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Optimization of Green Sysnthesis Nanoparticles Using Plant Extract and their Application

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ABSTRACT:

Researchers have shown considerable interest in developing a green synthesis method for silver nanoparticles (AgNPs) with targeted antimicrobial properties. In this study, AgNPs were successfully synthesized using a green approach and characterized using UV-Vis spectroscopy, SEM, and FTIR analysis. At a silver nitrate (AgNO3) concentration of 0.1 mM, stable AgNPs were produced, confirmed by a color change from yellow to brown and spectrophotometric analysis showing an optical density peak at 417 nm, indicative of plasmon absorbance. SEM analysis revealed the morphology and size of the AgNPs, while FTIR spectroscopy provided insight into their structural properties. The synthesized AgNPs exhibited potent antibacterial activity against both Gram-positive and Gram-negative bacteria, suggesting their potential as effective antimicrobial agents.

Keywords: plant extract, silver nanoparticles, antibacterial activity

1. Introduction:

Nanotechnology, especially biotechnology, is an emerging field of interdisciplinary research. Nanoparticles' importance, and their synthesis methods, nanoparticle research has become unavoidable in the modern world. Nanotechnology builds, manages, and uses materials at nanometer scales. Nanotechnology has enhanced scientific knowledge and advanced technology in herbal medicine and medicinal plant biology. In addition to medicine and agriculture, nanotechnology has many applications [1]. By protecting, producing, and protecting crops and livestock, natural resources can be utilized in agriculture to develop nanotechnology. Biosynthesis of nanoparticles (NPs) or green synthesis of nanoparticles (NPs) has received worldwide attention in recent years because of their biocompatibility, low toxicity, and environmental friendliness. Synthesis of NP from bacteria, yeast, mold, microalgae, and plant extracts has many advantages, including minimal energy consumption, moderate technology, and no toxic chemicals. Nanotechnology is used to control plant diseases through nanoparticles. Nanoparticles are either pesticide carriers or individual carriers of pesticides. The technique of synthesizing nanoparticles can be achieved in several ways, including chemical electrochemistry, radiation and photochemistry, Langmuir-Blodgett, and biochemical procedures. Nanotechnology involves utilizing toxic and harmful chemicals to synthesize nanoparticles, which can pose biological risks. These chemical processes may also harm the environment. Green synthesis and other biological techniques are more popular. As a result of the complex procedures involved in maintaining microbiological cultures, it may be more beneficial to synthesize nanoparticles using a variety of plant materials and their extracts rather than using other biosynthetic processes. We have undertaken the present review to explore the methods of synthesizing nanoparticles from different sources and their applications to different fields based on the above-mentioned facts.

The synthesis of silver nanoparticles (AgNPs) through green methods utilizing plant extracts has garnered considerable attention due to the presence of bioactive compounds such as flavonoids, ketones, aldehydes, tannins, carboxylic acids, phenolics, and proteins. These compounds play a crucial role in the reduction of silver ions (Ag^+) to elemental silver (Ag^0), facilitating the production of AgNPs. The resulting nanoparticles exhibit diverse types, sizes, and morphologies, which are influenced by various experimental parameters including temperature, pH, reaction kinetics, and the adsorption of capping agents. These factors collectively impact the physicochemical properties of AgNPs, including size, shape, and stability, thereby affecting their performance in applications. Controlling these parameters to tailor the size, shape, and stability of AgNPs is imperative for optimizing their efficacy and functionality. Recent advancements in utilizing plants and plant-derived products for the green synthesis of metal nanoparticles underscore the importance of this research area in nanotechnology.

Nanotechnology holds significant promise across various domains, particularly in biotechnology where precise manipulation of atoms and molecules at the nanoscale enables groundbreaking advancements. One notable application lies in DNA silicon chips, exemplifying the integration and control achievable through nanomaterial fabrication. Beyond biotechnology, nanotechnology finds diverse applications spanning military defense, medicine, astronomy, and computing. Its versatility extends to the synthesis and fabrication of a wide array of products, ranging from everyday items like eyeliner, suntan lotion, sterile socks, to advanced materials like self-cleaning glass. In medicine, nanotechnology facilitates the precise delivery of drugs within the human body, enhancing accuracy and efficacy. Moreover, ongoing research suggests the potential of nano food in regulating consumption, offering novel solutions to dietary challenges. Thus, nanotechnology emerges as a transformative force with multifaceted implications across various sectors, promising innovative solutions to contemporary challenges.

