

Biological synthesis of silver nanoparticles using *Abelmoschus moschatus*

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Abelmoschus moschatus Medik (Family: Malvaceae) is commercially valued for the ambrette oil obtained from its seeds. This plant source of musk is an economical, ethical substitute for animal musk and a healthier alternative. Supplementary uses of plant parts would enhance the economic value of the plant. Nanoparticles of gold, silver, iron and zinc have received a great deal of attention due to the altered properties of the constituent metal. In the present study, stable silver nanoparticles were biosynthesized using an aqueous extract of *A. moschatus*. The obtained silver nanoparticles were analyzed using UV-Vis spectrophotometry, Dynamic light scattering, Energy dispersive X-ray spectroscopy and Fourier transform infrared spectroscopy. The antimicrobial activity of silver nanoparticles was also demonstrated against some Gram positive and Gram negative bacteria.

Keywords: *Abelmoschus moschatus*, antimicrobial activity, bioreduction, silver nanoparticles

Introduction

Nanoparticles of metals have been extensively studied for their potential applications in catalysis, biological labeling, biosensing, drug delivery, antibacterial and antiviral activity, and detection of genetic disorders, gene therapy and DNA sequencing¹. A collection of atoms bonded together with a structural radius of less than 100 nm can be defined as a nanoparticle². These particles have unique properties, which can be attributed to variation in specific characteristics, such as, size, shape and distribution³. Silver, aluminum, gold, zinc, carbon, titanium, palladium, iron and copper have been extensively used for the synthesis of nanoparticles. Nanoparticles of silver and gold have applications in diverse areas, such as, medicine, electronics, cosmetics, coatings, packaging and biotechnology. Silver nanoparticles can be prepared using several methods like sol-gel process, chemical precipitation, reverse micelle method, hydrothermal method, microwaves, chemical vapour deposition and biological methods^{4,5}. Biological methods are preferred as they are eco-friendly and cost-effective. Several biological systems including microorganisms^{6,7,8}, fungi⁹ and plants (green chemistry)^{10,11} have been used in the synthesis of nanoparticles. Plants have a rich diversity of phytochemicals with strong antioxidant properties mediating the production of nanoparticles^{10,11}. Silver

nanoparticles have been synthesized using leaf extracts (*Pelargonium graueolens*, *Glycine max*, *Murraya koengii*, *Azadirachta indica* & *Mangifera indica*), bark extract (*Cinnamon zeylanicum*) and flower extracts (*Calotropis procera*)¹².

Abelmoschus moschatus Medik (Family: Malvaceae), commonly called *Muskdana*, is a medicinal herb. The commercial use of the seed oil along with the traditional applications of its flowers, fruits and stem makes the plant economically important^{13,14}. The aim of the present study was to standardize a simple and cost-effective method for the synthesis of silver nanoparticles using leaf extract of *A. moschatus* as a reducing and stabilizing agent. Silver nanoparticles were also analyzed for antimicrobial properties against Gram positive and Gram negative bacteria.

Materials and Methods

Bioreduction with Plant Extract

Silver nanoparticles were prepared by modifying a method followed by Mason *et al*¹². The leaves of *A. moschatus* were rinsed thrice in distilled water and dried on paper towel. Samples of 5 g each were cut into fine pieces and boiled with 100 mL of sterile distilled water for 5 min. The crude extract was passed through Whatman filter paper No.1 and the filtrates were stored at 4°C for further use. The *A. moschatus* leaf extract (5 mL) was added to 95 mL of 10⁻³ M aqueous silver nitrate solution and incubated at 100°C for 10 min. Suitable controls were maintained throughout the conduct of experiment.

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Characterization of Silver Nanoparticle

The silver nanoparticles were characterized using several methods. The reduction of silver ions was monitored periodically at room temperature using a UV-Visible spectrophotometer (Varian, Cary 50) operated at a resolution of 1 nm between 200 nm and 800 nm ranges. Size distribution and mean size of the bio-reduced silver nanoparticles was measured using Dynamic light scattering (DLS) (Zetasizer Ver 6.34, Malvern, UK). The zeta-potential of the aqueous dispersions was determined using the appropriate accessory of Zetasizer Ver 6.34 (Malvern, UK).

The size and shape of the silver nanoparticles was also determined using Transmission electron microscopy (TEM). A drop of the silver nanoparticle suspension was loaded on carbon-coated copper grids and the solvent was allowed to evaporate under infrared light for 30 min. TEM measurements were performed on Philips model CM 200 instrument operated at an accelerating voltage at 200 kV.

