INNSPUB

International Journal of Biosciences | IJB |
ISSN: 2220-6655 (Print), 2222-5234 (Online)
http://www.innspub.net
Vol. 13, No. 5, p. 1-15, 2018

RESEARCH PAPER OPEN ACCESS

Green synthesis of silver nanoparticles by using bacterial extract and its antimicrobial activity against pathogens

Ihsan Ali[1], Tian Yong Qiang[1*], Nikhat Ilahi[2], Mian Adnan[3], Wasim Sajjad[2,4]

[1]*School of Chemical and Biological Engineering, Lanzhou Jiaotong University, PR China*

[2]*Department of Microbiology, Faculty of Biological Sciences, Quaid-I-Azam University, Islamabad 45320, Pakistan*

[3]*MOE Key Laboratory of Cell Activities and Stress Adaptations, School of Life Sciences, Lanzhou University, Lanzhou, 730000, PR China*

[4]*Key Laboratory of Petroleum Resources, Gansu Province / Key Laboratory of Petroleum Resources Research, Institute of Geology and Geophysics, Chinese Academy of Sciences, Lanzhou 730000, PR China*

Key words: Silver nanoparticles, Antimicrobial activity, Bacterial extract, Pathogens.

http://dx.doi.org/10.12692/ijb/13.5.1-15 Article published on November 01, 2018

Abstract

Nanotechnology is the amendment, alteration, and development of significant properties of metals in the form of nanoparticles having applications in numerous fields. This study was conducted to synthesize functionalized silver nanoparticles (AgNPs) using bacterial T10 strain isolated from unusual environment. Stable and well dispersed AgNPs were obtained extracellularly by using bacterial extract within 1 hour of incubation. Reduction of silver ion was checked by using UV–visible spectrophotometry, and characterization of the AgNPs were done by Scanning Electron Microscopy (SEM), Fourier Transform Infrared Spectroscopy (FTIR)and X-Ray Diffraction(XRD). These nanoparticles were mono-dispersed, spherical and about 46–52.7 nm in size. XRD peaks were corresponding to diffraction facets of silver planes. Capping of AgNPs with functional groups were confirmed through FTIR. The obtained AgNPs showed antimicrobial against clinical pathogens and synergistic effect with antibiotics. Antibacterial activity of obtained AgNPs was tested against clinical pathogens by coating AgNPs on bandage. Our results clearly propose the bacterial extracts is an excellent source for green synthesis of AgNPs and could be used against pathogens.

* **Corresponding Author:** Tian Yong Qiang ✉ tian2918@163.com

Introduction

Silver (Ag) is highly electrical and thermal conductor having soft and shiny shape. It is used in several forms as vessels, coins, foils, sutures, solutions, and colloids as lotions, ointments. Literature explained as earlier as 300 BC that Ag was used as therapeutic agent. The healing abilities of numerous metals are cited in old Aurvedic medicine book of medicinal literature "Charak Samhita" (Galib *et al.,* 2011). Ag was commonly used as antimicrobial agent until the discovery of antibiotics. Ag was famous active agent against pathogenic microorganisms due to its medical significance even the people did not know that microbes are causative agent of infection and food spoilage. It is the prominent tonic agent in medicine for infectious diseases. Ag is consider relatively having low threats factors than that of its risking (Mody *et al.,* 2010).

Nano-dimension materials (1-100 nm) have unique characteristics compared to the similar bulk form materials. Such characteristics are according to structural and physical properties of atoms, and elemental bulk materials due to differences in surface to volume ratio and physiochemical properties (Mody *et al.,* 2010). With improvement in nanotechnology, a huge sum of nanomaterials is seeming with exceptional characteristics, opening spectrum of applications and research prospects (Sharma *et al.,* 2009). Nanoscience is emerging interdisciplinary subject that based on the keyproperties of nano-size stuffs(Mohanpuria *et al.,* 2008).Nanoparticles (NPs) retain astonishing optical, magnetic, electronic and catalytic properties than the bulk material because of high surface area to volume ratio (Poulose *et al.,* 2014).Silver nanoparticles (AgNPs) catch more attention because of their chemical, physical, and biological properties that familiar to the catalytic nature and antibacterial activity and found applications in nanoscience (Fayaz *et al.,* 2010). They are used as antimicrobial agents in wound coverings (Nambiar and Bhathena, 2010; Singh and Singh, 2014) as topical creams (Tian *et al.,* 2007) and as anticancer agents. The appreciation of antimicrobial, imaging, catalytic and electrochemical applications of metallic nanoparticles is well documented (Schrofel *et al.,* 2014). Various common methods are experienced to formed metallic nanoparticles like physical and chemical. The above-mentioned methods likely give well-defined and pure results, but the use of chemicals is toxic, energy demanding, costly, and not fit for biological practices. The synthesis of NPs is trendy in the past three decades. Different methods include chemical reduction, ultrasonic-assisted reduction, microwave-assisted, photo catalytic reduction, irradiation reduction, and biochemical reduction, Bacterial-Induced.

There are several green routes to synthesis NPs of zero valent metals and salts emphasizing recent developments (Kharissova *et al.,* 2013). NPs synthesis by using bacterial extracts is economic, easily handled, and environmentally caring. *Bacillus cereus* and *Bacillus flexus* are responsible for the production of Anisotropic nanoparticles at room temperature an incubation period of 3–5 days having spherical and triangular shape (Sunkar and Nachiyar, 2012; Priyadarshini *et al.,* 2013). The stability and synthesis of AgNPs depend on cell-free culture supernatants of extremophiles especially psychrophilic (*Pseudomonas antarctica, Pseudomonas proteolytica, Pseudomonas meridiana, Arthrobacter kerguelensis,* and *Arthrobacter gangotriensis*) and mesophilic bacteria (*Bacillus indicus and Bacillus cecembensis*) (Shivaji *et al.,* 2011). The spore crystal mixture of *Bacillus thuringiensis* is used to synthesize AgNPs of mixed morphology (cubic and hexagonal) of size 15 nm (Jain *et al.,* 2010).The interaction of *Plectonema boryanum* UTEX 485 with aqueous $AgNO_3$ for 28 days spawned precipitation of spherical silver nanoparticles (Lengke *et al.,* 2007; Shahverdi *et al.,* 2007). The silver ions get reduced rapidly within 5 minutes with the addition of the cell filtrate of *Enterobacteriaceae (Escherichia coli, Klebsiella pneumonia,* and *Enterobacter cloacae*) to silver nitrate solution (Shahverdi *et al.,* 2007). The size and shape of the silver nanoparticles synthesized using microbes depend on the interaction of silver ions with bacteria (Morones *et al.,* 2005; Panáček *et al.,* 2006). *Pseudomonas stutzeri* AG259, reported in silver

mine, synthesized AgNPs of well-defined size and distinct morphology inside bacterial periplasmic space (Klaus *et al.,* 1999).The enzymes present in the microorganisms are responsible for the reduction of silver ions forming silver nanoparticles (Mukherjee *et al.,* 2001). The reduction of the silver ions occurs possibly by a nitrate-dependent reductase enzyme and showed efficient antibacterial activity against *Staphylococcus aureus, Salmonella typhi, Staphylococcus epidermidis,* and *Escherichia coli* (Klaus *et al.,* 1999).

Currently, microbial resistance to antibiotics is the main issue in medical sciences. The use of other therapeutic agents against such microbes is demand of the time. One of the possible solution for this problem is the use of AgNPs. The current study was aimed to use green approach of AgNPs synthesis by using bacterial extracts. For this purpose, bacteria from unusual environment was used for reduction of silver nitrate salts. The obtained AgNPs were characterized and its antimicrobial activity was evaluated against clinical isolates.

