"Green" Nanotechnologies: Synthesis of Silver Nanoparticles Using Catharanthus roseus Leaf Aqueous Extract & Its Antimicrobial Activity

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Abstract

Green chemistry synthesis of nanoparticles has become new area of interest in nanotechnology. In the present study an environmental friendly silver nanoparticles (AgNPs) were prepared using *Catharanthus roseus* aqueous extract. The plant's aqueous extract was mixed with silver nitrate solution for the production of AgNPs. The crystalline phase and morphology of AgNPs were examined by using UV-Vis spectroscopy, Fourier transform infrared (FTIR) spectra, Scanning Electron Microscopy (SEM) and Transmission Electron Microscopy (TEM) techniques. Additionally, the synthesized AgNPs were tested against *Escherichia coli and Staphylococcus aureus* to find out the antimicrobial properties of synthesized nanomaterials and it was concluded that the obtained AgNPs have a good antimicrobial activity and can be used for biological applications. This green synthesis provides an eco-friendly, economic and clean green synthesis method for developing the AgNPs

Keywords: Green synthesis, Silver Nanoparticles, Catharanthus roseus, Characterization, Antimicrobial activity.

1. Introduction

From the past few decades, Nanotechnology has become an emerging field of nanoscience that contributed in the development of many technologies. Among that development of nanocomposites has been gain more attention due to their wide applicability. Different types of nanoparticles viz., Gold, platinum, and silver nanoparticles are the common types of nanoparticles that have been used extensively in products that directly come in contact with the human body like soaps, shampoos, shoes, detergent, tooth paste, cosmetic products, and medical and pharmaceutical devices [1]. Among these, silver nanoparticles (AgNPs) are of great importance because ultrafine particles of silver were found to exhibited unique morphologies and characteristics at the nanometer (nm) scale [2]. These particles also have unique size-dependent optical, electrical and magnetic properties [3]. They have prospective application in various fields such as in catalysis [4,5], optics [6,7] electronics [8-10], water treatment and silver-based consumer products [11,12]. They can also used as antibacterial agents in biotechnology, bioengineering, textile engineering. Due to these characteristics and applicability Silver nanoparticles was found to be better than other metallic nanoparticles. Usually, synthesis of Ag nanoparticle is carried out by various methods like radiation, chemical or photochemical methods, electrochemical techniques, and Languir-Blodgett approaches [13] sonochemical [14], microwave assisted process [15] etc. Although these methods can successfully produce pure, well-defined nanoparticles however these methods are very expensive and time consuming and can be dangerous to human health and the environment [16] therefore, there is a need to develop a cost-effective and environmentally friendly method for rapid synthesis of nanoparticles. Green synthesis of AgNPs is an successful, effective and adaptable method for producing AgNPs using environmentally benevolent materials like plant leaf extract, fungi and bacteria [17,18]. In recent years, plant mediated biological synthesis of nanoparticles is gaining much more importance due to succession over other methods like it does not involve high pressure, energy and toxic chemicals. Furthermore, this process is a rapid, low cost, eco-friendly, and a single-step process [19]. These characteristics make this green synthesis process better than other methods. Many scientists have been reported the about the biosynthesis of silver nanoparticles (AgNPs) using extracts of leaves of different kind of plants, including Pterocarpus santalinus [20], Moringa oleifera [21], Duranta repens [22], Oleo europaea [23], Loquat leaf extract [24], Annona squamosa [25], Rhinacanthus nasutus [26] and Catharanthus roseus [27] etc. *Catharanthus roseus* is an erect procumbent or perennial herb which is grown in India, Australia, Africa, and Southern Europe. It is commercially use for medicinal purposes. *C. roseus* also has antibacterial, anti-inflammatory, antidiuretic, cytotoxic, antifertility, hyperglycemic, antifungal, anti-malarial, and antivirus properties [27]. It contains alkaloids, mainly of the indole type. It contain alkaloids vinblastine and vincristine due to these it has been used as anti-cancer drugs in the treatment of different types of cancers, such as lymphomas, Hodgkin's lymphoma, breast cancer, acute lymphocytic leukemia, soft tissue sarcomas, multiple myeloma, and neuroblastoma [28]. It has alkaloid alstonine which is helpful to reduce hypertension, and reserpine, ajamalicine, and serpentine are alkaloids with antiplasmodic and hypotensive properties [29]. In the present study silver nanoparticles were synthesized in the aqueous solution of silver nitrate with the help of leaves extracts of Catharanthus plant. After that the synthesized nanoparticles were synthesized using different types of characterization techniques. Then antimicrobial activity of these synthesized AgNPs was tested against two microorganisms - Escherichia coli and Staphylococcus aureus.

