Effect of BTCA crosslinking on functional properties of of textiles treated with biomasses from Annona Squamosa and Moringa Oleifera

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Research Article

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Abstract

Two natural extracts were produced with *annona squamosa* and *moringa Oleifera* leaves through the ethanol-based solvent. 100% cotton and 80%:20% polyester/cotton blends with an areal density of 113.5 g/m^2 and 101 g/m^2, respectively. Eight different samples were produced by coating on natural extract as raw form and BTCA cross-linked form on cotton and polyester fabrics. SEM investigation was also done for all the test fabrics. These coated fabrics were investigated for their antimicrobial activity, wicking properties, stiffness, and crease recovery properties. It was found that the BTCA cross-linked fabrics showed higher antimicrobial activity against gram-positive and gram-negative bacteria. Similarly, the percentage crease recovery angle was higher for the *annona squamosa* coated sample than *moringa Oleifera* leave extract in both cotton and P/C samples. Further, it was reported that no significant difference in stiffness values was found between the control samples of cotton and P/C against the treated samples. It was interesting to note that treating the fabrics with cross-linker has improved the vertical wicking property and brought the values closer to control fabric values. This could be interpreted as more hydroxyl groups in the cross linking compound have supported more water penetration.

1. Introduction

Cotton is the most commonly used fabric by consumers owing to its comfort, absorbency, and softness. It has been utilized for diversified clothing applications, including medical fabric. In the case of medical fabric, the probability of getting affected by bacterial infections is very high. These bacteria can survive for lengthy periods on hospital materials such as hospital privacy drapes, scrub suits, and lab coats[1]. Nowadays, much research is focused on eliminating infections on natural textiles, particularly those caused by antibiotic-resistant bacterial strains[2]. Numerous studies have shown that nanoparticles have a strong capacity to resist microbial activity entirely [3].On the other hand, finishing cotton fabric with nanoparticles is a major challenge. It has inherent limitations in terms of fixation of or adherence of nanoparticles to the surface of the fabric[4]. To overcome this limitation, surface enrichment or modification of cotton textiles through a cross-linking agent has become necessary [5]. According to *Liberato et al.* DMDHEU easy care finish exhibits exceptional cross-linking performance with natural fibre cost-effectively; nonetheless, the potential health and environmental risks of formaldehyde release are inevitable. So, various researchers have investigated polycarboxylic-based acetic compounds such as 1,2,3,4-butane tetracarboxylic acid (BTCA) as commercially viable cross-linking compounds [6]. Traditionally, cross-linking was done with compounds that release formaldehyde, which is not environmentally friendly. If a green cross-linking agent such as BTCA (Butane-1,2,3,4-tetracarboxylic acid) is used, it helps to better fix the nanoparticle onto the surface of the material without harming the ecosystem.

BTCA was used as a cross-linking agent to improve some of the desirable properties of the fibre, and it was discovered that a 10% (W/W) BTCA treatment considerably improved the drapability, crease recovery, and tensile strength of cross-linked cotton fabric[7]. According to Yang *etal.* BTCA, in conjunction with sodium hypophosphite (NaH_2PO_2), can give satisfactory bonding performance with cotton materials[8].

Markovic has successfully proved that BTCA-based polycarboxylic acid and precursor salt $CuSO_4$ supported the even distribution of nanoparticles over the larger textile substrate[9].Sharaf*etal.* applied the Cu nanoparticles after the cross-linking cotton fabric with BTCA and found that cross-linking not only improved the durability and easy care property of treated fabric but also improved the thermal stability of the fabric, and this functionalized fabric showed maximum antibacterial activity against *S. aureus* and *E. coli*. It was further noted that BTCA produced a multifunctional textile product for smart applications [10]. A surface active agent is very important during the nanoparticle coating of the cotton fabric. Xu *et al.*reported an improvement in the absorbability of copper nanoparticles by cotton fabric when treated with thioglycolic acid in the presence of citric acid. This process increased the thermal stability of the treated fabric and gave outstanding laundering durability of the nanoparticle coating to several washes [11].

In order to treat textile material with natural antibacterial and antifungal agents and move towards sustainable techniques, lost plant-based sources were used. Atal safaa. said that Annona squamosa leaves contain phytochemicals such as cyclopeptides, alkaloids, diterpenes, and acetogenins. These phytochemicals are employed in a variety of medical applications, including immunomodulatory, antiviral, antitumor, antioxidant, and antidiabetic treatments[12]. A range of phytoconstituents found in *Annona squamosa* seeds are the main source of synthesis of nano compounds[13].Manisha etal. noted that Annona squamosa leaves were traditionally used to treat a variety of conditions, including fever, vomiting, wound infections, dysentery, tumours, hypertension, thyroid, acne, heart disease, inflammation, diabetes, hair loss, dandruff, bleeding, sores infected by maggots, coughing, and inflammation[14].Mariya Malik and others. *Annona squamosa* leaves were used to synthesize the liver nanoparticle, which was found to have antibacterial, anti-cancer, and anti-diabetic properties[15]. Hussein Jihan and others. It has been reported that leaves of Moringa Oleiferara exhibit pharmacological activity and a range of therapeutic qualities for venomous bites, stiffness, diabetes mellitus, hepatotoxicity, anti-ulcer, diuretic, and antibacterial effects, as well as antioxidant and anti-inflammatory capabilities. Additionally, they created gold nanoparticles for a range of biological uses[16]. Using *Moringa Oleiferara* leaves, Gufran et al. synthesised green silver nanoparticles that have antifungal and anticancer properties[17].Parkash et al. used *Moringa Oleiferara* leaf to create an aqueous decoction that is enhanced with bioactive chemicals for medicinal use[18].Manosi etal. Synthesised Selenium nanoparticles by combining Sodium Selenite solution with *Moringa Oleiferara* leaf extract. The resultant material was able to affect Phaseolus vulgaris growth[19].The present investigation was carried out to determine the effect of BTCA cross-linking agents on the antibacterial and comfort properties of fabrics treated with a*nnona squamosa* and m*oringa Oleiferara leaves* extracts.

