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ANTIMICROBIAL ACTIVITY OF COMFORT RELATED PROPERTIES OF SILK TREATED WITH HERBAL EXTRACTS IN MAKING OF REUSABLE MASKS

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Abstract
The study was aimed at divulging an eco-friendly antimicrobial finish on 100 % silk woven fabric. The leaves' extract of *Azadirachata indica, Butea monosperma* and *Litche chinensis* were used as the development of eco-friendly antimicrobial finish. The antimicrobial property and comfort related property were checked before and after applying antimicrobial finish. In comfort related property absorbency & air permeability were checked. The ASTEM E2149 Shake Flask method was used to check antimicrobial finish and AATCC method was used for checking fabric property. One way ANOVA statistical test was applied for analysis of results. The FTIR and SEM results showed the presences of finish on fabrics. In comfort related property, absorbency and air permeability was increased. The results showed that antimicrobial finish made 100% reduction against microorganism up to 25 washes which can be used in making reusable masks fight against COVID- 19.

Keywords: Antimicrobial. Azadirachata Indica. Butea monosperma. Litchi chinensis. Silk.

1. Introduction

With the arrival of innovative technologies, customers are demanding textiles in relations of health, hygiene and cleanliness. They are demanding comfortable and clean textiles for making of reusable masks, so the need for antimicrobial fabrication has risen so that comfort property of silk fabric not deteriorate. The comfort property of fabric is most important in making reusable masks. The market for antibacterial fabrics has revealed double digit progression in the last few years. The role of textiles in microbial proliferation and appliance of antimicrobial action have risen (Wasif and Laga 2009). All over the world customers are demanding functional textiles as wrinkle, fade, water and microbial resistant. Amongst these, expansion of antimicrobial fabric finish is highly crucial and applicable (Sathianarayanan et al. 2010).

There are more than 450 plants in Pakistan which have the property of pigments or dyeing the textiles. Several of these plants are also used for colour removal, are categorized as therapeutic and a few of these have shown antimicrobial commotion (Chengaiah et al. 2010). Numerous works have been underway on natural antimicrobial agents but there are many issues which need to be explored. The bio products are complex in chemical structure and all parts don't have antimicrobial action. Therefore, to find out the antimicrobial property of different parts of plants is a major task.in this study leaves of trees are used as development of antimicrobial finish. Natural antimicrobial ingredients are obtained from insects, fungus, plants, lichens and molluscs which is used since earliest times. These ingredients have ant carcinogenic, antibacterial and anthelmintic properties (Uzkul and Alkan 2018). The microorganisms caused odour and pathogenic diseases when fabric have unswerving contact with skin. Furthermore, loss of colour, shades and loss of functional properties of fabric are the result of microbial attack. Antimicrobial treated fabrics are important for medical, hygienic, mask for COVID and dresses which need no or least laundry (Afraz et al. 2019).

The application of these antimicrobial agents on to the fabric is a major task, the reason is most of the goods are not solvable in liquid. Another important issue is the durability of antimicrobial finish on fabric, which needs to be researched. However, research has been carried out on encapsulated technique (‘neem’ oil), in this way slow release of antimicrobial agents onto the fabric which may be a good invention in the area of bio fabrics (Ghoranneviss et al. 2011). In that study the antimicrobial finish should not change the core physical properties of the treated fabrics. For instance, air permeability of the cloth which is vital to the comfort of the wearers is reduced after coating the fabric surface with chitosan and so on. Such finish should also not alter other significant functional properties, such as bending rigidity and bending modulus which principally affect the textile stiffness and drape features. The change of the certain functional groups (which may be accountable for their antibacterial activity) throughout their fabric attachment can lead to the loss of their bioactivity on fabrics. Considering these limitations, further studies are recommended in the area of bioactive materials obtained from natural goods (Joshi et al. 2009). Antimicrobial finish works by the principle of limiting the growth of microorganism population. In this way quantity of unwanted by-products is reduced. The active principle which reduces the growth of bacterial population is known as “antimicrobial” (Varesano et al. 2011). This finish is applied for the purpose to reduce the transmission and development of pathogenic bacteria, to prevent bad odours due to microorganism degeneration and to reduce loss of an item’s suitability for use (Shaikh 2010).

