

## Production of Silver Nanoparticles by Marigold Extract

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### Abstract

Silver nanoparticles are widely used in manufacturing of different products considering their unique physical and chemical properties. Also they have been noticed in medical diagnosis and treatment because of their antibacterial properties. Physical and chemical methods of producing nanoparticles, are expensive and are not safe enough as toxic substances may remain in the final preparations. To solve this problem, biological production of nanoparticles is considered as an efficient alternative method. In present study, synthesis of silver nanoparticles via seeds, petals, roots, and hairy root extracts of *Calendula officinalis* were performed. These nanoparticles were characterized by means of spectrophotometer, particle size analyzer and transmission electron microscope. Nanoparticles which were synthesized by hairy root extract showed the highest absorption at 430 nm (1/6 a.u) and the smallest size (5/3 nm), in comparison to other examined particles. Results confirmed the better performance of hairy root extracts in the synthesis of silver nanoparticles.

**Keywords:** *Calendula officinalis*, Marigold, Hairy roots, Silver nanoparticles

### Introduction

Metal nanoparticles, similar to mass metals, show special and various electrical, magnetic, catalytic and light properties. These properties cause the widespread applications of nanoparticles in biomedical engineering, chemical, optical, electronic and life sciences. At present, various types of metal nanoparticles are produced by copper, titanium, magnesium, gold, and silver alloys and applied in a wide range of applications from preparation of equipment and tools for optical, catalytic and electronic industries to treat some cancers (Naveen et al., 2010). Among metal nanoparticles, silver nanoparticles play more prominent role because of their catalytic and antibacterial properties (Garrick and Pinches, 2006). Moreover, proper particle size and distribution are among special properties of silver nanoparticles. Chemical and physical methods for synthesis of colloidal silver nanoparticles, including laser-based production, chemical reduction, photochemical reduction and laser emitters-based production are still in progress and reform. They are often encountered with problems such as low stability of nanoparticles, controlling crystal growth and aggregation of particles by a little change in the temperature and/or pH (Bhainsa and Souza, 2006).

The antibacterial potential of nanoparticles is used in a large number of commercial products such as clothing, appliances, textile and weaving industries, water treatment, preparation of antimicrobial agents, and sunscreen lotions (Naveen et al., 2010).

Main reasons for the synthesis of silver nanoparticles via biological systems are their safe, simple, durable (Devina et al., 2010), eco-friendly, cost-effective and nontoxic properties (Thangaraju et al., 2012). Moreover, synthesis steps could be carried out at room temperature, without the need for providing high pressure or production of any toxic byproducts (Marchiol, 2012). David and Moldovan (2020) reported a green synthesis method for obtaining silver nanoparticles using *Viburnum opulus* fruit extracts. Their results confirmed the production of spherical and uniform-sized silver nanoparticles (AgNPs) with an average diameter of 16 nm.

Retrieval of nanoparticles from plant tissues is a tedious and expensive procedure, which needs the application of enzymes for degradation of plant cellulose tissue (Hu and Easterly, 2009). Therefore, the application of plant extracts, as substitutes of solid tissues, for small- or large-scale preparation of various metal nanoparticles is easier. For the first time, the extracts of leaves, stems, and roots of Cranesbills were applied for extracellular production

