IET Nanobiotechnology

Research Article

Nitrobacter sp. extract mediated biosynthesis of Ag_2O NPs with excellent antioxidant and antibacterial potential for biomedical application

ISSN 1751-8741
Received on 8th November 2015
Revised on 3rd April 2016
Accepted on 8th April 2016
doi: 10.1049/iet-nbt.2015.0097
www.ietdl.org

Gopalu Karunakaran[1,2] ✉, Matheswaran Jagathambal[3], Alexander Gusev[1,4], Nguyen Van Minh[1], Evgeny Kolesnikov[1], Arup Ratan Mandal[1], Denis Kuznetsov[1]

[1]*Department of Functional Nanosystems and High-Temperature Materials, National University of Science and Technology' MISiS', Leninskiy Pr. 4, Moscow 119049, Russia*
[2]*Department of Biotechnology, K. S. Rangasamy College of Arts and Science, Tiruchengode 637215, Tamil Nadu, India*
[3]*Department of Bio-chemistry/Bio-technology/Bio-informatics, Avinashilingam Institute for Home Science and Higher Education for Women, Mettupalayam Road, Bharathi Park Road, Coimbatore 641 043, India*
[4]*G. R. Derzhavin Tambov State University, 33, Internatsionalnaya Street, Tambov 392000, Russia*
✉ *E-mail: karunakarang5@gmail.com*

Abstract: In this study, extracellular extract of plant growth promoting bacterium, Nitrobacter sp. is used for the bioconversion of $AgNO_3$ (silver nitrate) into Ag_2O (silver oxide nanoparticles). It is an easy, ecofriendly and single step method for Ag_2O NPs synthesis. The bio-synthesized nanoparticles were characterized using different techniques. UV-Vis results showed the maximum absorbance around 450 nm. XRD result shows the particles to have faced centered cubic (fcc) crystalline nature. FTIR analysis reveals the functional groups that are involved in bioconversion such as C–N, N–H and C=O. Energy-dispersive X-ray spectroscopy (EDAX) spectrum confirms that the prepared nanoparticle is Ag_2O NPs. Particle size distribution result reveals that the average particle size is around 40 nm. The synthesized Ag_2O NPs found to be almost spherical in shape. Biosynthesized Ag_2O NPs possess good antibacterial activity against selected Gram positive and Gram negative bacterial strains namely Salmonella typhimurium, Staphylococcus aureus, Escherichia coli and Klebsiella pneumoniae when compared to standard antibiotic. In addition, Ag_2O NPs exhibits excellent free radical scavenging activity with respect to dosage. Thus, this study is a new approach to use soil bacterial extract for the production of Ag_2O NPs for biomedical application.

1 Introduction

Currently, nanotechnology is promising and upcoming area which deals with nanoparticles for various applications. In recent times, biosynthesis of nanoparticles has quickly augmented using different sources such as different microorganisms and plant extracts due to its cost effective, less time consuming and eco-friendly route [1]. Compare with almost all nanoparticles, silver oxide nanoparticles (Ag_2O NPs) comprise efficient and guarantee different applications in the area of nanotechnology such as catalytic, antibacterial properties and electrical conductivity [2].

There are different approaches which are widely used in the production of Ag_2O NPs, such as chemical and physical methods. However, these methods are not much promising due to the use of toxic chemicals as stabilising and reducing agents. Hence, the biosynthesis approach is the best reliable and eco-friendly approach for the synthesis of silver oxide nanoparticles [3]. The utilisation of bacterial extracellular extracts as stabilising and reducing agents was found to be more beneficial than the use of other bio sources because it is very easy to use and a more eco-friendly approach [4].

Several modern researches mainly focused to utilise different bacteria as prospective agents for the biosynthesis of silver oxide nanoparticles such as *Bacillus cereus* [5], *Chryseobacterium artocarpi* [6], *Lactobacillus casei* [7], *Escherichia coli* [8] and *Pseudomonas putida* [9]. However, till date, there is no evidence about the use of *Nitrobacter sp.* for the biosynthesis of Ag_2O NPs.

Nitrobacter sp. is a plant growth promoting bacterium which is Gram negative, rod shaped, chemoautotrophic and motile [10]. This bacterium is well known to have the capacity to promote the growth of plants by nitrogen fixation [11]. The bacterium requires different nitrite oxidoreductase enzymes for nitrogen fixation [12]. Thus, this essential soil bacterium can be a useful candidate for Ag_2O NPs production.

Thus, keeping the above findings in mind, we have planned our experiment for the synthesis of Ag_2O NPs using *Nitrobacter sp.* extracellular extract. This plan of research will provide a new area of biosynthesis using a novel, non-toxic and eco-friendly approach for the production of Ag_2O NPs. The produced Ag_2O NPs will also be used to analyse its antibacterial and antioxidant potentials.

