

BIOSYNTHESIS OF SILVER NANOPARTICLES BY LEAF EXTRACT AND ITS ANTIBACTERIAL ACTIVITY

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Abstract: The Biosynthesis of nanoparticles for biological processes is accelerating its prominence of research in the field of nanotechnology. Synthetic chemical compounds which are very dangerous and combustible in nature are usually used in synthesizing silver nanoparticles. The production of antibacterial silver nanoparticles using leaf extracts deals a ecofriendly biosynthesis process. Silver nanoparticles formation and their characteristics were detected using Ultraviolet visible spectrophotometry(UV-Vis), fourier transform infrared spectroscopy(FT-IR), scanning electron microscopy(SEM). The antibacterial activities were carried out against *Pseudomonas aeruginosa*, *Bacillus magaterium* strains by well diffusion method.

Keywords: silver nanoparticles, leaves, green synthesis, antibacterial activity.

1. INTRODUCTION

Uniqueness of nanoparticles in exhibiting physical and chemical properties along with their significant use in various applications (biology, chemistry, medicine, biotechnology, catalysis, electronics, material sciences, optoelectronics, optics and sensors attracts universal attention at present and in future^{1,2,3,4,5}. In precise colloidal silver exhibits antimicrobial properties as well as non-toxic and at ecofriendly. Silver ions are more lead than traditional antibiotics. Various methods are used to synthesized metal nanoparticles along with physical, chemical, photochemical, irradiative, electrochemical, and biological techniques^{6,7,8,9,10,11,12}. In spite of the fact that the majority of the strategies were fruitful in creating unadulterated and very much characterized nanoparticles but quite economical and non ecofriendly are pollute the environments. Biological synthesis heads among the other methods in synthesizing silver nanoparticles which proved to be economical, ecofriendly and easy in scaling up for large scale synthesis. Bacteria^{13,14}, fungi¹⁵ and plant extracts^{16,17,18} are used in reporting the biosynthesis of metal nanoparticles. Natural combination procedure gives a wide scope of ecofriendly philosophy, ease generation and least time. The urge of ecofriendly process in the synthesizing nanoparticles led towards Green chemistry and bioprocess¹⁰. The operation used for the composite of silver nanoparticles and

malignant synthetic compounds are utilized for the reduction procedure of substances, for example, citrates, NaBH₄ or ascorbates. In recent days, green bio-reduction strategies for the amalgamation of silver nanoparticles were adjusted by numerous analysts utilizing plant extract materials, like *Macrotyloma uniflorum*⁸, *Anacardium Mushroom* extract⁹, *Coleus amboinicus* lour¹⁰, *Medicago sativa*¹¹, and *Citrus sinensis* peel¹². The principle goals of this study were to synthesize the silver nanoparticles utilizing aqueous concentrate of leaves to describe the silver nanoparticle, where the Gram-positive and Gram-negative microorganisms antimicrobial properties can be analyzed using UV vis spectroscopy.

2. EXPERIMENTAL SECTION

2.1 Materials

The leaves *Azadirachta indica* (Neem), *Pongamia pinnata* (pongam tree) were collected from A.U college of engineering campus, Andhra University Visakhapatnam.

2.2 Silver Nitrate Preparation

Silver nitrate was utilized as progenitor for the formation of silver nanoparticles. Systematic category silver nitrate (AgNO₃) were prepared for 1.696mg of silver nitrate was weighed carefully and dissolved in 90 ml of Milli-Q-water. This fluid Silver nitrate arrangement was constantly arranged freshly three different concentrations (0.1M, 0.01M, 0.001M) of silver nitrate solution are prepared as shown in the figure below.



Fig. 1: Different concentrations of silver nitrate mixture

2.3 Preparation of Leaf Extract

Azadirachta indica (Neem): Newly harvested leaves of *Azadirachta indica* (Neem) were washed many times with water. 10gms of the leaves were cleansed under running water pursued by distilled water and after that air dried. The leaves were chopped into fine pieces and put into 500 ml glass beaker for boiling purpose. 200ml of double distilled water was added into the beaker. Then the beaker was placed on electric hot plate for boiling. Then the solution was boiled for one hour at 60°C. The extract was isolated and filtered with (5nm filter paper pore size) whattman filter paper. Then the mixture was utilized for the depletion of silver ions (Ag⁺) to silver nanoparticles (Ag⁰).

