Silver nanoparticles synthesized using leaf extract of *Azadirachta indica* exhibit enhanced antimicrobial efficacy than the chemically synthesized nanoparticles: A comparative study

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Abstract

A wide variety of methods have synthesized silver nanoparticles (Ag-NPs) in the recent past; however, biological methods have attracted much attention over the traditional chemical synthesis method due to being non-hazardous and eco-friendly. Here, a detailed and systemic study was performed to compare two different synthesis routes for Ag-NPs, that is, the chemical and the biological; their possible outcomes have also been described. Ag-NPs were synthesized chemically (cAg-NPs) using a chemical reductant and biologically (bAg-NPs) by using aqueous leaf extract of *Azadirachta indica* (neem). The synthesized nanoparticles were characterized using UV-visible spectrophotometry, FT-IR, EDX, and TEM. The average particle sizes (APS) of cAg-NPs were found to be 8 and 13 nm and of bAg-NPs to be 19 and 43 nm under different AgNO₃ concentrations. The antimicrobial tests of differently sized NPs were performed against *Escherichia coli* (Gram −ve) and *Staphylococcus aureus* (Gram + ve). The results revealed that bAg-NPs of APS 43 nm were highly antimicrobial against both the tested bacterial stains followed by cAg-NPs of 8 nm. We found the effect of cAg-NPs to be size-dependent, whereas bAg-NPs showed a more significant antimicrobial effect than cAg-NPs.

Keywords

Nanoparticles, chemical synthesis, biological synthesis, antimicrobial tests

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Introduction

Metallic nanoparticles (NPs) such as silver have been a focus of interest for many scientific communities due to their exclusive properties such as small size, high reactivity, and high surface area to volume ratio. Previously Ag-NPs have been utilized in optoelectronics, catalysis, chemical, and biomedical fields. They showed very promising broad-spectrum as antimicrobial drug applications under biomedical fields due to their high surface area to volume ratio. Several theories regarding the antimicrobial mechanism of Ag-NPs may include their anchorage towards the bacterial cell wall, release of Ag ions, thereby penetrating and causing membrane damage and leading to cell death. Some other mechanisms may involve generating reactive oxygen species (ROS) by respiratory enzyme inhibition causing damage to the cell membrane. Nanoparticles exhibit different sizes, shapes, and morphology. All these properties are strongly influenced under different experimental conditions such as temperature, pH, the kinetics of interaction between metal salts and reducing agents, nature, and adsorption of capping agents, etc. Several physical, chemical, and biological synthesis routes have been proposed to synthesize Ag-NPs. The chemical approach is the most popular one for synthesizing Ag-NPs by using various organic and inorganic chemical reductants or by physicochemical reductants. In the chemical method, sodium borohydride (NaBH₄) is the most common chemical reductant used for the synthesis, while trisodium citrate (TSC) is used to stabilize the size Ag-NPs. The chemicals used for the synthesis and stabilization are toxic, expensive, and often lead to non-eco-friendly by-products. However, it offers a short period for the synthesis containing fewer impurities. Recently biological methods have emerged, as they provide a better alternative platform for the synthesis. These methods are cost-effective, environment-friendly, energy-efficient, non-hazardous, easier to handle, and scaled up for large-scale synthesis. Biological methods involving plant extracts have a significant advantage: they are simple, free from toxic chemicals, provide natural capping agents, and are quick in reaction compared to microbes. They are also largely distributed in the environment. *Azadirachta indica* of the family Meliaceae, commonly called “neem,” is a well-known medicinal plant in India, and its leaves are used as a household remedy against various bacterial and viral infections. Some of the active components of neem leaves are terpenoids, phenolics, tannins, flavonoids, essential oils, etc. Previously it was suggested that these phytochemicals are directly involved in the bioconversion of silver salts to silver NPs. Synthesis of Ag-NPs via neem leaf extract is more advantageous as the active components present in the leaf enhance their antimicrobial activities through capping, thereby making medically relevant NPs (Figure 1).