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of gold nanoparticles. Shankar et al. (2003) reported the biological reduction of gold ions to gold nanoparticles using geranium leaf extract. Also, they were produced triangular and spherical gold nanoparticles, by lemon juice (Shankar et al., 2004). Gold nanoparticles were synthesized from the reaction of Neem extract with  $\text{HAuCl}_4$  (Chloroauric acid) in a period of two hours. The formation of nanoparticles was approved by UV-Vis-IR spectroscopy analysis and observation of color changes. It seems that nanoparticles which were produced by this method, in addition to spherical structures, also tend to have thin flat structures. These flat particles which were mainly in the form of triangular and to a lesser extent in hexagonal shape, had 50-100 nm size (Shankar et al., 2004). Silver nanoparticles can be also prepared from Aloe Vera extract. Developed nanoparticles had spherical shape and were in 15/5 nm size (Chandran et al., 2006). Leela and Vivekanandan (2008) synthesized silver nanoparticles using leaf extracts of *Helianthus annuus* and *Basella alba*. They showed that *Helianthus* has a comparably stronger potential for fast reduction of silver ions. They also reported that Poly-L and freaking loop water-soluble components, are primarily responsible for the decline and stabilization of silver nanoparticles. Other studies, have been shown that various parameters including plant source, organic compounds of leaves' extracts,  $\text{AgNO}_3$  concentration, temperature and the presence of pigments are effective in the development of nanoparticles (Leela and Vivekanandan, 2008). Bar et al, (2009) made silver nanoparticles using *Jatropha curcas*, as a reducing agent. These nanoparticles were identified by high-resolution transmission electron microscopy (HRTEM), X-ray diffraction and UV-Vis spectroscopy. As it was confirmed by HRTEM imaging, particles with 10 to 20 nm size ranges are usually stabilized by the cyclic peptides. Zargar et al, (2011) synthesized silver nanoparticles using methanol extract of *Vitex negundo* as a reducing and stabilizing agent, and investigated their antimicrobial properties. Changing solution's color from yellowish green to dark brown was considered as the determinant of nanoparticles formation. Spectrum UV-Vis experiments, indicated broadband SPR in two peaks of 442 and 447 nm.

Gnana Jobitha et al, (2012) were produced silver nanoparticles using *Elettaria cardamomum* and investigated their antimicrobial properties. Formation of silver nanoparticles was confirmed by pale yellow to dark brown color changes and the presence of a unique peak in the range of 460 nm, which was detected by UV spectrophotometer. Also,

antibacterial properties of these nanoparticles against pathogenic bacteria including *Bacillus subtilis* and *Klebsiella planticola* were reported. Shafaghat in 2014 used methanol extract of *Viburnum lantana* leaves for biosynthesis of silver nanoparticles and assessed their biological activity against gram-positive and gram-negative bacteria. Results indicated the strong antimicrobial effects of silver nanoparticles by rapid reduction of  $\text{Ag}^+$  ions to  $\text{Ag}^0$  as the mechanism of action.

Marigold extract has been used previously for the synthesis of silver nanoparticles. Marigold with scientific name *Calendula officinalis* belongs to the Asteraceae family. This medicinal plant is cultivated throughout the temperate and sunny regions of the world and could be localized easily. Chidambaram et al, (2014) synthesized silver nanoparticles using petal extracts of marigold. Baghizadeh et al, (2015) were applied calendula seed extracts for silver nanoparticle synthesis. Rodino et al, (2019) have been produced silver nanoparticles via green synthesis, using vegetal extracts of pot marigold flowers (*Calendula officinalis*), and investigated their application for healthy storage of fruits.

## Materials and Methods

### Plant extracts preparation steps

The silver nitrate was purchased from the Merck Company (Germany) and Marigold seeds were purchased from Isfahan Pakan seed Company. Marigold seeds were planted in the pots while they were kept in the greenhouse until flowering time. Sterile seedlings, were applied for preparation of Marigold hairy roots, which were then grown in a liquid  $1/2$  B5 medium to collect hairy root extracts. In addition, roots, petals, and seeds of Marigold were used for preparation of aqueous extracts. 10 grams of roots, petals, seeds and hairy roots were washed by deionized water. Then the samples were dried, powdered, and boiled in 100 ml of deionized water for 10 minutes before filtration by Whatman paper (No. 1). Final preparations, were kept in the refrigerator for downstream applications.

### Synthesis and purification of silver nanoparticles

The aqueous extracts, with two different volumes (5 and 10 ml) were poured into 100 ml Erlenmeyer flasks and brought to the volume of 100 ml by the addition of Silver nitrate solution ( $1 \times 10^{-3}$  M), for converting the  $\text{Ag}^+$  ions to  $\text{Ag}^0$  ones. They were kept at room temperature for 24 hours.

