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Silver nanoparticles prepared by colloid assisted biofabrication methodology using soilless-grown *Moringa oleifera* in Armenia

Anna Tadevosyan[1], Mahsa Daryadar[1*], Anahit Tovmasyan[1], Armenuhi Asatryan[1], Anahit Hakobjanyan[1]

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[1]Department of Plant Nourishment and Productivity, G.S. Davtyan Institute of Hydroponics Problems, National Academy of Sciences, Yerevan, Armenia

Abstract

In this study, green synthesis assisted colloidal solution system was used to prepare monodispersed spherical silver nanoparticles from *hydroponic Moringa oleifera Lam*. leaf extract. The *Moringa oleifera* leaf extract used has shown great potential in the biosynthesis of silver nanoparticles for medicinal and biotechnological applications. The formation of silver nanoparticles was observed by UV-Vis spectroscopy with a surface plasmon resonance band at 425 nm after 1 hour of preparation. Transmission electron microscopy (TEM) imaging shows that the nanoparticles are spherical in shape with an average size of 20 nm. The atomic force microscopy (AFM) image showed that the Ag nanoparticles were spherical with good monodispersity, confirming the TEM results. The biomedical use of the green synthesized silver nanoparticles from medicinal plants as antimicrobial and cytotoxic agents can be suggested by their strong bioactivity and synergistic effect of *Moringa oleifera* and silver nanoparticles.

Keywords: Hydroponics, Green chemistry, *Moringa oleifera*, Silver nanoparticles, Colloid solution, Armenia

1. Introduction

Green chemistry is a field of chemistry and chemical engineering that focuses on designing products and processes that minimize or eliminate the use and generation of hazardous substances, similar to sustainable chemistry or circular chemistry [1,2]. Bio-engineering is also considered to be a promising technique for the achievement of green chemistry goals. A number of important process chemicals can be synthesized in engineered Bioactive Ingredients on the basis of the plant extract [3-5]. In the course of research in this field, the Moringa tree (*Moringa oleifera Lam.),* also called the Tree of Life, has attracted much attention as a source of valuable active ingredients for the synthesis of nanomaterials especially nanoparticles. Owing to the presence of a broad spectrum of bioactive compounds, the plant has powerful antioxidant, antibacterial, firming, astringent and anti-inflammatory properties [6-7]. The leaves of the Moringa tree have been found to contain flavonoids such as myricetin, quercetin, kaempferol, isorhamnetin or rutin. They also contain phenolic acids. Fresh leaves are a good source of carotenoids. These include lutein, beta-carotene and zeaxanthin. The Moringa tree is also a good source of vitamins C and A. The plant's active ingredients have been shown to have beneficial effects on human skin and are a good alternative to synthetic ingredients. Among several metallic nanoparticles involved in biomedical applications, silver nanoparticles are one of the most important and fascinating nanomaterials [8,9]. Among metal nanoparticles such as Pt, Pd, Au, Ag nanoparticles are important in nanoscience and nanotechnology, especially in nanomedicine [10-13]. Recently, green synthesis of silver nanoparticles using *Moringa oleifera* and its efficacy against gram-negative bacteria targeting quorum sensing and biofilms reported by Haris et al. [14]. Mohammed and Hawar studied green biosynthesis of silver nanoparticles from *moringa oleifera* leaves and its antimicrobial and cytotoxicity activities [15]. In our previous research, Moringa plants were grown in soil and hydroponic systems to evaluate their adaptability to the climatic conditions of Armenia. Growth characteristics, yield, antioxidant activity and antimicrobial properties were investigated [16].

In continuing our previous work, in this study, we evaluated the effectiveness of the synthesis of silver nanoparticles with a unique combination of the green synthesis based on the aqueous extract of *Moringa oleifera* leaves extract and PEG surfactant-based micelles as nanoreactor. The prepared colloidal Ag nanoparticles were characterized with UV-Vis, XRD, TEM, and AFM analysis

Corresponding Authors: Mahsa Daryadar
daryadarmahsa0@gmail.com

2. Materials and Methods

2.1. Materials

Poly (ethylene glycol) (PEG) 35000 daltons as surfactant used in this experiment has the highest purity and was procured from Sigma-Aldrich and $AgNO_3$ as a metal source to synthesis silver nanoparticles was procured Merck. The leaves of *Moringa oleifera* were collected from the Hydroponic station of G.S. Davtyan Institute of Hydroponics Problems, National Academy of Sciences, Yerevan, Armenia.