Previously some reports have shown the synthesis of Ag-NPs by the chemical and biological method combined with anti-fungal drugs; however, their brief comparisons are needed to be explored. Therefore in the present study, we synthesized Ag-NPs by two different methods, that is, the chemical (using NaBH₄ & TSC) and the biological (using aqueous extract of *A. indica*); characterized both...
types of Ag-NPs and also assessed them for their antimicrobial activity on two selected bacteria, that is, *E. coli* (Gram −ve) and *S. aureus* (Gram + ve).

**Experimental**

**Materials**

Silver nitrate (AgNO₃ ≥ 99.9% pure) was purchased from Qualigens fine chemicals India, sodium borohydride (NaBH₄ ≥ 99.9% pure), and trisodium citrate (TSC ≥ 99.9% pure) was purchased from SRL Mumbai (India). The antimicrobial studies were carried out using two bacterial strains *E. coli* (ATCC 25922) and *S. aureus* (ATCC 25923). The nutrient broth was purchased from Himedia Lab. Ltd., Mumbai, and was used for inoculum preparation. Nutrient media (Himedia Lab Ltd., Mumbai) was used as a solid medium for supporting bacterial growth.

**Synthesis of Ag-NPs**

(i) By chemical method (cAg-NPs): For the chemical synthesis of Ag-NPs, AgNO₃ was chemically reduced with NaBH₄ and stabilized with TSC. In the first beaker, 0.5 ml of 1 mM AgNO₃ and 0.5 ml of 10 mM TSC were added simultaneously in 18.5 ml distilled water, 0.5 ml of 10 mM NaBH₄
was dripped at approximately one drop per second at 10°C. Similarly, in the second beaker, 0.5 ml of 10 mM AgNO₃, 0.5 ml of 10 mM TSC were added in 18.5 ml distilled water. Both the beakers were allowed for continuous stirring for 5 min and maintained at 10°C. After 5 min, 0.5 ml of 10 mM NaBH₄ was added. The color of the solution changed from colorless to yellow indicating NPs formation.

(ii) By biological method (bAg-NPs): For the biological synthesis of Ag-NPs, fresh leaves of neem were collected from the university campus. The leaves were washed thoroughly with distilled water and air-dried. These leaves were ground, and a fine powder was obtained. Thirty grams of dried powder were boiled in 100 ml of phosphate buffer, pH 8.0 for 30 min. After cooling at room temperature, it was centrifuged at 6000 rpm for 10 min and then filtered. The obtained filtrate was then stored at 4°C for further experiments. In the first beaker, 9 ml extract of neem leaves was mixed with 36 ml of 1 mM AgNO₃ solution in the 1:4 ratios. In another beaker, 9 ml extract was mixed with 36 ml of 10 mM AgNO₃ solution in the same ratios under aseptic conditions. Both the beakers were kept in a shaking water bath at 37°C in the dark for 5 h. A change in color intensities was observed after 5 h indicating NPs formation. The colloidal solution of all the obtained NPs were centrifuged at 20,000 rpm for 30 min, pellets were washed thrice, and were resuspended in sterile deionized water for further use.

Instrumentation

UV-visible spectroscopy: UV-Visible spectral analysis was done by using Shimadzu UV-spectrophotometer (UV-1800). UV-Vis absorption spectrophotometer with a resolution of 1 nm between the absorbance ranges of 200–700 nm was used to investigate the formation of Ag-NPs.

FT-IR analysis: FT-IR spectra were recorded using Perkin Elmer FT-IR instrument operating at a resolution of 400–4000 cm⁻¹ in % transmittance mode. The pellets were dried, mixed with KBr pellet, and analyzed on the instrument. FT-IR analysis was mainly performed to study the functional groups present in the Ag-NP formation. Therefore the technique was used for the qualitative assessment of synthesized nanomaterial.