Materials and methods

All the chemicals and reagents used in the present study were of analytical grade and obtained from Merck and Sigma-Aldrich Chemical Co.

Sampling site

Soil samples were collected from forests of Zhaobi Shan Xining, Qinghai, PR China (37°569.72°N' 101°41'28.98°E) (Fig. 1).

Fig. 1. Sampling site, Zhaobi Shan Xining, Qinghai (Source: Google earth).

The site is thick forest and very important due to enriched flora, which is believed to be ecologically important. This site was selected due to its unusual appearance and absence of anthropogenic activities. Appropriate spots were selected for sampling and standard microbiological protocols were adopted during sampling. Sterilized polythene zipper bags were used for sample collection. The color of the soil was completely dark brown up to one feet underground and surface, subsurface and bottom layers were sampled.

Physical parameters such as temperature and pH were reported. The samples were placed in ice box and brought back to laboratory and stored at -4°C till further processing.

Isolation of bacteria

Different soil samples were mixed, and 5 g soil sample was mixed in 100 mL distilled water for proper extraction of bacterial cells embedded in the soil aggregates. After serial dilution, the samples were inoculated on nutrient agar plates and incubated at 37°C for 24 hours. Colonies were observed and purified based on morphology and further purified on separate plates. Pure colonies of isolates were preserved at -80°C by using 15% glycerol in preserved tubes along bacteria. In current study, 10 bacterial strains were isolated from the forests of Zhaobi Shan Xining.

Screening for AgNPs synthesis

All the isolates were screened for the green synthesis of AgNPs. Freshly grown bacterial extract was obtained in nutrient broth and mixed with silver nitrate salt solution. The best producer (Isolate T10) of AgNPs was selected based on color change and spectrophotometer readings and further used for the synthesis of AgNPs.

Characterization of isolate

The best AgNPs producing bacteria was molecularly characterized based on 16S rRNA gene sequencing. For this purpose, bacterial DNA was isolated by using commercially available DNA isolation kit according to manufacturer instruction for DNA extraction. The extracted DNA was resuspended in 70 µL TE buffer mixed with RNase and its quantity and quality was assessed on 0.8% (w/v) agarose gel and by Nano Drop ND-1000 spectrophotometer (Nano Drop Technologies, Wilmington, USA) and stored at -4°C for subsequent analysis. 16S rRNA gene was amplified and sequenced commercially. For the identification of bacterial isolates, sequencing of 16S rRNA gene was performed. 16S rRNA gene was amplified using 27F' (5'-AGAGTTTGATCCTGGCTCAG-3') and 1494R' (5'-CTACGGCTACCTTGTTACGA-3') bacterial primers. Reaction mixture of PCR was 20 µL consisted of 1 µL DNA sample, 2 µL of deoxynucleotide triphosphate (dNTP), reverse and forward primer 2 µL each, PCR buffer 2 µL, 0.5 µL ex taq DNA polymerase and distilled water 10.5 µL. Initially, reaction mixture was incubated at 95°C for 4 minutes, and 30 amplification cycles were performed at 94°C for 40 seconds, 55°C for 60 seconds, and 72°C for 1 minute. Further, the reaction was incubated for 7 minutes at 73°C.

This purified PCR product was sequenced by using, 518F' (5'-CCAGCAGCCGCGGTAATACG-3') and 800R' (5'-TACCAGGGTATCTAATCC-3'). Sequencing was carried out at Beijing Genomics institute (BGI),PR China.

Sequence was checked for chimera using check-chimera program of the Ribosomal Database Project (RDP)(http://rdp.cme.msu.edu/seqmatch/seqmatch_intro.jsp) and compared with the deposited sequences in public database Gen Bank (NCBI) by using BLAST search program. Closely related sequences to our sequence obtained from the Gen Bank database were aligned with unknown sequence by using Bio Edit 6.0. Phylogenetic tree was constructed via maximum Likelihood method with robustness of 1000 bootstrap value in MEGA 6.0 (Tamura and Nei, 1993).

AgNPs biosynthesis

Freshly grown bacterial strain extract in broth was used for AgNPs synthesis. 100 mL of cell free extract in crude form was added to 100 mL of 10 mM concentration of silver nitrate ($AgNO_3$) (1:1) in 250 mL Erlenmeyer flask. The solution was incubated for 24 Hours at 40°C at 150 rpm.

The reaction process was performed in dark to avoid photochemical reactions. The color change of the $AgNO_3$ solution from colorless to dark brown was observed, which is the preliminary confirmation of silver salt reduction and AgNPs synthesis. Further, confirmation was done by withdrawn 1 mL aliquots with different interval of time and absorption was measured with on UV-Vis spectrophotometer at 423 nm due to Surface Plasmon Resonance (SPR), it is a reliable and accurate analytical assessment for AgNPs confirmation. The obtained AgNPs were purified through repeated centrifugation at 10,000 rpm for 20 min and used for further processing.

Optimization of physical parameter

Variables like effect of temperature (15, 25, 30, 50 °C), pH (5.5, 6, 6.5, 7.0) and silver nitrate concentration (5 mM, 10 mM, 15 mM and 20 mM) were optimized for maximum AgNPs synthesis by observing single variable at a time. Confirmation of maximum AgNPs synthesis was carried out with maximum absorbance measured at 423 nm.

Characterization of AgNPs

X-Ray diffraction (XRD)

The obtained AgNPs were concentrated via centrifugation and dry at room temperature. The crystal structure characterization of AgNPs were done through x-ray diffraction (XRD) spectrometry (Panalytical x-ray diffract meter CuK alpha 20-80). XRD was performed at 45 kV voltage and 40 A current having Cu-Ka1 radiation source and k= 1.5405 A° (0.1541 nm). The size was determined by using Debye Scherrer equation.

$$D = k\lambda \div \beta Cos\theta$$

K= orthorhombic shape constant and its value is 0.9.

λ = represents the wavelength of x-rays (0.1541 nm)

β=FWHM (full width at half maximum) of any diffracted peak.

D= is the particle diameter size.

Scanning electron microscopy (SEM)

The surface morphology and size of the AgNPs were examined using a Scanning Electron Microscope (SEM). AgNPs sample was loaded onto specimen holder. SEM micrographs of samples were taken.

Fourier Transform Infrared Spectroscopy (FTIR)

The AgNPs residues were washed with Milli-Q water to wash out attached moieties from the surface. The completely dried powder sample was placed into the sample holder and FTIR spectra were recorded in the range 4000-400 cm^{-1} in FTIR spectrophotometer (Tensor 27, Bruker, equipped with ZnSe ATR). The peaks of FTIR were identified and showed in wave number (cm^{-1}).

Biomedical Applications of AgNPs

Antibacterial activity of the greenly synthesized AgNPs was checked against several pathogenic bacteria and fungi. Details of the tested organisms are given in Table 1.

The antibacterial activity was checked by using disc method. Bacteria was suspended in sterilized normal saline to ensure the turbidity was comparable to 0.5M McFarland solution. Inoculum from saline suspension was poured on MHA plate to obtain a bacterial count of 10^6 CFU per mL. A sterile swab was used to evenly distribute bacterial culture and left to dry for 15 minutes before use in the test. AgNPs of 10 mg/mL concentration was made and small discs of Whatman filter paper was dipped for 15 minutes and dried before use. Antibiotic disc and AgNPs adsorbed discs were placed on the plate by using sterile forcep. One negative control a disc without any material was used in parallel. Plates were placed at 37 °C for 24 hours' incubation and zones of inhibition were measured in mm.

