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In situ green synthesis of antibacterial copper nanocomposite cotton fabrics using *Achyranthes aspera* leaf extract

Jaswanth Seetha[1], Umamahesh Mallavarapu[2*], Arundathi Mesa[3]

[1]Department of Chemistry, Jawaharlal Nehru Technological University, Anantapur, Anantapuramu-515002, India.
[2]Department of Chemistry, Rajeev Gandhi Memorial College of Engineering & Technology, Nandyal-518501, India.
[3]Department of Genetics and Genomics, Yogi Vemana University, Kadapa-516005, India.

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ABSTRACT

Nanomaterials are finding the diversity of application at the leading edge in emerging field of nanotechnology. Copper nanoparticles (CuNPs) were *in situ* generated on the surface of cotton fabrics, using *Achyranthes aspera* leaf extract by environmentally benign green synthesis. The structural and morphological properties of synthesized nanocomposite cotton fabrics (NCFs) were characterized by different spectral studies such as Fourier-transformation infrared (FTIR), scanning electron microscopy (SEM) coupled with energy-dispersive X-ray primary and derivative thermogravimetric (TG-DTG), differential scanning calorimetry (DSC), and X-ray diffractometer (XRD). The molecular functionalities of hydroxyl groups in polyphenols of *A. aspera* leaf extract were identified from FTIR absorption spectrum, and they are responsible for the bioreduction of Cu^{+2} into Cu^{0} for the formation of CuNPs. The average size of the formed CuNPs from SEM studies was found to be 95 nm. The formed CuNPs were exhibited Face centered cubic (FCC) crystalline structure, and it was confirmed by XRD studies. TG-DTG analysis publicized the thermal stability of NCFs. The tensile strength of NCFs was higher than normal cotton fabrics. These NCFs exhibited good antibacterial properties which considered for making aprons and wound dressing materials in medicine and for packing materials.

INTRODUCTION

Nanocomposites are gaining importance in our daily life as an emerging field of nanotechnology, which utilizes in pharmaceutical, textile, industrial, biological, and biomedical applications (Jia *et al*., 2012; Zare and Shabani, 2016). In medicine, metal nanoparticles (MNPs), such as copper, silver, titanium, gold, and platinum, are showing good biological properties (Dizaj *et al*., 2014; Nasrabadi *et al*., 2016; Palza *et al*., 2015; Zain *et al*., 2014). In specific, copper nanoparticles (CuNPs) were proved to have potential antibacterial, antifungal, and mechanical properties (Bagchi *et al*., 2013; Viet *et al*., 2016). In recent times, researchers have been shifted toward bioreduction of metal ions into MNPs, due to simple, cheaper in cost, eco-friendly, and easy technique to synthesize. Now, researchers are employing bioreduction method to prepare MNPs, utilizing plant parts such as leaves, stem, peel, and root extracts (Dubey *et al*., 2010; Raut *et al*., 2013; Surendra *et al*., 2016; Tahir *et al*., 2015). The preparation of nanoparticles in polymer matrices results in agglomeration and apart inferior properties (Werner *et al*., 2008). To overcome this agglomeration, it is better to shift to *an in situ* generation method for preparing MNPs in cotton fabrics. Hence, researchers are using *in situ* generation method to synthesize the MNPs in cellulose cotton fabrics (Muthulakshmi *et al*., 2017; Rao *et al*., 2018) and prepared cellulose nanocomposite films (Sivaranjana *et al*., 2017). The main objective of the authors is to synthesize the copper nanocomposite cotton fabrics (NCFs), using *Achyranthes aspera* leaf extract at appropriate conditions.

Achyranthes aspera plant species belong to *Amaranthaceae* family and is widely available in Asia and Africa, commonly called as uttareni in south India. It is used for the repossession of waste fields. In India, the seeds and leaves of uttareni plant were used for human consumption and in religious ceremonies (Ragupathy *et al*., 2008; Ragupathy *et al*., 2009). In Ayurveda, it is utilized for the treatment of boils, skin eruptions, stomach ache, and piles (Dwivedi *et al*., 2008). The leaf extract was formed to be antiperoxidative and prothyroidic

*Corresponding Author
Umamahesh Mallavarapu, Department of Chemistry, Rajeev Gandhi Memorial College of Engineering & Technology, Nandyal-518501, India.
E-mail: mahesh1962rgm@gmail.com

in rats (Tahiliani *et al*., 2000). Hence, the authors utilized the leaf extract of *A. aspera* as a reducing agent to generate the CuNPs in NCFs by *in situ* generation method. The synthesized NCFs were characterized by scanning electron microscopy (SEM) along with energy-dispersive X-ray (EDX), X-ray diffractometer (XRD), and Fourier-transformation infrared (FTIR) spectroscopy and thermal properties by derivative thermogravimetric (TG-DTG) and differential scanning calorimetry (DSC) analysis. The mechanical properties such as tensile strength and tensile strain were calculated by a universal testing machine (UTM).