2 Materials and methods

2.1 Materials used

Silver nitrate ($AgNO_3$) was procured from Sigma Aldrich (ACS reagent) with 99% purity and was used without any additional purification steps. Mueller Hinton Agar, Stanier's medium and 2, 2-Diphenyl-1-picrylhydrazyl (DPPH) were procured from HiMedia Laboratories, Mumbai, India for the analysis of antibacterial and antioxidant activities.

2.2 Biosynthesis of Ag_2O NPs

Nitrobacter sp. (NCIM 5067), the bacterium used in the experiment was obtained from the National Collection of Industrial

Microorganisms (NCIM), Pune, India. Stanier's modified medium (250 ml), a special medium for the bacterium was inoculated with the culture, followed by incubation at 37°C for 24 h in a shaker at 300 rpm. After incubation, the medium was centrifuged at 6000 rpm for 30 min to collect the supernatant (extracellular extract). The obtained supernatant was further used for the biosynthesis of Ag_2O NPs.

The extracellular extract and 0.1 M silver nitrate were mixed in the volume ratio of 1:1 and was incubated for 24 h under dark condition for the bioconversion. After 24 h of incubation, the change in colour from light brown to dark colloidal brown was observed (Fig. 1). The colloidal brown solution was precipitated by centrifugation at 6000 rpm for 30 min. The supernatant was removed and the obtained pellet was re-suspended 3 times in deionised water and centrifuged again at 6000 rpm for 30 min to achieve a clear supernatant. Thus, the obtained final pellet was dried in a hot air oven for 24 h at 60° C. The dried powder was further crushed using mortar and pestle into a fine powder and kept in a dry container and used further for its complete characterisation.

2.3 Characterisation of Ag_2O NPs

To study the different properties of Ag_2O NPs, the powder was subjected to different characterisation techniques. The bioconversion of Ag_2O NPs was confirmed via evaluating the wave length of the powder suspension by UV-Visible spectrum under spectrophotometer (LAMBDA 35, PerkinElmer, USA). The scanning range for the sample was between 200–800 nm at a speed of 480 nm per minute. The nature and the crystalline phase of the reduced Ag_2O NPs samples were identified by X-ray powder diffraction patterns (X'Pert PRO; PANalytical, the Netherlands) using CuKα as a radiation (l51.54060 Å) source. The diffractometer was scanned in the 2θ range from 10° to 80° at scanning rate of 5° min^{-1}. The observed peak positions and the relative intensities of the powder pattern were identified in comparison with the reference powder diffraction data.

The presence of functional groups and chemical bonds on the surface of Ag_2O NPs was investigated using Fourier transform infrared (FTIR) spectrophotometer (Spectrum 100; PerkinElmer, USA). The sample spectrum was recorded between the ranges of 4000–400 cm^{-1} at a resolution of 4 cm^{-1}. The presence of silver was confirmed by energy dispersive spectrum (EDS) (EDX-720; Shimadzu, Japan). The samples were focused directly at 10 mm/5 mm on a thin film of Mylar without further sample preparation. Particle size distribution (PSD) was used to determine the particle size using particle size analyser (Nanophox; Sympatec, Germany) using the well-known dynamic light-scattering technique (DLS). Transmission electron microscopy (TEM) was used to figure out the dimension and the form of Ag_2O NPs. The sample was analysed using high resolution TEM (TEMCM200; Philips, USA). For analysis, the well dispersed samples were loaded on the copper grids and scanned at 120 kV. Particle size was then manually calculated by taking into account, the maximum and minimum particle size as marked in the observed image.

2.4 Antibacterial activity

Antibacterial activity of the synthesised Ag_2O NPs was determined against selected Gram positive and Gram negative clinical bacterial strains (collected from NCIM, India) such as *Salmonella typhimurium* (NCIM 2501), *Staphylococcus aureus* (NCIM 2127), *E. coli* (NCIM 2065) *and Klebsiella pneumoniae* (NCIM 2883) using Kirby–Bauer disk diffusion method [13] in Muller–Hinton Agar medium. Sterile media (10 ml) was prepared and poured into petri dishes. Once the medium got solidified, 100 µl of freshly prepared overnight inoculums were spread on to it, using sterile swabs. Sterile filter disc was loaded over the solid medium, separated at equal distance. The prepared nanoparticles suspension was loaded over the disk under aseptic condition. The plates were subsequently incubated at 37°C for 24 to 48 h after which the zone of inhibition (in mm diameter) was measured and tabulated.

