RESEARCH ARTICLE

Preparation of antibacterial nanocomposite cotton fabrics with in situ generated silver and silver oxide nanoparticles by bioreduction using Moringa oliefiera leaf extract

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ABSTRACT

Aqueous *Moringa Oliefiera (MO)* leaves extraction is employed as a reductant to generate silver nanoparticles (AgNPs) and silver oxide nanoparticles (Ag_2ONPs) in cellulose fabrics by *in situ* technique. The biosynthesized nanocomposite cotton fabrics (NCFs) were analysed by X-ray Diffraction (XRD), Differential Scanning Calorimetry (DSC), Scanning Electron Microscopy (SEM) associated Energy Dispersive X-ray (EDX) spectroscopy, Fourier Transform Infrared (FT-IR) and Thermogravimetric Analysis (TGA) methods. The shape and mean size of AgNPs in NCFs were found to be globular and 82 nm, respectively and their formation in NCFs was established by SEM studies. EDX analysis established the presence of silver metal. The XRD analysis revealed that the obtained silver based nanoparticles were crystalline in nature. The TG and DTG analysis showed that the obtained NCFs were thermally stable. These NCFs exhibited good antibacterial activity against Gram negative (G^{-ve}) *Pseudomonas aeruginosa (P. aeruginosa)* and Gram positive (G^{+ve}) *Staphylococcus aureus (S.aureus)* bacteria. The mechanical properties such as modulus, tensile strain and stress of NCFs were also tested, utilizing universal testing machine (UTM). The modulus was found to be 276.4 MPa. These NCFs can be used in medicine for making antibacterial napkins, wound dressing bandage cloth, etc., and as packaging materials.

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INTRODUCTION

Nano materials play a key role in widening the applications in diversified fields on account of their larger boundary surface to volume ratio, prosperity of the human life and protection of the environment [1,2]. The present day researchers have drawn their attention towards ecofriendly methodologies due to the lower cost, no emission of pollutants and ease of preparation for the bio generation of metal nanoparticles (MNPs) and metal oxide nanoparticles (MONPs) [3-8]. The earlier chemical and physical methodologies employed for the synthesis of MNPs, leading to agglomeration and inferior properties. Now, the trend is shifting towards the *in situ* generation of MNPs in polymer matrices as it minimizes agglomeration and imparts good properties to the nanocomposites [9]. Plant species parts such as bark, flowers, leaves, seeds etc., were employed for bio synthesis of copper and silver nanoparticles (CuNPs and AgNPs) bimetallic nanoparticles (BMNPs) on NCFs, utilizing *in situ* method by a few researchers [10-14]. The metallic silver exhibits good antibacterial property which can be considered in medical applications [15-18]. Hence, the authors used environmentally benign *in situ* methodology, utilizing *MO* leaf broth in aqueous medium as a reducing and stabilizing agent for the generation of silver based nanoparticles in NCFs. *MO* is commonly known as drumstick tree in southern parts of India and most parts of the tree are considered in medical and commercial applications. In particular, the *MO* leaves are used in anti-tumor, hypotensive, cardio protective and wound healing etc. applications. Further, it has nutritional values (vitamin A and C). The *MO* leaves are also rich in polyphenols, flavonoids, calcium, iron, folic acid and β-carotene [19-21]. So, the authors utilized the leaf extract of *MO* for bio reduction of Ag^+ ions to silver based nanoparticles in NCFs. The prepared silver based nanoparticles on NCFs were characterized by FT-IR, SEM connected with EDX, TG-DTG & DSC analysis and XRD. The NCFs were also tested for their antibacterial and mechanical properties. The author's main aim was to prepare antibacterial NCFs that can be considered for medical applications such as bed materials in hospitals, napkins, and bandage cloths for wound dressing and packaging materials.

EXPERIMENTAL SECTION

Materials

Nutrient Agar (AR), Silver nitrate ($AgNO_3$, AR), supplied by Aldrich Chemical Ltd. Mumbai, India, white cotton cloth purchased from local market and the bacterial cultures from nearby medical college, were employed in the present work.

Preparation of aqueous leaf extract of MO

Fresh *MO* leaves were picked before sun rise from the tree and the leaves were cleaned thoroughly with deionized water for several times to remove the dust and dirt present on the surface. These were then desiccated at lab temperature and chopped in to small pieces. 100g of these pieces were taken in a glass vessel with 900mL of double distilled water. The glass vessel was then placed on a magnetic stirrer at 80 ^{o}C for about 20 min at a constant stirring rate of 300rpm. The aqueous MO leaf extract obtained was greenish yellow in color. It was riddled and centrifuged to obtain a clear solution. It was laid in bottles and kept in refrigerator at 4 ^{o}C to avoid fungus formation and for further utilization.

Preparation of Matrix

The white cotton fabrics (CFs) were washed with condensed water and then dried. They were made into pieces of dimension 140 mm x 100 mm. These pieces were immersed in *MO* leaf extract taken in 250mL glass beakers. They were rested on a magnetic mixer for a day (24h) at 25 ^{o}C with a

stirring rate of 300 rpm. After this, the white colored CFs changed to greenish yellow because of propagation of *MO* leaf solution into them. The CFs were rinsed and cleaned with distilled water for 3-4 times to eliminate the undiffused leaf extract contaminants on it and then dried to use them as matrix.