A variety of research works are available on the biosynthesis of nanoparticles using plant extract, *solanum trilobatum, vitex negundo, Cardiospermum halicacabum, euphorbia thymifolia* , on the basic available scientific literature. Nanotechnology has increased food quality, world food production, plant protection, detection of plant, animal and human disease [1]. These nanomaterial's are still a matter of discussion due to their newfound abilities which prove their uses in many fields and are considered a great choice for dealing with antimicrobial resistant microbes. Owing to their size and composition, these nanoparticles can be of various dimensions. Nanoparticles, nanotubes, Nano sheets, nanowire arrays are all used in various applications. Most of the available drugs for treating various cancers have organic nanoparticles which involve liposomes, micelles, notably cytarabine enclosed in a liposome for treating acute myeloid leukemia [2]. Iron oxide nanoparticles have also been implemented in treating prostate, pancreatic and glioblastoma cancer, approved in the year 2010. Metals like zinc nanoparticles and metal oxide nanoparticles, along with lipid based have also been used in treating SARS-Cov-2, a viral disease that shook the world. Silica and related NPs are also of great importance in medicinal field, silica based folic acid NPs are used as antitumor drugs [3] [4]. Ceramic NPs have been found to be involved in photo degradation of dyes, catalysis applications like photo catalysis [5]. Nanoparticles have two different approaches on how it can be synthesized, top down or bottom up, in a top-down based synthesis, bigger molecules are destroyed into smaller ones with optimization methods can produce spherical NPs in perfect size required to do the required function. Ideally iron oxides can give out 20-50 nm sized NPs when done in this method as reported by Priyadarshana G and coworkers in the year 2015 [6]. Laser irradiation also produces uniform NPs with physiochemical properties [7].

2. Materials and Methods

Analytical grade chemicals were obtained from Hi-Media, E. Merck, SRL and Sigma chemicals. Purchasing silver nitrate ($AgNO_3$) was done through Himedia Lab Pvt. Limited (Mumbai, India).

Identification of Plant

Asystasia gangetica was collected from Arimalam Pudukkottai ,Tamil Nadu And identified at the Siddha Central Research Institute (Central Council for Research in Siddha, Chennai, Ministry of AYUSH, Government of India)Anna Govt. Hospital Campus, Arumbakkam, Chennai – 600106,

Sample Collection

The Asystasia gangetica leaves were washed with distilled water and shade dried for a few days. Dry leaves were milled into a fine powder with a mixer grinder and sieved to remove coarse particles. In order to prevent moisture from entering the powder, it was stored in moisture-free containers [8].

Figure1:Asystasia gangetica

Preparation of Plant Leaf Extract

A conical flask was filled with 100 ml of double distilled water and 10 grams of the above powdered leaves were dissolved separately. The mixture was cooled to room temperature after boiling at 55°C for 30 minutes [9]. Aqueous leaves extracts from the extracts were filtered, and they were then refrigerated and used to synthesize silver nanoparticles (AgNPs) [1].

Synthesis of silver nanoparticles:

An aqueous extract of the plants was added to separate test tubes containing 4 ml of sterilized distilled water for the synthesis of silver nanoparticles (AgNPs). The above solution was enhanced with 1mM silver nitrate (AgNO3)[10]. Observed color changes after 24 hours of undisturbed storage at room temperature.