2.5 Antioxidant activity

Antioxidant activity of the Ag_2O NPs was analysed by following DPPH method available in literature with minor modifications [14]. Different masses of Ag_2O NPs such as 1, 5, 10 and 100 mg was taken and mixed with 1.7 ml of freshly prepared DPPH solution and vortexed for about 3 min. Control (C) tube contained only DPPH reagent whereas Ag_2O NPs added tubes served as test (T). The mixture was then incubated for 30 min at room temperature followed by centrifugation at 1000 rpm for 2 min. The absorbance of the supernatant was then measured using UV–Vis spectrophotometer (U-2900/2910, Japan) at 517 nm using methanol as blank solution. The inhibition percentage of the Ag_2O NPs was calculated by applying the formula as given below

$$\text{Inhibition percentage (\%)} = \frac{C_A - T_A}{C_A} \times 100$$

whereas, C_A (control) and T_A (test) absorbance.

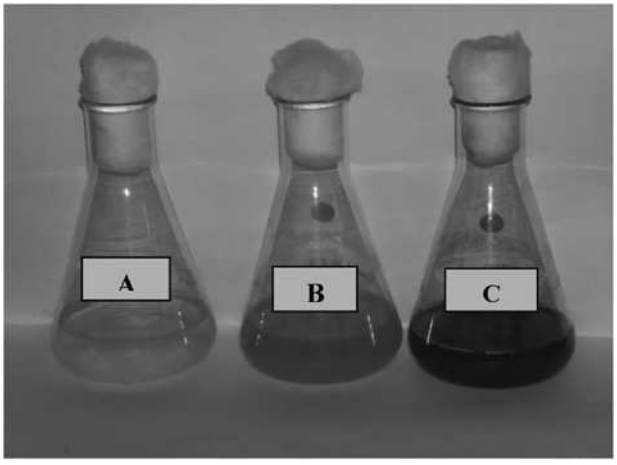

Fig. 1 *Biosynthesis of Ag_2O NPs, A: $AgNO_3$, B: Extracellular extract and C: Ag_2O NPs*