In situ generation of silver based nanoparticles in NCFs

Aqueous $AgNO_3$ source solutions of five different concentrations (1mM, 2mM, 3mM, 4mM and 5mM) were prepared separately in dark room to avoid oxidation of silver as per standard procedure. Each piece of matrix was dipped in 250 mL beakers covered with tin foil containing 200 mL of prepared $AgNO_3$ source solutions. At room temperature, these beakers were continuously stirred at a revolution of 250 rpm for 24h. The color of the matrix changed from greenish yellow to brown. The color change preliminarily specifies the development of silver based nanoparticles in NCFs. These NCFs were rinsed in distilled water and cleaned number of times to eliminate the unreacted $AgNO_3$ and desiccated. The persistent color of NCFs confirms the formation of silver based nanoparticles.

Characterization

The FT-IR studies were carried by recording the spectrum of NCFs, white cloth, matrix utilizing BRUKER ALPHA-II (BRUKER optics Inc. Billerica, MA.USA) FT-IR spectrophotometer with 32 scans at a resolution of 4 cm^{-1} in the wavenumber range of 4000 cm^{-1} to 400 cm^{-1}. The SEM correlated with EDX spectra were obtained, utilizing JEOL JSM-IT500 (JEOL Ltd., Japan) microscope operated at 5.0kV. The X-ray diffractometer (RIGAKU MINI FLEX-600) was employed to study the crystalline nature at a scanning rate of 4^o/min in the $2\theta = 10^o$ to 80^o range, operated at 30 mA and 40 kV. The TG, DTG and DSC studies were conducted to distinguish the thermal stability of NCFs utilizing PERKINELMER-STA600 (Westborough, MA, United States) thermobalance analyzer in nitrogen atmosphere at a heating rate of 10 ^{o}C /min and the temperature range of 35 ^{o}C to 700 ^{o}C. INSTRON-3369 (Norwood, MA 02062-2643, USA) Universal testing machine using ASTM D 638 specifications was employed to study the mechanical properties of NCFs. The antibacterial activity was studied by standard disc method. AATCC 100 standard test was conducted to test the pathogenic microbial cultures of *P. aeruginosa* (AATCC 27853) and *S. aureus* (AATCC 6538). Image J software was used to measure the formed zone of inhibition.

RESULTS AND DISCUSSION

Appearance of persistent color

The images of NCFs, using 1-5mM aqueous $AgNO_3$ source solutions were photographed and the obtained digital images are depicted in Fig. 1c-g. For differentiation, the white cloth and matrix pictures are also shown in Fig. 1a and Fig.1b respectively. As per the visual observation, the matrix was greenish yellow in color (Fig. 2b). The color of the NCFs changed to brown owing to the generation of silver based nanoparticles. The color of the NCFs deepened with increasing concentration of the $AgNO_3$ source solutions as shown in Fig. 1. The color of the NCFs was not diminished even with number of times of washings. The persistent visual observation of color change preliminarily establishes the formation of silver based nanoparticles in NCFs. For further confirmation, the SEM images were recorded.

SEM Analysis

The confirmation of presence of bio generated silver based nanoparticles in the NCFs

was carried by SEM studies. SEM and correlated EDX digital photographs were recorded for NCFs. For clarity, the SEM images of the NCFs made using 1mM (minimum) and 5mM (maximum) $AgNO_3$ solutions are shown in Fig. 2a and b respectively while their corresponding EDX spectra are shown in Fig. 2c and d respectively. To measure the size of formed silver based nanoparticles using 1mM (minimum) and 5mM (maximum) $AgNO_3$ solution on the surface of NCFs, Smart Tiff program was used. The histograms representing the size distribution of silver based nanoparticles in the NCFs prepared using 1 mM and 5 mM source solutions are shown in in Fig. 2e and f as histograms, respectively. From Fig. 2a and b, it is clear that the silver based nanoparticles in NCFs were spherical in shape and orderly dispersed with a slight assortment at some places. The peak at 3keV (Fig. 2c and d) was related to metallic silver [22] and approves the presence of silver metal in NCFs. From Figs. 2e and f, it can be seen that in the case of NCFs prepared using 1 mM and 5 mM source solutions, the silver based nanoparticles were generated in the size range of 71-100nm and 41-130nm, respectively. Further, the average size of the generated nanoparticles in these two cases was found to be 78nm (1mM) and 84nm (5 mM). Further, it is revealed from Fig. 2b that in the case of the NCF prepared using 5 mM source solution, more number of silver based nanoparticles was generated. Even though, *in situ* generation is expected to overcome agglomeration, at some places, formation of clusters could not be avoided due to the generation of more number of silver based nanoparticles in NCFs. A similar observation was also made by other researchers [23]. Hence, the SEM coupled with EDX confirmed the formed silver based nanoparticles in NCFs.