This current study by use of eco-friendly materials will make it practical alternative to synthetic product based antibacterial textiles. Such substances can serve as biocide material in the field of medicinal textiles. This study offered valuable evidence on woven, reusable, antibacterial silk cloths which could be use for party wear dresses which can be used for common public (Hong et al. 2012). The silk fabric is used mostly in formal wear, which is not washed regularly as compared to cotton fabric.so there is need to apply a finish on silk fabric which make it antimicrobial resistant. Now a days with the development of fashion industry same fabric face masks are used to protect against COVID-19. So, it is need of the day to make silk fabric used for apparel face masks must be antimicrobial resistant (Raja 2011). There was deficiency for this type of research in the field of clothing industry. Silk is most luxury fibres used in fabric industry but too a biopolymer of excessive interest, due to its biocompatibility, it applied in biomedical and biotechnological procedures (Leal-Egaña and Scheibel 2010).

2. Material and Methods

The leaves of trees were selected based on their reported medical property such as *L. chinensis*, *B. monosperma* and *A. indica* (Susmitha at al. 2013). The antimicrobial finish was applied on silk fabric and microorganism presence were tested before and after applying the finish. The binder was used in experiment to increase durability of finish and it was taken from CHT Pakistan (private) limited. Microorganisms’ presence and fabric properties were checked by pre-test, post-test control group design. Antimicrobial finish was applied by making 50% concentration solution. Durability of antimicrobial finish to washes was checked by repeated number of wash cycle i.e., 5 washing intervals up to 25 washes. The unfinished five-yard 100% silk fabric which was consisted on plain weave and 75 GSM weight was taken from Nishat Textile Limited Lahore.

Fabric Preparation

In National Textile University (NTU) Pakistan, the 100% silk fabric first washed by using 2g/l detergent (Name and commercialized). The temperature of washing process was 90°C and fabric was processed for one hour. After washing silk fabric was bleached by using 2g/l H_2O_2, 1g/l wetting agent and 1g/l Na_2CO_3. Temperature of the process was 70°C. Silk fabric was treated for one hour in bleaching solution.

Leaves of Plants and Extraction

The leaves of trees were collected from Botanical Garden of Government College University, Lahore Pakistan. The leaves were washed and shadow dry for two months (mentioned light intensity and duration). Then it was ground into very fine powder by using stainless steel grinder. The distilled water was autoclaved at 110°C, then in Laminar flow hood the leaves powder was mixed in distilled water by using ratio of 200 ml leaves extract, 150 ml distilled water and 50 ml poly urethane binder. The mixture was put for seven days and it was stirred twice a day. First this solution was filtered by using muslin cloth then by use of Whatman filter paper, after that the filtered extract was concentrated by use of rotary film evaporator (Model and manufacturer).

Development and Application of Finish

The silk fabric sample was cut in three meter length and one third meter in width and label it. The untreated silk sample is the control group on which no finish was applied while on treated sample antimicrobial finish was applied. The finish ratio was prepared by using 200 ml leaves extract, 150 ml distilled water and 50 ml poly urethane binder. The antimicrobial treatment was applied in National Textile University by using pad dry cure machine, on which drying temperature is 120 °C for two minutes and curing temperature is 150 °C for three minutes. After applying antimicrobial treatment, the presence of microorganism were checked in Centre of Excellence in Micro Biology by using ASTEM E2 149 Shake Flask method. The absorbency of fabric and air permeability as comfort related property were checked in National textile university, Faisalabad on control and treated samples. The absorbency was measured by using AATCC 79-2000 method and air permeability was measured by D 737 – 04 standard test methods. The sustainability to home laundry was checked in NTU by five washing with the interval of up to 25 washes.

3. Results

Microorganisms Testing of Silk Fabric

The results of microorganisms' reduction on treated and untreated silk fabric with *A. indica, B. monosperma* and *L. chinensis* are given in Table 1.

Table 1. Quantitative analysis test results of treated and untreated silk samples.

	Untreated	*A. indica*	*B. monosperma*	*L. chinensis*	Reduction %
	Reading after 22 hours				
1 st reading	0	0	0	0	100%
2nd reading	0	0	0	0	100%
3rd reading	0	0	0	0	100%
	Reading after 6 days				
1st reading	5	0	0	0	100%
2nd reading	1	0	0	0	100%
3rd reading	5	0	0	2	60%

Table 1 shows that there were no microorganism's growth found after 22 hours even after 6-day interval on A. indica, B. monosperma and L. chinensis treated fabric. Result revealed that treated silk fabric showed 100% reduction as compared to control group. But on the other hand, after six days interval two colonies were observed on silk fabric treated with L. chinensis while on untreated samples 11 colonies were observed. So, A. indica and B. monosperma made 100 % reduction while L. chinensis made 60 % reduction in microbial growth.

