




## Research Article

# Azadirachta indica-Derived Silver Nanoparticle Synthesis and Its Antimicrobial Applications

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Advancement of biologically driven experimental procedure for the generation of metal nanoparticles is a major aspect in the domain of nanotechnology. Herein, we synthesized one pot silver nanoparticles (AgNPs) using *Azadirachta indica* (Neem plant) via green synthesis method. The alkaloids and flavonoids in the plant extract served as capping and reducing agent to develop silver nanoparticles. The characterization of synthesized AgNPs was performed using analytical techniques such as UV-visible spectroscopy, FTIR, and SEM. The synthesized AgNPs were further impregnated onto a cotton cloth and the antimicrobial studies were performed. The AgNPs embedded in cotton fabric were found to show zone of inhibition against the fungi *Candida albicans*, gram-negative bacteria *Escherichia coli*, and gram-positive bacteria *Staphylococcus aureus*. This experimental study suggests that the AgNPs impregnated on cotton cloth could have great potential for application in smart nanobandages for wound dressing, medical textiles, sports clothing, etc.

## 1. Introduction

Nanotechnology is the interdisciplinary science which involves chemistry, physics, biology, material sciences, and the wide range of the engineering disciplines. Nanotechnology is the science and engineering of devices and materials on the scale of atoms or small groups of atoms having material size ranged from 0.1 to 100 nm [1]. These nanoscale ranged groups of atom possess various characteristics such as physical strength, magnetism, optical effects, chemical reactivity, and electrical conductance because of its small

size. The change in all the parameters or properties is due to two reasons. Firstly, very large surface area-to-volume ratio, and secondly, quantum mechanics change at nanoscale [2]. Nanotechnology has two approaches, i.e., top-down and bottom-up approach. In top-down approach, the structural size is diminished from larger to smaller, while in bottom-up approach the individual molecules or atoms get changed into nanostructures [3].

Metal nanoparticles such as silver, platinum, gold, and palladium are utilized for many purposes in industry and research. Amongst all these, AgNPs have been extensively

used due to its unique physical and chemical features, which consists of high electrical conductivity and thermal, optical, antimicrobial, and biological properties [4]. Owing to its unique properties, AgNPs are widely used in biomedical applications such as wound dressings [5], antiseptic fabrics [6], topical creams [7], and sprays [8]. AgNPs exhibit biocidal effect against microorganisms through interruption of the membrane followed by disruption of their enzymatic activities [9].

AgNPs are synthesized through three methods, i.e., chemical, physical, and biological. Chemical and physical methods have many disadvantages. Physical methods involve high energy and cost, hence is mostly not preferred method for synthesis [10, 11]. Chemical synthesis involves hazardous chemical which are hazardous to environment [12]. Preferred method for synthesis is by biological method as it is simple, rapid, nontoxic, dependable, and has greener approach [13]. Biological synthesis includes synthesis by microorganisms [14], plant extracts [15], and agricultural wastes [16]. Plant extracts contain biocompounds such as phenolic compounds, alkaloids, terpenoids, sugars, enzymes, and proteins which reduces metallic salts from positive oxidation state to zero oxidation state [17]. When the AgNP synthesis happens in the presence of plant extract, not only the silver salts are reduced but also the plant extracts act as capping agent [18]. The capping of AgNPs avoids the nanoparticle agglomeration, reduces toxicity, and enhances antimicrobial action.

In the current study, we have studied the use *Azadirachta indica* (neem) leaf extract for AgNP synthesis. Neem belongs to the Meliaceae family and is found extensively in India [19]. *Azadirachta indica* leaves are widely available in the subcontinent and also have rapid growth. Neem leaves contain polyphenolic flavonoids named  $\beta$ -sitosterol and quercetin which are known for its antibacterial and antifungal activities [20]. Neem leaves and its components were reported to have anti-inflammatory, immunomodulatory, antiulcer, anti-hyperglycemic, antioxidant, antimutagenic, anticarcinogenic, antimalarial, antifungal, antibacterial, and antiviral properties [21]. The neem leaves were further selected as the reducing agent in the biological synthesis of AgNPs because of its antimicrobial nature, and thus, we tend to enhance its antimicrobial efficacy by developing AgNPs. The neem leaf extract not only has the potential to reduce the silver salts to AgNPs but also stabilizes AgNPs [18].

Hence, in the current study, we examined the antibacterial activity of AgNPs entrapped on greige cotton cloth against gram-positive (*S. aureus*) and gram-negative bacteria (*E. coli*). Additionally, antifungal activity was also checked for *C. albicans*. The silver nanoparticles entrapped cloths were washed for 25 times and each of the successive washed clothes was checked for its antimicrobial activity. Formation of AgNPs was detected using UV-Vis spectroscopy. The size and morphology of AgNPs was confirmed by scanning electron microscopy (SEM) and transmission Electron Microscopy (TEM). Entrapment of AgNPs on cloth and size was confirmed by SEM. Fourier-transform infrared spectroscopy (FTIR) studies were performed for AgNPs entrapped cloth.

## 2. Materials and Methods

**2.1. Materials.** Silver Nitrate ( $\text{AgNO}_3$ ), Potato dextrose agar (PDA), Potato dextrose broth (PDB), Nutrient broth (NB), Agar Agar Type-1, and Itraconazole were procured from HIMEDIA Pvt. Ltd., Mumbai, India. Ampicillin and Itraconazole were used as positive control for antibacterial and antifungal studies, respectively. During the experiment, double distilled water was used. The leaves of *Azadirachta indica* were collected from Bhatan village, Mumbai, India. Culture of *Staphylococcus aureus* of strain 6538P and *Escherichia coli* was used for antibacterial test. *Candida albicans* was used for studying antifungal activity of the cloth. Fabric cotton cloth was collected from a local shop in Mumbai, India.

**2.2. Extraction of Plant Extract.** *Azadirachta indica* leaves harvested locally were washed with tap water and rinsed with double distilled water several times with a view to clear out any dust or unwanted particles that might restrict adhesion of  $\text{Ag}^+$  ions during the synthesis process. The washed leaves were dried, accurately weighed, and finely cut for the preparation of leaf extract. The mixture was then boiled for 30 minutes in double distilled water to release intracellular material into the solution. Following it, the mixture was cooled and filtered through Whatman filter paper. The filtrate obtained was thus stored at  $4^\circ\text{C}$  and later used for biosynthesis of silver nanoparticles.

**2.3. Synthesis of Silver Nanoparticles Using Neem Plant Extract.** The filtered suspension obtained was further treated with  $\text{AgNO}_3$  solution for AgNP synthesis. The entire process was carried out on a magnetic stirrer at fixed RPM. The bio-reduction of silver ions in the solution was observed at regular time interval by scanning the UV-Vis spectra at a range from 200 to 900 nm. The synthesized AgNPs obtained were further subjected to centrifugation and washing for removal of unreacted  $\text{Ag}^+$  ions. Synthesized AgNPs were kept at  $4^\circ\text{C}$  for further analysis.

**2.4. Impregnation of Silver Nanoparticles on Cotton Cloth.** Cloth material was washed at room temperature (RT) with double distilled water, dried, and cut to the dimension of  $4\text{ cm} \times 4\text{ cm}$ . The cut clothes were immersed in plant extract for 2 h at RT and then dried. These plant extract entrapped clothes were immersed in the  $\text{AgNO}_3$  solution for 1 hour at RT and were removed and dried. The clothes were further analyzed by means such as SEM, FTIR, and antibacterial examinations.