Adsorption of AgNPs onto Bandages

The antibacterial activity of the AgNPs coated bandage was checked. AgNPs were suspended in 100 mL distilled water and after sonication for 30 minutes; a sterile piece of bandages of 10mm×10mm size was soaked in this solution for 15 minutes.

The antibacterial activity of these AgNPs incorporated pieces were checked against all the test organisms (Table 1). Lawn of the test organisms were prepared as described above by using MHA plates. After that each piece of bandage was carefully placed with the help of sterile forcep in the middle of plates. For control, pieces without AgNPs were used. The zone of inhibitions was measured in mm and compared with

Clinical and Laboratory Standards Institute (CLSI).

Synergistic Effect of AgNPs

Synergistic effect of AgNPs along with different antibiotics was estimated against test organisms by using agar well diffusion method in Muller Hinton agar (MHA) plates. Bacterial cultures were suspended

in sterilized normal saline to ensure the turbidity was comparable to 0.5M McFarland solution.

Inoculum of 100 µL from saline suspension was poured on MHA plate to obtain a bacterial count of 10^6 CFU per mL. By using sterile swab, evenly distributed the bacterial culture. Four wells were created at proper distance using borer.

The AgNPs suspension (100 mg/ mL) was loaded in first well. Antibiotic solution was made and loaded into second well. Third well was loaded with 100 mL of AgNPs followed by 2 µg of antibiotic suspension. Fourth well was loaded with 100 µL of distilled water and left for some time to allow the sample set properly in the wells assuring an enhanced diffusion into the media. The plates were then placed at 37 °C for 24 hours' incubation and zones of inhibition were monitored and measured in mm. Synergistic effect was calculated by using the formula;

$$Synergistic\ effect\ (\%) = B - A/A \times 100$$

Whereas A= zone of inhibition for antibiotic

B= zone of inhibition for AgNPs plus antibiotic

Results

Isolation of microbes

Our findings showed that the strain T10 shared 99% similarity with *Shigella flexneri* strain ATCC 29903.

Table 1. Test organisms used for screening of antibacterial activates of AgNPs.

S. No	Test Organism	Nature	Description
1	*Candida albican*	Fungus	Microbiology Laboratory of Lanzhou second Hospital, Lanzhou, Gansu, PR China
2	*Staphylococcus aureus*	Gram positive	Microbiology Laboratory of Lanzhou second Hospital, Lanzhou, Gansu, PR China
3	*Klebsiella pneumonia*	Gram Negative	Microbiology Laboratory of Lanzhou second Hospital, Lanzhou, Gansu, PR China
4	Klebber	Gram Negative	Microbiology Laboratory of Lanzhou second Hospital, Lanzhou, Gansu, PR China
5	Methicillin resistant *Staphylococcus aureus* (MRSA)	Gram Positive	Microbiology Laboratory of Lanzhou second Hospital, Lanzhou, Gansu, PR China
6	*E. coil*	Gram Negative	Microbiology Laboratory of Lanzhou second Hospital, Lanzhou, Gansu, PR China

Table 2. Antimicrobial activity of AgNPs against pathogens.

Clinical isolates	AgNPs conc.	Antibiotics conc.	Zone of inhibition (mm)	
			AgNPs	Antibiotics
Candida albican	10 mg/mL	Nystatin (5 µg/mL)	40	35
Staphylococcus aureus	10 mg/mL	Oxacin (13µg/mL)	22	12
Klebsiella pneumonia	10 mg/mL	Amikacin (13µg/mL)	24	20
Klebber	10 mg/mL	Ampicillin (7 µg/mL)	33	18
Methicillin resistant *Staphylococcus aureus* (MRSA)	10 mg/mL	Ampicillin (5 µg/mL)	18	8
E. coil	10 mg/mL	Sulfametaxazole (10 µg/mL)	40	25

The constructed phylogenetic tree based on the obtained sequences exhibited that the T10 strain formed a clade with several other species of the genus (Fig. 2).

AgNPs Biosynthesis

Strain T10 was used for extracellular AgNPs biosynthesis. Reduction was noticed when 10 mM $AgNO_3$ solution was mixed with cell free supernatant with same ratio (1:1), the rapid change in color from pale white to deep reddish brown (Fig. 3). Further, AgNPs biosynthesis confirmation was carried out by using UV-Vis spectrophotometric analysis and maximum absorbance was reported at 420 nm after 1-hour incubation (Fig.4).

Table 3. Synergistic effect of AgNPs with different antibiotics against different pathogens.

Strains	Antibiotics In synergism	AgNPs in synergism	Zone of Inhibition in mm			%age Synergism
			AgNPs	Antibiotics (Abt)	AgNPs+Abt	
Candida albican	Nystatin (2.5 µg/mL)	5 mg/mL	20	35	50	42.85
Staphylococcus aureus	Oxacin (6.5µg/mL)	5 mg/mL	11	12	20	66.66
Klebsiella pneumonia	Amikacin (6.5µg/mL)	5 mg/mL	12	20	35	75
Klebber	Ampicillin (3.5 µg/mL)	5 mg/mL	16.5	18	28	55.55
MRSA	Ampicillin (2.5 µg/mL)	5 mg/mL	9	8	16	100
E. coil	Sulfametaxazole (5.0 µg/mL)	5 mg/mL	20	25	44	76

Optimization of Parameters for AgNPs synthesis

By using strain T10 for AgNPs synthesis, different conditions were optimized. Cell free supernatant was treated with silver nitrate solution and single parameter was checked at the same time. Maximum AgNPs synthesis was observed at 30°C temperature, pH 6.5, and silver nitrate concentration of 10 mM (Fig. 5).

The maximum absorbance reported at 420 nm indicated the synthesized AgNPs were highly stabled and well dispersed.

Characterization of AgNPs

X-Ray diffraction (XRD)

The XRD findings showed different peaks (Fig.6). These peaks were gained at 2Θ angels of38.06°, 44.1°, 64.44°, and 77.16° are matching to diffraction facets of Ag planes of 111, 200. 220, and 311 respectively. The findings exhibited that the AgNPs were contain of pure crystalline Ag. The result was in line with reference of Ag diffraction pattern having cubic structure in Joint Committee on Powder Diffraction Standards (JCPDS File No. 04-0783). Size of the particles was determined by using Debye Scherrer equation.

$$D = k\lambda \div \beta Cos\theta$$

The values of β for each peak was determined by using originlab 2017. The size of particles was in the range of 46–52.7 nm in size.

Scanning Electron Microscopy (SEM)

SEM analysis confirmed the synthesis and shape of AgNPs. The scanning micrograph at resolution of 0.2 µm, and 100 nm, confirmed the AgNPs synthesis and shape was almost spherical and well dispersed (Fig. 7). These micrographs exhibit the aggregates as well as individual AgNPs particles.

FTIR Spectroscopy Analysis

Findings of FTIR were analysed for identification the functional groups that acted as a capping and reducing agents during biosynthesis of AgNPs (Fig.8). The obtained spectra showed strong band at 1636, 1596, along with other bands. These sharp bands suggest N-H vibration bending obtained from primary or secondary amines present in cell filtrate. The band at 1376cm^{-1}showedthe C-N stretching from aromatic amines. The peak at 2919cm^{-1} shows the stretching of aldehyde C-H, this band could be due to OH functional groups that might have arrived from alcohols or phenols in filtrate.

