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Green nanoparticles synthesized from roots of *Datura metel* and evaluation of anti microbial activity

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ABSTRACT

Metal Nanoparticles are explored in recent years as an alternative approach to effectively kill drug resistant pathogenic microorganisms. Silver nanoparticles (AgNPs) are the metal of choice for anti microbial agents. In this study, phytochemicals extracted from roots of *Datura metel* using n-hexane as solvent by Soxhlet extract technique. The extract were treated with silver nitrate and kept in the dark conditions for 48 hrs, for the appearance of colour change. The Plasmon peak was observed at 448nm. The physical appearance of these silver nanoparticles was characterized using ultraviolet-visible spectroscopy, FT-IR, SEM, EDAX, TEM and X-ray diffraction techniques. Scanning electron microscopy (SEM) showed the clustered and irregular shapes of AgNPs, with a mean size of 50-85 nm. EDAX results confirmed the presence of silver nanoparticles in the adsorption peak of 2.30 keV. Nanoparticles average size determined by TEM studies and in identified in the range of 50-80nm. The antibacterial activity of the synthesized silver nanoparticles was tested against two micro-organisms using the disk diffusion method. The results reveal that silver nanoparticles synthesized using *Datura metel* root extract have potential antibacterial activity against *Pseudomonas aeruginosa, Escherichia coli.*

KEYWORDS: Roots of *Datura metel*, silver nanoparticles, Characterization, anti-microbial Activity.

1. INTRODUCTION

In the last decade there had been tremendous developments in nanotechnology science, which resulted in the establishment of several new techniques for research. Nanotechnology has achieved the rate of combing all living organisms-plants, animal and microorganisms [1]. The special characteristics of nanomaterials and their biologic effects suggest that nanoparticles may become potential alternative treatments of disease [2]. Silver has long been recognized as having inhibitory effect on microbes present in medical and industrial process. The most important application of silver and silver nanoparticles is in medical industry such as topical ointments to prevent infection against burn and open wounds[3]. The exceptional characteristics of AgNPs have made them applicable in various fields like biomedical applications[4]. Currently, sustainability initiatives that use green chemistry to improve and/or protect our global environment are focal issues in many fields of research. The development of cost efficient and ecologically benign methods of synthesis of nanomaterials still remains a scientific challenge as metal nanoparticles are of use in various applications [5]. Amongst various approaches utilized in the synthesis of nano-particles, green approach i.e. using plant extracts for the synthesis of nanoparticles, the active compounds which help in reduction of metallic ions to corresponding nanoparticles get enveloped around the synthesized nanoparticles via very weak bonds like Wander waal's forces of attraction. Generation of nanoparticles using plant extracts are amongst the most researched topics in nanotechnology. There are various advantages of using plant extracts for nano-particles synthesis such as: **(a)** It eliminates the tedious and time consuming process of maintaining cell cultures [8]. **(b)** The process is simple and eco- friendly [9].

Datura metel (L) (Figure1) also called as Indian Thorn Apple belongs to the family solanaceae which consists of 85 genera and 2500 species

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worldwide. A variety of phyto chemicals have been found to occur in *Datura metel*. These phytoconstituents comprises alkaloids, flavonoids, phenols, tanins, saponins and sterols. The whole plant is antiseptic, narcotic, sedative and is useful for asthma [6]. It is also used in the treatment of burns, calm cough and to treat laryngitis and treachitis [7].The demand of alkaloid content in the past posed its application as a subject for biomedical research is vast nowadays.Although there are some reports on the synthesis of AgNPs from leaves [10,11], flowers [12], to the best of our knowledge till date there is no report available on *Datura metel* root n-hexane extract for the reduction of metal salts to get nanoparticles. As part of our contributions to the growing interest of biomediated synthesis of AgNPs, we report for the first time an inexpensive synthesis of AgNPs by green route at room temperature, stabilized in situ using *Datura metel* root extract and tested for their anti-microbial activity.

Figure 1: *Datura metal* plant materials

2. MATERIALS AND METHOD

2.1. Chemicals:

All chemicals and reagents had analytical grade. Silver nitrate, n-hexane with high purity purchased from SD Fine Chemicals, India.

2.2. Apparatus and Instruments:

The conventional Soxhlet extraction apparatus was used, which consists of a condenser, a Soxhlet chamber, and an extraction flask. The extractor thimble was permeable one with 44 mm internal diameter and 200 mm external length. The rotary evaporator was used for evaporation of solvent of extracted material.