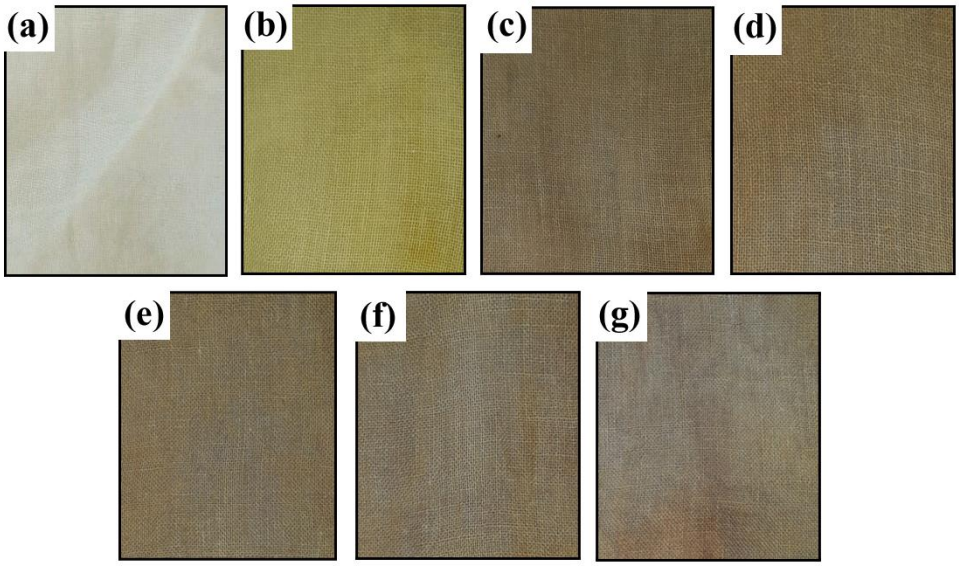

Fig. 1. Digital photographs of cotton fabric (a); Matrix (b); NCFs with *in situ* generation of AgNPs (c-g)

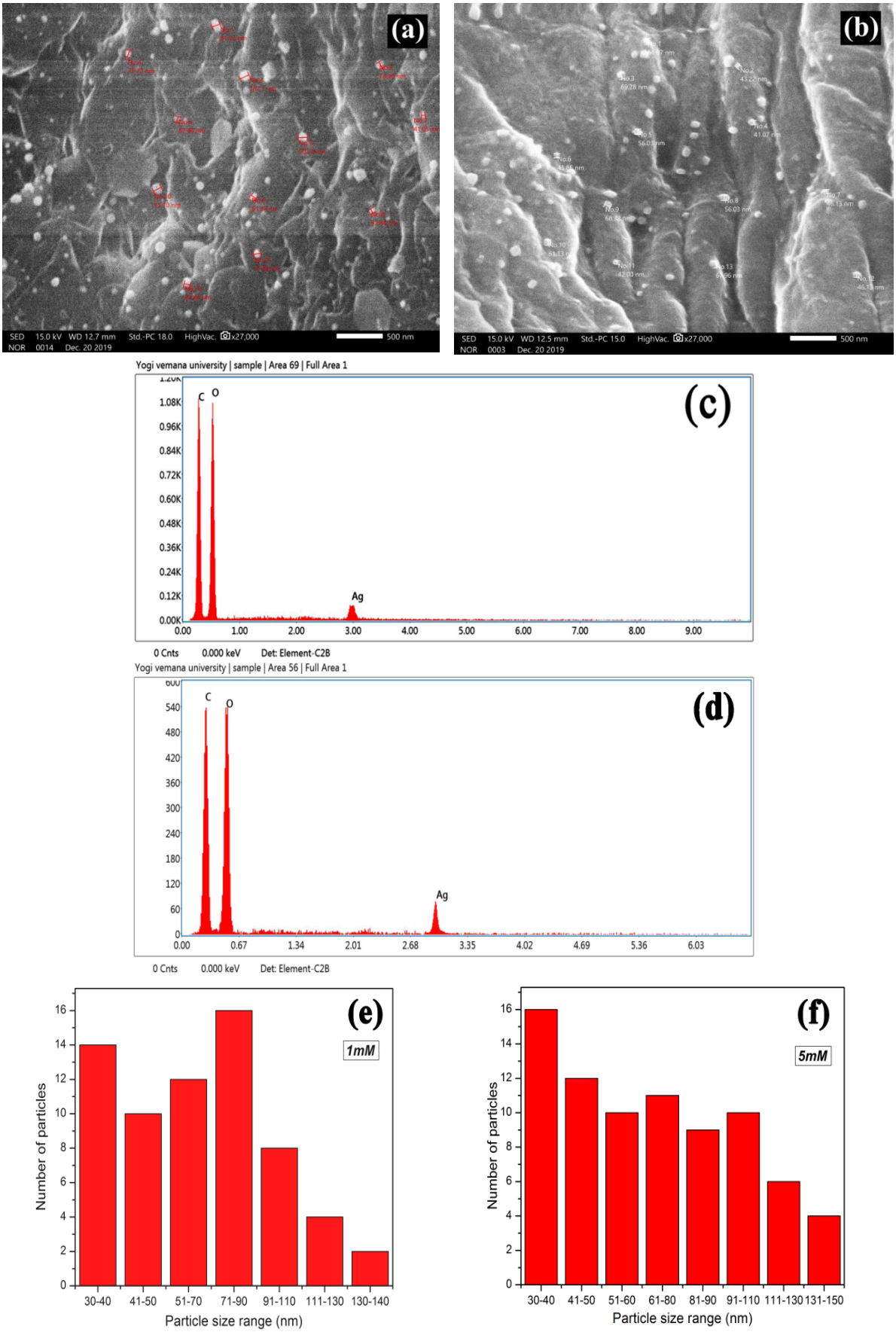

Fig. 2. Digital SEM images, EDX photographs and Histograms of 1mM (a,c,e) and 5mM (b,d,f) AgNPs in NCFs