Table 2 shows that A. indica and B. monosperma and L. chinensis plant extracts have effect on microorganism's presence of silk fabric as compared to control group. One Way ANOVA showed that the difference in antimicrobial finish between control group (M=1.83, SD=2.48), the first experimental group A. indica (M=.00, SD=0.00), second experimental group B. monosperma (M=0.00, SD=0.00) and third experimental group L. chinensis (M=.33, SD=0.816) were statistically significant ($F=2.69$, $p=0.074$, $\eta^2= 0.288$). Results revealed that control group scored significantly higher than the experimental groups. However, the two experimental groups, A. indica and B. monosperma did not differ significantly as compared to L. chinensis. The significant difference between control group and the experimental group is also evident from the big difference in the mean values and remarkable difference in standard deviation (control=2.48, A. indica=0.00, B. monosperma=0.00, L. chinensis=0.816).

Table 2. Effect of Antimicrobial finish on Microorganisms presences of silk fabric.

	Plant Name			Mean Difference (I-J)		Std. Error	Sig.[b]
Microorganisms presences	Control vs Experimental (*A. indica*)			1.833*		.755	.025
	Control vs Experimental (*B. monosperma*)			1.833*		.755	.025
	Control vs Experimental (*L. chinensis*)			1.500		.755	.061
	Control Group		*A. indica*		*B. monosperma*		*L. chinensis*
	Mean	SD	Mean	SD	Mean	SD	Mean	SD
	1.83	2.48	.00	.00	.00	.00	.33	.816