2.3. Sampling and extraction: The fresh sample of *Datura metal* root powder was collected at the end of May in local area agricultural fields. The samples were ground in grinding mill with particle size of less than 2 mm **(Figure 1)**. The raw grinded sample was sealed and stored in desiccators for further usage. 25gm homogenized *Datura metal* root sample was extracted with 100 ml n-hexane for 1hour. The extraction was repeated for 3 times and then the extracts were filtered through Whatman filter paper no 42. Then the filtered extract was stored in refrigerator at 4°C for further use in synthesis of silver nanoparticles.

2.4. Green synthesis of silver nanoparticles: For the synthesis of AgNPs, 10 ml n-hexane extract (1g in 100ml double distilled water) of Datura Metal root powder was added to 90 ml 1 mM solution of silver nitrate in 250 ml conical flask and kept at room temperature for 4 hours. The primary detection of synthesized AgNPs was carried out in the reaction mixture by observing the colour change of the medium. The AgNPs solution thus obtained was purified by repeated centrifugation at 2,000 rpm for 20 minutes on Remi Magnetic shaker. The supernatant was discarded and the pellet was dissolved in double distilled water. The AgNPs were confirmed by colour change. The bioreduction of Ag^+ ions was monitored by periodic sampling by the UV spectrophotometer. The AgNPs in the freeze-drying bottle were suspended in ultrahigh purity water for all characterization methods and antibacterial assays. During biosynthesis of silver nanoparticles when leafextract was added to 100 ml of 1 mM $AgNO_3$salt, theionization took place as follows:

$$AgNO_3(aq) \leftrightarrow Ag^+(aq) + NO_3^-(aq)$$
$$e^- + Ag^+ \rightarrow Ag$$

It is assumed that the silver ions enter inside the plant cell via the H^+ATPase protein embedded in the thylakoid membrane by an electro genic pump [10]. Synthesis of silver nanoparticles is a photochemical reaction.

2.5. Characterization techniques

- UV-Vis spectral analysis was done by using Elico UV-Vis spectrophotometer. The reduction of pure Ag^+ ions was monitored by measuring the UV-Vis spectrum of the reaction medium at 72 hrs, after diluting a small aliquot of the sample into distilled water.
- FTIR analysis was done using Perkin Elemer Spectrum-1, and was used to identify the chemical constituents in the region of 400-4000 cm^{-1} of the Ag-NPs

- **XRD measurement:** XRD measurements of Ag-NPs were cast into glass slides were done by Phillips PW 1830 instrument. The operating voltage of 40 kV and current of 30 mA with Cu kα radiation of 0.1541 nm wavelength, in the 2θ range 10- 80º, step size 0.02/θ.
- The morphology of the Ag-NPs was analyzed using an SEM. The powdered Ag-NPs were uniformly spread and sputter coated with platinum in an ion coater for 120 seconds, then observed by SEM JEOL-JSM 6360 MODEL, JAPAN). The size distribution of the nanoparticle was obtained by counting 150 particles from an enlarged SEM image.32 Elemental analysis of the powdered Ag-NPs was conducted using an EDX detector (EDS, EDAX Inc., Mahwah, NJ, USA) attached to the SEM machine.
- **TEM analysis of Ag-NPs:** Sample for TEM analysis was prepared, as mentioned in IR sample preparations. The sample was first sonicated (Vibronics VS 80) for 5 minutes. Ag-NPs were loaded on carboncoated copper grids, and solvent was allowed to evaporate under Infra light for 30 minutes. TEM measurements were performed on Phillips modle CM 20 instrument, operated at an accelerating voltage at 200 kV.
- The antibacterial activity of the synthesized AgNPs was determined using the disk diffusion method. Two types of pathogenic bacteria *Pseudomonas aeruginosa, Escherichia coli*. were tested. Pure cultures of the micro-organisms were subcultured on Mueller-Hinton agar. Each strain was swabbed uniformly onto individual agar plates using sterile cotton swabs. Sterile paper disks were placed on the agar plates, and 10 µL of 100 µg/mL (w/v) samples were applied to the disks. Gentamicin (30 µg per disk) was used as control agent. All the plates were incubated at 37°C for 18–24 hours. The tests were repeated three times. The zone of inhibition, which appeared as a clear area around the disks, was measured.

