

RESEARCH PAPER

Green synthesis of silver nanoparticles using the aqueous extract of *Viscum album* Fruit

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ABSTRACT

The size of nanoparticles is in a range of 1-100 nm. In addition to the composition and structure of the material, the size of the particles is also one of the factors affecting the properties of materials. Nowadays, green chemistry and its benefits encourage researchers in the environmental biosynthesis of metallic nanoparticles. In this study, a rapid, simple and green method for the synthesis of silver nanoparticles is proposed using aqueous extract of *viscum album*. The synthesized silver nanoparticles were characterized using UV-Vis spectroscopy, scanning electron microscopy (SEM), X-ray diffraction (XRD) and Fourier transform infrared spectroscopy (FTIR) methods. The results of SEM, XRD and UV-Vis techniques confirm the synthesis and formation of uniform and spherical shape of silver nanoparticles with the average particle size of about 40-70 nm. The aqueous extract of *viscum album* demonstrates the strong potential for reducing silver ions and producing silver nanoparticles using a highly cost-effective, clean, non-toxic, eco-friendly method that can be produced on a large scale. Silver synthesis with aqueous extract of tree parasite was studied in this study.

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INTRODUCTION

The field of nanotechnology is one of the most promising areas in modern material science and technology which has grown very rapidly all over the world for the past decades. It is defined as the science in the design, synthesis, characterization and application of nanoscale materials [1,2]. Because of thermal conductivity, chemical stability, catalytic and biological activities, the synthesis of silver nanoparticles is very important [3,4]. Size, shape, distribution and surface area to volume ratio of the silver nanoparticles act as affecting factors on their biological activities such as antibacterial, antifungal and antioxidant[5].

Up to now, large number of preparation and

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synthesis procedures including physical, chemical, electrochemical, and photochemical methods have been studied and reported for the silver nanoparticles [6,7]. Although these methods are very successful to produce well-defined silver nanoparticles, each of them has some disadvantages such as high cost and time of synthesis, the use of hazardous and harmful toxic chemicals, release of hazardous by-products in environment, difficulty in purification, low rate of material conversions and high energy consumption [8,9].

So, using the nontoxic and environmentally sustainable synthesis processes is attractive especially if they are intended for medical applications [10]. The use of plant extracts in synthesizing of the metal nanoparticles has more advantages over other



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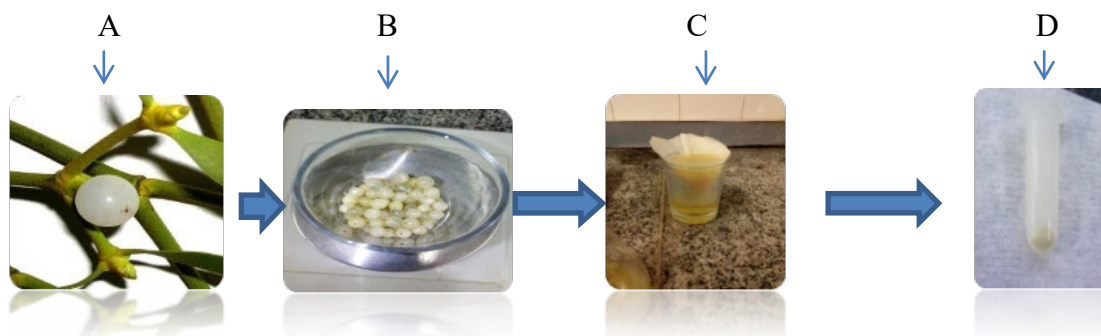


Fig. 1. Pictures of the aqueous extract preparation steps: (A) shows viscum album , (B) shows fruit of viscum album , (C) shows the aqueous extract of viscum album fruit , (D) shows the dried apple extract.

methods due to the elimination of harmful reagents, less reaction time, cost effectiveness, ease of scale up, economic viability and most importantly ecofriendly [11,12,13]. In the synthesis of nanoparticles by the plant extracts, the extract could act both as reducing agents and stabilizing agents [13,14]. The major natural products responsible for the spontaneous reduction of metal ions are flavonoids, terpenoids, carboxylic acids, quinones, aldehydes, ketones and amides [15,16].

A number of plants studied for the synthesis of silver nanoparticles in different sizes and shapes are *cardiospermum halicacabum* L. [17], *olea europaea* [18], *ziziphora tenuior* L [19], *justicia adhatoda* [20], *nasturtium officinale* [21], *syzygium cumini* [L. [22], lemon [23], garlic [24], *tribulus terrestris* [9] *pimpinella anisum* L. [10], *eucalyptus hybrid* [25], *chenopodium album* [16], *petroselinum crispum* [26], *aloe vera* [27], *securinega leucopyrus* [28], *matricaria chamomilla* [29], *dracaena mahatma* [30], etc.