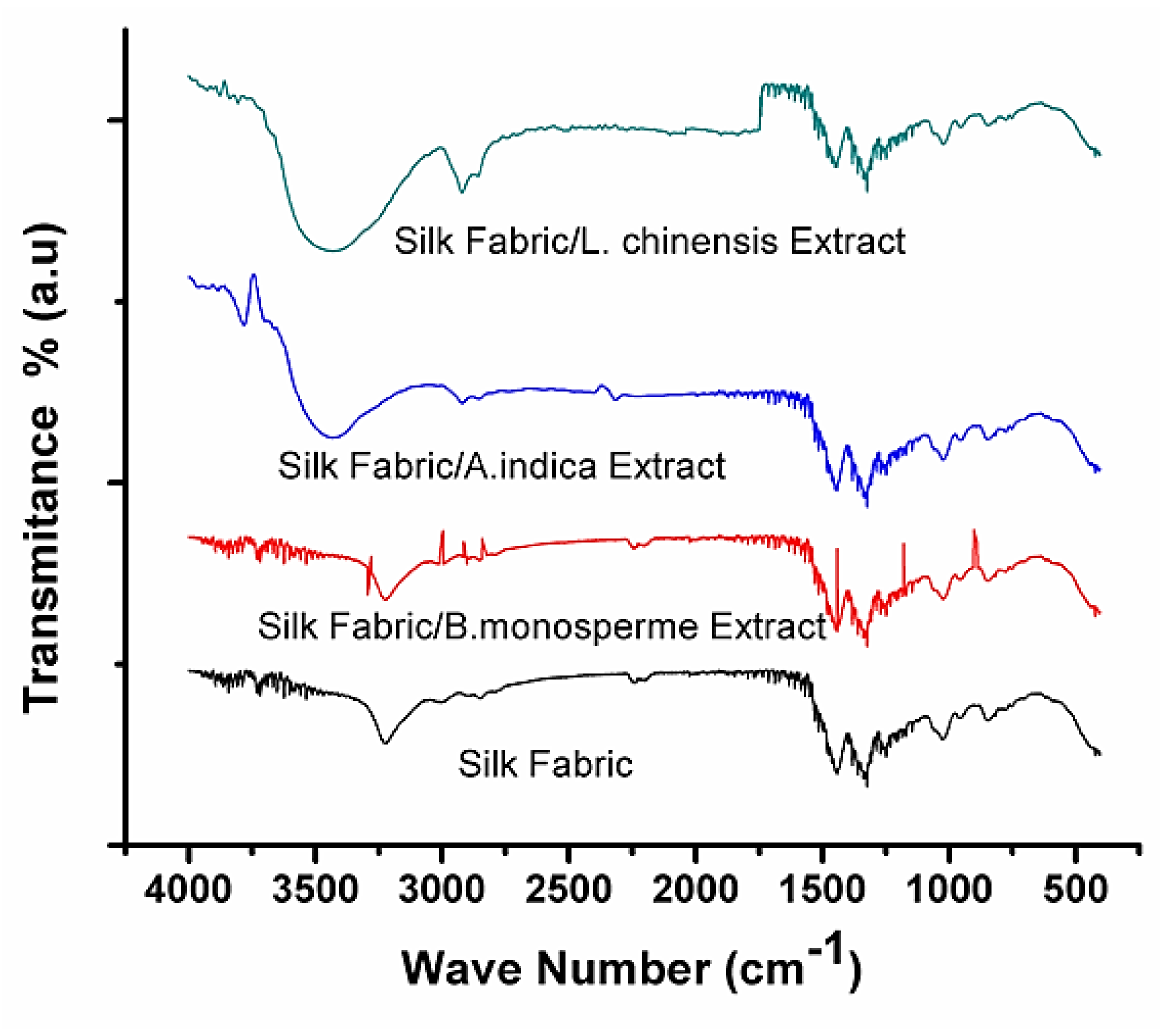

Figure 1. FTIR of treated and non-treated samples.

Silk polymer is composed of sixteen different amino acids compared with the twenty amino acids of wool polymer. Three of these sixteen amino acids namely, alanine, glycine and serine, make up about four-fifth of the silk polymers` composition. The IR spectrum cleared that the absorption band at ≈ 3275 cm-

1assigned to N-H stretching of the serine amino acid in silk samples, and the band at ≈ 2920 cm-1assigned to C-H stretching. The signal at v = 1443 cm-1 assigned to C-C stretching in ring of tyrosine and the band ≈ 1231 cm-1 assigned to C-N stretching in only found in silk samples spectrum. The antimicrobial finish has significantly applied on silk as A. indica, B. monosperma and L. chinensis have made some changes in the wavelength of fabric. So, this finish significantly affects the silk fabric. FTIR Spectra of untreated vs treated silk fabrics was showed in Figure 1.

Figure 2 represented the result of treatment of extract on silk fabric. Figure a is the SEM photograph of untreated polyester fabric, Figure b is *A. indica*, Figure c is *L. chinensis*, and Figure d is *B.* monosperma processed silk fabric. The treated silk fabric showed presence of finish as compared to untreated fabric. So, with the SEM analyses, it is evident that the treatment is done on the surface of the fabric.