3. RESULTS AND DISCUSSION

Reduction of the aqueous Ag^+ ions during exposure to the extract of *Datura metel* was easily followed by UV-Vis spectroscopy. It was observed that silver NPs exhibited reddish-brown colour in water, as shown in Figure 1(inset). The colour appeared due to excitation of surface plasmon vibrations in the metal nanoparticles [10]. UV-visible spectrum of the aqueous medium containing silver ions showed a peak at 448 nm corresponding to plasmon absorbance of silver nanoparticle as shown in Figure 2.

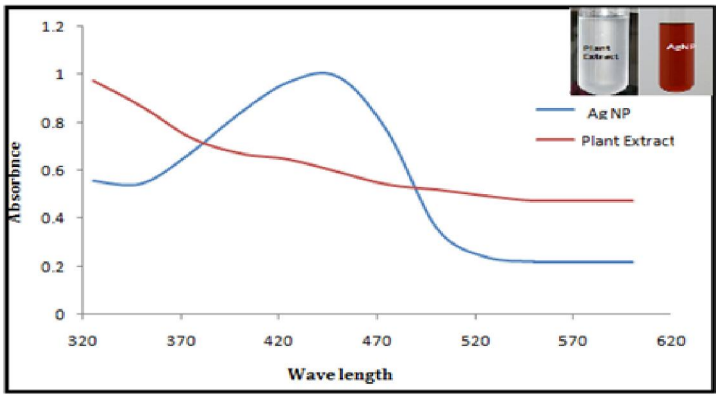

Figure 2: UV-Vis absorption spectrum of silver nanoparticles biosynthesized from Acacia and color change during the reduction of silver particles (Inset)

FT-IR spectra (Figure 3) of *Datura metel roots* AgNPs display a number of absorption peaks, reflecting its complex nature. A peak at 3360 cm^{-1} results due to the stretching of the N–H bond of amino groups and indicative of bonded hydroxyl (-OH) group. The strong absorption peak at 2930cm^{-1} could be assigned to –CH stretching vibrations of $–CH_3$ and $–CH_2$ functional groups. The shoulder peak at 1732 cm^{-1} assigned for C=O group of carboxylic acids. The peak at 1604 cm^{-1} indicates the fingerprint region of CO, C–O and O–H groups, which exists as functional groups of *Datura metel* roots extract. The intense band at 1083 cm^{-1} can be assigned to the C-N stretching vibrations of aliphatic amines. FTIR study of the *Datura metel* rootsAgNp's indicates that the carboxyl (-C=O), hydroxyl (-OH) and amine (N-H) groups of *Datura metel* roots extract are mainly involved in reduction of Ag^+ to AgO nanoparticles supported by the FT IR signal in the figure 3.

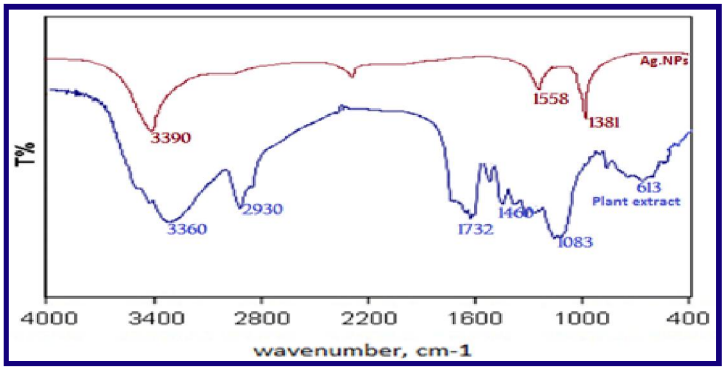

Figure 3: FTIR spectra of plant extract and synthesized silver nanoparticles (Plant extract, &plant AgNPs).

The crystalline nature of the synthesized AgNPs was investigated by XRD and the corresponding XRD diffractogram is shown in Figure 4. The five peaks 2θ value at 33.28, 46.40, 58.24, 68.71 and 78.24 which correspond to lattice planes at (111), (200), (142), (220), (311) have indexed as face centered cubic crystal structure of Ag NPs. The XRD pattern results are corroborated with the data base of JCPDS file no. 87-0720 and biosynthesized Ag NPs respectively [13]. The performed Ag NPs XRD values were calculated (Debye Scherer equation) by the particles size ranges of the silver from 55-80nm respectively.