Pongamia pinnata (Indian beech): Fresh leaves of *Pongamia pinnata* (Indian beech) were finely chopped into small pieces. 10 gms of the leaves were weighed using Electronic weighing machine. Then the chopped leaves were put into 500 ml measuring glass alongside 200 ml of double refined water. Then the solution was subjected to boiling for 1 hour at 60°C. The extract was isolated and filtered with (5nm filter paper pore size) whattman filter paper. At that point the solution was put away in fridge at 4°C for development use in mix of silver nanoparticles.

2.4 Bio-Synthesis of Silver Nanoparticles

Azadirachta indica (Neem) leaf extract: The Silver nitrate acts as a precursor in this synthesis. 0.01M of aqueous mixture of Silver nitrate was readied and the equivalent is utilized for the amalgamation for silver nanoparticles. 10ml leaf extract of *Azadirachta indica* (Neem) was added to aggressively stirred 10 ml of fluid blend of 0.01M silver nitrate and 80 ml of refined water and kept at room temperature. The leaf concentrate acts both as diminishing operator and stabilizing agent. Instantly after the inclusion of extract of Neem to AgNO₃(10⁻³ M) aqueous mixture a light yellowish color appears which is further changes to yellowish orange and then to dark brown color after 1 and half an hour. The colour change determine the development of silver nanoparticles (AgNPs). Then solution is then put through to centrifuge for 60 min at room temperature with 1500 rpm. Then supernatant was segregated and stored in refrigerator at 4°C for characterization and antibacterial studies. The same concentrations were sonicated using an ultrasonic bath and the results were recored at every 5mins using UV visible spectroscopy. The same concentrations were tested for light induced synthesis and the results were recored at every 5mins using UV visible spectroscopy.



Fig.2: Colour at the start of the reaction (left) and at the end of the reaction (right)

Pongamia pinnata (Indian beech) leaf extract: The Silver nitrate acts as a precursor in this synthesis. The leaf concentrate acts both as diminishing operator and stabilizing agent. 0.01M of aqueous mixture of Silver nitrate was arranged and utilized for the blend of silver nanoparticles. 10ml of leaf extract of *Pongamia pinnata* (Indian beech) was added to 10 ml aqueous mixture of 0.01M silver nitrate and 80 ml of distilled water and kept at room temperature. Promptly after the insertion of extract of Indian beech to AgNO₃ (10⁻³M) aqueous solution a light yellowish color appears which is further changed to reddish brown color after 10 hours. The color change shows that the development of silver nanoparticles (AgNPs). Then the arrangement is then treated with centrifuge for 60 min. at room temperature with 1500 rpm. Then supernatant was isolated and stored in refrigerator at 4°C for characterization and antibacterial studies. The same concentrations were sonicated using an ultrasonic bath and the results were recored at every 5mins using UV visible spectroscopy. The same concentrations were tested for light induced synthesis and the results were recored at every 5mins using UV visible spectroscopy.

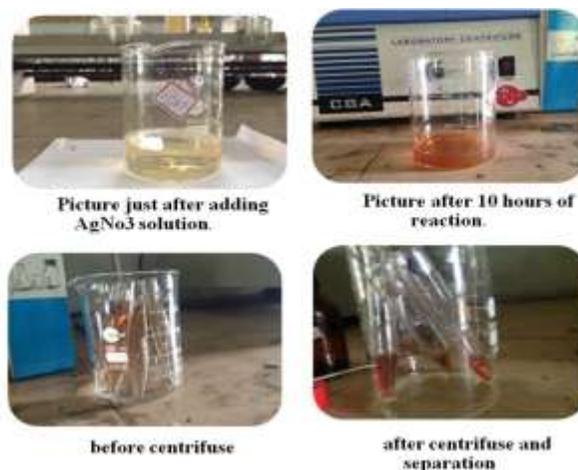


Fig.3: Pictures of Synthesis Process

2.5 Silver nanoparticles recovery

The colloidal response blends holding nanoparticles were centrifuged with 1500 rpm for 60 min. The pellets which acquired after centrifugation were wiped with 70 % ethanol and dried in oven at a temperature 25°C for 24h. The dried powder was segregated cautiously from centrifuge tubes and put away in test vials for further assessment.

