# Facile Green Synthesis of Silver nano particles for Antimicrobial Applications

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Abstract:

This paper describes a facile eco-friendly technique for the synthesis of silver nanoparticles(AgNPs) from aqueous solution of silver nitrate using flower extract of Catharanthus roseus in a single-pot process. Formation of stable AgNPs at different concentrations of $AgNO_3$ gave mostly spherical particles with a diameter ranging from 5 to 40 nm. Here the aqueous extract contains Alpha-tomatine (α-tomatine) , a steroidal saponine as the major phyto chemical and which acts as structure directing agent for the formation of nano spheres of silver. It was observed that the use of makes a fast and convenient method for the synthesis of AgNPs and can reduce silver ions into silver nanoparticles within 2 min of reaction time without using any severe conditions. AgNPs was characterized by UV-visible spectroscopy, scanning electron microscopy( SEM), Transmission electron microscopy(TEM), Atomic force microscopy(AFM) and X-ray diffraction (XRD). The UV–vis spectra gave surface plasmon resonance for synthesized silver nanoparticles at 420 nm. The XRD analysis showed that the AgNPs are crystalline in nature and have face-centered cubic geometry. Further, the AgNPs showed an effective antibacterial activity toward S.aureous pathogen.

Keywords: Biosynthesis, Silver nanoparticles, Antibacterial activity.

## Introduction:

Recently, particular emphasis has been placed on the size and shape controlled synthesis of noble metal nanoparticles which was motivated by the requirements to tune their properties to achieve practical applications like biosensing, catalysis, optics, data storage, solar energy harvesting, antimicrobials, and biomaterial production.[1] Silver nanoparticles (AgNps) are of particular interest owing to their unusual size and shape dependent intrinsic properties.[2] The face-centered cubic (fcc) structure of silver metal confers its tendency to nucleate and grow into NPs with their surfaces bounded by the lowest-energy (111)facets resulting in the formation of nanospheres, anisotropic nanowires and rods.[4] In the recent years green synthesize of silver nanoparticles using plant extract has gained much interest from chemist and researchers. It possesses many

advantages over chemical, physical, and microbial synthesis because there is no need of the elaborated process of culturing and maintaining the cell, hazardous chemicals, high-energy requirements, and wasteful purifications.

. Nanocrystalline silver particles have been found tremendous applications in the fields of high sensitivity biomolecular detection, diagnostics, antimicrobials, therapeutics, catalysis and micro-electronics.[17] Silver nanoparticles are one of the most vital and fascinating nanomaterials among several metallic nanoparticles involved in biomedical applications. These nanoparticles are reported to be non toxic to human and most effective against bacteria, viruses and other eukaryotic micro-organisms at very low concentration and without any known side effects.[13] Plants with their antioxidant or reducing properties are usually responsible for the reduction of metal compounds into nanoparticles. Recent reports of plants towards production of nanoparticles is said to have advantages such as easily available, safe to handle and broad range of biomolecules such as alkaloids, terpenoids, phenols, flavaniods, tannis, quinines etc, are known to mediate synthesis of nanoparticles.[25]

Reports are available for the synthesis of silver nanoparticles using plant extracts such as Helianthus annuus, Basella alba, Oryza sativa, Saccharum officinarum, Sorghum bicolor, and Zea mays [11]; pine, persimmon, ginkgo, magnolia, and platanus leaves [12]; Jatropha curcas seeds [13]; Acalypha indica leaf [14]; banana peel [15]; Chenopodium album leaf [16]; Rosa rugosa [17]; Trianthema decandra roots [18]; Ocimum sanctum stems and roots [19]; Sesuvium portulacastrum leaves [20]; Murraya koenigii (curry) leaf [21]; Macrotyloma uniflorum seeds [22]; Ocimum sanctum (Tulsi) leaf [23]; Garcinia mangostana (mangosteen) leaf [24]; Stevia rebaudiana leaves [25]; Nicotiana tobaccum leaf [26]; Ocimum tenuiflorum, Solanum trilobatum, Syzygium cumini, Centella asiatica, and Citrus sinensis leaves [27]; Arbutus unedo leaf [28]; Ficus benghalensis leaf [29]; mulberry leaves [30]; and Olea europaea leaves [31].

Herein we report the synthesis AgNPs using the flower extracts of Catharanthus roseus for reduction of Ag+ ions to Ag0 nanoparticles from silver nitrate solution within 2 min of reaction time at ambient temperature. It was also shown that the average size of silver nanoparticles can be controlled to 5 to 40 nm by varying the concentration of silver nitrate and the volume of leaf extract. Further, biosynthesized silver nanoparticles are found to be highly effective against Escherichia coli bacteria.

