Green Synthesis of Silver Nanoparticles Using 
Azadiracta indica and Gardenia gummifera 
Plant Extract

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Authors' contributions

This work was carried out in collaboration among all authors. All authors read and approved the final manuscript.

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ABSTRACT

In material science, nanotechnology is considered as most common active area of research. Nanotechnology deals with the nanoparticles having a size range of 1-100nm used in medicinal chemistry, atomic physics. Nowadays, the use of metal nanoparticles in areas such molecular diagnostics and drug delivery, imaging, sensing has been of great scientific interest as a result of their peculiar optoelectronic and physicochemical properties. Thus synthesis of metal nanoparticles through effective, environmental friendly and cheaper approach is very important in nanotechnology research. In the current study Silver nanoparticles (AgNPs) were incorporated utilizing Azadiracta indica and Gardenia gummifera plant separate alongside their antimicrobial action. Silver had been a subject of interest from past a very long time because of its physical and chemical properties. Further depiction of mixed nanoparticles was finished by using Scanning electron microscopy (SEM) and Transmission electron microscopy (TEM). The combined AgNPs were found with the size scope of 60-80nm. Antimicrobial movement of AgNPs was in contrast to S. aureus, E. coli and Pseudomonas aeruginosa. Thus the results indicate that the AgNPs may have an application in medical science.

Keywords: Azadiracta indica; Gardenia gummifera; green synthesis; nanotechnology; nanoparticles.

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1. INTRODUCTION

In the current materials engineering, nanotechnology is among the most active areas of study. Nanotechnology is also an area that progresses daily and affects all aspects of human life as well as generates an increasing sense of excitation in life sciences, particularly biomedical and biotechnological devices. The jargon “nanoparticles” is often used to define the average particle size of 1 to 100 nm [1]. Nowadays, the use of metal nanoparticles in stream of molecular diagnostics as well as drug delivery, imaging, sensing has been of great scientific interest as a result of their peculiar optoelectronic and physicochemical properties [2]. Nanoparticles synthesis can be done by 2 approaches - Top to bottom and bottom to top (Fig. 1). Thus synthesis of metal nanoparticles through effective, environmental friendly and cheaper approach is very important in nanotechnology research.

With the expanding need to limit the utilization of natural danger substances, as portrayed in the Green Chemistry Principles, the union of nanoparticles utilizing organic creatures has gotten expanding consideration in the course of the most recent decade. Biosynthetic systems include living creatures like microbes, growths and plants. Blend of nanoparticles utilizing these natural elements has been effectively recorded yet plants have discovered key application as amalgamation by utilizing plants is more secure, more ecological cordial, less expensive, more straightforward and relatively speedier contrasted with organism helped union.

There are numerous significant uses of AgNPs (Fig. 2). It has a wide scope of biomedical applications including antibacterial, antimalarial, larvicidal, hostile to skin inflammation, against dandruff, against plasmodial, against malignant growth, against wound contamination and clinical gadgets, including bone concrete, careful instruments, careful cover, and synergist movement. It is applied to materials, home water cleansing frameworks, clinical gear, makeup, hardware, and apparatuses for family use. Other than their antimicrobial attributes, AgNPs show solid optical qualities which make the nanoparticles appropriate for natural detecting and imaging. AgNPs have been used in conductive inks, adhesives, and sealants for a variety of electronic devices due to its elevated conductivity. AgNPs are additionally utilized as impetuses for various substance responses, like oxidation of styrene [3].