### 2.5. Characterization of Silver Nanoparticles

**2.5.1. UV-Vis Spectrophotometer.** The progress of AgNP synthesis was observed using UV-Visible spectrophotometer (SHIMADZU UV-1800). The absorption spectrum was recorded in the range of 200-900 nm.

**2.5.2. Scanning Electron Microscopy.** The surface morphology of AgNPs was characterized by SEM which was performed using VEGA3TESCAN. Different samples including

plain cotton cloth, cotton cloth with Neem extract, and cotton cloth impregnated with AgNPs before and after washing were irradiated with an electron beam at 10 kV. Before analysis, samples were gold coated through sputter deposition due to the fact that gold has higher conductivity and does not interfere with investigation of AgNPs.

**2.5.3. FTIR.** FTIR was used to recognize the biomolecules of the Neem leaf extract which plays crucial role in capping, reducing, and stabilizing the AgNPs. FTIR spectra were obtained for the plain cloth, cloth impregnated AgNPs, cloth impregnated AgNPs washed with water, cloth impregnated AgNPs washed with mild detergent, and cloth impregnated AgNPs washed with strong detergent by using BRUKER spectrophotometer in the spectral range of 4000-400  $\text{cm}^{-1}$  having a resolution of 4  $\text{cm}^{-1}$ . The fundamental component of the universal attenuated total reflectance sample holder was a ZnSe/diamond composite. The FTIR analysis was studied to validate the presence of AgNPs before and after washing steps.

**2.6. Washing of Impregnated Silver Nanoparticle Clothes.** To confirm the leaching of the AgNPs from the impregnated cotton cloth, they were further subjected to 25 cycles of washing process with distilled water and detergents (strong and mild detergent). Upon the first cycle of washing, clothes were dried, ironed, and then again subjected to second cycle of wash and this was repeated till 25 cycles. Later antibacterial and antifungal activities were carried on AgNPs impregnated cloth before and after washing steps.

### 2.7. Antimicrobial Assay

**2.7.1. Antibacterial Activity of Entrapped Silver Nanoparticle on Cloth.** Antibacterial activity was performed against two bacterial stains, i.e., gram-positive *Staphylococcus aureus* and gram-negative *Escherichia coli* bacteria. Loop full of both bacterial cultures was taken from stock culture and inoculated for 24 h in nutrient broth at 37°C. Spread plate technique was used to streak the sterile nutrient agar plates for both the fresh cultures. The impregnated AgNPs on cotton cloth of dimension 4 cm × 4 cm were subjected to 25 cycles of distilled water wash and each of these cloth pieces of 1 cm × 1 cm dimension was placed on the streaked nutrient agar plates to check its antibacterial activity. Same procedure was followed for mild detergent washed clothes and strong detergent washed clothes. Later, the petri plates were incubated at 37°C for 24 h followed by observation of zone of inhibition. Positive control used for both the cultures was ampicillin which was entrapped on cotton cloth of dimension 1 cm × 1 cm.

**2.7.2. Antifungal Activity of Entrapped Silver Nanoparticle on Cloth.** Antifungal activity was performed against *Candida albicans*. Loop full of fungal culture was taken from stock culture and inoculated for 48 h in potato dextrose broth at 37°C. Swabbing was performed on sterile potato dextrose agar plates with the fresh culture. The impregnated AgNPs on cotton cloth of dimension 4 cm × 4 cm were subjected to 25 cycles of distilled water wash and each of these cloth

pieces of 1 cm × 1 cm dimension was placed on the streaked nutrient agar plates to check its antifungal activity. Same procedure was followed for mild detergent washed clothes and strong detergent washed clothes. Later, the plates were incubated at 37°C for 24 to 48 h. The zone of inhibition was also observed. Itraconazole discs were used as positive control for antifungal studies.

## 3. Results and Discussion

### 3.1. To Analyze the Formation, Size, and Shape of AgNPs

**3.1.1. UV-Vis Spectroscopy.** For different aqueous  $\text{AgNO}_3$  concentrations, comparative studies were carried out to examine the effect of different amounts of leaf biomass on bioreduction rate of AgNPs. The quantity of leaf extract showed a significant role in size dispersion of AgNPs. The plant extract showed peak at 280 nm but no peak was observed between 400 nm and 500 nm. The reduction of the silver ions into AgNPs in the presence of plant extract can be observed through change in color. The leaf extract solution color changed from yellowish green to brown and became darkish brown gradually with time on addition of  $\text{Ag}^+$  ions due to the surface plasmon resonance (SPR).  $\text{Ag}^+$  ions reduction occurred rapidly in the presence of neem leaf extract and AgNP synthesis was completed in 1 h. The change in the absorbance was noted down every 10 minutes interval but there was no change in absorbance after 1 h indicating no further formation of nanoparticles as observed in Figure 1. The maximum absorption peak at 420 nm was observed in the UV-Vis spectrophotometer analysis indicating the formation of AgNPs. This broad SPR peak has been well studied for AgNPs with the size ranged from 10 to 100 nm [18].

### 3.2. To Confirm Impregnation of AgNPs on the Cloth

**3.2.1. FTIR Analysis.** The FTIR spectra were measured for the plain cloth and impregnated cloth with AgNPs (Figure 2). The spectrum for plain cloth showed bands at 3302.23  $\text{cm}^{-1}$ , 3257.11  $\text{cm}^{-1}$ , and 3336.32  $\text{cm}^{-1}$  which corresponds to carboxylic O-H stretching, alcoholic O-H stretching, and N-H stretching for secondary amine, respectively. The band located at about 1103.43  $\text{cm}^{-1}$  and 1023.74  $\text{cm}^{-1}$  can be allocated to C-O-C or C-O functional groups. The spectrum for impregnated cloth with AgNPs showed broad intense band at 3335.10  $\text{cm}^{-1}$  which can be attributed to the N-H stretching frequency which arises from the peptide linkages available in the proteins of the neem leaf extract and 1635.65  $\text{cm}^{-1}$  band for amide I bonds for proteins (carbonyl C=O stretching). The amide I bond in the spectrum might have originated from the proteins available in the leaf extract which act as capping ligands of the synthesized nanoparticles. These proteins contained in leaf extract could be flavanones or terpenoids which help in reducing and stabilizing the synthesized AgNPs, whereas the band around 1600  $\text{cm}^{-1}$  was not visible in the spectrum of plain cloth.