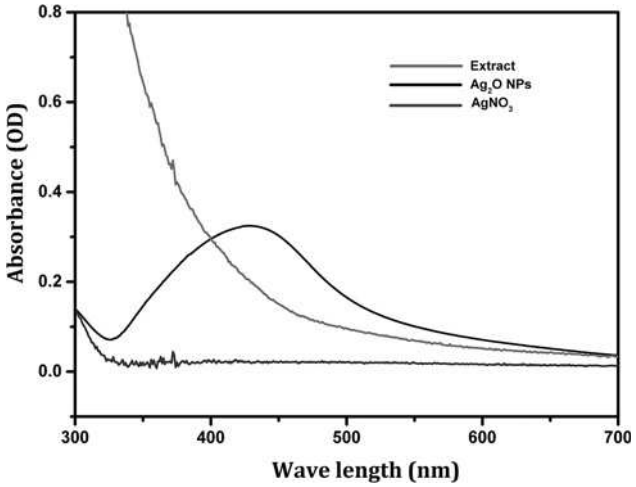

Fig. 2 *UV-Visible absorbance of Ag_2O NPs*

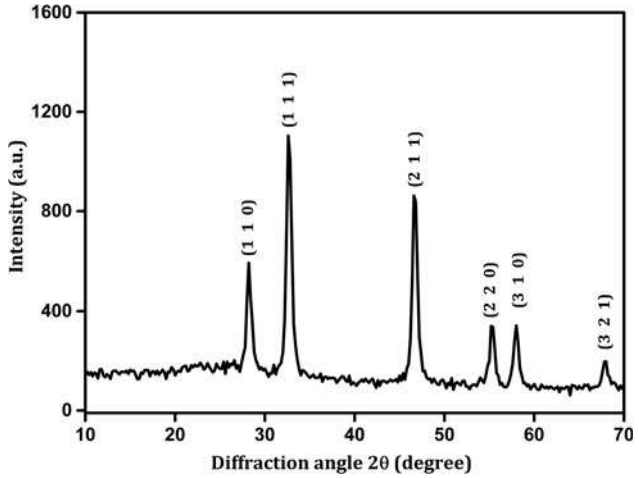

Fig. 3 *XRD pattern of Ag_2O NPs*

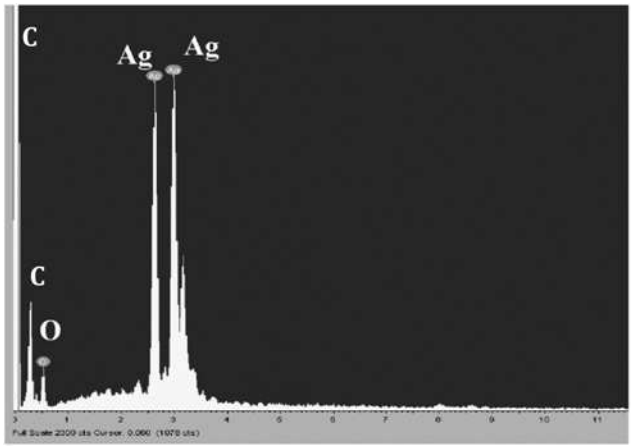

Fig. 5 *Elemental analysis (EDS) of Ag_2O NPs*

3 Results and discussion

3.1 UV-Visible analysis

After 24 h of incubation of the extracellular extract and 01 M silver nitrate mixture, the change in colour (Fig. 1) of the solution from yellow to dark brown indicated the bioconversion of silver nitrate into Ag_2O NPs. The change in colour might be due to the changes in the excitation energy of the particle's surface plasma resonance. Similar result was observed for *Bacillus licheniformis* and *Fusarium oxysporum*, used for the production of silver nanoparticles [15, 16]. In addition, the formation of nanoparticles may be attributed to the nitrate reductase enzyme or the proteins in the supernatant [17, 18]. The prepared powder was dispersed in deionised water for UV-Visible analysis. UV-Visible analysis (Fig. 2) showed that the wavelength for silver nanoparticles production ranged between 350 to 450 nm whereas for extracellular extract and $AgNO_3$ no absorption peak was observed. The shift of colour is due to the variation in shape and size of the silver nanoparticles [19].

3.2 XRD analysis

The XRD analysis result shown in Fig. 3 revealed the crystalline nature of the prepared Ag_2O NPs. The observed 2θ values were well matched with ICDD standard data (JCPDS File No: 43-0997), which clearly indicated that the synthesised nanoparticles (Ag_2O) had cubic crystal system. XRD pattern exhibited typical reflections from (110), (111), (211), (220), (310), and (321) Miller's planes at ~28°, ~32°, ~48°, ~54°, ~58° and ~68°, respectively, and correlated with the literature results [20].

3.3 FTIR analysis

FTIR result as represented in Fig. 4 revealed the presence of functional groups in the synthesised Ag_2O NPs. The presence of proteins was observed in the range of 1036–1150 cm^{-1}. The signature of phenols was indicated in the band 1761 cm^{-1}. Presence of esterified groups was revealed in the peak of 1330 cm^{-1} [21]. Peak at 3456 cm^{-1} indicated the existence of hydroxyl group from phenol. In addition, an asymmetrical band of methyl and methylene was observed at 2411 and 2733 cm^{-1} [21]. An intense sharp peak between 817–1761 cm^{-1} was due to the reduction of Ag^+ to Ag^0. Thus, from the above observations, it was clear that the functional groups in the supernatant could be responsible for the bioconversion of Ag_2O NPs. These functional groups might have acted as reducing, capping and stabilising agents during Ag_2O NPs formation. This result coincided with a study in which *Lactobacillus casei* was being used in the biosynthesis of silver nanoparticles [7].

3.4 Energy-dispersive X-ray spectroscopy (EDAX) analysis

EDAX analysis shown in Fig. 5 showed the strong signals due to surface plasma resonance at 2.98 keV. EDAX results clearly demonstrated the synthesis of Ag_2O NPs. The surface of the Ag_2O NPs showed the presence of oxygen and carbon which might be

