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INVESTIGATION OF ANTIMICROBIAL ACTIVITY OF COTTON AND BLENDED FABRICS TREATED WITH NATURAL BIOACTIVE AGENTS.

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Abstract

The natural bioactive agents were found to be highly effective as antimicrobial agents and successfully inhibit the bacterial growth. In the present study, natural bioactive agents were extracted from aloe vera sap and catechu by using organic solvent (Ethylene) by maceration method. The above bioactive agents (5% & 10 % owf) were applied on plain cotton and polyester/cotton woven fabrics by the pad-dry-cure process. For fixation of the finishing agents, Glutaraldehyde (8% owf) was used as a cross-linking agent along with Sodium hypophosphite (2% owf) as the catalyst. Evaluation of the antimicrobial activity of untreated and treated fabrics was performed quantitatively by percentage reduction test (AATCC-147-1998) against test organisms gram-positive bacteria staphylococcus aureus (ATCC 6538) and gram-negative bacteria Escherichia coli (ATCC 11230). The results indicate that treated fabrics exhibit above 80% antimicrobial activity against S. aureus and E. coli bacterial strains. After 10 washes the efficacy of antimicrobial activity was reduced by 10-20%.

Key words: antibacterial finishing, bioactive agents, crosslinking, bacterial growth

1. Introduction

Microorganisms, particularly bacteria, can cause infection, disease, odors, health concern along with the problems of deterioration, and staining of textile products. For these reasons, it is highly desirable that the growth of microbes on textiles be minimized during their use and storage. This paper present the studies on the evaluation of antimicrobial properties of fabrics treated with extracts of natural plant based compounds such as Aloe vera sap and Catechu. Aloe vera gel contains large number of constituents mainly mucopolysaccharides, enzymes, fatty acids, sterols, prostaglandins, amino acids and a wide variety of minerals and vitamins. It also contains some bioactive compounds such as salicylates, acemannan, lupeol, magnesium lactate, β-sitosterol, campestrol and anthraquinones (Joshi et al 2009).

Aloe vera is an ornamental and medicinal plant. Whole leaf components found to have direct antibacterial properties due to the presence of bioactive compounds such as anthraquinones and saponin. Polysaccharides in aloe vera plant have bacterial activity through the stimulation of phagocytic leucocytes to destroy bacteria. It was observed that the antimicrobial agents of aloe vera gel can kill or greatly reduce or eliminate the growth of various microbeswhich includes *Staphylococcus aureus, Klebsiella pneumoniae, Streptococcus pyogenes, Pseudomonas aeruginosa, Escherichia coli, Propionibacterium acne, Helicobacter pylori* and *Salmonella typhi.* Aloe leaf contains about 200 active ingredients including 75 nutrients, 20 minerals, 18

amino acids and 12 vitamins. Aloe Vera has been used in most of all the cosmetic products and healing wounds and burns.

Different polysaccharides are found in Aloe Vera which include glucomannan with different molecular weight, an acetylated glucomannan, galactogalacturan, galactoglucomannan with different composition as well as acetylated mannan or acemannan. Acemannan a long chain polymer consisting of randomly acetylated linear D-mannopyranosyl units has immunomodulation, antibacterial, antifungal antitumor properties. Wasif and Rubal, treated cotton fabric with aloe vera extract at 5, 10, 15, 20 and 25 gpl concentrations and glyoxal of 100 gpl was used as a cross linking agent. It was observed that the treated fabric exhibited excellent antimicrobial property compared to untreated (Wasif et al 2007) . A study conducted by Vyas, Ingale, Mukhopadhyay, and Saraf (2008) revealed the effect of aloe vera gel on antimicrobial properties of cotton fabric. They observed that aloe was effectively inhibiting the microbial growth on treated cotton fabric. The fabric finished with aloe vera and neem combination was found to be durable.

Catechu is found in several species of acacia trees which is generally used as a food additive, astringent, tannin, and dye. The catechu is high in natural vegetable tannins. Cutch is used for dyeing of wool, silk, and cotton and give a yellowish-brown. It gives olive-browns with a copper mordant and gray-browns with an iron mordant. Catechu also known as cutch, black cutch, and is extracted from acacia trees used as a food additive, astringent and dye. Under the name *cutch*, it is a brown colour dye used for dyeing wool, silk, and cotton. Rajjni Singh *et al* have studied bactericidal properties of some available dye powders, namely Acacia catechu, Quercus infectoria, Kerria lacca, Rubia cordifolia and Rumex maritimus, against some common microbes. They applied these dyes on wool fabrics with a view to develop protective clothing. Acacia catechu (at 9.2%) was found to be effective against all microbes tested except Pseudomonas aeruginosa. A reduction of 10-15% in bacterial growth is observed which is found to be insufficient (Singh R et al 2005).

