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ORIGINAL ARTICLE

Phyto-mediated synthesis of silver nanoparticles from *Melia azedarach* L. leaf extract: Characterization and antibacterial activity

Ansar Mehmood [a,*], Ghulam Murtaza [a], Tariq Mahmood Bhatti [b], Rehana Kausar [a]

[a] *Department of Botany, City Campus, University of Azad Jammu and Kashmir (UAJK), Muzaffarabad -13100, Pakistan*
[b] *Department of Chemical and Material Engineering, Pakistan Institute of Engineering and Applied Sciences (PIEAS), Islamabad - 45650, Pakistan*

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Biological methods;
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Abstract Nowadays different methods for the synthesis of metal nanoparticles are under consideration due to their useful applications in different fields. These include physical and chemical methods but both of these are time-consuming, expensive and environmentally toxic. Biological methods are of great advantage due to their non-toxic and large scale synthesis. In the present study, leaves extract of *Melia azedarach* L. was used as a reducing agent of silver ions to silver nanoparticles. Bio-reduction was monitored by colour change using UV–Visible spectroscopy which revealed absorption peak at λ_{max} 482 nm. Scanning electron microscopy and energy dispersive X-ray spectroscopy were utilized to identify characteristics of synthesized particles. The synthesized particles were spherical with size ranging from 34 to 48 nm. The antibacterial activity of silver nanoparticles against commonly found bacteria was assessed to find their potential use in silver containing antibacterial products.

1. Introduction

Nanotechnology deals with the manufacturing of materials at atomic level to gain distinctive properties, which can be rightfully manipulated for preferred applications (Gleiter, 2000). Nanoparticles due to their controlled size and composition got fundamental and technological attention because they offer solutions to technological and environmental challenges in the fields of catalysis, water treatment, medicine and solar energy conversion. Therefore, the formation and utilization of nanostructures from 1 to 100 nm is a promising area of research (Dahl et al., 2007; Hutchison, 2008).

Hazardous wastes from different physical and chemical processes are contributing factors for global warming and climate change. This alarming condition induced a worldwide awareness and attempt to reduce these generated wastes. Therefore, green chemistry and chemical processes are

* Corresponding author. Tel.: +92 3465387843.
E-mail address: ansar.mehmood321@gmail.com (A. Mehmood).
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increasingly integrated in science and industry for sustainable development (Anstas and Warner, 1998). There are different methods for the synthesis and stabilization of metal nanoparticles including chemical and physical methods (Balantrapu and Goia, 2009; Tripathi et al., 2010), photochemical reaction in reverse micelles (Rodriguez-Sanchez et al., 2000), electrochemical techniques (Patakfalvi and Dekany, 2010) and recently green chemistry (Taleb et al., 1998). Synthesis and characterization of nanomaterials through green approach is an emerging area in nanotechnology from the past few decades, due to their uses in the fields of chemistry, physics, medical and biology.

In biological method, the use of plants for the synthesis of metal nanoparticles did attract the attention of scientists for having a quick, cost effective, environmentally-friendly and a one-step method for the biosynthesis process (Kowshik et al., 2003). Previous literature revealed that several plants have been used for the synthesis of silver nanoparticles like *Euphorbia prostrate* (Zahir et al., 2012), *Mollugo nudicaulis* (Anarkali et al., 2012), *Calotropis gigantea* (Vaseeharan et al., 2012), *Epipremnum aureum* (Saha et al., 2012), *Padina tetrastromatica* (Jegadeeswaran et al., 2012), *Cissus quadrangularis* (Alagumuthu and Kirubha, 2012), *Spinacia oleracea* and *Lactuca sativa* (Kanchana et al., 2011).

Synthesis of nanoparticles by using plant extract can be beneficial in different human contacting areas like cosmetics, medicines and food (Parashar et al., 2009). Silver is well known for its antimicrobial effect in medicine and industrial products (Reda et al., 2011). Silver nanoparticles can be used in preventing and controlling HIV infection as they interact with the HIV-1 virus (Elechiguerra et al., 2005). These are frequently applied in topical ointments to treat infection against burn and open wounds (Lok et al., 2007). Due to their antimicrobial activity, silver nanoparticles are employed in textile fabrics, as food additives, in plastics and packages. Another application of silver nanoparticles in consumer products such as room sprays, laundry detergents, and wall paints has already been reported (Sun and Xia, 2002).

