



## Exploring antifungal activity of organically synthesized silver nanoparticles from leaf extract of *Datura metel*

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### Abstract

Silver nanoparticles were synthesized by eco friendly route from the leaf extract of *Datura metel* under different temperature conditions. Presence of nanoparticles was characterized by colour changes of reaction mixture and confirmed using UV-Vis Spectroscopy, Transmission Electron Microscopy (TEM) and Scanning Electron Microscopy (SEM). The diameter of synthesized silver nanoparticles was found within the range of 18-30 nm. The antifungal activities were assessed by measuring the zone of inhibition by these organically synthesized silver nanoparticles. The biggest zone of inhibition was observed against *A.niger* (11 mm) followed by *Penicillium* sp. (9 mm) at 4.5 ml concentration. The maximum activity of these particles was found in case of extracts synthesized at 60°C against *A.niger*.

**Keywords:** Antifungal properties, Silver nanoparticles, SEM, TEM.

### Introduction

Nanotechnology is an environmental friendly approach that synthesizes particles in the size range of 1-100 nm. The silver nanoparticles can be synthesized in different shapes and sizes [1].

Nanotechnology has led to economic growth, improves national security and provides small size structure with simplicity and low costs. For biosynthesis of nanoparticles different methods via Chemical and Biological involving oxidation and reduction reactions are used. Chemical method is less preferable due to its high toxicity level as compared to Biological method as the later is easily scaled up, safer and highly cost effective being used for diverse purposes [2, 3]. These metallo nanoparticles are used extensively in the field of Medicine, Biotechnology, Bioengineering and Electronics etc. [4]. These nanoparticles are used in antimicrobial and anti-inflammatory assessment studies [5]. Due to their unique physical and chemical properties, Dynamic light scattering, Scanning Electron Microscopy, Transmission Electron Microscopy and Atomic Force Microscopy techniques are used for determining nanoparticles shape and size [6, 7].

*Datura metel* is a medicinal plant and has insecticidal, herbicidal, antimicrobial, anticancer, anti-inflammatory and anti-rheumatoid activities [8]. It contains high amount of secondary metabolites which include alkaloid compounds and various phytochemicals. *Datura metel* seeds and leaves are used to treat skin rashes, ulcers, bronchitis and diabetes. Leaves of *Datura metel* are used as local applicant for allergy, eczema, painful tumours and glandular inflammation such as mumps. Roots of the plant comprise huge amount of atropine and scopolamine accumulated in aerial parts [9, 10].

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## Material and Methods

### Collection of Leaves

*Datura metel* leaves were collected from the local fields. The collected leaves were washed and cleaned with distilled water to remove dust and dried with absorbent paper. After drying, leaves were chopped into small pieces with sterilized knife and crushed in pestle mortar.

### Extract Preparation

Dispense 30 gm of crushed leaves material in 5 times distilled water and percolated for 2 hrs on hot plate at 100°C temperature. Then leaf extract was filtered using Whatman Filter Paper No.1. The filtrate was collected in a sterilized conical flask and stored at 4°C. The pH of leaf extract was determined using digital pH meter.

### Biosynthesis of Silver Nanoparticles

#### a. Reagents and Chemicals

1M silver nitrate was dissolved in 1000 ml distilled water.

#### b. Synthesis process

During synthesis of silver nanoparticles, both the filtered leaf extract and silver nitrate solution were mixed in clean sterilized flasks. For reduction reaction of silver ions at different concentrations, plant extract and silver nitrate solution were observed at different temperature conditions.

Take two sterilized conical flasks. Add 2.5 ml and 4.5 ml of plant extract respectively to 50 ml of freshly prepared silver nitrate solution in each. These were kept on water bath for 1-2 hours at 100°C temperature. The other extract of same plant was kept at 60°C temperature at two different concentrations (2.5 ml and 4.5 ml) following the same methodology.

### Characterization of Silver Nanoparticles

#### Preliminary Analysis

**a) Change in colour:** Presence of AgNPs was confirmed by colour change i.e. greenish to brown.

**b) UV-Vis Spectroscopy Analysis:** Absorbance was monitored at 540 nm using UV-Visible Spectroscopy. Bioreduction of pure  $Ag^+$  to  $Ag^0$  was monitored by measuring the UV-Vis Spectrum by sampling aqueous extract augmented with silver nitrate and kept at different experimental conditions (60°C, 100°C) at concentration of 2.5ml and 4.5ml, respectively.

#### Confirmatory Analysis

**a) TEM (Transmission Electron Microscopy)** technique was used for elucidation of size of AgNPs. Aliquot of AgNPs solution was placed on carbon coated copper grid and allowed to dry under ambient conditions. TEM image was recorded on Model H-7650 TEM machine at voltage of 80 KV in Punjab Agricultural University (PAU), Ludhiana.



