Green synthesis of non-cytotoxic silver nanoparticles using Solanum nigrum leaves extract with antibacterial properties

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Abstract

Green synthesizes of silver nanoparticles is a promising method in material science and nanotechnology today. Silver nanoparticles (AgNP) were synthesized using a high-efficiency, cost-effective green and simple method at room temperature using Solanum nigrum leaves extract. The biosynthesized AgNP was validated and classified using spectroscopy profiles from ultraviolet-visible spectroscopy, scanning electron microscope, Fourier-transform infrared spectroscopy and x-ray diffraction. FTIR spectra indicated the presence of biological molecules in AgNP synthesis and UV-visible spectra revealed a surface resonance peak of 420 nm corresponding to AgNP formation. The antimicrobial potential of these synthesized nanoparticles is evaluated against the bacteria Escherichia coli (MCC 2079) and Staphylococcus aureus (MCC 2408), both of which are important foodborne pathogens. Ciprofloxacin and Clotrimazole were used as positive controls antibiotic. The nanoparticles were shown to have strong antibacterial efficacy against the strains examined. Their antibacterial function allows them to be included in antimicrobial formulations. By using the MTT assay, the biocompatibility of Solanum nigrum silver nanoparticles were found non-toxic to mouse fibroblast cell lines (L929 is one of the first in continuous culture to be created. The L strain was generated from a male C3H mouse’s usual subcutaneous areolar and adipose tissue.) at lower concentrations.

Keywords: Silver nanoparticle; Solanum nigrum; MTT assay; Mouse fibroblast cell lines

1. Introduction

Nanotechnology is an interdisciplinary field of study that combines fundamental techniques from a variety of disciplines, including chemistry, engineering, physics, and biology, in order to create novel methods for manipulating and producing nanoparticles (NPS) [1, 2, 3]. Nanoparticles have at least one dimension and a diameter of one to one hundred nanometers. Nanotechnology is the study, development, and use of a broad range of nanoparticles. Despite the fact that these methods are not ecologically friendly, noble metals such as gold, silver, platinum, and others are routinely utilized in the creation of nanoparticles utilizing a mixture of chemical and physical techniques. A non-toxic and ecologically sustainable approach for synthesizing nanoparticles is critically needed. Yeast, microorganisms, spores, and plant extracts, among other biological processes, are increasingly often utilized in green biosynthesis approaches for the creation of nanoparticles. Numerous efforts have been conducted to biosynthesize silver nanoparticles with controlled physicochemical characteristics using plant extracts. Silver nanoparticles (AgNP) have been shown to have important biological functions in treatments [4, 5, 6, 7]. The goal of this study was to explore into the usage of plant extracts in the green synthesis of AgNP. Solanum nigrum, often known as European black nightshade, is a flowering plant of the Solanum family native to Eurasia but now found in the Americas, Australasia, and South Africa. Ripe berries and fried leaves of edible strains are consumed in certain locations, while plant parts are utilized as folk treatments in others. The plant has been used as a medicine for thousands of years, going back to ancient Greece. It is a narcotic plant with sedative, analgesic, and sudorific properties. Because of its varied composition and toxicity, internal usage has

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waned in Western herbalism, although it is still used externally to treat herpes zoster. It is controversial if the Solanum nigrum leaves and berries are harmful. However, this plant is produced as a food crop in a number of nations. The toxicity of Solanum nigrum varies according to its surroundings and species. In various traditional Indian treatments, Solanum nigrum is used. Dysentery, gastrointestinal difficulties and fever are treated with infusions. The juice of the plant is utilized for the healing of ulcers and skin problems. The fruit is used as a laxative, appetite stimulant and tone to heal allergies and to "over-hunger." The roots of the plant are juiced and used to treat asthma and whooping cough. Solanum nigrum is a natural plant in oriental medicine because to its antitumorigenic, antioxidant, anti-inflammatory, hepatoprotective, diuretic, and antipyretic effects. Numerous studies have indicated that the plant reduces the establishment of cervical cancer in mice. Solanine, the plant’s main element, suppresses the growth of cancer cells in situ, including breast and pancreatic cancer cells. Its anticancer action is mostly reliant on the activation of various cellular and molecular pathways, which results in cell and molecular apoptosis and autophagy, as well as tumor metastasis suppression [8]. Because of its solvent-free nature and low toxicity, plant-based nanoparticle production has gained popularity in recent years. Furthermore, their synthesis is much quicker and less costly [9, 10]. Bacteria, yeast, and fungi have been demonstrated to produce AgNP during the biosynthetic process [11, 13]. These nanoparticles have been shown to be beneficial in a wide range of biological applications [3, 12, 14]. Metal nanoparticles may be found in shampoos, soaps, detergents, fragrances, and toothpaste, as well as pharmaceutical and medical products. As a consequence, they have direct communication with human processes [15, 16, 17]. To the best of our knowledge and expertise, Solanum nigrum has not been employed in the production of silver nanoparticles. This work tried and described the green synthesis of AgNP from Solanum nigrum. The same method was used to assess their effects on biological processes.

