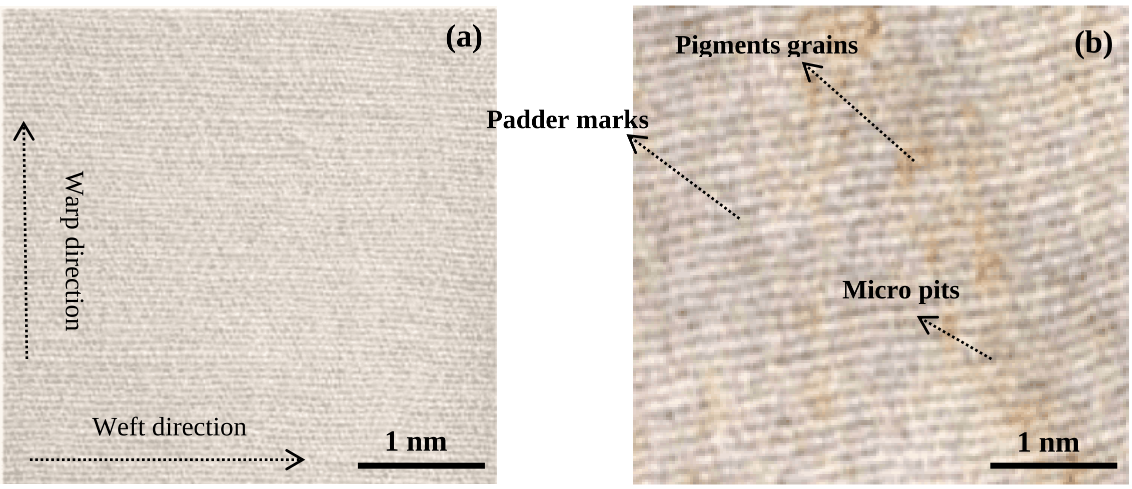

Fig. 7 Microscopic images of 100% cotton fabric (a) Before application of antimicrobial agent, (b) After padding and curing process

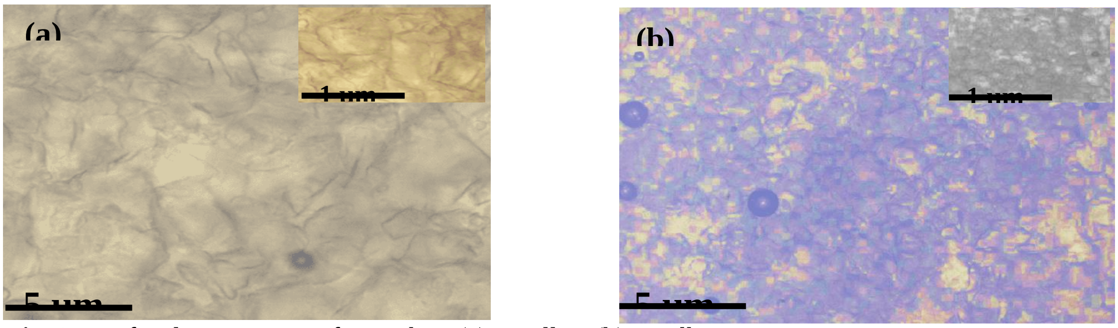

Fig. 8 Confocal microscopy of microbes, (a) Bacillus, (b) *C. albicans*

Table 5. Antibacterial activity of *Melia azedarachayan* Extract with subsequent washings (with and without binding agent)

AATCC 100-2004(Quantitative) ***Melia azedarach*ayan** Extraction Samples (10g/100ml)	No of Washes	Staphylococcus (Gram Positive) %	*C. albicans* %	Bacillus (Gram Negative) %	*E. coli* (Gram Negative) %
10g/100ml	0	78	11	65	74
	5	60	7	57	58
Before Bindering	10	53	5	48	51
	20	48.5	3	41	46.5
	0	80	12	68	77
	5	77.5	8	61	73.5
After bindering	10	68.8	7	57	64.1
	20	63.2	4	49	61.2