FT-IR Analysis

The FT-IR spectral studies were carried to identify the possible bio molecules which are responsible for constructional changes in NCFs. The recorded FT-IR spectra of cotton cloth, matrix and NCFs are shown in Fig. 3. Both white cloth and matrix exhibited similar peaks (Fig. 5a) which are overlapping with each other and a slight increase in the matrix intensity. It indicates that both the white cloth and matrix possess similar molecular functionalities. i.e., no changes are added by MO leaf extract in the matrix. From Fig. 5b, there is no change in peak positions of absorption bands of NCFs. However, some changes in their intensities are observed. From Fig. 5b, the peaks at 3285and 2903cm^{-1} were related to characteristic –OH (hydroxyl) functional group of alcohol and phenolic compounds, and alkaline C-H stretching associated with lipid molecules present in MO leaf extract, respectively. The other peak at 1635 cm^{-1} was due to Amide-II bond from proteins. The peaks at 1434 and 1319 cm^{-1} were assigned to methylene (CH_2) bending oscillations of cellulose and CH_2 wagging mode. Another peak at 1016 cm^{-1} was assigned to skeletal resonance of cellulose in C-O-C pyranose ring. The earlier workers also noticed similar type of absorption bands in some other systems [19, 24, and 25]. Further, the absorption bands of NCFs exhibited similar peak at 3285 cm^{-1}. The intensity of peaks of NCFs was lower than the matrix and gradually decreased with increase in concentrations of $AgNO_3$ source solutions. This indicates that the hydroxyl functional groups present in the matrix were utilized in the bioreduction of Ag^+ ions in to Ag^0 [26, 27].

XRD spectral analysis

The XRD study was employed to know the effect of crystallinity on NCFs by the generated silver based nanoparticles. The diffractograms of XRD were recorded for the matrix and NCFs and as an example, the diffractograms of the matrix and the NCF prepared using 5mM source solution are presented in Fig. 4a. From Fig. 4a, it can be observed that the obtained diffractograms are overlapping one over the other and both had the common main peaks at 2θ =15.2º, 17.0º, 22.9ºand 34.5º, corresponding to the planes (101), (10-1), (002) and (040) of cellulose-I structure [28, 29]. The intensity of the peak of NCF (5mM) was higher than that of the matrix indicating that the generated silver based nanoparticles increased its crystallinity. For observing the peaks conforming the produced silver based nanoparticles, the diffractogram of NCF using 5mM $AgNO_3$ solution was expanded in the 2θ=25º to 80º range (Fig. 4b) which indicates low intensity peaks at 2θ=28.13º and 32.45º, correlating with (110) and, (111) planes respectively, indicating the formation of Ag_2ONPs which are in agreement with the standard values mentioned in JCPDS 76-1393. Besides, some more additional peaks at 2θ = 38.35º, 46.35º and 77.56º correspond to the reflections from the (111), (200) and (311) planes of AgNPs respectively. These values and their miller indices are in agreement with the standard values of AgNPs (JCPDS file No. 04-0783). Similar observation was also reported by the earlier workers [30, 31]. The X-ray diffractogram can be clearly indexed to a face centered cubic structure (FCC) of Ag. Thus the NCFs prepared in this study had both AgNPs and Ag_2ONPs.

TG-DTG and DSC analysis

The thermal stability of biosynthesized NCFs with in situ generated silver based nanoparticles was tested by TG, DTG and DSC analyses. The primary and derivative thermograms of the matrix and the NCFs are presented in Fig. 5a and Fig. 5b, respectively. It is understood from Fig.

5a and b, both matrix and NCFs undergo deterioration in two stages. The initial stage of thermal decay was perceived at temperature variation between 40 ºC-150 ºC. This degradation was due to the elimination of water molecules and volatile constituents present in NCFs and matrix. The second phase of deterioration was initiated at 260 ºC and ended at 402 ºC. The inflection temperature (related to high decay rate) of NCF was found to be a little lower than that of the matrix (Fig. 5b). Similar occurrence was noticed by earlier researchers during the preparation of nanocomposites with silver based nanoparticles [12, 13]. The highest thermal decay temperature of matrix (380 º C) was found to be decreased to a minimum value (372 ºC) in the case of the NCFs. However, no general trend of decrease was observed in case of NCFs, using 1-5mM source solutions. It was due to agglomeration of silver based nanoparticles in some parts. For further confirmation, DSC analysis was also carried along with TG and DTA and presented in Fig. 5c. The DSC analysis reveals that an exothermic reaction occurred in NCFs and it is responsible to raise the sample temperature with lesser heat. Hence, the formed silver based nanoparticles in NCFs possessed FCC crystalline structure was confirmed by DSC. These results are in accordance with XRD analysis.