2 Martials and methods

2.1 Materials

Fabric: For the application of the above compounds plain cotton and polyester/cotton woven fabrics were used in this study. The test fabrics were sourced locally and their construction particulates are shown in Table 2.1

Table. 2.1 Construction particulars of test fabrics

Sl. No.	Fabrics	Particulars		
		Ends/cm x Picks/cm	Yarn count (Tex)	Fabric Weight (GSM)
1	Cotton Fabric	16X14	12X13	113
2	Polyester/ cotton blended fabric (67/33)	16X13	14X12	115

Antimicrobial agents: **A**ntimicrobial extracts used for this study were extracted from the following natural plant materials (Fig.2.1 & Fig 2.2).

1. Aloe vera sap
2. Catechu powder

Fig. 2.1 Aloe vera plant and sap

Fig.2.2 Catechu plant and Catechu powder

2.2 Experimental methodology

2.2.1 Extraction of active compound

Green leaves and the respective plant parts were separated and were first dried in shade, later at 40ºC in a drier. Later these plant materials were powdered into fine particles using a laboratory grinding machine **(Fig. 2.3).** This powdered plant materials were used to prepare the plant extract using organic solvent.

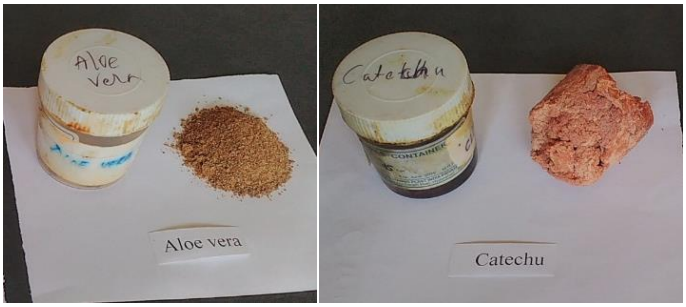


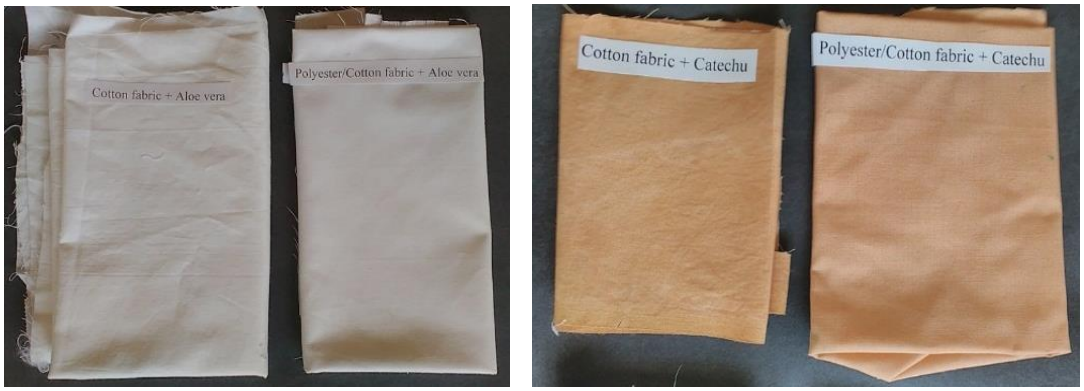
Fig. 2.3 Plant materials in powder form and extracts of Aloe vera & Catechu

The active compound was extracted from dried plant materials by maceration method using methanol as solvent for 5 days at room temperature and stored in dark**..** The extract was filtered using Whatman filter paper IV. The solvent was then distilled under reduced pressure in a rotary evaporator until it become completely dry.