Then, silver nanoparticle solutions were centrifuged at 13,000 rpm for 15 minutes (Sigma 14-1, Germany). The upper solution was poured out in

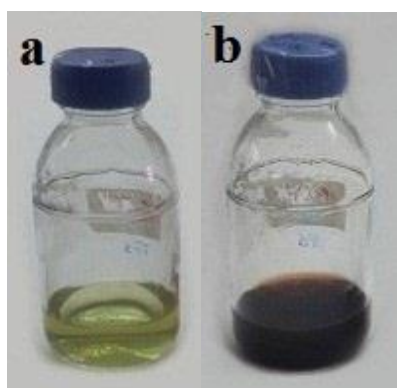
each case and the silver nanoparticles which were precipitated at the bottom of the tube, mixed with deionized water and centrifuged again. This procedure was repeated for three times to increase the purity of nanoparticles. Then, the best samples were selected based on their appearance properties, such as intensity of darkness and applied for further experiments.

### Characterization of silver nanoparticles

The revival of pure  $\text{Ag}^+$  ions was evaluated through UV-Vis spectroscopy. The UV-Vis spectrum analyses were performed using Array Photonix Ar 2015 spectrophotometer in the range of 300-350 nm wavelengths. In order to determine the size distribution of synthesized nanoparticles, Particle Size Analyzer (Vasco, France) was applied. To do this, samples were diluted (10 folds) with distilled water prior to analyses. Also, for more precise determination of particle sizes and morphological study of nanoparticles, transmission electron microscopy (Leo 912AB) was carried out at a voltage of 120 kV. TEM samples were prepared on carbon coated copper grids by dropping AgNPs colloidal solutions and the following drying steps under vacuum.

### Results and Discussion

At first, plant extracts were pale yellow or green but following the addition of silver nitrate solution, their color changed to dark brown in 24 hours. As demonstrated in figure 1, this color changes were apparently visible (Figure 1).



**Figure 1.** Color differences between (a) Marigold extract solutions and (b) Marigold extracts in the presence of silver nitrate solutions.

Plant extracts, which were kept in the absence of silver nitrate solution and didn't show any color changes, were considered as controls. The establishment of dark brown color after mixing the extracts with silver ions was a clear indicator of the

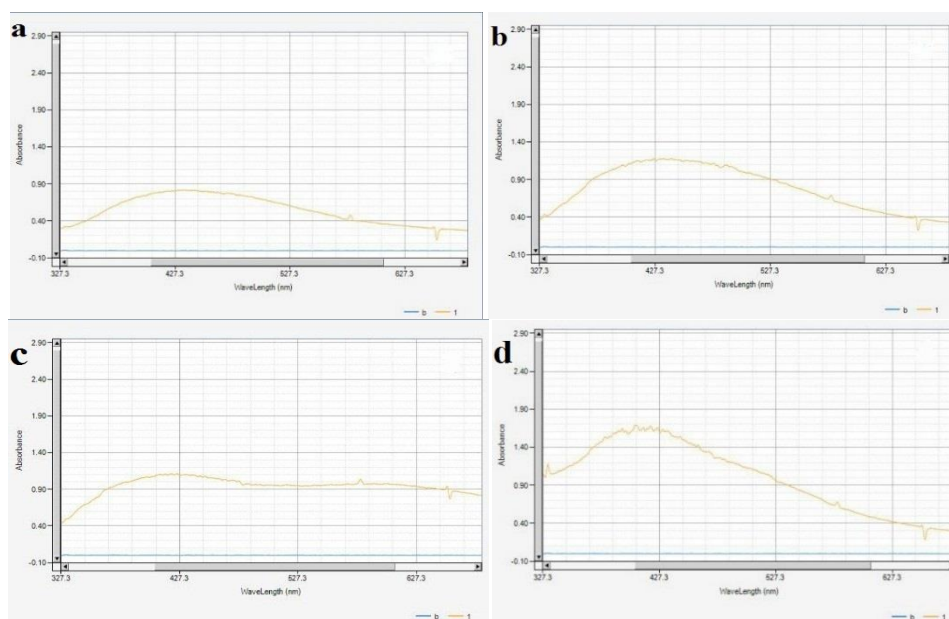
metal ions reduction and the formation of silver nanoparticles in the environment. The addition of plant extracts to silver nitrate solution at a ratio of 10 to 100 was the best and produced relatively darker nanoparticle solutions.

In general, if color changes are associated with the formation of sediments, so they will show the formation of large particles; but, when color changes happened without any sedimentation, it could be concluded that synthesized nanoparticles have a very small particle size distribution. During our experiments no sedimentation was observed in nanoparticle solutions of marigold extracts.