**Experimental:**
**Materials and Methods:**

The genus Catharanthus (also known as periwinkle) belongs to the family Apocyanaceae a variety of which is extensively used in traditional medicine.Generally, it is known as Vinca rosea, Ammocallis rosea and Lochnera rosea. The flowers of the plant *Catharanthus rosesus* were collected from local area on the basis of cost effectiveness, ease of availability and medicinal property. The plant was identified taxonomically.

**Figure 1. *Catharanthus rosesus***

**Preparation of plant extract:**

The flowers of *V. rosea* plant were washed with tap water followed by rinse with distilled water thoroughly to remove dust. The aqueous extract was prepared by taking15g of thoroughly washed and finely cut *V. rosea* flowers in a 250 ml Erlenmeyer flask with 100 ml of sterile distilled water and then boiled the mixture for 1hr at 60°C to obtain bioorganic compounds from flowers The extracts were then filtered through Whatman No. 1 filter paper and centrifuged for 10 minutes at 10000 rpm, the supernatant was collected and refrigerated at 4°C. The filtrate act as reducing and stabilising agent.The extracts obtained were subjected to phytochemical screening for identification of various phytoconstituents as per the phytochemical methods analysis[1]

**Preparation of AgNPs:**

1mM aqueous solution of sliver nitrate ($AgNO_3$) was prepared and used for flower extract mediated biosynthesis of silver nanoparticles. To 500ml of silver nitrate solution 25 ml of *V. Rosea* aqueous extract was added while stirring for reduction into silver ions and the reaction mixture was kept at room temperature for

24-48h. The formation of reddish brown colour was observed after suitable incubation time at room temperature AgNPs was characterized by UV-visible spectroscopy, SEM, AFM, XRD and TEM.

**Results and Discussion:**

The recent development and implementation of new technologies have led to new era, the nano revolution which unfolds role of plants in bio and green synthesis of nanoparticles. Employing plants towards synthesis of nanoparticles are emerging as advantageous compared to microbes with the presence of broad variability of bio-molecules in plants which can act as capping and reducing agents and thus increases the rate of reduction and stabilization of nano particles. Here, we report an inexpensive, eco-friendly and rapid synthesis of silver nanoparticles by reduction process using flower extracts of *Catharanthus roseus*. The flower petals, seeds and other parts of this plant exhibit antioxidant properties. It has multiple applications in foods, cosmetics and pharmaceutical industries. Besides antioxidant activity, these extract exhibits antiallergic, anti-inflammatory, antimicrobial, anti-thrombotic, cardio protective and vasodilatory effects. The phytochemical analysis of th extract revealed the presence of bioactive compounds and the results are shown in table 1.

**Table 1.Phytochemical Screening results of the aqueous extract**

| Extract Tests | Flower (Aqueous) |
|---|---|
| **Saponins** | + |
| **Flavonoids** | + |
| **Alkaloids** | + |
| **Carbohydrates** | - |
| **Tannin** | + |
| **Glycosides** | + |

(+) indicates the presence of phytochemical constituents

(-) indicates the absence of phytochemical constituents

AgNPs was prepared from aqueous solution of silver nitrate using flower extract of Catharanthus roseus in a single-pot process. As the flower extracts were mixed with the aqueous solution of the silver ion complex, the solution exhibited a colour change from yellow to reddish brown (Figure.2) which directly indicates the formation of AgNPs..

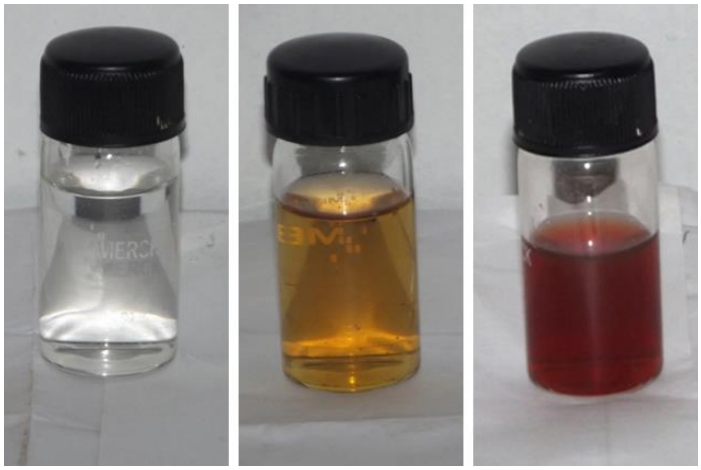

**Fig. 2. Visual appearances containing the aqueous extract of *V. rosea* flower and $AgNO_3$ Solution.**

It is well known that AgNPs exhibit brown colour in water due to excitation of surface Plasmon vibration in metal nanoparticles (Abhishek *et al.*, 2014). The UV-Vis absorption spectrum of the AgNPs is shown in figure3. A peak located between 400 to 465 nm was observed and it reveales the formation of AgNPs.