Silver nanoparticle synthesis optimized by various parameters

AgNPs were synthesized most rapidly and maximally by optimizing the parameters of time, pH, temperature, and silver nitrate concentration. [14]

Effect of different pH

The pH of 2, 4, 6, 8, 10, 12, and 14 were observed at different wavelength to achieve synthesis of Nanoparticles. The absorbance of synthesized nanoparticles was noted. The reaction from 1 to 60 minutes was carried out to achieve the optimal AgNP production rate. To adjust the pH of the solution 0.1 M of hydrogen chloride and 0.1 M of sodium hydroxide were used.[14]

Effect of different temperature

The temperature of synthesized nanoparticles were optimized under following conditions; 25 °C, 50° C, 75°C, 100°C. The absorbance of the synthesized nanoparticles were monitored and noted.[14]

Effect of silver nitrate concentration

The Concentration of $AgNO_3$ was optimized with the standard concentration of plant extract. The concentrations used are as follows, 1: 0.5, 1:1, 1:1.5, 1:2, 1:2.5 and 1: 3. . [15]

Characterization of synthesized Nanoparticles:

UV-Visible vis spectroscopy

The reduction of silver ions from silver nitrate (AgNO3) to silver nanoparticles (AgNPs) was monitored on a Systronic UV-Visible spectrophotometer 2200 by measuring the absorbance as a UV-Vis spectrum. Detection of silver nanoparticles (AgNPs) synthesized by [11] was performed by measuring the absorbance between 400 and 700 nm.

Scanning Electron Microscopy

Green synthesized silver nanoparticles were analyzed using SEM. Using carbon counted tape, a thin film of dry fine powder of green synthesize silver nanoparticles powder was applied to the grid. Excess sample was removed with blotting paper.[12]

FTIR(Fourier transform infrared spectroscopy)
Biological biomass residues were removed by centrifugation at 12,000 rpm for 20 minutes. A redispersion of the pellet in distilled water followed by centrifugation was performed on the pellet again. Repeat this step 2–3 times. FTIR spectroscopy measurements were conducted after samples were dried and ground with KBr pellets. KBr pellets were measured in the different reflectance mode on a Thermo-Nicolet-Avatar 370 instrument.
Applications of synthesized AgNPs
Antimicrobial activity
Assays were conducted to determine the antibacterial potential of the synthesized AgNPs obtained from leaf extract against staphylococcus aureus and Escherichia coli. Antibacterial activity was determined using the agar plate well diffusion method [13]. A sterile petriplate was filled with 20 ML of MHA agar medium. A sterile cotton swab was used to spread the test bacterial cultures onto Muller Hinton agar media. Then, the wells were labeled as A, B, C and D. The A well was loaded with 30 µL of AgNO3 (1 mM); B and C wells were loaded with 30 µL of plant extract and synthesized AgNPs; D well was repared with 30 µL of standard . The plates were incubated at 37 °C for 24 hours, and the inhibition zone around the wells was recorded.

3. Result and Discussion:

Uv spectrophotometry
It appears that the extracts changed color over time after mixing with silver nitrate solutions. This may be related to the reduction of silver ions, which activates the Surface Plasmon Resonance (SPR) of the AgNPs. The UV spectrum showed a peak at 417 nm, indicating that nanoparticles formed within a stable range. By increasing concentration, the surface Plasmon resonance shifted toward red shift, resulting in stable particles because of larger functional ligands. On large concentrations, however, we did not observe a peak in our UV-VIS spectra because the extract we prepared was too thick. By reducing the concentration, we shifted the SPR towards blue, which resulted in smaller particles having a greater surface area and are more stable. A clear peak was obtained by diluting the extract, but the peak position remained the same.[16] We optimized AgNPs for further characterization. The optimized AgNPs could potentially be used in various applications such as catalysis, sensing, drug delivery, and antimicrobial coatings due to their stability, controllable size, and increased surface area.

Figure2: Color change was indicate the presence of silver nanoparticles

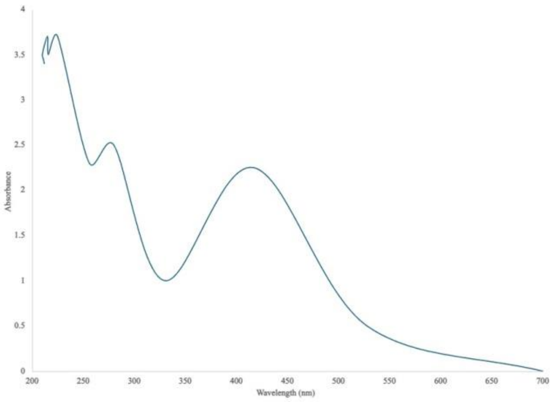


Figure 3: UV spectral analysis of biosynthesized AgNPs showed an absorption peak at 417 nm

Effect of p^H

One of the major environmental factors that affect pH is the production of Ag nanoparticles. Ag nanoparticles smaller quantities of production were observed in an acidic range. A wider range of peaks was detected. When the pH increased, absorbance peaks became narrower and sharper, which indicated that smaller and spherical Ag nanoparticles were being produced. As the peak showed symmetrical distribution at pH 7.0 this also confirmed that nanoparticles were uniformly distributed. It was observed that the maximum peak intensity corresponding to 1.33 occurred at 417 nm at pH 7.0, indicating the presence of higher concentrations of Ag nanoparticles present in the solution.

