

BIOSYNTHESIS OF SILVER NANOPARTICLES IN *LAGERSTROEMIA SPECIOSA* (L.) PERS AND THEIR ANTIMICROBIAL ACTIVITIES

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ABSTRACT

Objective: To synthesize silver nanoparticles (AgNPs) using the leaf extract of *Lagerstroemia speciosa* and to determine their antimicrobial activity.

Methods: AgNPs were synthesized by mixing the plant leaf extract with AgNO₃ solution and incubated in the dark condition.

Results: In the present study, the synthesis of AgNPs was achieved by using the aqueous leaf extract of *L. speciosa* (L.) Pers in 0.5 mM AgNO₃ solution. The synthesized AgNPs was characterized by using UV- visible spectrophotometer, Fourier Transform Infrared (FT- IR) spectroscopy and Field Emission Scanning Electron Microscopic (FESEM). The technique is initially to synthesize AgNPs the reaction mixture was incubated separately at different temperatures at 30°C, 40°C, 50°C, 60°C, 70°C and 80°C. Absorbance spectrum was measured in UV-visible spectrophotometer. The samples were incubated at 70°C and 80°C showed peak absorbance at 435 nm and 432 nm respectively. Antimicrobial activity of synthesized AgNPs was carried out using Streptomycin and Ketoconazole as control for bacteria and fungi respectively. The results show that the growth inhibitory zone of 0.6 - 2.0 cm for bacterial strains (*Staphylococcus aureus*, *Proteus vulgaris*, *Pseudomonas aureginosa* and *Klebsiella sp.*) and 0.7 cm - 1.8 cm for fungal strains (*Aspergillus flavus*, *Aspergillus niger*, *Curvularia sp.* and *Cladosporium sp.*). The biosynthesis of AgNPs from *L. speciosa* appears to be Eco-friendly method in nature.

Conclusion: To summarize, in this study we have successfully synthesized AgNPs using medicinally important *L. speciosa* as potential reducing and stabilizing agent. The biosynthesis of silver nanoparticles had several advantages in pharmaceutical applications as well as large scale commercial production.

Keywords: *L. speciosa*, AgNPs, Antimicrobial activity, Bacteria, Fungus, FESEM.

INTRODUCTION

Over the past few years, synthesis of metal nanoparticles has become an important research topic in modern material science due to their distinctive potential applications in the field of electronics, magnetics, optoelectronics, information storage and drug delivery [1, 2, 3, 4, 5]. The AgNPs have potential applications in the Bio medical field. Biological synthesis of AgNPs has several advantages over physical and chemical methods due to its cost effectiveness, compatibility for medical and pharmaceutical applications as well as large scale commercial production [6].

It has been already proved that biosynthesis of crystalline AgNPs with the size ranging from 20-30 nm using *Acalypha indica* leaf extract showed potential antimicrobial activity against water borne bacterial pathogens [7]. Apart from its antimicrobial activity nowadays biogenic AgNPs have expanded towards anticancer applications. A recent study revealed that AgNPs synthesized using *Melia azedarachand* displayed stupendous cytotoxicity against human cervical carcinoma cells (HeLa) and *in vivo* Dalton's Ascites Lymphoma (DAL) mice model [8]. The research in nanotechnology highlights the possibility of green chemistry route to produce technologically important nanomaterials. In recent times, prevalence resistance to anti-microbial agents has emerged as a major health problem [9]. Biosynthesis of metallic nanoparticles is an eco-friendly process and important step in the field of applied nanotechnology [10].

There is a commercial demand for nanoparticles due to their wide applicability in various areas such as electronics, catalysis, chemistry, energy and medicine [11]. In the present generation of nanoparticle synthesis various plant and microbial entities [12] have been screened for AgNPs synthesis towards biomedical applications. Recently, several pharmacologically important plants such as *Morinda citifolia* [13], *Rauvolfia tetraphylla* [14] and *Terminalia catappa* [15] were used as nanofactory system to synthesis rapid, eco-friendly and narrow size range of AgNPs. In the present study *L. speciosa* (L.) Pers, which belongs to the family Lythraceae was used

as a reducing and stabilizing agent for AgNPs synthesis. The plant products such as leaves, stem, root and seeds possess medicinal properties. In particular, the leaf extract of *L. speciosa* was used to synthesize AgNPs from silver nitrate. Synthesized AgNPs were characterized by using UV- Visible Spectrophotometer, Fourier Transform Infrared spectroscopy (FT-IR) and Field Emission Scanning Electron Microscope (FESEM). After characterization the synthesized AgNPs have been screened for their anti-microbial activity against bacterial and fungal pathogens.

