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Green Synthesis of AgNps using *C.roseus* Leaf Extract to Evaluate Antimicrobial Activity Against Clinical Pathogens

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Abstract
Aims: Simple, rapid production of silver nanoparticles by green synthesis using *C.roseus* leaf extract and evaluating its anti-microbial property against clinical pathogens. **Methods:** Characterisations were performed by UV-Vis spectrophotometry, FTIR spectroscopy, Scanning Electron Microscopy (SEM) and antimicrobial activity was determined against *Staphylococcus aureus* and *Escherichia coli*. **Results:** UV-Vis spectrum analysis ensured reduction of silver ions, forming silver nanoparticles. FTIR analysis showed presence of proteins, ester groups of chlorophyll. SEM images confirmed the almost spherical shape of the nanoparticles and the synthesized nanoparticles were found sensitive against the mentioned clinical pathogens. **Conclusions:** Silver nanoparticles prepared in this eco-friendly green synthesis method showed the significant antimicrobial activity against clinical pathogen.

Keywords
Catheranthus roseus, FTIR, Green synthesis, Pathogen, SEM, Silver nanoparticle.

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1. INTRODUCTION:
In modern day's nanotechnology is thought to be the base of new upcoming technological advancement. Major breakthrough is happening in research and development of nanotechnology. The nanoparticles have at least one of its dimensions between 0-100 nm. Distinctive features like catalytic, magnetic, optical and electrical properties are observed in metal nanoparticles, so they are of great interest [1, 2].

Improved and completely new properties are exhibited by nanoparticles in comparison with bulk materials of larger particles. These properties are caused mainly due to distribution and particle morphology variation. With the decrement in particle size, nanoparticles offer higher surface area to volume ratio. Silver (Ag) has a great importance as nanoparticles (Nps) due to its physical, chemical, biological properties and medical applications. Properties like antimicrobial and catalytic activity of silver nanoparticles (AgNps) can be linked with specific surface areas [3-5]. Increment of biological effectiveness may be seen with increase in specific surface area due to elevation of surface energy. Nobel metal nanoparticles like that of gold, silver and platinum are abundantly used in products involving direct contact with the human body, like, toothpaste, shampoos, detergent, shoes, soaps and cosmetic products, along with pharmaceutical and medical applications.

Silver nanoparticles can be synthesised by a number of approaches like facile method [6], electrochemical [7], sonochemical [8], thermal decomposition of silver compounds [9], microwave assisted process [10] and recently green synthesis [11]. There is an urgent need for development of environment friendly nanoparticles synthesis process as because many of those production procedures involve high energy requirements, toxic chemicals, low material conversions, wasteful and tedious purifications.

Plant extract or micro-organisms employed bio-synthetic methods have been found as easily available and sample alternative to physical and chemical methods [12, 13]. Nanoparticles synthesis using plant materials could have more advantages because elaborate processes like multi step purification, intracellular synthesis or microbial cell culture maintenance are not required.

Here in this study green synthesis of silver nanoparticles, reducing the silver ions of the silver nitrate solution by the leaf Extract of *c.roseus*. Further characterisations of green synthesized nanoparticles were studied, and antimicrobial activity was found against different clinical pathogenic bacteria.

2. MATERIALS AND METHODS:

2.1. Materials:

Silver Nitrate was purchased from Merck (Mumbai, India). The culture media used for bacterial culture was purchased from Hi-Media (Mumbai, India). *C.roseus* leaves were harvested from Jadavpur University Campus, Kolkata, India for silver nanoparticle synthesis.

*2.2. Preparation of leaf extract***:**

Fresh leaves of *C.roseus* were collected from Jadavpur university campus, Kolkata, India and washed with double distilled water. Clean leaves were cut into small pieces and transferred it to 500 ml conical flask. Chopped leaves were boiled with 100 ml distilled water for 10 minutes. After condensation, the liquid was used for nanoparticles synthesis.

*2.3. Green synthesis of AgNps***:** 3 mM silver nitrate solution was prepared. For the green synthesis of AgNps, 80ml of silver nitrate solution (3 mM) was mixed with 20 ml of plant extract. The mixture was heated at 80°C for 15 minutes. A colour change from greenish yellow to dark brown indicates the presence of silver nanoparticles. Finally, pellet of AgNps was collected by centrifugation at 10,000 rpm for 5 minutes and dried.