3. Material and Methodology

Two types of fabrics, namely 100% cotton and an 80%:20% P:C blend were used for the study. The specifications of plain-woven cotton (100%) fabric were indicated as ends per inch of 97, picks per inch of 85, an areal density of 113.5 $g/m^{2,}$ and a thickness of 0.29 mm. Similarly, the specifications of P/C blend are ends per inch of 60, picks per inch of 70, an areal density of 101 g/m^2, and a fabric thickness of 0.28 mm. The slected fabrics were scoured and bleached with 2 to 5g/l of sodium carbonate and 2.5g/l Hydrogen peroxide.

3.1 Preparation of methanolic leaf extracts

Fresh leaves of custard apple(*Annona squamosa)* and *Moringa Oleiferara* were collected from Osmania University campus, Hyderabad, India, and washed with distilled water, dried under shade for a week, and then pulverized in a grinder. The powder obtained was sieved for homogeneity. The active biomolecules in the finely ground leaf powders were extracted through the Soxhlet apparatus, where one part of leaf powder and 15 parts of solvent (methanol) was used. The dried leaf powders (27 gm each) were packed separately in two muslin cloth packs to keep in the Soxhlet thimble, which is placed inside the Soxhlet extractor[20]. Methanol (400 mL) was taken in the round bottom flask of the Soxhlet apparatus. The solvent is heated through the isomantle. Upon heating at 60ºC, methanol evaporates and moves through the apparatus to the condenser. The condensate drips into the extractor. When the solvent level reaches the siphon, it then falls into the flask. This is one cycle of extraction. Extraction was done for nine hours. The extract obtained was in dark greenish colour and was used for padding the test fabrics. Figure 1 shows the Soxhlet unit during the crude extracting process from *Annona squamosa* and *moringa Oleiferara* leaves.

3.2 Cross-linking fabrics with BTCA

According to Hu*etal.* test fabrics were cross linked with Butane-1,2,3,4-tetracarboxylic acid (BTCA) in the presence of a sodium hypophosphite catalyst (SHP). The fabrics were soaked in a solution containing 80 g/L (BTCA) and 36.6 g/L (SHP)for an hour, dried at 80ºC for 5 minutes, and cured at 120ºC for 2 minutes. The mechanism of BTCA cross-linking is presented in Fig. 2.

During the curing process, three different BTCA molecules are present in the cellulose cross-linking system. In BTCA, two neighboring carboxyl groups dehydrate to active anhydride, which then esterifies with hydroxyl in cellulose. The grafted BTCA forms a second anhydride and proceeds to esterify with cellulose to form a cross bond. Finally, BTCA forms two ester-bonded hydroxyls in cellulose. As a result, each BTCA can only generate two ester linkages with hydroxyls in cellulose[21].

3.3 Fabric coating

The pre-treated cotton and P/C fabrics were prepared with 500 x 500 cm dimensions. Eight different fabric samples were prepared; among them, four samples were produced with a coating of custard apple(*Annona squamosa)* and *Moringa Oleiferara* leaf extracts on raw cotton and P/C fabrics, and the remaining four samples were produced by the coating of custard apple(*Annona squamosa)* and *Moringa Oleiferara* leaf extracts on 3% BTCA cross-linked cotton and P/C fabrics. During this process, the custard apple(*Annona squamosa*) and *moringa Oleiferara* extract concentration was kept at 8% (V/W) for all the samples. The material-to-liquid ratio of 1:20 was maintained through a pad–dry–cure method at 50^{o} C for 30 min. After the padding process, the fabric samples were dried and cured at 180^{o} C for 3 min. The sample plan is shown in Table 1.

Table 1
Experimental sample plan

Sample No	Code	Description
1	C|CAME	Cotton fabric padded with custard apple methanolic leaf extract
2	PC|CAME	PC blend padded with custard apple methanolic leaf extract
3	C|MME	Cotton fabric padded with moringa methanolic leaf extract
4	PC|MME	PC blend padded with moringa methanolic leaf extract
5	C|BTCA|MME	Cross-linked cotton fabric padded with moringa methanolic leaf extract
6	PC|BTCA|MME	Cross-linked PC blend fabric padded with moringa methanolic leaf extract
7	C|BTCA|CAME	Cross-linked cotton fabric padded with custard apple methanolic leaf extract
8	PC|BTCA|CAME	Cross-linked PC blend fabric padded with custard apple methanolic leaf extract

4. Characterization

4.1 FTIR

Infrared spectroscopy analysis was done on all the treated fabrics through a Horiba FT-210 spectrophotometer with a potassium bromide pellet. This analysis used to confirm the chemical coating on the surface of the fabric samples.

4.2 Antibacterial activity through AATCC 147 method

The antimicrobial activity of the treated fabric sample was analyzed as per the AATCC 147 method. This technique is a relatively rapid and straightforward qualitative method for determining the antibacterial activity of diffusible antimicrobial agents on textile materials. This method can provide the effectiveness of the material to combat gram-positive and gram-negative bacteria. In this parallel streak method, the bacterial inoculum is gently streaked five times (60 mm in length) about 10 mm apart from each other without refilling the inoculum loop. The objective is to measure the size of the zone of inhibition(ZOI). Zone of inhibition (ZOI) is a clear area where there is no growth of the bacteria on the agar surface. The microbes such as *Staphylococcus aureus* (Gram-positive) and *Escherichia coli* (Gram-negative) were considered as the test organisms.