MATERIALS AND METHODS

Preparation of *L. speciosa* using leaf extract

Healthy leaves of *L. speciosa* (L.) Pers were collected from Botanical Garden of our Department Tiruchirappalli, Tamilnadu, India. Collected leaves were washed thoroughly 2-3 times in running tap water followed by sterile distilled water. After that, leaves were shade dried at room temperature for two weeks, then powdered using kitchen blender. From that 0.750 g of leaf powder was weighed and mixed in 100 ml of double distilled water and the mixer was boiled in heating mantle at 60°C for 10 to 20 minutes. After that, the mixture was filtered through Whatman No.1 filter paper and stored at 4°C for further study.

Synthesis of silver nanoparticles

For AgNPs synthesis, the reaction mixture was prepared by blending 10 ml of leaf broth with 90 ml of AgNO₃ (0.5 mM) solution in a 250 ml borosil conical flask for the reduction of Ag⁺ ions. The effect of temperature on AgNPs was studied by the reaction in water bath at different temperature (30°C to 80°C). This set up was incubated in dark (to minimize the photo activation of silver nitrate) at 37°C. The synthesis of silver nanoparticles were characterized and confirmed by UV-Visible Spectrophotometer, FT-IR and FESEM analysis [16].

UV-visible spectrophotometer analysis

The reduction of pure Ag⁺ ions from AgNO₃ was monitored by measuring the UV-Visible Spectrum of the different temperature at

30° C -80° C. The bio-reduction of silver ions in aqueous solution was monitored by UV-Visible Spectra of between 220-1100 nm. UV-Visible Spectral analysis was done by using UV-Visible spectrophotometer (Lambda 35).

FT-IR analysis

The bio-reduced silver nitrate solution was centrifuged at 10,000 rpm for 15 mins. The pellet was made to dry by using 5 ml of ethanol to get rid of the free proteins or enzymes that are not capping the AgNPs. The dried pellet was kept at room temperature to obtain particles/crystals of Ag nanoparticles. The dried nanoparticles were analyzed by FT-IR spectrum.

FESEM analysis of AgNPs

The small stainless steel coupons of size 1 x 2 cm were mechanically polished to mirror finish and then subjected to clean and finally by immersion in 10% nitric acid for 5 minutes at 60°C water bath followed by gentle cleaning with trichloroethylene. These coupons were autoclaved at 121°C at 15 psi for 15 minutes. A drop of aqueous solution containing the silver nano-materials (Black color) was placed on the sterile stainless steel coupon and is air dried to apply gold sputtering. These plates were examined at magnification [10 k] by Field Emission scanning electron microscope [Model – ZESIS].

Study of antimicrobial activity

The antibacterial activities of synthesized AgNPs were carried out by disc diffusion method. Nutrient agar plates and Potato dextrose agar plates were prepared, sterilized and solidified. After solidification, various bacterial cultures namely *Staphylococcus aureus*, *Proteus vulgaris*, *Pseudomonas aeruginosa*, *Klebsiella sp.* and fungal cultures *Aspergillus flavus*, *Aspergillus niger*, *Curvularia sp.* and *Cladosporium sp.* were swabbed on plates. The sterile disc were placed on plates by dropping the various concentrations (μ l) of Bacteria (25, 35, 45, 55, 65, 75, 85 and 95) and Fungus (60, 70, 80, 90 and 100 μ l) in Ag nanoparticles solution over the sterile disc. All the plates were incubated at 37°C for 24 hours with bacteria and four days with fungus and inhibition zones were measured.