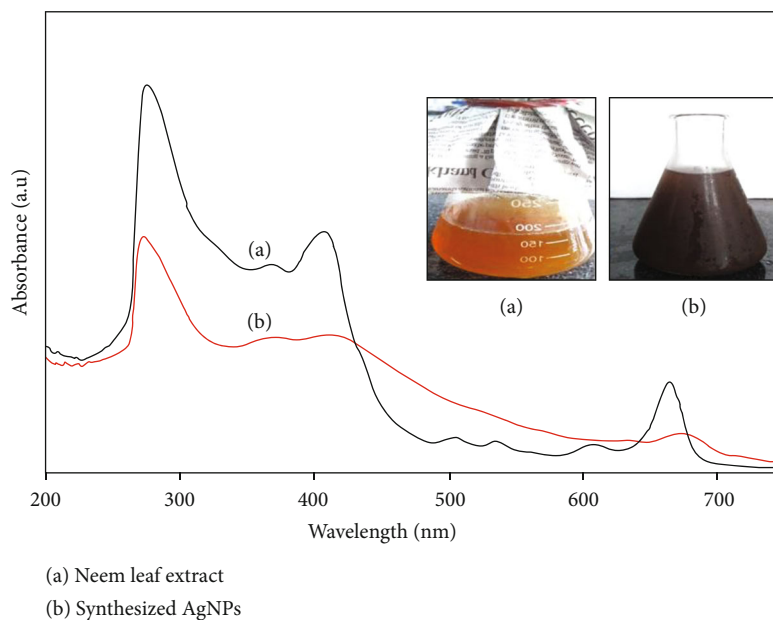


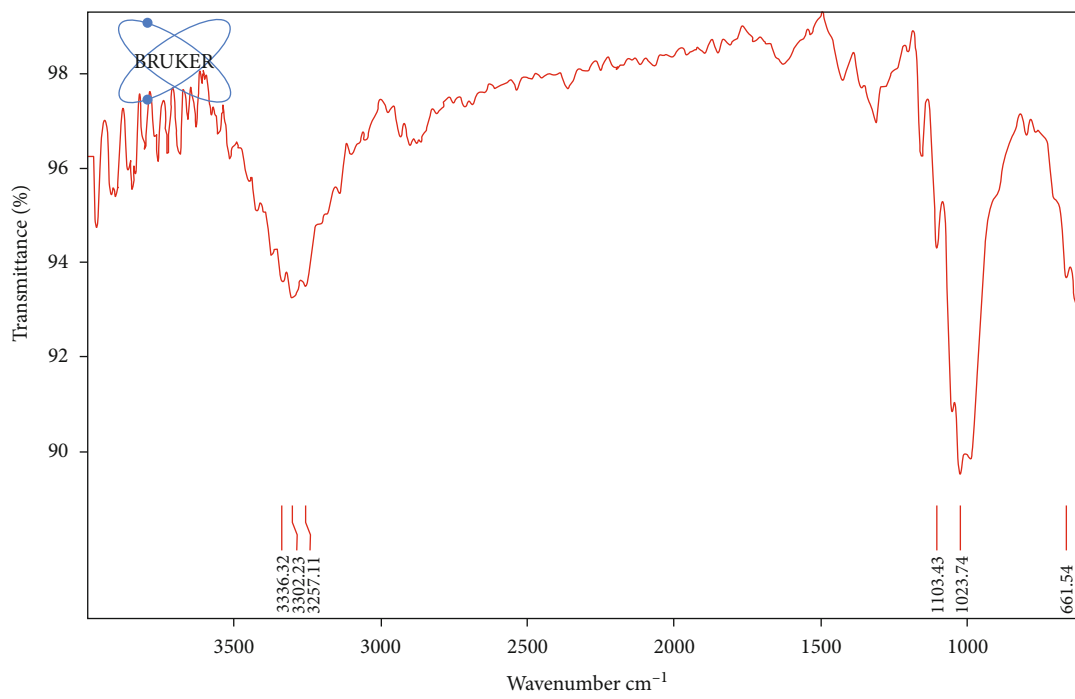
FIGURE 1: UV-Vis spectra of (a) neem leaf extract and (b) synthesized AgNPs.

**3.2.2. SEM Analysis.** The surface morphology of plain cotton cloth, cotton cloth impregnated with AgNPs, cotton cloth impregnated with AgNPs (after 25 cycles of distilled water wash), cotton cloth impregnated with AgNPs (after 25 cycles of mild detergent wash), and cotton cloth impregnated with AgNPs (after 25 cycles of strong detergent wash) was analyzed using SEM. The micrograph obtained in Figure 3 clearly confirmed the presence of AgNPs from Figures 3(b)–3(e), whereas no AgNPs were observed in Figure 3(a). Thus, we clearly demonstrate the impregnation of AgNPs on cotton cloth and adherence of AgNPs were still observed even after 25<sup>th</sup> washing with water or detergent. The antibacterial and antifungal results obtained clearly justify the results.

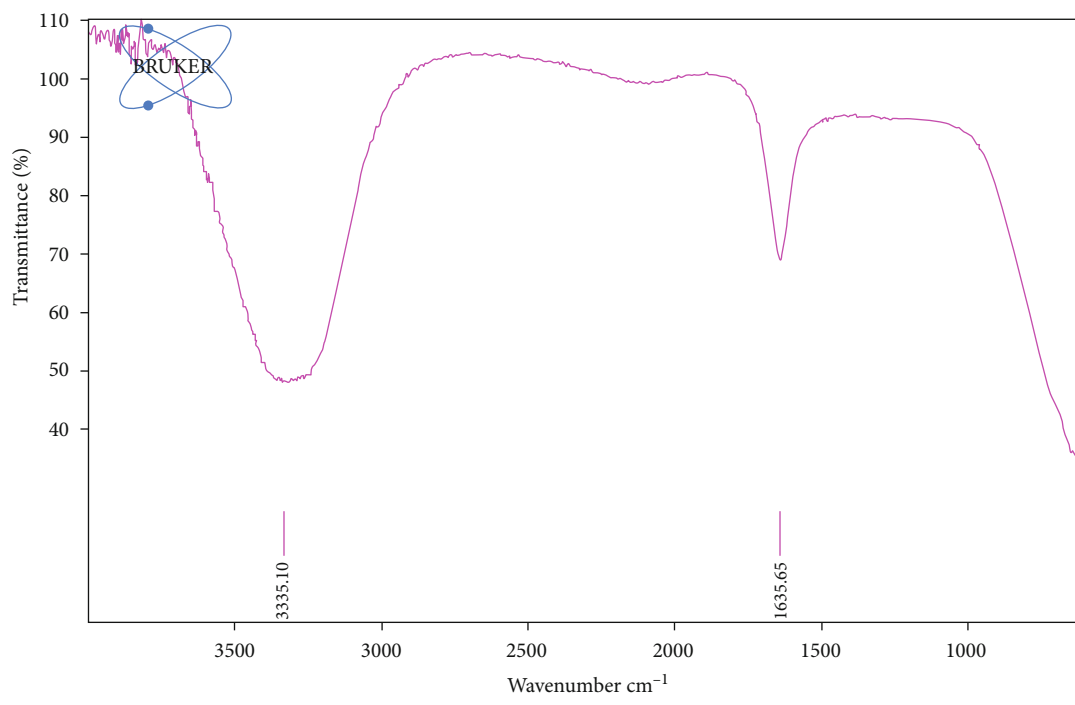
**3.3. Antibacterial Activity.** The two strains of bacteria *E. coli* (gram-negative) and *S. aureus* (gram-positive) are most commonly associated with infected wounds which were selected for antibacterial studies. Both the strains were exposed to clothes impregnated with AgNPs before and after 25 cycles of washes (mild and strong detergent) to confirm its antibacterial property. The effect of synthesized AgNPs on both gram-positive and gram-negative bacteria could indicate possible mode of action. AgNPs are known to display extensive range of antibacterial effects via different biochemical pathways. The AgNPs entrapped 1 cm × 1 cm cloth were immediately tested for respective antimicrobial activities against gram-negative (*E. coli*) (Figures 4(a) and 4(b)) and gram-positive (*S. aureus*) (Figures 5(a) and 5(b)) bacterial strains showing the respective zone of inhibition. Based on the zone of inhibition produced, it can be confirmed that the AgNPs demonstrated excellent antibacterial activity till 25 washes against both *S. aureus* and *E. coli*. The high surface to

volume ratio and small size of nanoparticles produce a significant bactericidal effect which permits the AgNPs to penetrate the cell wall of the bacteria and trigger the cell death [22, 23]. AgNPs when interact with the bacterial cell membrane having proteins along with sulfur compounds, the silver ion further attacks metabolic chain of bacteria and DNA molecules further causing the cells get damaged and died [24]. Thus, the results show that biologically synthesized AgNPs when embedded on cloth have antibacterial activity against gram-positive and gram-negative bacteria. Due to antimicrobial activity of AgNPs, coated cloth can be used as a potential fabric material for front-line health workers, sportspersons, military people, etc.