Mechanical properties

The industrialists and researchers are attracted towards making high strength, small size and light weight nanocomposite materials [32, 33]. The performance of NCFs with induced mechanical properties (tensile strength and strain) was studied by universal testing machine. For comparison, both matrix and cotton fabric were also tested in the present study. The tensile stress and strain curves of NCFs made with 1mM and 5mM, matrix and cotton fabric are presented in Fig. 6. The experiment was carried by taking the average of five values and the calculated average Young's modulus values are given in Table 1. The silver coated NCFs showed high tensile stress and low strain than matrix and white cotton fabric. It is also observed that an increase in tensile strength and decrease in strain were proportional to the concentration of $AgNO_3$ solutions (1-5mM) used in the preparation and reached a maximum value at 5mM. From Table 1, it is evident, in the case of NCF (1mM) showed the strain at 0.14% and tensile stress of 17.11 MPa at a maximum load of 59.88 N (cotton fabric strain=0.19% and tensile stress=12.28 MPa at maximum load of 58.33N matrix and strain=0.21% and tensile stress =12.40 MPa at maximum load of 55.80 N) and for NCF (5mM), the tensile strain and tensile stress were found to be 0.16 % and 17.47 MPa respectively at a maximum load of 78.62 N. The calculated modulus value of NCF (5mM) was found to be 276.36 MPa. The improved mechanical properties of NCFs can be employed in packaging applications.

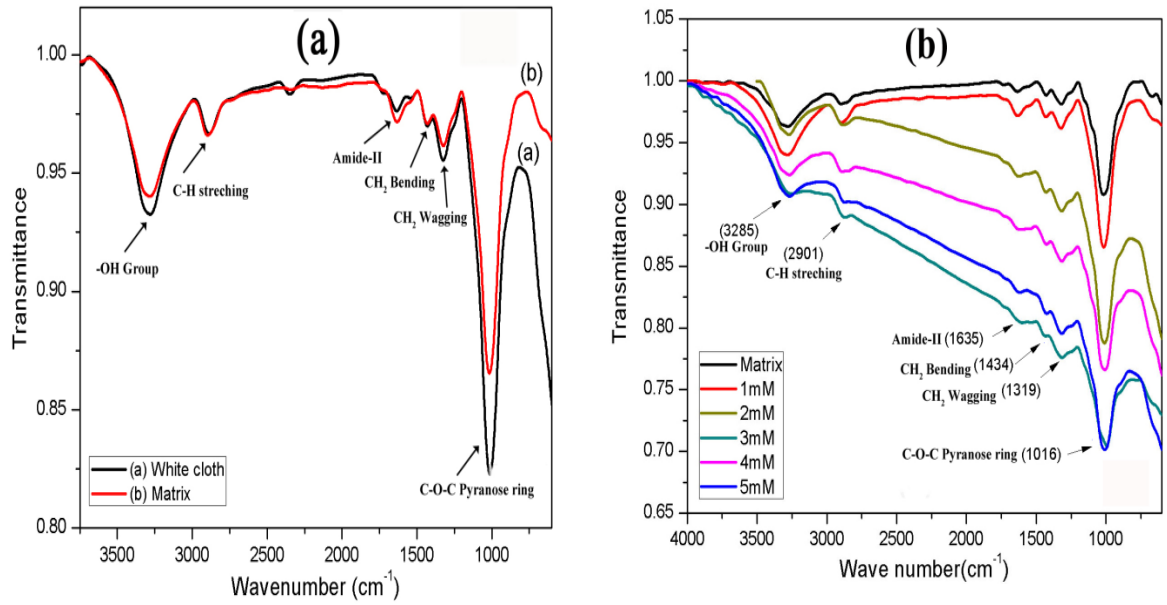

Fig. 3. FTIR spectra of (a) white cloth and matrix; (b) Matrix and Silver based nanoparticles generated NCFs