The ZOI was measured using the formula

W = (T-D)/2

where, W = clear zone of inhibition in mm

T = Total diameter of the test fabric

D = Diameter of the test specimen in mm

4.3 Stiffness

Stiffness can be measured quantitatively using the Eureka stiffness tester using the ASTM D1388 Standard test procedure. The bending length of the fabrics and flexural rigidity are calculated based on cantilever principles. The face and back of both ends of each test specimen are tested, making for four readings per sample. The average of the readings is reported.

4.4 Crease Recovery Angle

Crease recovery is a fabric feature that allows it to recover from deformations through folding. The crease recovery angle of the test fabrics was evaluated using the Sasmira crease recovery tester (India) as per AATCC Test Method 66-2003. The crease recovery angle was measured in both the face and back side of the fabric, and the average reading was reported.

5. Results and Discussion

5.1 FTIR

The FTIR spectra of cotton and P/C blended coated with custard apple leaf and moringa leaf extracts in different conditions were shown in Figs. 3 & 4.

This analysis confirmed the esterification reaction between the carboxylic acid of BTCA and cellulose structure. The band peek at 1720 was found in BTCA-coated cotton and P/C blended samples alone. It confirmed that the ester carbonyl group in BTCA formed the covalent bond, and the intensity of such a peak is higher for cotton samples than P/C blend samples[22]. Similarly, the raw cotton fibre has not shown a peak at 2926 cm^{-1}, but H-OH bond between the cellulose and BTCA has created such a peak. Further, this peak gets narrow because of the decrease of H-bonded OH in BTCA cross-linked samples. Further, a new peak formation was found at 1020 cm^{-1}, which was interpreted with a C = O bond in the carboxyl group, which was formed due to the formation of chemical bonding between natural extract and BTCA compound with cellulose structure[23].

5.2 Effect of BTCA cross-linking on the ZOI of the *moringa* extract-coated fabrics

Two types of test fabrics were used in the research study, namely cotton and P/C blend (80:20), with the aim of understanding the differences in the antibacterial activity of the padded fabrics after three wash cycles (5,10,15). The test fabrics (Cotton and P/C blend)were padded with *Moringa* extract with and without BTCA cross-linking. Further, the two sets of fabrics were tested for antibacterial activity. The results of the antibacterial activity of these specimens are shown in Table 2 and Fig. 3.

Table 2
Effect of BTCA cross-linking on the ZOI of the *moringa* methanolic leaf extract padded cotton fabrics

Sample Code	**ZOI of unwashed fabrics**		**ZOI after 5 Washes**		**ZOI after 10 Washes**		**ZOI after 15 Washes**	
	E.Coli	***S.aureus***	***E.Coli***	***S.aureus***	***E.Coli***	***S.aureus***	***E.Coli***	***S.aureus***
C|MME	3.5	5.5	3.5	5.5	2.5	4.5	2.5	4.5
C|BTCA|MME	6	6.5	5	6	5.5	6.5	4.5	6
PC|MME	3.5	5.5	2.5	4.5	2.5	3.5	2.5	2.5
PC|BTCA|MME	6.5	6	6.5	5.5	5	5.5	4.5	4.5

(a) ZOI of cotton and PC blend fabrics padded with moringa methanolic extract - unwashed, 5 washes, 10 washes, 15 washes – *E.coli*	(b) ZOI of cotton and PC blend fabrics padded with moringa methanolic extract- unwashed, 5 washes, 10 washes, 15 washes – *S.aureus*

Figure 5. Antimicrobial activity of cotton and PC blend fabrics padded with moringa leaf extract

It is clear from Table 2 and Fig. 5 that the zone of inhibition after cross-linking was much better when compared to non-crosslinked fabric. The ZOI of the cotton and P/C blend fabrics remained stable even after 15 washes for both *E.coli* and *S. aureus*. It can be seen that there was almost a 50% increase at all three stages – unwashed, 5 washes, 10 washes, and 15 washes, since the cross-linked fabric created better bonding with the natural extract. Hence, its fastness properties were also comparatively higher than the non-cross-linked fabric[24].

5.3 Effect of BTCA cross-linking on the ZOI of the fabrics coated with custard apple leaf extract

Two test fabrics, namely cotton and P/C blend (80:20) were padded with custard apple leaf extract with and without BTCA cross-linking. Further, the two sets of fabrics were tested for antibacterial activity after three wash cycles (5,10,15). The results of the antibacterial activity of these specimens are shown in Table 3 and Fig. 4.

Table 3

Effect of BTCA cross-linking on the ZOI of the custard apple methanolic leaf extract on cotton and P/C blend fabrics

	ZOI of unwashed fabrics		**ZOI after 5 Washes**		**ZOI after 10 Washes**		**ZOI after 15 Washes**	
Sample Code	***E.Coli***	***S.aureus***	***E.Coli***	***S.aureus***	***E.Coli***	***S.aureus***	***E.Coli***	***S.aureus***
C|CAME	5.5	6.5	4.5	5.5	3.5	4.5	3.5	4.5
C|BTCA|CAME	6.5	6.5	5.5	6	5.5	6.5	5	6.5
PC|CAME	6.5	6.5	5.5	5.5	4.5	5.5	3.5	4.5
PC|BTCA|CAME	5.5	6.5	5.5	6	4	6.5	4.5	6.5

ZOI of cotton and PC blend fabrics padded with custard apple methanolic extract - unwashed, 5 washes, 10 washes, 15 washes – *E.coli*	ZOI of cotton and PC blend fabrics padded with custard apple methanolic extract- unwashed, 5 washes, 10 washes, 15 washes – *S.aureus*

Figure 6. Antimicrobial activity of cotton and P/C blend fabrics padded with Custard apple leaf extract