Energy dispersive X-ray spectroscopy (EDS) analysis was used mainly to determine the bulk elemental composition of the sample materials. The silver nanoparticle drop was coated on a copper grid and analyzed on JEOL-MODEL 6390 SEM instrument equipped with a Thermo EDAX attachment.

The Fourier transform infrared spectroscopy (FT-IR) measurements of biosynthesized silver nanoparticles and leaf extract were carried out to recognize the chemical change of the functional group involved in the bioreduction. The range of reflection mode used was from 4000 cm^{-1} to 400 cm^{-1} with a resolution of 4 cm^{-1} .

Antibacterial Assay

The biosynthesized silver nanoparticles from *A. moschatus* leaf extract were tested for antimicrobial activity by the agar cup method against bacterial species, such as, *Escherichia coli*, *Pseudomonas aeruginosa* (Gram negative), *Staphylococcus aureus* and *Bacillus subtilis* (Gram positive). The axenic bacterial strains were cultured in sterile Luria Bertani broth, added evenly into aliquots of Muller Hinton molten agar and plates were prepared. Wells were punched into the agar plates and 80 μL of the silver nanoparticle suspension as well as the appropriate controls were added to each well. The zone of inhibition was measured after incubating the plates at 37°C for 24 h.

Results and Discussion

Boiling aqueous silver nitrate solution with the *A. moschatus* leaf extract at 100°C changed the colour of the solution from transparent to brownish yellow, which is a characteristic of silver nanoparticles due to excitation of surface plasmon resonance (Fig. 1)¹⁵. A spectrophotometric analysis of the silver nanoparticle suspension revealed a broad peak from 402-425 nm, indicating that the silver nanoparticles formed were polydispersed (Fig. 2).

The size of the synthesized silver nanoparticles was determined by dynamic light scattering measurements and the physical stability of the silver nanoparticles

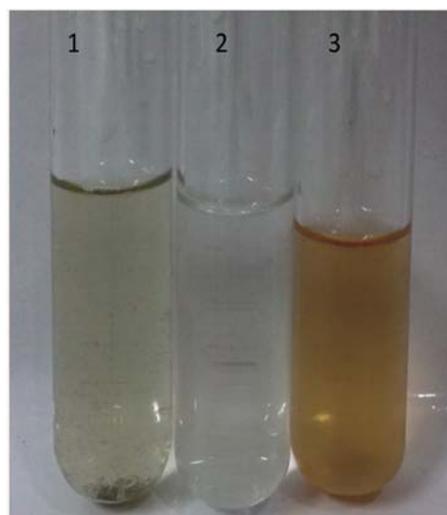


Fig. 1—Silver nanoparticles synthesized using *A. moschatus* leaf extract: Plant leaf extract (1); Silver nitrate (2); & Silver nanoparticles (3).

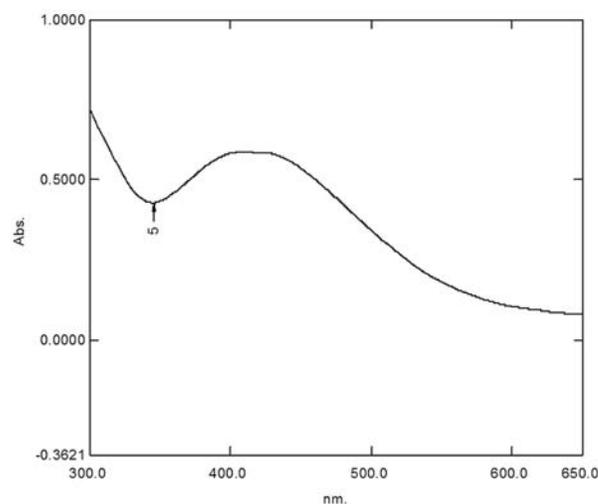


Fig. 2—UV spectrophotometry data from 300-650 nm of synthesized silver nanoparticles.

was evaluated in terms of zeta potential. The electrophoretic mobility and stability of the silver nanoparticle suspensions can be inferred from the zeta potential values¹⁶. The zeta potential value (-30 mV) of silver nanoparticles indicated a good physical stability of the particles probably due to interparticle repulsion (Table 1).

The Z-average represents the hydrodynamic diameter of the particle and not the real particle size¹⁷. The hydrodynamic diameter is affected by the environment surrounding the particle and is calculated with the assumption that it is an isotropic spherical particle (Table 1). Hence the particle size was also determined by TEM. The TEM micrograph (Fig. 3) of the synthesized silver nanoparticles showed that the nanoparticles were spherical in shape and the particle size ranged from 5.79 to 16.06 nm in diameter. The selected area electron diffraction (SAED) pattern (Fig. 4) confirmed the crystalline nature of silver nanoparticles.