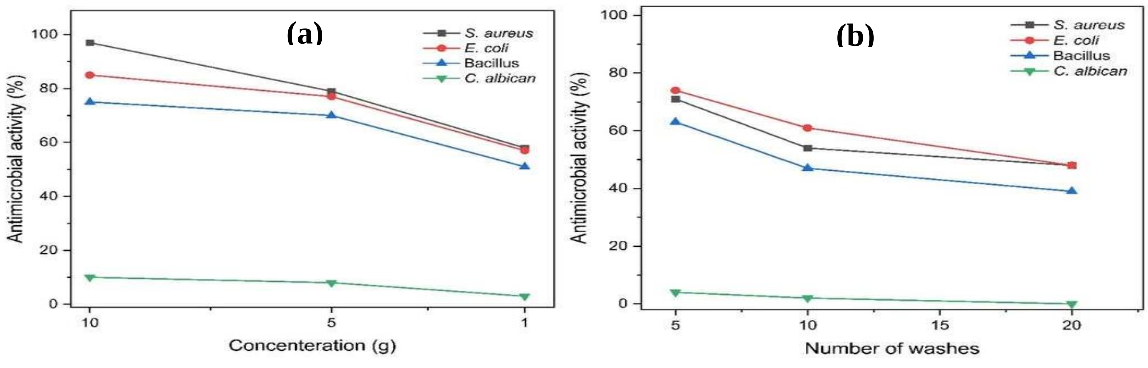

Fig. 9 (a) Effect of extract concentrations on anti-microbial activity of *Melia azedarach*ayan, (b) Effect of number of washings on anti-microbial activity of *Melia azedarach*ayan

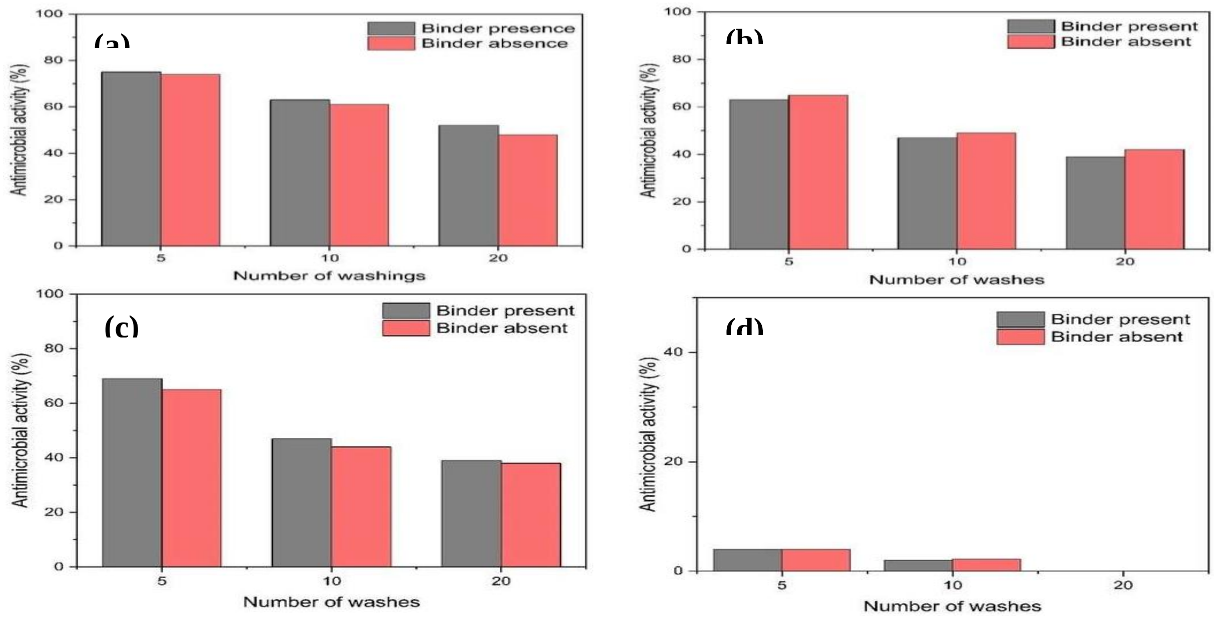

Fig. 10 Effect of binder on antimicrobial activity against (a) *E. coli*, (b) *S. aureus*, (c) Bacillus, (d) *C. albican*

Table 6. Quantitative antibacterial activity of *Melia azedarach*ayan extracts samples with different washings