It can be observed from Table 3 and Fig. 6 that the zone of inhibition after cross-linking was much better when compared to non-cross-linked fabric. The ZOI of the cotton and P/C blend fabrics remained stable even after 15 washes for both *E.coli* and *S. aureus*. It can be seen that there was almost a 50% increase at all three stages – unwashed, 5 washes, 10 washes, and 15 washes. Since the cross-linked fabric created better bonding with the natural extract, its fastness properties were comparatively higher than the non-cross-linked fabric[24]. The ZOI of the untreated and treated fabrics almost doubled even after several washes and appeared to remain stable. The BTCA treatment appeared to have strengthened the ZOI. The results of this study are in concurrence with Koruyucu*etal.*, 2021, where the synergistic effect of the BTCA on the antibacterial wash durability of the fabrics coated with copper (I), copper (II)oxide particles, and zinc oxide particles was measured and reported[25]. The research of Sennur et al. further confirms that the cotton fabrics were functionalized with BTCA, which enhanced properties such as pilling, crease resistance, and flame retardant properties[26].

5.4 Crease Recovery Angle

All the test fabrics were studied for their crease recovery angle and comparatively analysed for the influence of BTCA cross-linking. Table 4 indicates the crease recovery angles of the face and back sides of different treated fabrics and the percentage change from non-cross-linked fabric structure to cross-linked fabric structure.

Table 4
Crease recovery angle in cotton and PC blend fabrics padded with natural extracts

Crease Recovery Angle (CRA)	C| CAME	C| BTCA| CAME	PC| CAME	PC| BTCA| CAME	C| MME	C| BTCA| MME	PC| MME	PC| BTCA| MME
Face side CRA	82.50	87.50	77.50	82.50	85.00	87.50	77.50	80.00
% change	-	6.06	-	6.45	-	2.94	-	3.23
Back side CRA	80.00	90.00	80.00	85	82.50	87.50	75.00	82.50
% change	-	9.09	-	9.68	-	2.94	-	6.45

According to Table 4. the CRA values of all the test samples were more than 77° for PC fabric and more than 80° for cotton fabric. These CRA values are comparatively less than other coating agents on the respective fabrics[27][28]. Further, it is understood that the change in back side CRA for fabrics coated with custard apple *methanolic* leaf extract was found to be higher when compared with those coated with moringa methanolic leaf extract.Several studies confirm that any surface coating on the textile fabric would modify its CRA significantly. In this case, percentage of change due to BTCA cross linking was comparatively lesser than other kind of surface treatments. Sathianarayan *et al*., (2009) also studied the crease recovery angle (CRA) of cotton fabric padded with methanolic extract of Tulsi and Pomegranate. The CRA was verified in three methods, namely direct, micro-encapsulated, and cross-linking methods. CRA showed a 32% improvement in encapsulated Pomegranate extract and about 20% in encapsulated Tulsi extract fabrics. It was reported that the cross-linking has increased the CRA angle significantly[29].

5.5 Vertical wicking

The vertical wicking behavior of all the test specimens was analysed, and the respective height values of each sample are presented in Fig. 7.

From Fig. 5, it can be noted that vertical wicking in cotton fabrics padded with methanolic extract samples decreased when compared to the control fabric, which can be attributed to the surface coating prohibiting the water penetration of the fabrics. It is interesting to note that treating the fabrics with BTCA cross-linker has improved the vertical wicking and brought the values closer to control fabric values, which can be interpreted as the presence of more hydroxyl group in the BTCA compound has supported more water penetration[7]. In contrast, all the surface-coated PC blend fabrics improved vertical wicking compared with the control sample. It is interpreted that the moisture absorbency of polyester fibre was very less, but the surface coating has given support to improve this moisture-wicking capability. Zhang *et al*., studied the wicking behavior of polyester fabrics after cross-linking with BTCA, where it was reported that the wettability of the polyester fabric increased after cross-linking. This research work authenticates the findings of Zhang *etal.*[30].

5.6 Stiffness

The stiffness parameters of all the test specimens are presented in Table 5 and Fig. 8.

Table 5
Stiffness details of fabric samples padded with natural extracts

Sample Code	Length of overhang (in cm)	Bending length (in cm)	Sample Code	Length of overhang (in cm)	Bending length (in cm)
Cotton - Control	2	1	PC - Control	1.7	0.85
C|BTCA	2.2	1.1	PC|BTCA	1.9	0.95
C|MME	2.1	1.05	PC|MME	2	1
C|BTCA|MME	2.2	1.1	PC|BTCA|MME	2	1
C|CAME	2.2	1.1	PC|CAME	2	1
C|BTCA|CAME	1.9	0.95	PC|BTCA|CAME	2	1

Figure 6 indicates the bending length of all the test samples. No significant difference was found between the control samples of cotton and P/C against the treated samples. It is further reported that the BTCA treatment did not change the stiffness of the fabric.

6. Conclusion

This study optimized the coating of *annona squamosa* and *moringa Oleiferara* leaves extract on cotton fabric using a cross-linking agent. BTCA is a non-formaldehyde-based cross-linking agent used in cellulose structures. Because of increased environmental awareness among global communities, most textile value-addition processes are transformed into environmentally friendly ones. For this experiment, fabric substrates such as 100% cotton and 80%:20% polyester/cotton blends were chosen. Eight distinct samples were created, each with or without cross-linking agents. The antibacterial activity, wicking characteristics, stiffness, and crease recovery properties of these coated fabrics were examined. The cross-linked textiles were found to have enhanced antibacterial activity against both gram-positive and gram-negative bacteria. The ZOI values of BTCA cross-linked *moringa* methanolic leaves extract coated cotton and P/C blends were 6 to 6.5 cm, which was more than 50% higher than non-BTCA cross-linked fabric.Similarly, ZOI values of 5.5 to 6 cm were found in BTCA cross-linked custard apple leaves extract coated cotton and P/C blends, which was more than 20% higher than non-BTCA cross-linked fabric.Further, the CRA value of BTCA cross-linked *moringa* methanolic and custard apple leaves is higher than that of non-cross-linked fabrics, which were 6 to 9% for cotton and 2.5 to 4% for P/C. BTCA cross-linker has improved the vertical wicking properties. The difference in bending length of values of all the treated samples was shown to be insignificant. It is concluded that the BTCA cross-linking method can be used to achieve a natural extract coating on cotton and other cellulosic fabrics to produce superior functional qualities.