EXPERIMENTAL

Materials

White cloth procured from local market, penta hydrated copper sulfate ($CuSO_4.5H_2O$), Sigma Aldrich, Mumbai, and *A. aspera* leaves collected from local fields were employed in the present study.

Achyranthes aspera leaf extract preparation

The fresh leaves of *A. aspera* were picked before sunrise from the plants and cleaned thoroughly several times with double distilled water to remove all the impurities such as dust and dirt present on their surface, dried, and cut in to small pieces. About 900 ml of distilled water was taken in a cleaned glass vessel, and 100 g of chopped *A. aspera* leaves was weighed and poured into glass vessel. It was kept on magnetic stirrer and heated up to 80°C for 20 minutes with a stirring rate of 300 rpm. The obtained extract was filtered by using filter cloth followed by Whatman no. 1 filter paper, stored in reagent bottles, and kept at 4°C in the refrigerator.

Preparation of matrix

About 250 ml of prepared *A. aspera* leaf extract was taken in cleaned 250-ml beakers. The washed and dried white cotton fabrics cut into pieces of 9 cm × 27 cm were immersed in beakers and placed on a magnetic stirrer with a constant stirring rate of 300 rpm in room temperature for 1 day. The leaf extract was diffused on to the surface of cotton fabrics. These cotton fabrics were removed from the leaf extract, rinsed and eroded with condensed water for more than two times to remove non-adhered organic impurities on its surface, dried, and used as a matrix.

Preparation of CuNPs in matrix by in situ method

The dissimilar concentrations of aqueous penta hydrated copper sulfate ($CuSO_4.5H_2O$) precursor solutions (250, 125, 25, 5, and 1 mM) were prepared. About 250 ml of each concentrated solution was taken separately in 250-ml beakers, and two pieces of matrices were immersed in each precursor solution. These beakers are labeled and placed on a magnetic mixer for 1 day with a mixing rate of 300 rpm at room temperature. The color changes from greenish yellow to greenish brown. After 24 hours, these NCFs taken out, rinsed, and cleaned carefully with double distilled water most of the times and dried. After many washings, the color remains unchanged, specifying the formation of stable CuNPs in NCFs.

Characterization

The SEM pictures along with EDX of NCFs were documented, using JEOL JSM-IT500 scanning electron microscope. The FTIR spectra were recorded, using BRUKER ALPHA-II spectrophotometer. The XRD spectrum was also carried out to validate the morphology of the prepared CuNPs in NCFs, using RIGAKU MINI FLEX 600. A standard INSTRON-3369 UTM was utilized to calculate the mechanical strength of the synthesized NCFs, for comparing matrix and white cellulose cotton fabric. The antibacterial activity of the matrix and CuNP cotton fabrics were studied for their antibacterial activity against Gram-positive and Gram-negative bacteria by standard disc method (Natarajan *et al*., 2005).

RESULTS AND DISCUSSION

Preliminary conformation of CuNPs in NCFs

The initial visual conformation of generated CuNPs on the surface of NCFs has done with the persistent change of color. The digital images of NCFs generated with different concentrations of source solutions (1, 5, 25, 125, and 250 mM), white cellulose fabric, and matrix are shown in Figure 1. The greenish brown color intensity of the NCFs was gradually deepened with an increased concentration of source solution (Fig. 1c–g).The preliminary observation of the change of persistent color and not diminished even after several washings specifies the generation of *in situ* generated CuNPs in NCFs.

