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Beijerinckia indica Extracellular Extract Mediated Green Synthesis of Silver Nanoparticles with Antioxidant and Antibacterial Activities against Clinical Pathogens

Gopalu Karunakaran, Matheswaran Jagathambal, Nguyen Van Minh, Evgeny Kolesnikov, Denis Kuznetsov

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Abstract**—**This work investigated the use of *Beijerinckia indica* extracellular extract for the synthesis of silver nanoparticles using $AgNO_3$. The formation of nanoparticles was confirmed by different methods, such as UV-Vis absorption spectroscopy, XRD, FTIR, EDX, and TEM analysis. The formation of silver nanoparticles (AgNPs) was confirmed by the change in color from light yellow to dark brown. The absorbance peak obtained at 430 nm confirmed the presence of silver nanoparticles. The XRD analysis showed the cubic crystalline phase of the synthesized nanoparticles. FTIR revealed the presence of groups that acts as stabilizing and reducing agents for silver nanoparticles formation. The synthesized silver nanoparticles were generally found to be spherical in shape with size ranging from 5 to 20 nm, as evident by TEM analysis. These nanoparticles were found to inhibit pathogenic bacterial strains. This work proved that the bacterial extract is a potential eco-friendly candidate for the synthesis of silver nanoparticles with promising antibacterial and antioxidant properties.

Keywords**—**Antioxidant activity, antimicrobial activity, *Beijerinckia indica,* characterisation, extracellular extracts, silver nanoparticles.

I. Introduction

AgNPs play a significant role in the field of biology and medicine due to their significant and diverse properties like catalysis, magnetic, and antimicrobial activity [1]. Many methods, such as chemical reduction, polyol process and radiolysis have been employed for the synthesis of AgNPs, but the concern for environmental contaminations is also boosted [2]. There is a need for green chemistry that includes a clean, nontoxic, and environment-friendly method of nanoparticle synthesis for commercial applications [3]. The use of bacterial extracellular extracts as stabilizing and reducing agents was found to be more beneficial than the use of other bio sources because it is very easy to use and an eco-friendlier approach [4]. In recent years, more attention is paid on bacterial strains such as *Pseudomonas putida* [5], *Lactobacillus casei* [6], *Escherichia coli* [7], *Nitrobacter sp.* [8], and *Bacillus cereus* [9] as effective candidates for the biosynthesis of AgNPs. However, to the best of our knowledge till now, there is no evidence about the utilisation of *Beijerinckia indica* for the green synthesis of AgNPs.

Beijerinckia indica is one of the best nitrogen fixing, aerobic acidophilic bacterial strains [10]. It promotes the growth of plants and hence used as bio-fertilizer [11]. The bacterium fixes the nitrogen in the plant by the action of nitrite oxido-reductase enzymes [12]. In the present study, the beneficial microbe *Beijerinckia indica* extracellular extract is used for the biosynthesis of silver nanoparticles. It is a novel, eco-friendly, and low cost method for the synthesis of silver nanoparticles. The prepared silver nanoparticles were characterized and analyzed for its antibacterial activity against pathogenic bacterial strains.

II. Materials and Turning tests

The silver nitrate ($AgNO_3$) was procured from Reachem, Russia, 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.

The bacterium used in this study, *Beijerinckia indica* (NCIM 2096) was obtained from NCIM, Pune, India. 250 ml medium was prepared and the culture was inoculated into it and incubated at 37 °C for 24 hours followed by centrifugation at 6000 rpm for 30 mins to collect the extracellular extract. The extracellular extract and 0.1 M silver nitrate were mixed in the ratio of 1:1 (v/v) and were incubated for 24 hours under dark condition for bioconversion. After 24 hours 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 mins. The supernatant was removed, and the obtained pellet was re-suspended three times in deionized water and centrifuged again at 6000 rpm for 30 mins to obtain a clear supernatant. Thus, the obtained final pellet was dried in a hot

Gopalu Karunakaran is with the Department of Functional Nanosystems and High-temperature Materials, National University of Science and Technology "MISiS", Russia, Moscow, No. 4, Leninsky Prospect, Moscow, Russia and with the Department of Biotechnology, K. S. Rangasamy College of Arts and Science, Tiruchengode-637215, Tamil Nadu, India (corresponding author, phone: +7-985-663-75-69, e-mail: karunakarang5@ gmail.com).

Matheswaran Jagathambal is with the 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.

Nguyen Van Minh, Evgeny Kolesnikov and Denis Kuznetsov are with the Department of Functional Nanosystems and High-temperature Materials, National University of Science and Technology "MISiS", Russia, Moscow, No. 4, Leninsky Prospect, Moscow, Russia.

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air oven for 24 hours at 60 °C. The dried pellet was into a fine powder crushed using mortar and pestle and kept in a dry container and used further for its complete characterization and analysis.