**3.4. Antifungal Activity.** The AgNPs entrapped 1 cm × 1 cm cloth were similarly tested for antifungal activity against *C. albicans* showing the respective zone of inhibition. The AgNPs coated cloth showed excellent antifungal activity even after washing the cloth with strong and mild detergent. Based on inhibition zone produced, it can be assumed that the synthesized AgNPs exhibited antifungal activity till 25 washes against *C. albicans* (Figures 6(a) and 6(b)) and (Figures 7(a) and 7(b)). AgNPs might have got attached and penetrate the cell membrane of the fungi by creating pores or “pits” in the membrane causing the leakage of the intracellular components out and it is also reported that AgNPs get attached to the respiratory sequence causing inhibition cell division and ultimately cell death [25, 26]. Therefore, it is expected that the neem leaf extract synthesized AgNPs coated fabric can be used in medical applications such as uniform for health workers, undergarments, bedding linen, and towels since it has antifungal activity against harmful fungal pathogens.



(a)



(b)

FIGURE 2: FTIR spectra for (a) plain cloth and (b) cloth impregnated with AgNPs.

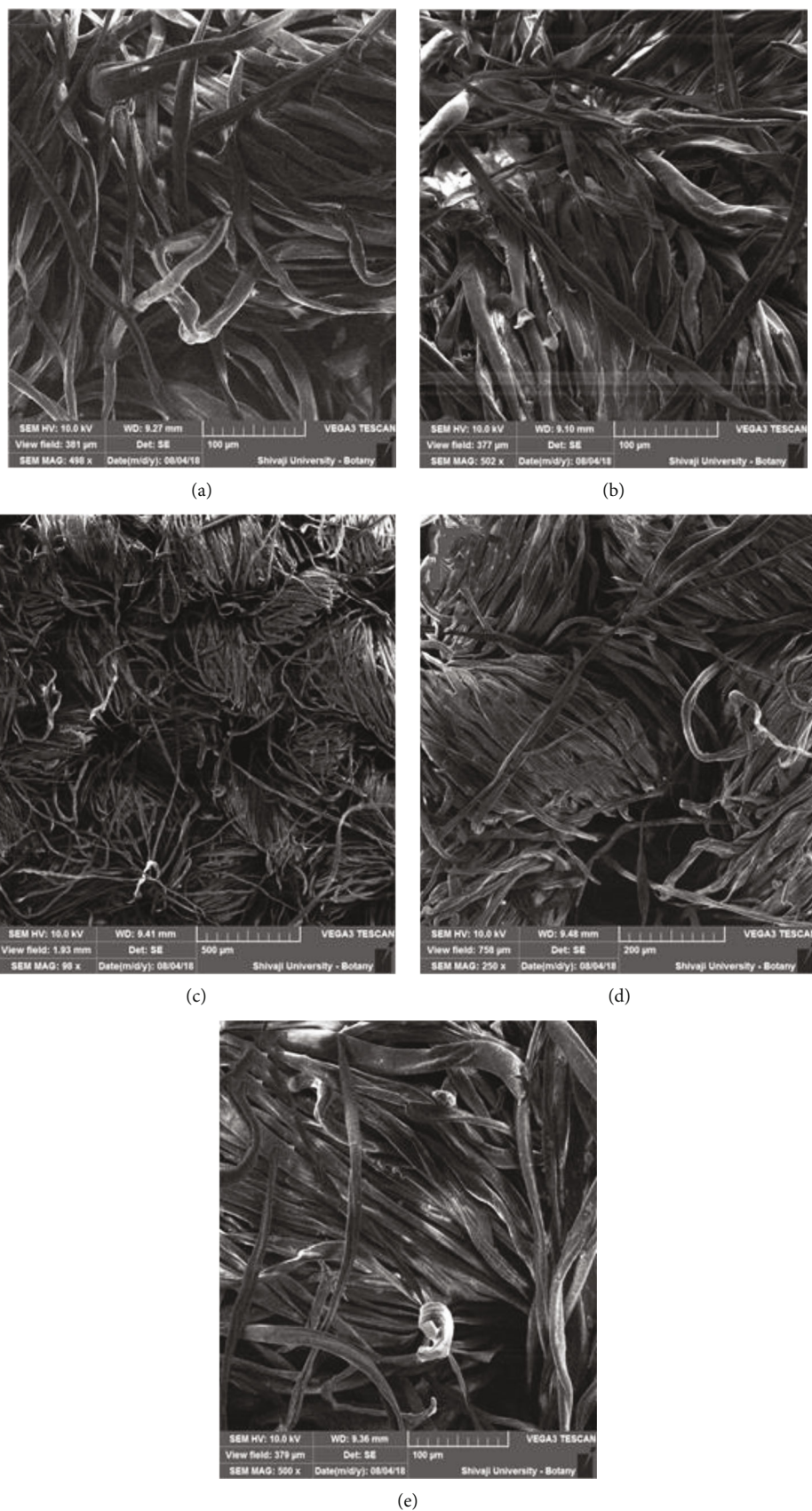
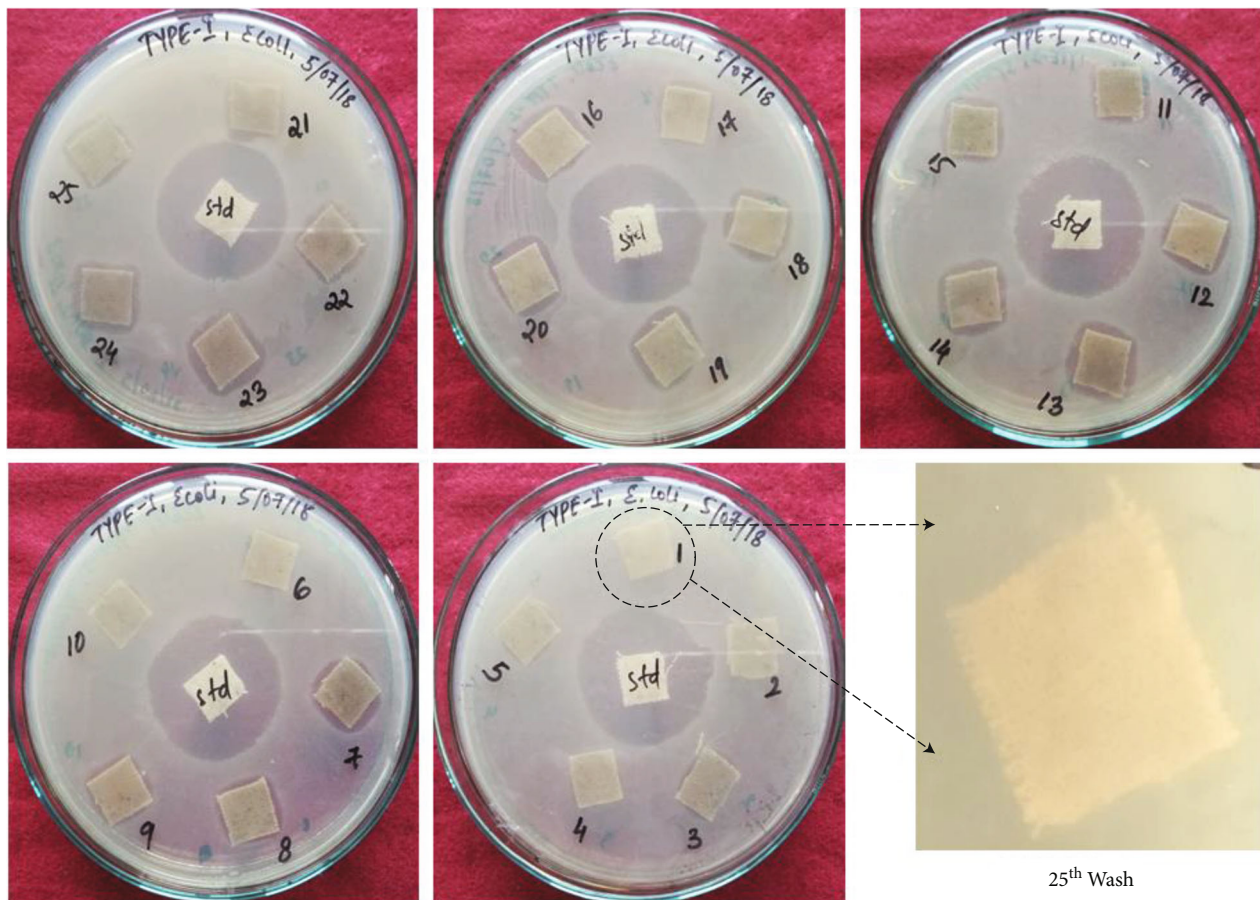