![Fig. 1. Methods of synthesis of nanoparticles](image-url)
Silver nanoparticles’ chemical synthesis incorporates synthetic mixtures, for example, sodium borohydride to lessen Ag+ to AgNPs; nonetheless, because of its related harmfulness, compound techniques are frequently not required, because of low biocompatibility. Green blend is viewed as an elective methodology for integrating the AgNPs, likewise called biogenic union. The union interaction starts after the plant extricates have been hatched with silver salts (generally silver nitrate is utilized). The mix of decent AgNPs is a 2-venture measure including firstly the reduction of Ag+ particles to Ag°, followed by cluster and change inciting the course of action of oligomeric AgNP packs. The decrease interaction happens within the sight of organic impetuses, Green amalgamation of AgNPs through plant helps in dye removal (Fig. 3) [4]. Considering the presence of cell fortifications and the proteins, the plant eliminate fills in as reducing experts similarly as settling expert during the advancement of metal nanoparticles. A large number of active ingredients present in *Azadiracta indica* and *Gardenia gummifera* played a significant role as a reducing agent and a capping agent [5] [6]. It would also be of great interest to study and explore these various plants for a greener formation of the AgNPs.

### 1.1 Review of Literature

The literature study revealed the method for green synthesis of AgNPs using plant extract.

Sivaiah Kummara and others, study showed the Synthesis, depiction, biocompatible as well as anticancer development of green and artificially mixed AgNPs. The objective of this investigation oversees imaginative methodology for the biosynthesis of AgNPs using *Azadirachta indica* remove as reducing subject matter expert. Such mixed nanoparticles were depicted with the help of TEM (Transmission electron microscopy), DLS (Dynamic Light Scattering), and FTIR (Fourier Transform Infrared Spectroscopy) [8]. Sujata Patra and others, through the utilization of *Butea monospermm* (BM) leaf remove just as the execution in malignancy treatment, were introduced in this examination to exhibit a green union of AgNPs. Yet, combined nanoparticles were described utilizing diverse investigation techniques. Au and AgNPs were balanced out in the supports and biocompatible to the malignant growth cell lines, as demonstrated by discoveries. The conveyance of restorative items dependent on blended nanoparticles utilizing doxorubicin has a restraint of malignancy cell spread. Accordingly, finding recommend that...
AgNPs are useful for the therapy of cell malignant growth [9].

M. Balamurugan and others, study uncovered the combination and portrayal of antibacterial AgNPs by utilizing Coriander sativum therapeutic spice and the orchestrated AgNPs were described by UV-obvious spectroscopy and XRD. Antibacterial movement was tried against E. coli, Klebsiella pneumonia, Pseudomonas aeruginosa, Proteus vulgaris, and Staphylococcus aureus. Integrated nanoparticles displayed yellowish – earthy colored tone, appearance of yellowish – earthy colored showed the development of AgNPs [10]. P.P.N. Vijay Kumar et al, study showed the green blend of AgNPs by utilizing Boerhaavia diffusa plant extricate go about as a decreasing specialist. Ecofriendly combined AgNPs portrayal was finished with by SEM, UV–obvious spectroscopy, X–beam diffraction (XRD), TEM (Transmission Electron Microscopy) and Fourier change infrared (FT–IR) spectroscopy methods. XRD and TEM examination uncovered that orchestrated AgNPs round fit as a fiddle with the size of 25nm. Antibacterial trial of the orchestrated AgNPs was tried against bacterial microbe, for example Pseudomonas fluorescens [11].

Mansour Ghaffari-Moghaddam et al (2014), study uncovered the blend of AgNPs utilizing Crataegus douglasii natural product concentrate and go about as a diminishing specialist. Biosynthesized AgNPs were checked by UV-noticeable and further portrayed by SEM. Examining electron microscopy pictures showed AgNPs with size of 29.28nm and practically round fit as a fiddle. Antibacterial properties of incorporated AgNPs were affirmed against the Escherichia coli and Staphylococcus aureus [12]. Palanivel Sathishkumar et al. disclosed a study that uncovered the AgNPs were integrated utilizing Coriandum sativum leaf remove and the properties of incorporated nanoparticles were portrayed by utilizing UV-vis spectroscopy, FTIR, XRD strategies. Also, further enemy of skin break out and hostile to bosom malignant growth viability of biosynthesized nanoparticles were surveyed again unique cell lines, for example, Propionibacterium acnes MTCC 1951, human bosom adenocarcinoma (MCF-7) cell line and Malassezia furfur MTCC 1374. The green combined AgNPs size was discovered to be ~37nm. Results reasoned that orchestrated AgNPs utilizing C. sativum have a huge potential in biomedical application like enemy of skin break out, against bosom malignant growth as well as antidandruff disease [13]. Partha P. Duttaa et al (2017), in this examination antimalarial capability of AgNPs were blended by utilizing Syzygium jambos (L.) Alston (Myrtaceae) leaf and bark separate. Further combined AuNPs and AgNPs were portrayed by zeta potential, TEM, XRD, and FTIR [14].