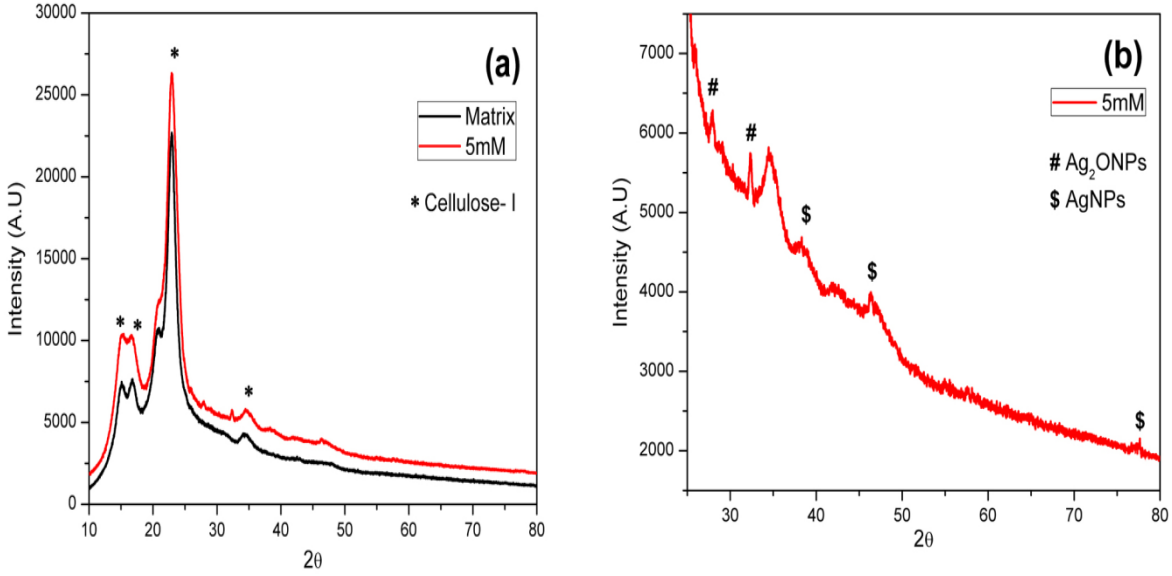

Fig. 4. XRD spectra of (a) matrix and NCF (5mM); (b) expanded diffractogram of 5mM NCF in 2θ = 25° to 80°