2.2.2 Application

The natural extracts were applied (5, 8 & 10 % owf) to the cotton and polyester/cotton blended fabrics separately using liquor ratio 1:30 along with glutaraldehyde (8%) and Sodium hypophosphite (2%) by conventional exhaust process. To show the effect of cross linking agent on wash durability of the finish, fabrics were also applied with the extract (5%) without glutaraldeyde. The finishing bath was heated at 40-50^{o} C and kept for 25-30 min. The fabric was taken out, squeezed and dried at 85^{o} C for 5 min and then cured at 120^{o} C for 2 min. **Fig. 2.4** show the photographs of treated fabrics.

- Extract : 5, 8 & 10 % owf
- Glutaraldehyde (cross linking agent) : 8 %
- Sodium hypophosphite (Catalyst) : 2 %
- Liquor ratio : 1:30
- Temperature : 40 - 50°C
- Time : 25-30 min
- Drying temperature : 60-80 °C
- Curing Temperature : 100 - 120℃



(a) **(b)**

Fig.2.4 Photographs of cotton and polyester/cotton fabrics treated with extracts of

(a) Aloe vera (b) Catechu

2.2.3 Evaluation of Antimicrobial activity

For the study test bacteria Staphylococcus aureus (S. aureus) (gram positive) ATCC 6538 and Escherichia coli (E. coli) (gram negative) ATCC 11230 were used (section 3.2.3). Evaluation of antimicrobial activity for untreated and treated fabric samples was carried out by both qualitative and quantitative methods.

Antimicrobial activity by qualitative method: The untreated and the treated samples were tested and antimicrobial activity was determined by using agar diffusion test AATCC 147-2004

Antimicrobial activity by quantitative method: The untreated and the treated samples were tested and antimicrobial efficiency was determined in terms of % reduction of bacterial growth using modified colony counting method (quantitative) (AATCC test method 147-1998).

2.2.4 Wash durability

The finished fabric samples were subjected to multiple washes using lander-o-meter as per ISO 6330-1984E. The fabric samples were then subjected to antimicrobial tests.

2.2.5 Phytochemical analysis

Phytochemical analysis of the plant extract was carried using standard procedure to verify the presence or absence of various phytocompounds.

2.2.6 FTIR spectra analysis

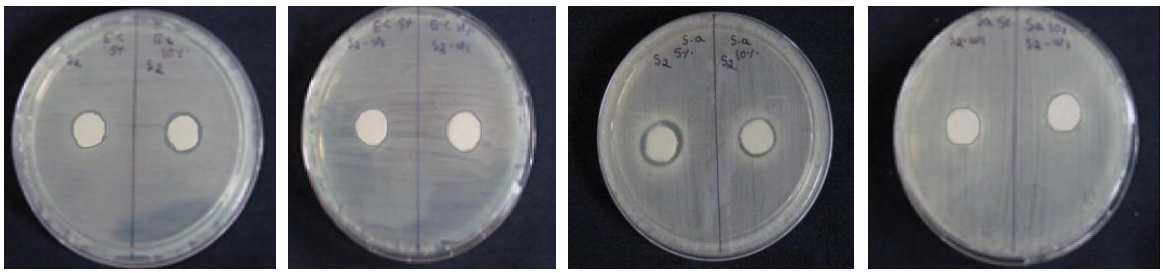
Characterization of chemical nature and functional group present in the natural dyed cotton and polyester/cotton blended fabrics was evaluated by BRUKER ALPHA FT-IR spectrometer overhead ATR attenuated Total Reflectance Unit (A537) using wavelength 600-4000^{-1}.

3 Results and discussions

3.1 Antimicrobial activity by Agar diffusion test (Qualitative) (AATCC 147-2004)

The petri plates containing both treated and untreated fabric samples after incubation were taken for observing the zone of inhibition. The sample which gives the higher zone of inhibition has more microbial resistance and vice versa. The zone of inhibition of the control strain and the test strains i.e., the diameter of circular zone is observed.

The effectiveness of finish depends on the strain of bacteria and concentration of antimicrobial agent used. The Escherichia Coli being strong microbe gives less zone of inhibition compared to Staphylococcus aureus.