Transmission electron microscopy (TEM): For the analysis of external morphology, crystalline nature as well as the size of the synthesized Ag-NPs, TEM was performed using JOEL-JEM-2100 TEM instrument on carbon-coated copper grids. For analysis, a drop of prepared sample was placed on a copper grid and was allowed to dry prior to loading onto the specimen holder. Samples were operated at an acceleration voltage of 200 kV.

Energy-dispersive X-ray spectroscopy (EDX): To analyze the elemental composition of the synthesized NPs, EDX spectroscopy was carried out. The results were
analyzed based on a set of peaks emitted by a particular element in the electromagnetic emission spectrum.

**Antimicrobial activity**

Antimicrobial assessment of Ag-NPs was performed on two selected bacteria *E. coli* (Gram −ve) and *S. aureus* (Gram + ve), by agar well diffusion method. Nutrient media and nutrient broth were made according to the standard protocol. The sterile nutrient broth was inoculated with *E coli* and *S. aureus* strains and kept in an incubator overnight at 37°C. Media plates were prepared by pouring the nutrient broth on Petri plates and kept overnight, allowing them to solidify. Hundred μl of fresh overnight grown cultures were taken and spread on media plates using a sterile spreader so as to cultivate bacteria. Agar was punched with the help of a sterile borer to create 8mm wells. Wells were sealed with 50 μl of soft agar to prevent leakage of the NPs from the bottom. The colloidal solution of NPs was loaded into respective wells with different volumes (20, 40, 60, & 100 μl). Plant extract of *A. indica* was also loaded in one of the wells and served as a control. In order to maintain equal concentrations in terms of particle number the concentrations of 2 and 3 mg/ml were selected for cAg-NPs and 5 and 8 mg/ml for bAg-NPs. All the plates were incubated at 37°C for 24 h and zones of inhibition (ZOI) were measured around the wells. The bacterial sensitivity was determined on the basis of obtained average ZOI around the wells.

**Statistical analysis**

Statistical analysis was performed for determining the significant differences in the antimicrobial activities of cAg-NPs and bAg-NPs towards the tested bacterial strains using Graph Pad Prism version 7.04 for windows. One-way ANOVA was performed, and a *p*-value of < 0.05 was considered to be significant.

**Results and discussion**

**Characterization studies**

Silver nanoparticles were synthesized at two different concentrations of AgNO₃ and were analyzed by UV-Vis spectra of surface Plasmon resonance (SPR) band. In Figure 2(a) AgNO₃ showed a peak at 220 nm. In the chemical method, as soon as Ag-NPs starts forming in the reaction mixture upon chemical reduction of 1mM AgNO₃, the color of the solution changed from colorless to yellow and showed absorption maxima at 400 nm (Figure 2(b)), thereby indicating nanosilver formation which is due to surface Plasmon resonance vibrations on its surface.²⁰ In the second case, on increasing AgNO₃ concentration from 1 to 10 mM, there is an increase in absorption intensity at 400 nm. Parallel changes have been observed in the color intensities in both cases, and it increases with an increase in the time of incubation. In the biological method, plant extract of *A. indica* was added to a
1 mM aqueous solution of AgNO₃ and incubated for 4–5 h. The color of the reaction mixture changed from colorless to light yellow then to reddish-brown. The resultant Ag-NPs formed in the reaction mixture showed a characteristic peak at 450 nm (Figure 2(d)) due to the surface Plasmon vibrations existing within NPs. The results coincide with the previously reported literature showing absorbance within the range of 420–450 nm.²¹ Broadening of the peak indicates that particles are larger in size²² compared to the above method and are well dispersed in nature. In the second case, it was also observed that upon the increase in AgNO₃ concentration, the color intensity of the solution changes and becomes dark brown upon prolonged incubation. The shifting up of absorbance peaks with increased AgNO₃ concentration signifies changes in particle size due to more excitation of SPR, which increased with an increase in time duration.²³ This implies that the absorbance of Ag-NPs depends upon two factors one is AgNO₃ concentration, and the other is the time of incubation. UV–Vis spectroscopy results indicate that 90% of the reduction and stabilization process is completed within a few minutes in the case of the chemical method. Particles to be smaller in size due to narrower peaks.
The biological method takes around 4–5 h for the process to get completed. Existing particles to be larger in size due to peak broadening (Figure 2).