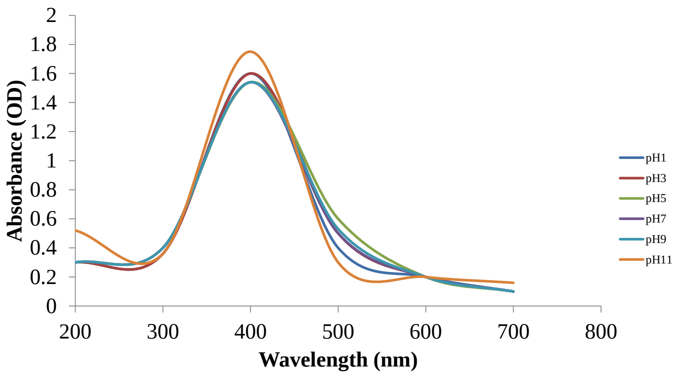


Figure 4:Effect of pH in Green synthesized nanoparticles

Effect of different temperature

A key controlling factor in the formation of Ag nanoparticles is temperature. Peaks shifted towards lower wavelengths with increasing temperature from 25°C to 50°C, indicating a reduction in particle size. The nanoparticles of Ag were found in a variety of sizes at low temperatures. A narrower range of Ag nanoparticles was found in the solution at a higher temperature, which indicated a narrow range of Ag nanoparticles. Highest and narrowest peak The results were obtained at 75°C. At 418 nm, there was a maximum intensity indicating a large number of Ag nanoparticles in the solution The peak was found to be most symmetrical at 40°C, indicating that the biosynthesised Ag nanoparticles were monodisperse.

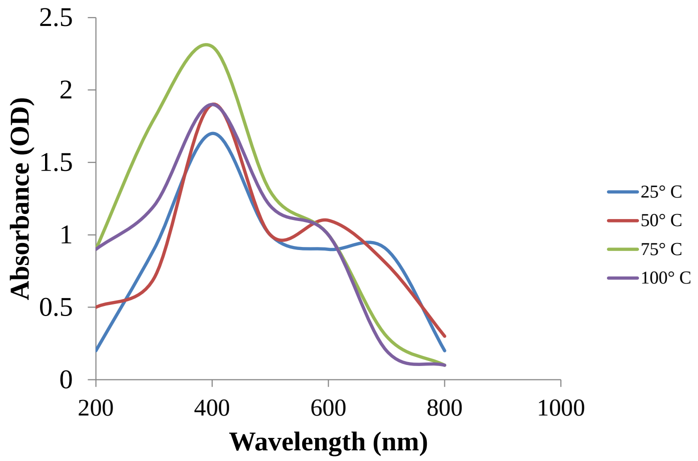


Figure 5: Effect of temperature in Green synthesized Nanoparticles

Effect of silver nitrate concentration:

A study of the effect of AgNo3 concentration on the production of Various concentrations of Ag nanoparticles were evaluated. During the past few years, there has been a gradual increase in Producing nanoparticles of Ag by adding 1.5 mm of AgNO3 effort Depending on the peak intensity, this value is the maximum The concentration of AgNO3 was 1.5 mm in the presence of 2.149 The higher production was found to be at 417 nm Nanoparticles of Ag . As a result of an increase in Peak distortion was observed for AgNO3 concentrations As a result, the symmetry and peak flattened out, indicating Non-uniformity.