Declarations

Ethical Approval

Not applicable

Competing interests

The author(s) declared no potential conflicts of interest with respect to the research, authorship, and/or publication of this article

Authors' contributions

Sirisha Deepthi Sornapudi: Experimental work, sample preparation, visualization

Srinivas Manchikatla : characterization , characterization

Meenu Srivastava : Material sourcing, writing support

H.Samuel Thavaraj: Editing and Proof Checking

B.Senthil kumar: manuscript writing, coordination

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Figures

Figure 1

Methanolic leaf extracts of leaves of *Annona squamosa* and *moringa Oleiferara*

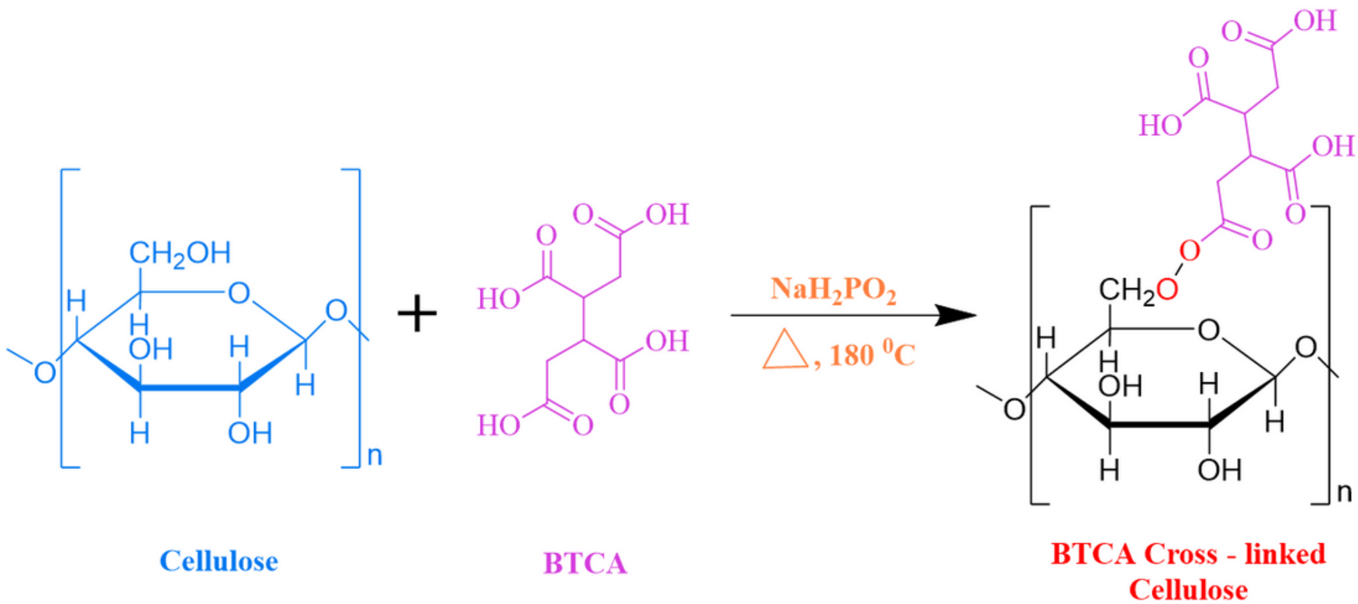


Figure 2

Reaction mechanism of BTCA and cellulose[21].