The long-term stability of cAg-NPs and bAg-NPs were also compared from UV-Vis spectral analysis (Figure 3). The absorption spectra of both the synthesized NPs were recorded after 3 months of synthesis and were compared with the absorption spectra recorded initially. In the case of cAg-NPs, there was no change in absorption spectra even after 3 months of storage, thereby signifying its stable character; however, bAg-NPs showed a small shift in peak towards higher wavelength due to aggregation among the particles upon prolonged storage, thus becoming unstable (Figure 3).

To further enumerate the formation of cAg-NPs as well as bAg-NPs, FT-IR analysis was carried out, which confirms the presence of some functional groups. The characteristic peaks of cAg-NPs were observed at 3438.20 cm\(^{-1}\), 2924.17 cm\(^{-1}\), 2854.06 cm\(^{-1}\), 1634.22 cm\(^{-1}\), and 670.52 cm\(^{-1}\) (Figure 4). The observed peak at 3438.20 cm\(^{-1}\) may be assigned to O-H bond stretching, while the peak at 1634.22 cm\(^{-1}\) may be assigned to amide C = O stretching. The peaks at 664.62 cm\(^{-1}\) and small shoulder peaks at 2924.17 and 2854.06 cm\(^{-1}\) can be assigned to C-H stretching vibrations, which may be due to the citrate capping agent on the Ag-NPs surface\(^9,^{11}\) being surrounded by a set of C = O and OH groups.

Biologically synthesized silver nanoparticles showed a similar set of peaks at 3438.16 cm\(^{-1}\), 2074.56 cm\(^{-1}\), 1634.57 cm\(^{-1}\), and 668.17 cm\(^{-1}\); however, they also showed a small shoulder peak at 1049.50 cm\(^{-1}\). The dual role of A. indica leaf extract as a reducing as well as capping agent was confirmed at 3438.16 cm\(^{-1}\), 1634.57 cm\(^{-1}\), 1049.50 cm\(^{-1}\), and 2074.56 cm\(^{-1}\), which determined the presence of OH, amide C = O as well as small C-O, and C = C bond stretching involved in

Figure 3. Stability analyses of: (a) cAg-NPs and (b) bAg-NPs by UV-vis spectroscopy.
Ag-NP formation (Figure 4). The observed amide C = O stretching vibrations may be due to the presence of amide group, raised by carbonyl stretch of proteins, whereas OH, C-O, C = C stretch may be attributed to the phytochemicals such as alkaloids, terpenoids, flavonoids, phenolics, etc. which are abundantly present in the leaf extract. Therefore, FT-IR analysis demonstrates the presence of proteins and phytochemicals involved in capping Ag-NPs hence providing stability to them.  