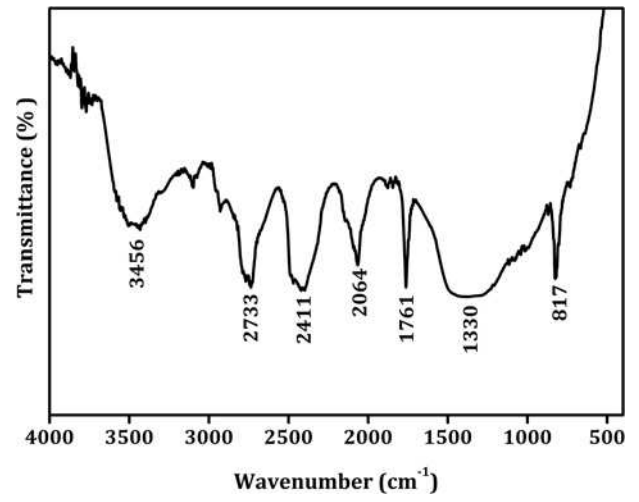

Fig. 4 *FTIR spectrum of Ag_2O NPs*

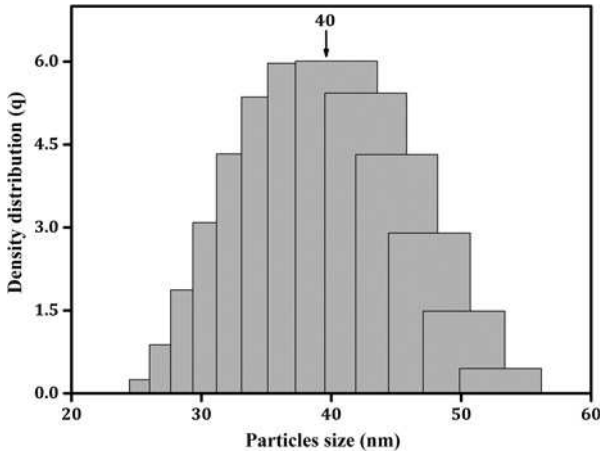

Fig. 6 *PSD of Ag_2O NPs*

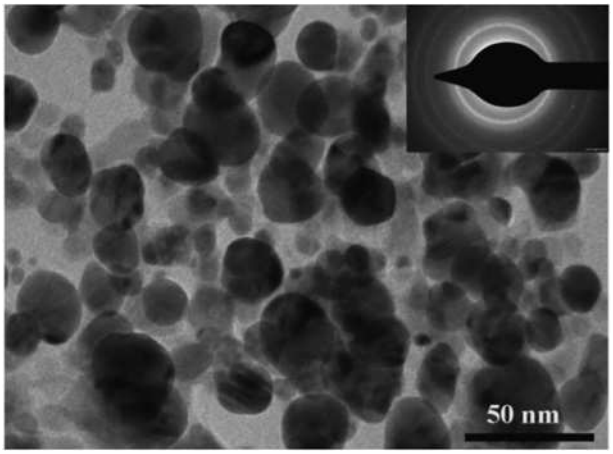

Fig. 7 *TEM image of Ag_2O NPs*

due to the influence of organic molecules in the extract used for synthesis. The two peaks observed below 1 keV indicated the presence of carbon as previously reported [22, 23]. The obtained result was in well agreement with the available reports wherein silver nanoparticles was synthesised using *E. coli* [8] and *Pseudomonas putida* [9].

3.4 DLS analysis

To determine the dimension and distribution of the Ag_2O NPs, DLS method was employed. The sample graphs (Fig. 6) clearly depicted the average PSD of Ag_2O NPs was to be 40 nm, respectively.

3.5 TEM imaging

TEM analysis (Fig. 7) of Ag_2O NPs samples reveals that the nanoparticles share ellipsoidal and spherical morphology. The diameter of the particles is found to be in the range of 20 to 40 nm. The respective patterns in SAD are obtained with diffraction rings with the spots and d-spacing indexed as a crystalline structure [faced centred cubic (fcc)] according to JCPDS file 43-0997. In the analysis, it is evident that the Ag_2O NPs appears to have good crystalline structure, as the patterns of SAD reveal a strong existence of bright spots along the crystal orientations appearing in the diffraction rings. The measurement of the diameters from the centre along the rings was consistent with the d-spacing and corresponds with fcc phase of the ions of silver as determined in the XRD.

3.6 Antibacterial activity

The antibacterial potential of the prepared Ag_2O NPs was analysed using well known disk diffusion method. The observed results are depicted in Fig. 8 and given in Table 1. It is clear that, as the concentration of the Ag_2O NPs was increased, there was an increase in the zone of inhibition when compared with the standard antibiotic streptomycin. The antibacterial property also depended on the type of the bacterium used. In our study, we have used two Gram positive bacteria (*S. typhimurium* and *S. aureus*), and two Gram negative bacteria *(E. coli and K. pneumoniae*). The result revealed difference in antibacterial activity of the Ag_2O NPs with respect to the bacterium used. The Gram positive bacteria *S. typhimurium* and *S. aureus* showed less zone of inhibition that is of 12 ± 0.54 mm and 12 ± 0.28 because of the presence of thick peptidoglycan on the surface of the cell, which inhibited the entry of Ag_2O NPs inside the bacterial cell [24]. In contrast, Ag_2O NPs