European mistletoe (*viscum album* L.) is an evergreen, perennial, epiphytic, and hemiparasitic shrub. It is also a plant pathogen, a source of pharmaceutical compounds and a symbol in mythology [31]. It weakens more than 450 woody plant species [32], and is widely distributed in Europe. It was introduced to North America and Canada at the beginning of the 20th century [33].

In the present study, for the first time, we report a suitable green route for the synthesis of silver nanoparticles using aqueous extract of *viscum album* as a reducing and stabilizing agent.

The primary requirement for the green synthesis

The primary requirement for the green

synthesis of AgNPs is silver metal ion solution and a reducing biological agent. In most cases, reducing agents or other constituents present in the cells act as the stabilizing and cap-ping agents, so there is no need of adding capping and stabilizing agents from outside.

EXPERIMENT

Preparation of plant extract

Viscum album was collected from Tuscistan , Gorgan, Iran and the fruit was separated from the stems and leaves. About 20 g of fruit was dipped into a beaker containing 100 ml of Millipore water. The content of beaker was mixed at temperature ($T = 30^{\circ}\text{C}$) and the extraction was allowed to proceed during 30 min. The aqueous extract was filtered using Whatmann filter paper and then centrifuged at 8,000 rpm for 30 min to remove heavy biomaterials. The extract was stored at 4°C until used. Fig.1 Pictures of the aqueous extract preparation steps: (A) shows viscum album, (B) shows fruit of viscum album, (C) shows the aqueous extract of viscum album fruit, and (D) shows the dried apple extract

Synthesis of silver nanoparticles

For the synthesis of silver nanoparticles, 10 ml of aqueous extract was slowly added to 50 ml of 10 mM aqueous silver nitrate solution for the reduction of Ag^+ to Ag. The solution was allowed to react at temperature 25°C , and kept for 2 h. The color change was noted by visual observation indicating the formation of silver nanoparticles. The completion of the reaction was

monitored by UV-visible spectroscopy. Fig.2 Pictures of silver nanoparticle synthesis steps are:



Fig. 2. Pictures of silver nanoparticle synthesis steps are:(A) 10 mL of viscum album fruit extract and 50 mL of 10 mM aqueous silver nitrate solution, after 30 min.(B) 30 mL of extract with 10 mM aqueous silver nitrate solutions after 2 h under ultrasonic.

(A) 10 mL of viscum album fruit extract and 50 mL of 10 mM aqueous silver nitrate solution, after 30 min.(B) 30 mL of extract with 10 mM aqueous silver nitrate solutions after 2 h under ultrasonic.

Characterization of silver nanoparticles

The synthesized silver nanoparticles were confirmed and characterized by the following methods; UV-Vis spectroscopy, scanning electron microscopy (SEM), X-ray diffraction (XRD) and Fourier transform infrared spectroscopy (FT-IR). UV-Vis spectra of synthesized silver nanoparticles were monitored as a function of reaction time on a spectrophotometer (PG, UK) in 400-500 nm range operated at a resolution of 10 nm. SEM analysis was done using Hitachi S-4500. The crystalline nature of silver nanoparticles was studied by XRD analysis using an X-Ray diffractometer (Bruker AXS D8 ADVANCE). The FTIR spectroscopy was done using a Perkin Elmer infrared spectrometer (model TENSOR 27Bruker) in the range 400-4000 cm^{-1} .

RESULTS AND DISCUSSION

The green synthesis of silver nanoparticles was done using aqueous extract of Mistletoe and aqueous silver nitrate solution. Visual observation, UV-Vis, XRD, SEM, and FTIR studies confirmed the bio-reduction of aqueous silver ions to silver