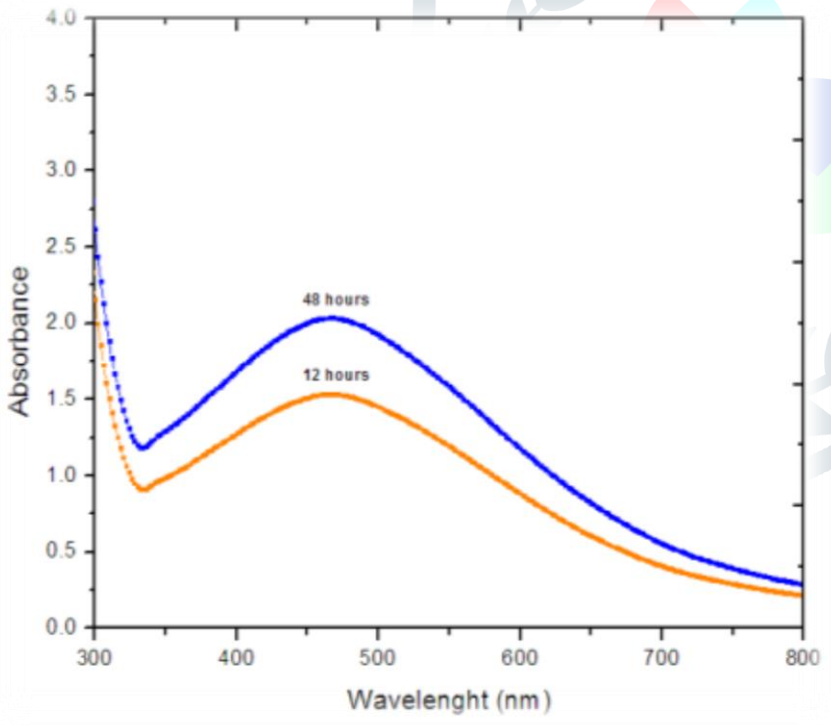


**Figure. 3. Absorption spectrum of AgNPs synthesized by aqueous extract of *Vinca rosea.***

The particle size of the formed AgNPs was measured by dynamic light scattering method. The average particle size was obtained as 100nm. The size distribution is as shown in figure 4.

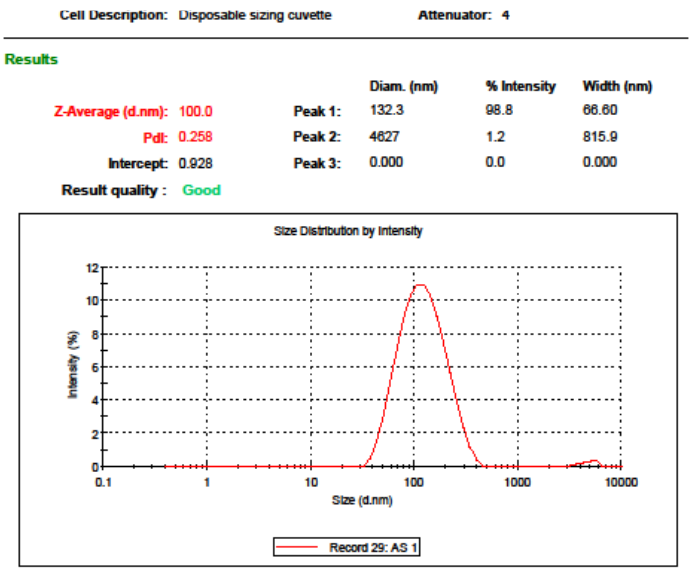


**Figure. 4. Size distribution of AgNPs**

The morphology of nanoparticles was determined by SEM, AFM and TEM. The images showed the formation of nano spherical AgNPs having diameter 5-40nm. The images are shown in Figure 5.