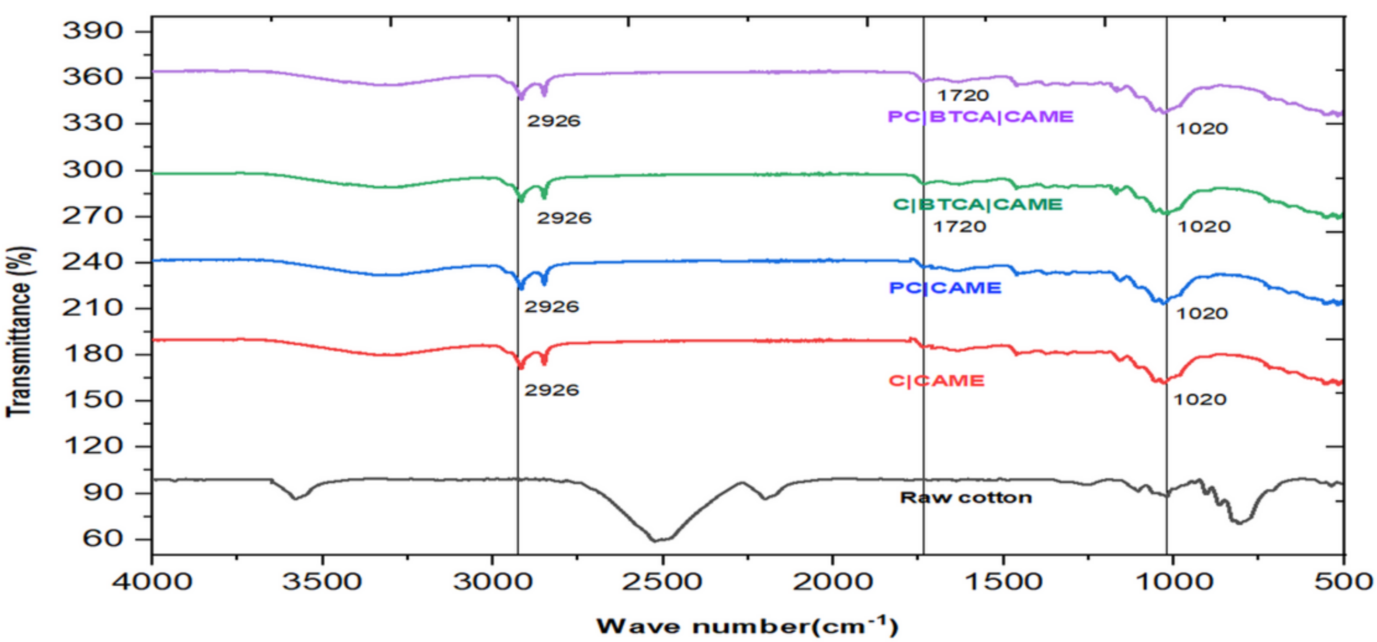


Figure 3

FTIR spectra for custard apple leaf extract coated samples

