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JIGNASA STUDENT STUDY PROJECT

ON

GREEN-SYNTHESIS OF SILVER NANOPARTICLES USING ALTERNANTHERA SESSILIS (LINN.) LEAF EXTRACT AND THE STUDY OF ANTIOXIDANT, PHOTOCATALYTIC AND ITS ANTIBACTERIAL, ANTIFUNGAL ACTIVITIES.

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GREEN-SYNTHESIS OF SILVER NANOPARTICLES USING *ALTERNANTHERA SESSILIS* (LINN.) LEAF EXTRACT AND THE STUDY OF ANTIOXIDANT, PHOTOCATALYTIC AND ITS ANTIBACTERIAL, ANTIFUNGAL ACTIVITIES.

ABSTRACT

Green synthesis of nanoparticles has gained notable significance in the recent time because of the use of cost-effective and eco –friendly procedure. In the present study, one such simple cost-effective and eco-friendly biosynthesis of silver nanoparticles {AgNPs} was developed. The synthesis of AgNPs from aqueous leaf extract of *Alternantera sessilis* was assessed by varying different reaction parameters like concentration of plant extract, the ratio of reactants, temperature and reaction time. Characterization using UV-Visible Spectrophotometry revealed a Surface Plasmon Resonance [SPR] peak at 429nm confirming the formation of AgNPs. Further, characterization of the AgNPs was carried out using X-ray Difractometer{XRD} ,it reveals the presence of face-centered cubic structure of AgNPs and confirms its crystalline nature and The crystallite size of the synthesized particle was 22.29 nm which was calculated using peak broadening profile of [111] peak at 37.72^{o}. Nanoparticle analyzer helps in determining the size of the particle and size distribution, The SEM image of AgNPs synthesized from aqueous leaf extract of *Alternantera sessilis* the particles are predominantly spherical in shape and The TEM results revealed well dispersed and mostly spherical AgNPs. Maximum particles were in the size range of 10 - 20 nm and few particles were found above the range of 30 nm .The Fourier Transform Infrared Spectroscopy {FTIR} study explained that Bimolecules in Alternantera sessilis leaf extract have acted as the reducing and stabilizing agents during the synthesis. The in-vitro antimicrobial activity of the AgNPs was investigated against Bacillus subtilis, Escherichi coli, Klebsiella pneumonia and staphylococcus aureus. In the present study, AgNPs showed greater antimicrobial activity in comparison with the standard antibiotic Ampicillin. The activity exhibited by AgNPs was found to be dose-dependent. With increase in concentration of the AgNPs, there was also a simultaneous increase in the zone of inhibition showing an increase in theAntibacterial activity exhibited by the green synthesized AgNPs. The catalytic activity of the AgNPs thus synthesized was investigated by studying the degradation of methylene blue dye by sodium borohydide. The present study revealed significant catalytic activity of AgNPs in presence of sodium borohydride against methylene blue dye, and found that, dye completely degraded with in 25mints.

AgNPs synthesized from aqueous leaf extract of Alternantera sessilis showed effective antimicrobial and catalytic properties. The developed method can be used has substitute for the physical and chemical methods used for synthesis of AgNPs.

INTRODUCTION

The outbreak of research in nanotechnology captured the attention of the scientific community. Metal nanoparticles have found applications in various fields of medicine, science, engineering, technology, biosensing and forensic science.[1-10] The synthesis of metal nanoparticles like silver, gold, palladium, platinum and other metals using physical[11, 12] and chemical methods[13, 14] have already been reported. However, these methods are associated with high risk credited to contamination by chemical precursor, toxicity of organic solvents and formation of toxic by-products.[15] Therefore, development of alternative greener methods of synthesis of metal nanoparticles has become the need of the hour. Microorganisms such as bacteria, fungi, algae, viruses and plants have been widely used for the synthesis of metal nanoparticles.[16] India, being a country with rich biodiversity, green synthesis of metal nanoparticles from plant extracts can be employed effectively. Owing to the easy availability, less maintenance and easy handling, plant extracts have been preferred over microorganisms for synthesis of metal nanoparticles.[17, 18] Formation of silver and gold nanoparticles from plants was first reported by Jose-Yacaman and co-workers.[19, 20] Among the various metal nanoparticles, AgNPs are promising. Due to the wide range of applications conferred to them, they are effectively being synthesized using green chemistry approach. AgNPs show biomedical applications such as antimicrobial [21], anti-inflammatory [22], antiviral [23], antitumor activity [24] and many more. Besides their medical uses, AgNPs are also used in clothing [25, 26], food industry [27], paints, electronics and other fields. They also exhibit catalytic activity in degradation of organic dyes. [28]

