Available online at www.ijpab.com

DOI: http://dx.doi.org/10.18782/2320-7051.7327

ISSN: 2320 – 7051

Int. J. Pure App. Biosci. **7 (1):** 247-252 (2019)

Research Article

International Journal of Pure & Applied Bioscience

OPEN ACCESS

Antibacterial Effects of Green Synthesized AgNPs from *Datura metel* Leaf Extracts

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Received: 12.01.2019 | Revised: 16.02.2019 | Accepted: 13.02.2019

ABSTRACT

The silver nanoparticles were synthesized by biological means using Datura metel leaf extracts prepared at different temperature ranges (0^0C, 60^0C and 100^0C). Synthesis of silver nanoparticles was confirmed by formation of brown color of reaction mixture and characterized using Calorimeter, Transmission Electron Microscopy (TEM) and Scanning Electron Microscopy (SEM). The diameter of synthesized silver nanoparticles prepared at 60^0C was found within the range of 18-30 nm. Pseudomonas sp. and Escherichia coli were isolated from soil samples using specific media. Datura metel leaves extracts were used to evaluate their antibacterial effects at two different concentrations (2.5 ml and 4.5 ml) on Pseudomonas sp. and Escherichia Coli. The biggest halo formation was observed at 4.5 ml concentration of the extracts prepared at 60^0C.

Key words: *Antibacterial activity, Datura metel, Silver nanoparticles, SEM, TEM.*

INTRODUCTION

The use of silver nanoparticles is on high demand due to their better optical communication, microbial, chemical and electrical properties. Various approaches have been used to synthesize silver nanoparticles in different shapes and sizes[1]. Organically synthesized nanoparticles are much more economical than chemically synthesized ones and it has led to economic growth & improved national security[2]. The characterization of silver nanoparticles is essential because the physical and chemical properties of these particles influence the biological properties[3]. Scanning Electron microscopy and Transmission electron microscopy are used to determine the shape and size of nanoparticle[4]. *Datura metel* is a medicinal plant and has insecticidal, herbicidal, antimicrobial, anticancer, anti-inflammatory and anti-rheumatoid activities[5]. Leaves of *Datura metel* are used as local applicant for allergy, eczema, painful tumors and glandular inflammation such as mumps[6,7]. The silver nanoparticle of *Datura metel* show high Antibacterial activity against Gram negative bacteria[8].

Cite this article: Kaur, J., Gupta, N., Kaur, M. and Chatli, A. S., Antibacterial Effects of Green Synthesized AgNPs from *Datura metel* Leaf Extracts, *Int. J. Pure App. Biosci.* **7(1):** 247-252 (2019). doi: http://dx.doi.org/10.18782/2320-7051.7327

The present study was undertaken with the

OBJECTIVES

Synthesis and characterization of silver nanoparticles and their antibacterial properties.

MATERIAL AND METHODS

Isolation of Microorganisms

Escherichia coli and *Pseudomonas* sp. were isolated from soil samples of Ludhiana by using Modified King's B and MacConkey agar media, respectively. These organisms were preserved by subculturing on Nutrient agar slants.

Preparation of *Datura metel* Leaves extract

Fresh *Datura metel* leaves were obtained from nearby local fields of Ludhiana. The collected leaves were washed and cleaned with distilled water to remove external dirt impurities present in them and dried with paper toweling. After drying, leaves were chopped into small pieces with sterilized knife and crushed.About 30 gm of crushed leaves were dispensed in 5 times distilled water and then the extract was percolated at 100°C temperature for 2 hrs. Then leaf extract was filtered using Whatman Filter Paper No.1 to remove insoluble macromolecules. The resultant 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. The reaction mixture was incubated in the dark at 30°C to avoid the photo activation of silver nitrate under static conditions.

b. Synthesis process

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

Two sterilized conical flasks of 50 ml capacity each were taken. Prepared two different concentrations (2.5 ml and 4.5 ml) of leaves extracts. These extracts were added to 50 ml of freshly prepared silver nitrate solution and kept on water bath for 1- 2 hours at 60^0C and 100°C temperatures, respectively. The reference temperature was taken as 0^o C

Characterization of Silver Nanoparticles

a) Change in color: Presence of silver nanoparticles was confirmed by change in color i.e. greenish to brown.

b) Calorimetric Analysis: Absorbance was monitored 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 at 540 nm. Optical density as reference was also monitored for Silver nanoparticles prepared at 0^0C at 4.5 ml concentration.

c) TEM (Transmission Electron Microscopy) technique was used for determination 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.

