Research Article

Analysis of Antibacterial Activity of Biogenic Silver Nanoparticles Using Leaf Extracts of *Amaranthus caudatus*

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Accepted on: 20-08-2016; **Finalized on:** 31-10-2016.

ABSTRACT

Development of new antimicrobials for the management of infectious diseases becomes a matter of serious concern. In this critical situation, one of the most reliable and promising alternative therapeutic agent is silver nanoparticles (SNPs). Keeping the biological perspective in mind, we synthesized silver nanoparticles in an eco-friendly approach, using *Amaranthus caudatus* leaf extract as reducing and stabilizing agent. The reaction process was simple and was monitored by Ultraviolet–visible spectroscopy (UV–vis). The green synthesized crystalline silver nanoparticles were characterized and confirmed by various analytical techniques, such as, Field-emitter Scanning electron microscope (FE-SEM) equipped with EDAX (energy dispersive X-ray), X-ray diffraction (XRD). Resultant metal nanoparticles showed significant antibacterial effects against both Gram-positive and Gram-negative bacteria which increases with increase of particle dose. From the results obtained it is suggested that biosynthesized NPs can address the medical concerns and could be used effectively food safety applications also.

Keywords: *Amaranthus caudatus*, Silver nanoparticles, Characterization, Antibacterial activity.

INTRODUCTION

The application of nanoscale materials and structures may provide solutions to technological and environmental challenges in the areas of solar energy conversion, catalysis, medicine and water treatment.[1,2] In view of the rapid progress of application of nanomaterials in different fields, there is a growing need to develop clean, nontoxic, simple and environmentally friendly procedures for the synthesis and assembly of NPs.[3] Consequently, researchers in the field of nanoparticle preparation turned their attention towards biological systems.[4] Nowadays, green chemistry procedures using various biological systems such as yeast, fungi, bacteria and plant extract[5–7] for the synthesis of nanoparticles are commonly used. Among them, the use of plants for the fabrication of nanoparticles is a rapid, low cost, eco-friendly and a single step method for biosynthesis process.[8] The usage of plants can also be suitably scaled up for large-scale synthesis of nanoparticles in a controlled manner according to their size, shape and dispersity, with controlled physicochemical properties. Moreover, the use of plants in process of nanoparticles synthesis is more beneficial than other processes since the nanoparticles are produced extracellularly.[9–11] currently, the metallic nanoparticles are thoroughly being explored and extensively investigated as potential antimicrobials. The antimicrobial activity of the nanoparticles is known to be a function of the surface area in contact with the microorganisms. The small size and the high surface to volume ratio i.e., large surface area of the nanoparticles enhances their interaction with the microbes to carry out a broad range of probable antimicrobial activities.[12]

Among the noble metal nanoparticles, silver nanoparticles are effective antibacterial agents and possess a strong antibacterial activity. In the present investigation we report the biosynthesis of highly stable silver nanoparticles using *Amaranthus caudatus* leaf extract endowed with significant antibacterial properties. Studies were carried out on both strains of Gram- positive and Gram- negative bacteria. *Amaranthus caudatus* Linn. Belongs to the family Amaranthaceae, occurs naturally in Southern Asia. *A.caudatus* is a small herbs, known for its high anti-oxidant, anti-hypercholesterolemic, anti-atherogenic, anti-arthritic and anti-microbial properties. Various type of phytoconstituents such as Gallic acid (GA), Caffeic acid (CA), Rutin (RU), Ferulic acid (FA) and Quercetin (QU) present in *A.caudatus* are mainly responsible for their medicinal properties.[13]

MATERIALS ANS METHODS

Collection of plant material and chemicals

The fresh plant material was collected from local market of Tambaram, Chennai, India. The plant was botanically identified as *Amaranthus caudatus* L. and authenticated by LEO Chem Research and Pvt. Ltd., Bangalore. Silver nitrate (AgNO3) from Thomas Baker (Mumbai, India), Nutrient agar, Mueller-Hinton broth, and Mueller-Hinton agar medium were purchased from Hi-Media (Mumbai, India).