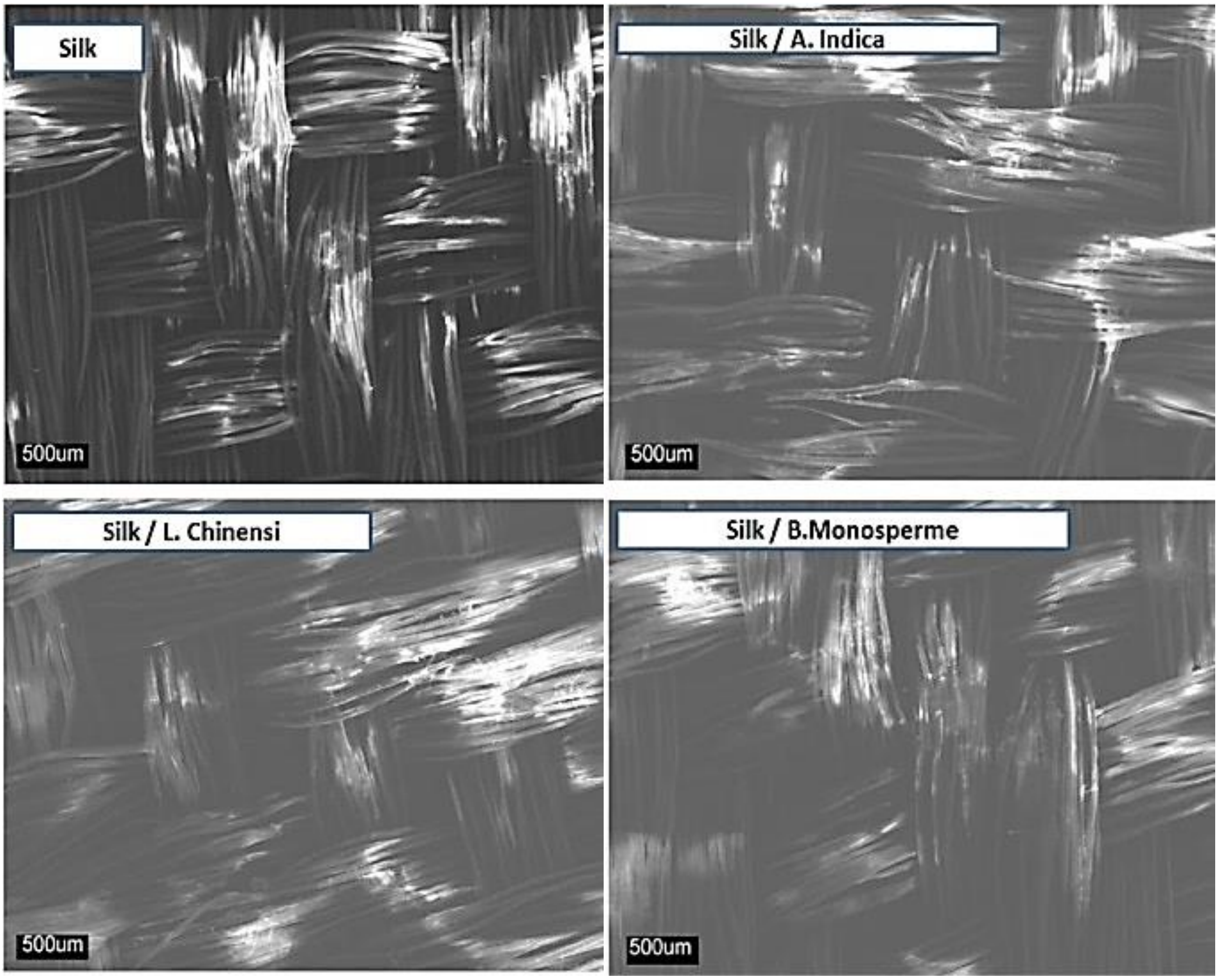

Figure 2. SEM micrographs of untreated and treated silk fabric. Result of treatment of extract on silk fabric. Silk - SEM photograph of untreated polyester fabric; Silk treated with *A. indica*; Silk treated with *L. chinensis* and Silk treated with *B. monosperma*.

Effect of Antimicrobial Finish on Comfort Property of Silk Fabric

The results of comfort related property (absorbency, air permeability) on treated and untreated silk fabric with *A. indica, B. monosperma* and *L. chinensis* are given below*:*

ANOVA was applied to find the significance difference of *A. indica, B. monosperma, L. chinensis* and control group plants extract on absorbency, air permeability face and air permeability back of silk fabric. The result of F-test indicates that there was significant difference of Antimicrobial finish on absorbency (.000) of silk fabric and the effect size was large (η^2=.92). The F-test of air permeability face was (.001) indicates that there was significant difference and effect size was large (η^2=.86) and F test of air permeability back indicates that there was significant difference (.015) and effect size was large (.71). The F-test of air permeability (face + back) (.004) indicates that there was significant difference and effect size was large (η^2=.552).

Table 5 shows that *A. indica*, *B. monosperma* and *L. chinensis* leaves extracts antimicrobial finish have effect on absorbency of silk fabric as compared to control group. One way ANOVA showed that the difference in antimicrobial finish between control group (M=2.40, SD=.55), the first experimental group *A. indica* (M=12.20, SD=1.485), second experimental group *B. monosperma* (M=11.00, SD=3.94) and third experimental group *L. chinensis* (M=27.60, SD=7.02) were statistically significant (F=30.10, p=0.000, η^2=.92). Results revealed that control group scored significantly low than the experimental groups. However, the three experimental groups' *A. indica*, *B. monosperma* and *L. chinensis* antimicrobial finish significantly affects the absorbency of silk fabric. The significant difference between control group and the first, second and third (*A. indica*, *B. monosperma*, *L. chinensis*) experimental group was also evident from the big difference in the mean values and remarkable difference in standard deviation (control=.55, *A. indica*=1.48, *B. monosperma*=3.94, *L. chinensis*=7.02).