(a) **(b)** **(c)** **(d)**

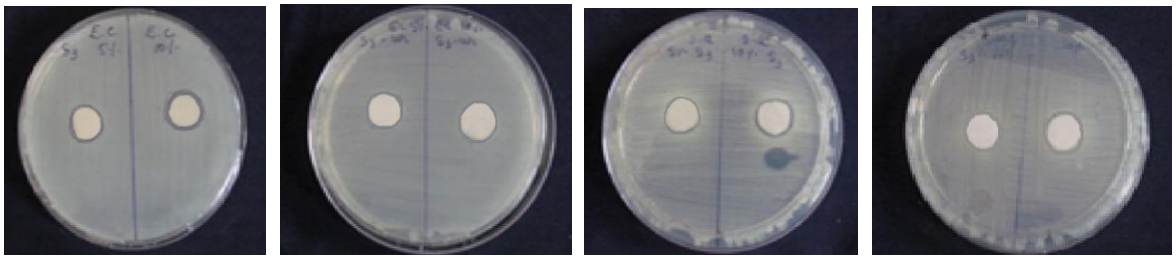
Fig. 3.1 Photographs showing zone of inhibition for Aloe vera treated fabric (a) E. coli before wash (b) E. coli after wash (c) S. aureus before wash and (d) S. aureus after wash

While evaluating the antimicrobial activity of treated fabrics a clear zone of inhibition was observed for both the test organisms. Untreated samples show clear growth of bacteria under them and no zone of inhibition indicating that the control fabric samples by themselves do not inhibit microbial activity. Generally, the bigger the inhibition zone the higher is the activity. In the present study it was observed that the treated fabrics show excellent antimicrobial activity as is evident from the complete absence of microbial growth underneath the treated fabric samples.

Fig.3.1 show the photographs of petri plates containing both treated and untreated fabric samples after incubation. A clear zone of inhibition around the unwashed treated fabric samples indicate a good resistance to bacterial attack. Zone of inhibition was absent for treated fabric samples after they were subjected to multiple wash cycles (20 cycles). The absence of zone around the sample does not necessarily mean an absence of antimicrobial activity. It means the antimicrobial agent is bound to the fibre and no growth was observed under the sample. As

active compound is not leached, there was no bacterial growth around the sample. Further, it was also observed that there was no bacterial growth underneath the fabric discs and indicates the presence of antimicrobial activity (for both unwashed and washed fabrics). This suggest that due to crosslinking the active compound is bound (not leached) and inhibit the bacterial growth.

From the Fig 3.1 it is observed that the fabric finished with aloe vera extract is effective and shows good resistance to growth of both Gram positive and Gram negative microbes. The absence of zone of inhibition after washing indicate removal unfixed antimicrobial agent during washing. When a cross linking agent is used the antimicrobial compound is bound to the substrate and hence prevent leaching out.



(a) **(b)** **(c)** **(d)**

Fig. 3.2 Photographs showing zone of inhibition for Catechu treated fabric (a) E. coli before wash (b) E. coli after wash (c) S. aureus before wash and (d) S. aureus after wash

The above Fig.3.2 shows a clear zone of inhibition and the complete absence of growth underneath the sample indicate that catechu extract used in the finish is effective. The zone of inhibition after washing is found to be small indicating removal of unfixed active agent during washing. However there was no microbial growth underneath the samples. This suggests that the active compound is bound to the fabric and effectively inhibit the bacterial growth.

3.2 Antimicrobial properties by Suspension method (Quantitative) AATCC 100–2004

In the present study the cotton and polyester/cotton fabrics were treated with natural antimicrobial agent extracted from plant parts of Aloe vera, and Catechu. The percent reduction in number of colonies (CFU/ml) in treated and untreated fabric samples are given in the **Tables 3.1 to 3.2** and from **Fig 3.3 to 3.4**.

From the results it may be observed that the reduction of antimicrobial activity of fabrics treated with catechu extract was 86%, and aloe vera extract 82%. With respect to material there is a negligible difference in the antimicrobial activity of treated cotton and polyester/cotton fabrics. It is also observed that the percentage reduction of bacterial growth is increased as the concentration of the antimicrobial agent in the fabric is increased (Ravindra et al 200).