AATCC-147-2004(Qualitative)				
	S. aureus (AATCC 6538)	*E. coli* (AATCC4352)	Bacillus (AATCC 6538)	*C. albican*
Fabrics	Inhibition Zone(mm)			
***Melia azedarach*ayan Extract Treated Swatches**	3.5±0.04mm[a]	4.5± 0.04 mm	3.8 ± 0.04 mm	0 mm and heavy growth
Untreated Swatches (Control Sample)	0 mm and No growth reduction	0 mm and No growth reduction	0 mm and No growth reduction	0 mm and No growth reduction

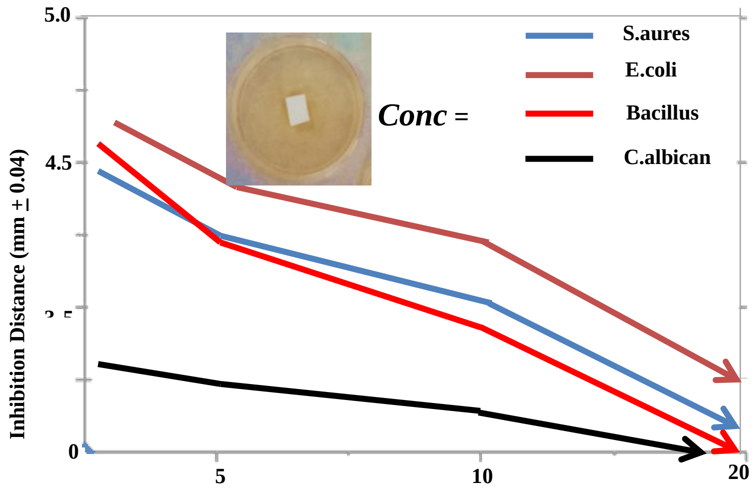

Fig. 11 Graphical performance of antimicrobial activity according AATCC-147-2004 (Qualitative) method

2.7 Effect of binder on fixation

To improve the attachment of extract normally the process of mordanting is used. In this study the effect of potash alum as a binder in mordanting process was evaluated (Table 5). Since it forms co-ordination bond with the cellulose (cotton fabric in our case) as shown in the Fig. 6a. The 100% cotton fabrics were pre-mordanted with the binder potash alum (5g/L) at 100° C Fig. 6 and the antimicrobial activity was checked after subsequent washing as discussed before.

2.8 Characterization of mordanted cotton fabric

2.8.1 FTIR spectra

The potash alum treated 100% cotton fabric was also analyzed for functional groups using FTIR spectrum in the region from 4000 to 500 cm^{-1}.

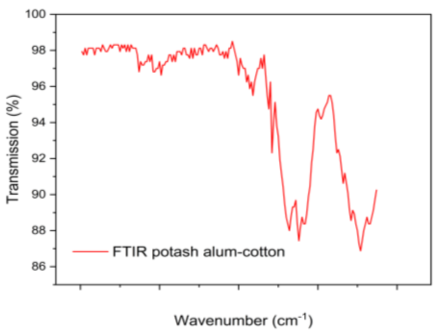

Fig. 12 FTIR spectra of potash alum treated 100% cotton fabric

2.8.2 SEM analysis

SEM analysis was conducted to understand the morphological characteristics of binder untreated and treated 100% cotton fabric. Fig. 8 (a) shows the morphological and surface characteristics of untreated fabric at different magnifications level. Fig. 8 (b) has shown the morphological and surface characteristics of treated fabric at different magnifications level.

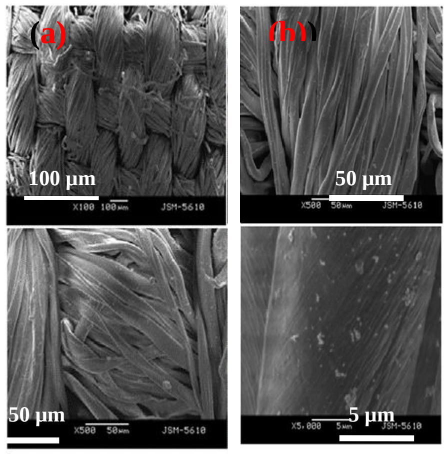

Fig. 13. **SEM analysis of 100% cotton fabric, (a) before treatment, (b) After treatment**