2. Material and methods

2.1. Collection and extraction of plant material

Fresh Solanum nigrum plant leaves were collected from Bangalore and washed with sterile water. In addition, 70 g of dry plant material was weighed and soaked in 500 mL of methanol before being processed for 5 hours in a Soxhlet apparatus at 100°C. The extracted solvent was boiled, then cooled, filtered with Whatman filter paper, and condensed for future testing [16].

2.2. Silver nanoparticles synthesis using Solanum extract

A newly prepared aqueous solution of silver nitroargent (AgNO₃, 1 mM) was utilized to synthesize silver nanoparticles for AgNP production. Solanum nigrum leaves extract (5 mL) and silver nitrate solution (45 mL) were blended. The synthesis of silver nanoparticles at room temperature for production of silver nanoparticles was conducted to investigate temperature influence. The initial color changes, as well as the pH change, were registered.

2.3. Characterization of the synthesized Ag nanoparticles

In ratios, 1mM AgNO₃ solution was taken. To 20 mL of a newly made 1 mM AgNO₃ solution in a conical flask, 10 mL of leaf extract was added to this ratio. The sample absorption was estimated using a UV-Visible spectrophotometer after twenty-four hours within 200-700 nm range. Many of the percentages are examined using the same procedure. For the appropriate experiment, the best Solanum nigrum leaf extract ratio was chosen: 1mM AgNO₃ (1:4) solution [5, 8].

Particle size distribution was determined by dissolving silver nanoparticles in chloroform and measuring their distribution in the liquid using a computer-controlled particle size analyzer. The computer received 2 mL of the study, and the measurements were shown as readings and a graph [3].

SEM is a kind of electron microscope, which makes a sample image by using an electron beam. During interaction with atoms in a sample, electrons emit a spectrum of signals that show the topography and structure of the sample surface. The resolution of the microscope for the scanning electron (SEM) exceeds 1 nm.

FTIR spectroscopy was used to determine the various functional groups in the substance. To evaluate FTIR, the silver nanoparticle solution was centrifuged for 15 minutes at 10000 rpm. The pellet has been washed with 10 mL of deionized water three times to remove unattached proteins or enzymes that do not cap silver nanoparticles. The pellet was vacuum dried before being analyzed using FTIR [11].
2.4. Antimicrobial Assay

The antimicrobial function of synthesized nanoparticles was tested using the agar well diffusion method against *Escherichia coli*, *Staphylococcus aureus* (MCC 2408) and other bacteria (MCC 2079). Mueller-Hinton agar was used to develop bacteria, while YE Potato Dextrose agar was used to grow yeast. Fresh overnight colonies of inoculum (200 L) were spread on agar plates, and 20 L of different concentrations of test sample and standard were added through 6 mm diameter wells. Ciprofloxacin and Clotrimazole were employed as positive controls. Phosphate buffer (PBS) was utilized as a negative regulator. At 37°C overnight the plate was then incubated. To test the anti-microbial behavior of the synthesized nanoparticle, the diameter of the inhibition region generated the following day was evaluated [15].