**b) SEM (Scanning Electron Microscopy)** was used for the confirmation of shape of the silver nanoparticles. Aliquot of silver nanoparticle solution was analysed at Sophisticated Analytical Instrument Labs, Thapar University in Patiala. Aliquot solution of AgNPs was placed on Aluminium grid and SEM image was recorded.

#### Antifungal Activity

Potato Dextrose Agar (PDA) Media was prepared, sterilized and solidified in petriplates. After solidification of PDA, two fungal isolates (*Aspergillus niger* and *Penicillium* sp.) were cultured by spread plating technique. Sterilised filter paper discs were dipped in silver nanoparticle solution (2.5 ml and 4.5 ml) along with control and placed on cultured petriplates. These plates were incubated at  $25\pm 2^{\circ}\text{C}$  for 6-7 days. Halo zones (zone of inhibition) indicating antifungal activity was measured.

#### Results and Discussion

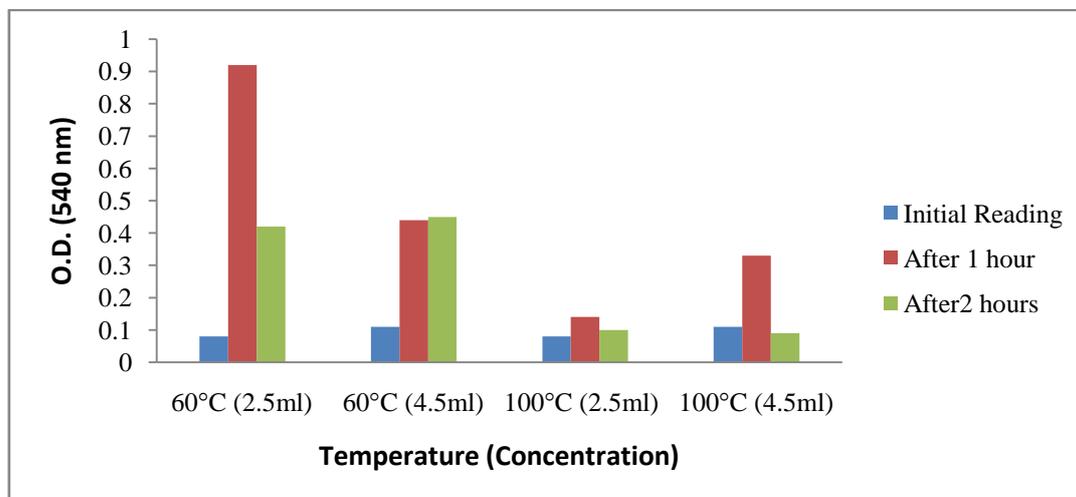
*Datura metel* leaf extract was used for production of AgNPs. Silver ions were reduced into AgNPs when leaf extract was mixed with silver nitrate solution. Reduction process was characterized by immediate changes in colour from light green to brownish at different temperature conditions, which indicated the formation of AgNPs.

**Table 1. Change in colour of solution during silver nanoparticle synthesis**

Colour Change		
Solution	Before Reduction	After Reduction
<i>Datura metel</i> leaf Extract	Light green	Initial- Berry colour After 1 hour- light brown After 2 hour- Dark brown
Silver nitrate solution	Colourless	Nil

#### UV-Visible spectroscopy

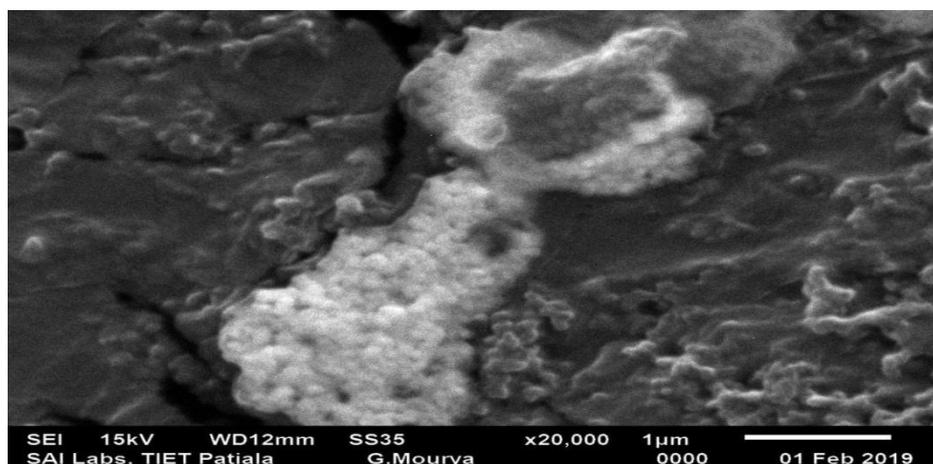
UV-Vis spectral analysis was done by using UV-Vis spectroscopy at 540 nm. (**Figure 1**) The maximum absorbance of *Datura* leaf extract was observed at  $60^{\circ}\text{C}$  temperature after 1 hour but it was decreased after 2 hours indicating the activity of extract between the periods of 1-2 hours.