To study the different properties of AgNPs, the powder was subjected to different characterization techniques. The bioconversion of AgNPs was confirmed via evaluating the wave length of the powder suspension by UV-visible spectrum using 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 AgNPs samples were identified by X-ray powder diffraction patterns (X'Pert PRO; PANalytical, the Netherlands) using CuKα as a radiation source (l.5154060 Å). The powder was undergone scanning between the range of 10° to 80° (5° min^{-1} scanning rate) under 2θ. The obtained results were compared with references [8].

The presence of functional groups and chemical bonds on the surface of AgNPs 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. Transmission electron microscopy (TEM) was used to figure out the dimension and the form of AgNPs. The sample was analyzed using high resolution TEM (TEMCM200; Philips, USA), for which the well dispersed samples were loaded on the copper grids and scanned at 120 kV.

Antibacterial activity of the synthesized AgNPs 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), *Escherichia coli* (NCIM 2065) *and Klebsiella pneumoniae* (NCIM 2883) using Kirby-Bauer disk diffusion method [13] in Muller- Hinton agar medium. Sterile medium (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 cotton swabs. Sterile filter disc was placed 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 ºC for 24 to 48 hours after which, the zone of inhibition (in mm diameter) was measured and tabulated.

Antioxidant activity of the AgNPs was analyzed by following DPPH method available in literature with minor modifications [14]. Different masses of AgNPs 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 AgNPs added tubes were served as test (T). The mixture was then incubated for 30 min at room temperature followed by centrifugation at 1000 rpm for 2 mins. 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 AgNPs was calculated by applying the formula as given below, where C_A and T_A are control and test absorbance, respectively:

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

III. Results and Discussion

The change in colour (Fig. 1) of the solution from yellow to dark brown following the incubation of the extracellular extract and 01 M silver nitrate mixture for 24 hours indicated the bioconversion of silver nitrate into AgNPs. The color change might be due to the changes in the excitation energy of the particle's surface plasma resonance. Similar result was observed for the microbial strains *Bacillus licheniformis* and *Fusarium oxysporum*, which were used for AgNPs production [15], [16]. In addition, nitrate reductase enzyme or the bacterial proteins in the supernatant might have facilitated the formation of nanoparticles [17], [18]. The prepared powder was dispersed in deionized water for UV-Visible analysis. AgNPs production was confirmed by absorption between 350 to 450 nm (Fig. 2), whereas the extracellular extract and $AgNO_3$ exhibited no absorption peak. The shift in colour is due to the variation in shape and size of the AgNPs [19].

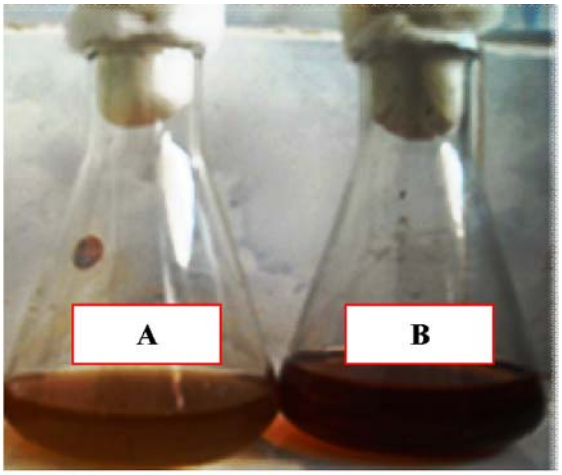


Fig. 1 Biosynthesis of AgNPs, A: Extracellular extract and B: AgNPs formation

The XRD analysis of the synthesized AgNPs, as shown in Fig. 3, revealed its crystalline nature. The observed 2θ values very well coincided with ICDD standard data (JCPDS File No: 43-0997), clearly indicating the cubic crystal system. XRD pattern exhibited Miller's planes at ~35^0, ~45^0, ~55^0, and ~65^0 respectively and correlated with the literature results [20].

FTIR result as depicted in Fig. 4 revealed the presence of various functional groups in the synthesized AgNPs. The presence of proteins was observed in the range of 1036-1150

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cm^{-1}. The band observed at 1761 cm^{-1} and 1330 cm^{-1} indicated the presence of phenol and esterified groups, respectively [21]. The signature of hydroxyl group was confirmed by the peak at 3456 cm^{-1}. In addition, an asymmetrical band of methyl and methylene was observed at 2411 and 2733 cm^{-1} [21]. The intense sharp peak between 817-1761 cm^{-1} is attributed to the reduction of Ag^{+} to Ag^{o}. The above observations clearly showed that the functional groups in the supernatant participated as reducing, capping and stabilizing agents during AgNPs formation. This result coincided with a study, in which *Lactobacillus casei* was being used in the biosynthesis of AgNPs [7].