Hence, synthesis of AgNPs is being carried out extensively.

Materials and Methods

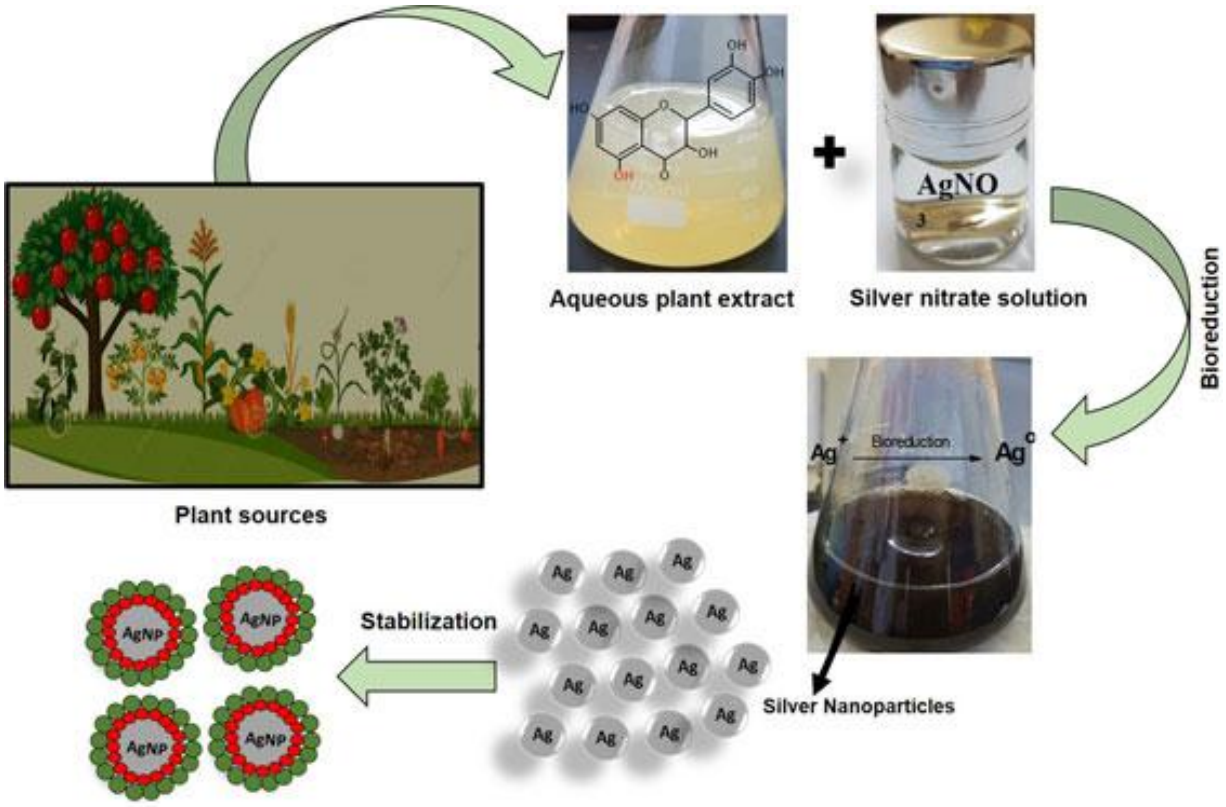
Experimental: Silver nitrate was obtained from Finar Production Company. All the glassware used in the present work was sterilized in hot air oven before use.