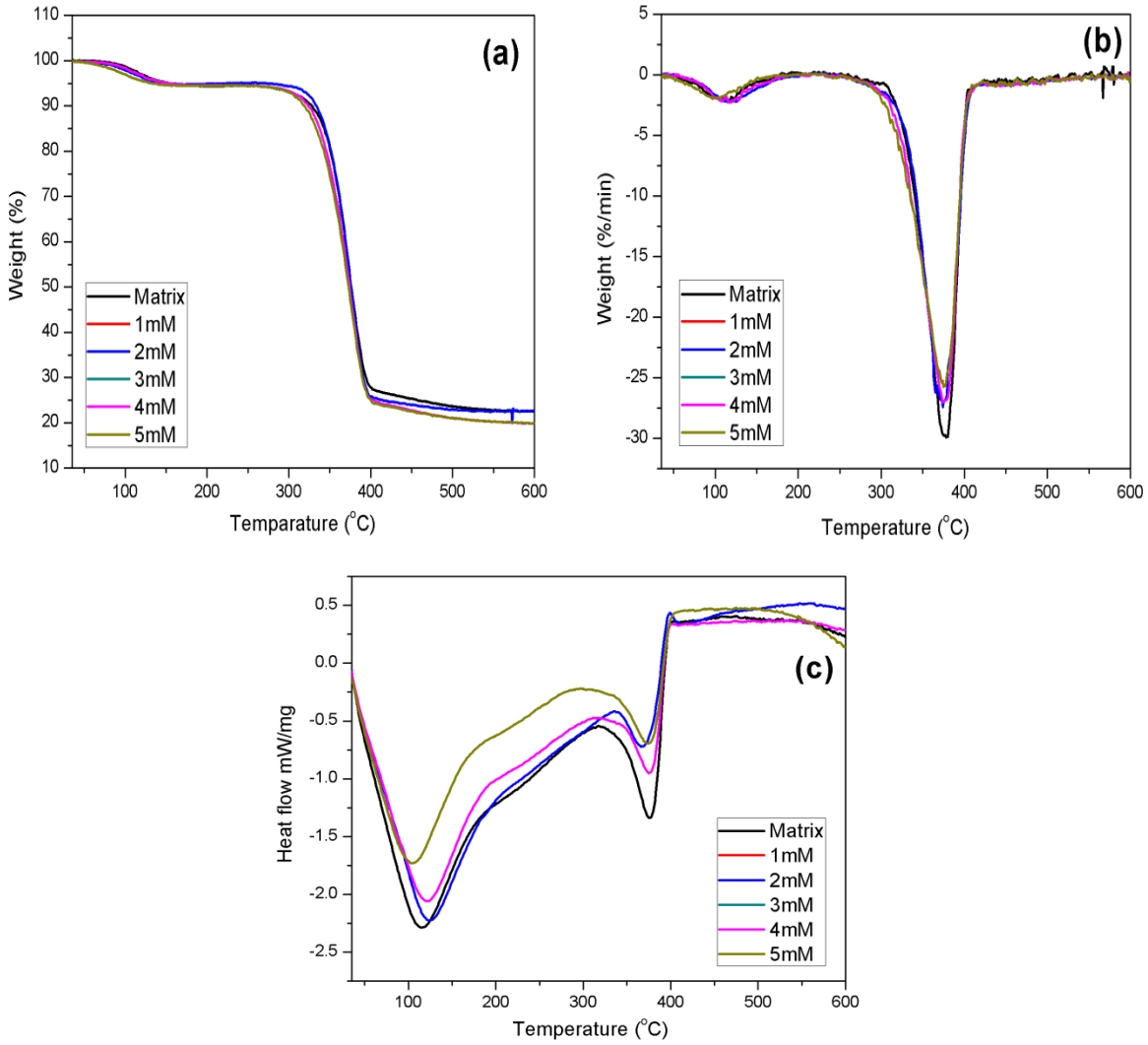

Fig. 5. (a) TG, (b) DTG and (c) DSC analysis of NCFs

Antibacterial studies and mechanism

It is an established fact that silver metal causes the death of pathogenic bacteria (G^{-ve} and G^{+ve}). In order to confirm whether the NCFs with in situ generated silver based nanoparticles, also exhibit annihilation of bacteria or not, the antibacterial studies were carried. P. aeruginosa and S. aureus were used in the present study to test the antibacterial activity of NCFs by disc method. The calculated killing area of both bacteria by generated silver based nanoparticles in NCFs, along with white cloth and matrix were photographed and are presented in Fig. 7 and the corresponding measured zone of inhibition are given in Table 2. It indicates that the NCFs exhibited good antibacterial properties.

The formation of AgNPs was confirmed by performing the antibacterial activity of NCFs with repeated washings (5, 10, 20 and 30) and the obtained histograms are presented in Fig. 8. Even after 30 washings, both bacteria did not exhibit appreciable decrease in zone of bacteria killing. The persistent zone of inhibition by NCFs confirms the formation of silver based nanoparticles. Earlier researchers also reported the similar observation [34, 35].This can be considered in medical applications as antibacterial pillow and bed materials in hospitals, surgical aprons, napkins, bandage cloths etc.

For killing action of AgNPs on human

pathogenic bacteria, a possible mechanism was proposed and it is depicted in Fig. 9. Importantly, the NCFs actively participated in the damaging of cytoplasmic layer of the bacteria followed by apoptosis. Further, the silver based nanoparticles in NCFs enter into the bacterial cell and damage the protein and enzymatic content of the bacteria. This may destroy the respiratory system of the bacteria, cell division and cell death [36].

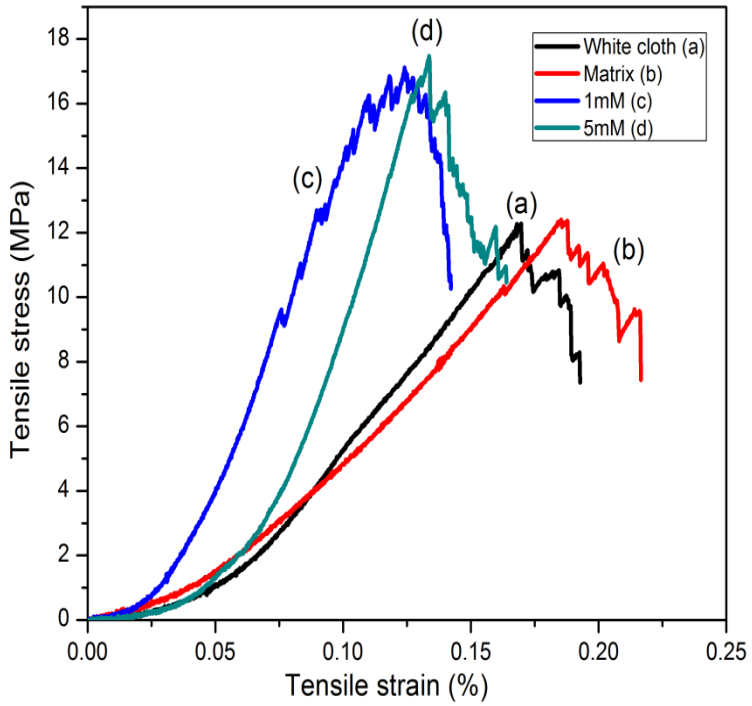

Fig. 6. Tensile strain and stress curves of (a) white cotton fabric; (b) matrix; (c) NCF (1mM); (d) NCF (5mM)

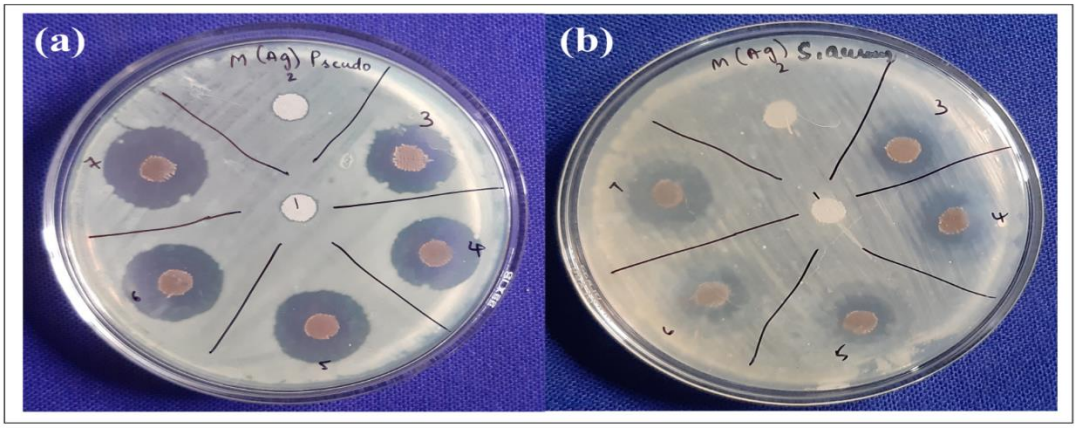

Fig. 7. Digital photographs of Antibacterial activity of NCFs against *P. aeruginosa* (a) and *S. aureus* (b)