In this article, we described a simple one step method for the synthesis of silver nanoparticles by the reduction of aqueous silver ions using leaf extracts of *Melia azedarach* (Family, Meliaceae), at room temperature without using any additive protecting the silver nanoparticles from aggregation. The use of leaf extract of *M. azedarach* for reduction of silver ions was previously unexploited. The leaves of *M. azedarach* are available as it is the part of native flora of Muzaffarabad, Azad Jammu and Kashmir, Pakistan. *M. azedarach* is a deciduous tree with purplish, reddish bark found in tropical and subtropical regions of the world. It grows up to a height of about 15 m but most commonly found plants are of height about 9 m. The leaves are compound with alternate arrangement. Fragrant flowers are produced in spring. It has mucilaginous and sticky fruits with hard seeds. Locally leaves of this plant are used as a remedy of fever. Meliacine is a compound found in leaves of this tree which is effective against herpes simplex type 1 (Villamil et al., 1995).

The applications of metal nanoparticles have received tremendous attention recently besides their synthesis. Because of increase of bacterial resistance to antibiotics, silver nanoparticles have been acknowledged as antimicrobials due to their competent antimicrobial property and constancy (Rai et al., 2009). As a novel approach to silver nanoparticles, phytosynthesis has been investigated to fabricate silver nanoparticles with antibacterial action. Several biobased silver nanoparticles have been used to evaluate their antibacterial property using paper disc diffusion or broth medium (Sathishkumar et al., 2009; Krishnaraj et al., 2010; Nabikhan et al., 2010). To investigate the latent application of plant-based silver nanoparticles in the development of antimicrobial materials, the antibacterial activity of silver nanoparticles was carried out by agar disc diffusion method against *Escherichia coli*, *Klebseilla pneumonia*, *Staphylococcus aureus*, *Pseudomonas aeruginosa* and *Proteus spp*. Significantly, production of sliver nanoparticles by rapid bio-reduction of silver ions by the *M. azedarach* leaf extract and their antibacterial test in this work may provide valuable technical parameters for industrialization of the biosynthetic technique and further antibacterial application of the silver nanoparticles.

2. Materials and methods

2.1. Plant extract

Fresh leaves of *M. azedarach* L. (Fig. 1) were collected and washed with tap water as well as with distilled water three times each. Five grams of air dried leaves were cut into fine pieces and boiled for 10 min in microwave oven to get leaves extract. The extract was cooled at room temperature and then filtered by using Whattman filter paper No. 1.

2.2. Silver nitrate solution

Silver nitrate (Merck) solution of 4 mM was prepared by dissolving appropriate amount of silver salt in distilled water and stored in amber colour bottle.

2.3. Synthesis of silver nanoparticles

For the synthesis of silver nanoparticles, 100 ml of leaf extract was mixed with 100 ml of silver nitrate aqueous solution in an Erlenmeyer flask and kept at room temperature. A change in

Figure 1 *Melia azedarach* L.

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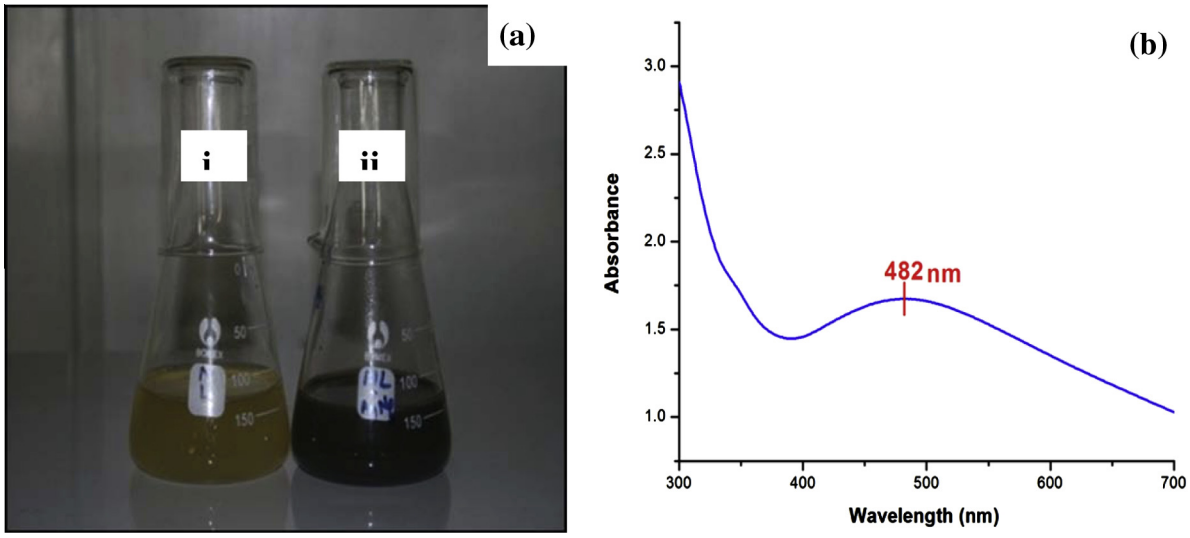