2.4. UV-Vis spectrum study:

UV visible spectrum study (Carry 60 UV-Vis, Agilent Technologies) was carried out for the reaction mixture against distilled water as a blank. The spectrum analysis was performed at a resolution from 200 nm to 800 nm.

2.5. FTIR study:

The sample was prepared with KBr (Purchased from Merck, Mumbai, India). Thin pellet of sample was made by hydraulic Pellet Press and proceed to FTIR study (PerkinElmer spectrometer, with range of 4500-500 cm^{-1} at a resolution of 4 cm^{-1}).

*2.6. SEM study***:**

FE scanning electron microscope (FE-SEM) study was done using JEOL JSM 6700-F machine. Very little amount of sample dropped on a carbon coated copper grid and the extra solution was removed. Thin film of the sample was dried and subjected to analysis.

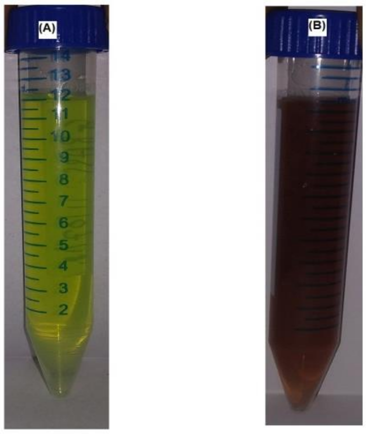
*2.7. Antimicrobial activity assessment***:**

Green synthesis of AgNps was studied for antimicrobial activity against clinical pathogenic bacteria *Staphylococcus aureus* and *Escherichia coli*. Antimicrobial activity assessment was performed using Agar well diffusion array method [14]. Overnight bacterial culture was prepared in Luria Broth. 100 µl of AgNps were filled into each well and placed for overnight incubation at 37°C.

3. RESULT AND DISCUSSION:

3.1. Visual examination:

Leaf extract of *C.roseus* was mixed with Silver Nitrate solution. Then the initial greenish yellow colour changed to dark brown colour within 15 minutes. The visual colour change indicated the green synthesis of silver nanoparticles (Figure1).



(A) C. roseus leaf extract (B) Synthesized AgNPs

Figure1: Visual observation of colour change before (A) and after (B) the reduction process of silver ions to silver nanoparticles using green synthesis method

3.2. UV-Vis spectrum analysis:

Reduction of silver ions was ensured by UV-Vis spectrum analysis and characteristic absorption peak was seen at 434 nm. The spectrum of synthesized silver nanoparticles was given in Figure2.

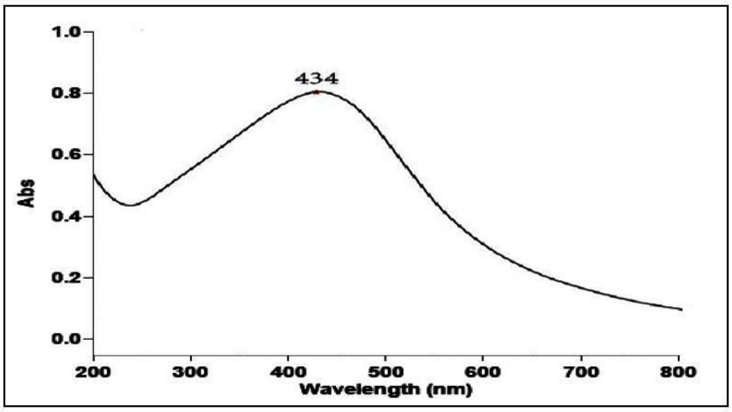


Figure2: Absorption spectrum of UV-Vis spectrophotometric analysis of silver nanoparticles synthesization.

3.3. FTIR Analysis:

FTIR analysis was done to identify the bio-molecules liable for the reduction of silver ions. FTIR spectrum of the *C.roseus* leaf extract was shown in Figure3 and notable peaks were observed at 1381, 1585 and 1724 cm-1. The peak 1585 cm-1 was arrived due to proteins, amide I band (possibly chlorophyll) [15, 16]. Another peak at 1381 cm-1 and 1724cm-1 was arises from ester groups of chlorophyll [17] and stretching vibrations of carboxylate ion of amino acid free carboxylate group.