TEM studies were further performed using JOEL- JEM 2100 TEM instrument to reveal particle size and morphology of the synthesized Ag-NPs. As shown in Figure 5(a) and (b), highly magnified TEM images of cAg-NPs reveal particle sizes to be in the range 3–14 nm with an average diameter existing within 8 ± 0.5 nm at 1 mM AgNO₃ precursor, 7–16 nm with an average diameter of 13 ± 1.3 nm at 10 mM AgNO₃ concentration. All cAg-NPs were well dispersed in nature, mostly spherical in shape, smaller in number, and were size-controlled. In Figure 5(c) and (d) most of the bAg-NPs appear to be spherical in shape, having a bead-like structure, much larger in size, uniformly distributed, and without aggregation. Particle sizes were observed to be in the range 9–42 nm with an average diameter of particles existing within 19 ± 0.6 nm at 1 mM AgNO₃ precursor concentration which were in agreement with earlier reports whereas particle size range increased to 20–76 nm when 10 mM AgNO₃ concentration was used during the synthesis with an average diameter of particles existing around 43 ± 0.6 nm. TEM studies reveal that the sizes of bAg-NPs are larger compared to the cAg-NPs. This may be due to the fact that bAg-NPs interact with several other phytochemical constituents, which increase their diameter. Thus, synthesis by chemical method offers better control over the size and nucleation of Ag-NPs compared to the biological approach. The size of the NPs can be explained on the basis of the nucleation rate of silver particles that tend to aggregate into larger particles owing to a rise in the increase in the
Figure 5. TEM micrograph is showing: cAg-NPs (a) synthesized with 1 mM AgNO$_3$, (b) with 10 mM AgNO$_3$; bAg-NPs (c) synthesized with 1 mM AgNO$_3$, and (d) with 10 mM AgNO$_3$. Histograms showing the range of particle-size distribution.
collision frequency upon an increase in AgNO₃ concentration; hence change in morphology can also be observed (Figure 5).

The formation of both cAg-NPs and bAg-NPs was also confirmed from EDX spectra. The profile showed the presence of Ag in both the samples with a small percentage of carbon depending upon the surrounding molecules (Figure 6). No additional impurities were detected in either cAg-NPs or bAg-NPs (Figure 6).

**Comparison of antimicrobial activities of cAg-NPs and bAg-NPs**

The antimicrobial activity of both cAg-NPs and bAg-NPs was determined, and a sensitivity test was carried out using the agar well diffusion method against both Gram −ve and Gram + ve bacteria. All the plates were incubated at 37°C for 24 h, bacterial growth inhibition was measured after the overnight incubation (Figure 7), and antimicrobial activities were determined as shown in Table 1.

Both cAg-NPs and bAg-NPs synthesized under different AgNO₃ concentrations demonstrated variable antimicrobial activity against both *E. coli* and *S. aureus*. It can be inferred from Table 1 that cAg-NPs which were smaller in size (8 nm), were found to be more active towards bacteria compared to large-sized particles (43 nm), thereby indicating size-dependent antimicrobial activities.²⁶–²⁸ However, in the case of bAg-NPs, small-sized particles (19 nm) showed moderate activity, whereas large-
sized particles (43 nm) showed high activity towards the bacteria, while no activity was demonstrated by plant extract alone. This may be due to the fact that large-sized particles have better adsorption of phytochemicals on their surface that enhanced their antimicrobial activity; nevertheless, the plant extract alone did not

Table 1. Antimicrobial activity against *E.coli* and *S. aureus*.

| Method of synthesis | Concentration of AgNO₃ (mM) | Concentration of Ag-NPs (mg/ml) | Particle size using TEM | *E. coli* | *S. aureus* |
|---------------------|-----------------------------|--------------------------------|------------------------|-----------|-------------|
|                     |                             |                                |                        | Sensitivity | Sensitivity |
| Chemical 1          | 1                           | 2                              | 8 ± 0.5                 | MS +       | S ++        |
| 10                  | 3                           | 13 ± 1.3                       | 7–16                   | MS +       | MS +        |
| Biological 1        | 1                           | 5                              | 19 ± 0.6                | MS +       | MS +        |
| 10                  | 8                           | 43 ± 0.6                       | 20–76                  | VS +++     | VS +++      |
| Plant extract alone | –                           | –                              | –                      | NS 0       | NS 0        |

NS: not sensitive; MS: moderately sensitive; VS: very sensitive; S: sensitive.