Figure 2 (a) Colour of plant extract before and after adding $AgNO_3$ and (b) UV–Visible spectrum of treated solution after 6 h.

colour was observed after mixing plant extract and silver solution. The reduction of silver in the colloidal solution was monitored by periodic flame atomic absorption spectrometry analysis. The mixture was centrifuged at 14,000 rpm for 4 min and the supernatant was subjected for flame atomic absorption spectrometry (FAAS) analysis after 0, 3 and 6 h of reaction.

2.4. Characterization

The synthesized silver nanoparticles were characterized by using different techniques including UV–Visible Spectroscopy, scanning electron microscopy and energy dispersive X-ray spectroscopy.

2.4.1. UV–Visible spectroscopy

The reduction of silver ions in the colloidal solution was confirmed by UV–Visible spectroscopy. A small aliquot of sample was taken in a quartz cuvette and observed for wavelength scanning between 300-700 nm with distilled water as a reference. PerkinElmer Lambda 950 UV/Vis spectrometer was used for UV Visible spectroscopy.

2.4.2. Scanning electron microscopy

Surface morphology of silver nanoparticles was demonstrated by scanning electron microscopy. The sample was prepared by centrifuging colloidal solution after 6 h of reaction at 14,000 rpm for 4 min. The pellet was redispersed in deionized water and again centrifuged. The process was repeated three times and finally washed with acetone. The purified silver nanoparticles were sonicated for 10 min for making the suspension and then a drop from the suspension was placed on the carbon coated copper grid. The sample was kept under lamp until completely dry. The prepared sample was subjected to SEM analysis by using Jeol JSM-6490A Analytical Scanning Electron Microscope at National University of Science and Technology Islamabad.

2.4.3. Energy dispersive X-ray spectroscopy

The centrifuged material contained pure silver which is determined by EDX. It is used to determine the composition and also the crystalline nature of material nanoparticles. The SEM and EDX were carried out both on same instrument.

2.5. Antibacterial action

The antibacterial activity of the silver nanoparticles was evaluated against *E. coli*, *K. pneumonia*, *S. aureus*, *P. aeruginosa* and *Proteus spp*. Paper disc diffusion method was used to test the antibacterial activity of silver nanoparticles. Different concentrations of silver nanoparticles were used to determine the minimum inhibitory concentration. After placing discs, agar plates were incubated at 37 °C for 24 h and then the zone of inhibition (mm) was measured. One millimolar $AgNO_3$ solution was used as control. Three replicates were used for each treatment and ANOVA was done by using MS Excel software.

3. Results and discussion

When the leaves extract was mixed with silver nitrate solution its colour started to change (Fig. 2a). It is previously known that silver nanoparticles in aqueous solution exhibit dark brown colour. A change in colour occurred because of excitation in surface Plasmon resonance wherein, it can be an indication of the formation of silver nanoparticles. Our results are similar to the previous work where the colour of fresh suspension of *Vitex negundo* and silver nitrate solution was also dark brown (Zargar et al., 2011). Further confirmation was done by using UV–Visible spectroscopy. A small aliquot from the reaction mixture was taken in a quartz cuvette and observed for absorption spectrum. It was noted that colloidal solution after 6 h of reaction showed absorption peak at 482 nm (Fig. 2b). It was proven to be a good technique for the confirmation of silver nanoparticles in the solution. The result of the UV–Visible spectrum was well correlated with previous work where the absorption peak was observed at 475 nm for silver solution having the leaf extract of *Memecylon edule* (Elavazhagan and Arunachalam, 2011).