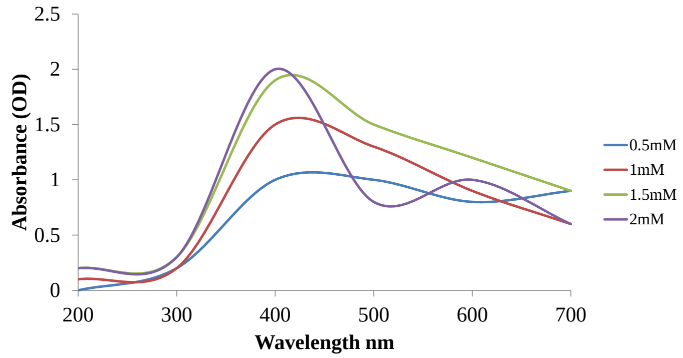


Figure 6: Effect of silver nitrate in Green synthesized Nanoparticles

FTIR:

The research utilized Fourier transform infrared (FTIR) spectroscopy to elucidate the role of secondary metabolites in the reduction and capping of silver nanoparticles (AgNPs) within leaf extracts. The FTIR spectra of AgNPs exhibited prominent absorption peaks at specific wavenumbers, indicative of the presence of phytoconstituents. Notably, shifts in these peaks were observed, reflecting the involvement of various functional groups in nanoparticle stabilization. For instance, the peak at 3269.69 cm–1, attributed to phenolic compounds' O–H or N–H stretching, shifted to 2918.08 cm–1, suggesting alterations in molecular interactions. Additionally, characteristic peaks associated with methylene groups, triterpene saponins, alkenyl or aromatic C═C stretches, and −C–O stretching of phenol or tertiary alcohols were identified, further underscoring the diverse molecular components involved in AgNP synthesis. The presence of polyphenolic groups, including triterpenoids, alkaloids, steroids, and tannins, within the leaf extracts corroborated their role in facilitating AgNP formation. Notably, our findings revealed distinct phytochemical profiles in Asystasia gangetica leaves, highlighting the variability in nanoparticle synthesis mechanisms among plant species. These results underscore the intricate interplay between phytoconstituents and AgNP synthesis, offering insights into green nanoparticle fabrication processes.

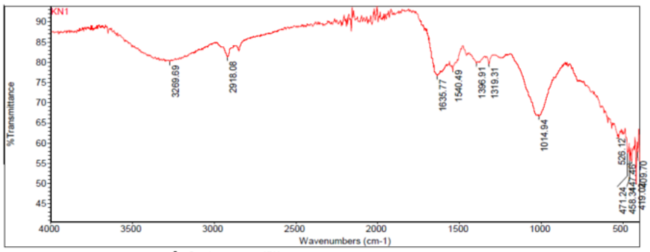


Figure 7: FTIR analysis of synthesized AgNPs indicates the presence of various functional groups

SEM:

Silver nanoparticle green synthesis was demonstrated using SEM. The SEM images confirmed that metal particles are nanosized. At 11500X, the silver nanoparticles were magnified at 10 kV, and their average diameter was 60nm–344nm, indicating that the particles had a spherical shape. Silver nanoparticles agglomerate into larger sizes on the SEM images due to their scattering structure. It is also correlated with the observed broad absorbance peak area. The compounds in the plant extract act as a ligand which stabilizes the formed silver nanoparticles. The silver nanoparticles shape and size were photographed using SEM.

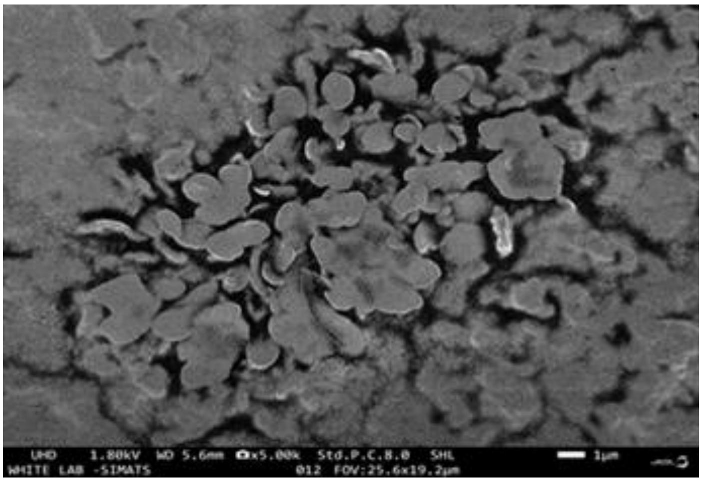
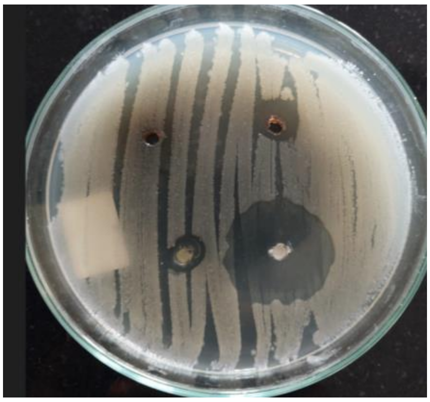