Table 1—Dynamic light scattering (DLS) data of silver nanoparticles and their zeta potential values

Sample	Z-average (nm)	Zeta potential (mV)
Silver nanoparticles	103.00	-30.0

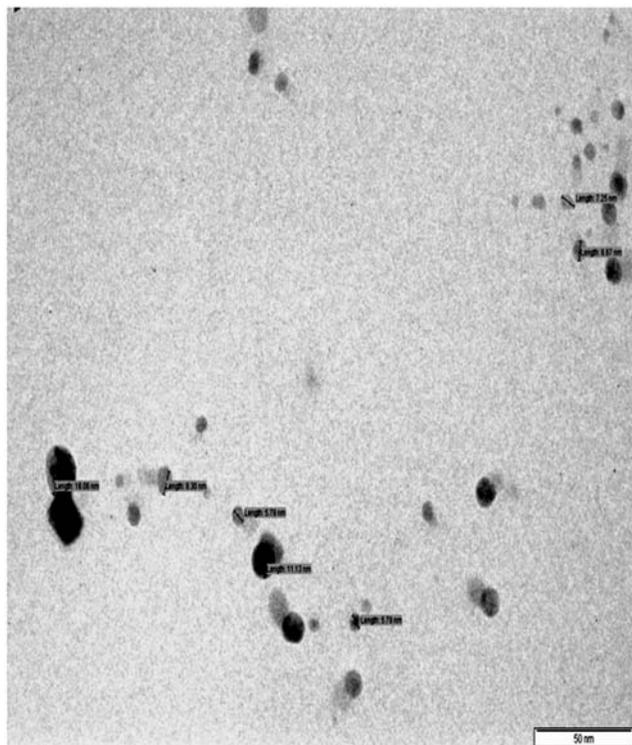


Fig. 3—TEM micrograph of synthesized silver nanoparticles.

The EDS spectrum of silver nanoparticles (Fig. 5) showed a peak around 3 keV confirming the presence of metallic silver¹⁸. Some impurities like Na, C & O were also observed which may be a contribution of plant extract.

FT-IR spectrum analysis (Fig. 6) was used to determine the nature of interaction between the extract and silver nanoparticles. Absorbance peaks were observed at 445.27, 668.97, 1384.21, 1640.19, 2067.72, 2921.22, 3456.81 and 3861.67 cm^{-1} in the colloidal solution after bioreduction, indicating that biomolecules having carbonyl groups may be responsible for the reduction and stabilization of silver nanoparticles.

Silver nanoparticles have been recognized for their antimicrobial properties. The biosynthesized silver nanoparticles were analyzed for their antimicrobial activity against *E. coli*, *P. aeruginosa*, *S. aureus* and *B. subtilis* by agar cup method. The results of

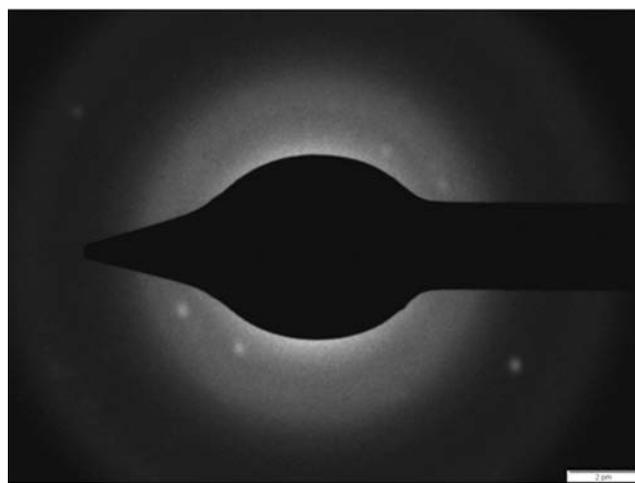


Fig. 4—SAED pattern of synthesized silver nanoparticles.

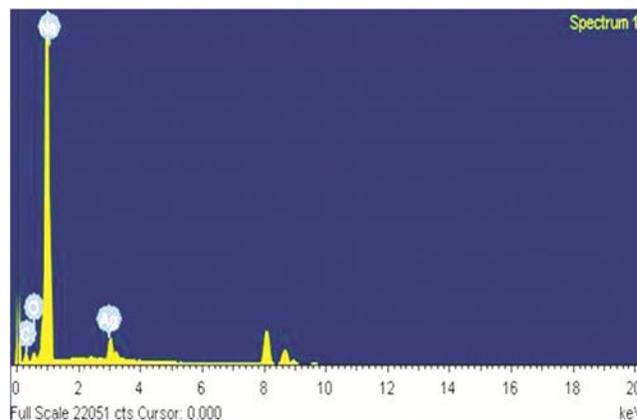


Fig. 5—EDS report of synthesized silver nanoparticles.