The amount of silver ions in the treated solution was analysed by periodic flame atomic absorption spectrometry. The sample was withdrawn after 0, 3 and 6 h of reaction. The

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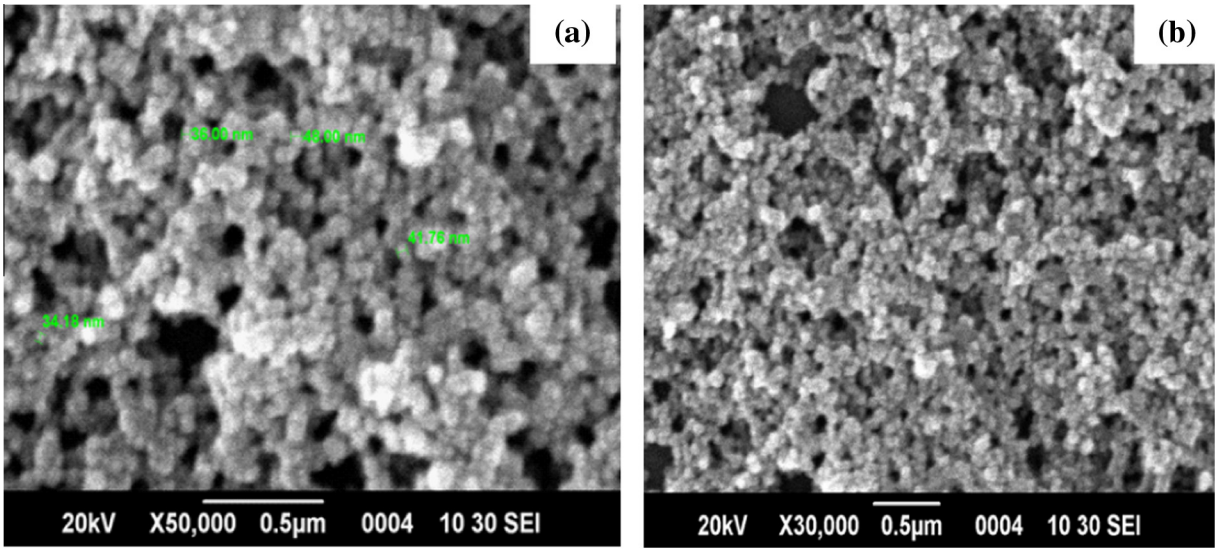

Figure 3 (a) SEM micrograph of silver nanoparticles at ×50,000 and (b) ×30,000.

mixture soon after the addition of leaves extract in the silver nitrate solution contained 216 ppm/ml silver ions. After 3 and 6 h, the silver ions reduced to 53 and 22 ppm/ml respectively. It showed that it is a quick process to get large amount of silver nanoparticles. The studies of Singhal et al. (2011) indicated that the conversion of silver ions into silver nanoparticles was completed in almost 8 min but in present work it was comparatively slow and took about 6 h. It may be due to variability of bio-molecules present in plants.

The synthesized silver nanoparticles were further characterized by SEM analysis. SEM determines the surface morphology and size of particles. It was noted that the particles were predominantly spherical in shape. The particles other than the spherical shaped were also present. The size ranges from 34 nm to 48 nm (Fig. 3a and b). The different sizes of particles may be correlated with the variable shapes. Our results have good resemblance with Elavazhagan and Arunachalam (2011).

The presence and crystalline nature of silver nanoparticles in the material were observed by EDX analysis. It is well know that silver nanocrystals show typical optical absorption peak approximately at 3 keV due to surface Plasmon resonance (Magudapatty et al., 2001). Fig. 4 showed the absorption peak at 3 keV region which revealed that nanoparticles were formed exclusively of silver with crystalline nature. EDX peak for Cd was also noted and it is suggested that it may be due to the presence of precipitates in the plant material (Table 1).