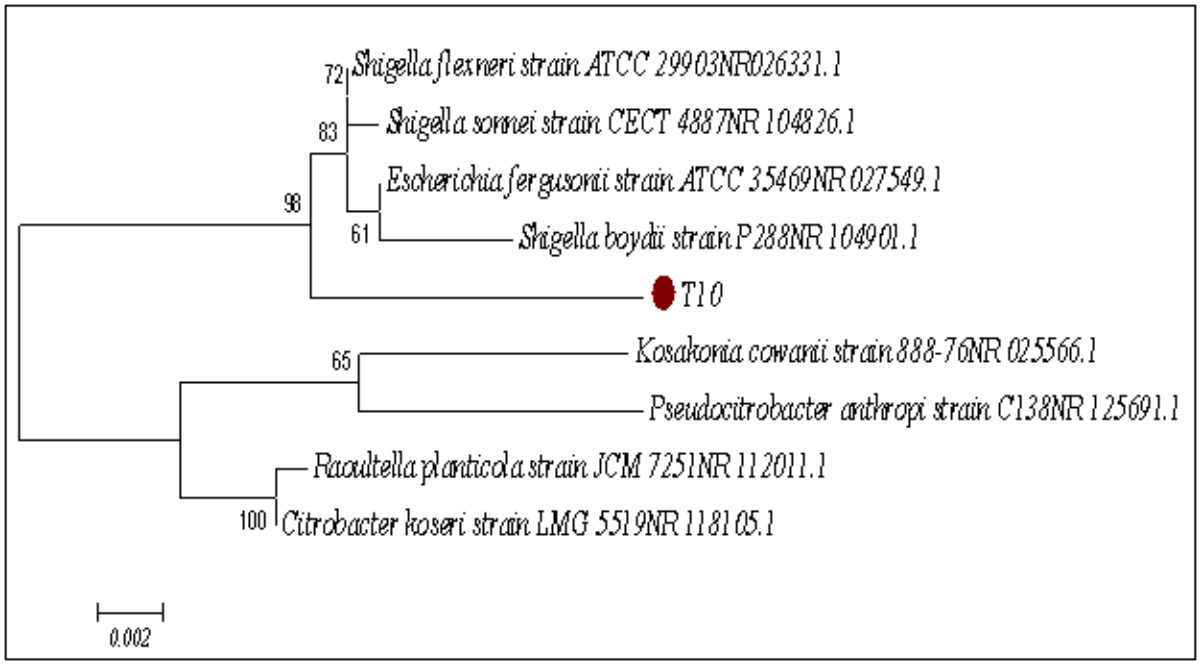

Fig. 2. Molecular Phylogenetic analysis of AgNPs synthesis strain T10 by Maximum Likelihood method on the bases of 16S rRNA with reference sequences from Gen Bank. Evolutionary analyses were conducted in MEGA6.

Antibacterial Activities of AgNPs

The diameters of zone of inhibition (ZOI) by AgNPs were measured in millimeter (mm) against different pathogens are shown in Table 2. Results showed that the antimicrobial activity of AgNPs against pathogens were quite effective.

Maximum ZOI was 40 mm against *Candida albicans* and least was 8 mm against MRSA. All the zones produced by AgNPs were higher than control antibiotics (Table 2).

Antibacterial Activities of AgNPs onto Bandage

The ZOI diameters by AgNPs adsorbed on the surface of bandage were measured in mm against pathogens (Fig.9 and 10). Results showed that the AgNPs adsorption ability on bandage was encouraging. Maximum ZOI was against *Candida albican* (33 mm) and least was against *Staphylococcus aureus* (14 mm).

Synergistic Effect of AgNPs with Antibiotics

The synergistic activity of AgNPs were checked with antibiotics against pathogens. Effective synergistic effect of AgNPs was reported against MRSA (100%) as shown in Table 3, the least activity was against *Candida albicans* that was 42.85%.

Discussion

Bacterial isolates from unusual environments where anthropogenic activities are negligible play crucial role in biotechnology. Several studies are conducted on bacterial use for AgNPs synthesis (Qian *et al.,* 2013; Netala *et al.,* 2016; Dong *et al.,* 2017).This study has significance since bacterial extract was used for AgNPs synthesis. There is no previous study to use

bacterial strains from unusual forest environment for AgNPs synthesis.

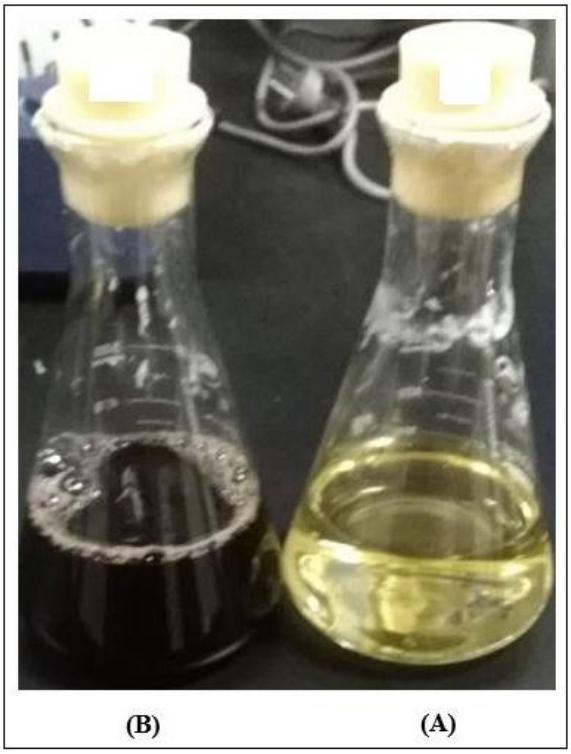

Fig. 3. (A) Control without silver nitrate, (B) color change to deep blackish after treatment with silver nitrate solution.

The bacterial strain T10 isolated from thick forests environment was evaluated for the synthesis of AgNPs synthesis. The 16S rRNA gene sequences showed similarity with *Shigella* spp that is used for the AgNPs synthesis for the first time.

Bacterial strain T10 successfully synthesized AgNPs that were confirmed through color change (Manikprabhu and Lingappa, 2013), and UV-visible spectrophotometer based on its characteristic surface plasmon resonance.

This shows that the bacterial extract contains several enzymes that reduced silver nitrate to AgNPs. Sharp peaks were obtained at 420 nm, which shows highly stable and dispersed AgNPs synthesis. Present results are in line with (Sunkar and Nachiyar, 2012; Dong *et al.,* 2017) they successfully used endophytic bacteria for AgNPs synthesis. Like our findings, Sunkar and Nachiyar, (2012) and Dong *et al.,* (2017) studied AgNPs synthesis by using endophytic bacteria and the AgNPs were synthesized after 72 and 3 hours of incubation respectively, however, in current study after one hour of incubation complete reduction of silver nitrate was achieved and no further increase in UV reading was observed.