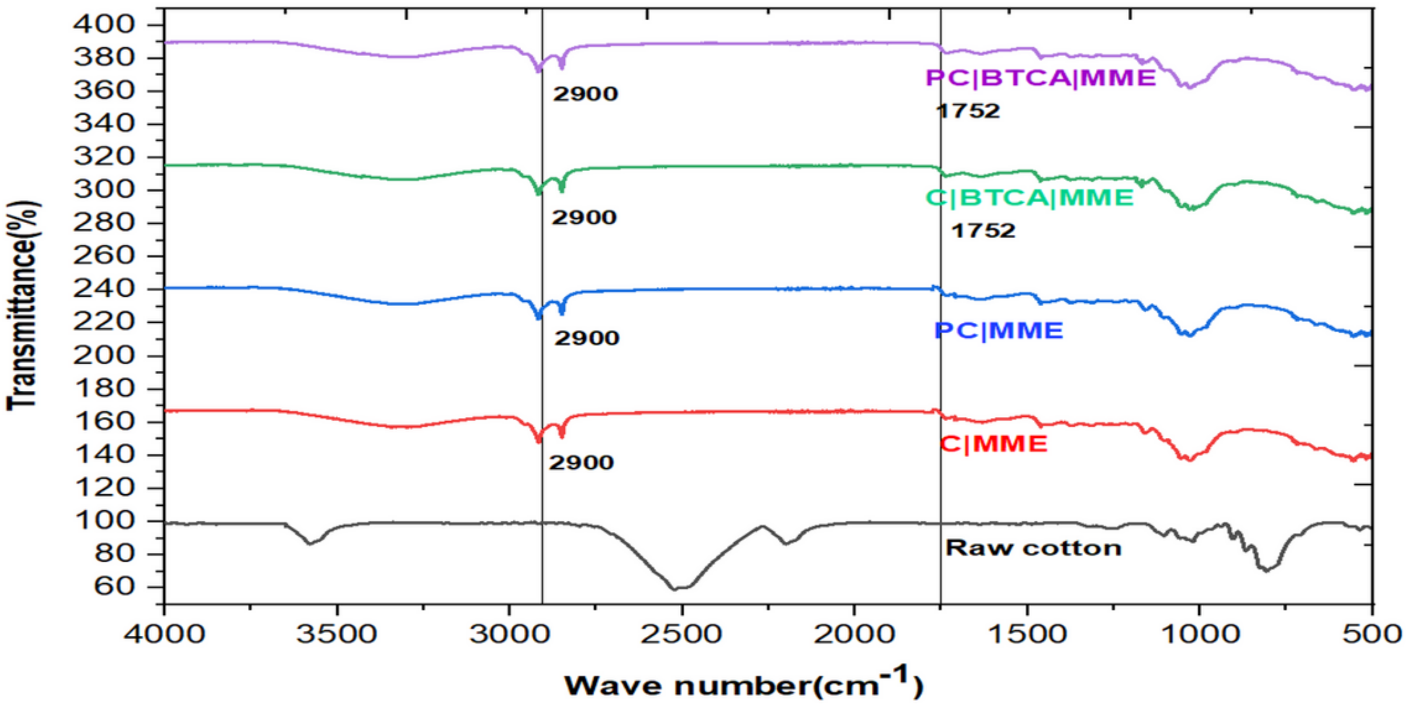
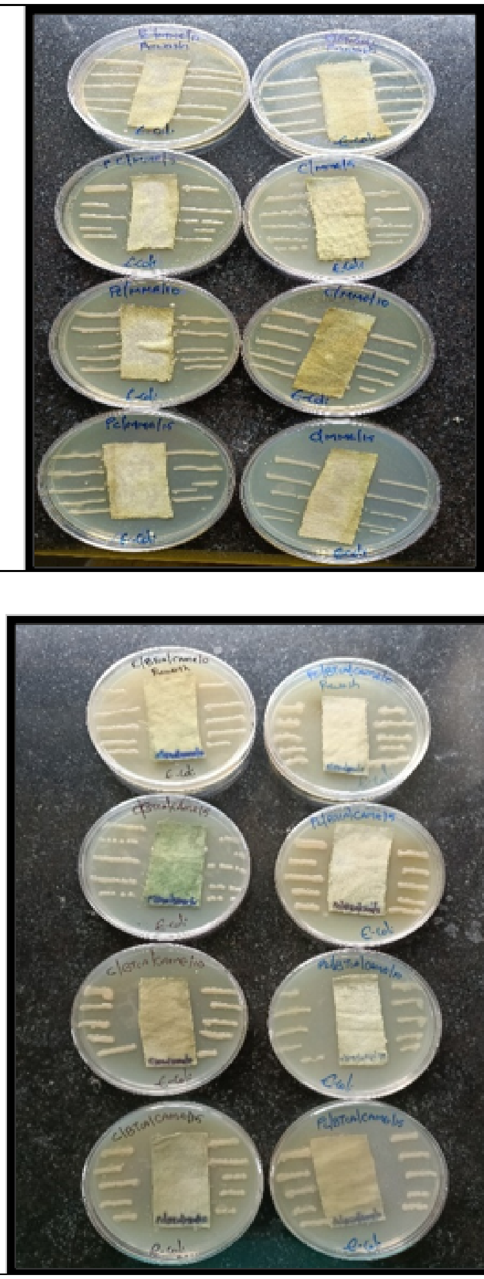
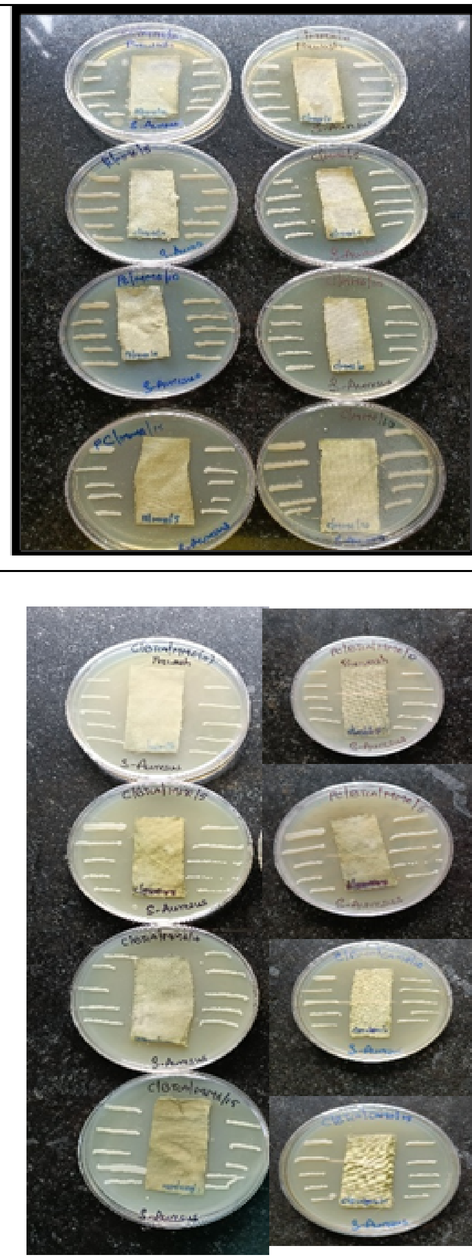