In the afore-mentioned work carried out by researchers, although the green synthesis of AgNPs using different plants extracts showed a better result for the treatments of various diseases but still lacked to prove their potent in terms of providing effective treatment. Therefore there is a need to develop a formulation with enhanced efficacy and bioactivity. The present research work comprises of Azadiracta indica that possess immense anticancer, anti-inflammatory, antibacterial, antiviral, antioxidant and wound healing properties. Gardenia gummifera also used herein possess remarkable antibacterial and anti-inflammatory properties therefore there is need to develop alternative approach to synthesize the AgNPs. Therefore, it can be interpreted that combination of such potent extracts with AgNPs will aid in treating the diseases caused by pathogenic agents.

2. METHODOLOGY

2.1 Design

Plant selection was based on literature survey, Azadiracta indica and Gardenia gummifera were considered as the green source for AgNPs synthesis and the plants collected from the native location. After collection of plant, extract of plant was prepared and the AgNPs was synthesized by using the same. Then synthesized nanoparticles was characterized by various analytical techniques; TEM (Transmission Electron Microscopy) and SEM (Scanning Electron Microscopy) and Antimicrobial activity of the synthesized AgNPs was evaluated.

2.1.1 Preparation of plant extract

The plants gathered from the place where they lived were carefully washed with tap water and water in order to remove particles of dust. After washing, the plant parts were crushed in blender and stored for further process. 1gm of dried plants were boiled for 10 minutes in 100 ml solvent and then the extract was filtered using vacuum filtration assembly.
2.1.2 Synthesis of silver nanoparticle by using plant extract

Silver nitrate (AgNO3) watery arrangement (1mm) was arranged and utilized for amalgamation of AgNPs. After this 10ml of plant remove was poured in 95ml of 1mm watery arrangement of silver nitrate (AgNO3) and bubble at 80° C for 15mins. At that point particles settled down were washed completely with refined water to eliminate the concentrate and dried in the hot air boiler. The synthesized nanoparticles were stored for future purposes.

2.2 Sample

- Azadiracta indica
- Gardenia gummifera

2.2.1 Antimicrobial activity by well diffusion method

Antimicrobial action of Synthesized AgNPs utilizing Azadiracta indica and Gardenia gummifera were tried against the P. aeruginosa, S. aureus, and E. coli by well dispersion strategy. Organic entity unadulterated culture was subcultured on Muller – Hinton stock on a shaker at 200 rpm at 35°C. Utilizing sterile q-tip each strain was swabbed proportionately on the individual plates. Wells of the size of 6 mm were delivered utilizing gel cut on Muller – Hinton agar plates. Utilizing micropipette, the nanoparticles arrangement 50μl, and 100μl test was filled in different wells on all plates. After 18 h brooding interval at 35°C, the different restraint zone levels were determined.

2.2.2 Instrument

Characterization of AgNPs was finished by conducting scanning electron microscopy (SEM) and transmission electron microscopy (TEM). Examining electron magnifying instrument delivered an engaged electron pillar over the outside of test to make picture. Delivered electron bar communicate with test and create different signs to get data about the surface geology and arrangement of the example. Transmission electron magnifying instrument delivered a light emission on the example to create a picture. With the assistance of TEM, surface morphology and the size of particles were examined and worked at a sped up voltage of 120Kv.