Test results showed that there is a slight decrease in the percentage reduction of bacterial growth after 10 and 20 washing cycles. Direct comparisons of all the values can be easily seen in

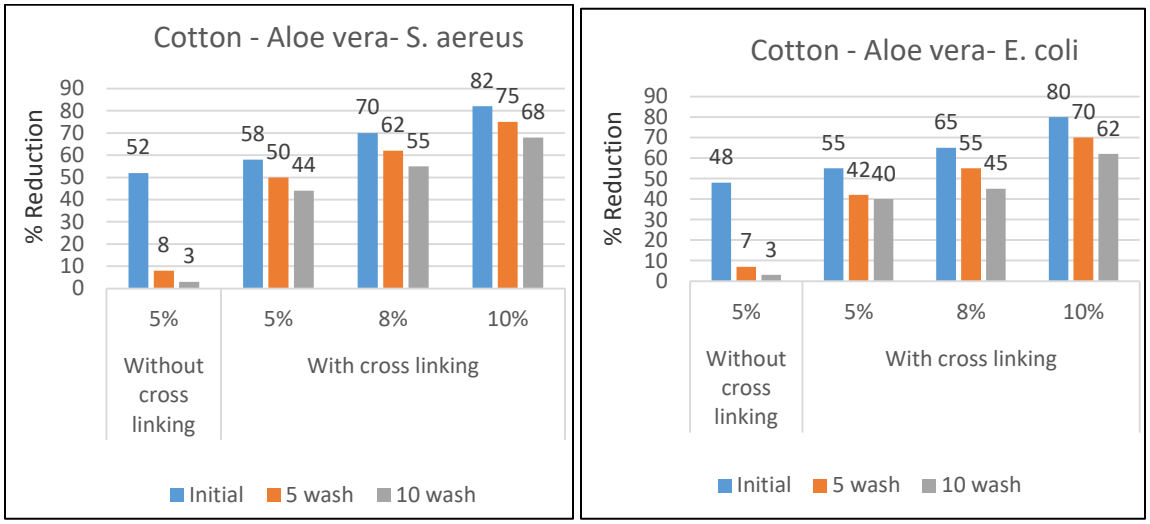
the graph plotted below to understand the level of antimicrobial resistance of the finished samples.

Aloe vera

Table 3.1 Antimicrobial activity of fabrics treated with aloe vera extract

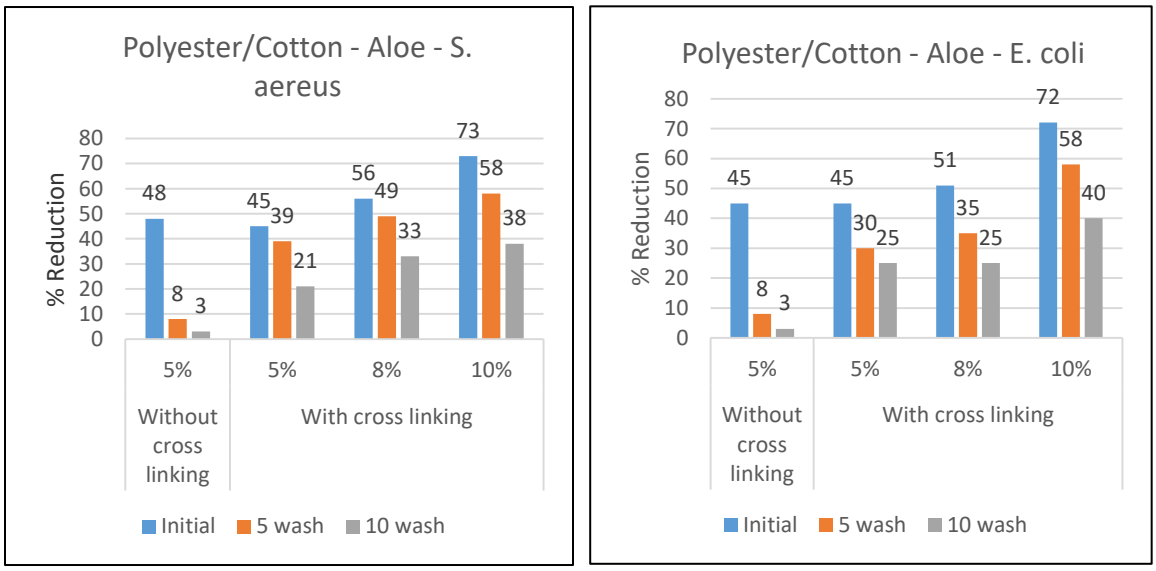
Fabric	Conc. % owf	Reduction of bacterial growth in %					
		S. aureus			E. coli		
		Initial	10 wash	20 wash	Initial	10 wash	20 wash
Cotton	5.0 Without cross linking agent	52	8	3	48	7	3
	5.0	58	50	44	55	42	40
	8.0	70	62	55	65	55	45
	10.0	82	75	68	80	70	62
Polyester /cotton	5.0 Without cross linking agent	48	8	3	42	6	2
	5.0	45	39	21	42	28	24
	8.0	56	49	33	49	33	24
	10.0	73	58	38	70	56	38