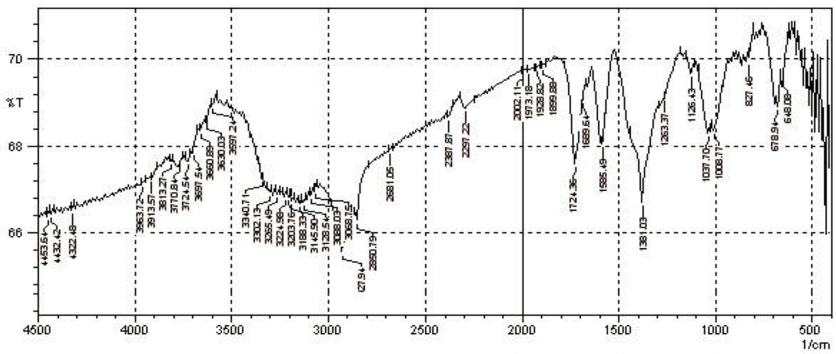


Figure3: FTIR spectra of green synthesized silver nanoparticles using *C.roseus* leaf extract

3.4. SEM Analysis:

The Scanning Electron Microscope image analysis provides information regarding morphological phenomena of the synthesized nanoparticles. SEM images of green synthesized silver nanoparticles were shown in Figure4. The SEM image of silver nanoparticles was aggregated with diameter range 30-70 nm. SEM image confirmed the almost spherical shape of the nanoparticles.

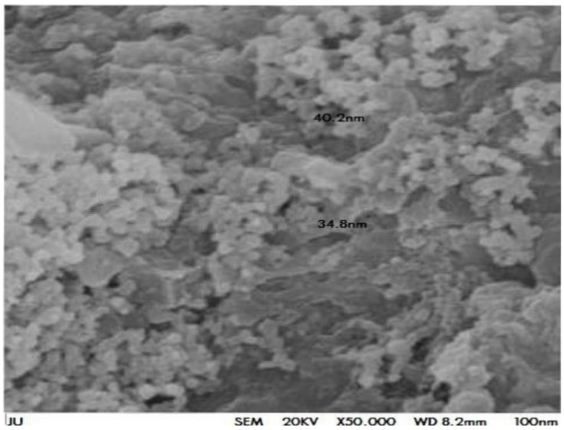


Figure4: Scanning electron microscopic image of synthesized silver nanoparticle in colloidal condition at different nanometer scale

3.5. Antimicrobial activity analysis:

Green synthesis silver nanoparticles were subjected to exhibit antimicrobial properties against clinical pathogenic bacteria, (A) *Escherichia coli* (Gram negative) and (B) *Staphylococcus aureus* (Gram positive). In Figure5 the synthesized nanoparticles showed the antimicrobial activity against the mentioned pathogens and thus were found sensitive to them. However, the zone of inhibition was observed better against *E. coli* than *S.aureus.*

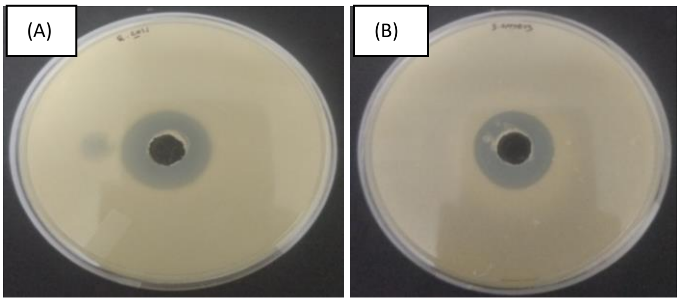


Figure5: The antimicrobial activity of silver nanoparticles against (A) *E. coli* and (B) *S.aureus*

Antimicrobial efficiency of green synthesized silver nanoparticles was reported in this study, but still remains to expurgate the accurate effect of the nanoparticles on essential cellular mechanism like DNA, RNA and protein synthesis.

4. CONCLUSION:

In this study, we have green synthesized AgNps using the *C.roseus* leaf extract. Visual representation of colour change to dark brown confirmed the presence of silver nanoparticles. The reduction of silver ion was confirmed by UV Vis spectrum analysis at 434 nm with maximum absorbance. Presence of functional group and synthesized particle shape, size was determined by FTIR and SEM analysis respectively. Synthesized nanoparticles showed the significant antimicrobial activity against *Escherichia coli* and *Staphylococcus aureus*. This green synthesis method of preparing silver nanoparticles is quite cheap and fast. We believe that, this eco-friendly process for the silver nanoparticles synthesis is an emergent and considerable step in the field of nanoscience and nanotechnology.

CONFLICT OF INTEREST STATEMENT:

We have declared that we don't have any conflict of interest.