Figure 7. Well diffusion tests for Ag-NPs synthesized by two different methods against *E. coli* (Gram –ve) and *S. aureus* (Gram +ve). 1, 3, and 5, 7 represent growth inhibition by cAg-NPs (synthesized with 1 mM and 10 mM AgNO₃ respectively), whereas 2, 4, and 6, 8 represent growth inhibition by bAg-NPs (synthesized with 1 mM and 10 mM AgNO₃ respectively). Wells contains different volumes of Ag-NPs (20, 40, 60, & 100 μl). The significant antibacterial effect is highlighted in the broken circle.
show the activity. However, phytochemicals have been reported to be antimicrobial in some cases.29

The obtained results for bAg-NPs are also well supported by previous studies, which demonstrated that the antimicrobial activity of Ag-NPs was only due to particles alone but not due to plant extracts. However, their activity can be influenced by the capping agents present in them.18,21 It has been suggested that small-sized Ag-NPs having a large surface area to volume ratio allows them to closely interact with the microbial membrane causing structural changes in the cell membrane like its permeability hence causing contact-dependent death of the cell.30–32 In our studies, bAg-NPs showed different scenarios concerning their antimicrobial activities. Despite having large sizes, they showed high activity towards the selected Gram +ve and Gram –ve bacteria. It can be speculated that phytochemicals present in the extract may be responsible for not only controlling the size and shape of the particles but also affecting their activities by fully occupying surfaces through weaker binding. In the case of bAg-NPs there are two factors that may be responsible for enhanced antimicrobial activities. One may be either due to greater occupancy of phytochemicals on the particle surface that improved their binding affinity towards bacterial cell membrane or the excessive Ag+ release (due to a change in chemical environment) inhibited the bacterial growth.29,33,34 As described earlier, some environmental factors may also influence the antimicrobial activities of bAg-NPs, such as a change in pH, temperature, and ionic strength.8,16,35 However, further studies are required to understand the role of phytochemicals in enhancing the antimicrobial activity. The differences in the obtained inhibition zones by cAg-NPs and bAg-NPs were found to be statistically significant ($p < 0.05$). As a result, significant differences have been found in the antimicrobial activities of different sized cAg-NPs and bAg-NPs evaluated with one-way ANOVA where the P-value comes out to be 0.008.

**Conclusion**

Two methods for the synthesis of Ag-NPs have been compared in this study, that is, the chemical and the biological, using different concentrations of AgNO3. The chemical method took a short time for the synthesis as the reduction process was completed in just a few minutes and particles were quite stable for few months. At the same time, the biological method took a longer period of time for synthesis since the reduction process was completed in 4–5 h. However, in the biological method, there is no requirement for additional capping agent for the size stabilization of Ag-NPs as plant extracts themselves provides natural capping to the NPs. From TEM studies, the average sizes of bAg-NPs were observed to be 19 and 43 nm, respectively, whereas the average sizes of cAg-NPs were 8 and 13 nm synthesized with differently designed conditions. For the synthesis of size-controlled, more stable, uniformly distributed particles, the chemical method is desired using a strong chemical reductant such as NaBH4, whereas for the synthesis of variable-sized, widely distributed particles biological method using plant extracts can be
preferred. Our results suggest that cAg-NPs with smaller APS of 8 nm have a significant antimicrobial effect against both \textit{E. coli} (Gram –ve) and \textit{S. aureus} (Gram + ve), whereas bAg-NPs can have a more prominent effect even with larger APS of 43 nm. Therefore Ag-NPs mostly exhibit size-dependent antimicrobial activity with few exceptional factors such as the chemical environment of the synthesized NPs as well as nature and adsorption of capping agent used in the synthesis process. Thus, in this study, the biological method was found to be competitive to the chemical method of synthesis with promising antimicrobial potential besides other factors such as easy to synthesize, non-toxic in nature, safer to handle, cheap, and potential to be applied for large scale synthesis.

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\textbf{Author’s contributions}

KK was involved in the laboratory analysis and interpretation of data. SJ reviewed and approved the final manuscript.