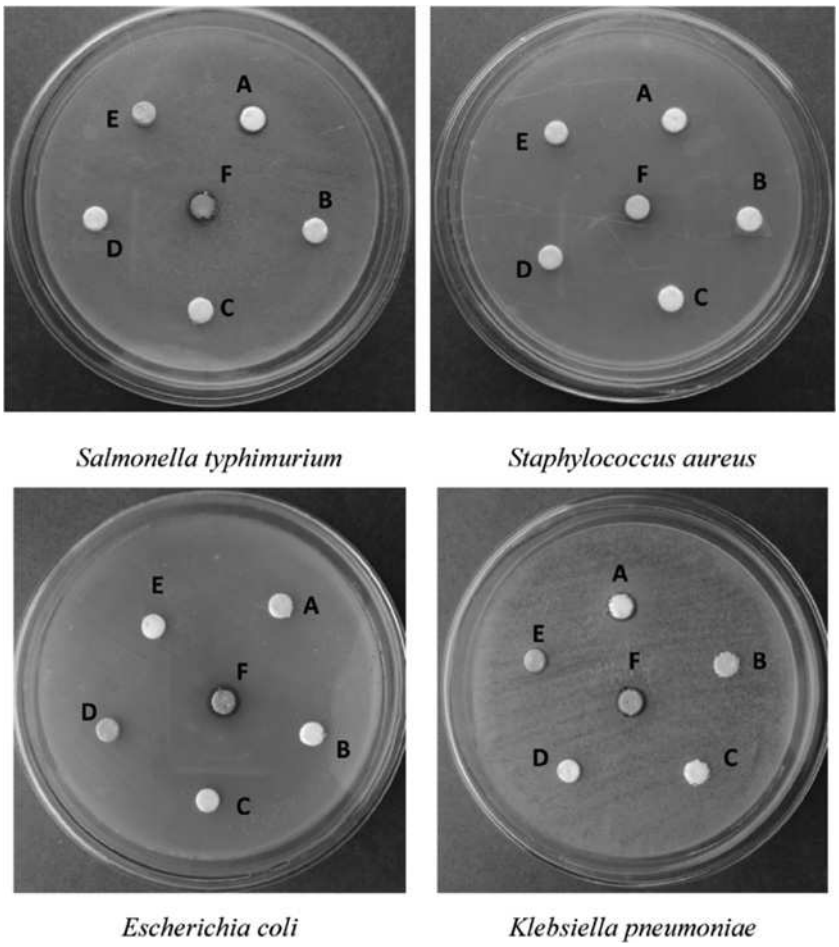

Fig. 8 *Antibacterial activity of Ag_2O NPs (A: 100 μg/ml antibiotic, B: Extracellular extract, C&D: only disk, E: distilled water, F: 100 μg/ml Ag_2O NPs)*

IET Nanobiotechnol., 2016, Vol. 10, Iss. 6, pp. 425–430

Table 1 Antibacterial activity of Ag_2O NPs synthesised from *Nitrobacter sp.* extracellular extract

Microorganisms	Zone of inhibition [Mean ± SD (mm)]	
	Streptomycin (100 µg/ml)	Ag_2O NPs (100 µg/ml)
S. typhimurium	12 ± 0.45	12 ± 0.54
S. aureus	11 ± 0.16	12 ± 0.28
E. coli	10 ± 0.47	14 ± 0.15
K. pneumoniae	12 ± 0.58	13 ± 0.45

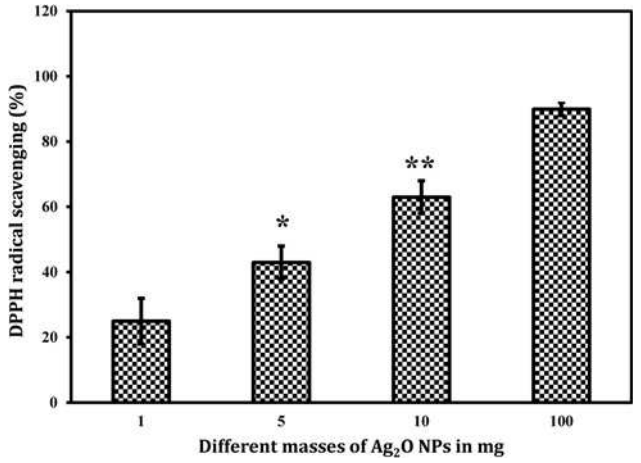

Fig. 9 *Antioxidant activity of Ag_2O NPs (*, ** Represents level of significant at $p < 0.05$)*

found to exhibit higher order of inhibition against Gram negative bacteria *E. coli and K. pneumoniae*, which was about 14 ± 0.15 mm and 13 ± 0.45 mm size. This was because, Gram negative bacterium possess thin layer of peptidoglycan, which made it easier for the Ag_2O NPs to enter the cell and kill it [25]. Thus, this result clearly showed that, antibacterial activity not only depends on the type of particle, but also on the surface composition of the tested bacterium. In a recent study, it was shown that the antibacterial activity was due to the electrostatic interaction between the bacterial cell wall and Ag_2O NPs [26].

3.7 Antioxidant activity

The free radical scavenging activity of synthesised Ag_2O NPs is shown in Fig. 9. It was observed that as the concentration of the Ag_2O NPs increased, its scavenging activity also increased correspondingly: 25% (1 mg), 43% (5 mg), 63% (10 mg) and 90% (100 mg). Similar antioxidant activity was being reported for iron and nickel oxide nanoparticles [27, 28]. In living systems, free radicals are being generated continuously due to various biochemical reactions. Biosynthesised Ag_2O NPs will be very helpful to scavenge these free radicals [29]. The small size with high surface area of these particles helps them to react easily with the free radicals and thereby scavenging them. Currently in the field of biomaterial and medicine, generation of free radicals is being considered a serious issue [30]. Hence, these Ag_2O NPs could be used as an additive in biomedical applications to scavenge the free radicals.