Preparation of plant extract

The fresh leaves of *A.caudatus* were thoroughly washed under running tap water, shade dried for 4–5 d. Then the dried materials were homogenized to fine powder and kept in sterile plastic air-tight container for further use. For extraction, about 100g of plant powder was mixed

with 300 ml of solvent (water). The extraction was carried out by continuous percolation method using Soxhlet apparatus for 36 h accompanying with occasional shaking and stirring. The extract was underwent a coarse filtration by muslin cloth followed by a filtration through Whatman filter paper. Each extract was concentrated by distilling off the solvent and evaporated to dryness under vacuum. The crude extracts were used for further analysis.

Biosynthesis of silver nanoparticles

Aqueous solution (1mM) of silver nitrate (AgNO3) was prepared and used for the synthesis of silver nanoparticles. 3 ml of extract of *A. caudatus* was added to 40 ml of 1mM AgNO3 solution with continuous stirring and mild heating. The reaction mixture was kept at room temperature (37°C) into dark room condition until the color change was arisen. The reaction solution color changes have observed for the characterization of silver nanoparticles.

Characterization of synthesized silver nanoparticles

To verify reduction of silver ions, the solution was scanned in the range of 300–700 nm using a double-beam UV-visible spectrophotometer (Shimadzu, UV-1800) with water as the reference. Further characterization was done using X-ray diffraction technique. It was performed on an X-ray diffractometer (X'Pert Pro a Analytical) operated at 45 kV and 40 mA using dried silver nanoparticles. The pattern was recorded by Cu Kα radiation radiation in a θ–2θ configuration. The morphology of the AgNPs was examined using scanning electron microscopy (ZEISS Ultra FE-SEM). Thin films of the sample were prepared on a carbon coated copper grid by dropping a very small amount of the sample on the grid. The extra solution was removed using a blotting paper and then the film on the SEM grid was allowed to dry under a mercury lamp for 5 min. The presence of elemental silver was determined by energy dispersive X-ray (EDAX) spectrometric analysis using SEM instrument equipped with EDAX attachments.

Test microorganisms and preparation of bacterial inoculums

To evaluate the antibacterial potential the following test microorganisms, Gram-positive bacteria including Staphylococcus aureus, Bacillus subtilis, Gram-negative bacteria including Pseudomonas aeruginosa, Klebsiella pneumoniae were procured from local hospitals and the pure cultures of bacteria were maintained on Nutrient agar (pH 7.2-7.4) slant at 4°C. Bacterial inoculums were prepared by growing cells in Mueller Hinton Broth (MHB) for 24 h at 37°C. These cell suspensions were diluted with sterile MHB to provide initial cell density of about 1×107 CFU/ml.

Screening of antibacterial property in synthesized nanoparticles

Antibacterial assay was carried out on Muller Hinton Agar (MHA). Screening of antibacterial activity was performed by well diffusion technique.[14] The MHA plates were seeded with 1% of the standardized inoculums of each test organism. Once agar was solidified, 6 mm diameter uniform well was cut using sterile standard cork borer on the surface of the MHA. The cut agar discs were removed by vacuum device. 100µl of different concentrations (10, 20, 30, 40 and 50µg/ml) of AgNPs solutions were introduced using sterilized dropping pipettes into the each well and allowed to stand for 1h at room temperature to diffuse and incubated at 37°C for 24h. The Inhibition Zone Diameter (IZD) was measured by antibiotic zone reader.[15] three replicates were maintained for each extract against each of the test organism.

RESULTS AND DISCUSSION

Today nanomaterials are at the primary stage of fast developing nanotechnology phase. Nanomaterials are facilitating modern technology to deal with nano-sized objects, their unique properties especially size-dependent one makes them superior materials and essential in different human activities.[16] In this present research, the green synthesis of silver nanoparticles (AgNPs) through plant extracts were carried out using aqueous leaf extract of *Amaranthus caudatus* and the biosynthesized particles were used to study the bactericidal effects.