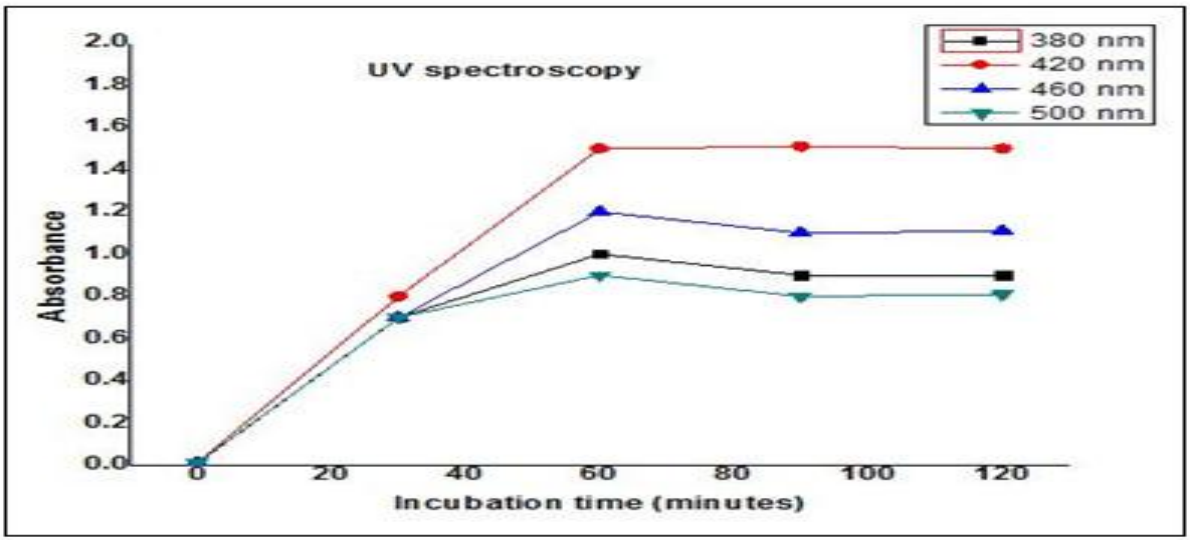

Fig. 4. UV-visible spectra of bio-based synthesis of silver nanoparticles at different time periods and different wavelength.

It shows the reducing reaction was very rapid and the enzymes were well dispersed in the solution. This way could be useful for rapid production of AgNPs. These results exhibit the presence of proteins and enzymes

into extract solution that perform silver ion reduction. Some studies have found the existence nicotinamide adenine dinucleotide, reduced form (NADH) and NADH-dependent nitrate reductase enzymes, which are important factors in the biosynthesis of AgNPs and capping the AgNPs to prevent the particles to agglomerate and stabilize the media (Duran *et al.,* 2007; Basavaraja *et al.,* 2008). SEM micrographs suggested that the AgNPs synthesized in this study were spherical in shape, which is notable success as synthesis of small size nanoparticles both in green and chemical methods is quite difficult. SEM not only confirmed the AgNPs synthesis during reaction, but also provided the insight analysis of AgNPs. Our results are in line with the findings of (Dong *et al.,* 2017).

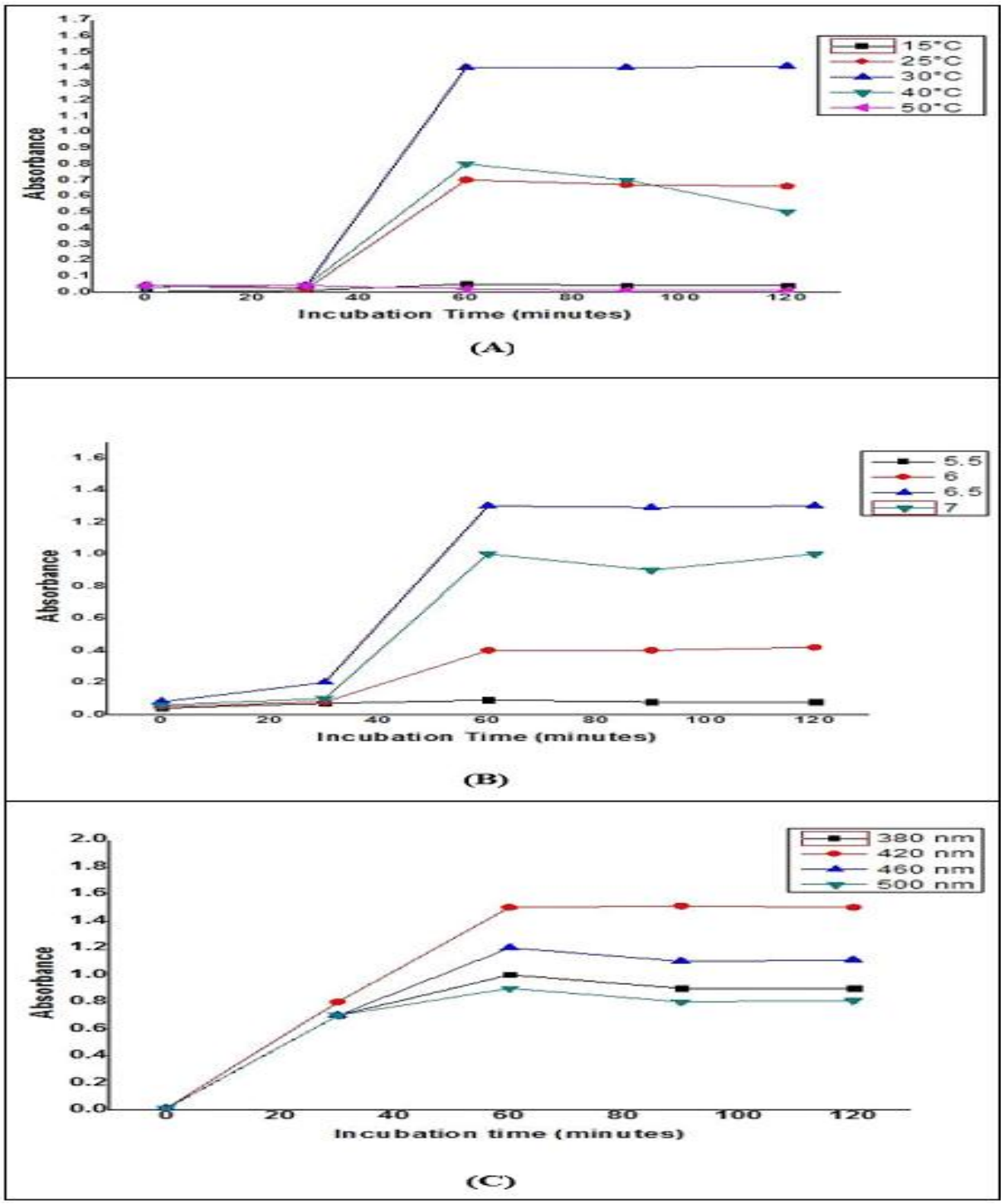

Fig. 5. Effect of different parameters on AgNPs biosynthesis through T10 isolate, (A) effect of temperature, (B) effect of pH, (C) effect of nitrate concentration.

The FTIR results showed the presence of functional groups associated with AgNPs. This shows that the AgNPs synthesized by using T10 strain are capped by several proteins and metabolites that are originally arrived from bacterial extract. It has been studied that biological molecules perform dual functions such as formation and stabilization of AgNPs (Sunkar and Nachiyar, 2012). In this study, we used the extracellular bacterial filtrate for AgNPs synthesis and the entire process was free from any sort of poisonous or toxic chemicals and solvents. This can lead towards the green and natural synthesis of AgNPs.