2.9 Surface microscopy

The microscopy of microbes and the treated fabric has been observed under the digital microscope with optical laser microscopy to reveal the surface modification after treatment with antimicrobial pitch. Fig. 9 (a) has revealed the changes on the surface of the fabric before and after application. The color of the fabric surface has changed and pigments have clearly covered the cellulosic area of 100% cotton fabric. The padder marks and micro pits on the surface at the swatch. Fig. 9 (b) shows some yellow grains of pigments after the dry and fixation process. Fig. 9 (b) reveals that the pitch *Melia azedarach*ayan has fixed and bonded with the cellulosic fabric. Laser micrographs are shown in Fig. 10, in which it can be seen that in most of the impregnated microbes and agar behavior belong to the gram-positive bacterium. There are specialized growing hyphae. As for bacillus subtilis in Fig. 10 (a), it can be concluded that there were better results for growing the bacteria. Fig. 10 (b) reveals the impregnation for the case of *C. albicans*. The confocal method shows the generation of agar through bacteria at a pitch.

3. Results & Discussion

The antimicrobial activity of the finished samples was evaluated after being subjected to several wash cycles through the test method ISO: 6330.2012E. For this study type, A machine (FOM 71S Wascator) with1B & 5B procedure was used as recommended by ISO standard.

The same above domestic washing procedures were repeated for each wash. The standard ECE detergent was used with sodium perborate without optical brightener & Enzymes (3% owf). Fig. 5 shows the antimicrobial activity on 100% cotton fabric after subsequent washings. The gram-positive and gram-negative bacteria behave differently. Better antimicrobial activity was observed against gram-negative *E. Coli* due to the presence of their thin cell wall. The cell wall of gram-positive bacteria is quite thicker. The detailed results of the quantitative test have been presented in Table 5.

Potash alum is considered to be an optimal binder for the 100% cotton fabrics. It forms a coordination bond between the oxygen atom in the glycosidic bond and aluminum Fig. 6(a). In order to check the effect of potash alum as binder to see the improvements in the binding ability of our extract. After the pre-mordanting, the fabric was characterized by using FTIR and SEM. FTIR showed the characteristic metal and cotton peak at 886.32 cm^{-1} confirming the formation of co-ordination bond and ultimately successful coating Fig. (7). SEM analysis before the binder-extract treatment showed smooth surface of the cotton fabric Fig. 8 (a) and the analysis after the application of binder showed the coarse surface of the fabric confirming the coating of the fabric Fig. 8 (b).