Visible Observation of silver nanoparticles:

AgNPs were successfully synthesized using aqueous leaf extract of *A.caudatus* with 1mM silver nitrate in a continuously stirred and heated mixture solution. The colorless reaction mixture slowly changed to a yellowish-brown color suspension after several minutes of reaction (Figure 1). The visible color change indicates the formation of silver nanoparticles. This may be as a result of AgNO3 reduction and stimulation of Surface Plasmon Resonance (SPR) which is the characteristic property of the AgNPs.[17] No precipitation was observed. The color change was stable even after completion of the reaction.

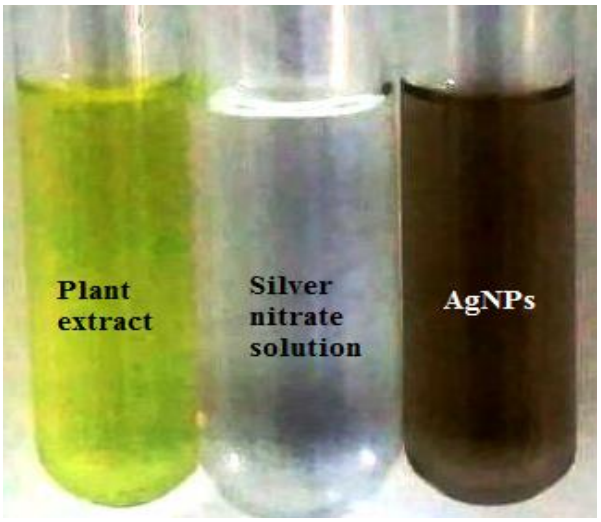

Figure 1: Color change of plant leaf extracts to yellowish-brown suspension during formation of AgNPs

Characterization of silver nanoparticles

UV-vis spectrum analysis

The UV-vis spectroscopy is one of the most widely used simple and sensitive technique for the analysis of silver nanoparticles synthesis. In particular, absorbance in the range of 420–450 nm has been used as an indicator to confirm the reduction of Ag+ to metallic Ag.[18, 19] the absorption spectrum (Figure 2) of the yellowish-brown silver nanoparticles solution showed a surface plasmon vibration band with a maximum of 425 nm, indicating the presence of spherical Ag nanoparticles. The metal nanoparticles have free electrons, which give the SPR absorption band, due to the combined vibration of electrons of metal nanoparticles in resonance with light wave. Due to the tiny dimensions, silver nanoparticles have distinctive color in colloidal solution.[20] it is well known that colloidal silver nanoparticles exhibit absorption at the wavelength from 390 to 420 nm due to Mie scattering.[21] hence, the band at 420-430 nm can be attributed to the property of Mie scattering. This may not include the protecting agent, because the Mie scattering responds only to the silver metal.[22]

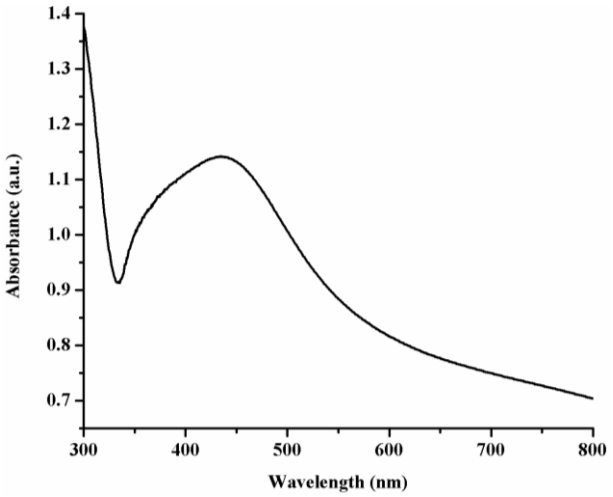

Figure 2: UV-vis absorption spectrum of biosynthesized AgNPs using *Amaranthus caudatus* leaf extract