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

Antibacterial activity

Antibacterial assay of *Datura metel* leaves extract was observed by Disc diffusion method. Nutrient Agar media was used for growth of Bacteria. Isolated Bacterial strains (*E. coli* and *Pseudomonas*) were cultured by spread plate technique. Sterile Disc were dipped in silver nanoparticle extract and placed on cultured nutrient media plate and incubated at 28±2°C for 48 hours. Zone of inhibition (Halo zone) was measured in mm.

RESULTS AND DISCUSSION

Synthesis of Silver nanoparticles

Datura metel leaf extract is used for production of silver nanoparticles. Silver ions reduced into silver nanoparticles when leaf

extract was mixed with silver nitrate solution. The pH of the extract was found to be acidic which is inhibitory to bacterial growth. Similarly inhibitory effect of reduced pH to bacterial growth has been observed by various scientists[9]. Reduction process was characterized by immediate change in color from light green to brownish at different time and temperatures, which indicated formation of silver nanoparticles. Before the reduction reaction color of extracts was light green and after reduction reaction the color of reaction mixture changed (initial light green to berry) and after 2 hours berry to dark brown color.

Calorimeter

The absorbance of extract was found to increaseat 60^0C at 4.5 ml concentration with increase in time from 0-2 hours. Similar pattern wasobserved at referencetemperature (0^0C) at 4.5 ml concentration (**Figure 1**).