A better anti-microbial effect was observed against gram-positives at high concentrations. Table .5 reveals the comparison binder treated and untreated, it is observed that the binding agent works well for the furtherance of domestic washing against the antimicrobial activity. Improvement of results has been achieved by adding the amount of binding agent. The lowest antimicrobial activity was generated against *C. albican*. This is possible due to the fungal nature of *C. albican* and the presence of chitin in the cell wall which has been shown in Fig. 9 & 10 (b). The energy accumulated in the surface molecules of water is manifested as surface tension. As shown in Fig 5 (a) *M. azedarachayan* has excellent antimicrobial properties. The pitch solution has shown potent antimicrobial agents like flavones and many polyethoxylated compounds. The effects of concentration of extracts were analyzed at 1g, 5g, and 10g /100ml for both gram-positive and gram-negative microbes. The best antimicrobial performance was achieved at 10g /100 ml against all microbes. This is obvious because of the increased concentrations of the active ingredients. For Staphylococcus, *E. coli*, and Bacillus microbes a gradual decrease was observed in Fig 11 (a). (10 g) shows the better antimicrobial activity as compared to less concentration (1g). It is mainly due to the presence of alkaloids, which are having antibacterial potential. Antimicrobial performance is reduced as we reduce the concentration from 10g to 1 g/ 100 ml. The treated fabrics were found to have wash durability up to 20 wash. In the case of antimicrobial activity, after each wash cycle, the antibacterial activity decreases gradually and that is evidenced at the end of the 20th wash cycle Table 5. The percentage reduction value reduced gram-negative and gram-positive bacteria respectively. Fig. 11 has represented the effect of concentration and washes on antimicrobial activity. Fig. 11 (a) has shown the effect of concentration on different microbes which are used in the analysis of microbial activity. It has been revealed with the help of graphical values that the highest concentration should be greater in antimicrobial activity. As previously proved that higher concentration is related to the great value of the antimicrobial activity, Fig. 11 (b) has revealed that when the number of washes increases the decline seen in antimicrobial activity. This is mainly due to the fact that the forces of attraction between the pitch and cotton fabrics are mainly governed by Van der Waals forces. The washing process is the method of cleaning the surface of a solid material with a liquid bath that involves a physicochemical activity other than simple water or some mixture. It is exceptionally improved cleaning effect of a liquid bath generated by the presence of a particular substance, the detergent. When a dirt particle is removed from a substrate during laundry, an adhesive link between both the particle and the fiber is broken. The attractive (primarily Van der Waals) forces and the contact area between the soil particle and the fiber surface determine the strength of this bond and, as a result, the energy needed to remove the particle. This also explains the decrease in antimicrobial activity of the final finishes. The interaction between the applied pitch and the cotton cloth diminishes after every wash, and the microbial activity decreases with consecutive washing. Surface tension exists at the liquid-air contact while interfacial tension exists at the liquid-liquid or liquid-solid interfaces of water. Water cannot penetrate and wet fabrics due to interfacial tension. The electric forces of attraction (particularly hydrogen bonding) work in all directions in a body of water, keeping each molecule in balance. However, there are no forces acting from the air side at the water's surface, therefore the equilibrium is Fig. 12 is showing the relation between the presence and absence of binder with the antimicrobial activity after subsequent washes. It is concluded from the results that the binder increases the retaining of antimicrobial properties of the 100% cotton fabrics. The maximum retaining percentage was observed in the case of E. coli Fig. 12 (a) and the minimum was observed in the case of *C. albicans* Fig. 12 (d). However, a negligible difference was observed in the case of Bacillus Fig. 12 (c).

Table. 6 shows have shown the qualitative results, where all samples had been tested for antimicrobial activity along with subsequent washings with and without binder. After 20 washes, the antimicrobial activity was reduced for Staphylococcus and *C. albicans.* Other microbes have shown maximum resistance to antimicrobial Fig. 13 has represented the antimicrobial activity analysis against the method AATCC-147-2004 which belongs to qualitative analysis. The graph revealed that increasing the washing number should be decreasing the inhibition zone value. Fig. 13 has clearly identified that 10 g liquor ratio samples are resistant to the microbes, but initial washing numbers only. As the detergents worked by weakening the Van der Waals forces between the dirt particles and the 100% cotton fabrics so it is also decreasing the interactions between the applied pitch and the fabrics. This is the reason why subsequent washings are decreasing the antimicrobial activity.

CONCLUSION

Anti-bacterial capabilities were discovered in the coating of 100% cotton fabric made from plant natural materials. *Melia azedarach*ayan, a useful source of waste biomass, includes an antimicrobial ingredient that may be used to make medical textiles resistant to bacteria. Only C. *albicans* was shown to be resistant to the prepared textile. Each time a garment is washed, it loses its ability to fight against germs. As a result of this friction, active agents were converted to natural extracts. Using potash alum as binder, we are able to get around this problem. It is via the application of a binder that dyes are kept in place on the cloth. A process' ultimate fastness and cost criteria will always guide the selection of binders. The vast majority of textile binders are the result of polymerization addition. For pigment printing, the binder film has a 3D structure, but the third dimension is of minor relevance. Anti-microbial properties were studied after a series of washing methods. The antibacterial activity decreases with each wash, as seen by the resulting wash results. Van der Waals forces are disrupted during washing cycles, causing natural extracts to lose their active ingredients. We successfully overcome this difficulty with the aid of binders. Our developed antimicrobial 100% cotton fabrics find their applications in biomedical applications like wound dressings, and they can also be used as the gown material in the hospital by the staff for the prevention of diseases. These fabrics can also be used for the making of sportswear for athletes to prevent microbial infections passively.

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Conflicts of interest

"There are no conflicts to declare"

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تشطيب مضاد للميكروبات صديق للبيئة للأقمشة القطنية باستخدام عوامل نشطة بيولوجيًا من مستخلص التوت **Melia azedarachayan** الجديد وأدائها بعد عمليات الغسيل اللاحقة

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