SEM analysis

The surface morphologies of the synthesized NCFs by *in situ* generation method were studied by SEM analysis. The digital SEM photographs of NCFs made using 1 mM (minimum) and 250 mM (maximum) source solutions were recorded and are shown in Figure 2a and b, corresponding EDX spectra in Figure 2c and d, and size distribution histograms in Figure 2e and f. The formed CuNPs in NCFs were found to be spherical in shape with an average size of 95 nm, using Smart Tiff program. The presence of metallic copper was confirmed by the appearance of the energy peaks in the EDX spectra (Fig. 2c and d). The EDX spectrum of NCFs made using 1 mM (minimum) showed only one peak at an energy level of 1 keV due to the lower concentration of $CuSO_4.5H_2O$. However, the EDX spectrum of 250 mM (maximum) exhibited energy band peaks at 1 and 8 keV, due to the formation of more number of CUNPs as reported by earlier workers (Sadanand *et al*., 2017). The remaining NCFs made using 5 mM onward which are not shown in figure also exhibited two energy peaks at 1 and 8 keV. Hence, the generation of CuNPs on NCFs was confirmed by SEM and EDX spectra.

FTIR spectral studies

To probe the interaction between the different molecular functionalities present in *A. aspera* leaf extract and NCFs, FTIR spectral analysis was carried out as shown in Figure 3. The spectra of white cellulose cotton fabric and matrix (Fig. 3a) were overlapping with each other, showing similar chemical groups in them. The FTIR spectra of matrix and CuNPs in NCFs of all concentrations are shown in Figure 3b. In Figure 3b, it is evident that both matrix and NCFs were exhibited a similar intensity band peak at 3,279 cm^{-1} corresponding to hydroxyl groups (−OH) present in polyphenolic and alcoholic compounds. The other band peak at 2,889 cm^{-1} is due to C-H symmetric and asymmetric stretching of alkenes present in the leaf extract. The remaining absorption band peaks at 1,635, 1,431, 1,323, and 1,013 cm^{-1} are due to N-H bending vibrations of amides, N-O asymmetric

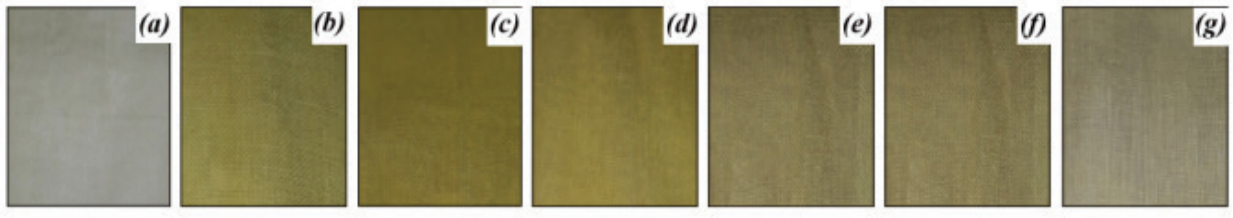

Figure 1. Digital images of (a) cellulose cotton fabric, (b) matrix, and (c–g) different concentrations of NCFs.