Biosynthesized silver nanoparticles were subjected to antibacterial activity against selected microbes. Fig. 5a–e show the zones of inhibition of different concentrations (10 μg/ml, 5 μg/ml and 2.5 μg/ml) of silver nanoparticles against *E. coli* (a), *K. pneumonia* (b), *S. aureus* (c), *P. aeruginosa* (d) and *Proteus spp* (e). Highest activity of silver nanoparticles (10 μg/ml) was observed against *S. aureus* (11.67 ± 0.33 mm) and *K. pneumonia* (11.33 ± 0.33 mm). While moderate activity was observed against *E. coli*, *P. aeruginosa* and *Proteus spp* (9.33 ± 0.67 mm). In case of minimum inhibitory concentration, silver nanoparticles of 2.5 μg/ml showed marginal inhibitory effect against *E. coli* and *K. pneumonia*. The 5 μg/ml

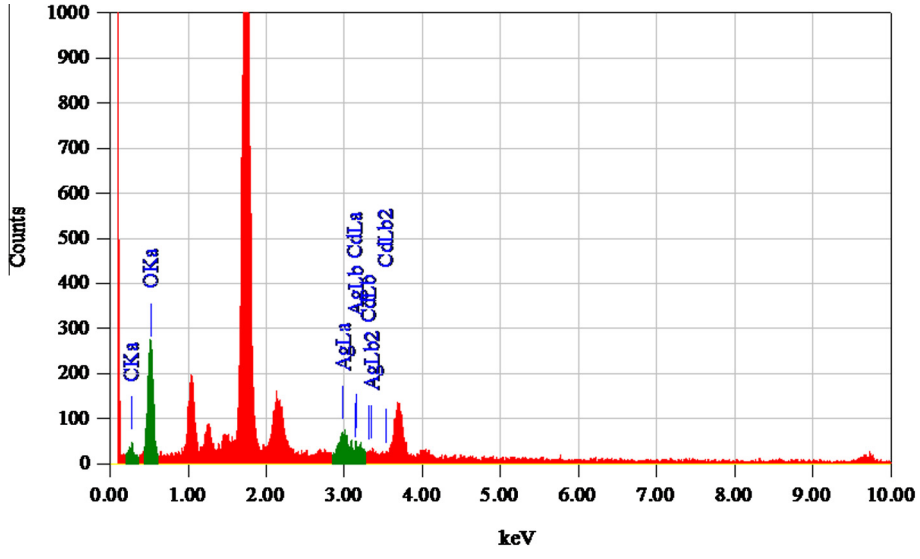

Figure 4 EDX spectrum of material containing silver nanoparticles.

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Table 1 EDX elemental micro-analysis of the silver nanoparticles.

Element	Mass (%)	Atom (%)
C K	9.34	13.76
O K	57.73	83.8
Ag	13.51	2.22
Cd	1.42	0.22

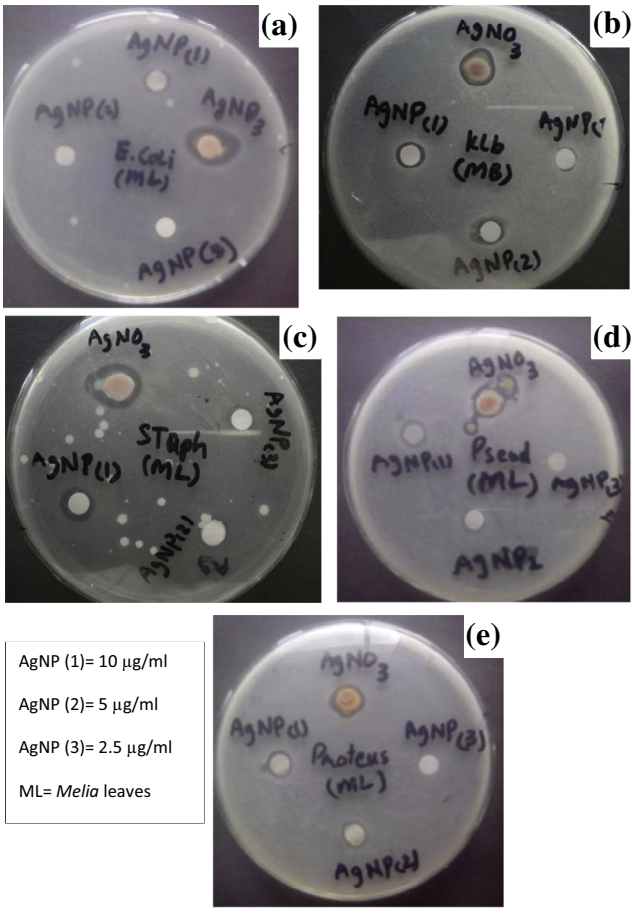

Figure 5 Zones of inhibition of silver nanoparticles against (a) *Escherichia coli*, (b) *Klebsiella pneumonia*, (c) *Staphylococcus aureus*, (d) *Pseudomonas aeruginosa* and (e) *Proteus spp* respectively.