2. Materials and Methods

2.1 Chemical and Instrumentation

All reagents used in the present work were of analytical grade. Methanol and Silver Nitrate ($AgNO_3$) solution was supplied by E. Merck, Darmstadt, Germany. Digital pH meter (DB 1011 India), Sartorius CP224S analytical balance (Gottingen, Germany) and ultra sonic cleaner (Frontline FS 4, Mumbai, India) were used during the study. Adsorption measurements were recorded on a Systronics spectrophotometer 166 (India) over the wavelength range 325–990 nm. Zeiss EVO 50 instrument was used for Scanning Electron Microscope (SEM) analysis and Transmission Electron Microscopy (TEM) were performed on Philips model CM 200 instrument operated at an accelerating voltage at 200 kV.

2.2 Preparation of Catharanthus roseus leaf extract

Fresh leaves of Catharanthus roseus were collected from local area of the Indore, India and washed several times with tap water. The leaves were crushed, and sieved to obtain mesh sizes. Thereafter, Sieved biomass was treated with hydrogen peroxide (30%) at 50°C for 24 h to oxidize the adhering organic impurities and washed repeatedly with doubly distilled water. After that 50 g of the leaves were mixed with 20 ml methanol and 80 ml distilled water in a 100 ml beaker. The solution was boiled for 2 h. at 70^0C. Now it was filtered and allowed to stand at room temperature for cooling. Finally, thus obtained mixture was stored at 4°C for further use.

2.3 Preparation of Silver Nitrate ($AgNO_3$) solution

3 mM of $AgNO_3$ solution was prepared by dissolving 0.5097 gm $AgNO_3$ in double distilled water. Then stored in Amber colored bottle to avoid auto oxidation of silver.

2.4 Green synthesis of Silver Nanoparticles

50 ml of leaf extract of Catharanthus roseus was taken in a 500 ml conical flask. Now 250 ml of freshly prepared 3mM $AgNO_3$ solution was added to the same flask and solution was heated up to 80°C for 5 minutes. The color change occurs which is the primary indication of the formation of Silver nanoparticles. After this the solution was centrifuged at 10000 rpm for 20 minutes and the supernatant was transferred to a clean beaker for further settlement of particles. Following procedure was repeated again and again untill we didn't get the clear solution. Now the solution was microfuged and the flask was incubated in dark at room temperature for 24 hrs to get dried silver nanoparticles. The particles obtained were further characterized using different characterization techniques.

2.5 Particle Characterization

Developed AgNPs were characterized using different techniques. The morphology of the nanoparticles has been observed using Scanning Electron Microscopy (SEM) and Transmission Electron Microscopy (TEM) techniques. Fourier transformation infrared (FTIR) spectrometer has been used to find out the bounding or interaction of element to element. UV-Vis spectroscopy was used to examine the size and shape of the developed nanoparticles. Adsorption measurements were recorded on a Systronics spectrophotometer over the wavelength range 330–630 nm.

2.6 Antimicrobial Assay

The evaluation of antimicrobial activity was done by using agar diffusion method against two microorganisms - Escherichia coli and Staphylococcus aureus. For the agar diffusion method agar was used as nutrient for cultivation of the microorganisms. On the petri plates the fresh grown culture of the microorganisms (50 µL) was spread and the hole was created in the media. Then, a specific amount of synthesized AgNPs was incorporated in this hole using a sterile instrument. After that all the plates were allowed to stand at room temperature for 15 min for the prediffusion and incubated at 350°C for 24 h.

2.7 Design of experiment

The experiment has been performed in two parts: synthesis of the silver nanoparticles (AgNPs) and evaluation of the antimicrobial activity of synthesized AgNPs. Initially, the extract of leaves was prepared by following the washing and grinding process Thereafter, antimicrobial activity of these particles were evaluated against two microorganisms - Escherichia coli and Staphylococcus aureus.