X-ray diffraction analysis

The formation of crystalline silver nanoparticles was confirmed using X-ray diffraction analysis. The comparison of XRD spectrum results with standards confirmed the crystalline nature of silver nanoparticles formed strong peaks at 2θ values of 32.17°, 47.44°, 67.66°, 77.12° and 85.87° corresponding to (111), (200), (220), (311) and (222) of Bragg reflections for silver metal, respectively (Figure 3). The crystallite size of AgNPs can be calculated by using Debye-Scherer's formula (1). The average size of synthesized AgNPs was found to be 16-23 nm from XRD data and using Debye-Scherer equation.

$$D = K\lambda / \beta \cos\theta \qquad (1)$$

Whereas, D is the crystal size of AgNPs, λ is the wavelength of X-ray source used (1.541A°), β is the full width at half maximum of the diffraction peak, K is the constant of Debye-Scherer equation with value from 0.9 to 1 and θ is the Bragg angle.[23] The X-ray diffraction peaks were found to be broad around their bases indicating that the silver particles are in nanosizes and it can be stated that the obtained silver nanoparticles had a high purity.

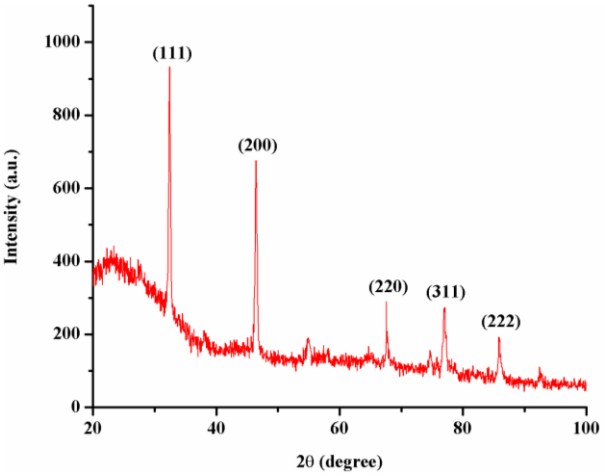

Figure 3: X-ray diffraction pattern of prepared silver nanoparticles

FE-SEM analysis

While the absorption spectra provide solid evidence of nanoparticles formation, the shape and size of the resultant particles were elucidated with the help of SEM. The size, morphology and distribution of silver nanoparticles was analyzed by FE-SEM. Representative SEM images of NPs synthesized by incubating aqueous leaf extract with 1mM AgNO3 are depicted in Figure 4. FE-SEM micrographs indicated that the NPs were monodisperse and spherical in nature with 16–23 nm in diameter. The image showed the presence of an amorphous shell surrounding these phyto-inspired nanoparticles. Biomolecules are known to cover the particle surface and thus contribute toward NP stability.[24]

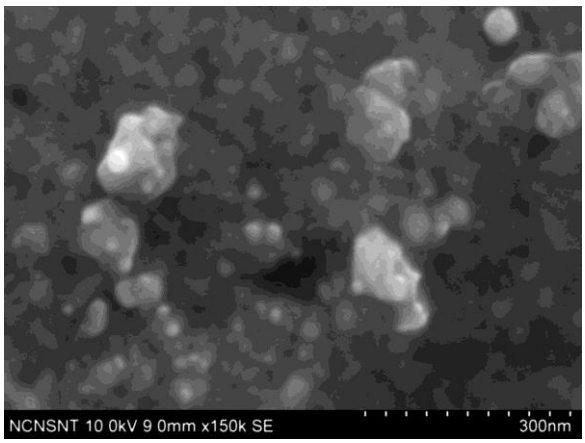

Figure 4: SEM image of AgNPs synthesized by leaf extract of *Amaranthus caudatus*

EDAX analysis

EDAX characterization has shown absorption of strong silver signal along with other elements, which may be originate from the biomolecules that are bound to the

surface of nanosilver particles. From EDAX spectra, shown in Figure 5, it is clear that silver nanoparticles reduced by leaf extract of A. caudatus. Silver nanocrystallites display an optical absorption band peak at approximately 3 keV, which is typical of the absorption of metallic silver nanocrystallites due to surface. Presence of carbon and oxygen indicates that the extracellular organic moieties are adsorbed on the surface of the metallic nanoparticles.