concentration of silver nanoparticles showed 8.67 + 0.33 mm and 10.67 ± 0.33 mm against *E. coli* and *K. pneumonia* respectively (Fig. 6). While against *S. aureus*, *P. aeruginosa* and

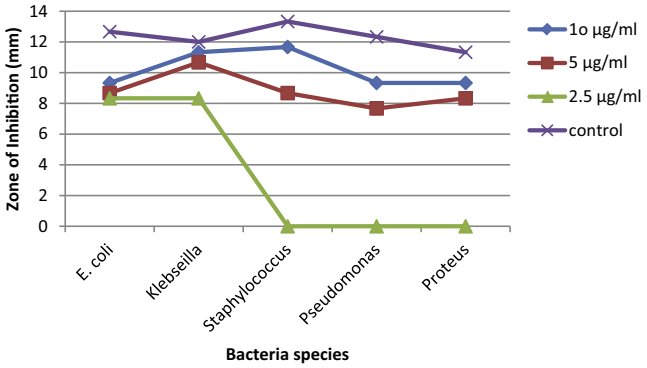

Figure 6 The minimum inhibitory concentration of silver nanoparticles against tested bacteria.

Proteus spp the minimum inhibitory concentration was 5 µg/ml (Table 2). The 2.5 µg/ml concentration did not show any activity against *S. aureus*, *P. aeruginosa* and *Proteus spp*. It was noted that an increase in silver nanoparticle concentration, also increased antibacterial activity. The exact mechanism of inhibition of bacterial growth by silver nanoparticles is not completely understood. However, Sondi and Sondi (2004) demonstrated that the antibacterial activity of silver nanoparticles on gram-negative bacteria was dependent on the concentration of Ag Nanoparticles and was closely linked with the development of pits in the cell wall of bacteria. Previously it was reported by Shrivastava et al. (2007) that the effect of silver nanoparticles on *S. aureus* is much less. However, in our case, silver nanoparticles showed higher antibacterial effect against *S. aureus* as compared to other tested bacteria. Recently silver nanoparticles are widely used in coatings, textiles and wood flooring as antibacterial agents. These biogenic synthesized silver nanoparticles showed high antibacterial activity which may be used in these materials.

4. Conclusion

Silver nanoparticles were synthesized by applying environmentally safe method which minimizes the addition of hazardous wastes in the environment. The synthesized nanoparticles were spherical, 34–48 nm in size, crystal in nature and showed absorption spectrum at 482 nm characterized by using different techniques. Determination of antibacterial effect of silver nanoparticles against tested bacteria reveals that silver nanoparticles are highly active as antibacterial agent. In comparison to conventional antibiotics, the nanoparticles provide more chemical, catalytic, physical and thermal activities due to larger surface area to volume ratio. The current study could be a helpful contribution to the following fields: health,

Table 2 Antibacterial activity of silver nanoparticles synthesized from leaf extract of *M. azedarach* (mm).

AgNPs concentration (µg/ml)	Zone of inhibition – mm (mean ± standard error)				
	E. coli	*K. pneumonia*	*S. aureus*	*P. aeruginosa*	*Proteus spp*
10	9.33 ± 0.67**	11.33 ± 0.33**	11.67 ± 0.33**	9.33 ± 0.67**	9.33 ± 0.33**
5	8.67 ± 0.33**	10.67 ± 0.33**	8.67 ± 0.33**	7.67 ± 0.33**	8.33 ± 0.67**
2.5	8.33 ± 0.33	8.33 ± 0.33	–	–	–
$AgNO_3$	12.67 ± 0.33	12 ± 0.58	13.33 ± 0.67	12.33 ± 0.67	11.33 ± 0.33

** Significant at $P = 0.01$.

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environment, energy, information technology and cosmetics. Moreover, silver nanoparticles may be proven safer because of their non resistant nature in comparison to conventional antibiotics.

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