



## Research Article

# Biogenic Synthesis, Characterization of Silver Nanoparticles from *Candelula Officinalis* Extract and Evaluation of their Antimicrobial Activity

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**Received:** Apr. 28, 2015; **Accepted:** Jun. 9, 2015; **Published:** Jun. 18, 2015.**Citation:** Rashmi Arora and Anita Grewal. Biogenic Synthesis, Characterization of Silver Nanoparticles from *Candelula Officinalis* Extract and Evaluation of their Antimicrobial Activity. Nano Biomed. Eng. 2015, 7(2), 47-51.**DOI:** 10.5101/nbe.v7i2.p47-51.

## Abstract

Synthesis of silver nanoparticles from root extract of *Candelula officinalis* (marigold) as a biomaterial is reported in the present study. Plant root extract has water soluble organic materials which help in the reduction of silver ions and stabilization of AgNPs. Aqueous solution of silver nitrate was treated with root extract of *C. officinalis* for the formation of AgNPs. The UV-Visible spectroscopy indicated an absorption peak at 423 nm, which indicates synthesis of AgNPs. Further characterization by Dynamic Light Scattering (DLS) showed size distribution profile of synthesized silver nanoparticles (AgNPs). Transmission electron microscopy (TEM) demonstrated the presence of spherical AgNPs in range of 14-40 nm. The formed silver nanoparticles showed good antimicrobial activity against various microorganisms viz. *Eshcherichia coli*, *Pseudomonas aeruginosa*, *Bacillus subtilis*, *Candida albicans*. AgNPs showed the maximum activity against *Pseudomonas aeruginosa* with a inhibition zone of 22 nm. Thus, the present study provides evidence of simple, rapid and environmental friendly route to synthesize AgNPs using aqueous root extract of *Candelula officinalis*.

**Keywords:** Silver nanoparticles; *Candelula officinalis*; TEM; DLS; UV-Vis; Antimicrobial activity

## Introduction

Nanotechnology deals with the production, manipulation, and use of material that ranges from less than a micron in size. A nanometer is 1 billionth of a meter or roughly the length of 3 atoms side by side [1]. Bionanotechnology on the whole deals with the interaction of biology, chemistry, material sciences, engineering, and medicine. Silver nanoparticles are of immense use due to their size, catalytic properties, and antimicrobial behavior [2]. Remarkable advances are

made in the field of biotechnology and nanotechnology to harness the benefit of life sciences, health care and industrial biotechnology [3, 4]. Nanomaterials may provide solutions to technological and environmental challenges in the areas of solar energy conversion, catalysis, medicine and water treatment [5]. This increasing demand must be accompanied by “green” synthesis methods. The green synthesis of nanoparticles, on the other hand, concerns the transformation of biological systems to create new devices on a nanoscale. These are produced through

various physical and chemical processes however, all of these methods have detrimental effects due to increased size, high energy and capital investment requirements, and the use of toxic chemicals [6, 7]. Keeping in view the disadvantages entailed in both processes, we have adopted biogenic methods for the synthesis of AgNPs. These methods are clean and less expensive, require less energy, and employ ambient conditions [8]. There are many examples of synthesizing silver nanoparticles using plant/plant extract because they possess large variety of metabolites like terpenoids, flavones, aldehydes, ketones etc for the bioreduction involved in biosynthesis [9]. Silver has long been recognized as having inhibitory effect on microbes. Ag NPs are supposed to be the new generation of antimicrobials as they are being used in many antimicrobial preparations [10], therapeutics [11] and pharmaceutical applications [12] antiviral and HIV studies [13] applications.

*Candelula officinalis* (Marigold) is a common garden herb which belongs to family Asteraceae. *Calendula officinalis* is a good source of natural antioxidants, which contains many different radical scavenger components providing protection against harmful-free radicals. It is used for the treatment of inflammation and skin wounds [14, 15]. It is having medicinal importance due to presence of oleanolic acid which provide health benefits, protective effects against the development of cancer, chemotherapy and radiation therapy adverse effects, inhibition of existing tumour cells, anti-inflammatory activity and antioxidant activity [16]. Thus, we report here synthesis, characterization and antibacterial activity of AgNPs using root extract of *Candelula officinalis*.