2.5. Cell Viability Assay/MTT Assay

MTT test was used to assess the cytotoxicity of *Solanum nigrum* AgNP in mouse fibroblastic cell line L929. Cells were frozen one week before the experiment to allow for acclimatization in a culture flask with DMEM (Dulbecco modified Eagle's Media) culture medium supplemented with 1% antibiotic solution and 10% foetal bovine serum (FBS) and kept in a Co2 incubator at 37°C. When the cells reached ideal confluence, they were planted on 96 well culture plates in accordance with the MTT assay’s specifications. After treatment with various dosages of AgNP and incubation of the cells in a Co2 incubator, cell viability was evaluated for 24 hours at 37°C. Following incubation, the spent media was withdrawn and 200l of new media supplemented with 10 l of MTT (3-(4, 5-dimethylthiazolyl-2), 5-diphenyltetrazolium bromide) was administered to each well. Following incubation, the medium was added with 200l of DMSO, and the optical density (OD) was calculated to be 540nm [1].

\[
\text{Viability (\%)} = \frac{NT}{Nc} \times 100
\]

Where, NT and Nc represent the absorbance of nanoparticle-treated and negative control cells, respectively.

3. Results and discussion

3.1. Silver nanoparticle formation

In the biosynthesis of AgNP, a leaf extract of *Solanum nigrum* was used. When dark green aqueous leaf extract was added to AgNO3 solution, the color changed to dark brown, perhaps owing to the surface plasmon resonance feature of silver (Figure 1). The color transition is caused by the Surface Plasmon Resonance phenomenon. The combination of metal nanoparticles in resonance with light waves produces the band of absorption for the SPR. Metal nanoparticles also contain free electrons. A UV-vis spectroscopy research showed more than that [18].

![Figure 1 Indicating the brown colour and synthesis of nanoparticles](image)

3.2. UV-vis spectroscopy analysis

According to UV visible spectroscopic studies, the maximum absorbance of *Solanum nigrum* leaves extract generated AgNP was 400 nm. *Solanum* leaf extract and 1mM AgNO3 solution were used in different ratios. The UV-vis spectrometer analysis data from the samples are shown in Figure 2. The extract’s secondary metabolites operate as a reducing and capping agent for AgNP production, drastically lowering the production of silver nitrate into silver ions. Previous study backs up this data by using a green method to create silver nanoparticles that are less toxic to people [14]. A ratio of 1:4 was chosen for further study since absorbance begins to decrease beyond that level.
Figure 2 UV-vis spectroscopy for silver nanoparticles synthesized using *Solanum nigrum* methanolic leaf extract

3.3. FTIR analysis

The surface and function groups, as well as the interaction with AgNP, were identified using FTIR spectroscopy. FTIR measurements have been utilized to identify and stabilize biomolecules employed in the manufacture of cap metal nanoparticles. Figure 3 illustrates the spectrum acquired from 4000 to 500 cm\(^{-1}\) sample research. When comparing FTIR analytical peaks with normal peaks, the many functional groups in the sample were discovered and summarized. The 3500 cm\(^{-1}\) band creates O-H and H-bound drinks and phenols in the FTIR spectrum of silver nanoparticles. Pieces of roughly 2250 cm\(^{-1}\) reveal the (-C=C-) binding of (N-H) 1650 cm\(^{-1}\) and the (-C=C-H) binding of Alkenes of 690 cm\(^{-1}\).

![FTIR graph showing the functional groups predicted in silver nanoparticle](image)

Figure 3 FTIR graph showing the functional groups predicted in silver nanoparticle

The binding of (-C=C-) was shown. As a result, proteins and metabolites comprising functional components like terpenoids were added to the nanoparticles synthesized. We have shown that amino acid residues and carbonyl protein groups are more capable of attaching metals which prevent protein clumping via interactions and medium stabilization with metal nanoparticles (i.e., capping of silver nanoparticles). This means that living molecules can produce and stabilize silver nanoparticles in watery situations. The surface of metal nanoparticles is absorbed by flavanones, depending on the kind of carbonyl. If additional strong ligation agents at an adequate concentration are absorbed,
flavanones might be adsorbed on metals nano surfaces, maybe by contact with carbonyl groups or electrons. There may be a decrease in carbohydrates due to the existence and production of metal nanoparticles. Terpenoids are also intended to assist remove metal ions by transforming aldehyde groups into carboxylic acids in molecules. These problems cannot be dealt with until the different portions of the ion reduction have been isolated, categorized and separately analyzed [19, 20, 21].