**Figure 1: Absorbance (540 nm) of Silver nanoparticles treated *Datura metal* leaf extract at different concentrations and temperatures**

### SEM Analysis

SEM micrograph showed that silver nanoparticles are spherical shaped as shown in (Figure 2).

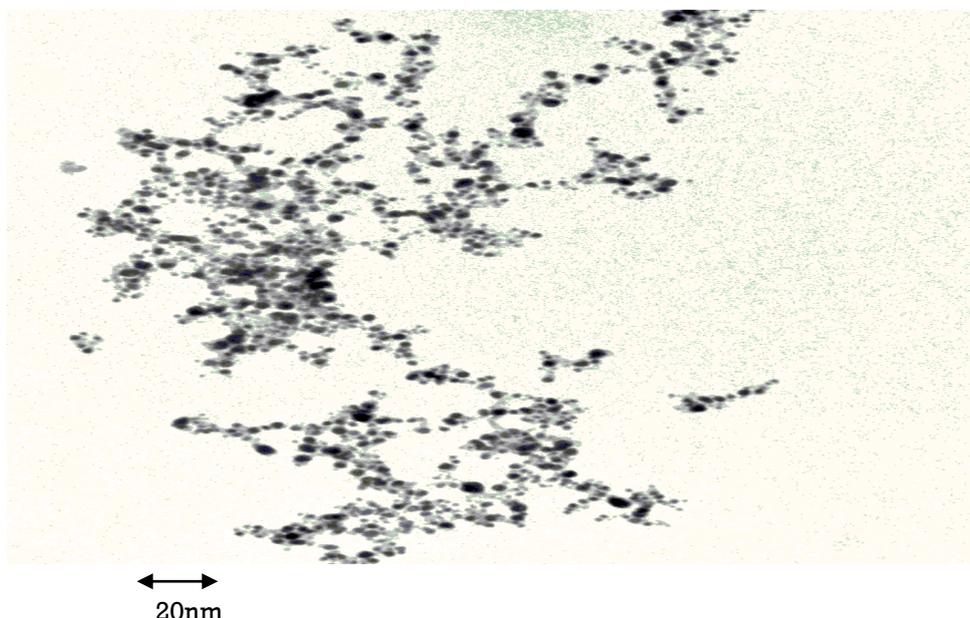


**Figure 2: SEM view of Silver nanoparticles**



### TEM Analysis

The size of synthesized silver nanoparticles was elucidated with the help of Transmission Electron Microscope as shown in Figure 3. The silver nanoparticles were of 20 nm size.



**Figure 3: TEM image of silver nanoparticles**

### Antifungal Assay

Antifungal tests provided evidence that silver nanoparticles formed in leaf extract showed sufficient activity against fungi (*A.niger* and *Penicillium* sp.). The nanoparticles extracted at different temperatures (60°C, 100°C) showed varied range of antifungal activities at 25±2°C for *A. niger* and *Penicillium* sp. At the extraction temperature of 100°C, their antifungal activity was reduced. Moreover when the concentration of extract was more the antifungal activity also increased. The maximum antifungal activity was recorded at 4.5 ml concentration at both the temperatures of extraction. At 60°C temperature greater zone of inhibition (11 mm) as compared to 100°C (8 mm) at 4.5 ml concentration was recorded in case of *A.niger*. On the other hand at 2.5 ml Concentration, halo zones were comparatively of smaller size. The zones of inhibitions were smaller in *Penicillium* sp than *A. niger* though in this fungus too, the maximum antifungal activity was recorded at 4.5 ml (9 mm) than 2.5 ml extract concentration (8 mm) as shown in figure 4 and figure 5. The antifungal activity may be due to production of some inhibitory compounds produced by leaf extracts of Datura [8] (Figure 6).