The experiment to grow *moringa* was carried out in an experimental field, Ararat Valley, Armenia, involving automatic irrigation hydroponic equipment (with a density of 6 plant m^{-2}) using the EBB and Flow hydroponic system. Temperatures in Ararat Valley ranged from -26.1 to 32.6 °C in winter and 37.5 to 42.6 °C in summer. The average annual rainfall was 325 mm. Ararat Valley stands 800-1000 m above sea level. Moringa seeds were sown in pots during March and April in the greenhouse (23-28 °C). Seedlings were transferred outdoors to a hydroponic automatic system at the end of April and the start of May. The seedlings were planted in the hydroponic vegetation vessels with a 2 m^{-2} surface.

2.2. Preparation of plant extract and synthesis of silver nanoparticles

Plant leaf extract was prepared by mixing 20 g of dried leaf powder with 100 mL of deionized water in 100 mL round bottom balloon flask and boiled for 20 minutes to give a light green solution. For the preparation of silver nanocolloid, a clear aqueous solution of PEG surfactant (0.05 M) was first prepared. A certain amount of $AgNO_3$ was added to the surfactant solution so that the concentration of $AgNO_3$ was adjusted to 0.01 mol/L. The solution was mixed to form nanomicelles containing Ag^+ ions for 10 min. For the reduction of Ag+ ions in nanoreactor reverse micelles, 5 mL of leaf extract was added dropwise to the above solution for 30 min with strong stirring and heated at 60-80 °C for 20 min. A color change from colorless to brown was observed, confirming the presence of silver nanoparticles (Fig. 1).

Fig. 1. A simple schematic route to synthesis Ag nanocolloid

2.3. Characterization

UV-Vis spectral analysis was performed using a UV-Vis spectrophotometer (Shimadzu UV2401 PC) to verify Ag nanoparticles formation. In this study, the silver nanocolloid was diluted by a small aliquot of the sample in distilled water after 1 h of reaction and monitored by measuring the UV-Vis spectrum to determine the surface plasmon resonance (SPR). The prepared sample was also used for Transmission Electron Microscopy (TEM) and atomic force microscopy (AFM). Transmission electron microscopy (TEM) is a valuable, widely used and important technique for the characterization of nanoparticles, providing quantitative measures of particle and/or grain size, size distribution and morphology. In this study, TEM (Philips CM12) is a microscopy technique used to determine the morphology and monodispersity of silver nanoparticles. In TEM analysis, a beam of electrons is transmitted through an ultrathin sample that has been coated with gold and interacts with the sample as it passes through. The image of the Ag nanoparticles was magnified and focused onto an imaging device by the interaction of the electronstransmitted through the sample. The Atomic Force Microscope (AFM) allows to characterize nanoparticles in 3D with sub-nanometer resolution Dual scope C-26. AFM is a relatively recently developed technique for imaging local surface features from the sub-micron to the nanometer length scale. In general, in this study, AFM was used to investigate the dispersion and aggregation of Ag nanoparticles, in addition to their size, shape, sorption and structure based on the noncontact mode. Crystal structure and particle size of the nanoparticles were determined by means of X-ray diffraction with (Philips PW1730/10).

3. Results and Discussion

3.1. UV-Vis analysis

The first characteristic indicator of the synthesis of silver nanoparticles is the change in color from a light yellow to a dark brown color (Fig. 1). The absorbance of the colloidal Ag nanoparticles after 1 h of preparation was measured by UV-Vis spectroscopy; the results showed that the maximum absorbance was 425 nm (Fig. 2). The color change indicates the occurrence of surface plasmon resonance (SPR) in the metal nanoparticles [17]; the color change was observed after the leaf extract was incubated with silver nitrate solution, and this is evidence for the reduction of silver ions to silver nanoparticles. The results suggested that the extract solution containing reducing compounds plays a significant role in the conversion of silver ions into Ag^0 nanoparticles. Another result of [17] is that the color change happened because the leaf extract contains terpenoids and flavonoids, which could be the surface active molecules stabilizing the nanoparticles as capping agent.