3.4. XRD analysis

The XRD range exhibited wide peaks with a diffraction of 380 degrees. These high peaks in Bragg might have been produced by a nanoparticle covered material. Intensive Bragg-reflections imply the presence of powerful X-ray dispersal centers generated by capping agents during the crystalline phase. The effects of particle size typically propagate to the peaks in solid XRD patterns. The larger peaks reveal lower particle sizes and reflect impacts on the nucleation of crystal nuclei and experimental environment growth.

The XRD analysis curve (or counts) is a two-theta scale intensity plot in this example. The exam is carried out using specialized software. The program compares the peaks recorded with different silver types. If the silver vertical shape is consistent with most peaks, the silver shape is considered present on the study sample. The examination sample shown in Figure 4 contains 80 percent Silver-3C.

![XRD Analysis curve of synthesized silver nanoparticle](image)

3.5. Analysis using SEM

SEM is a valuable instrument for assessing the morphology of synthesized nanoparticles with great spatial resolution. Figure 5 shows high-density Ag-NP synthesized by Solanum nigrum leaves extract from the SEM sample. Figure 5 clearly shows the formation of agglomerates of spherical bead-like AgNP with a standardized size distribution. The white individual spots in the SEM image are silver nanoparticles, whereas the larger spots are silver nanoparticle aggregates.

The majority of the silver nanoparticles are 5.2 nm in diameter and are circular and uniform Ag-NP with diameters varying from 4 to 6.5 nm. And when aggregated, the capping agent maintained that the nanoparticles stayed compact and did not come into contact with one another. Larger silver nanoparticles were found during SEM experiments, which may be the product of smaller ones aggregating. The synthesized nanoparticles are spherical, according to electron microscopy, which will be 20–40 nm in size for better applications.
Figure 5 SEM scans of the lyophilized silver nanoparticles revealed predominantly spherical particles with a size of less than 40 nm

3.6. Antibacterial disc diffusion analysis

When discussing antimicrobial experiments, it’s necessary to keep in mind that the outcomes are dose-dependent. In comparison to the control, it demonstrated positive inhibitory behavior toward the measured species. Well 1 has a concentration of 5 mg/mL, well 2 has a concentration of 10 mg/mL, well 3 has a concentration of 20 mg/mL, well 4 has a concentration of negative control, and well 5 has a concentration of 1 mg/mL Ciprofloxacin/Clotrimazole as a helpful monitor. To complement the findings, Table 1 shows the inhibition zones obtained with various concentrations of silver nanoparticles. According to the findings, the synthesized silver nanoparticle has a significant inhibitory activity against *Staphylococcus aureus* and a mild reaction against *Escherichia coli*. Biological tests on pathogenic bacterial strains and cell viability experiments showed that synthesized AgNP is non-toxic to mammalian cells and has good antibacterial activity. This is often used in conjunction with a synergistic combination of silver cores and capping layers comprising natural compounds with antimicrobial properties and is therefore regarded as a viable alternative to AgNP. Antibacterial activity of AgNP was observed against bacteria, and the antibacterial activity was dose-dependent [22, 23]. The antibacterial efficacy of AgNP was completely based on their concentration; higher levels of AgNP inhibited microbe development more effectively.

Figure 6 Petri plates marked with inhibition zones obtained by different organisms. Well 1 - 5mg/mL AgNP (20µL); Well 2 - 10mg/mL AgNP (20µL); Well 3 - 20mg/mL AgNP (20µL); Well 4(+) - PBS (Negative Control) (20µL) and Well 5 (+) - 1mg/mL Ciprofloxacin/Clotrimazole (Positive Control) (20µL).
Table 1 Inhibition zones obtained by different concentrations of silver nanoparticles

| Microorganism | Test Extract | Zone of Inhibition in mm | Positive control (+) | Negative control (-) |
|---------------|--------------|--------------------------|----------------------|----------------------|
|               |              | 5 mg/mL (1) | 15 mg/mL (2) | 25 mg/mL (3) |
| *S. aureus*   | AgNP         | 10 | 13 | 23 | 41 | - |
|               |              | 15 | 16 | 24 | 40 | - |
| *E. coli*     | AgNP         | 5 | 9 | 14 | 35 | - |
|               |              | 8 | 10 | 16 | 36 | - |