FIGURE 3: SEM image of (a) cotton cloth with plant extract only (top left). (b-e) AgNPs embedded onto the fibers of the cotton cloth.



(a)

FIGURE 4: Continued.

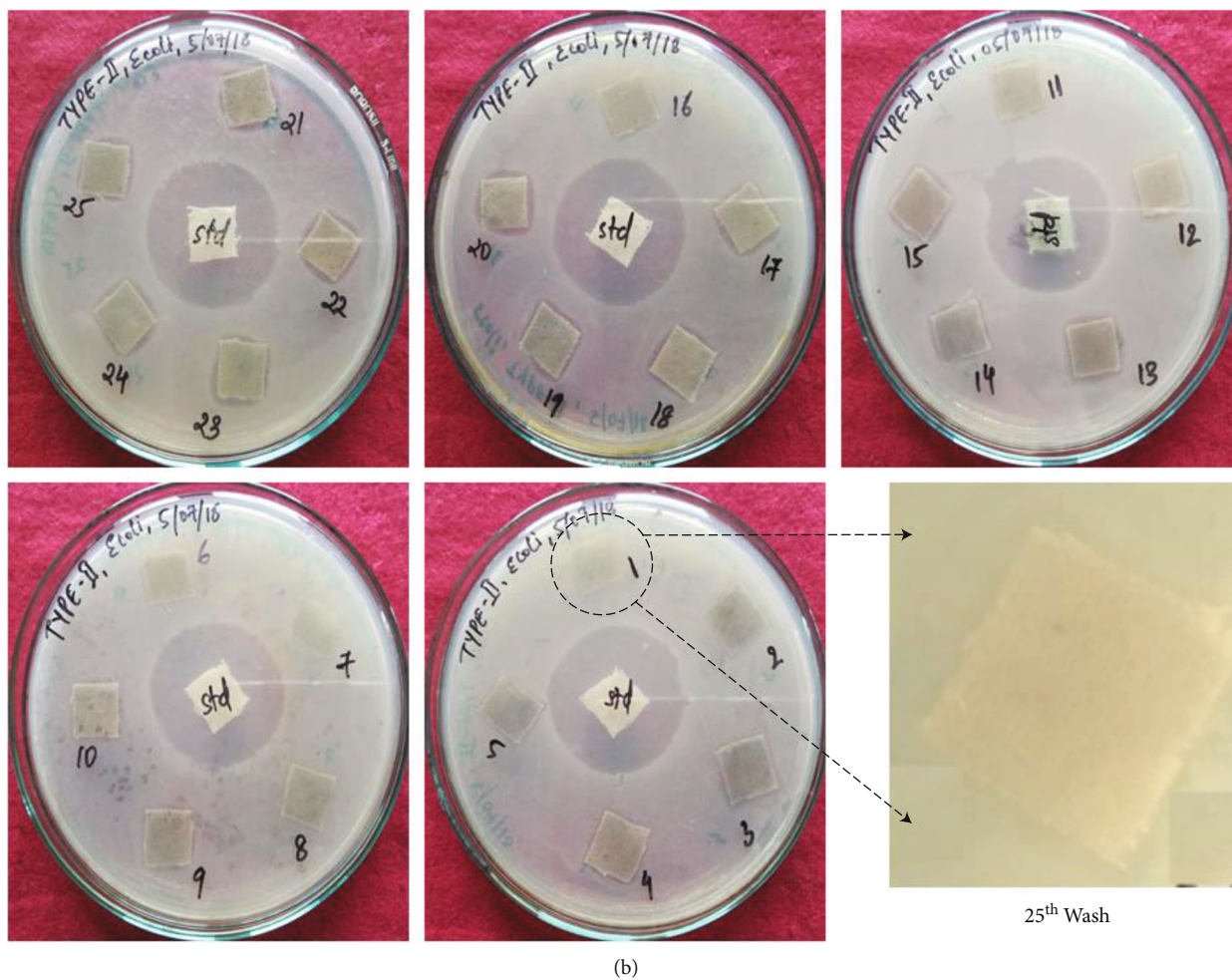
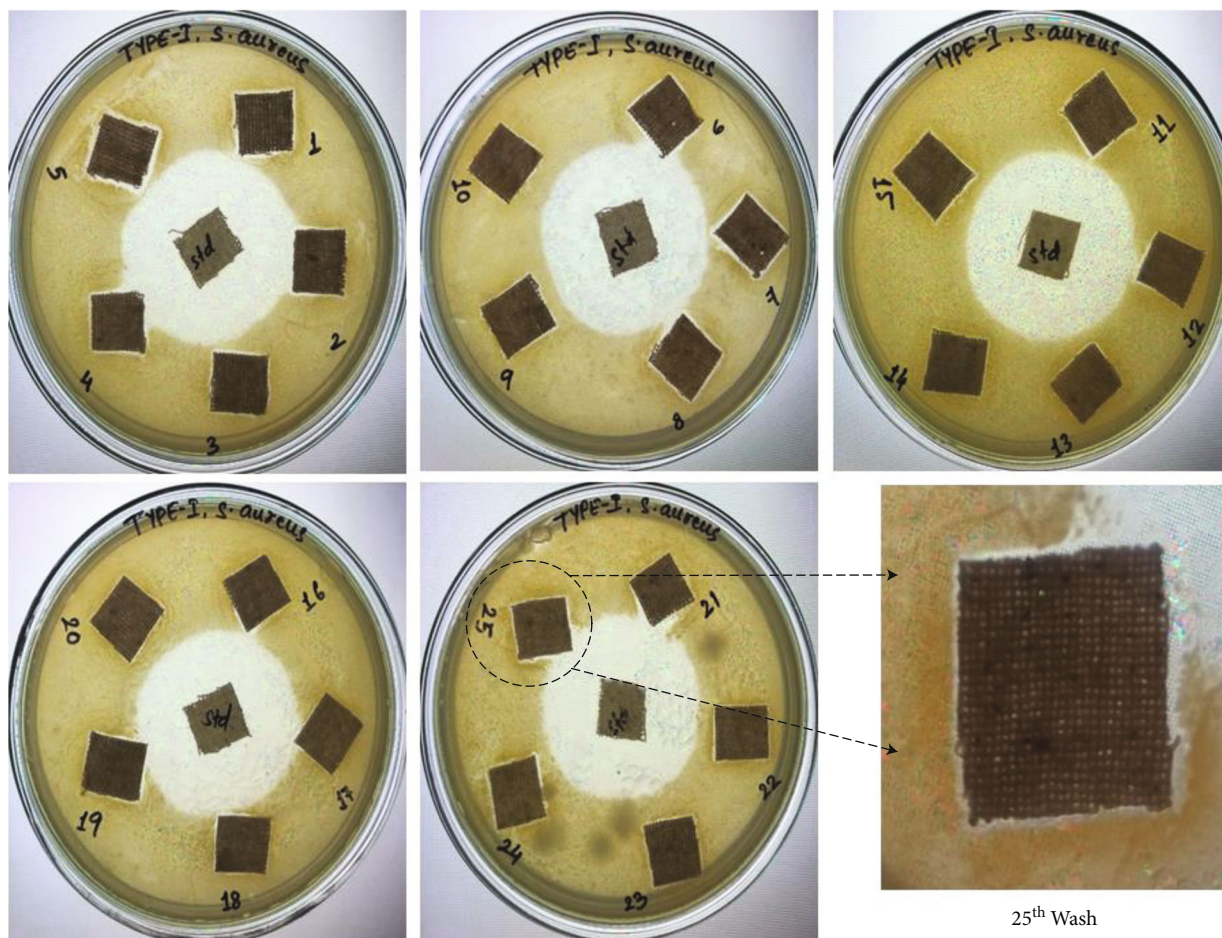