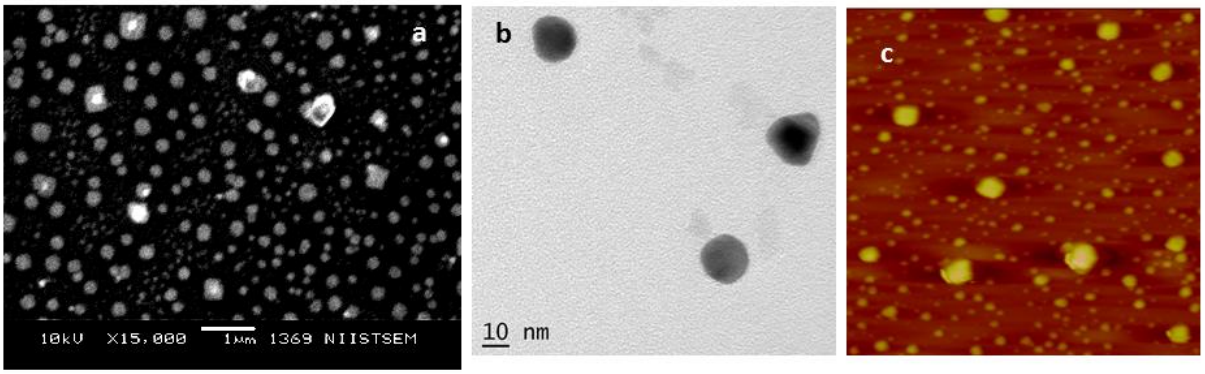


**Figure 5. SEM(a),TEM (b) and AFM (c) images of AgNPs.**

The X-ray diffraction pattern of AgNP is shown in Figure 6.The XRD analysis showed that the AgNPs are crystalline in nature and have face-centered cubic geometry. It exhibited peaks corresponding to (100), (200), (220) and (311) planes of the crystal.

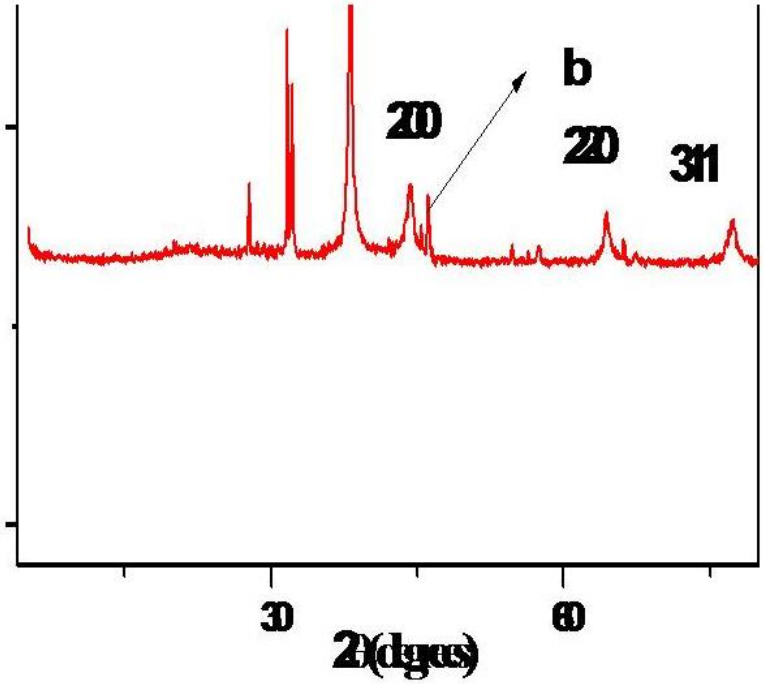


**Figure 6. XRD pattern of AgNPs**

Further, the AgNPs showed an effective antibacterial activity toward Escherichia coli pathogen.The antimicrobial effects of silver nanoparticles have been reported by many researchers.The antimicrobial effects of flower extract of *V. rosea* plant and their respective biologically synthesized silver nanoparticles (AgNPs) was evaluated by agar disc diffusion method using bacterial species *E. coli and Staphylococcus aureus.* Petriplates containing 20ml Muller Hinton Agar Medium were seeded with bacterial culture. Plates were placed with sterile paper discs having respective test samples. The plates were then incubated at 37°C for 24 hours. The antibacterial activity was assayed by measuring the diameter of the inhibition zone formed around the discs. The disc with AgNP shows wider zone of inhibition than with flower extract alone and is shown in Figure 7.


**Figure 7. Disc showing the anti bacterial activity of AgNps against S.aureus**

Fluorescent staining with Propidium iodide (specific for dead cells) and SYBR green (specific for live cells) was done for bacterial samples taken after incubation from both flasks and is shown in figure8 and figure 9. We got ~100% increase in number of dead cells and 83% decrease in number of live cells in test solution (with AgNps) with respect to the control (without AgNps).