4 Conclusions

In the present investigation, a search for new source for the production of Ag_2O NPs is successfully being carried out. This method is very rapid, eco-friendly, economical and easy for the synthesis of Ag_2O NPs. In addition, this is a single step method without the use of toxic chemicals and less time consuming. By this method, we have successfully synthesised Ag_2O NPs with unique size and morphology. The bacterial extracts act as reducing, capping and stabilising agents. FTIR analysis evidenced about the organic groups involved in the bioconversion of Ag_2O NPs. XRD pattern reveals that the produced Ag_2O NPs are crystalline with face centred geometry. From the EDAX spectrum analysis, it is clear that the prepared nanoparticle is Ag_2O NPs. TEM analysis reveals that the synthesised Ag_2O NPs found to be almost spherical in shape. The antibacterial susceptibility assay reveals that the Ag_2O NPs exhibits good antibacterial property against both Gram negative and Gram positive bacteria such as *S. typhimurium, S. aureus, E. coli and K. pneumoniae* when compared with standard antibiotic streptomycin. In addition, Ag_2O NPs possess good antioxidant potential which is dose dependent. Thus, these findings will serve as a base for the development of Ag_2O NPs into nano-medicine for the treatment of bacterial diseases, in future.

5 Acknowledgment

The authors gratefully acknowledge the financial support of the Ministry of Education and Science of the Russian Federation in the framework of Increase Competitiveness Program of NUST «MISiS» (№ К4-2015-017).

6 References

1 Dhand, V., Soumya, L., Bharadwaj, S., *et al.*: 'Green synthesis of silver nanoparticles using *Coffea arabica* seed extract and its antibacterial activity', *Mater. Sci. Eng. C*, 2016, **58**, pp. 36–43
2 Castro, L., Blazquez, M.L., Gonzalez, F., *et al.*: 'Biosynthesis of silver and platinum nanoparticles using orange peel extract: characterisation and applications', *IET Nanobiotechnol.*, 2015, **9**, pp. 252–258
3 Mittal, A.K., Tripathy, D., Choudhary, A., *et al.*: 'Bio-synthesis of silver nanoparticles using *Potentilla fulgens Wall.* ex Hook. and its therapeutic evaluation as anticancer and antimicrobial agent', *Mater. Sci. Eng. C*, 2015, **53**, pp. 120–127
4 Paul, B., Bhuyan, B., Purkayastha, D.D., *et al.*: 'Photocatalytic and antibacterial activities of gold and silver nanoparticles synthesized using biomass of *Parkia roxburghii* leaf', *J. Photochem. Photobiol. B Biol.*, 2016, **154**, pp. 1–7
5 Sunkar, S., Nachiyar, C.V.: 'Biogenesis of antibacterial silver nanoparticles using the endophytic bacterium *Bacillus cereus* isolated from *Garcinia xanthochymus*', *Asian Pac. J. Tropical Biomed.*, 2012, **2**, pp. 953–959
6 Venil, C.K., Sathishkumar, P., Malathi, M., *et al.*: 'Synthesis of flexirubin-mediated silver nanoparticles using Chryseobacterium artocarpi CECT 8497 and investigation of its anticancer activity', *Mater. Sci. Eng. C*, 2016, **59**, pp. 228–234
7 Korbekandi, H., Iravani, S., Abbasi, S.: 'Optimization of biological synthesis of silver nanoparticles using *Lactobacillus casei* subsp. casei', *J. Chem. Technol. Biotechnol.*, 2012, **87**, pp. 932–937
8 Ghorbani, H.R.: 'Biosynthesis of silver nanoparticles by *Escherichia coli*', *Asian J. Chem.*, 2013, **25**, pp. 1247–1249
9 Thamilselvi, V., Radha, K.V.: 'Synthesis of silver nanoparticles from *Pseudomonas Putida* NCIM 2650 in silver nitrate supplemented growth medium and optimization using response surface methodology', *Dig. J. Nanomaterials Biostructures*, 2013, **8**, pp. 1101–1111
10 Cakmakc, R.I., Aydin, D.F., Sahin, A.F.: 'Growth promotion of plants by plant growth-promoting rhizobacteria under greenhouse and two different field soil conditions', *Soil Biol. Biochem.*, 2006, **38**, pp. 1482–1487
11 Bock, E., Sundermeyer-Klinger, H., Stackebrandt, E.: 'New facultative lithoautotrophic nitrite-oxidizing bacteria', *Arch. Microbiol.*, 1983, **136**, pp. 281–284
12 Aamand, J., Ahl, T., Spieck, E.: 'Monoclonal antibodies recognizing nitrite oxidoreductase of *Nitrobacter hamburgensis*', *Appl.ied and Environ. Microbiol.*, 1996, **67**, pp. 2352–2365
13 Bauer, A.W., Kirby, W.M., Sherris, J.C., *et al.*: 'Antibiotic susceptibility testing by a standardized single disk method', *Am. J. Clin. Pathol.*, 1966, **45**, pp. 493–496
14 Serpen, A., Capuano, E., Fogliano, V., *et al.*: 'A new procedure to measure the antioxidant activity of insoluble food components', *J. Agric. Food Chem.*, 2007, **55**, pp. 7676–7681
15 Kalimuthu, K., Deepak, V., Ramkumarpandian, S., *et al.*: 'Extracellular biosynthesis of silver nanoparticles by the culture supernatant of *Bacillus licheniformis*', *Mater. Lett.*, 2014, **62**, pp. 4411–4413
16 Ahmad, A., Mukherjee, P., Senapati, S., *et al.*: 'Extracellular biosynthesis of silver nanoparticles using the fungus *Fusarium oxysporum*', *Colloids Surf. B*, 2003, **28**, pp. 313–318
17 Morones, J.R., Elechiguerra, J.L., Camacho, A., *et al.*: 'The bactericidal effect of silver nanoparticles', *Nanotechnology*, 2005, **16**, pp. 2346–2353