3. RESULTS AND DISCUSSION

3.1. CHARACTERISATION OF SILVER NANOPARTICLES

- UV-visible spectrophotometer analysis

UV-visible spectrophotometric studies is used to follow and confirm the production of silver nanoparticles. The depletion of unadulterated Ag⁺ ions from AgNO₃ was observed by measuring the UV-Visible Spectrum. The bio-decrease of silver particles in fluid arrangement was checked by UV-Visible Spectra of range 220-800 nm. The absorbance spectra of the AgNP were examined by utilizing an UV 2400 PC arrangement spectrophotometer. AgNP showed reddish yellow color in water because of excitation of the restricted surface plasmon shaking of the metal nanoparticles. Synthesized nanoparticles is the surface plasmon resonance (SPR) groups are influenced by the shape, size, piece, morphology and dielectric condition.

Azadirachta indica (Neem) AgNPs

The UV-visible spectrum of Azadirachta indica (Neem) extract does not show any peaks in the range from 250 to 700 nm. The change in colour results in formation of AgNPs. Addition of extract to silver nitrate mixture brought appreciable change in the shade of colored solution. A peak was seen at about 443nm for the synthesized nanoparticles. This wavelength will be suitable for the biologically synthesized nanoparticles.

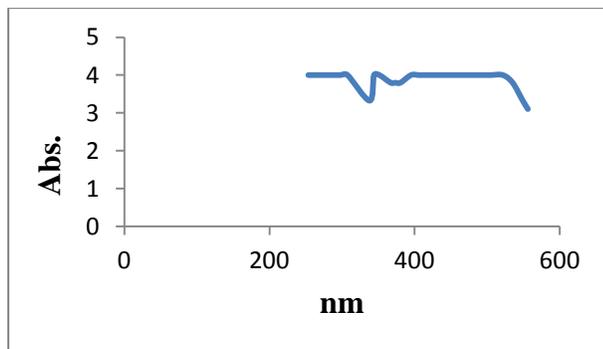


Fig.4: Graph representing UV analysis of AgNPs of Azadirachta indica (Neem) leaf extract

Pongamia pinnata (Indian beech)AgNPs:

The UV-visible spectrum of Pongamia pinnata (Indian beech) extract does not show any peak in the range from 200 to 800 nm. The change in colour results in of the formation of AgNPs. Addition of extract to silver nitrate mixture cause appreciable change in the shade of colored solution. A peak was observed at about 454nm for the synthesized nanoparticles. This wavelength will be suitable for the biologically synthesized nanoparticles.

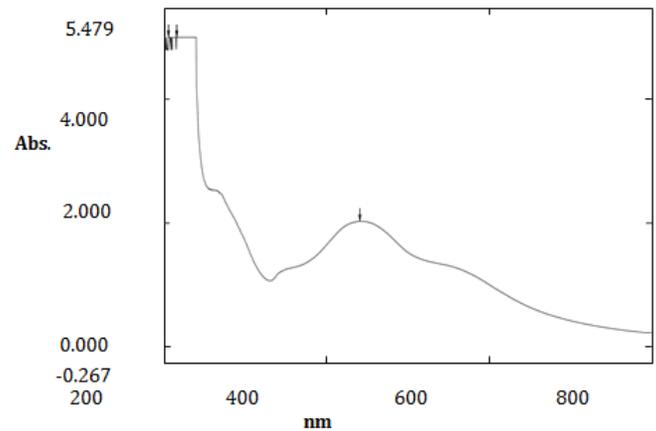
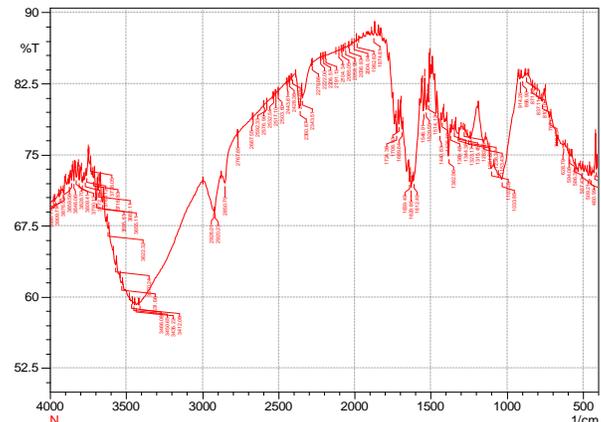


Fig.5: Graph representing UV analysis of AgNPs of Pongamia pinnata (Indian beech) leaf extract

- FT-IR analysis

The bioreduced arrangement was centrifuged with 10,000rpm for 60 mins. The pellet dried by utilizing 5 ml of ethanol to dispose of the free proteins or catalysts that are not delegated the AgNPs. The dried pellet was kept at room temperature to secure particles or precious stones of silver nanoparticles (AgNPs). The studies of dried nanoparticles were performed by FT-IR spectrum.