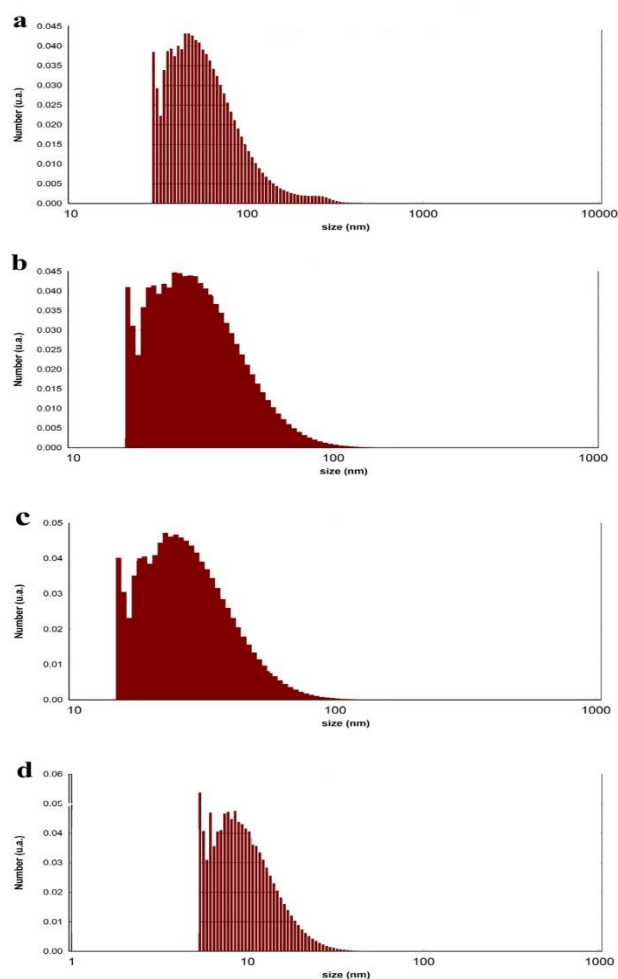
### Formation and stability of silver nanoparticles

To confirm the formation and proper stability of silver nanoparticles, their absorption spectrum was read after 24 hours in the range of 300-350 nm using a spectrophotometer (Unico Gene Company, USA). Results of UV-Vis analyses (Figure 2) showed that the aqueous extracts of various plant organs of Marigold, were produced different concentrations of silver nanoparticles. The highest absorbance at 430 nm was 1.6 a.u (Absorbance unit) and recorded for the nanoparticles which were synthesized using hairy root extracts, while, the lowest was obtained for nanoparticles synthesized by seed extracts (0.8 a.u). Absorptions of 1.5 a.u and 1.17 a.u were respectively observed at 430 nm for nanoparticles synthesized by petal and root extracts. Marchiol et al, (2014) used extracts of different organs (leaves, stems and roots) of various plants including *Brassica juncea*, *Festuca rubra* and *Medicago sativa* for synthesis of silver nanoparticles. Extracts of different plant organs indicated different performances during synthesis of nanoparticles. In *Brassica juncea* and *Festuca rubra*, root extracts showed a better performance, while, in *Medicago sativa*, leaf extracts were the best. In the present study the potential of various organ extracts of marigold (leaves, petals, seeds, roots and hairy roots) for production of silver nanoparticles were assessed comparatively, and the priority of hairy root extracts were confirmed.

The results of particle size analyses were shown in Figure 3. Results indicated that nanoparticles which were synthesized using hairy root and root extracts had the smallest size, respectively. The size of smallest and largest nanoparticles synthesized were equal to 5.4 and 22.4 nm in hairy root extracts, 14.8 and 61.7 nm in root extracts, 16.2 and 67.6 nm in petal extracts, and 29.5 and 67.6 nm for seed extracts. As demonstrated in Figure 3 (C, D), nanoparticles synthesized by hairy root and root extracts of marigold had the smallest size.



**Figure 2.** The results of UV-Vis analyses of silver nanoparticles synthesized by seed (a), petal (b), root and (c), hairy root (d) extracts of Marigold.