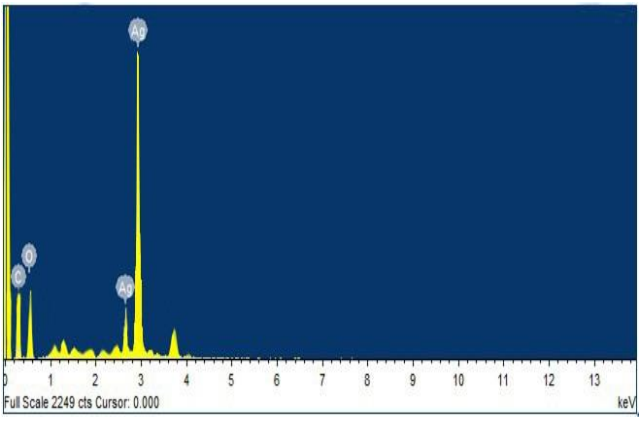

Figure 5: EDAX spectrum of biogenic AgNPs

Evaluation of antibacterial activity of biosynthesized AgNPs

Biosynthesized silver nanoparticles were used for antibacterial activity against pathogenic bacteria by standard zone of inhibition techniques, with increasing concentration of particles in the well. The AgNPs showed inhibition zone against all Gram-positive and Gram-negative bacteria, but antibacterial activity of AgNPs are more significant against Gram-negative bacteria than the Gram-positive one. Maximum clearance zone was found to be 20.19 mm in K. pneumoniae and minimum of 10.3 mm in S. aureus among all studied bacteria (Figure 6).

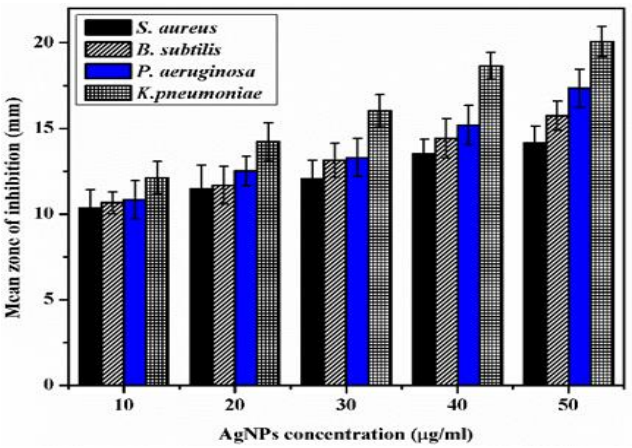

Figure 6: Antibacterial activities of silver nanoparticles against Gram-positive and Gram-negative bacteria

The Gram-negative bacteria showed maximum zone of inhibition which may due to the cell wall of Gram-positive bacteria composed of a thick peptidoglycan layer, which consisting of linear polysaccharide chains cross linked by short peptides thus forming more rigid structure leading to difficult penetration of the silver nanoparticles compared to the Gram-negative bacteria where the cell wall possesses thinner peptidoglycan layer [25]. The changes in the local electronic structure on the surface of the smaller sized particles lead to the enhancement of their chemical reactivity leading to high bactericidal effect[26].

CONCLUSION

In conclusion, we have demonstrated the simple biological way for synthesizing the silver nanoparticles using leaf extract of Amaranthus caudatus. The characterization of reduced silver metal was done by UV–vis, XRD, FE-SEM with EDAX. The results of SEM suggested that the plant extract have played an important role in the stabilization of silver nanoparticles. X-ray diffraction (XRD) spectra reveal a high degree of crystallinity of the SNPs. The spherical silver nanoparticles proved as effective antibacterial agents and the activity was in a dose-dependent manner. This process could be easily scaled up for the industrial applications, such as medicine and therapeutics, synthetic textiles and food packaging products, to increase the yield of the nanoparticles significantly, which undoubtedly would establish its commercial viability.

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Source of Support: Nil, **Conflict of Interest:** None.