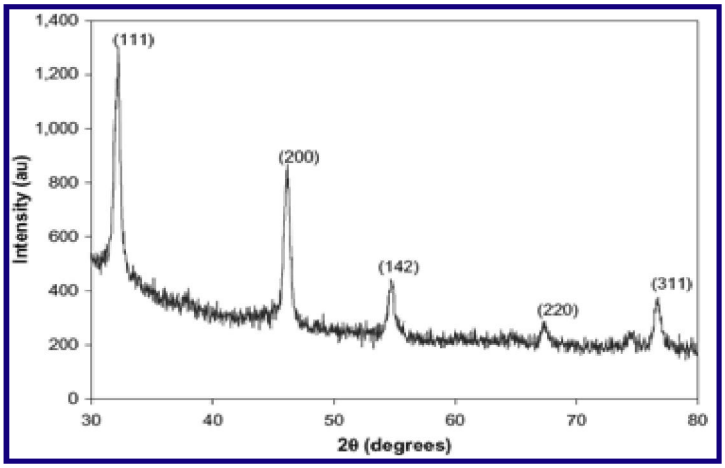

Figure 4: X-ray diffraction (XRD) patterns of synthesized AgNPs.

Scanning electron microscopy (SEM) and energy-dispersive microanalysis (EDX) to gain further insight into the features of the silver nanoparticles, analysis of the sample was performed using SEM and EDX techniques. Scanning electron microscopy provided further insight into the morphology and size details of the silver nanoparticles. Comparison of experimental results showed that the diameter of prepared nanoparticles in the solution was about 50-85 nm.The EDX spectrum recorded in the spot-proûle mode demonstrated strong signals from the silver atoms while weaker signals from C, O, Cl, Si atoms were recorded Figure 5. It is a qualitative X-ray EDX spectrum corresponding to arbitrary nanoparticles. The analysis conûrms that the nanoparticles are pure silver ion.

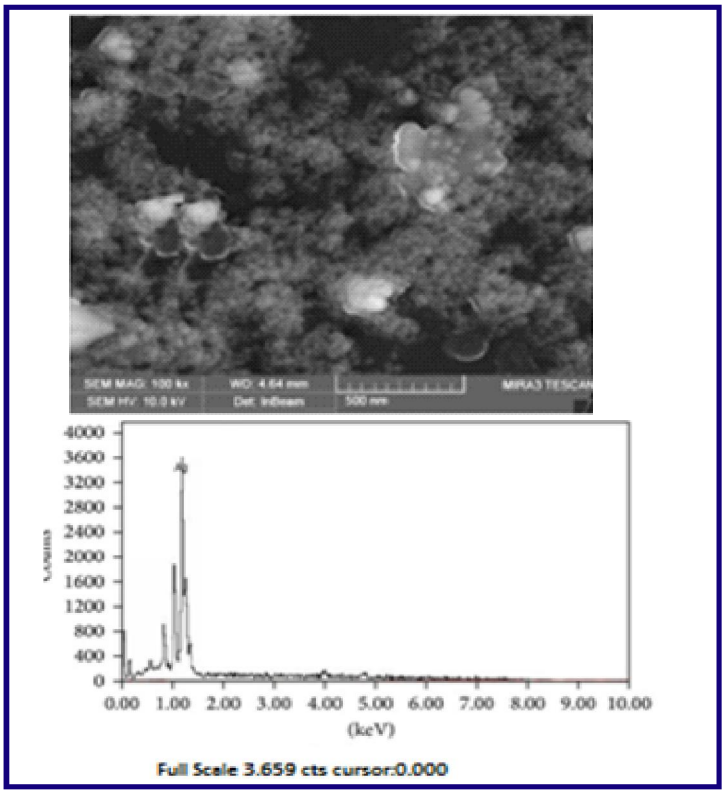

Figure 5: SEM micrograph of Acacia Bark extract-silver nanoparticles and corresponding EDX spectra

TEM images of AgNPs derived from the extract of Datura root extract shown in Figure 6. The morphology of the NPs was predominantly spherical. Some of the NPs were found to be oval. Such variation in shape and size of nanoparticles synthesized by biological systems is common [5]. TEM analysis showed that most particles had a size of about 64nm. Figure 6 shows selected area electron diffraction pattern (SAED) of the silver nanoparticles. The silver particles are crystalline, as can be seen from the selected area diffraction pattern recorded from one of the nanoparticles in the aggregate. SAED spots that corresponded to the different crystallographic planes of face-centered cubic (fcc) structure of elemental silver are seen in Figure 6.

