International Journal of Advanced Research in Biological Sciences
ISSN: 2348-8069
www.ijarbs.com
DOI: 10.22192/ijarbs Coden: IJARQG(USA) Volume 6, Issue 3 - 2019

Research Article

DOI: http://dx.doi.org/10.22192/ijarbs.2019.06.03.013

Biosynthesis and characterization of AgNPs and their antibacterial effects

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Abstract

The Silver nanoparticles (AgNPs) were biosynthesized from the leaf extracts of *Syzygium cumini* by ecofriendly means. Synthesis of $AgNP_S$ was preliminary characterized by color change and calorimetric readings. Their spherical shape was further confirmed by Scanning Electron Microscopy (SEM). The nanoparticles synthesized from the leaf extract prepared at 60^0C were 50 nm in diameter as observed by Transmission Electron Microscopy (TEM) studies. *Escherichia coli* and *Pseudomonas* sp. were isolated from soil samples using the MacConkey Agar Media and Modified King's B Agar Media, respectively. The antifungal activities of these Silver nanoparticles were studied by determining halo zones against *E. coli* and *Pseudomonas* sp**.** isolates.

Keywords: Biosynthesis, Calorimeter, Silver Nanoparticles, SEM, TEM.

Introduction

Nanotechnology constitutes strategies for synthesis of new nanomaterials. Nanoparticles represent completely new and improved properties based on specific characteristics such as size distribution and morphology (Logeswari and Abraham, 2015). Nanoparticles are synthesized by two different ways: Chemical and Biological. Chemical synthesis of nanoparticles leads to synthesis of environmentally toxic byproducts but production of silver nanoparticles from green extracts including bark, leaf, root etc is non toxic and has effective stability (Prasad et al., 2012). Thus, the best and the most ecofriendly way to synthesize nanoparticles is a biological method. Biological way is simple, fast and economical. Moreover, this green technology does not involve any toxic chemicals (Zhang et al., 2016). The nanoparticles are characterized by using SEM, TEM and UV-Vis spectroscopy. In this present study, Silver nanoparticles were synthesized using the leaf extract of *Syzygium cumini*, which is a traditional medicinal tropical plant. *Syzygium cumini* commonly referred to as Jamun or Java Plum, is an evergreen tree belonging to family of Myrtaceae (Banerjee and Narendhiranrakanan, 2011). Silver nanoparticles can be inhibitory to various microorganisms (Salomoni et al., 2017).

Materials and Methods

A. Collection of leaves

Syzygium cumini leaves were collected from local fields of District Ludhiana, Punjab, India. These leaves were washed multiple times with distilled water. These were kept at room temperature overnight for air drying. After drying, leaf samples were

chopped into fine and small pieces. The chopped leaves (30 gm) were added in 150 ml of distilled water and were placed on hot plate for percolation at 100°C. After the desired period of 1 hour 30 mins approximately, samples were filtered through Whatman filter paper No. 1 to get the leaf extract and stored at 4°C.

B. Preparation of $AgNO_3$ solution

For the preparation of 1000 ml Silver nitrate solution, 1 ml (0.169gm) $AgNO_3$ was added to known amount of double distilled water and the volume was made to 1litre. The solution was mixed thoroughly and was stored in dark bottle in order to prevent auto oxidation of silver. 2 ml and 4 ml each of leaf extract were mixed with 50 ml of Silver nitrate solution. These two solutions were kept on hot water bath at 60°C and 100°C, respectively. The standard solution was prepared by mixing 4 ml of leaf extract with 50 ml of Silver nitrate solution and was kept at 0^0C.

C. Characterization of Silver Nanoparticles

a. For preliminary analysis

1. Change in color

Color change of extract by the addition of $AgNO_3$ was recorded after regular intervals.

2. Calorimeter

The Silver Nanoparticles (2 ml and 4 ml extract concentration at 60^0C and 100^0C) were quantified at 670 nm by the use of Calorimeter. Optical density of reference (Standard) extract was also monitored (4 ml concentration at 0^0C) at same wavelength.

b. Confirmatory Analysis

Following techniques were used to examine silver nanoparticles:

1. Scanning Electron Microscopy (SEM)

The sample was placed on aluminium grid for drying. Scanning Electron Microscopy was used to identify the shape of nanoparticles at SAI (Sophisticated Analytical Instrumental) Labs, Thapar University, Patiala.