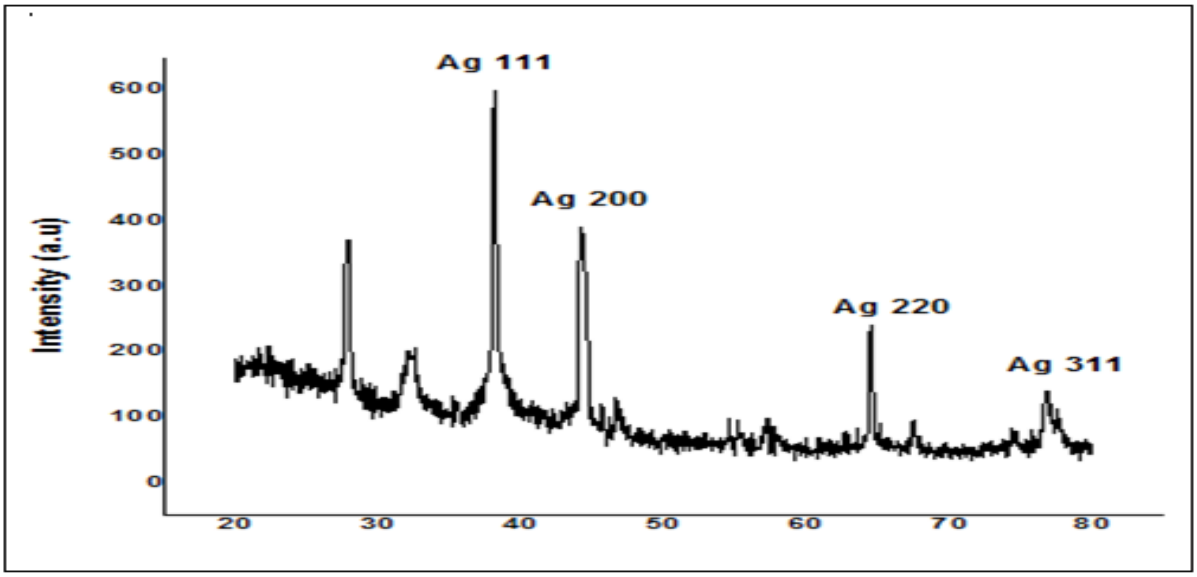

Fig. 6. X-ray diffraction patterns of AgNPs synthesized by using T10 bacterial strain.

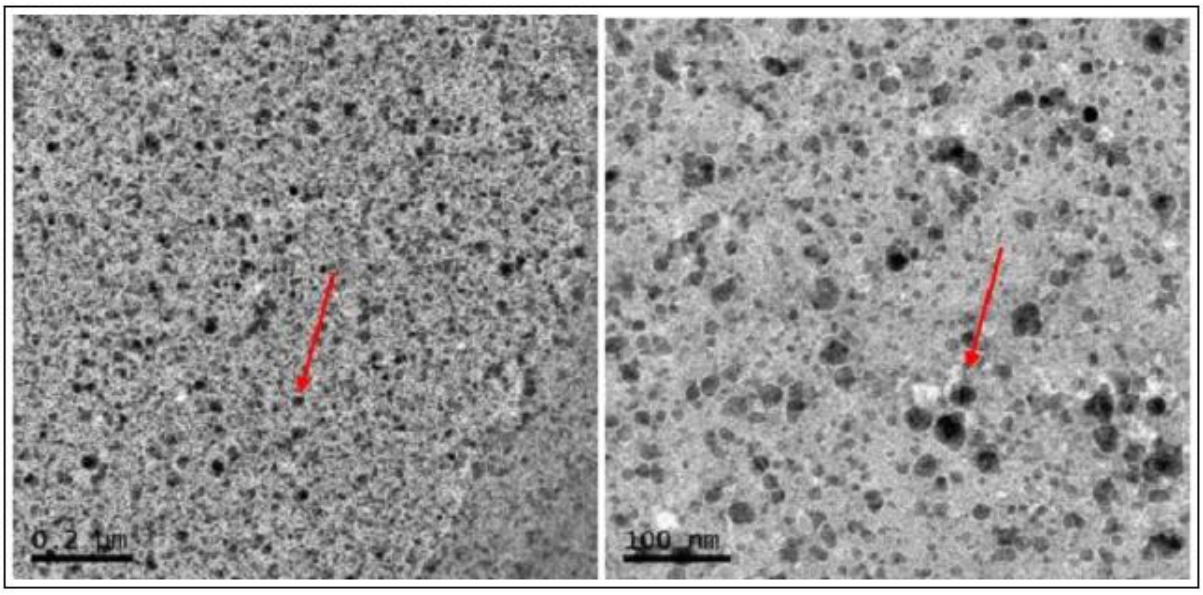

Fig. 7. SEM micrographs exhibited the biosynthesis of AgNPs by T10 bacterial strain.

The pattern of XRD showed distinct peaks corresponding to 111, 200, 220, and 311 planes of Ag. The diffraction pattern obtained was agreed with the reference in database Joint Committee on Powder Diffraction Standards (JCPDS File No. 04-0783). The findings showed the crystalline nature of Ag rather than amorphous with the particle size ranged from 46–52.7 nm. Strong peak at 38.06° and 44.1° is attributed to 111 and 200 facets, respectively, of the centered face cubic silver structures, while the diffraction peaks intensity of other facets, (220) and (311), were very weak.

Interestingly, the intensity ratio here between 200 and 111 peaks is low. These results showed that the obtained AgNPs are dominated by 111 facets. Our findings are in line with (Sunkar and Nachiyar, 2012; Qian *et al.,* 2013; Netala *et al.,* 2016).

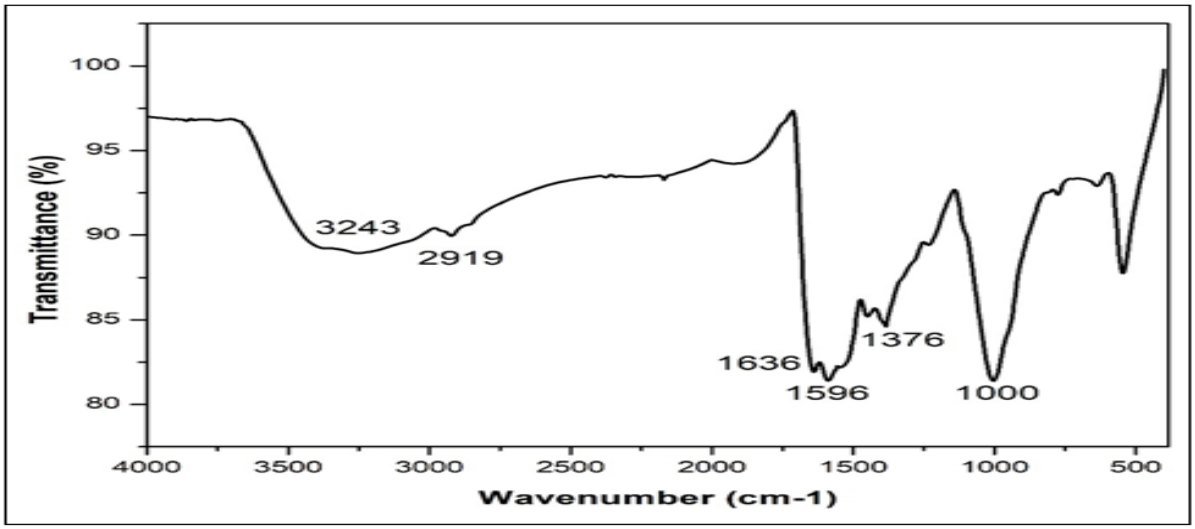

Fig. 8. FTIR spectrum of the AgNPs synthesized by using T10 bacterial strain.