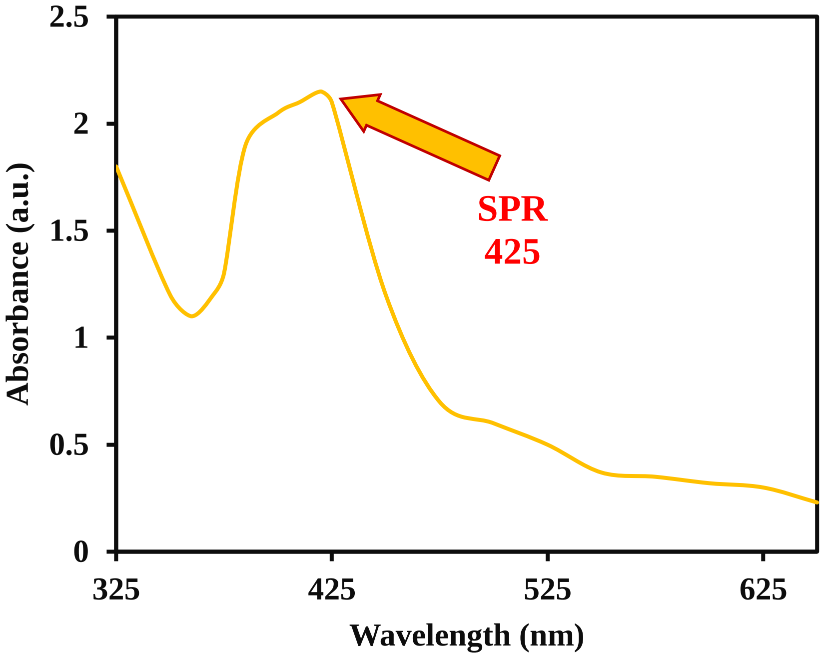


Fig. 2. The UV-Vis spectrum of silver nanoparticles

3.2. XRD study

The crystal structure and particle size of the nanoparticles were determined by means of X-ray diffraction. The results (Fig. 3) showed the formation of a semi-cubic crystal structure with diffraction angles of 38.52, 44.39, 64.89 and 77.68. These angles correspond to the crystal planes of the Braggs reflection patterns at 2θ, and these patterns correspond to (111), (200), (220) and (311). The diffraction peaks were compared with the standard data (JCPDS file no. 04-0783), which showed that the face-centered cubic (FCC) of the silver nanoparticle solution and the crystal size were estimated by the Debye-Scherrer equation, and this result is in agreement with the results of study of Mayachar et al. [18].

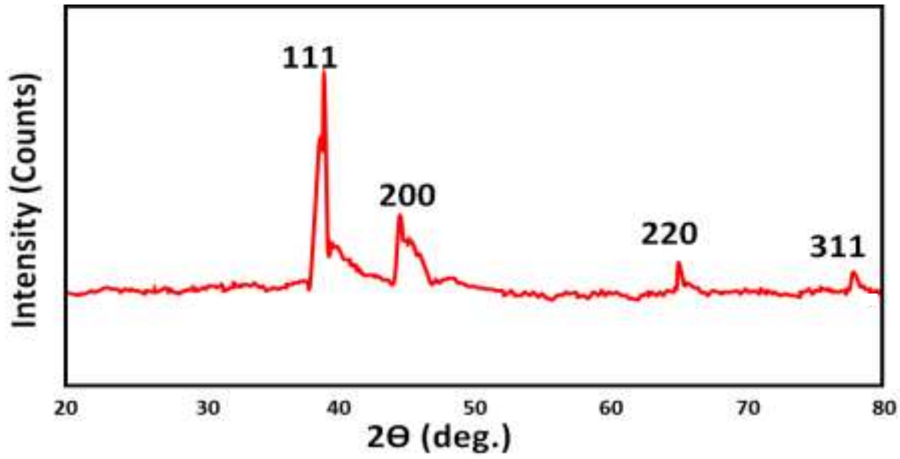


Fig. 3. The XRD pattern of silver nanoparticles

3.3. TEM investigation

Fig. 4. presents the TEM image of the prepared silver nanoparticles in green colloid solution system. The result obtained from the TEM image shows the dispersity, size distribution, and morphology of synthesized Ag nanoparticles based on the colloid solution as soft template method. As can be seen from Fig. 4 nanoparticles are spherical in the range of 12–60 nm, and the average size was about 20 nm.