2.8 Quality assurance/quality control

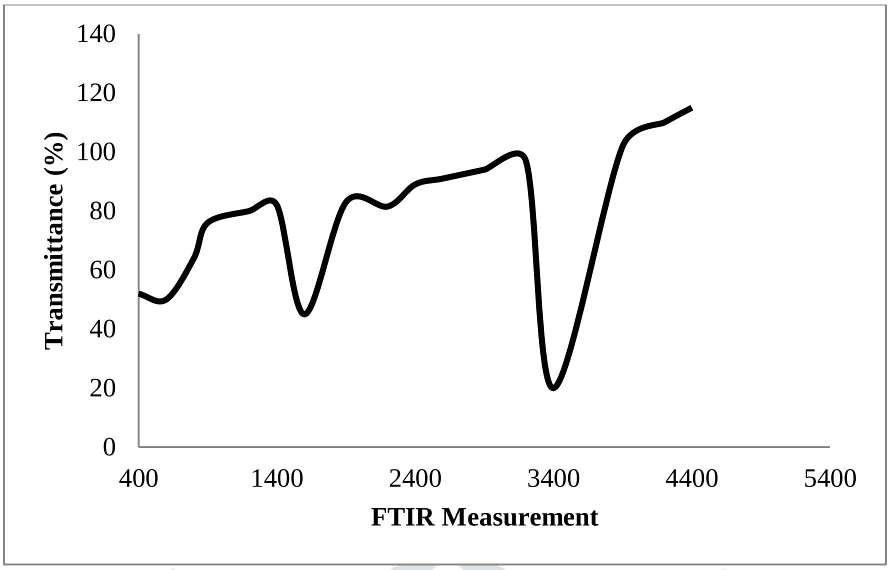
All the experiments were performed with proper concentration and accuracy. All glasswares used in the study were prepared by soaking in 5 % HNO_3 solution for a period of 3 days before being doubly rinsed with double distilled and oven dried. Double distilled water was used throughout the experiments.

3. RESULTS AND DISCUSSION

The developed Silver nanoparticles were characterized using the following techniques:

3.1 Fourier Transform Infrared (FTIR) Analysis

Fourier Transform Infrared (FTIR) Spectroscopy is used to find the exact chemical structure and presence of the functional groups in the developed material. The FTIR spectra of AgNPs have been shown in Fig. 1. The FTIR signals of AgNPs were observed at 3410, 2004, 1600, and 1208. The absorption peak at 1208 was due to the C–O stretching which was possibly caused by the presence of carboxylic acid and the peak occurred at 1600 was due to the N–H stretching, which confirms the presence of amide groups. The strong absorption peaks at 3410 were caused by the N–H bond for amine groups, which were used for the stabilization of AgNPs. However, the presence of the amide group characteristic proteins/ enzymes is responsible for the reduction of $AgNO_3$ to Ag [30].



3.2 Visual Properties analysis

UV-Vis spectroscopy has been used to study the reduction of silver ions into aqueous solution of silver nanoparticles. Due to the surface plasmon resonance, the color of solution changes from yellow to brown when we added leaf extract of Ocimum Sanctum to aqueous solution of $AgNo_3$. This spectroscopic technique can be used to study the size and shape of the developed nanoparticles. In this technique it was observed that the absorbance peak increased according to the intensity with time and at a definite wavelength it got constant. UV-Vis absorption spectra were monitored at 455 nm as shown in Fig.1.

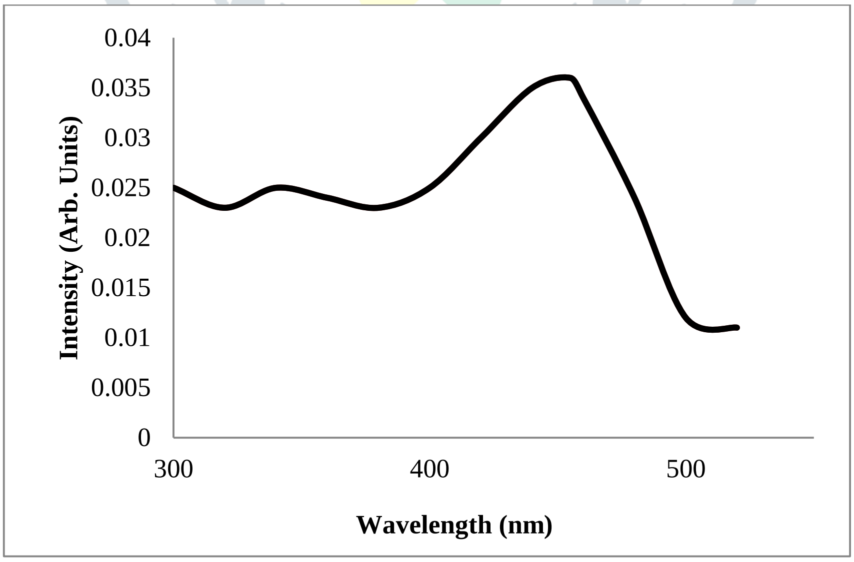


Fig 1: UV-Visible spectra of AgNPs.