The percentage reduction of bacterial colonies increased as concentration of extract in the recipe is increased **(Table 3.1).** The aloe vera treated cotton fabric show 52% reduction without cross linking agent where as it is increased to 58% when cross linking agents used. It means that cross linking agent used in the recipe also contribute to the antimicrobial activity. In case of aloe vera treated fabrics without using cross linking agent, the antibacterial activity is completely reduced to as low as 3%. This indicate the importance of cross linking agent in fixing the active compound to the fibre substrate. Compared to aloe vera treated cotton fabric the aloe vera treated polyester/cotton fabrics is found to be less effective **(Fig. 3.3 & 3.4).**



(a) **(b)**

Fig 3.3 Antibacterial activity of Aloe Vera extract cotton fabric (a) S. aureus (b) E. coli



(a) **(b)**

Fig 3.4 Antibacterial activity of Aloe Vera extract for polyester/cotton fabric (a) S. aureus (b) E. coli

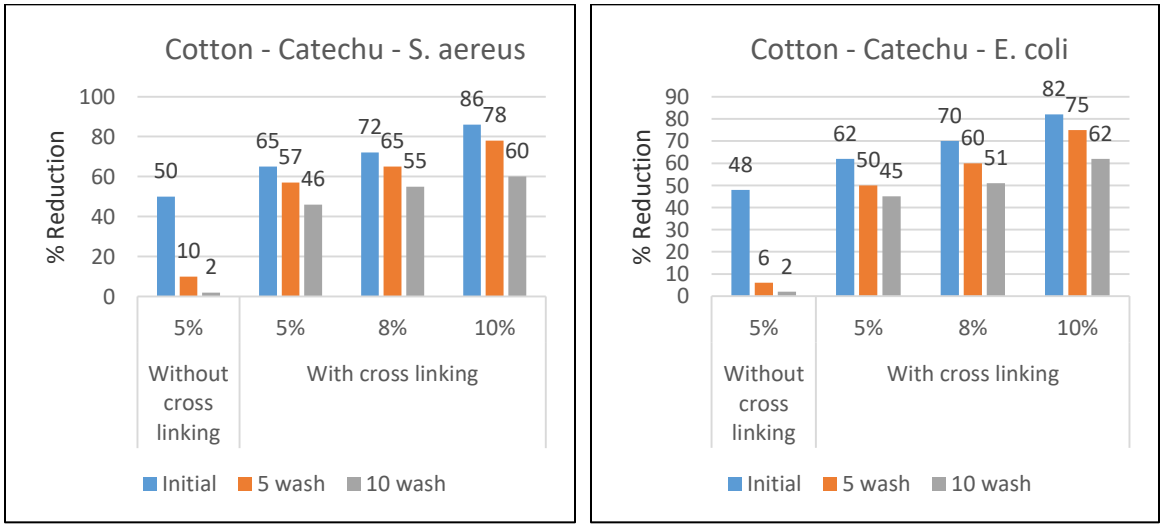
Although there is slight reduction (about 10-20%) in the antimicrobial efficiency after 20 washes, still the treated fabrics were found to be useful for the production of health care products like hospital clothing, and domestic hygiene purpose. The fabrics treated with these extracts can be used for products which need frequent washes.