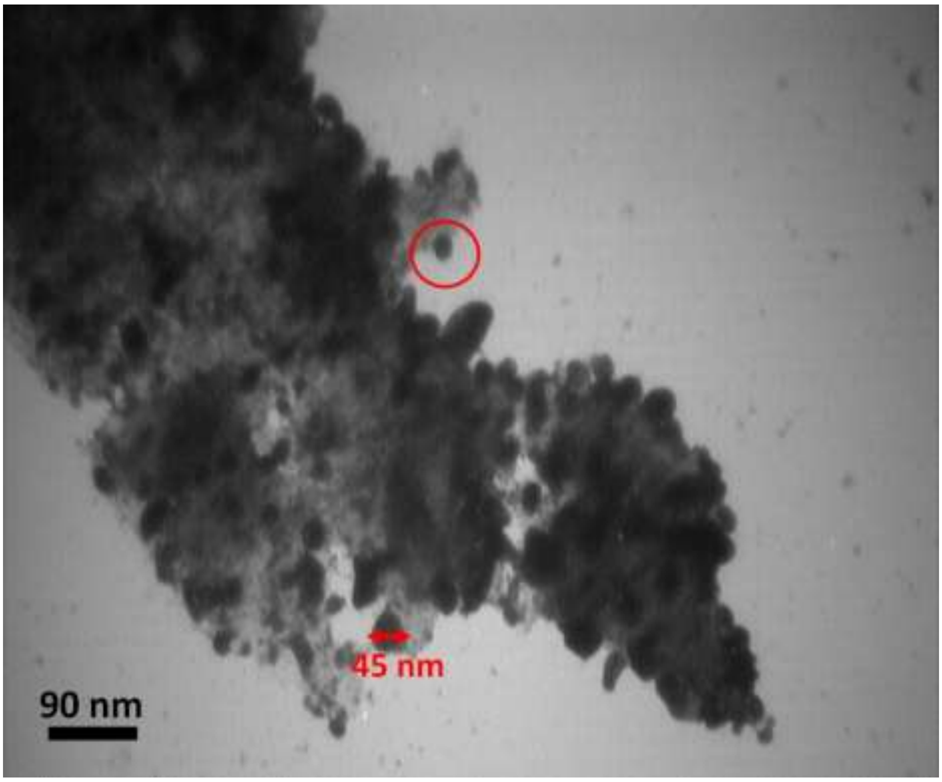
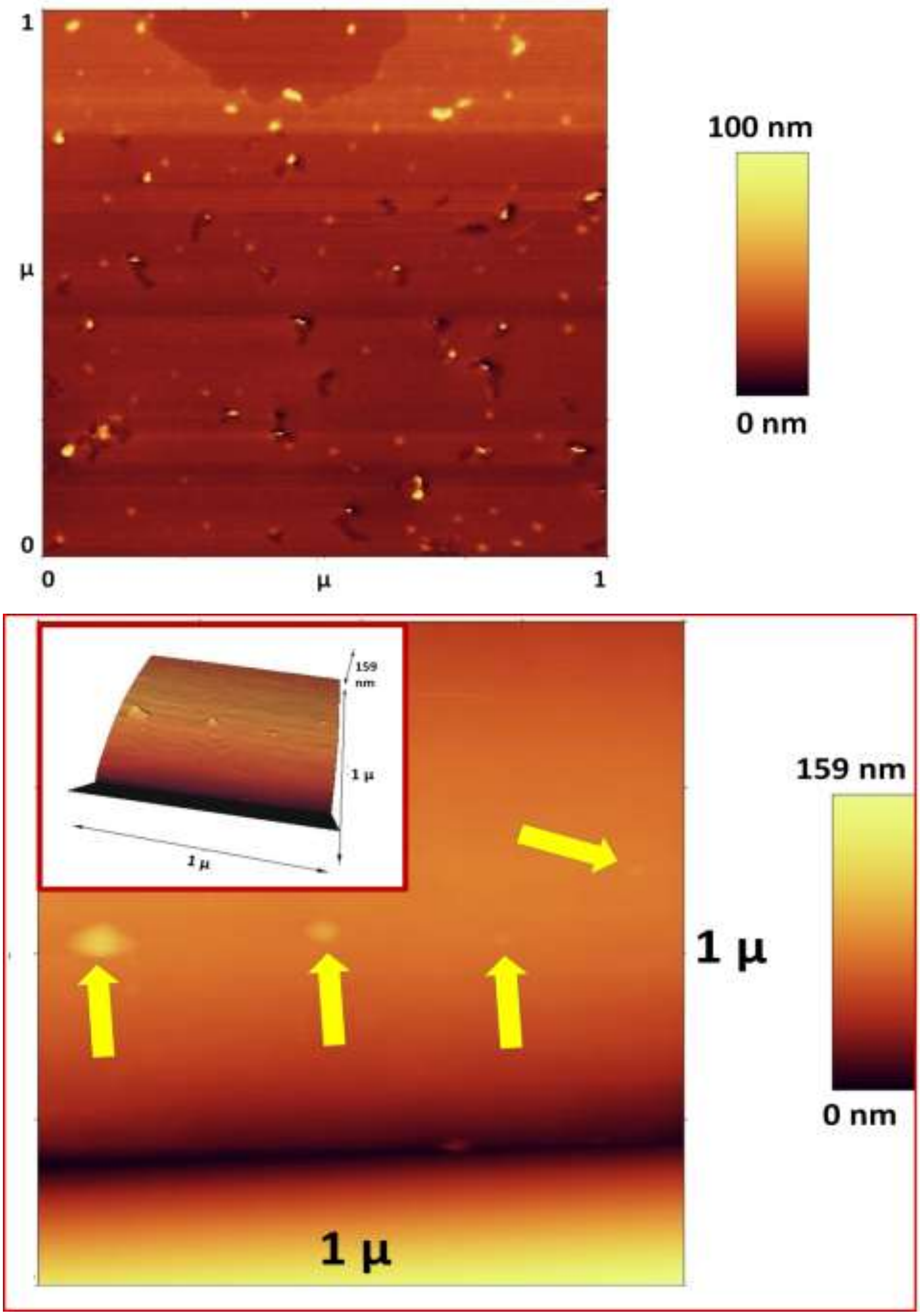