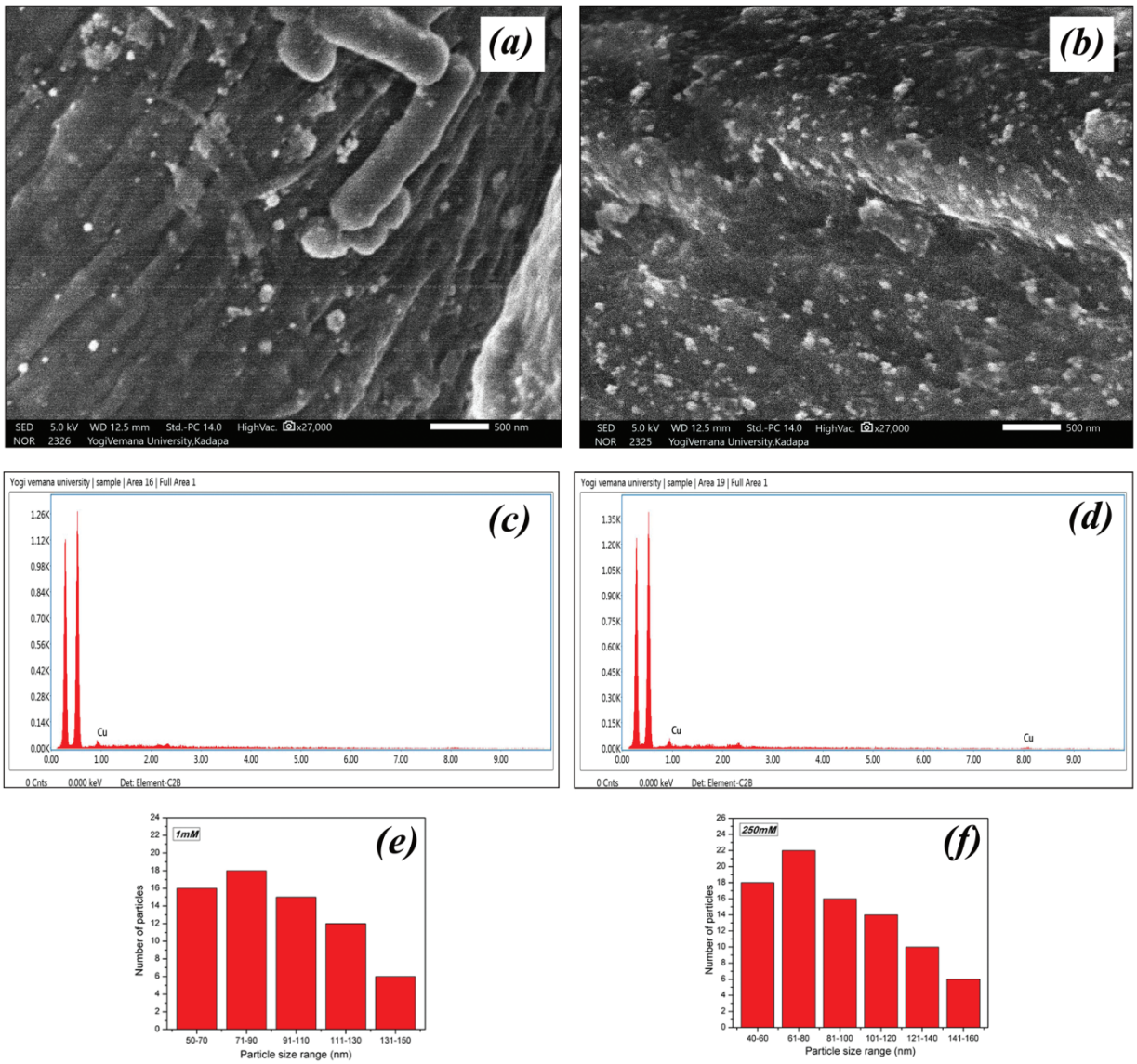

Figure 2. SEM along with EDX and histograms of NCFs made using 1 mM (a, c, and e) and 250 mM (b, d, and f) $CuSO_4.5H_2O$ source solutions, respectively.

stretching vibrations of nitro compounds, C-N stretching vibrations of aromatic compounds, and C-O stretching vibrations of aromatic and aliphatic compounds (Elumalai *et al*., 2016). These observations reveal that there will be an increase in the number of –OH and other functional groups in the matrix, due to the diffusion of *A. aspera* leaf extract into the cotton fabric. The increase in the number of hydroxyl functionalities in the matrix is responsible to involve the *in situ* generation of CuNPs in the matrix. The main absorption band peak at 3,279 cm^{-1} for the NCFs was lower than that of the matrix, noticing that the consumption of hydroxyl (−OH) functional groups was reduced the Cu^{+2} ions into CuNPs. The same observation was reported by earlier workers (Amara *et al*., 2019) during the biosynthesis of CuNPs on NCFs using red sander powder extract as a reducing agent.