Characterization of silver nanoparticles by UV-Vis Spectroscopy

It is obvious that silver nanoparticles in aqueous solution show yellowish brown color due to excitation of surface plasmon vibrations of silver

nanoparticles [34]. When the extract of Mistletoe was mixed with the aqueous solution of the silver ion, it started to change the color from colorless to yellowish brown during the time, indicating the formation of silver nanoparticles. In contrast, the color of control solution (without silver nitrate ions) remains unchanged during this period of the experiment (Fig. A, after 30 and 120). The intensity of color was directly proportional to the formation of nanoparticles (Fig. B, after 30). With increasing the extract concentration, the color of the reaction solution changed to reddish brown and finally to colloidal brown, leading to the formation of well-defined and stable silver nanoparticles (Fig. C, after 30 and 120 under ultrasonic wave). So, when the concentration of the biological material mediating nanoparticle synthesis is increased, higher contents of the biomolecules are involved in the metal reduction. Fig. 3 Pictures of the reaction solutions including: (A) Control solution containing 10 mL of viscum album extract and 30 mL of doubled distilled water (without silver nitrate ions), first after 30 min, and then after 120 min. (B) 10 mL of viscum album fruit extract and 50 mL of 10 mM aqueous silver nitrate solution, after 30 min. (C) 30 mL of extract with 10 mM aqueous silver nitrate solutions after 2 h under ultrasonic.

On the other hand, UV-Vis spectroscopy is generally recognized as an important technique to examine the metal nanoparticles in aqueous suspensions [35]. The UV-Vis spectrum of silver nanoparticles was recorded after 15, 30, 45 Minutes from the initiation of reaction between the extract of viscum album fruit and aqueous silver nitrate

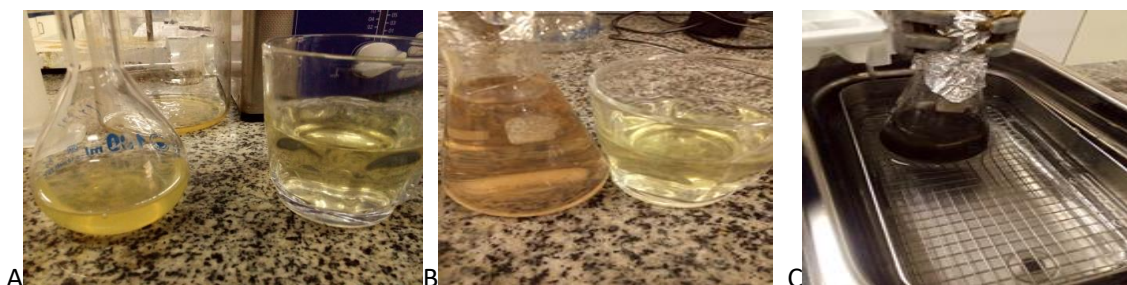


Fig. 3. Pictures of the reaction solutions including: (A) Control solution containing 10 mL of viscum album extract and 30 mL of doubled distilled water (without silver nitrate ions), first after 30 min, and then after 120 min. (B) 10 mL of viscum album fruit extract and 50 mL of 10 mM aqueous silver nitrate solution, after 30 min. (C) 30 mL of extract with 10 mM aqueous silver nitrate solutions after 2 h under ultrasonic

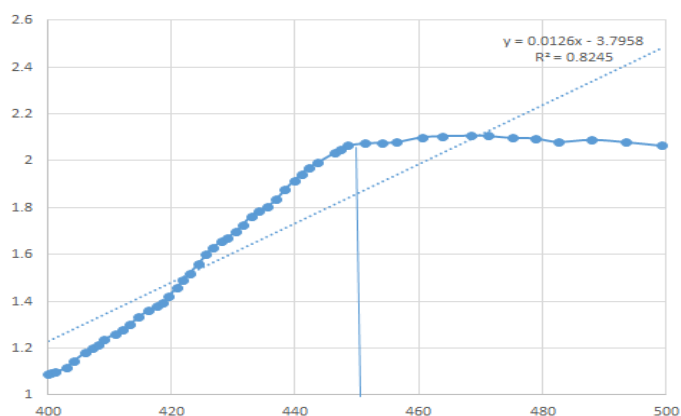


Fig. 4. UV-Vis spectra of the colloidal silver nanoparticles synthesized using the extract of viscum album fruit and aqueous silver nitrate solution recorded at reaction times.

solution.

As shown in Fig. 4, the UV-visible spectra show an absorption band at about 450 nm corresponding to the absorbance of silver nanoparticles and steadily increases in intensity as a function of time of reaction without any shift in the peak wavelength [19]. The spicy brown color in the reaction solution results from absorption of the colloidal silver nanoparticles in the visible region of the electromagnetic spectrum (380-500 nm) due to the excitation of their surface plasmon vibrations [36]. The UV-visible absorption spectra of silver nanoparticles exhibit characteristic surface plasmon resonance bands centered at 425 nm. The location of the surface plasmon resonance peak on the lower end of the absorption range (380-500 nm) indicates that the colloidal dispersion was primarily composed of small spherical silver nanoparticles which are in agreement with SEM results.