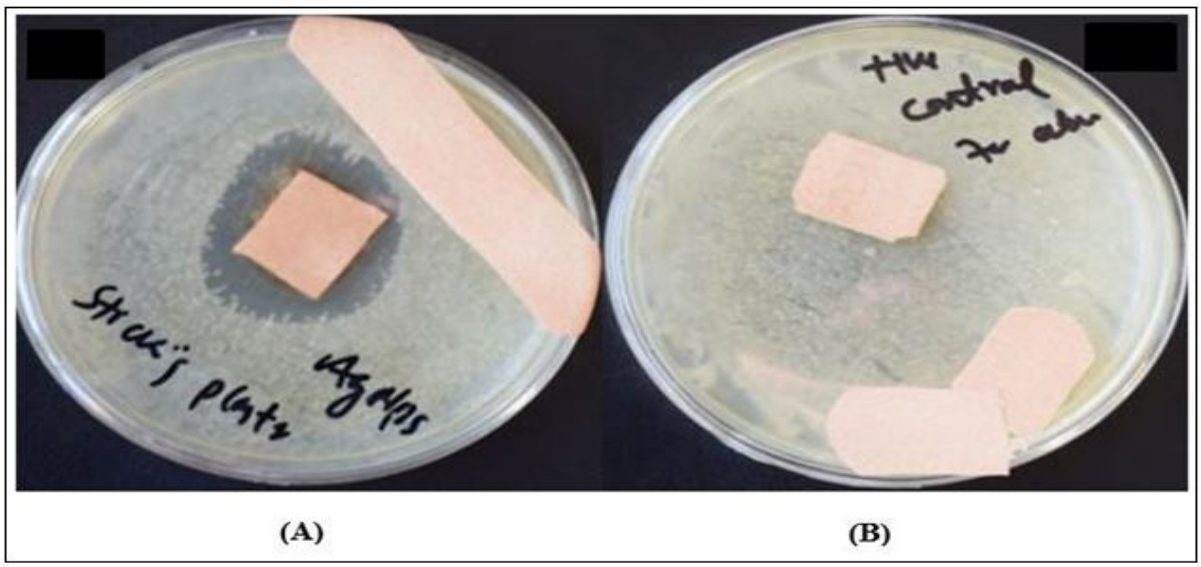

Fig. 9. Antimicrobial activity of AgNPs adsorbed bandage (A) AgNPs adsorbed bandage (B) Control without adsorb AgNPs.

Resistance emergence against antibiotics is an alarming threat to mankind. MRSA is an important pathogen with high rate of resistant against multiple antibiotics. Our findings showed that MRSA was susceptible to 100 µg/mL of AgNPs. It exhibits that the colloidal Ag at low concentration are powerful antibiotics against wide range of microorganism (Singh *et al.,* 2008). The obtained AgNPs were also effective against other pathogens including fungi. Small AgNPs having large surface area are effective antimicrobial agents even at low concentration. Antibacterial activity of AgNPs against bacterial pathogens were also reported by (Batal *et al.,* 2013; Dong*et al.,* 2017). In this study synergistic effect of AgNPs with antibiotics was also evaluated.

The findings revealed significant results and could be used to overcome antibiotics resistance. Our results were similar with the findings reported by (Rai *et al.,* 2012; Rahim and Mohamed, 2015).

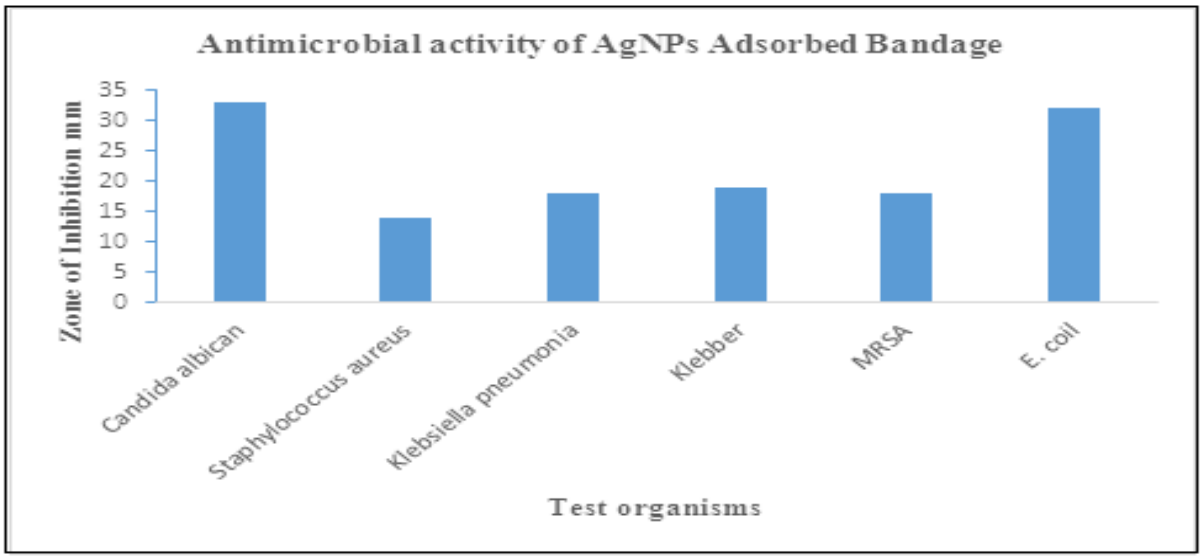

Fig. 10. Antibacterial activity of AgNPs adsorbed on to bandage against different pathogens.

The possible mechanism that are involved in antibiotics and AgNPs synergism is the formation of complexes between both antibiotics by binding the functional groups such as amino and hydroxyl groups of antibiotics to large surface area of AgNPs (Fayaz *et al.,* 2010). Our results are encouraging, and it can help in minimizing the antibiotic dose usage which is the main reason of resistance.

The obtained AgNPs were successfully adsorbed on to bandage and their antimicrobial activity against pathogens were checked. The results against pathogens were quite encouraging. This showed that the AgNPs are easy to adsorb on to bandages that could be used to prevent or cure infection and to enhance the healing process of wounds. Our results are similar with (El-Rafie *et al.,* 2014; Emam *et al.,* 2015; Balakumaran *et al.,* 2016) they successfully adsorbed AgNPs on to cotton and evaluated its biomedical applications. The adsorption of AgNPs is not only restricted to bandage but also can be used for other fabrics such as cotton, silver socks, to prevent skin pathogens and smell causing flora resides on skin.

Acknowledgment

We are thankful to department of life sciences, Lanzhou University to provide some experimental facilities. We extend our thanks to Waheed Ahmed from Physics department Lanzhou University for SEM analysis.

References

Balakumaran M, Ramachandran R, Balashanmugam P, Mukeshkumar D, Kalaichelvan. 2016. Mycosynthesis of silver and gold nanoparticles optimization characterization and antimicrobial activity against human pathogens. Microbiological Research **182,** 8–20.

Basavaraja S, Balaji S, Lagashetty A, Rajasab Z, Venkataraman A. 2008. Extracellular biosynthesis of silver nanoparticles using the fungus Fusarium semitectum. Materials Research Bulletin **43,** 1164–1170.

Batal EL, Amin AI, Shehata MA, Merehan MAH. 2013. Synthesis of silver nanoparticles by *Bacillus stearothermophilus* using gamma radiation and their antimicrobial activity. World Applied Sciences Journal **22,** 1–16.