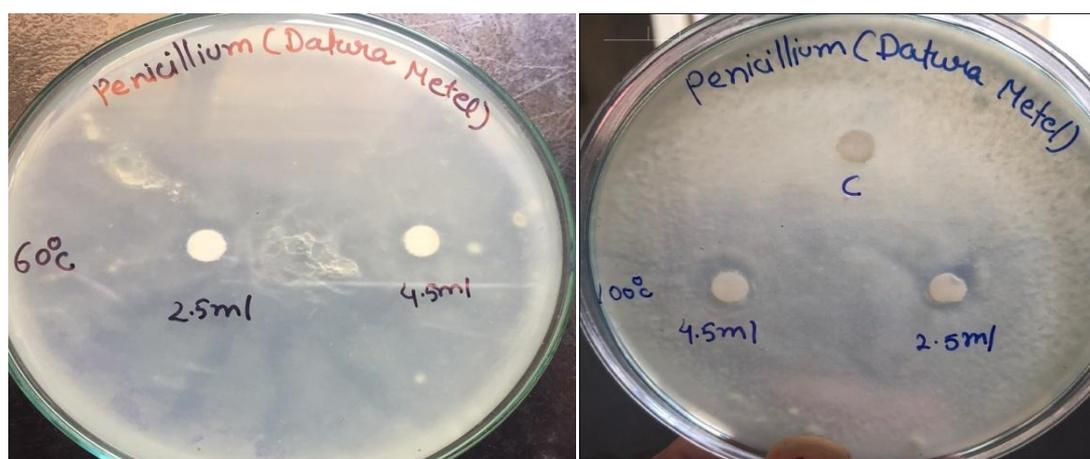


**Table3. Effect of AgNPs produced from *Datura metel* leaf extract against *A.niger* and *Penicillium sp.***

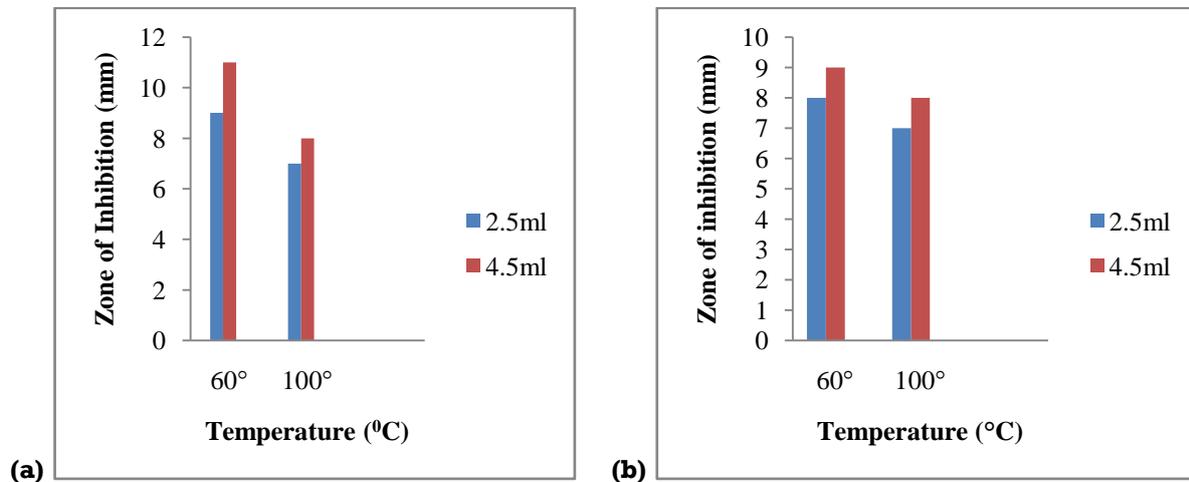
Temperature	60°C		100°C	
Concentration	2.5ml	4.5ml	2.5ml	4.5ml
Fungal Isolates	Zone of inhibition in (mm)			
<i>Aspergillus niger</i>	9	11	7	8
<i>Penicillium sp.</i>	8	9	8	9



**Figure 4: Antifungal activity of Silver nanoparticles against *Aspergillus niger* by disc diffusion method (2.5 ml ,4.5 ml of AgNPs)**



**Figure 5: Antifungal activity of Silver nanoparticles against *Penicillium sp.* by disc diffusion method (2.5 ml, 4.5 ml of AgNPs).**



**Figure 6: Halo zones (mm) against (a) *A.niger* (b) *Penicillium sp.* by *Datura metel* extracts at different temperatures (60°C and 100°C)**

## Conclusion

It has been reported that *Datura metel* leaf extract was successfully used for green synthesis of silver nanoparticles. Reduction of metal ions with reducing agent of Silver nitrate from leaf extracts leads to formation of silver nanoparticles. Silver nanoparticles were characterized by colour change and UV- Vis Spectrophotometer readings. The confirmatory analysis was performed by Transmission Electron Microscope (TEM) and Scanning Electron Microscope (SEM). Further the *Datura* plant mediated synthesized silver nanoparticles were used to test antifungal activities.

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