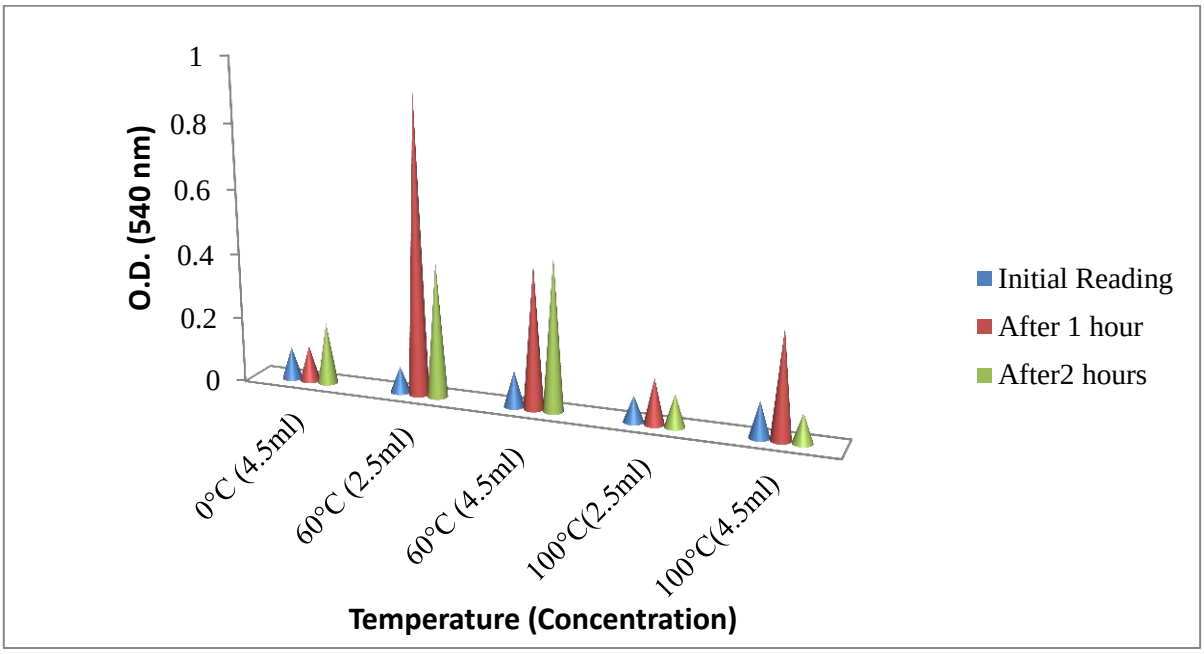


Fig. 1: Silver nanoparticles treated *Datura metel* leaf extract absorbance (540nm) at different concentrations (2.5 ml and 4.5 ml) and temperature conditions

SEM Analysis

The shapes of resultant nanoparticles were elucidated with the help of SEM. SEM micrograph suggested that silver nanoparticles were spherical in shape (**Figure 2**). Our results were in consonance with the results of[10].

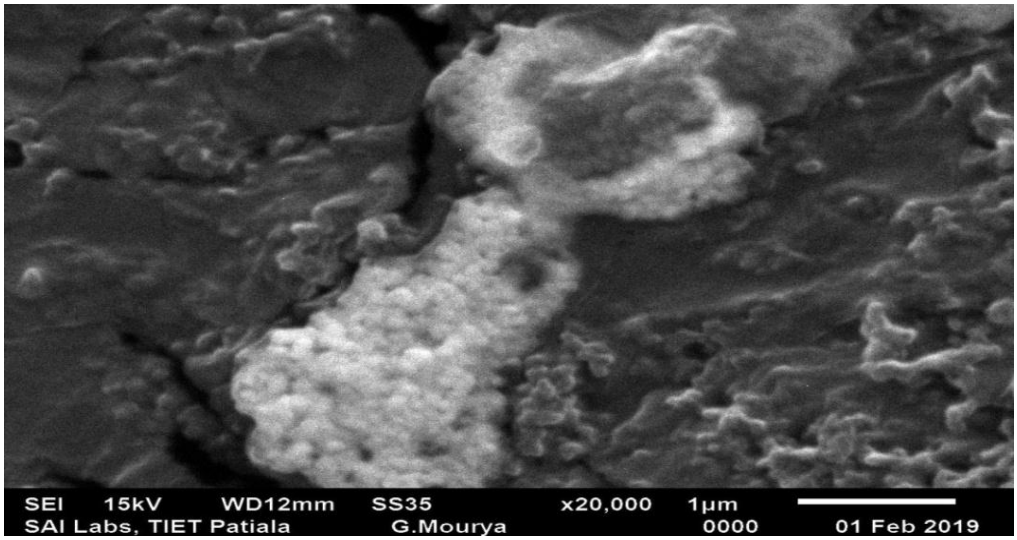
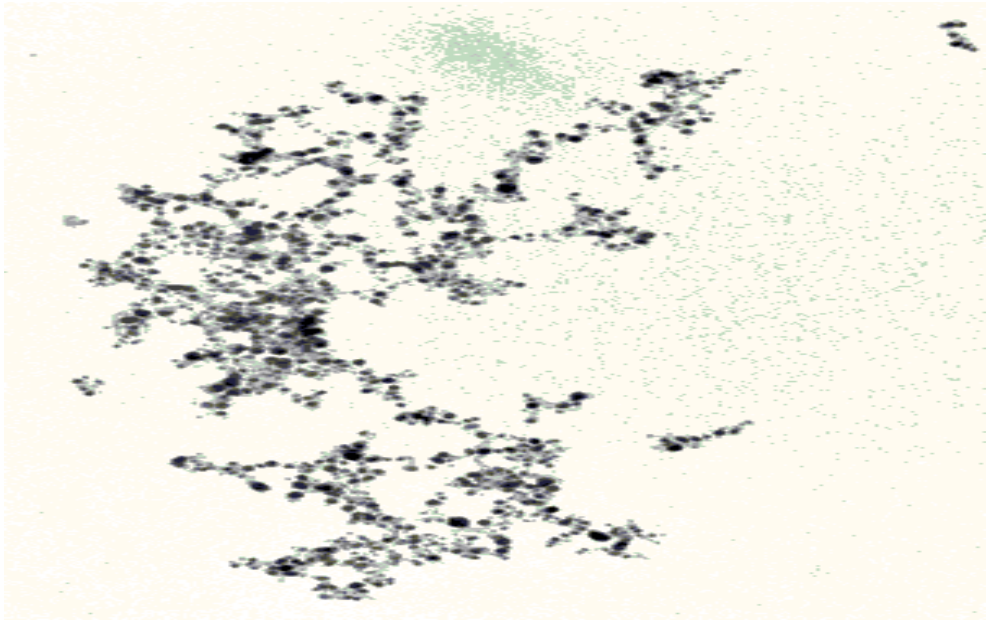