Table 3 also shows that *A. indica*, *B. monosperma* and *L. chinensis* leaves extracts antimicrobial finish have effect on air permeability face of silk fabric as compared to control group. One way ANOVA showed that the difference in antimicrobial finish between control group (M=1123.20, SD=174.76), the first experimental group *A. indica* (M=1396.00, SD=213.38), second experimental group *B. monosperma* (M=1432.00, SD=60.99) and third experimental group *L. chinensis* (M=1572.00, SD=78.55) were statistically significant (F=16.52, p=0.001, η^2=0.86). Results revealed that control group scored significantly lower than the experimental groups. However, the three experimental groups' *A. indica*, *B. monosperma* and *L. chinensis* antimicrobial finish significantly affects the air permeability face of silk fabric. The significant difference between control group and the first, second and third (*A. indica*, *B. monosperma*, *L. chinensis*) experimental group was also evident from the big difference in the mean values and remarkable difference in standard deviation (control=174.76, *A. indica*=213.38, *B. monosperma*=60.99, *L. chinensis*=78.55).

Table 3 also shows that *A. indica*, *B. monosperma* and *L. chinensis* leaves extracts antimicrobial finish have effect on air permeability back of silk fabric as compared to control group. One way ANOVA showed that the difference in antimicrobial finish between control group (M=1041.00, SD=224.06), the first experimental group *A. indica* (M=1396.00, SD=143.46), second experimental group *B. monosperma* (M=137800, SD=135.54) and third experimental group *L. chinensis* (M=1406.00, SD=214.31) were statistically significant (F=6.54, p=0.015, η^2=0.71). Results revealed that control group scored significantly lower than the experimental groups. However, the three experimental groups' *A. indica*, *B. monosperma* and *L. chinensis* antimicrobial finish significantly affects the air permeability back of silk fabric. The significant difference between control group and the first, second and third (*A. indica*, *B. monosperma*, *L. chinensis*) experimental group was also evident from the big difference in the mean values and remarkable difference in standard deviation (control=224.06, *A. indica*=143.46, *B. monosperma*=135.54, *L. chinensis*=214.31).

Table 3 also shows that *A. indica*, *B. monosperma* and *L. chinensis* leaves extracts antimicrobial finish have effect on air permeability (face + back) of silk fabric as compared to control group. One way ANOVA showed that the difference in antimicrobial finish between control group (M=1082.10, SD=193.37), the first experimental group *A. indica* (M=1396.00, SD=170.05), second experimental group *B. monosperma* (M=1405.00, SD=97.72) and third experimental group *L. chinensis* (M=1489.00, SD=146.39) were statistically significant (F=6.581, p=0.004, η^2=0.552). Results revealed that control group scored significantly lower than the experimental groups. However, the three experimental groups' *A. indica*, *B. monosperma* and *L. chinensis* antimicrobial finish significantly affects the air permeability (face + back) of silk fabric. The significant difference between control group and the first, second and third (*A. indica*, *B. monosperma*, *L. chinensis*) experimental group was also evident from the big difference in the mean values and remarkable difference in standard deviation (control=193.37, *A. indica*=170.05, *B. monosperma*=97.72, *L. chinensis*=146.39). The antimicrobial finish increases the air permeability of fabric as compared to control group. The reason was that antimicrobial finish opens the pores between warp- and weft yarns. The *indica*, *B. monosperma* and *L. chinensis* have made some changes in the properties of fabric. So, this finish significantly affects the comfort related properties of silk fabric and increased the absorbency and air permeability of silk fabric.

Table 3. Effect of Antimicrobial finish on Absorbency, Air permeability face and back of silk fabric.

	Plant Name	Mean Difference (I-J)	Std. Error	Sig.[b]
Absorbency	Control vs *A. indica*	-9.333*	2.309	.004
	Control vs *B. monosperma*	-6.333*	2.309	.025
	Control vs *L. chinensis*	-21.333*	2.309	.000
Air permeability face	Control vs *A. indica*	-261.333*	78.250	.010
	Control vs *B. monosperma*	-391.333*	78.250	.001
	Control vs *L. chinensis*	-528.000*	78.250	.000
Air permeability back	Control vs *A. indica*	-395.000*	104.285	.005
	Control vs *B. monosperma*	-365.000*	104.285	.008
	Control vs *L. chinensis*	-368.333*	104.285	.008
Air permeability	Effect of Antimicrobial finish on Air permeability (face+back) of silk fabric			
	Control vs *A. indica*	-313.900*	98.636	.006
	Control vs *B. monosperma*	-322.900*	98.636	.005
	Control vs *L. chinensis*	-406.900*	98.636	.001