3.3 Surface morphology and characteristics

Scanning electronic microscopy (SEM) study is one the most popular, primary, and widely used characterization technique used for the study of surface properties and morphology of biosorbent material. SEM study tells about porosity and texture of biosorbent material. The morphological structure of developed AgNPs was observed under SEM (Zeiss EVO 50 instrument) and the micrograph is shown in Fig.1 which indicates that It has a porous texture having small cavities on the surface or quite rough surface and there are asymmetrical shaped pores present.

Fig 2: SEM image of AgNPs.

3.4 Transmission electron microscopy (TEM)

Transmission electron microscopy study has also been done to find out the shape, crystal structure and particle size of developed silver nanoparticles. The micrograph of the TEM analysis of has been showed in Fig. 3. The morphology of nanoparticles is basically spherical and the vacuums are well-dispersed with size ranging from 30-60 nm.

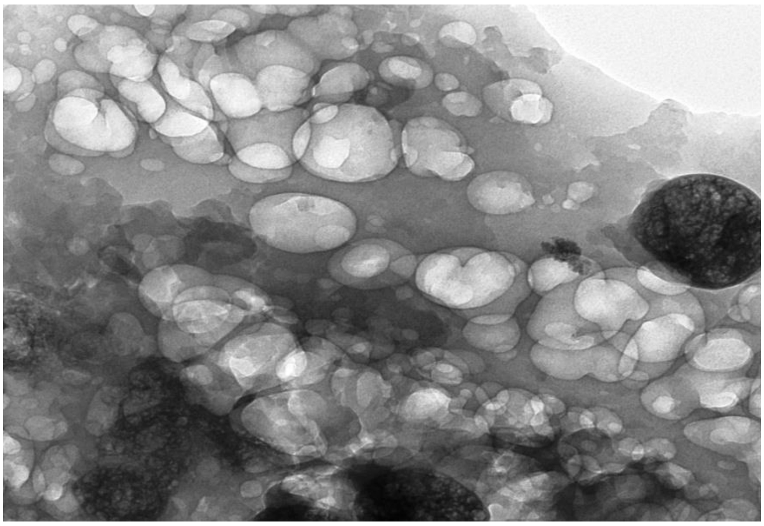


Fig 3: TEM image of AgNPs.

4. Antimicrobial Activity Analysis

Antimicrobial activity of synthesized AgNPs using *Catharanthus roseus*, was observed against Escherichia coli and Staphylococcus aureus bacteria. For this disc diffusion method was used. The outcomes showed that AgNPs exposed effective antimicrobial activity against both the bacteria. This method is used to find out the sensitivity of bacterial strains with a zone of inhibition towards antibiotics and results are tabulated in mm (**Table 1)**. The results showed that the synthesized AgNPs revealed good inhibition against both Escherichia coli and Staphylococcus aureus bacterial strains.

Table 1: Antimicrobial activity of synthesized AgNPs using Catharanthus roseus leaf extract

Name of Microorganism	Zone of Inhibition in mm		
	Venomycin (10µg/disc)	Concentration of Silver Nanoparticles (µL)	Silver Nanoparticles
Escherichia Coli	30	10	1.8
	30	20	2.2
	30	30	2.5
Staphylococcus Aureus	30	10	1.5
	30	20	1.9
	30	30	2.1

5. Conclusions

The Biosynthesized AgNPs were immense attention because they have eco-friendliness, economic prospects, and feasibility and Short time for synthesis. The developed AgNPs were characterized using different techniques namely: UV-Vis, SEM, TEM and FTIR and after that their antimicrobial activity has been checked against *Escherichia coli* and *Staphylococcus Aureus* bacterial strain. These particles may have extensive series of applications in nanomedicine, catalysis medicine essentially for the pharmaceutical industries. Mainly they can be used for development of new formulations against the microbial strains which are developing resistance to traditional antibiotics.

6. References

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