## Materials and Methods

### Preparation of Plant Extracts

The roots of *Calendula officinalis* (marigold) collected from the campus of Kurukshetra University, Kurukshetra were thoroughly washed several times with running water to remove the dust particles. They were placed in a hot air oven for drying at 70° overnight for 4-5 days. Dried parts were ground to fine powder and used for preparing the plant extract. The extract was prepared by taking 20 g of powder in 100 ml deionized water in a 250 ml of conical flask. This mixture was boiled for 10 min. using hot plate and filtered with whattman No.1 filter paper. The extract was preserved inside a refrigerator for future use. Then

the solution was used for the reduction of silver ions ( $\text{Ag}^+$ ) to silver nanoparticles ( $\text{Ag}^0$ ).

### Cultures

*Escherichia coli* (MTCC No. 40), *Bacillus subtilis* (MTCC No. 441), *Candida albicans* (MTCC No. 3017), *Pseudomonas aeruginosa* (MTCC No. 424) used for antimicrobial assay of AgNPs were obtained from IMTECH, Chandigarh, India.

### Synthesis of silver nanoparticles

Still up to date there is no proposed mechanism for the synthesis of silver nanoparticles. The hypothetical method behind synthesis is an enzymatic reaction in which plant extract contains the complex of reducing enzymes which reduce the chemicals such as silver nitrate into silver ions and nitrate ions. Silver nitrate solution of (1 mM) was prepared and used for synthesis of silver nanoparticles. 4 ml of plant extract was added to 16 ml solution of 1mM  $\text{AgNO}_3$  for reduction into  $\text{Ag}^+$  ions, incubated at 80 °C in a water bath for 10 mins. The colour of the solution changed to brown red indicating the synthesis of silver nanoparticles.

### Characterization of silver nanoparticles

#### UV-Visible spectroscopic analysis

UV-Vis absorption spectroscopy is the branch of spectroscopy where the absorption of light by molecules that are in a gas or vapor state or dissolved molecules/ions can be measured. It investigates the absorption between the wavelength limits 190 nm and 1100 nm where absorption of the electromagnetic radiation is caused by the excitation of bonding and non bonding electrons of the ions or molecules. The reduction of metallic  $\text{Ag}^+$  ions was observed by subjecting small aliquots from the reaction mixture for measurement of the absorbance by UV-Visible spectrophotometer which showed absorption peak at 423 nm that indicates the synthesis of silver nanoparticles.

#### Transmission electron microscopy

TEM uses an electron beam to prepare an image of a sample. High energy electron beam falls upon ultrathin samples allow for image resolutions that are of the order of 1-2 Å. Scanning under TEM (Philips CM-10) was done to find out shapes and sizes of synthesized silver nanoparticles (AgNPs).

## Dynamic light scattering

The Dynamic light scattering (DLS) or photon correlation spectroscopy method was used to obtain the size distribution profile of nanoparticles present in suspension or solution. Dynamic Light Scattering Analyzer is required for DLS analysis.

## Evaluation of antibacterial activity

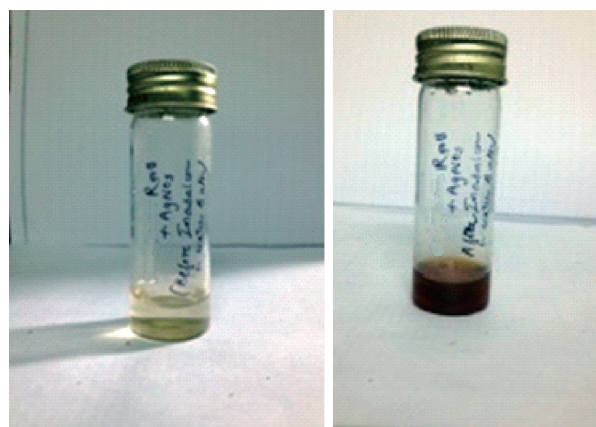
Effect of biosynthesized silver nanoparticles was analyzed against human pathogens by agar well diffusion method. In this method, three wells were cut in nutrient agar plates with the help of sterile 1 ml tips. Pathogens (*Bacillus subtilis*, *Staphylococcus aureus*, *Pseudomonas aeruginosa*, *Escherichia coli*, *Candida albicans*) were spread on agar plates by spread plate method. After that, in one well 2 to 20  $\mu$ l plant extract was filled. 2nd well was filled with  $\text{AgNO}_3$  (Positive control) and 3rd well was filled with the mixture of  $\text{AgNO}_3$  and extract. Then, the plates were incubated at 37 °C for 24 h. After incubation, the zones of inhibition were measured and activities were evaluated by increase in fold area activity.