RESULTS AND DISCUSSION

Synthesis of AgNPs using leaf extract of *L. speciosa*

The leaf extract of *L. speciosa*, using 10 ml of aqueous leaf extract was added into 90 ml of 0.5 mM silver nitrate (AgNO_3) solution. The result exhibited reddish brown colour (Figure 1). The colour change determined the synthesis of silver nanoparticles (AgNPs). It was well known that Ag nanoparticles exhibits reddish brown color in aqueous solution due to the excitation of surface Plasmon Vibrations. The nanoparticles were not have indirect contact even within the aggregates, indicating stabilization of the nanoparticle by capping agent [10]

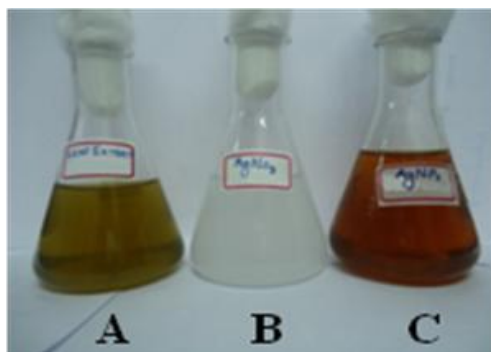


Fig. 1: (A) *L. speciosa* leaf extract (B) AgNO_3 solution (C) synthesized AgNPs

Characterization of AgNPs UV-visible spectrophotometer analysis

The synthesis of AgNPs by the reduction of aqueous metal ions during the exposure of different temperature from 30°C to 80°C with leaves extract of *L. speciosa* easily monitored by using UV-Visible Spectrophotometer. Efficient synthesis was noticed at 70°C and 80°C and the peak absorbance was noticed at 435 nm and 432 nm respectively (Fig. 2 and 3).

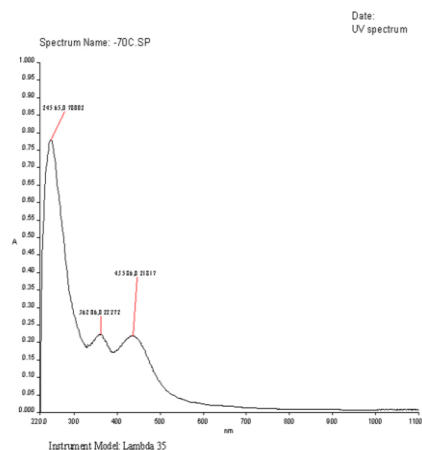


Fig. 2: UV-Visible spectrophotometer AgNPs absorption at 70°C

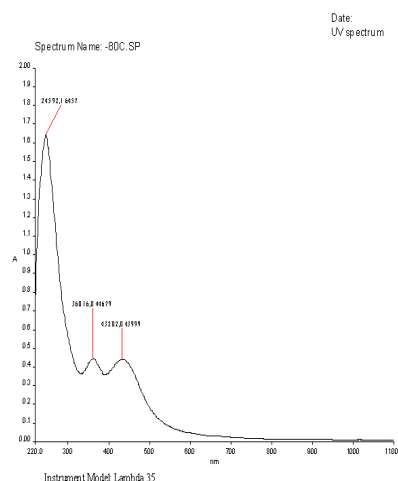


Fig. 3: UV-Visible spectrophotometer AgNPs absorption at 80°C

FT-IR analysis AgNPs

FT-IR measurement was carried out to identify the possible biomolecules for capping and efficient stabilization of the metal nanoparticles synthesized by *L. speciosa* broth. The FT-IR Spectrum of AgNPs is shown (Fig. 4). The peak value at 3440.58 cm^{-1} corresponds to Alcohols, Phenols and primary Amine group (O-H and N-H). The peak value at 2579.45 cm^{-1} corresponds to Carboxylic acids and their derivatives (C=O). The peak value 1618.84 and 1063.35 cm^{-1} corresponds to Primary Amine groups. The peak value at 1333.16 cm^{-1} corresponds to Alcohol and Phenols (O-H). Finally peak value at 623.45 cm^{-1} corresponds to Alkynes group (C-H). Analysis of FTIR studies were confirmed that the carbonyl group from the amino acid residues and proteins has stronger ability to bind metal indicating that the proteins could possibly form the metal nanoparticles [6].