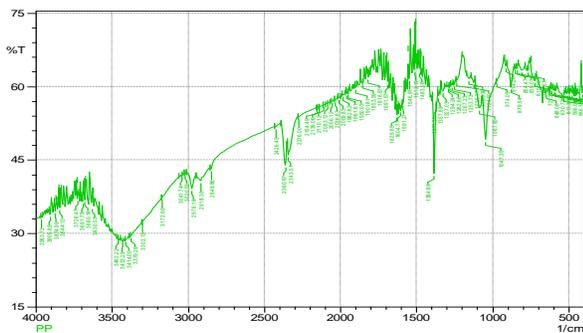


Fig.6:Graph representing FTIR spectrum analysis of AgNPs

Graph shows the FTIR spectrum of AgNPs. The FTIR showed the presence of bands at 1620 cm^{-1} , 1633 cm^{-1} and 1637 cm^{-1} corresponding to silver nanoparticles prepared from the extracts of *Azadirachta indica* (Neem), *Pongamia pinnata* (Indian beech) respectively. Synthesized AgNPs are recognized as amide I and appear due to a carbonyl stretch in the amide linkages of the proteins. The FTIR results thus stipulate that the optional structure of the proteins isn't influenced as a outcome of reaction with the Ag^+ ions or official with the silver nanoparticles. This outcome suggests that the biological molecules could play out a capacity for the arrangement and adjustment of AgNP in a aqueous medium. It is outstanding that proteins can bind to AgNP through free amine groups in the proteins and finally the adjustment of the AgNP by surface-bound proteins is a possibility.

The conceivable biomolecules for topping and proficient adjustment of the silver nanoparticles were measured using FTIR by *Azadirachta indicia* (Neem), *Pongamia pinnata* (Indian beech) respectively. The silver nanoparticles (AgNPs) FTIR spectrum is given above. The Alcohols, Phenols and primary Amine group (O-Hand N-H) corresponds at the peak value 3440.58 cm^{-1} from the above FTIR spectrum. Carboxylic acids and derivatives are identified at the peak value 2579.45 cm^{-1} from the above FTIR graph. The peak value at 1618.84 and 1063.35 cm^{-1} represents to Primary Amine groups. 1333.16 cm^{-1} peak gives Liquor and Phenols (O-H) and final peak value 623.45 cm^{-1} represents Alkynes group C-H). The more grounded capacity of the carbonyl gathering from the amino corrosive buildups and proteins to tie metal were

affirmed by examined consequences of FTIR thinks about which thusly demonstrating that proteins could shape the silver nanoparticles.

• **Scanning Electron Microscopy (SEM) Analysis**

This examination was performed to know the size and state of the silver nanoparticles which are biosynthesized. Scanning Electron Microscopy examination was performed using FEI Quanta 200 SEM machine. On a carbon secured copper work a slender films of the example were set up by just dropping a very humble amount of the model on the system, extra plan was removed using a smearing paper. Then the film on the network was permitted to dry and the pictures of nanoparticles were caught.