Figure8: SEM analysis of biologically synthesized AgNPs

Antimicrobial activity of green synthesis of silver nanoparticles:

The study was investigated the antimicrobial activity of silver nanoparticles (AgNPs) against pathogenic bacterial strains, including gram-negative Escherichia coli and gram-positive Staphylococcus aureus, through a well diffusion assay. Ampicillin served as the control group alongside plant extracts, AgNO3, and synthesized AgNPs. The findings revealed that all synthesized AgNPs exhibited significant efficacy against both bacterial strains, surpassing the antibacterial activity of AgNO3 and plant extracts. Specifically, the inhibition zones for AgNO3 Nanoparticles against E. coli and S. aureus were measured at 1.4mm and 0.8mm, respectively. Notably, the observed antibacterial effects were solely attributed to the biosynthesized nanoparticles, as neither plant extracts nor AgNO3 demonstrated antibacterial activity against the tested strains.

The precise mechanism underlying the antibacterial action of AgNPs remains elusive; however, several hypotheses have been proposed. It is speculated that AgNPs induce antimicrobial effects by generating reactive oxygen species, releasing Ag+ ions that interact with sulfhydryl groups to disrupt protein structures, or physically damaging bacterial cells upon attachment. Remarkably, AgNPs synthesized from Asystasia gangetica exhibited potent inhibition against both gram-positive and gram-negative bacteria, suggesting that the antimicrobial activity of AgNPs remains consistent regardless of differences in bacterial cell wall composition. These findings underscore the broad-spectrum antimicrobial potential of AgNPs and contribute to our understanding of their mechanisms of action against pathogenic bacteria.



(A. Plant extract; B. $AgNO_3$; C. Antibiotic; D. AgNPs) Figure9: Antimicrobial activity of biologically synthesized AgNPs (*Staphylococcus aureus)*

(A. AgNPs ; B. Plant extract; c:Antibiotic ; D. $AgNO_3$)

Figure10: Antimicrobial activity of biologically synthesized AgNPs (*E.Coli)*

4. Conclusion

In conclusion, this study presents a novel approach for the synthesis of silver nanoparticles (AgNPs) using aqueous extracts of Asystasia gangetica. By employing room temperature reduction of silver nitrate with Asystasia gangetica extract, a straightforward, efficient, and environmentally friendly method for AgNP synthesis was achieved. Through an extensive literature review, we optimized a protocol to yield AgNPs rapidly and in high quantities.
The green synthesis of AgNPs using Asystasia gangetica extract ensures the absence of toxic compounds, making them suitable for biomedical applications where safety and compatibility with biomolecules are paramount. The presence of proteins, flavonoids, and phenols in the

extract contributes to the stabilization and green synthesis of AgNPs, enhancing their potential for medical applications.
Characterization of the synthesized AgNPs plays a crucial role in determining their suitability for various applications. Our study underscores the importance of simple, low-cost, and environmentally friendly synthesis methods for AgNPs, offering potential avenues for future biological research. Moreover, the versatility of AgNPs synthesized from Asystasia gangetica extract opens possibilities for their application in catalysis and other fields. Overall, this study lays the foundation for further exploration of herbal plant-based nanoparticle synthesis and its diverse applications in biomedicine and beyond.

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Conflict of Interest
Authors claim that there is no conflict of interest.
Author's Contribution
All authors have equal have made equal contributions
Funding
Nil
Ethical Statement and Informed Consent
Does not apply

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