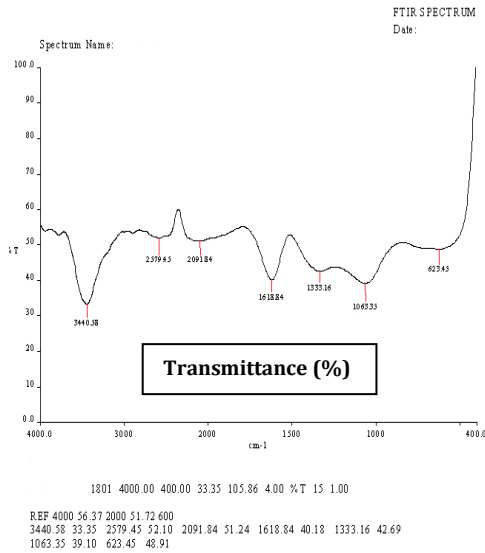
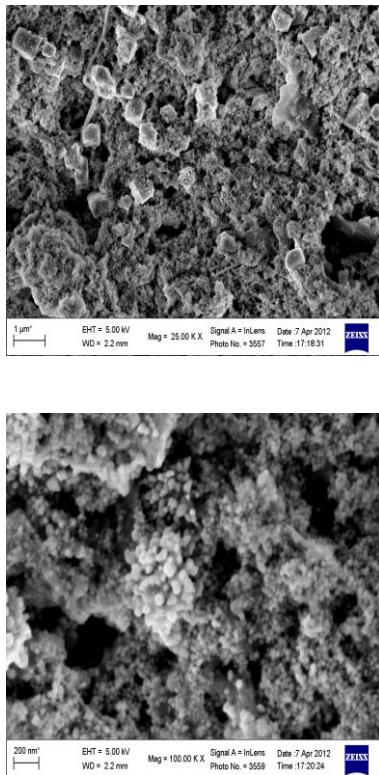


Fig. 4: FT-IR Analysis of AgNPs

FESEM analysis AgNPs

The AgNPs (Fig. 4) were observed spherical shape of the particles under the Field Emission Scanning Electron Microscope. These nanoparticles were analyzed in different magnifications 25X, 35X, 100X and 230X.



The SEM image of silver nanoparricles were synthesized from plant extract were assembled on to the surface due to the interactions such as hydrogen bond and electrostatic interactions between the bio-organic capping molecules bound to the Ag nanoparticles [10]. Similar phenomenon has been reported previously, where the SEM micrograph shows crystalline spherical AgNPs [8].

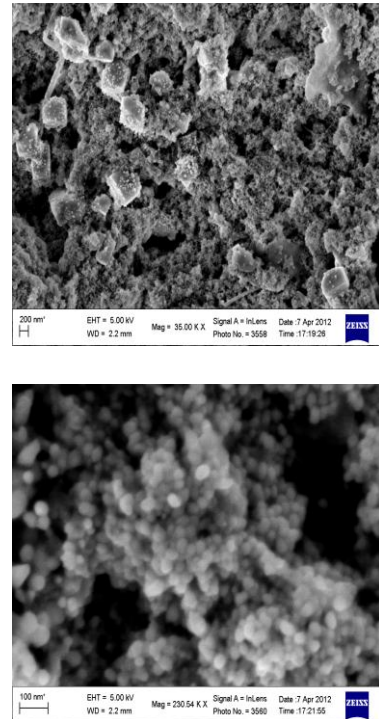


Fig. 5: FESEM analysis of synthesized silver nanoparticles

Study of antimicrobial activity AgNPs

The inhibitory activities of AgNPs against different pathogens have been tabulated (Table 1 and 2) and compared with two standard antibiotics viz. Streptomycin and Ketoconazole. The zone of inhibition (cm) of Ag nanoparticles activity against various microorganisms was ranged from 0.6 – 2 for bacteria (Fig. 6 a-d) and 0.7 - 1.8 for fungi (Fig. 7 a-d).