Plant material*:* A. sessilis is a profusely branched perennial herb that grows spreading over ground, simple leaves with small and white flowers in axillary clusters and distributed throughout India in moist places, growing wild and often cultivated. Plant material contains 5% of iron i.e., 16.7mg/100g and a good amount of alpha – tocopherols and beta – tocopherols. Fresh leaves of A. sessilis were collected from gadwal area, telangana, India. The plant specimen was identified and certified by Dept. of Botany.

Preparation of leaf extract: The fresh leaves weighing 150gms of A. sessilis were washed several times with distilled water to remove dust, cut into fine pieces and were crushed into 300 ml sterile distilled water and filtered through Whatman No.1 filter paper. The filtration process was repeated and used for synthesis of silver nanoparticles.

Synthesis of nanoparticles: 1mM aqueous solution of silver nitrate was prepared and used for the synthesis of silver nanoparticles. 10 mL of leaf extract was added into 90 mLof 1mM silver nitrate. The primary detection of synthesized silver nanoparticles was carried out in the reaction mixture by observing the color change from greenish to dark brown and optical density (O.D) at different time intervals were taken for 6 hours, using a UV–Visible Spectroscopy. Then the solution is stored in dark at room temperature for 24 hours for the complete settlement of nanoparticles. After 24 hours, the reaction mixture was centrifuged at 10,000 rpm for 10 minutes. The suspension concentrated by repeated centrifugation. The supernatant was replaced by 10 ml of distilled water each time and suspension stored for antibacterial assays and for the optical measurements.Colour change before and after formation of Nanoparticles

Colour change before and after formation of Nanoparticles



Biological synthesis of silver nanoparticles.

Extraction of silver nanoparticles:

UV-Visible Spectra Analysis: The bio reduction of reaction mixture of pure silver ions was observed by measuring the UV- Vis Spectrum at different time intervals taking 1mL of the sample, using 1mL of distilled water as blank. UV – VIS Spectrometer UV – 2450 (Shimadzu, Japan) was used.

XRD Analysis: The suspension which is stored for optical measurements was purified by repeated centrifugation at 10,000 rpm for 10 minutes and freeze dried. The freeze-dried silver nanoparticles were analyzed by using Xpert PRO, X – ray diffractometer (PAN analytical BV) to determine the characterization of the nanoparticles by operation at a voltage of 40kv and the intensity of the diffracted x rays is measured as a function of the diffraction angle 2. The crystalline domain size was calculated from the width of the XRD peaks, using the Scherer formula Where D is the average crystalline domain size perpendicular to the reflecting planes, λ is the x-ray wavelength, β is the Full Width at Half Maximum (FWHM), and is the diffraction angle.

EDS Analysis: Energy Dispersive Spectroscope Analysis determined the presence of elemental silver. In order to carry out EDS Analysis, thin films of the sample were prepared on a carbon coated copper grid by just dropping a very small amount of the sample on the grid and performed on JEOL JSM – 6610LV SEM instrument equipped with a thermo EDS attachment.

TEM Analysis: Transmission Electron Microscopic (TEM) Analysis was performed with JEOL 1200 EX instrument operating at 120kv voltage. Thin film of the sample were prepared on a carbon coated grid by dropping a very small amount of the sample on the grid, extra solution was removed using a blotting paper. Later on, film on the TEM grid was allowed to dry by placing it under a mercury lamp for 5 minutes for the characterization of size and shape of synthesized silver nanoparticles.

PS Analysis: The particle size ranges of the nanoparticles were determined by using Particle Size Analyzer (nano particle SZ – 100, horiba, Japan). Particle sizes were arrived based on measuring the time dependent fluctuation of scattering of laser light by the nanoparticles undergoing Brownian motion.

Antibacterial Assays: The antibacterial assays by agar diffusion method was done against human ophthalmic pathogenic bacteria like Staphylococcus aureus, Pseudomonas aeruginosa lawn cultures on Mueller Hinton (MH) agar plates using turbidity standards and fresh overnight cultures were used for inoculating silver nanoparticles. 10µL of sample was inoculated on MH agar plates. The zone of inhibition around area of silver nanoparticle was measured after 18–24h with Antimicrobial Sensitivity Measuring scale and the absence of growth on the plates around the silver nano particle area confirmed antibacterial activity. Here we report the effect of silver nanoparticles extracted from A. sessilis, when they are attached to ocular antibiotics like Gatifloxacin and Tobramycin in the ratio of 1:0.5, 1:1 and 1:2 against S. aureus and P. aereginosa.