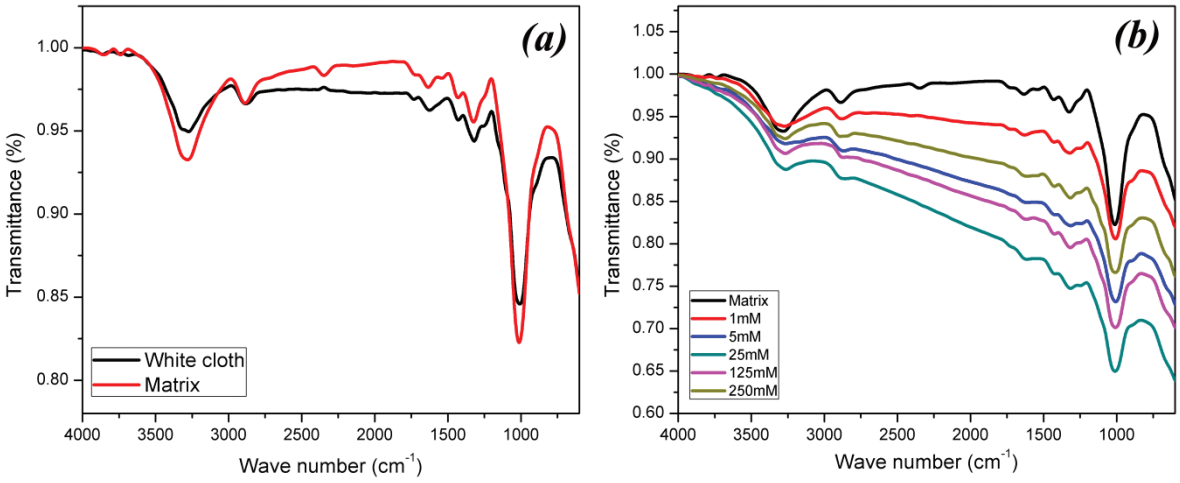

Figure 3. FTIR spectra analysis of (a) matrix and cellulose fabric and (b) NCFs (all concentrations).

XRD analysis

To study the nature of crystallinity of the generated NCFs, the XRD analysis was carried out. The recorded diffractogram of matrix and NCF made using 250 mM (maximum) is shown in Figure 4. Both matrix and NCFs were exhibited the same peaks at 2θ = 15.11˚, 17.01˚, 22.89˚, and 34.28˚, corresponding to the planes (101), (10-1), (002), and (040), respectively, related to cellulose-I structure (Zhou *et al*., 2017). In addition, the NCF exhibited various peaks at 2θ = 42.59˚, 50.31˚, and 73.18˚, corresponding to the lattice planes (111), (200), and (220), respectively, related to CuNPs. The remaining peaks at 2θ = 46.01˚, 64.28˚, and 70.07˚, corresponding to the planes (111), (200), and (220), respectively, related to Cu_2ONPs (Jadhav *et al*., 2011; Salavati-Niasari *et al*., 2009). The formed CuNPs are found to be face-centered cubic lattice structure, confirmed the formation of *in situ* generated CuNPs in NCFs.

TG-DTG and DSC analysis

To explain the thermal stability of formed CuNPs in NCFs, TG-DTG and DSC studies were carried out. The primary and derivative thermogram curves of NCF [1 mM (minimum) and 250 mM (maximum)] source solutions and matrix are shown in Figure 5a and b. Both matrix and NCFs exhibited a thermal degradation in two stages. The initial phase of deterioration was noticed between 44°C and 179°C, owing to the vaporization of volatile compounds and moisture in NCFs and matrix. The next phase of degradation was detected in the temperature range of 271˚–372°C and 308°C–408°C for NCFs and matrix, respectively. In Figure 5a and b, it is noticed that the thermal stability of NCFs was lesser than matrix, owing to the catalytic activity of CuNPs. A similar behavior was also noticed by earlier researchers (Sadanand *et al*., 2016). DSC studies were also carried along with TG-DTG to explain the phase transfer of NCFs, and the obtained DSC spectra are shown in Figure 5c. It is also revealed that the degradation of NCFs was lesser than matrix, indicating an exothermic reaction which is utilized to increase the temperature. These results confirmed that the generated CuNPs in NCFs were crystalline in nature and in accordance with X-ray diffraction spectral studies.

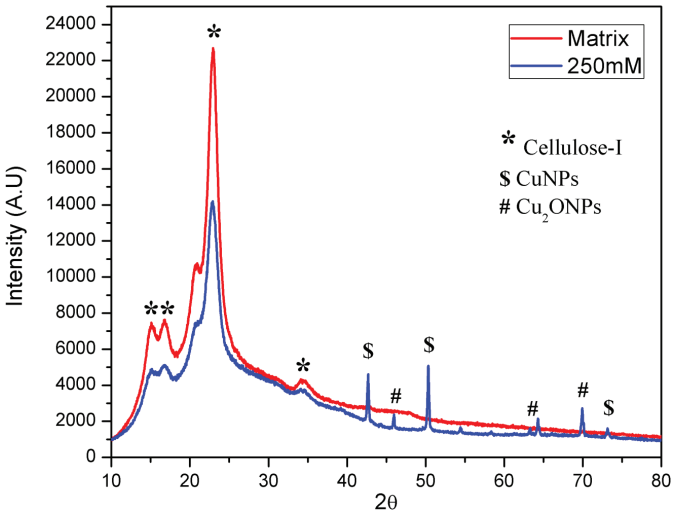

Figure 4. XRD spectra of matrix and NCF (250 mM).