18 Kalimuthu, K., Suresh Babu, R., Venkataraman, D., *et al*.: 'Biosynthesis of silver nanocrystals by *Bacillus licheniformis*', *Colloids Surf. B Biointerfaces*, 2008, **65**, pp. 150–153
19 Kiruba Daniel, S.C.G., Abirami, J., Kumaran, S., *et al*.: 'Microbicidal tissue paper using green synthesized silver nanoparticles', *Current Nanosci.*, 2015, **11**, pp. 64–68
20 Jeeva, K., Thiyagarajan, M., Elangovan, V., *et al*.: '*Caesalpinia coriaria* leaf extracts mediated biosynthesis of metallic silver nanoparticles and their antibacterial activity against clinically isolated pathogens', *Ind. Crops Prod.*, 2014, **52**, pp. 714–720
21 Nabikhan, A., Kathiresan, K., Anburaj, K., *et al*.: 'Synthesis of antimicrobial silver nanoparticles by callus and leaf extracts from saltmarsh plant, *Sesuvium portulacastrum* L.', *Coll. Surf. B Biointer.*, 2010, **79**, pp. 488–493
22 Das, B., Dash, S.K., Mandal, D., *et al*.: 'Green synthesized silver nanoparticles destroy multidrug resistant bacteria via reactive oxygen species mediated membrane damage', *Arab. J. Chem.*, 2015, Article in press, http://dx.doi.org/10.1016/j.arabjc.2015.08.008
23 Saravanakumar, A., Ganesh, M., Jayaprakash, J., *et al*.: 'Biosynthesis of silver nanoparticles using Cassia tora leaf extract and its antioxidant and antibacterial activities', *J. Ind. Eng. Chem.*, 2015, **28**, pp. 277–281
24 Jayachandra Reddy, N., Nagoor Vali, D., Rani, M., *et al*.: 'Evaluation of antioxidant, antibacterial and cytotoxic effects of green synthesized silver nanoparticles by *Piper longum* fruit', *Mater. Sci. Eng. C*, 2014, **34**, pp. 115–122
25 Shrivastava, S., Bera, T., Roy, A., *et al*.: 'Characterization of enhanced antibacterial effects of novel silver nanoparticles', *Nanotechnology*, 2007, **18**, pp. 225103–225111
26 Abbaszadegan, A., Ghahramani, Y., Gholami, A., *et al*.: 'The effect of charge at the surface of silver nanoparticles on antimicrobial activity against gram-positive and gram-negative bacteria: a preliminary study', *J. Nanomaterials*, 2015, pp. 1–8, ID 720654
27 Paul, S., Saikia, J.P., Samdarshi, S.K., *et al*.: 'Investigation of antioxidant property of iron oxide particlesby 1′-1′ diphenylpicryl-hydrazyle (DPPH) method', *J. Magn. Magn. Mater.*, 2009, **321**, pp. 3621–3623
28 Saikia, J.P., Paul, S., Konwar, B.K., *et al*.: 'Nickel oxide nanoparticles: a novel antioxidant', *Colloids Surf. B.*, 2010, **78**, pp. 146–148
29 Watal, G., Watal, A., Rai, P.K., *et al*.: 'Biomedical applications of nano-antioxidant', *Methods Mol. Biol.*, 2013, **1028**, pp. 147–151
30 Pham-Huy, L.A., He, H., Pham-Huy, C.: 'Free radicals, antioxidants in disease and health', *Int. J. Biomed. Sci.*, 2008, **4**, pp. 89–96