Antimicrobial assay

The antibacterial activity of the synthesized AgNPs was studied and assessed against Bacillus subtilis, *Escherichia coli*, *Klebsiella pneumoniae* and *Staphylococcus aureus* strains by Disc Diffusion assay method. The prepared nutrient media was poured into the sterilized petriplates and the bacterial strains were inoculated on the nutrient media separately and spread with a spreader. Then sterile discs dipped into different concentrations of AgNPs i.e., 5 µl, 10 µl, 15 µl and 20 µl were placed on the inoculated petriplates with a positive control, Ampicillin [10µg/10ml]. After 24 hours of incubation at 37^0C, the formation of zone of inhibition was observed and measured

Fourier Transform Infrared Spectrophotometer

The functional groups and composition of AgNPs was analyzed using FTIR spectroscopy. The Fourier Transform Infrared Spectrophotometer [SHIMADZU IR Prestige21 FTIR Instrument] was used to get the FTIR spectra of the synthesized AgNPs by KBr pellet method. The scan was carried out in the range of 250 - 4000 cm-1.

Scanning Electron Microscopy

The morphology of the AgNPs was characterized by Quanta 400 (FEI, The Netherland) Scanning Electron Microscope [SEM].

Nanoparticle Analyzer

The size of the synthesized AgNPs and zeta potential was recorded at 25^0C using SZ 100 Nanoparticle Analyzer, Horiba Scientific, Germany.**Catalytic activity**

The catalytic activity of the prepared AgNPs was studied on the degradation of methylene blue [MB] dye by sodium borohydride [$NaBH_4$] by mixing 1 ml of $NaBH_4$ [0.025M] with 5ml of MB [0.167 x 10-4M]. 50µl of AgNP colloidal solution was added to this mixture and continuously stirred followed by recording of UV-VIS absorption spectra at regular intervals of time.

Determination of antioxidant activity DPPH (2'2-Diphenyl-1-picrylhydrazyl) assay

Free radical scavenging activity of biosynthesized AgNps determined by DPPH assay. The reaction mixture of dilution series (10-100 mg/ml) taking different volumes of biosynthesized AgNps (10-100µl) incubated with DPPH (3ml; 0.15 mM) solutions in methanol. The solution was allowed to stand for 30 minutes at room temperature. The biosynthesized AgNps when reacted with DPPH, a stable purple-colored free radical will convert into colorless compound α-α diphenyl β-picryl hydrazine. The extent of discolorations indicates the amount of DPPH scavenged. The absorbance will be measured at 517nm. Ascorbic acid will be used as a reference antioxidant or standard[28].

The percent inhibition of DPPH will be calculated using the formula:

The percent inhibition of DPPH **= [(C-T)/C] X100**

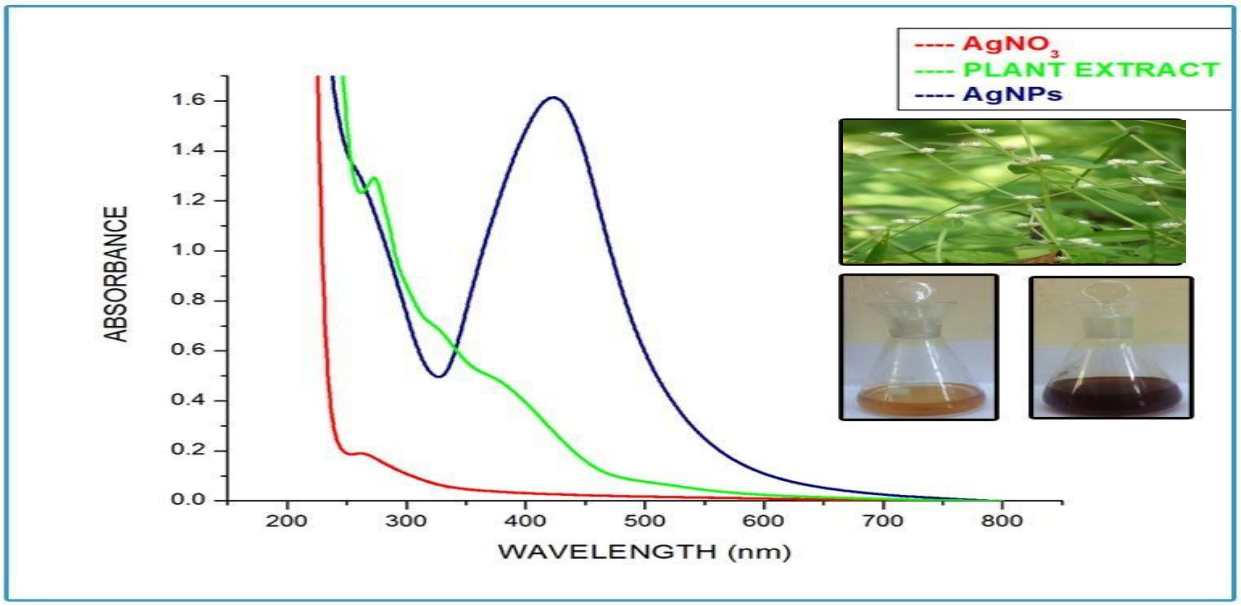
Where C is the absorbance of control and **T** is the absorbance of the test sampl

Results and Discussion

Table 1 Qualitative analysis for phytochemical in leaf extract.

S.No	Test for phytoconstituents	*Alternanthera sessilis (Linn.)* leaf extract
	Alkaloids (Dragendroff's test)	+
2	Flavonoids (Shinoda test)	+
3	Phenols (Lead acetate test)	+
4	Tannins (Ferric chloride test)	+
5	Glycosides (KellereKilliani test	+
6	Terpenoids (Leiberman-Burchards's test)	+

Figure 2. UV-Visible absorption spectrum of *Alternanthera sessilis* (Linn.) Silver nanoparticles at 10, 30 and 60 min. time interval at 60^{0}C temperature



UV-Visible absorption spectrum of *Alternanthera sessilis* (Linn.) Silver nanoparticles at 10, 30 and 60 min. time interval at 60^{0}C temperature

Antibacterial activity

The synthesized Silver nanoparticles exhibited excellent antibacterial activity against the bacterial pathogens *Escherichia coli* and *Staphylococci auerus* (Figure:4a). The present study clearly indicates that the synthesized Silver nanoparticles have good antibacterial action against Gram-negative bacteria than Gram-positive bacteria. Silver ions released by the nanoparticles may attach to negatively charged bacterial cell wall and rupture it, thereby leading to protein denaturation and cell death [32]. The antimicrobial activities of colloidal Silver particles are influenced by the particle dimension, the smaller the particles lead to the greater antimicrobial effects [33]. The zone of inhibition is higher in the case of *Escherichia coli* followed by *Staphylococci* (Figure:4a, 4b) when compared with standard *Streptomycin*. The zone of inhibition was measured with transparent ruler in millimeter and compared with the standard antibiotic streptomycin. The experiments were repeated thrice and mean values of zone of inhibition diameters were presented

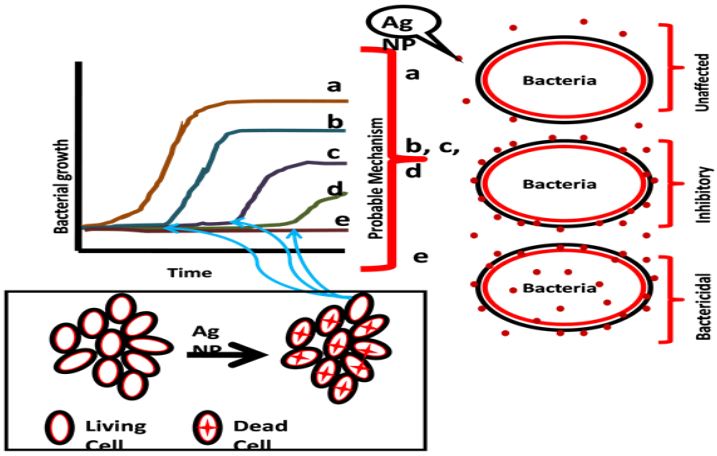
Zone of inhibition of Silver nanoparticles of *Alternanthera sessilis* (Linn.)