Mechanical properties

In recent days, metal nanocomposite material utilization was increasing day by day, due to their high strength, smaller size, and light weight (Gouda *et al*., 2010; Li *et al*., 2015). To study the mechanical properties of the synthesized NCFs, such as high load bearing capability, tensile stress (T. stress) and tensile strain (T. strain) were measured and obtained the curves of NCFs (1 and 250 mM), white cotton cloth and matrix are shown in Figure 6, and the obtained average of three values is given in Table 1 The white cellulose cotton fabric (T. strain = 13.56 MPa and T. stress = 0.26%) and matrix (T. strain = 13.43 MPa and T. stress = 0.24%) were exhibited almost equal tensile stress and strain values (Fig. 6), indicating that both have similar structure. The generated NCFs, such as 1-mM NCF (T. strain = 18.63 MPa and T. stress = 0.23%) and 250-mM NCF (T. strain = 20.26 MPa and T. stress = 0.20%), exhibited higher tensile stress values, compared with matrix/white cloth. The generation of CuNPs on the surface of cellulose fabrics was responsible for higher strength. The induced mechanical properties of NCFs can be employed as packaging materials.

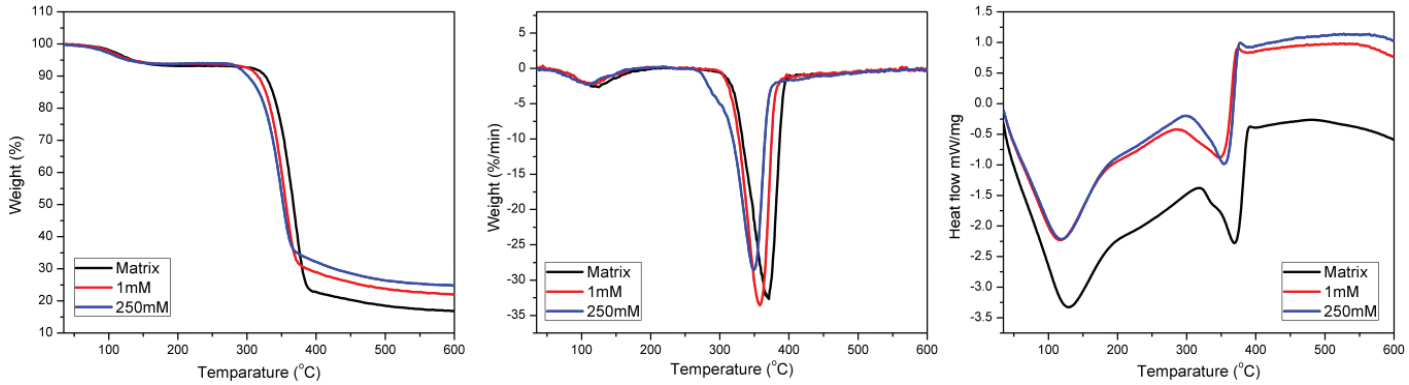

Figure 5. TG-DTG and DSC curves of matrix and NCFs (1 and 250 mM).

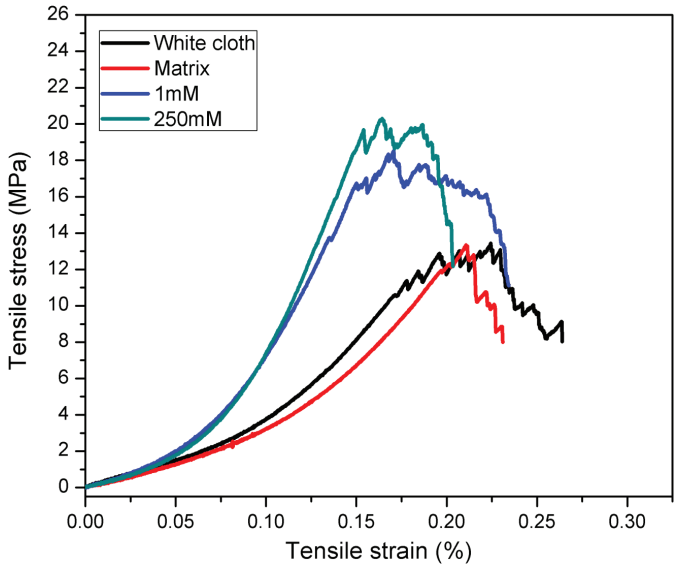

Figure 6. Mechanical properties curves of NCFs (1 and 250 mM) and matrix.