Figure 4

FTIR spectra for moringa leaf extract-coated samples



(a) ZOI of cotton and PC blend fabrics padded with moringa methanolic extract - unwashed, 5 washes, 10 washes, 15 washes – *E.coli*



(b) ZOI of cotton and PC blend fabrics padded with moringa methanolic extract- unwashed, 5 washes, 10 washes, 15 washes – *S.aureus*

Figure 5

Antimicrobial activity of cotton and PC blend fabrics padded with *moringa* leaf extract

ZOI of cotton and PC blend fabrics padded with custard apple methanolic extract - unwashed, 5 washes, 10 washes, 15 washes – *E.coli*	ZOI of cotton and PC blend fabrics padded with custard apple methanolic extract- unwashed, 5 washes, 10 washes, 15 washes – *S.aureus*

Figure 6

Antimicrobial activity of cotton and P/C blend fabrics padded with Custard apple leaf extract

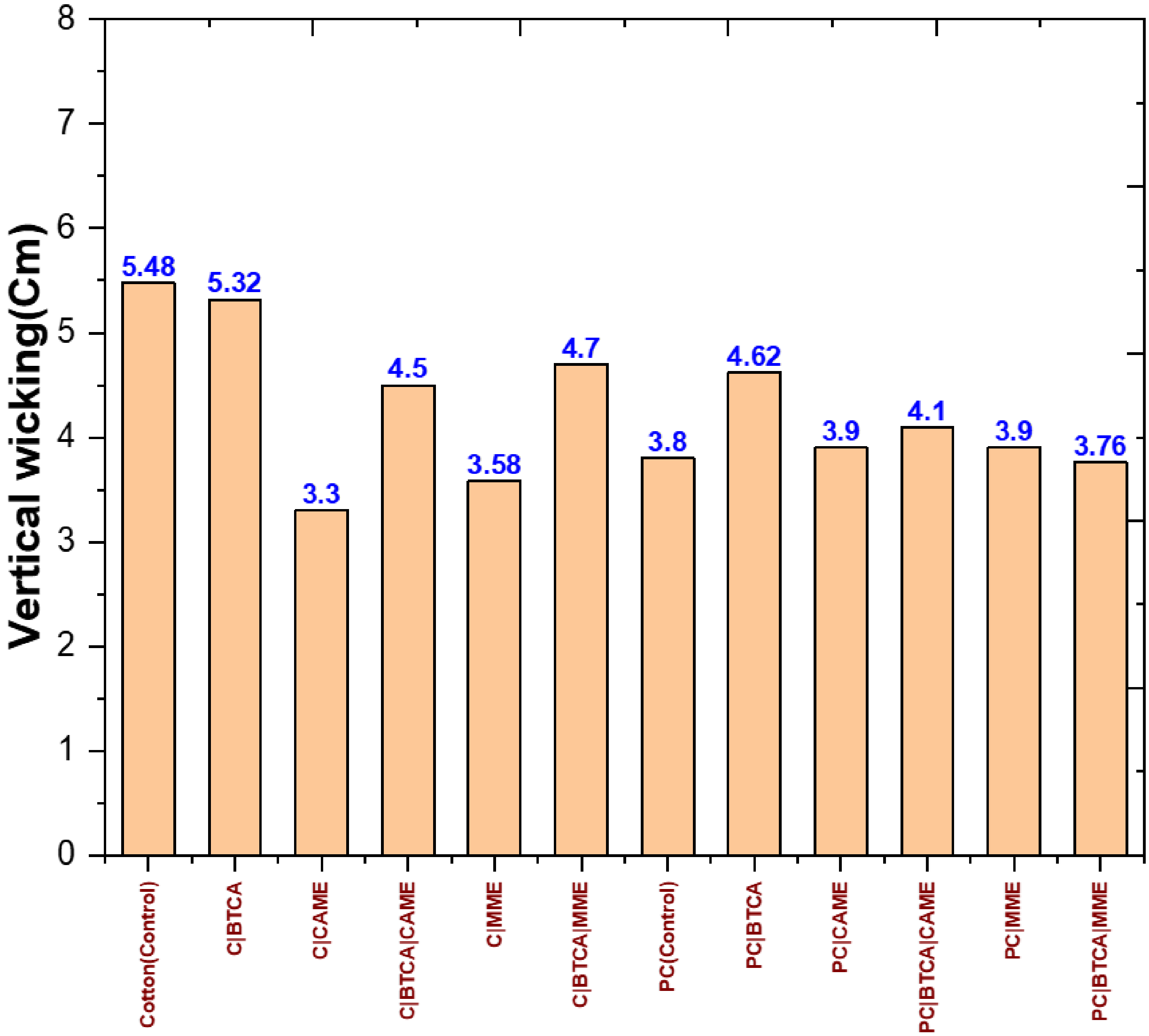


Figure 7

Wicking height of test specimens

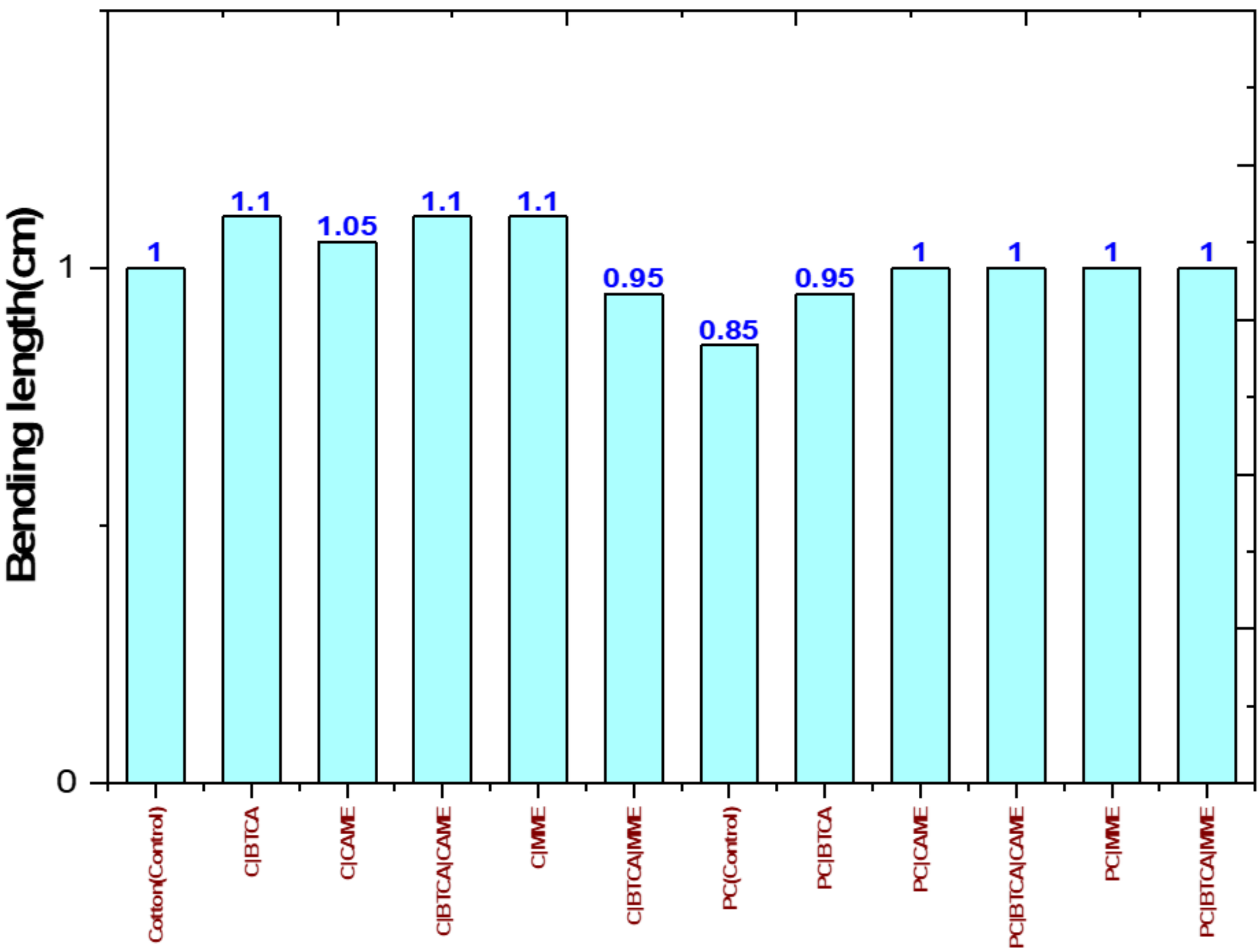


Figure 8

Bending length of test specimens