3.7. Cell viability assay Analysis:

The L929 normal mouse fibroblast cell lines were used to monitor the toxicity of AgNP made by *Solanum nigrum*. MTT is reduced to purple color formazan in this process by mitochondrial dehydrogenase enzymes found in viable cells. Since the mitochondrial dehydrogenase enzyme is inactive in dead cells, this reduction reaction does not take place, and therefore no blue pigment is formed. Figure 7 indicates that 80 percent of mouse fibroblast cells remain alive after 24 hours of *Solanum nigrum* AgNP therapy at 0.0095-0.156 mg/mL concentration, indicating that *Solanum nigrum* AgNP were non-toxic to normal cells at 0.156 mg/mL. As a result, the silver nanoparticles created in this investigation had no cytotoxic effect on normal mouse fibroblasts at the doses used in this work. As a result, it was determined that these nanoparticles might be employed as a cancer therapy at a lower dose, avoiding harm to normal cells and targeting just cancer cells. This characteristic of selectively targeting cancer cells may be facilitated by a capping agent derived from *Solanum nigrum*, which is claimed to possess a unique mechanism for specifically targeting cancer cells. If this research can be conducted in *vitro* in the future, a drug with minimal cytotoxicity may be directly injected into the human body. Numerous toxicity studies conducted on animal models have shown that conventionally manufactured nanoparticles are neither harmful in *vitro* nor in vivo [24, 25]. This study established the hypothesis that nanoparticles formed by the reduction of plants are non-toxic in nature.

![Cytotoxicity of silver nanoparticles against L929](image-url)
4. Conclusion

The use of Solanum nigrum leaves extract as an effective reducing and capping agent resulted in an efficient, rapid, and green synthesis of AgNP in this research. Temperature, AgNO3 concentration, stirring conditions and time were all investigated as factors influencing AgNP synthesis. The resulting AgNP is spherical and monocrystalline, with sizes varying below 40nm. The capping of the obtained AgNP was validated using FTIR spectroscopy. Silver nanoparticles produced in an environmentally friendly manner have great potential in biological applications. Disc diffusion was used to test the antibacterial activity of Solanum nigrum AgNP against Staphylococcus aureus and Escherichia coli. Finally, it has been shown that Solanum nigrum leaf extract may form extracellular Ag nanoparticles, and that the Ag nanoparticles are extremely stable in solution. As compared to the antibiotics, the shaped silver nanoparticles demonstrated significant antimicrobial activity. The SPR peak at 420 nm and the XRD pattern also confirmed the development of AgNP. This silver nanoparticle biogenesis reveals that they are feasible candidates for medicinal applications needing antibacterial activity and may therefore be employed to combat the spread of multidrug-resistant bacteria. The biocompatibility of Solanum nigrum silver nanoparticles (SnAgNP) were determined using the MTT assay, which revealed that 80 percent of mouse fibroblast cells remained alive after 24 hours of Solanum nigrum AgNP therapy at 0.0095-0.156 mg/ml concentration, indicating that Solanum nigrum AgNP were not toxic to normal cells at 0.156 mg/ml concentration (lower concentrations). They may be employed in antimicrobial compositions due to their antibacterial characteristics. In many studies papers the nanoparticles created were proven to be not cytotoxic and had strong antibacterial activity, which indicates that they might be used in many biomedical and pharmaceutical applications.

Disclaimer

The research reported in this manuscript has been done and prepared in the Department of Biotechnology, R.V College of Engineering, Bangalore-560059. We confirm that there is no conflict of interest exists in the institution mentioned for direct or indirect/known or unknown purpose and therefore, the above-said manuscript publication has no objection from the organization mentioned above. We confirmed that the above-mentioned manuscript has not been previously published and is not under consideration in the same or substantially similar form in any other peer-reviewed media. The research publication reported in this manuscript has not received any external funding. All authors listed have contributed sufficiently to the project, and all those who are qualified to be authors are listed in the author by-line.

Compliance with ethical standards

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Disclosure of conflict of interest

All authors declare no conflict of interest.
Statement of ethical approval
The research reported in this manuscript has been conducted in the Department of Biotechnology, R.V College of Engineering, Bangalore-560059, and India. Apart from this, we would like to mention that this research was performed to make scientific community aware of silver nanoparticles synthesized using *Solanum nigrum* as a potent drug for inhibiting various disease-causing microbes. The facts cannot be used to generate any funds or any other corrupt practices.

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