\textbf{Declaration of conflicting interests}

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\textbf{References}

1. Xu J, Han X, Liu H, et al. Synthesis and optical properties of silver nanoparticles stabilized by gemini surfactant. \textit{Colloids Surf A Physicochem Eng Asp} 2006; 273(1–3): 179–183.
2. Dong XY, Gao ZW, Yang KF, et al. Nanosilver as a new generation of silver catalysts in organic transformations for efficient synthesis of fine chemicals. \textit{Catal Sci Technol} 2015; 5(5): 2554–2574.
3. Królikowska A, Kudelski A, Michota A, et al. SERS studies on the structure of thioglycolic acid monolayers on silver and gold. \textit{Surf Sci} 2003; 532: 227–232.
4. Naz SS, Shah MR, Islam NU, et al. Synthesis and bioactivities of silver nanoparticles capped with 5-Amino-?-resorcylic acid hydrochloride dihydrate. \textit{J Nanobiotechnol} 2014; 12(1): 1–8.
5. Yan X, He B, Liu L, et al. Antibacterial mechanism of silver nanoparticles in Pseudomonas aeruginosa: proteomics approach. *Metallomics* 2018; 10(4): 557–564.
6. Prabhu S and Poulose EK. Silver nanoparticles: mechanism of antimicrobial action, synthesis, medical applications, and toxicity effects. *Int Nano Lett* 2012; 2(1): 1.
7. Das B, Tripathy S, Adhikary J, et al. Surface modification minimizes the toxicity of silver nanoparticles: an in vitro and in vivo study. *J Biol Inorg Chem* 2017; 22(6): 893–918.
8. Verma A and Mehata MS. Controllable synthesis of silver nanoparticles using Neem leaves and their antimicrobial activity. *J Radiat Res Appl Sci* 2018; 9(1): 109–115.
9. Iravani S, Korbekandi H, Mirmohammadi SV, et al. Synthesis of silver nanoparticles: chemical, physical and biological methods. *Res Pharm Sci* 2014; 9(6): 385–406.
10. Gong J, Liu H, Jiang Y, et al. In-situ synthesis of Ag nanoparticles by electron beam irradiation. *Mater Charact* 2015; 110: 1–4.
11. Guzmán MG, Dille J and Godet S. Synthesis of silver nanoparticles by chemical reduction method and their antibacterial activity. *Int J Mater Metall Eng* 2008; 2(7): 91–98.
12. Ahmad A, Mukherjee P, Senapati S, et al. Extracellular biosynthesis of silver nanoparticles using the fungus Fusarium oxysporum. *Colloids Surf B Biointerfaces* 2003; 28(4): 313–318.
13. Gavhane AJ, Padmanabhan P, Kamble SP, et al. Synthesis of silver nanoparticles using extract of neem leaf and triphala and evaluation of their antimicrobial activities. *Int J Pharm Bio Sci* 2012; 3(3): 88–100.
14. El-Naggar ME, Shaheen TI, Fouda MM, et al. Eco-friendly microwave-assisted green and rapid synthesis of well-stabilized gold and core–shell silver–gold nanoparticles. *Carbohydr Polym* 2016; 136: 1128–1136.
15. Korbekandi H, Iravani S and Abbasi S. Optimization of biological synthesis of silver nanoparticles using Lactobacillus casei subsp. casei. *J Chem Technol Biotechnol* 2012; 87(7): 932–937.
16. Ahmed S, Ahmad M, Swami BL, et al. A review on plants extract mediated synthesis of silver nanoparticles for antimicrobial applications: a green expertise. *J Adv Res* 2016; 7(1): 17–28.
17. Sharma VK, Yngard RA and Lin Y. Silver nanoparticles: green synthesis and their antimicrobial activities. *Adv Colloid Interface Sci* 2009; 145(1–2): 83–96.
18. Ahmed S, Saifullah Ahmad M, et al. Green synthesis of silver nanoparticles using Azadirachta indica aqueous leaf extract. *J Radiat Res Appl Sci* 2016; 9: 1–7.
19. Jamdagni P, Rana JS and Khatri P. Comparative study of antifungal effect of green and chemically synthesised silver nanoparticles in combination with carbendazim, mancozeb, and thiram. *IET Nanobiotechnol* 2018; 12(8): 1102–1107.
20. Amendola V, Bakr OM and Stellacci F. A study of the surface plasmon resonance of silver nanoparticles by the discrete dipole approximation method: effect of shape, size, structure, and assembly. *Plasmonics* 2010; 5(1): 85–97.
21. Banerjee P, Satapathy M, Mukhopahayay A, et al. Leaf extract mediated green synthesis of silver nanoparticles from widely available Indian plants: synthesis, characterization, antimicrobial property and toxicity analysis. *Bioresour Bioprocess* 2014; 1(1): 1.
22. Tripathy A, Raichur AM, Chandrasekaran N, et al. Process variables in biomimetic synthesis of silver nanoparticles by aqueous extract of Azadirachta indica (Neem) leaves. *J Nanopart Res* 2010; 12(1): 237–246.
23. Prathna TC, Chandrasekaran N, Raichur AM, et al. Biomimetic synthesis of silver nanoparticles by Citrus limon (lemon) aqueous extract and theoretical prediction of particle size. *Colloids Surf B Biointerfaces* 2011; 82(1): 152–159.