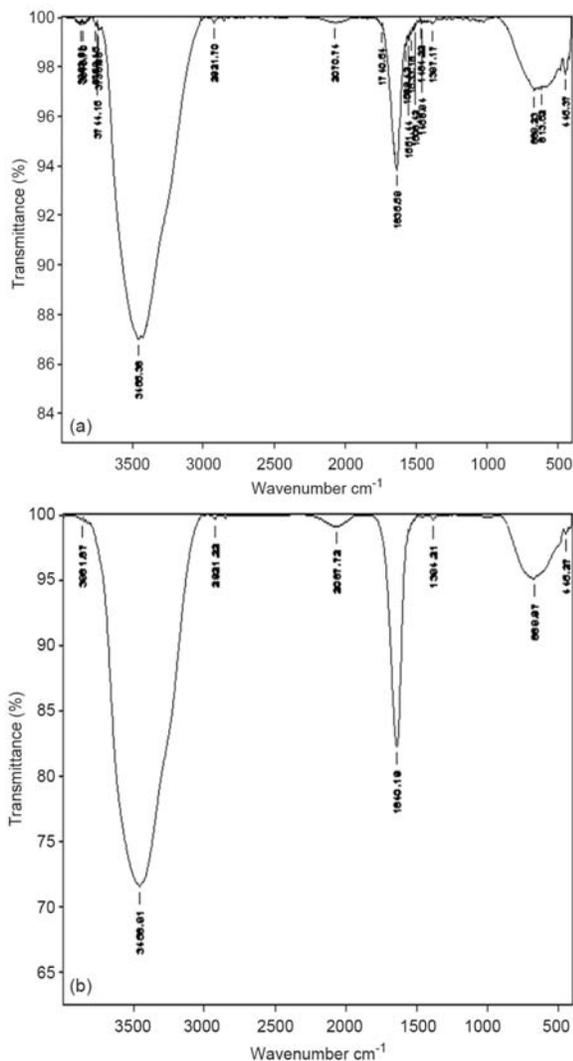


Fig. 6 (a & b)—FT-IR analysis of plant extract (a) and synthesized silver nanoparticles (b).

antimicrobial activity of aqueous *A. moschatus* leaf extracts and the prepared silver nanoparticles against various microbial strains are summarized in (Table 2). The prepared silver nanoparticles suspension was found most effective against *P. aeruginosa* (Fig. 7) but had no visible effect on *E. coli*. Moreover, the tested organisms were not susceptible to the leaf extract.

Conclusion

The *A. moschatus* leaf extract can be used as an effective reducing as well as stabilizing agent for the synthesis of stable silver nanoparticles. The size of the synthesized silver nanoparticles ranged from 5.79 to 16.06 nm and was determined to be spherical in shape. They showed antimicrobial activity against *P. aeruginosa*, *S. aureus* and *B. subtilis* but were not

Table 2—Antimicrobial effect of silver nanoparticles

Organism	Zone of inhibition (cm)
<i>Escherichia coli</i>	-
<i>Bacillus subtilis</i>	1.1
<i>Staphylococcus aureus</i>	1.1
<i>Pseudomonas aeruginosa</i>	2.0

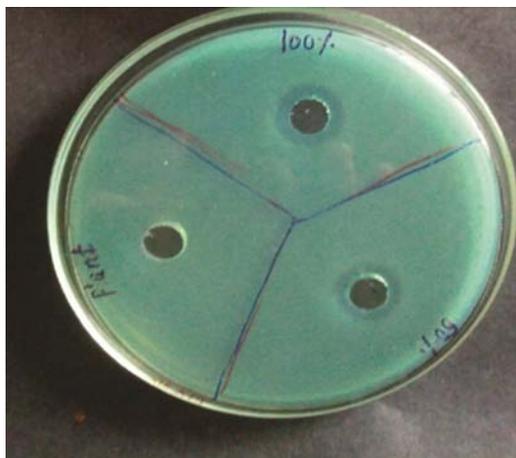


Fig. 7—Antimicrobial activity of synthesized silver nanoparticle (50 & 100%) suspension and plant extract against *P. aeruginosa*.

effective against *E. coli*. Further studies are needed to optimize the scaling up of the synthesis of silver nanoparticles.

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