## Results and discussion

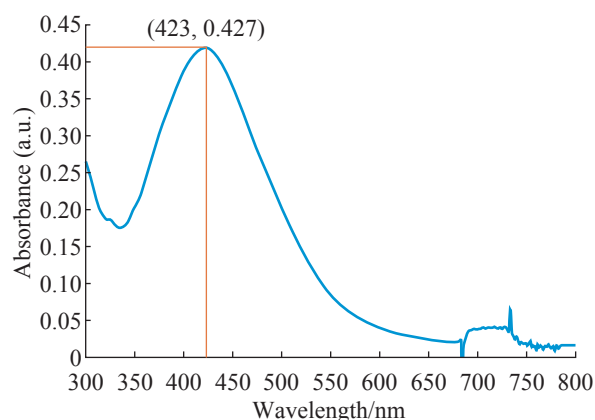
The reduction of silver ions into silver particles following exposure to plant extracts could be deciphered by a change in color. Synthesised silver nanoparticles of *C. officinalis* in the present study exhibited a dark yellowish color initially when the extract was treated with  $\text{AgNO}_3$  in ratio of 4:1 but changed to brownish red after incubation at 80 °C in water bath for 10 minutes (Fig. 1). The reason for colour change was the excitation of Surface Plasmon vibrations in metal nanoparticles [17, 11]. Optical absorption spectroscopy has proved to be a very useful technique for the analysis of nanoparticles.

In order to verify the synthesis of AgNPs, the test samples were subjected to UV-vis spectrophotometric analysis. The test sample (root extract treated with 1mM of  $\text{AgNO}_3$ ) was collected in aliquots from the reaction mixture and analysed to record their absorbance by UV-vis spectrophotometer. This analysis showed the sharp absorbance at around 423 nm (Fig. 2), which was specific for AgNPs [18, 19]. Li et al. [20] reported similar results, in which they observed that when sun-dried leaf extracts of *Capsicum* were challenged with aqueous silver ions, the reaction mixture containing AgNPs showed the absorption

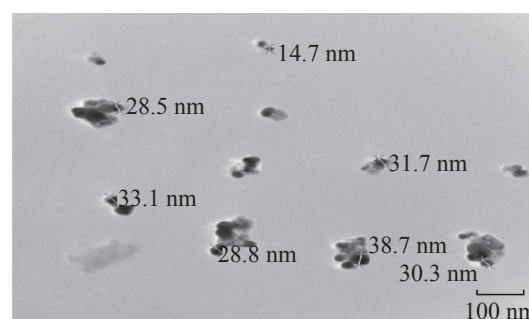
peak at about 420 nm due to the excitation of plasmon resonance vibration. Similarly TEM Analysis was done to find out the shape and size of biosynthesized silver nanoparticles in solution. TEM images of AgNPs revealing the average mean size of 14-40 nm (Fig. 3). It was observed that most of the Ag nanoparticles were spherical in shape in addition there are agglomerated



**Fig. 1** Change in colour of root extract of *C. officinalis* from transparent to reddish brown after treatment with 1 mM of  $\text{AgNO}_3$  solution.



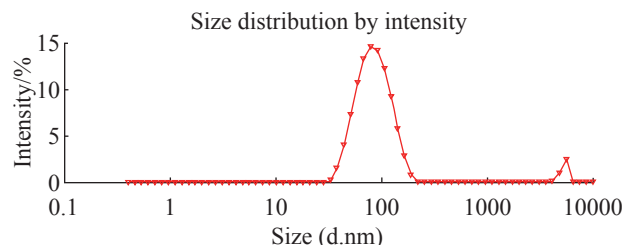
**Fig. 2** Uiv-Vis Absorption spectrum for silver nanoparticles synthesized by *Calendula officinalis* (marigold) root extract showing absorbance at 423 nm.