Table 1. Calculated tensile strength and stress values of NCFs at maximum loads.

Specimen for testing	T. Stress (MPa)	T. stain (%)	Max. load (N)	Young's Modules (MPa)
Cotton fabric	13.56	0.26	80.66	111.44
Matrix	13.43	0.24	90.08	119.69
NCF (1mM)	18.63	0.23	101.85	190.85
NCF (250mM)	20.26	0.20	121.82	239.93

Antibacterial activity

Antibacterial activity of *in situ* generated CuNPs on NCFs was tested by standard disc method, using Gram-positive *Staphylococcus aureus* and Gram-negative *Pseudomonas aeruginosa* bacteria. The zone of killing of both bacteria employed by NCFs using 1, 5, 25, 125, and 250 mM source solutions and the comparison of white cloth and matrix are shown in Figure 7, and the measured zone of inhibition is presented in Table 2. These values reveal that the NCFs were shown a good antibacterial activity as compared with matrix, and the white cellulose fabric does not exhibited any zone of inhibition (Fig. 7).The zone of inhibition was enhanced with an increase in the concentration of

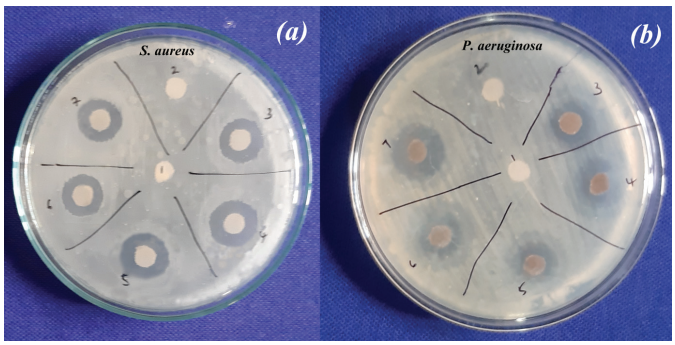

Figure 7. Antibacterial activity of NCFs and matrix against (a) *P. aeruginosa* and (b) *S. aureus*.

Table 2. Pathogenic bacteria killing zones are exhibited by NCFs against *P. aeruginosa and S. aureus*.

Tester name	Number of tester	width of formed zone (mm)	
		P. aeruginosa	*S. aureus*
White cloth	1	No formation	No formation
Matrix	2	No formation	No formation
1mM NCF	3	16	14
5mM NCF	4	18	14
25mM NCF	5	18	13
125mM NCF	6	17	13
250mM NCF	7	18	17

source solution by both bacteria (Table 2). Due to this antibacterial property, the generated CuNPs on NCFs were utilized in pharmaceutical applications such as wound dressing, aprons, antibacterial beds, and floor cleaning items.

CONCLUSION

In this work, CuNPs were prepared on the surface of cellulose cotton fabrics, using aqueous *A. aspera* leaf extract by *in situ* generation method. The obtained NCFs were characterized by different spectral analyses such as SEM, EDX, TG-DTG, DSC, FTIR, and XRD and tested for the mechanical and antibacterial properties. The formed CuNPs are globular in shape with an average size of 95 nm, proved by the SEM analysis. The confirmation of copper

metal present in the cotton fabric by observing the energy band peak forms the EDX spectrum at 1 and 8 keV. The FTIR analysis revealed that hydroxyl functional groups present in *A. aspera* leaf extract molecularities are responsible for the bioreduction of Cu^{+2} into Cu^{0}. The synthesized CuNPs in NCFs exhibited a better pathogenic bacteria killing activity against *P. aeruginosa* and *S. aureus*. Hence, these *in situ* generated NCFs can be utilized in medical field for making aprons, bandage cloths, napkins, and floor cleaning items and can be considering for packing materials.

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CONFLICT OF INTEREST

The authors declare that they have no conflicts of interest.

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None.

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