SEM analysis of silver nanoparticles

SEM has provided further insight into the morphology and size of the synthesized nanoparticles. The micrographs of the synthesized silver nanoparticles synthesized using the aqueous extract of viscum album fruit in optimum conditions are shown in Fig. 5(A). The result shows that the synthesized silver nanoparticles have spherical shape and slightly agglomerate (Clump). The particle sizes were found in the range of 40-70 nm

XRD pattern of silver nanoparticles

The crystalline nature of silver nanoparticles was confirmed by the analysis of XRD studies. The XRD pattern of the silver nanoparticles synthesized in optimum conditions is shown in Fig. 6. The peaks in the X-ray diffraction pattern are due to reflections from the (111), (200), (220), and (311)

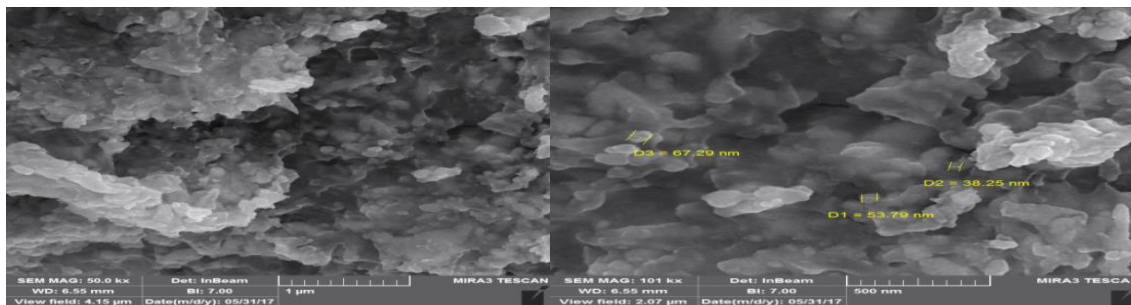


Fig. 5. SEM image of the synthesized silver nanoparticles

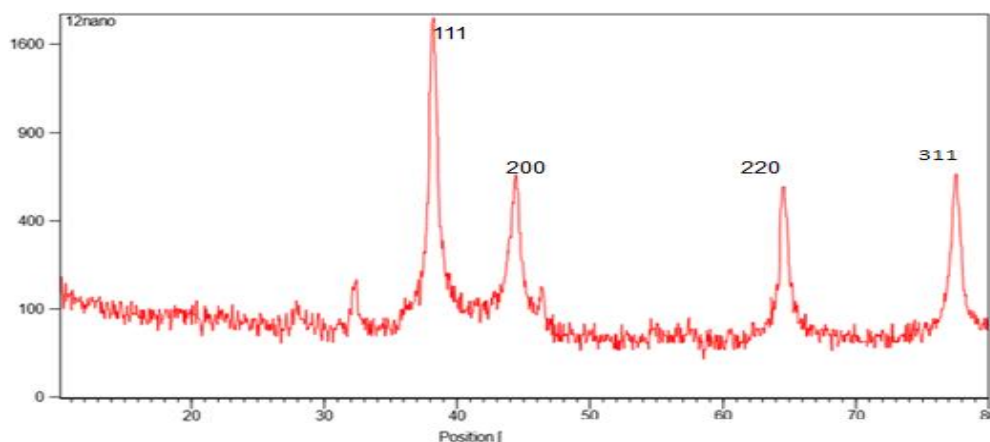


Fig. 6. XRD pattern of the synthesized silver nanoparticles

planes and confirm the crystalline structure of the synthesized silver nanoparticles. The peaks at 32.1 and 46.4, in Fig. 6, may be due to the metabolites present in the extract and responsible for silver ions reduction and stabilization of the resultant nanoparticles.

FT-IR analysis

FT-IR measurements were performed to identify the major functional groups on the extract of *Viscum album* fruit and their possible involvement in the synthesis and stabilization of silver nanoparticles. The spectra of the *viscum album* fruit before reaction with silver nitrate are shown in Fig. 7. The FT-IR spectrum of the extract of *viscum album* fruit shows several characteristic peaks; absorption band of O-H 3431.88 cm^{-1} , peaks at 2919.98 cm^{-1} for $-\text{CH}_2$ characteristic absorption band at about 2850.79 cm^{-1} for alkanes C-H stretching, peaks at 2361.95 cm^{-1} for band phosphine P-H and absorption band C=O at 1733.46 cm^{-1} for esters, band alkenes C=C or