Dong ZY, Manik PNR, Min Xiao, Hong-Fei W, Wael N, Hozzein Wei C, Wen-Jun L.2017. Antibacterial activity of silver nanoparticles against Staphylococcus warneri synthesized using endophytic bacteria by photo-irradiation. Frontiers in Microbiology **8,** 1-8.

Duran N, Marcarto P, Souza D, Alves GIH, Esposito E. 2007. Antibacterial effect of silver nanoparticles produced by fungal process on textile fabrics and their effluent treatment. Journal Biomedical Nanotechnology **3,** 203–208.

El-Rafie M, Ahmed HB, Zahran M. 2014. Characterization of nano-silver coated cotton fabrics and evaluation of its antibacterial efficacy. Carbohydrate Polymers **107,** 174–181.

Emam HE, Saleh N, Nagy KS, Zahran M. 2015. Functionalization of medical cotton by direct incorporation of silver nanoparticles. International Journal of Biological Macromolecules **78,** 249–256.

Fayaz AM, Balaji K, Girilal M, Yadav R, Kalaichelvan PT, Venketesan R. 2010. Biogenic synthesis of silver nanoparticles and their synergistic effect with antibiotics a study against gram-positive and gram-negative bacteria. Nanomedicine Nanotechnology Biology and Medicine **6,** 103–109.

Galib BM, Mashru M, Jagtap C, Patgiri BJ, Prajapati PK.2011. Therapeutic potentials of metals in ancient India: A review through Charaka Samhita. Journal of Ayurveda and Integrative Medicine **2,** 55–63.

Jain D, Kachhwaha S, Jain R, Srivastava G, Kothari LS. 2010. Novel microbial route to synthesize silver nanoparticles using spore crystal mixture of *Bacillus thuringiensis*. Indian Journal of Experimental Biology **48(11),** 1152–1156.

Kharissova OV, Rasika HV, Kharisov BI, Perez BO, Perez VMJ. 2013. The greener synthesis of nanoparticles. Trends in Biotechnology **31(4),** 240–248.

Klaus T, Joerger R, Olsson E, Granqvist CG. 1999. Silver based crystalline nanoparticles microbially fabricated. Proceedings of the National Academy of Sciences of the United States of America **96(24)**, 13611–13614.

Lengke MF, Fleet ME, Southam G. 2007. Biosynthesis of silver nanoparticles by filamentous cyanobacteria from a silver(I) nitrate complex. Langmuir **23(5),** 2694– 2699.

Manikprabhu D, Lingappa K. 2013. Antibacterial activity of silver nanoparticles against methicillin-resistant Staphylococcus aureus synthesized using model Streptomyces sp. pigment by photo-irradiation method. Journalof Pharmacy Research **6,** 255–260.

Mody VV, Siwale R, Singh A, Mody HR. 2010. Introduction to Metallic Nanoparticles. Journal of Pharmacy and Bio-allied Sciences **2,** 282–289.

Mohanpuria P, Rana NK, Yadav SK. 2008. Biosynthesis of nanoparticles technological concepts and future applications. Journal of Nanoparticle Research **10(3),** 507–517.

Morones JR, Elechiguerra JL, Camacho A, Holt K, Kouri JB, Ramírez JT, Yacaman MJ. 2005. The bactericidal effect of silver nanoparticles. Nanotechnology **16(10)**, 2346–2353.

Mukherjee P, Ahmad A, Mandal D, Senapati S, Sainkar SR, Khan MI, Parishcha R, Ajaykumar PV, Alam M, Kumar R, Sastry M. 2001. Fungus-mediated synthesis of silver nanoparticles and their immobilization in the mycelial matrix a novel biological approach to nanoparticle synthesis. Nano Letters **1(10),** 515–519.

Nambiar D, Bhathena ZP. 2010. Use of silver nanoparticles from *Fusarium oxysporum* in wound dressings. Journal of Pure and Applied Microbiology **4(1),** 207–214.

Netala VR, Kotakadi VS, Bobbu P, Gaddam SA, Tartte V. 2016. Endophytic fungal isolate mediated biosynthesis of silver nanoparticles and their free radical scavenging activity and antimicrobial studies. 3 Biotech **6,** 132.

Panáčk A, Kvitek L, Prucek R, Kolář M, Večeřá R, Pizúrová N, Sharma VK, Nevěčná TJ, Zbořil R. 2006. Silver colloid nanoparticles: synthesis, characterization, and their antibacterial activity. The Journal of Physical Chemistry B **110(33),** 16248–16253.

Poulose S, Panda T, Nair PP, Theodore T. 2014. Biosynthesis of silver nanoparticles. Journal of Nanoscience and Nanotechnology **14(2),** 2038–2049.

Priyadarshini S, Gopinath V, Meera Priyadharsshini N, MubarakAli D, Velusamy. 2013. Synthesis of anisotropic silver nanoparticles using novel strain *Bacillus flexus* and its biomedical application. Colloids and Surfaces B Bio-interfaces **102,** 232–237.

Qian Y, Yu H, He D, Yang H, Wang W, Wan X, Wang L. 2013. Biosynthesis of silver nanoparticles by the endophytic fungus Epicoccum nigrum and their activity against pathogenic fungi Bioprocess. Bioprocess Biosystem Engineering **36,** 1613–1619.

Rahim KAAA, Mohamed AMA. 2015. Bactericidal and antibiotic synergistic effect of nano-silver against methicillin-resistant Staphylococcus aureus. Jundishapur Journalof Microbiology **8(11)**.

Rai M, Deshmukh S, Ingle A, Gade A. 2012. Silver nanoparticles the powerful Nan weapon against multidrug-resistant bacteria. Journal of Applied Microbiology **112,** 841-852.

Schrofel G, Kratosova I, Safarık M, Safarıkov I, Raska L, Shor M. 2014. Applications of biosynthesized metallic nanoparticles-a review. Acta Biomaterialia **10(10),** 4023–4042.

Shahverdi R, Minaeian S, Shahverdi H, Jamalifar Nohi AA. 2007. Rapid synthesis of silver nanoparticles using culture supernatants of Enterobacteria a novel biological approach. Process Biochemistry **42(5),** 919–923.

Sharma VK, Yngard RA, Lin Y. 2009. Silver Nanoparticles Green Synthesis and Their Antimicrobial Activities. Advances in Colloid and Interface Science **145,** 83–96.

Shivaji S, Madhu S, Singh S. 2011. Extracellular synthesis of antibacterial silver nanoparticles using psychrophilic bacteria. Process Biochemistry **46(9),** 1800–1807.

Singh M, Singh S, Prasad S, Gambhir I. 2008. Nanotechnology in medicine and antibacterial effect of silver nanoparticles. Digest Journal of Nanomaterials and Biostructures**3,**115-122.

Singh R, Singh D. 2014. Chitin membranes containing silver nanoparticles for wound dressing application. International Wound Journal **11(3),** 264–268.

Sunkar S, Nachiyar CV. 2012. Biogenesis of antibacterial silver nanoparticles using the endophytic bacterium Bacillus cereus isolated from *Garcinia xanthochymus*. Asian Pacific Journalof Tropical Biomedicine **2,** 953–959.

Tamura K, Nei M. 1993.Estimation of the number of nucleotide substitutions in the control region of mitochondrial DNA in humans and chimpanzees. Molecular Biology and Evolution **10,** 512–526.

Tian J, Wong KK, Ho CM, Lok CN, Yu WY, Che CM, Chiu JF, Tam PK. 2007. Topical delivery of silver nanoparticles promotes wound healing. Chem Med Chem **2(1),** 129–136.