Catechu

Table 3.2 Antimicrobial activity of fabrics treated with catechu extract

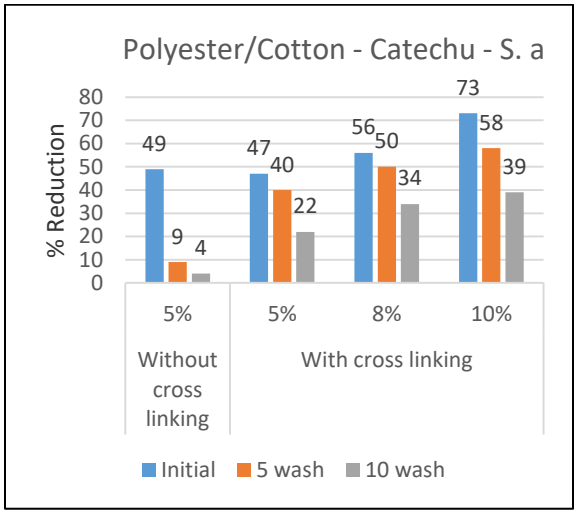
Fabric	Conc. % owf	Reduction of bacterial growth in %					
		S. aureus			E. coli		
		Initial	10 wash	20 wash	Initial	10 wash	20 wash
Cotton	5.0 Without cross linking agent	50	10	2	48	6	2
	5.0	65	57	46	62	50	45
	8.0	72	65	55	70	60	51
	10.0	86	78	60	82	75	62
Polyester /cotton	5.0 Without cross linking agent	49	9	4	44	7	2
	5.0	47	40	22	43	29	23
	8.0	56	50	34	50	34	24
	10.0	73	58	39	71	56	38

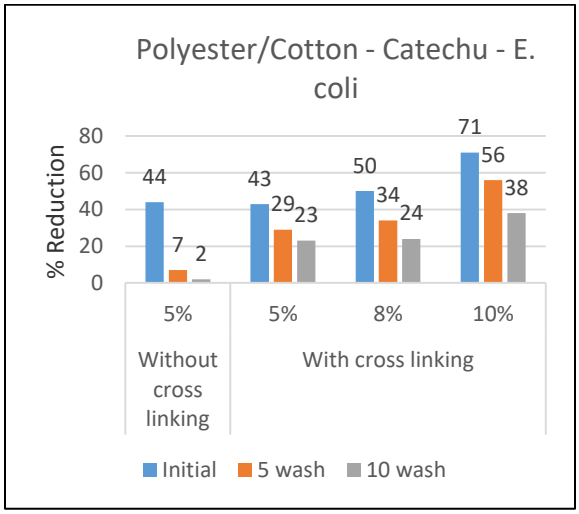
Tannins (tannic acid) present in catechu extract exhibit excellent antimicrobial activity. From the results in **Table 3.2** and in **Fig. 3.5 & 3.6** it is evident that the herbal finishes with 10% catechu extract showed 86% reduction against *S. aureus* and 82% against *E.coli* confirming the great potential of bioactive compounds for successful production of health care products.



(a) **(b)**

Fig 3.5 Antibacterial activity of Catechu extract for cotton fabric (a) S. aureus (b) E. coli





(a) **(b)**

Fig 3.6 Antibacterial activity of Catechu extract for polyester/cotton fabric (a) S. aureus (b) E. coli

It is also understood that the maximum concentration of 10% extracts would be sufficient to inhibit the growth of both gram positive and gram negative bacteria (Ravindra et al 2016). With respect to material there is a negligible difference in the antimicrobial activity of treated cotton and polyester/cotton fabrics.

3.3 Phytochemical analysis

The phytochemical screening of aloe vera showed the presence of polysaccharides (shown in **Table 3.3**). Catechu mixture is high in natural vegetable tannins and will dye silk, wool, and cotton a yellowish-brown. as shown in the table and it can be used as natural antimicrobial agent for finishing of textiles. The results in the present study suggests that these plant extract can be conveniently used for textile finishing and prevent the transfer of diseases caused by the tested microbes.

Table 3.3 Phytochemical analysis of herbal extracts

Sl. No	Herbal extract	Active antimicrobial compounds	Inference
01.	Aloe vera	Polysaccharides	+
02.	Catechu	Tannins	+