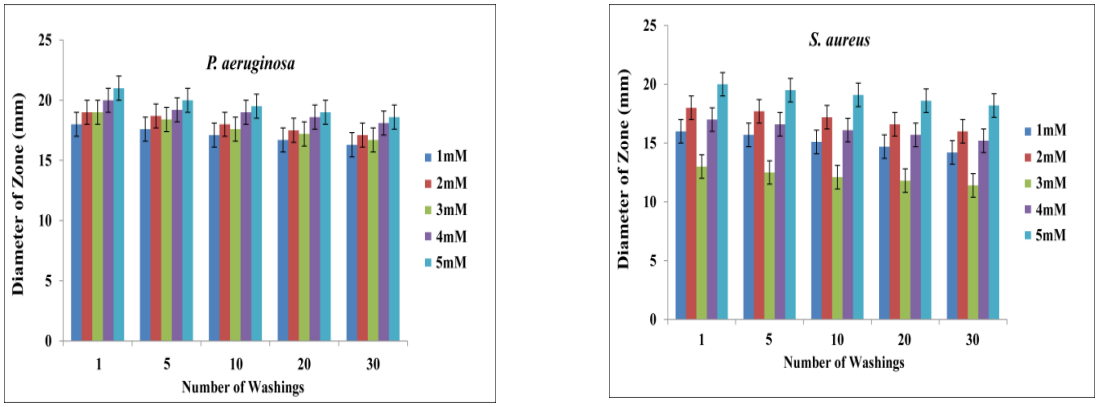

Fig. 8. Antibacterial zones exhibited by the NCFs before and after several washings

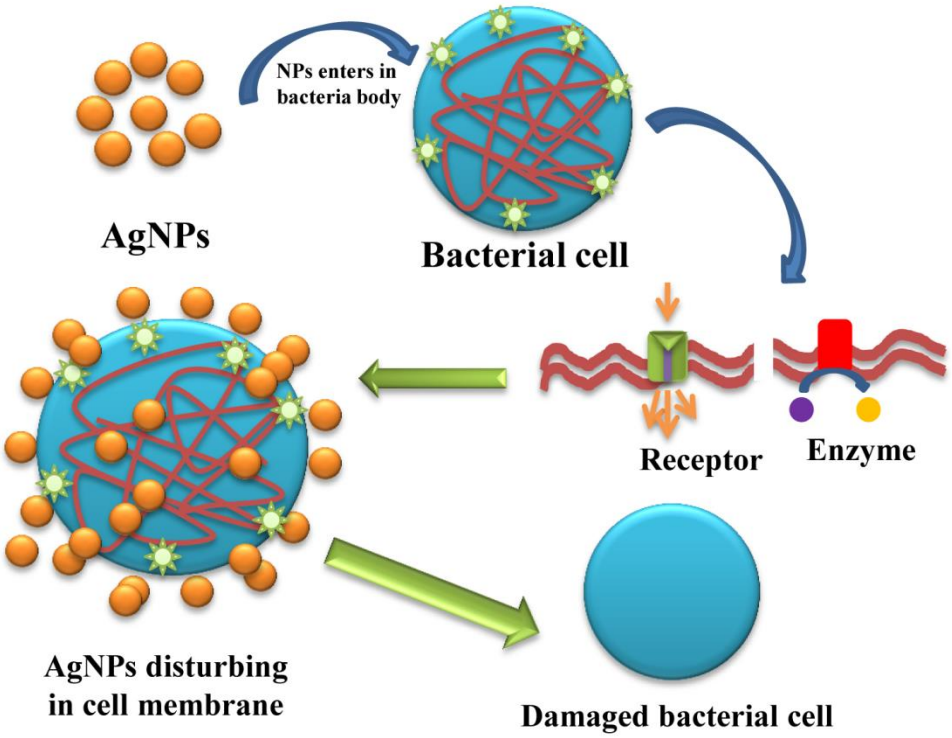

Fig. 9. Plausible mechanism of AgNPs in NCFs on bacterial strain

CONCLUSION

Silver based nanoparticles were in situ generated in NCFs by employing bio reduction method, using MO leaf extract. The different analyses were carried out to confirm the bio synthesized AgNPs in NCFs. Most of the formed globular shaped silver based nanoparticles in NCFs were with a mean size of 82 nm. The XRD studies indicated the generation of both AgNPs and Ag2ONPs with crystalline FCC lattice structure in the NCFs. The prepared NCFs possessed good mechanical properties and exhibited good antimicrobial properties. Thus, these materials can find applications in the field of medicine for preparing antibacterial napkins, hospital bed accessories, surgical aprons and packaging materials.

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CONFLICT OF INTEREST

The authors declare no conflict of interest.

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