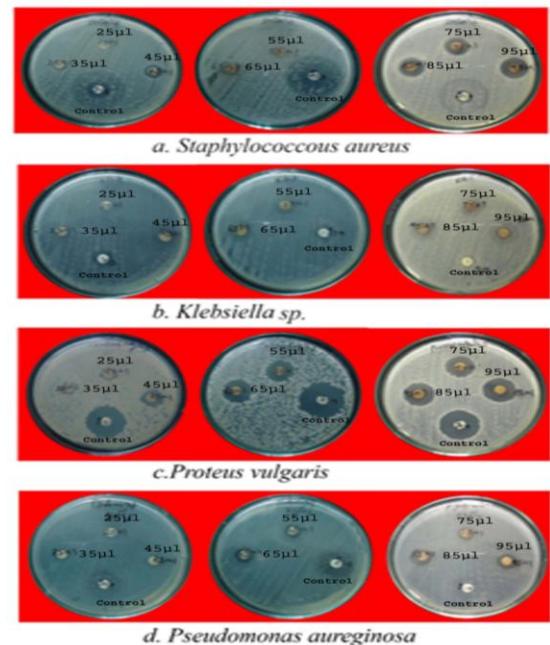


Fig. 6: Antibacterial activity of different concentrations of synthesized AgNPs (25-95 μl) and control (Streptomycin 10mg/ml).

The significant effect of AgNPs along with antibiotics was also checked various multiple drug resistance pathogenic bacteria and

fungus. The mechanism of the bactericidal effect of silver colloid particles against bacteria is not very well-known. AgNPs may attach to the surface of the cell membrane and disturb its power function such as permeability and respiration. It is reasonable to state that the binding of the particles to the bacteria depends on the surface area available for interaction.

Smaller particles having the larger surface area available for interaction will give more bactericidal effect than the larger particles [17]. Bactericide effects of ionic silver, the antimicrobial activity of colloid silver particles are influenced by the dimensions of the particles the smaller the particles, the greater antimicrobial effect. This suggests the possibility that the Ag nanoparticles may also penetrate inside the bacteria and fungi causing damage by interacting with electron phosphorous and sulphur containing compounds such as DNA. [18, 19].

Synthesized silver nanoparticles from plant species are toxic to multi drug resistant microorganisms. It shows that they have great potential in biomedical applications. More over the silver nanoparticles enhance the therapeutic efficacy and strengthen the medical values of Herbal plants [20]



Fig. 7: Antifungal activity of different concentrations of synthesized AgNPs (60-100µl) and control (Ketoconazole 10mg/ml).

Table 1: Antimicrobial activities of synthesized AgNPs from *L. speciosa* against different bacterial Pathogens

Test organisms	(Antibiotic Sterptomycin 10mg/ml)	Different Concentrations of Synthesized AgNPS (µl)							
		25	35	45	55	65	75	85	95
Control	2.3								
<i>Staphylococcus aureus</i>		0.6	0.8	1.0	1.1	1.4	1.4	1.5	1.6
Control	2.5								
<i>Proteus vulgaris</i>		0.9	0.7	1.2	1.7	1.8	1.7	2.0	1.8
Control	-								
<i>Klebsiella sp.</i>		-	-	-	0.7	1.0	1.2	1.5	1.6
Control	2.0								
<i>Pseudomonas aeruginosa</i>		-	-	-	1.0	1.2	1.0	1.4	1.4

Table 2: Antifungal activities of synthesized AgNPs from *L. speciosa* against different fungal Pathogens

Test organisms	Different Concentrations of Synthesized AgNPS (µl) (Antibiotic Ketoconazole 10mg/ml)	Zone of inhibition (cm)				
		60	70	80	90	100
Control	-					
<i>Cladosporium sp.</i>		0.9	0.7	1.0	1.2	1.3
Control	1.4					
<i>Aspergillus flavus</i>		0.7	1.0	1.3	1.5	1.6
Control	1.3					
<i>Aspergillus niger</i>		-	-	-	-	-
Control	0.8					
<i>Curvularia sp.</i>		1.2	1.4	1.6	1.5	1.8

CONCLUSION

To summarize, in this study we have successfully synthesized AgNPs using medicinally important *L. speciosa* as potential reducing and stabilizing agent. The biosynthesis of silver nanoparticles had several advantages in pharmaceutical applications as well as large scale commercial production. In addition the antimicrobial studies suggest that the silver nanoparticles obtained from *L. speciosa* can be used as potential drug candidature to treat various diseases.

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