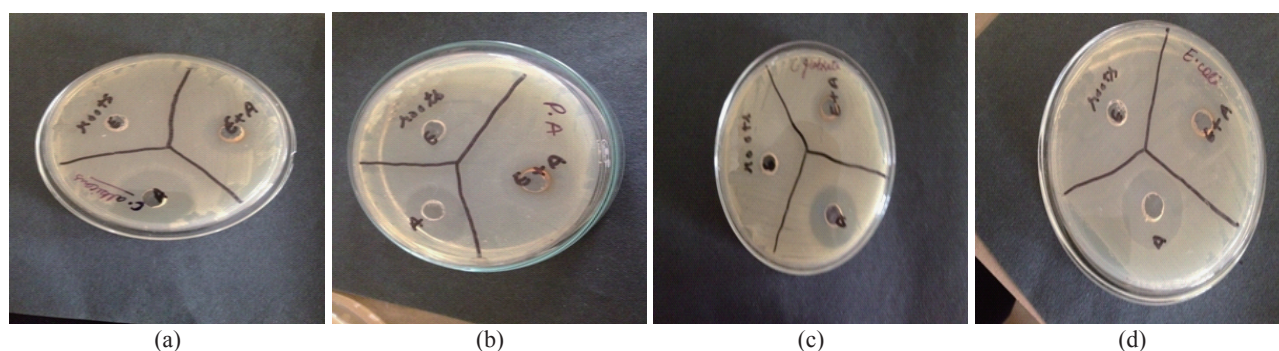
**Fig. 3** TEM images and size distribution of silver nanoparticles synthesized by reduction of  $\text{AgNO}_3$  with *C. officinalis* (Scale bar -100 nm).



		Size (d.nm)	Intensity (%)	Width (d.nm)
Z-Average (d.nm): 84.33	Peak 1:	87.34	96.4	31.14
PdI: 0.414	Peak 2:	5294	3.6	407.2
Intercept: 0.918	Peak 3:	0	0	0
Result quality: Good				



**Fig. 4** Graph between intensity (%) of light scattered from particles in suspension v/s diameter of silver nanoparticle synthesized from *C. officinalis* in nm.



**Fig. 5** Plates showing antimicrobial activity of AgNPs synthesized by *Calendula officinalis* (marigold) roots extract against *C. albicans* (a) and *P. aeruginosa* (b) *Bacillus* (c) and *E. coli* (d). (A:  $\text{AgNO}_3$ , E: Plant extract, A+ E: AgNPs).

silver nanoparticles, indicating possible sedimentation at a later time. Further FTIR analysis may be used to identify and to get an approximate idea of the possible biomolecules that are responsible for the reduction and stabilization of the AgNPs. Dynamic light scattering or Photon Correlation Spectroscopy is a technique used in material physics for determining the size distribution profile of metal based nanoparticles in solution. The study revealed that the average particle size of Ag nanoparticles synthesized from *Candelula officinalis* (marigold) root extract was in range of 40-400 nm with average size of approximately 80-90 nm (Fig. 4).

## Antimicrobial Activity of *C. officinalis* AgNPs

The antimicrobial activity of silver has been recognized by clinicians for over 100 years due to their growth inhibitory capability against microorganisms. The nanoparticles synthesized were used for antimicrobial activity against *Bacillus subtilis*, *Pseudomonas aeruginosa*, *Escherichia coli*, *Candida albicans*, it was found that the highest

inhibition was against *P. aeruginosa* with a inhibition zone of 22 mm (Fig. 5) whereas lowest was revealed against *B. subtilis*. These findings substantiate with the findings in which *S. aureus* showed the maximum sensitivity to Ag NPs, whereas *E. coli* was less sensitive [21]. Similarly, phytosynthesis of silver nanoparticles using *Pterocarpus santalinus* leaf extract and their antibacterial properties are also reported [22]. It is suggested that the antibacterial effect of Ag nanoparticles is associated with the peptidoglycan layer, although the precise mechanism of the inhibitory effects on microorganisms is not clear. Some have suggested that the positive charge on the Ag ion is crucial for antibacterial activity due to electrostatic attraction between the negatively charged cell membrane of the microorganism and the positively charged nanoparticles [23, 24]. There is an accumulation of Ag nanoparticles in the bacterial membrane causing an alteration in permeability, which results in cell death.

## Conclusions

It has been proved from above experiment that

root extract from *candelula officinalis* is capable of synthesizing silver nanoparticles extracellularly. Thus, it is an economic, quick, simple and environment friendly technique. Also Silver nanoparticles have several characteristics that make it currently among the most widely used nanoparticle in science. The characterization from UV-Vis, TEM, DLS support the stability of the biosynthesized nanoparticles. UV-vis spectra for *Calendula officinalis* (marigold) roots denoted a peak at 418 nm. TEM analysis of silver nanoparticles synthesized in present investigation confirmed the presence of spherical shaped nanoparticles in the size range of 40 nm. It may be inferred that root is the most relevant source for the extracellular synthesis of bionanoparticles. The great tendency of silver nanoparticles towards the toxicity for bacteria made them a good option for bio-medicinal applications.

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