Fig. 4. The TEM image of silver nanoparticles

3.4. AFM images

AFM images of Ag nanoparticles and their spherical morphology are shown in Fig. 5 (a-b). As reported in the American ASTM E2859-11 [19] and NPL GPG 119 [20] guidelines, two methods can be used to measure the diameter of spherical nanoparticles: (i) height of isolated nanoparticles dispersed onto a flat substrate and (ii) lateral distance of nanoparticles in a closely packed monolayer arrangement. Considering that the second method can be a source of error due to the dilatation of the tip and that a perfect densely packed monolayer arrangement of nanoparticles can be difficult to achieve, the first method is to be preferred. As can be seen from Fig. 5-a, based on the height of isolated nanoparticles dispersed onto a flat substrate, it is clear that the Ag nanoparticles are spherical with the highest dispersity and good size distribution.



(a)

(b)

Fig. 5. AFM images of Ag nanoparticles

4. Conclusion

In conclusion, it has been demonstrated that the aqueous extract of M. oleifera leaves is capable of producing Ag nanoparticles. The formation of silver nanoparticles has been observed by UV-Vis spectroscopy with a surface plasmon resonance band at 425 nm after one hour of preparation. The TEM and AFM images shows that the nanoparticles are spherical in shape. The average size of the nanoparticles is 20 nm. This simple method of obtaining silver nanoparticles has such an advantage as compatibility for pharmaceutical and medical applications.

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Conflict of Interest

The authors indicate no conflict of interest for this work.

Author information

*Corresponding Author: Mahsa Daryadar
E-mail address: daryadarmahsa0@gmail.com

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