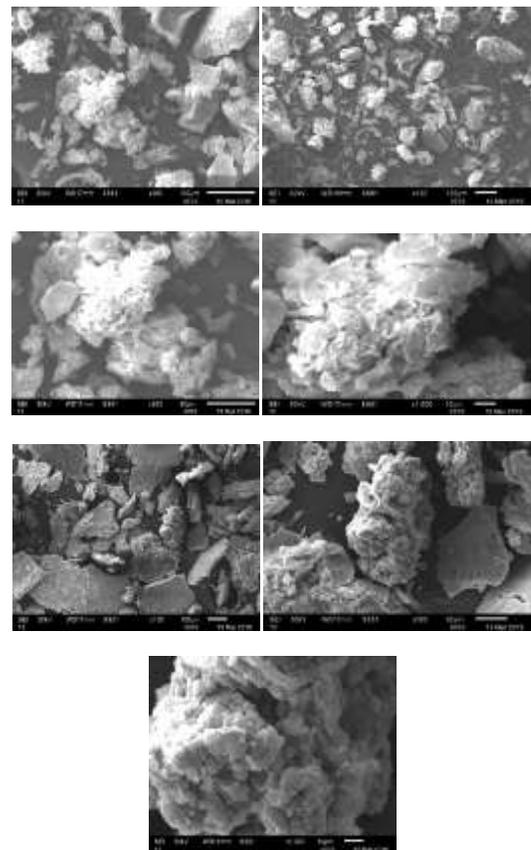


Fig.7: SEM images of nanoparticles synthesized from leaf extracts

3.2 STUDY OF ANTI BACTERIAL ACTIVITY

Antibacterial are those that destroys bacteria or suppresses their growth or their ability to reproduce. They are used to treat bacterial infections. In the current studies synthesis of silver nanoparticles has been done using a particular variety of leaf extracts. Afterward, the antibacterial action of the synthesized silver nanoparticles was tried utilizing both gram positive and gram negative organisms that is *pseudomonas auroginosa*, *bacillus magaterium*.

• **Microbiological assays:**

Antibacterial Activity:

Antibacterial activity of silver nanoparticles (AgNPs) was measured utilizing Gram positive and Gram negative pathogenic bacteria named Bacillus Magaterium (BM) and Pseudomonas Aeuroginosa by utilizing the agar well diffusion method. In this method bacterial cultures were inoculated on nutrient agar containing various contents of constituents. Later the medium was hardened and afterward the cotton was placed superficially and openings were punched with the bacterial suspensions. Synthesized nanoparticles (AgNPs) were filled into the wells created in the plates and the leaf extracts were utilized as a control. The samples were incubated for 24 h at a 37°C temperature. Zone of hinderance was observed around the well after the incubation period.

Anti bacterial activity results:

Antibacterial activity synthesized by the leaf extracts was investigated using Bacillus Megaterium(Gram Positive) and Pseudomonas Aeuroginosa(Gram negative) using the well diffusion method. The diameter of inhibition zones (mm) around each well with silver nanoparticle solutions is observed. The highest antibacterial activity of silver nanoparticles synthesized by Azadirachta indica (Neem)plant extract was found against Pseudomonas Aeuroginosa 11.6 mm and 13.9 mm was found against Bacillus Megaterium. The lesser antibacterial activity of silver nanoparticles synthesized by Pongamia pinnata (Indian beech)was found to be 8.2 mm against Pseudomonas Aeuroginosa and 9.5mmwas found against Bacillus Megaterium. The only leaf extracts has no antibacterial activity.



Fig.8: Antibacterial activity of silver nanoparticles using bacteria

Table 1.Anti-Bacterial activity

sample	Pseudomonas aeuroginosa (Gram negetive)	Bacillus magaterium (gram positive)
	Zone of inhibition	Zone of inhibition
Azadirachta indica (Neem) AgNP's	11.6 mm	13.9 mm
Pongamiapinnata (Indianbeech)AgNP's	8.2 mm	9.5 mm
Azadirachta indica (Neem) leaf extract	no activity	no activity
Pongamia pinnata (Indian beech)leaf extract	no activity	no activity

4. CONCLUSION

The study demonstrated the synthesis of silver nanoparticles from the leaf extracts of Azadirachta indica (Neem) and Pongamia pinnata (Indian beech).The photocatalytic activity is introduced to reduce the reaction time of biosynthesis. The particle sizes obtained by silver nanoparticles from Azadirachta indica (Neem)are 20-50nm range which are smaller when compared to silver nanoparticles prepared from Pongamia pinnata (Indian beech) leaf extract(70-90nm) as confirmed by SEM analysis. 443nm and 454nm absorption wavelengths were observed in UV-VIS spectroscopic analysis. This wavelength will be suitable for the biologically synthesized nanoparticles, FTIR studies were affirmed that the carbonyl group from the amino acid deposits and proteins has more grounded capacity to tie metal showing that the proteins could frame the silver nanoparticles. The silver nanoparticles synthesized from Azadirachta indica (Neem)has the maximum antibacterial activity against pseudomonas aeruginosa which when compared to silver nanoparticles prepared from Pongamia pinnata (Indian beech)leaf extract. In recent studies it is also stated in other studies that the leaf separates of Azadirachta indica (Neem) and Pongamia pinnata (Indian beech) have antimalarial activity and antihyperglycemic activity.

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