24. Chung IM, Park I, Seung-Hyun K, et al. Plant-mediated synthesis of silver nanoparticles: their characteristic properties and therapeutic applications. *Nanoscale Res Lett* 2016; 11(1): 1–4.

25. AbdelRahim K, Mahmoud SY, Ali AM, et al. Extracellular biosynthesis of silver nanoparticles using Rhizopus stolonifer. *Saudi J Biol Sci* 2017; 24(1): 208–216.

26. Agnihotri S, Mukherji S and Mukherji S. Size-controlled silver nanoparticles synthesized over the range 5–100 nm using the same protocol and their antibacterial efficacy. *RSC Adv* 2014; 4(8): 3974–3983.

27. Logaranjan K, Raiza AJ, Gopinath SC, et al. Shape-and size-controlled synthesis of silver nanoparticles using Aloe vera plant extract and their antimicrobial activity. *Nanoscale Res Lett* 2016; 11(1): 1–9.

28. Feng A, Cao J, Wei J, et al. Facile synthesis of silver nanoparticles with high antibacterial activity. *Materials* 2018; 11(12): 2498.

29. Murugan T, Wins JA and Murugan M. Antimicrobial activity and phytochemical constituents of leaf extracts of Cassia auriculata. *Indian J Pharm Sci* 2013; 75(1): 122.

30. Hajipour MJ, Fromm KM, Ashkarran AA, et al. Antibacterial properties of nanoparticles. *Trends Biotechnol* 2012; 30(10): 499–511.

31. Zhou Y, Kong Y, Kundu S, et al. Antibacterial activities of gold and silver nanoparticles against *Escherichia coli* and bacillus Calmette-Guérin. *J Nanobiotechnology* 2012; 10(1): 1–9.

32. Lok CN, Ho CM, Chen R, et al. Silver nanoparticles: partial oxidation and antibacterial activities. *J Biol Inorg Chem* 2007; 12(4): 527–534.

33. Tippayawat P, Phromviyo N, Boueroy P, et al. Green synthesis of silver nanoparticles in aloe vera plant extract prepared by a hydrothermal method and their synergistic antibacterial activity. *PeerJ* 2016; 4: e2589.

34. Zhao CM and Wang WX. Importance of surface coatings and soluble silver in silver nanoparticles toxicity to *Daphnia magna*. *Nanotoxicology* 2012; 6(4): 361–370.

35. Ajitha B, Reddy YA, Shameer S, et al. *Lantana camara* leaf extract mediated silver nanoparticles: antibacterial, green catalyst. *J Photochem Photobiol B* 2015; 149: 84–92.

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