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REFERENCE:
[1] Bar, H., Bhui, D.K., Sahoo, G.P., Sarkar, P., De, S.P. and Misra, A., Green synthesis of silver nanoparticles using latex of Jatropha curcas. *Colloids and surfaces: A Physicochemical and engineering aspects*, *339*(1-3), pp.134-139, (2009)
[2] Rassaei, L., Sillanpää, M., French, R.W., Compton, R.G. and Marken, F., Arsenite determination in phosphate media at electroaggregated gold nanoparticle deposits. *Electroanalysis: An International Journal Devoted to Fundamental and Practical Aspects of Electroanalysis*, *20*(12), pp.1286-1292, (2008)
[3] Bae, E., Park, H.J., Lee, J., Kim, Y., Yoon, J., Park, K., Choi, K. and Yi, J., Bacterial cytotoxicity of the silver nanoparticle related to physicochemical metrics and agglomeration properties. *Environmental Toxicology and Chemistry*, *29*(10), pp.2154-2160, (2010)
[4] Gurunathan, S., Kalishwaralal, K., Vaidyanathan, R., Venkataraman, D., Pandian, S.R.K., Muniyandi, J., Hariharan, N. and Eom, S.H., Biosynthesis, purification and characterization of silver nanoparticles using Escherichia coli. *Colloids and Surfaces B: Biointerfaces*, *74*(1), pp.328-335, (2009)
[5] Pal, S., Tak, Y.K. and Song, J.M., Does the antibacterial activity of silver nanoparticles depend on the shape of the nanoparticle? A study of the gram-negative bacterium Escherichia coli. *Applied and environmental microbiology*, *73*(6), pp.1712-1720, (2007)
[6] Nigam, N., Kumar, S., Ghosh, T. and Dutta, P.K., Preparation of Chitosan based silver nanocomposites by a facile method. In *International Conference on Optics and Photonics* (Vol. 139), (2009)
[7] Starowicz, M., Stypuła, B. and Banaś, J., Electrochemical synthesis of silver nanoparticles. *Electrochemistry Communications*, *8*(2), pp.227-230, (2006)
[8] Solano-Ruiz, E., Sato Berrú, R., Ocotlán-Flores, J. and Saniger, J.M., Synthesis of silver nanoparticles by sonochemical induced reduction application in SERS. In *Journal of Nano Research* (Vol. 9, pp. 77-81). Trans Tech Publications, (2010)
[9] Navaladian, S., Viswanathan, B., Viswanath, R.P. and Varadarajan, T.K., Thermal decomposition as route for silver nanoparticles. *Nanoscale research letters*, *2*(1), p.44, (2007)
[10] Sreeram, K.J., Nidhin, M. and Nair, B.U., Microwave assisted template synthesis of silver nanoparticles. *Bulletin of Materials Science*, *31*(7), pp.937-942, (2008)
[11] Begum, N.A., Mondal, S., Basu, S., Laskar, R.A. and Mandal, D., Biogenic synthesis of Au and Ag nanoparticles using aqueous solutions of Black Tea leaf extracts. *Colloids and surfaces B: Biointerfaces*, *71*(1), pp.113-118, (2009)
[12] Bhattacharya, D. and Gupta, R.K., Nanotechnology and potential of microorganisms. *Critical reviews in biotechnology*, *25*(4), pp.199-204, (2005)
[13] Mohanpuria, P., Rana, N.K. and Yadav, S.K., Biosynthesis of nanoparticles: technological concepts and future applications. *Journal of nanoparticle research*, *10*(3), pp.507-517, (2008)
[14] Perez C, Pauli M, Bazerque P. Antibiotic assay by agar well diffusion method. Acta Biol Med Exp.; 15:113–115, (1990)
[15] Cai, S. and Singh, B.R., Identification of β-turn and random coil amide III infrared bands for secondary structure estimation of proteins. *Biophysical Chemistry*, *80*(1), pp.7-20, (1999)
[16] Kóta, Z., Horváth, L.I., Droppa, M., Horváth, G., Farkas, T. and Páli, T., Protein assembly and heat stability in developing thylakoid membranes during greening. *Proceedings of the National Academy of Sciences*, *99*(19), pp.12149-12154, (2002)
[17] Tavitian, B.A., Nabedryk, E., Mäntele, W. and Breton, J., Light-induced Fourier transform infrared (FTIR) spectroscopic investigations of primary reactions in photosystem I and photosystem II. *FEBS letters*, *201*(1), pp.151-157, (1986)