FIGURE 4: Antibacterial activity of AgNPs against *E. coli* species after 25 washes (a) mild detergent and (b) strong detergent.





(a)

FIGURE 5: Continued.

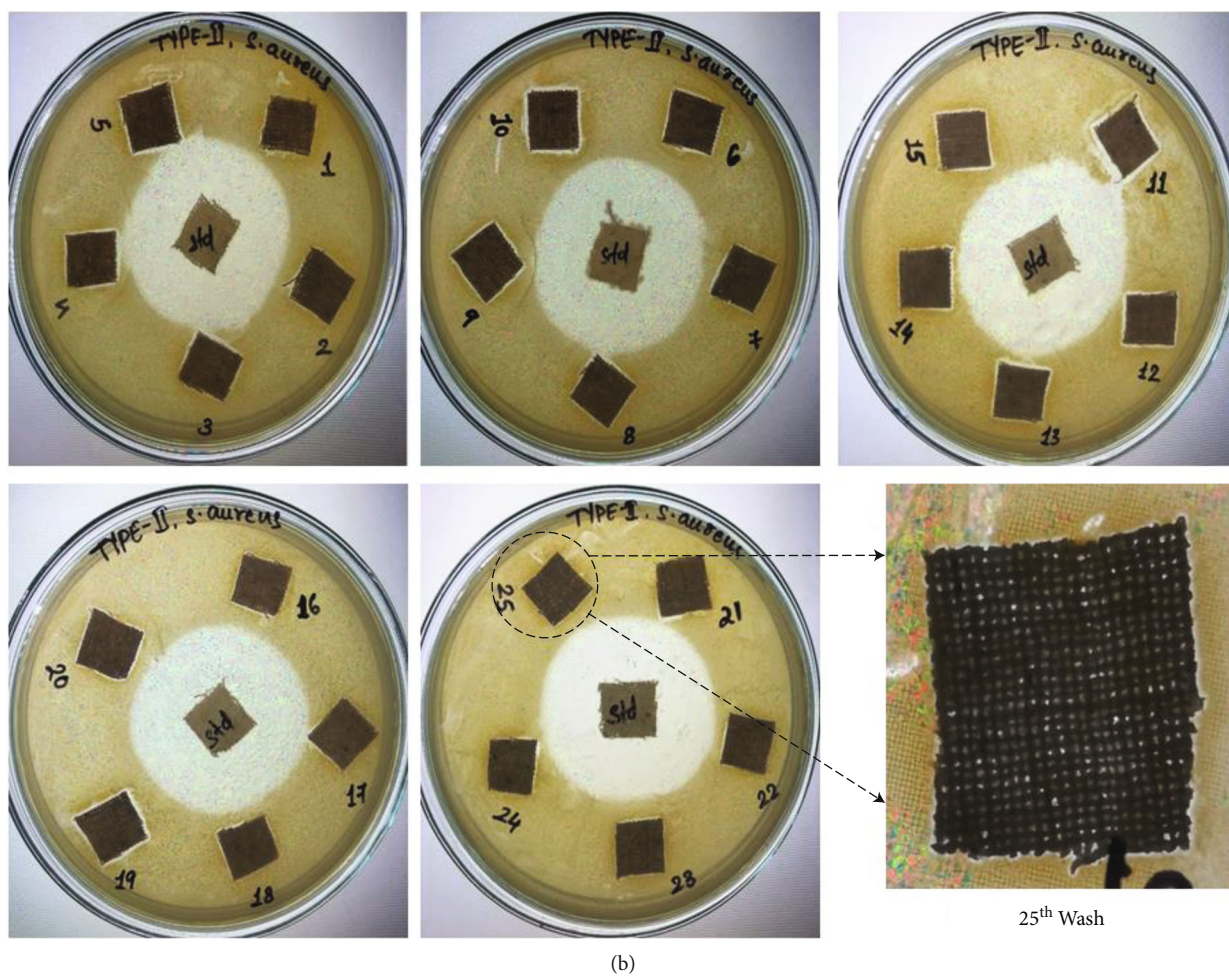
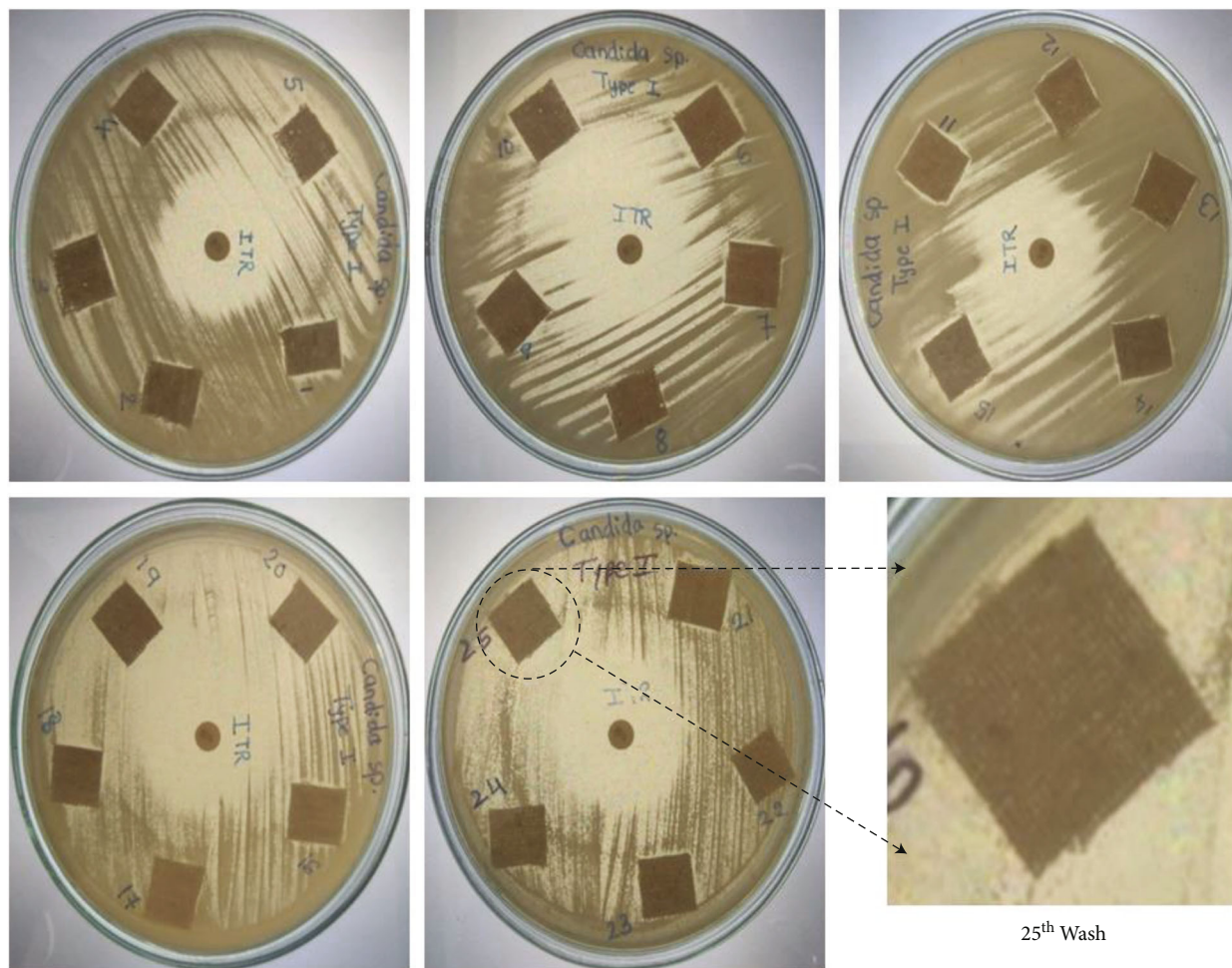