Fig. 2: SEM image of silver nanoparticles from *Datura metel* leaves extract

TEM Analysis

TEM micrograph suggests that the size of particles were 20 nm (**Figure 3**). Similar results were observed by[11].



20nm

Fig. 3: TEM image of silver nanoparticle biosynthesised from *Datura metel* leaf extract

Antibacterial Assay

Antibacterialtests provided evidence that silver nanoparticles formed in leaf extract showed sufficient activity against bacteria (*E.coli* and *Pseudomonas* sp.). Silver nanoparticles interacted with cell membrane and phosphorous containing DNA of bacteria and leading the cell death[12]. The nanoparticles extracted at different temperatures (0°C, 60°C, 100°C) showed varied range of antibacterial activities at 28±2°C for *E.coli* and *Pseudomonas* sp. At 60°C the reaction rate was increased due to increasing temperature asparticles size became smaller as compared to lower temperatures. At extraction temperature of 100°C, the nanoparticles condensed into clusters and increased their size and their antibacterial activity was reduced. Moreover, as the concentration of extracts was increased, the antibacterial activity also increased. The maximum antibacterial activity was recorded at 4.5 ml concentration at 60^0C temperature. At 60°C temperature, zone of inhibition was bigger (14 mm) thanthat at 100°C (11 mm) at 4.5 ml concentration in case of *E.coli*. On the other hand at 2.5 ml Concentration, halo zones were comparatively of smaller size. The zones of inhibitions were smaller in *Pseudomonas sp*. than *E.coli* though in this bacterium too, the maximum antibacterial activity was recorded at 4.5 ml (11 mm) than at 2.5 ml extract concentration (7 mm) as shown in Figure 4 and Figure 5. At 0^0C (reference temperature), the zones of inhibitions were smaller in size than that at 60^0C and 100^0C temperatures. It is justified by various scientists that low temperature is favorable to growth of nanoparticles but as the temperature increases the nanoparticles become smaller in size and their activity increases[13]. Out of these three temperatures, 60^0C is considered as optimal temperature for the formation of Silver nanoparticles thermophile conditions, nucleation of these nanoparticles starts.

Table 1: Effect of silver nanoparticles extracted from *Datura metel* leaves against *E.coli.* and *Pseudomonas* sp.

Temperatures	**0°C**	**60°C**		**100°C**	
Concentrations (ml)	**4.5**	**2.5**	**4.5**	**2.5**	**4.5**
Bacterial Isolates	Zone of inhibition (mm)				
E.coli	7	11	14	9	11
Pseudomonas sp.	5	7	11	7	9