C=O at 1641.72 , peaks at 1465.33 for alkanes and aromatic bands C-C or CH_2 and CH_3 , absorption bands N-O or S=O at 1377.65 , peaks at 1272.17 for band P=O phosphoramidate and absorption band at 1172 for S=O sulfonyl chloride, peaks at 1105.38 band C-O alcohols, absorption band P-OR esters at 1039.07 and peaks at 722.97 for band C-H rock alkanes, indicating that these groups are effective in the reduction of the silver ions to silver nanoparticles. The FT-IR spectrum of silver nanoparticles (reaction product) shows some of the peaks of the extract of *viscum album* fruit, such as O-H, $-\text{CH}_2$, C-H, P-H, C=O, are decreased and some peaks are lost, indicating that these groups are effective in the reduction of the silver ions to silver nanoparticle. The peaks observed at 3433.72 cm^{-1} ($-\text{OH}$), 2920.89 cm^{-1} (chelating compounds), 2851.85 cm^{-1} (C-H), 2365.72 cm^{-1} (P-H), 1642.83 cm^{-1} (C=O) and 1462.79 cm^{-1} (C-C, or CH_3) suggest the presence of flavanones or terpenoids which adsorbed on the surface of silver nanoparticles.

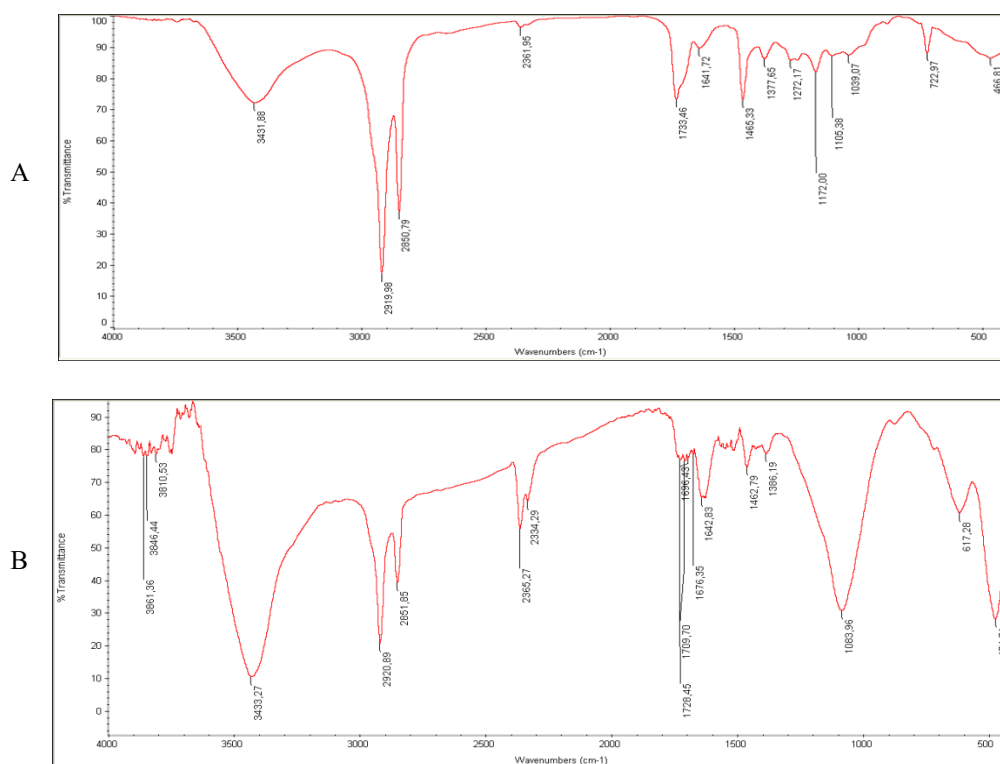


Fig. 7. FT-IR spectra of (A) the extract of viscum album fruit and (B) silver nanoparticles

CONCLUSION

Green synthesis of silver nanoparticles using plant extract has shown many advantages such as safe, non-toxic, and ecofriendly synthesis which can be manufactured at a large scale. The results in the present study show that the aqueous extract of viscum album fruit can act as a reducing agent as well as the capping agent that can be used for the synthesis of silver nanoparticles. Hence, water soluble metabolites containing functional groups are suggested to be responsible for the reduction and stabilization of silver nanoparticles. The XRD, and SEM techniques confirmed that the synthesized nanoparticles have spherical shape with an average size of about 50 nm.

CONFLICT OF INTEREST

The authors declare that they have no conflict of interest.

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