3. RESULTS AND DISCUSSION

3.1 Scanning Electron Microscope (SEM)

SEM supplied the details on surface morphology as well as nanoparticles synthesized in size. After test results have been obtained, the diameter of synthesized solvent nanoparticles has different sizes of nm. The results showed spherical particles with irregular form of some nanoparticles (Fig. 4).

3.2 Transmission Electron Microscope (TEM)

TEM has been generally utilized for distinguish the morphology, shape and size of the nanoparticles. A result shows that the incorporated nanoparticles are dominatingly round fit as a fiddle however a portion of the nanoparticles were found to be of unpredictable shape (Fig. 5).

Green blend of AgNPs utilizing Azadiracta indica and Gardenia gummifera shows diverse size going from 60-80nm of nanoparticles after portrayal with the Scanning electron magnifying lens and Transmission electron magnifying lens. Studies uncovers that most ideal techniques for green union of AgNPs is utilization of organic substances like microorganisms and plants, but
plants extract mediated synthesis is advantageous over microorganisms because of simple, safe and environment friendly approach. Plant extricates contains expansive varieties of metabolites that can be utilized in the decrease of metal particles. Amalgamation of AgNPs with *Azadiracta indica* and *Gardenia gummifera* can be utilized in wide scope of organic applications, for example, anticancer, antibacterial and so forth.

3.3 Antimicrobial Activity

Antimicrobial action of the AgNPs integrated utilizing *Azadiracta indica* and *Gardenia gummifera* was thought about in contrast to the various microbes like *P. aeruginosa, E. coli* as well as *S. aureus* by utilizing admirably diffusion technique. Table 1 shows the restrictions zone of defined diameter across each well with AgNPs. AgNPs made from *Azadiracta indica* as well as *Gardenia gummifera* were determined to be the most effective antibacterial activity against *E. coli* (32mm) and *S. aureus* (34mm) bacteria, respectively. AgNPs displayed viable antibacterial activity that differed from different salts due to their large surface area, which leads to enhanced interaction with microbes. The nanoparticles have become entangled in the cell film and have even reached the organisms. That microbial film binds sulfur-containing proteins, although AgNPs aid those proteins throughout the cell in the same way as aid blends including phosphorus, such as DNA. As AgNPs join the epithelial cells, they form a low sub-nuclear weight zone at the gatherings of the extremely tiny living beings, protecting the DNA again from silver particles. In an ideal world, nanoparticles cluster along the respiratory chain, with cell division triggering cell death. In bacterial cells, the nanoparticles emit silver particles, which boost bactericidal effect.
Table 1. Zone of inhibition against pathogenic bacteria

| Zone of inhibition (mm) against pathogenic bacteria | Stapliococcus aureus | Escherichia coli | Pseudomonas aeruginosa |
|----------------------------------------------------|---------------------|----------------|------------------------|
| Silver nanoparticles                               | 50µl                | 100µl          | 50µl                   | 100µl                  | 100µl                  |
| AgNPs                                               | 19                  | 34             | 20                     | 32                     | 17                     | 26                     |

4. CONCLUSION

Green blend of AgNPs by utilizing Azadiracta indica and Gardenia gummifera plant extract alongside their antimicrobial properties was accounted for in this investigation. Union of AgNPs was discovered to be effective on schedule and dependability. It shows to be a fast green, ecofriendly approach for combination and proficient method of AgNPs amalgamation. Thus, the total interaction fulfills every one of the details of a green substance measure. The primary advantages of ecofriendly AgNPs blend utilizing plant separate is that it is financially savvy, energy proficient, ensuring human wellbeing and climate prompting more secure items. This climate cordial strategy could be an option in contrast to the physical and compound utilized for the combination of AgNPs and along these lines it has an extraordinary potential to use in biomedical exploration and assume a huge part in clinical gadgets in future.

CONSENT

It is not applicable.

ETHICAL APPROVAL

It is not applicable.

COMPETING INTERESTS

Authors have declared that no competing interests exist.

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