FIGURE 5: Antibacterial activity of AgNPs against *S. aureus* species after 25 washes (a) mild detergent and (b) strong detergent.



(a)

FIGURE 6: Continued.

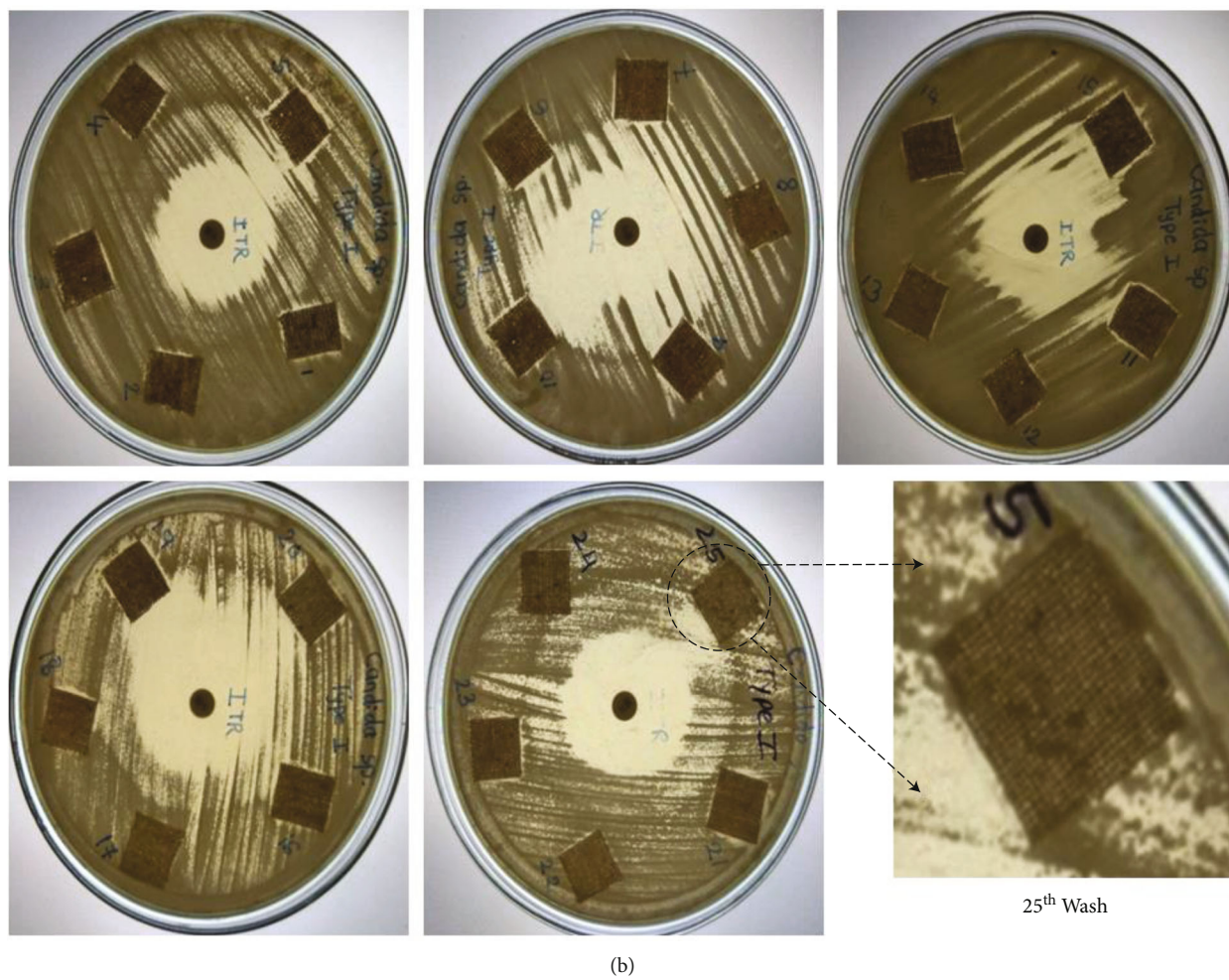
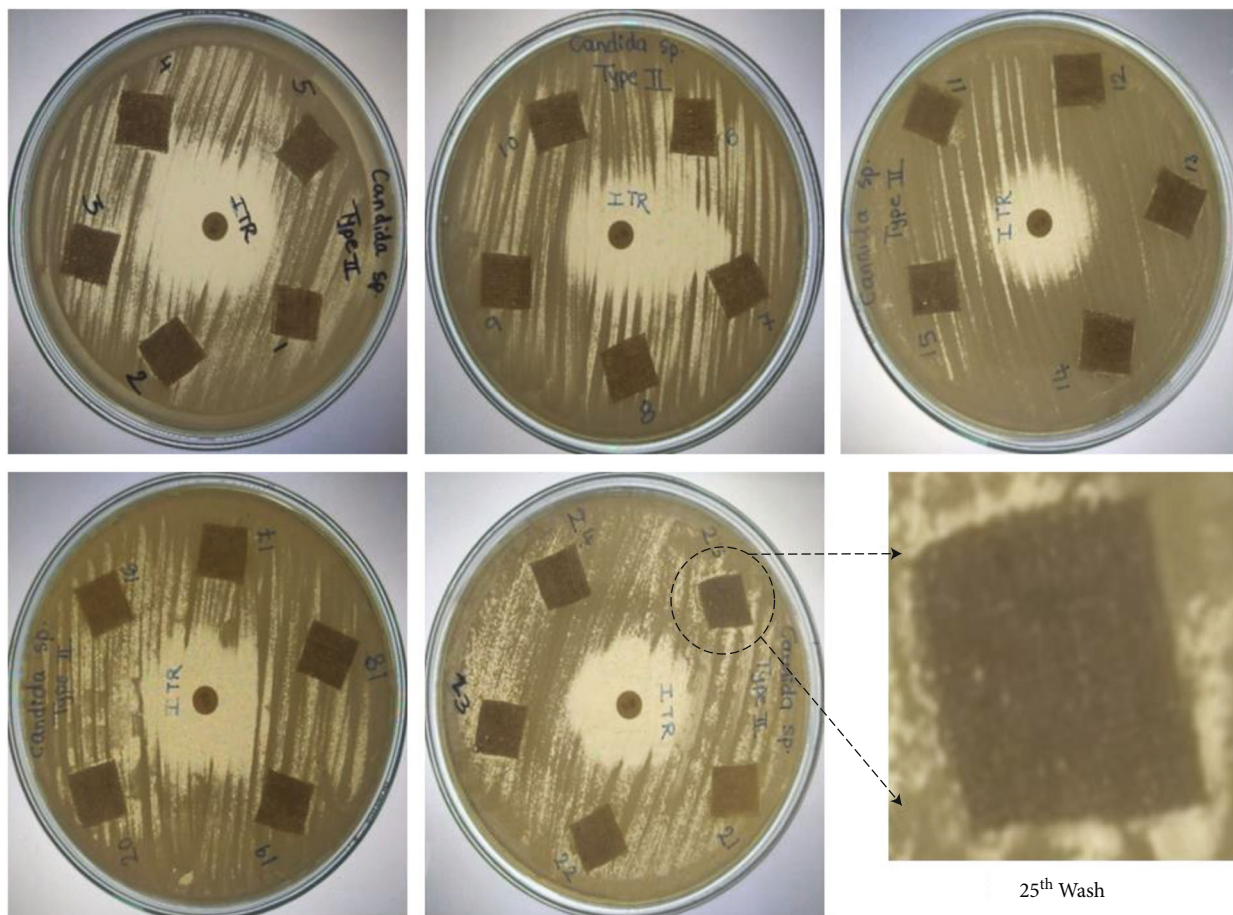