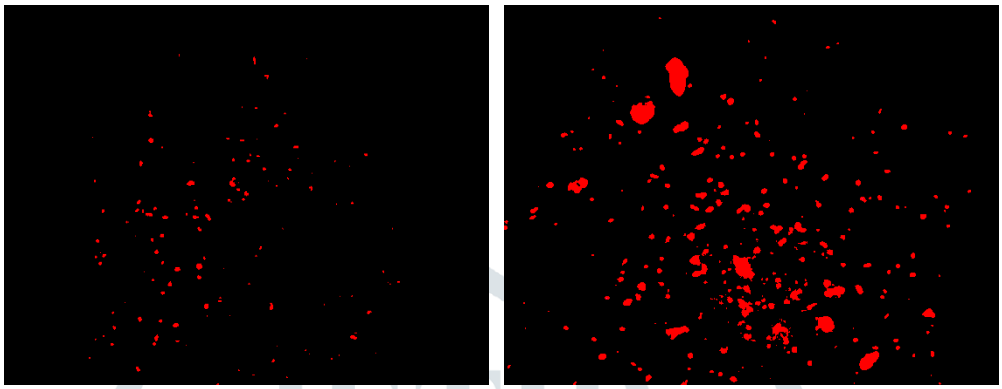

**Figure 8. Fluorescent micrograph of Propidium iodide stained *S.aureus* dead cells in control without Ag Nps and test with AgNps**

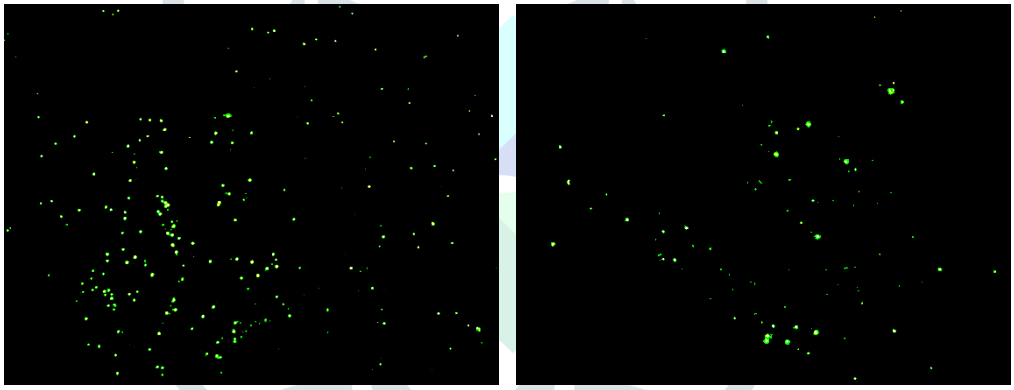

**Figure 9. Fluorescent micrograph of SYBR green stained *S.aureus* live cells in control without Ag Nps and test with Ag Nps**

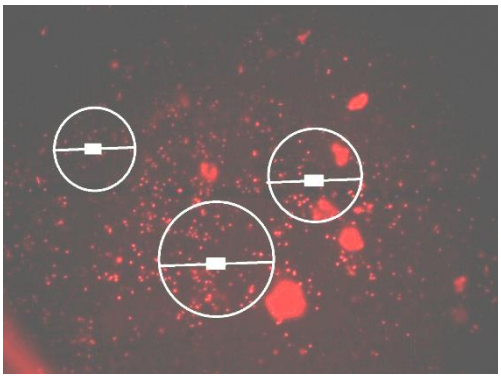

**Figure 10. Fluorescent micrograph of Ag Nps stained with Propidium iodide (specific for dead cells) for counting the population of dead cell**

Summary:

AgNP nano spheres with average diameter of 5-40nm was prepared by a one pot method using the flower extract of vinca rosea. The AgNPs was characterized using UV-visible spactra, dynamic light scattering, XRD, SEM, AFM and TEM. In the absorbtion spectra the sample exhibited an absorption maxima of 465nm corresponding to the surface plasmon resonance energy of AgNP spheres. In XRD pattern it showed the characteristic peaks of the planes of fcc crystal lattice. The morphological observations clearly designate the formation of AgNP nano spheres. Further its antibacterial activity against S.aureus was demonstrated using disc diffusion method and flourascent staining method. In disc diffusion the sample indicates greater zone of inhibition than those with flower extract alone. In staining method the sample exhibited 85% increase in dead cells with AgNP samples. Besides the method adopted is a simple , cheap, eco-friendly, green technique which explores the development of other nano materials for variety of appliacations.

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