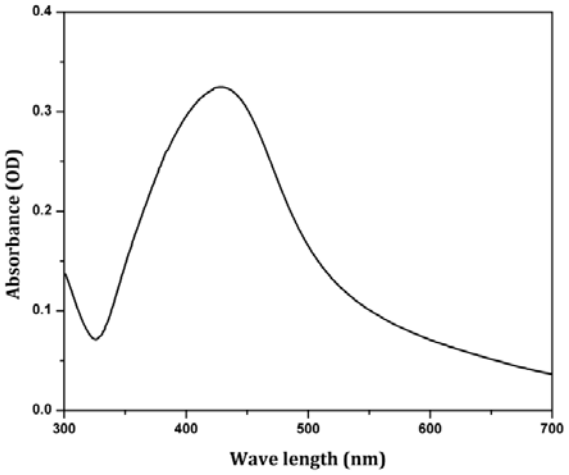


Fig. 2 UV-Vis absorbance of AgNPs

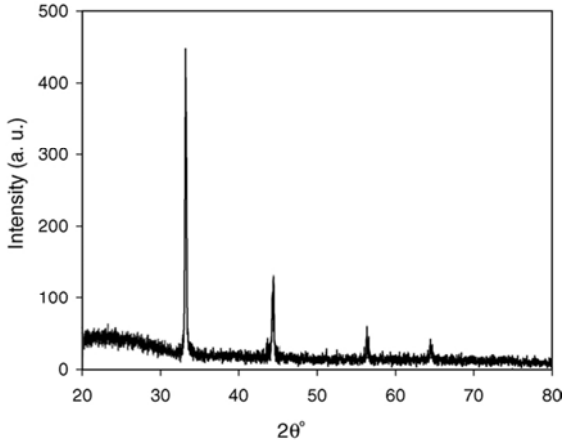


Fig. 3 XRD pattern of AgNPs

EDAX analysis (Fig. 5) showed the strong signals due to surface plasma resonance at 2.98 keV, clearly demonstrating the synthesis of AgNPs. The presence of oxygen and carbon might be 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 accordance with the available reports in which, *Escherichia coli* [8] and *Pseudomonas putida* [9] were used for AgNPs synthesis.

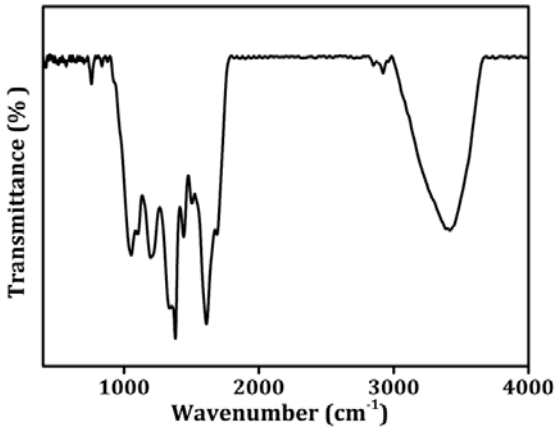


Fig. 4 FTIR spectrum of AgNPs.

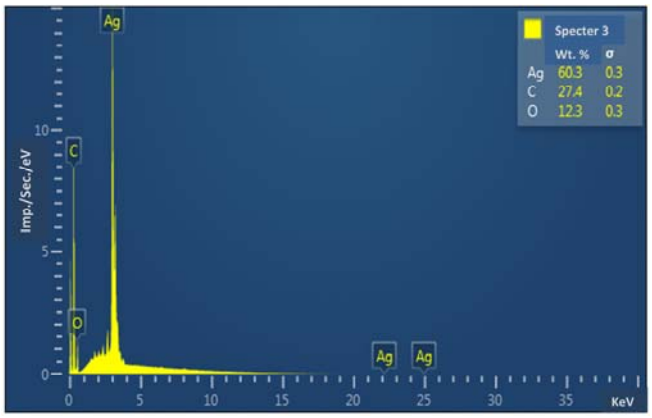


Fig. 5 Elemental analysis (EDS) of AgNPs

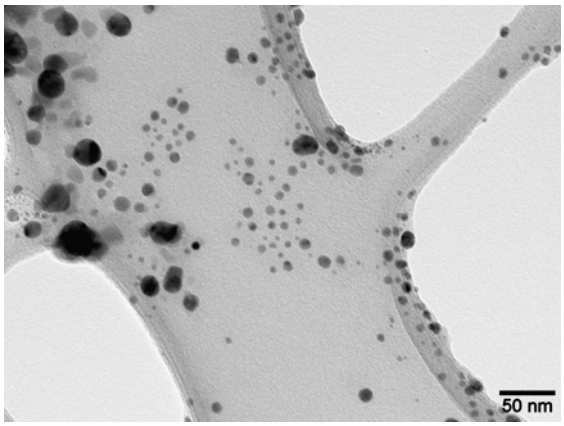


Fig. 6 Transmission electron microscopy image of AgNPs

TEM analysis (Fig. 6) of the synthesized AgNPs samples revealed their ellipsoidal and spherical morphologies. The diameter of the particles was in the range of 20 to 40 nm.