FIGURE 6: Antifungal activity of AgNPs against *C. albicans* after 25 washes of mild detergent (a) 48 h and (b) 72 h.



(a)

FIGURE 7: Continued.

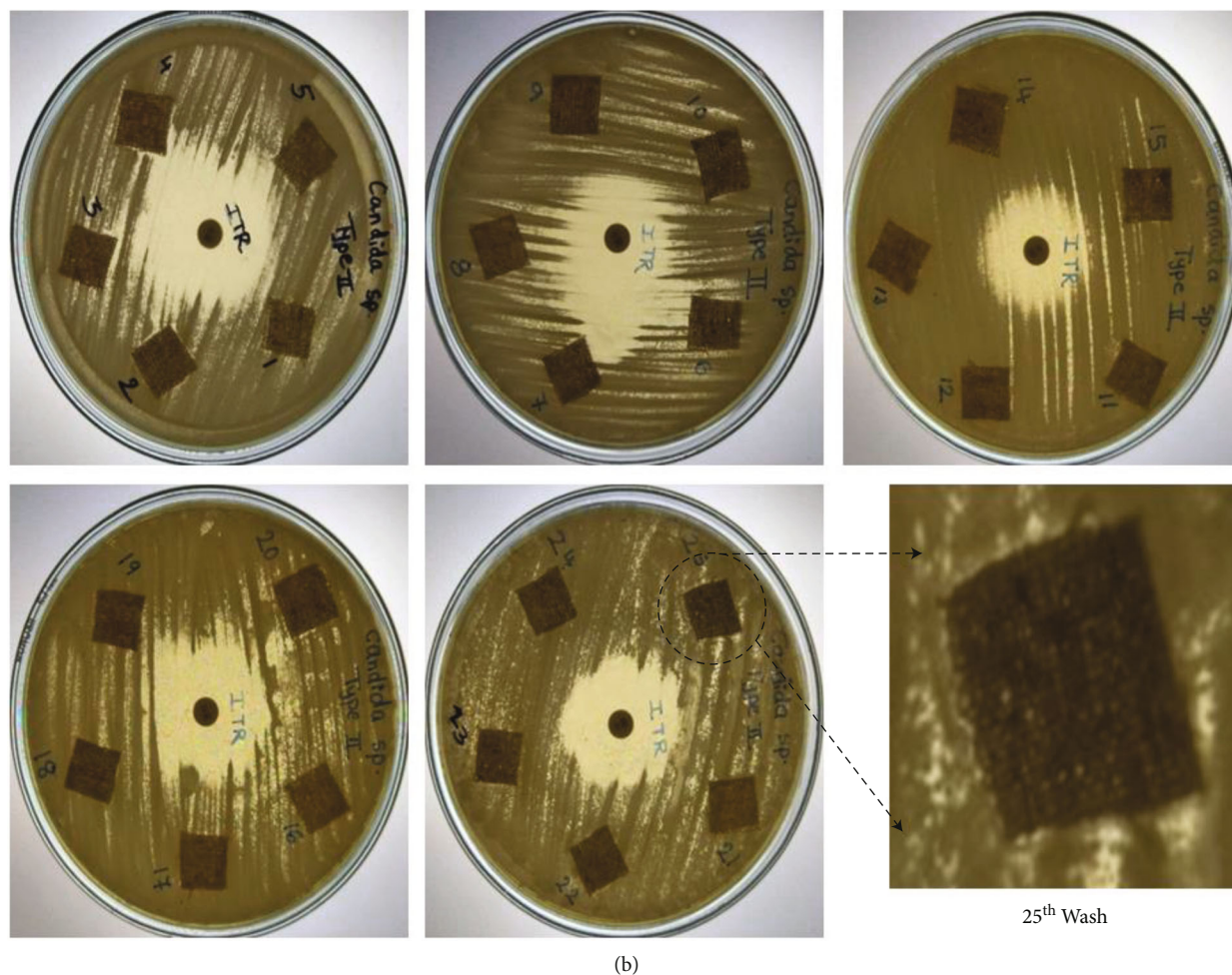


FIGURE 7: Antifungal activity of AgNPs against *C. albicans* after 25 washes of strong detergent (a) 48 h and (b) 72 h.

#### 4. Conclusion

The biological synthesis of AgNPs from *Azadirachta indica* leaf extract was successful. AgNPs produced a characteristic peak at in the range of 400–500 nm due to its SPR seen in UV-Vis spectrophotometer. The TEM and SEM analyses showed that the AgNPs produced were spherical and in the range of 20 nm–100 nm with a capping material around it. The FTIR spectra showed a significant peak of amide I bonds which confirms the presence of capping ligand which are the proteins available in neem leaf extract around the synthesized nanoparticles. AgNPs showed antibacterial activity against both gram-positive (*S. aureus*) bacteria and gram-negative (*E. coli*) and also antifungal activity against *C. albicans*. Thus, we can conclude that the cotton cloth with impregnated AgNPs has numerous medical applications where infections can easily occur like smart-bandages, bed sheets in hospitals, lab coats, and sanitary napkins.

#### Data Availability

All relevant data are included within the article.

#### Conflicts of Interest

All authors declare that there is no conflict of interest.

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