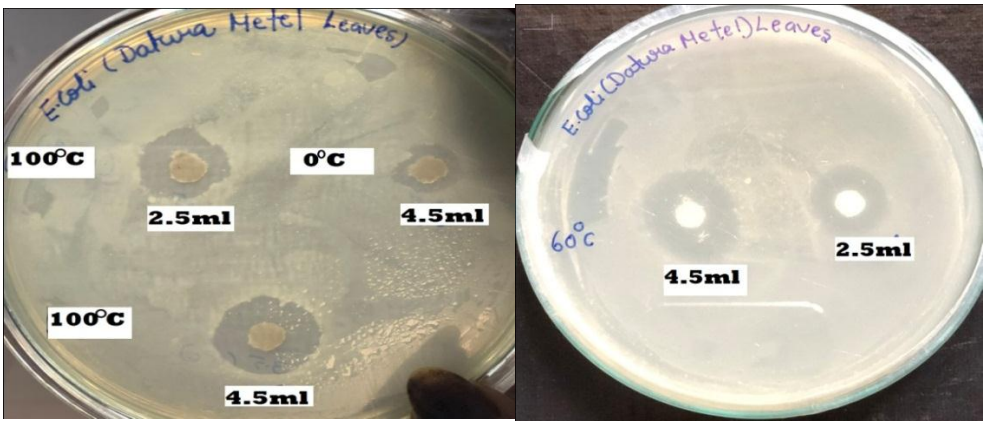


Fig. 4: Halo zones indicating antimicrobial activity at different temperatures and concentrations (against *E.coli*)

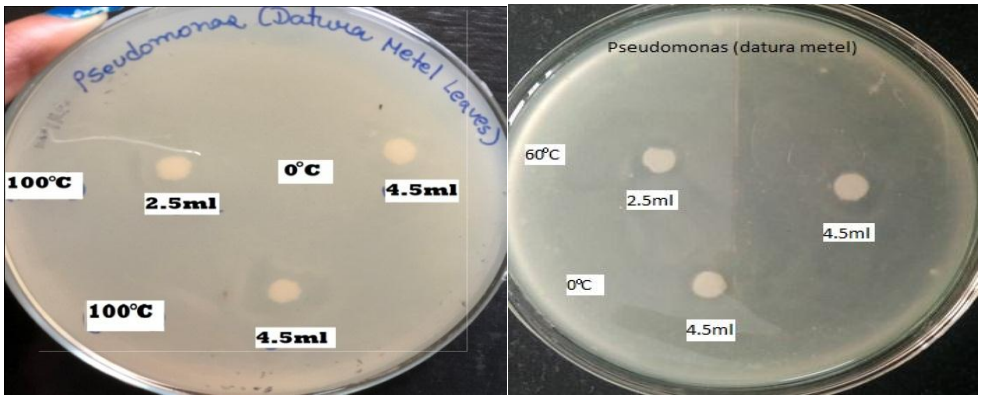


Fig. 5: Halo zones indicating antimicrobial activity at different temperatures and concentrations (against *Pseudomonas* sp.)

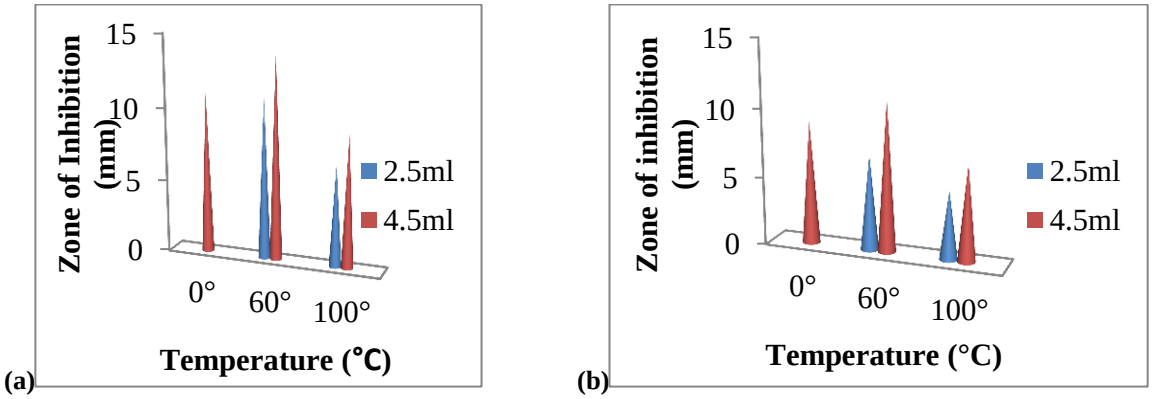


Fig. 6: Zone of inhibition (mm) against (a) *E. coli.* (b) *Pseudomonas* sp. by *Datura metel* extracts at different temperatures and concentrations