The antibacterial potential of the prepared AgNPs as done using disk diffusion method is given in Table I. An increase in the concentration of the AgNPs has increased the zone of inhibition. A comparison was made using streptomycin as standard antibiotic. For the present investigation, two Gram positive bacteria (*Salmonella typhimurium and*

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Staphylococcus aureus), and two Gram negative bacteria *(Escherichia coli and Klebsiella pneumoniae*) bacterial strains were used. AgNPs showed difference in antibacterial activity with respect to the type of bacterium used. A lesser zone of inhibition of about 13±0.63 mm and 13±0.25 mm was observed for *Salmonella typhimurium and Staphylococcus aureus* respectively, showing that the presence of thick peptidoglycan layer on their cell surface potentially inhibited the entry of AgNPs inside the cell [24]. To our surprise, AgNPs exhibited higher order of inhibition of about 15±0.32 mm and 14±0.33 mm against *Escherichia coli and Klebsiella pneumoniae*. This clearly demonstrated that the presence of thin peptidoglycan layer in Gram negative bacterium has made it easier for the AgNPs to enter and kill the bacterial cell [25]. In a recent study, it has been shown that the electrostatic interaction between the bacterial cell wall and AgNPs is involved in exhibition of their antibacterial activity [26].

TABLE I
ANTIBACTERIAL ACTIVITY OF AGNPS SYNTHESIZED FROM *BEIJERINCKIA INDICA* EXTRACELLULAR EXTRACT

Microorganisms	Zone of inhibition [Mean ± SD (mm)]	
	Streptomycin	AgNPs
S. typhimurium	11±0.65	13±0.63
S. aureus	11±0.16	13±0.25
E. coli	12±0.62	15±0.32
K. pneumoniae	12±0.32	14±0.33

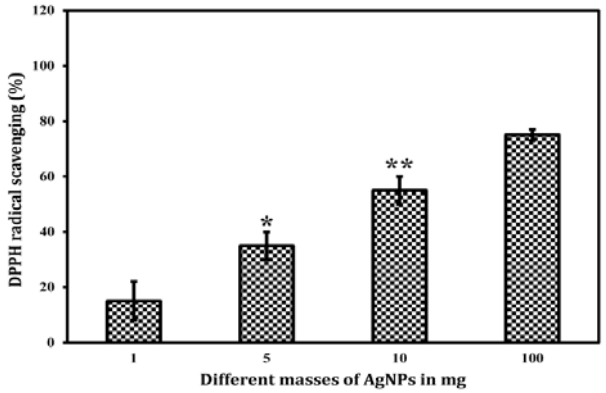


Fig. 7 Antioxidant activity of AgNPs (*, ** Represents level of significant at $p<0.05$)

The free radical scavenging activity of synthesized AgNPs is illustrated in Fig. 7. The result showed that the antioxidant activity enhanced in a concentration-dependent manner: 15% (1 mg), 35% (5 mg), 55% (10 mg) and 75% (100 mg). Similarly, iron and nickel oxide nanoparticles have exhibited strong antioxidant activity [27], [28]. Free radicals are being generated in living organisms in a continuous manner due to various biochemical reactions. Biosynthesized AgNPs will aid in scavenging these free radicals [29]. The high surface area of these nanoparticles facilitates them to react with the free radicals easily and hence scavenging them. In the current scenario, free radicals generation is considered as a serious issue in biomaterial and medicine fields [30]. Therefore, the biosynthesized AgNPs could be used as an additive in biomedical applications to solve this problem.

IV. CONCLUSION

In the present investigation, a search for new source for the production of AgNPs is successfully being carried out. This method is very rapid, eco-friendly, economical, and easy for the synthesis of AgNPs. In addition, it is a single step method, devoid of toxic chemicals and less time consuming. Using this method, we have successfully synthesized AgNPs with unique size and morphology. The components in the bacterial extract were served as reducing, capping, and stabilizing agents for nanoparticle generation. FTIR analysis evidenced the organic groups involved in the bioconversion of AgNPs. XRD pattern revealed the crystalline phase of the synthesized AgNPs. The EDAX spectrum analysis confirmed the generation of AgNPs. TEM analysis revealed the spherical morphology of AgNPs. The antibacterial susceptibility assay and antioxidant study demonstrated that AgNPs possessed excellent antibacterial and free radical scavenging activities in a dose-dependent manner. Thus, the findings of the present investigation clearly showed that AgNPs could be used in nano-medicine for the treatment of bacterial diseases, in future.

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