Against bacterial pathogens (a) *E. coli*. (b) S. aureus

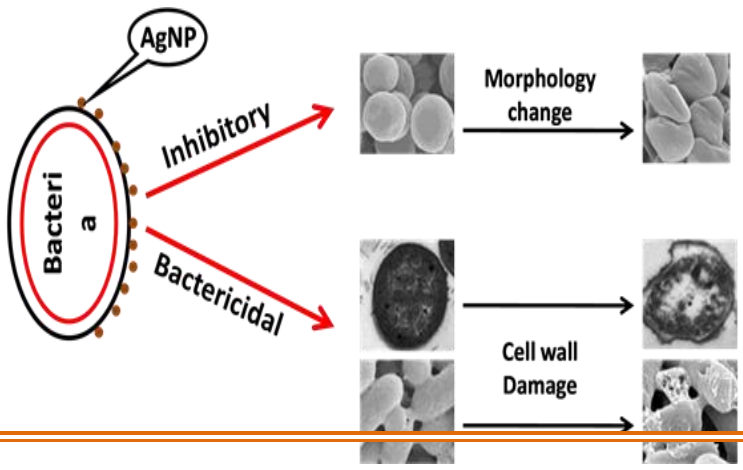
Antifungal activity

The Silver nanoparticles from *Alternanthera sessilis* (Linn.) posses strong antifungal activity against *Aspergillus niger* . The leaves of A. *sessilis* (Linn.) are used in the traditional medicine to cure eye infections [31] and have been reported as a great protectant against insect pests . In present study the Silver nanoparticles from *Alternanthera sessilis* (Linn.) exhibited moderate inhibitory effects. The antimicrobial activity of this plant was already reported .

Zone of inhibition of Silver nanoparticles of *Alternanthera sessilis* (Linn.) against Plant pathogen *Aspergillus Niger*.



Mechanism of antibacterial action of AgNPs



Conclusions

A facile method to synthesize Silver nanoparticles from the aqueous leaf extract of *Alternanthera sessilis* (Linn.) is reported. The present study reveals that the *Alternanthera sessilis* (Linn.) is a good source for synthesis of Silver nanoparticles at a faster rate. The formation of Silver nanoparticles was confirmed by the colour change within 30 minutes. The Silver nanoparticles were characterized by using UV-Vis. UV-Visible Spectra of the reaction mixture at different time intervals 10, 30 and 60 min. at 90^0C temperature was recorded. The UV absorption spectra were recorded at 427 nm and 432 nm respectively which confirmed the formation of polydispersed Silver nanoparticles. The preliminary phytochemical analysis of leaf extract revealed the presence of amino acids, carbohydrates, proteins, flavonoids, sterols, terpenoids and phenolic compounds. The antibacterial activity against *Escherichia coli* and *Staphylococci auerus* confirmed that the Silver nanoparticles are capable of rendering antibacterial efficacy. The leaves of *Alternanthera sessilis* (Linn.) contain camphor as the major chemical constituent which may lead to antifungal activity of Silver nanoparticles. Synthesis of Silver nanoparticles from the aqueous leaf extract of *Alternanthera sessilis* (Linn.) enhancing the importance of plant sources and implementing green chemistry for further research. Moreover their strong antimicrobial and antifungal activities, broad distributions and medicinal functions create them capable sources of natural bioactive compounds that could be used to invent new and more potent antimicrobial drugs of natural beginning. Our results strongly support the use of the studied plant as traditional medicine, and thereby it suggests that some of these plant extracts possess compounds with high-quality antibacterial and antifungal properties thus it can be used as antimicrobial agents in search of new drugs.

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