Microorganisms Testing After Fabric Washes

A summary of microorganisms testing after five washes interval is given in Table 4. Tables 4 shows presences of microorganism's colonies. "0" mean no colony of microorganisms and "1" mean presences of colony of microorganisms

The ASTM Shake Flask method was used to check antimicrobial finish. On untreated fabrics only those microorganisms were studied which were detected during the experiment. On 100% silk Gram –ve short thin rods and fungal hyphae were observed. On untreated silk fabric Gram –ve coccus and Gram + cocci were observed. The reason was that antimicrobial finish was effective against microorganisms. Crosstab statistical technique was used. The silk fabric showed 100% reduction against microorganism after successive washes, while on untreated fabrics microorganisms' presences was observed.

Table 4. Quantitative analysis of washes on silk sample.

Washes interval	Untreated	*A. indica*	*B. monosperma*	*L. chinensis*
0	0	0	0	0
5	1	0	0	0
10	0	0	0	0
15	1	0	0	0
20	0	0	0	0
25	1	0	0	0

4. Discussion

The presences of microorganisms were checked before and after applying antimicrobial finish up to 25 washing by five washes interval. The ASTM Shake Flask method was used to check antimicrobial finish. The results showed that antimicrobial finish made 100% reduction against microorganism up to 25 washes. In another study Chitosan and chitosan/PEG antimicrobial finish was applied on cotton fabric. The AATCC Standard Test Method was used for antibacterial testing against S. aureus. Results showed that antibacterial samples showed bacterial resistance up to 25 washes, while the samples laundered 50 time revealed no resistance against S. aureus. This study supports the present study that antimicrobial finish was effective up to 25 washes (Bonin 2008).

The *A. indica*, *B. monosperma* and *L. chinensis* were applied on 100% silk fabric. Before and after applying antimicrobial finish comfort related property (absorbency and air permeability) were studied. The antimicrobial finish *A. indica*, *B. monosperma* and *L. chinensis* increased both absorbency and air permeability as comfort related property of 100% silk fabric. In another study *A. indica* (Neem), Papaya, Mexican daisy leaves were selected for the antimicrobial finish, and it is tried on the silk fabric. The mechanical, comfort and antimicrobial activities of such herbs on silk fabric was evaluated. Five samples were cut at random for each of the finished concentration using template dimension. It shows that the absorbency of the finished sample was increased up to 8.8 Percent in drop test, 34.157 in sinking test (Malathy 2014).

The antimicrobial finish was applied on 100% silk fabrics. The FTIR and SEM results showed the presences of finish on fabrics. While in another study, ready for dyeing (RDF) silk fabric was treated with *Aloe vera* using 1,2,3,4-butanetetracarboxylic acid (BTCA) as crosslinking agent and sodium hypophosphite (SHP) as catalyst. The treated fabric with a concentration of 15 % of Aloe-Vera showed excellent antimicrobial properties. Since BTCA was used as a crosslinking agent, crease recovery angle, improved with minimal loss in breaking and tearing strength. The mechanism of treatment of Aloe-Vera is found to be chemical binding with silk and not simply of coating or impregnation. FTIR studies showed that the carboxyl side groups, and short chain amino acids side groups act as sites for BTCA crosslinking interalia chemical binding of Aloe-Vera. SEM studies revealed that no coating or tangible impregnation on the surface of the fiber is visible substantiating the chemical binding phenomenon (Chena and Chang 2007). This is further substantiated by the durability of the finish to dry cleaning of treated silk. Since Aloe-Vera is a natural product and BTCA is an eco-friendly resin, the treatment of silk with Aloe-Vera is eco-friendly in nature (Nadiger et al. 2015).

5. Conclusions

The antimicrobial finish *A. indica*, *B. monosperma* and *L. chinensis* increased both absorbency and air permeability as comfort related property of 100% silk fabric. The antimicrobial finish was applied on 100% silk fabrics. The FTIR and SEM results showed the presences of finish on fabrics. Fabrics properties were checked before and after applying antimicrobial finish. In comfort related property, absorbency and air permeability was increased. The presences of microorganisms were checked before and after applying antimicrobial finish up to 25 washes by five washes interval. The ASTM Shake Flask method was used to check antimicrobial finish. The results showed that antimicrobial finish made 100% reduction against microorganism up to 25 washes. So, this silk fabric can be used for formal wear dresses as well as face masks against COVID-19.

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