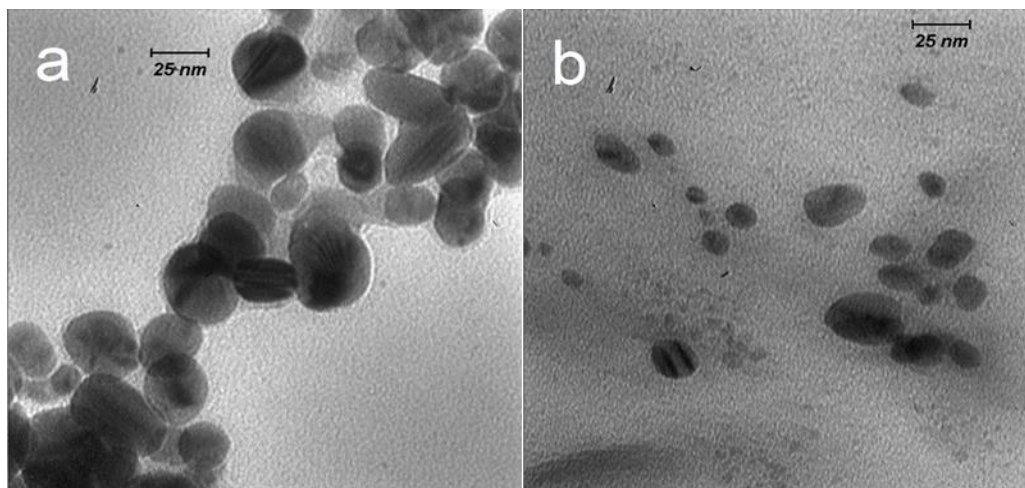


**Figure 3.** The results of nanoparticle size analyses obtained based on dynamic light scattering experiments (Cumulants mode) for silver nanoparticles synthesized by seed (a) petal (b) root (c) and hairy root (d) extracts of *Calendula officinalis*.

For more certainty and also to determine the morphological features of silver nanoparticles, they were investigated via transmission electron microscope.

The size of silver nanoparticles can be very different. This depends on materials applied to reduce silver ions, silver nitrate concentration, temperature conditions, and so on. In 2011, Darroudi et al, from Putra Malaysia University reported green synthesis and characterization of silver nanoparticles.

synthesized silver nanoparticles, vastly, depend on the used materials and synthesis conditions. TEM confirmed the desired spherical appearance for silver nanoparticles which were synthesized in present study (Figure 4).



**Figure 4.** Images obtained via transmission electron microscopy (TEM) for silver nanoparticles with spherical appearance, which were synthesized using root (a) and hairy root (b) extracts of *Calendula officinalis*, scale bar: 25 nm.

They showed that by increasing the temperature, smaller silver nanoparticles are produced. Also, silver nanoparticles which were obtained in gelatin solution were smaller than the ones prepared in sugar containing gelatin solution. In another study, *Catharanthus roseus* was applied for synthesis of nanoparticles and their properties were determined by spectrophotometer UV-VIS, SEM and X-Ray analyses. They reported the preparation of nanoparticles with a diameter between 48 and 67 nm.

This method was introduced as eyewitness to produce nanoparticles with added value for commercial and industrial applications (Mukunthan et al., 2011). Padalia et al. (2015) produced silver nanoparticles by *Calendula officinalis* extract. The size of the synthesized nanoparticles was between 10-90 nm and they had spherical, hexagonal, and irregular shapes. Chidambaram et al. (2014) prepared silver nanoparticles from petal extracts of *Calendula officinalis* with 2-20 nm size ranges. Baghizadeh et al. (2015), reported the synthesis of silver nanoparticles from marigold seed extracts with spherical shape and diameter of 5 to 25 nm. Most of the nanoparticles produced in this study were located between 5 to 10 nm, considering their size distribution. It seems that the size and shape of

## Conclusion

During last decades, silver nanoparticles have been attracted worldwide attention due to their unique physical and chemical properties. Considering disadvantages of physical and chemical methods of nanoparticle synthesis, green synthesis technologies were suggested as efficient alternatives. Different plant organs could be examined to achieve best results. Results from the present study, for the first time, confirmed that hairy root extracts of marigold had a better performance for production of desirable silver nanoparticles in comparison to other organs extracts.

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## Conflict of Interest

The authors report no conflicts of interest.

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