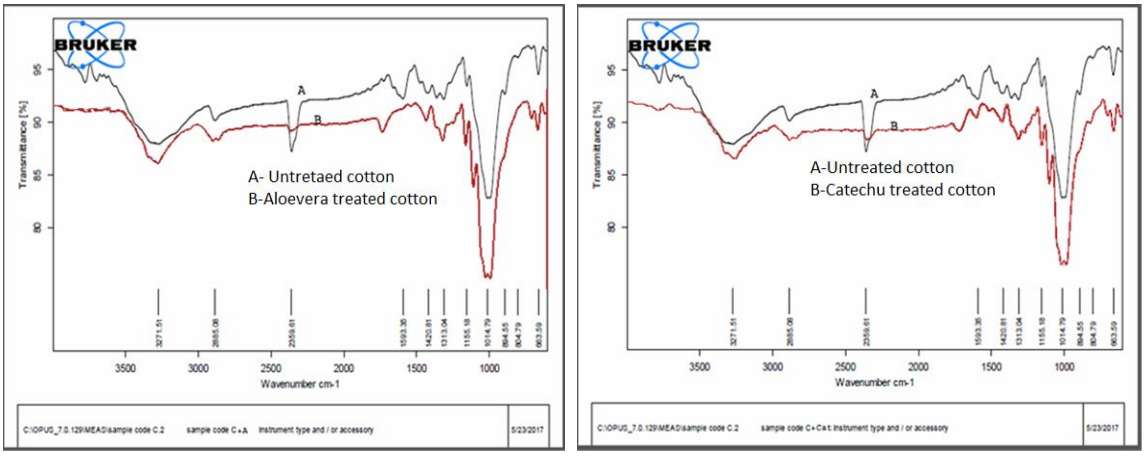
3.4 FTIR spectra analysis

Aloe vera

The hydroxyl group (O-H) and ether linkage (C-O-C) of polysaccharide units in Aloe vera extract shows strong intensities were detected by analyzing the FTIR spectrum. Broad band at 3271 cm^{-1} and strong band at 1020 cm^{-1} is due to OH and C-O-C groups of polysaccharide unit present in Aloe vera extract treated cotton fibers. The absorption band around 2893-2856cm^{-1} is due to C-H stretching, a characteristic of any natural fiber. Further, the peaks at 1,729 and 1,242 cm^{-1} ascertain the presence of o-acetyl ester. The sharp peak at 1,427 cm^{-1} (symmetrical COO^{-} stretching vibration) is most pronounced in the FT-IR spectrum of polysaccharide unit in aloe vera extract. Thus, it indicated that chemical modification had indeed occurred when the cotton fiber is treated with Aloe vera leaves extract **(Fig. 3.7a).**

Acacia catechu

The spectrum of cotton treated with Acacia catechu shows the following band characteristics at 3333-3267 cm^{-1}(Free O-H stretching vibration), the peak at 3050-2852 cm^{-1} is due to the C-H and -CH_2- stretching frequency of benzene skeleton and methylene group of tetrahydropyran moiety present in Acacia catechu molecule. The peaks are found at 1430, 1517, 1586 and 1608 cm-1 (benzene skeleton vibrations). The O-H in plane bending vibration occurs at 1430 cm^{-1}. The presence of bands at 1315 cm^{-1} and 1020 cm^{-1} (C-H, C-O stretching vibration). From this spectrum it indicates that Acacia catechu molecule is present in treated cotton fabric **(Fig. 3.7b).**



(a) **(b)**

Fig. 3.7 FTIR spectra of cotton fabric treated with (a) Aloe vera (b) catechu

Conclusion

Cotton and polyester/cotton fabrics were treated with Aloe vera and catechu extracts. The active compound from the respective plant parts was extracted by maceration method using methanol and the effectiveness of antimicrobial properties, were studied. Phytochemical analysis of the extracts was carried out to verify the presence of active compound in the plant extract which is responsible for anti-bacterial activity.

In all the treated fabrics without cross linking it was observed that a clear zone was formed around the treated samples. The unfixed extract leach out and a zone of inhibition was formed. The active compound was attached to the fibres due to cross linking and hence no bacterial growth below the fabric and around the fabric sample was observed. The complete absence of bacterial growth underneath the fabric indicate an effective antimicrobial activity of the extract present in the fabric.

The agents impart excellent antimicrobial property to cotton and polyester/cotton blended fabrics. The reduction percentage for aloe vera treated cotton fabric at maximum concentration of 10% was found to be 82% and 80% against S. aereus and E. coli respectively. Whereas for blended fabrics the bacterial reduction was 73% and 70% against corresponding test bacteria. Similarly for catechu treated cotton fabrics the bacterial reduction was 86% and 82% respectively and for blended fabric it was 73% and 71%.

Phytochemical investigation (Qualitative) confirmed the presence of active compounds in the respective plant extracts. Polysaccharides in aloe vera extract, tannins in catechu extract, Also FTIR spectra analysis confirm the presence of these compounds in the treated cotton fabric sample. Durability of the finish to washing in all the treated fabrics was found to be satisfactory and hence the treated cotton and polyester/cotton fabrics can be used for products which need frequent washes.

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