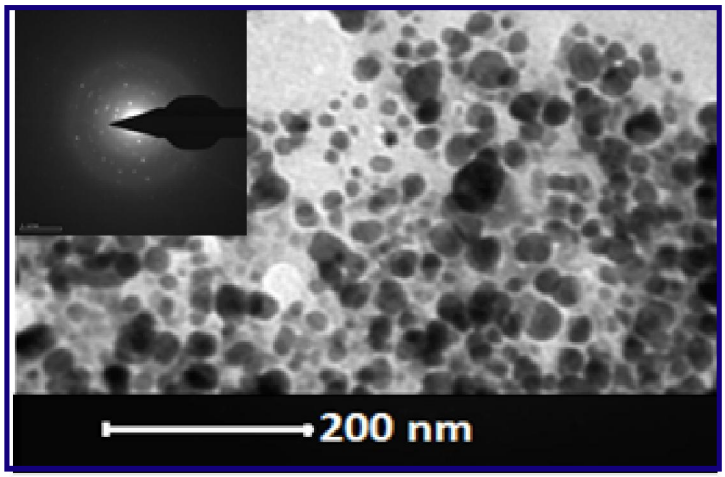

Figure 6: TEM micrographs of the synthesized silver nanoparticles [inset SAED image].

Antimicrobial activity: Disc diffusion method was used to assess antimicrobial activities of synthesized silver nanoparticles against *Pseudomonas aeruginosa & Escherichia coli*. Microbes were sub culture and incubated at 37°C for 24 h. Fresh cultures were taken and spread on Mackonkey agar (HiMedia) plates to cultivate bacteria. Sterile paper discs of 5 mm diameter were saturated with double distilled water (as control), plant extract and silver nanoparticles solution were placed in each agar plate and incubated again at 37°C for 24 hrs (Figure 7). At a concentration of 100 µg/mL, the AgNPs efficiently inhibited the growth of two bacterias. Figure 4 shows the antibacterial activity of AgNPs against *Pseudomonas aeruginosa & Escherichia coli*. Based on inhibition zones around the discs, the antimicrobial activities were measured saturated with plant extract and synthesized silver nanoparticle. The diameters of the inhibition zones for the all tested pathogens are listed in Table 1. Thus, our results show that AgNPs synthesized using an aqueous extract of *Datura metel* bark nanoparticles have potential antibacterial activity against *Pseudomonas aeruginosa*.

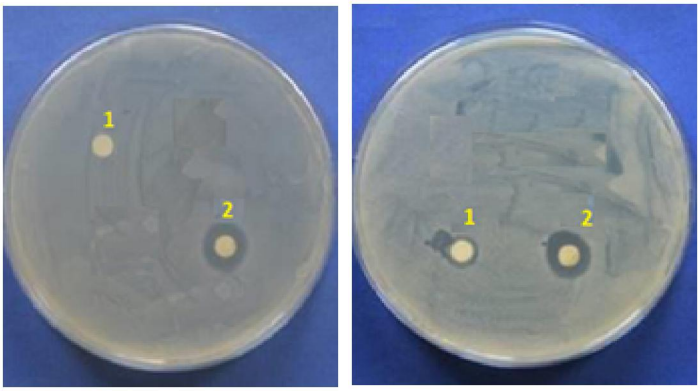

Figure 7: Antibacterial activity of AgNPs analyzed by disc diffusion method

Table 1: Zone of inhibition (mm) obtained by disc diffusion method

Components	Zone of inhibition(mm)	
	Pseudomonas aeruginosa	*Escherichia coli*
Control	NZ	NZ
Plant extract	8	7
Silver nanoparticle	16	12

4. CONCLUSION

The rapid synthesis of stable silver nanoparticles of average size ~80 nm using *Datura metel* root was demonstrated. Achievement of such rapid time scales for synthesis of silver nanoparticles makes it more efficient as a biosynthetic pathway, though there still remains some scope for further decreasing the reduction time periods to make it a viable alternative to chemical synthesis methods.The characteristics of the obtained silver nanoparticles were studied using UV-Vis, XRD, EDX, and SEM techniques. The resultsconfirmed the reduction of silver nitrate to silver nanoparticles with high stability and without any impurity.Comparison of experimental results showed that the average size of synthesized silver nanoparticles was about 80 nm. Applications of such eco-friendly nanoparticles in bactericidal, wound healing and other medical applications, makes this method potentially exciting for the large-scale synthesis of other inorganic materials. Toxicity studies of silver nanoparticles on human pathogen opens a door for a new range of antibacterial agents.

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