CONCLUSIONS

It has been concluded that *Datura metel* leaf extract was successfully used for biosynthesis 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 color changes and Calorimeter 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 antibacterial activities.

REFERENCES

1. Narani, J. S., Saha, K. C., Khan, R. A. and Sunny , H. M. S., Silver Nanoparticles Synthesis Properties, Application and Future Perspectives: A Short Review, IOSR *Journal of Electrical and Electronics Engineering*. **10:** 117-126 (2015).
2. Hassan, S., A Review on Nanoparticles: their Synthesis and type, *Research Journal of Recent sciences*. **4:** 1-3 (2015).
3. Zhang, F. X., Liu, G. Z. and Gurunathan, S., Silver nanoparticle: Synthesis, characterization, properties, Application and Therapeutical Approaches, *International Journal of Molecular Science*. **17(9):** 1534 (2016).
4. Iravani, S., Korbekandi, H., Mirmohammadi, V. S. and Zolfaghari, B., Synthesis of silver nanoparticle: Chemical, physical and biological methods, *Res. Pharm. Sci*. **9(6):** 385- 406 (2014).
5. Dhanalakshmi, M., Thenmozhi, S., Devi Manjula, K. and Kameshwaran, S., Silver Nanoparticles and its Antibacterial Activity, *International Journal of Pharmaceutical & Biological Archieve*. **4(5):** 819-826 (2013).
6. Monira, M. K. and Shaik, M. M., Review on *Datura metel*: A potential medicinal plant, *Global Journal of Research on Medicinal Plant & Indigenous*. **1:** 123-132 (2012).
7. Ojha, K., Akshaya, Jogeswari, R., Behera, Shikha and Nayak, P. L., Green Synthesis and Characterization of Zero valent Silver Nanoparticles From the leaf extract of *Datura Metel*, *International Journal of Pharmaceutical Research & Allied Sciences*. **2(1):** 31-35 (2013).
8. Guzman, M., Dille, J. and Gobet, S., Synthesis and antibacterial activity of silver nanoparticle against Gram positive and Gram negative bacteria, *Nanomedicine: Nanotechnology, Biology and Medicine*. **8(1):** 37- 45 (2011).
9. Chatli, S. A., Sood, S. and Beri, V., Effect of pH on inorganic phosphate solubilisation by microorganisms isolated from Trans Himalayan Region of Himachal Pradesh, *International Journal of Multidisciplinary Research & Development*. **2(9):** 31-35 (2015).
10. Gupta, N., Komal, R. and Chatli, S. A., Development and Evaluation of Hemolytic Efficacy of Guava (*Psidium guajava*) based green Nanoparticles, *International Journal of Research in Engineering, science and Management*. **1(12):** 2581-5792 (2018).
11. Gupta, N., Komal, R., Chatli, S. A., Expression of organically synthesized metallonanoparticles. LAP LAMBERT Academic Publishing, Mauritius, Pg.no. 1-80 (2018).
12. Logeswari, P., Silambarasan, S. and Abraham, J., Synthesis of silver nanoparticles using plants extract and analysis of their antimicrobial property, *Journal of Saudi Chemical society*. **19(3):** 311-317 (2012).
13. Liu, H., Zhang, H., Wang, J. and Wei, J., Effect of temperature on the size of biosynthesized silver nanoparticle: Deep insight into microscopic kinetic analysis, *Arabian Journal of Chemistry*. 1878- 5352 (2017).