2. Transmission Electron Microscopy (TEM)

It is used to determine the size of nanoparticles. The liquid sample was placed on carbon coated copper grid in favourable conditions which made it dry. Sample was viewed under model H -7650 at Department of Nanosciences and Electron Microscopy, Punjab Agriculture University, Ludhiana.

D. Antibacterial Efficacy

Antibacterial activity of nanoparticles was determined by Disc Diffusion method (Bauer et al., 1959) in which Nutrient Agar Media (NAM) was used for bacterial growth. The bacteria were cultured by spread plating. Sterile filter paper Disc (4 mm) was dipped in Silver nanoparticle extracts, placed on grown bacteria and incubated at 28±2°C for 48 hours. Halo zones (Zone of inhibition) were measured in mm.

Results and Discussion

Synthesis of Silver nanoparticles

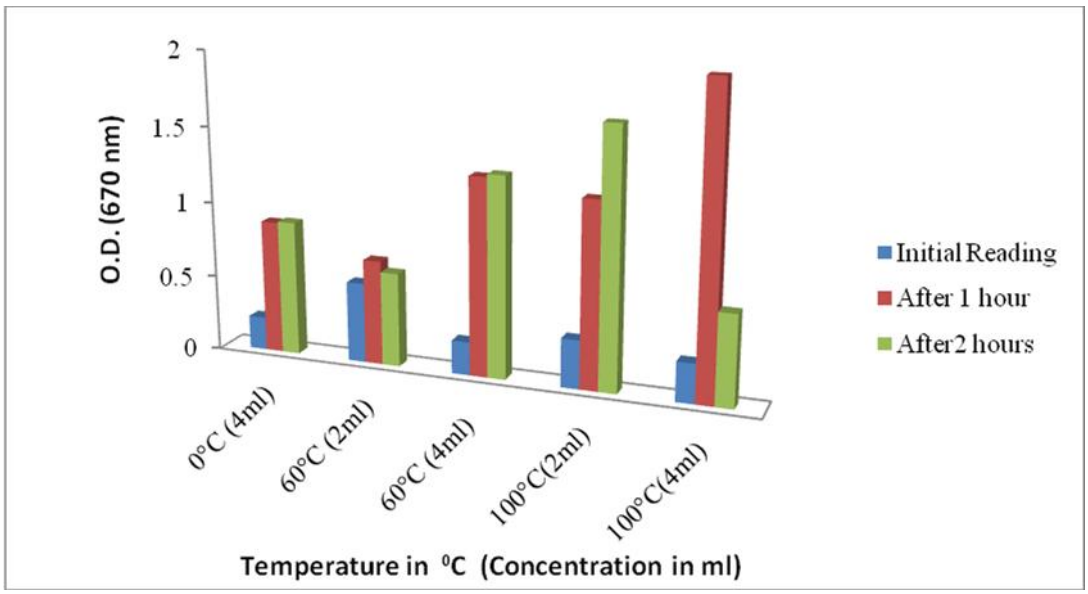
Jamun leaf extracts were utilized for the synthesis of Silver nanoparticles. Thus, colour changes represented the formation of nanoparticles. The $AgNP_S$ were formed due to the reduction of the silver ions upon interaction with the metabolites present in the leaf extracts which was evident by the ultimate light brown colour of the aqueous solution (Table 1).

Table 1: Colour Changes in the Extract after the formation of Silver Nanoparticles

Time Interval	Color Change
Initial	Light Rosewood
After one hour	Golden
After two hours	Brown

Calorimeter

The absorbance of extract was determined at 670 nm with time period of 2 hours at 60^0 C and 100^0C at 2 ml and 4 ml concentrations, respectively. The extract prepared at 0^0 C (4ml concentration) was taken as reference and its Optical Density (O.D.) was also taken (Graph 1). The increase in O.D. with time indicated increase in concentration of AgNPs. Our results were similar to the results of (Gupta et al., 2018).



Graph 1: Absorbance of leaf extracts prepared at 0^0C, 60^0 C and 100^0C (After 1hr and 2hrs)

SEM Analysis

The shapes of Silver nanoparticles were found to be spherical as observed by SEM micrographs (Fig 1). Our results were in consonance with the results of Gupta et al (2018).

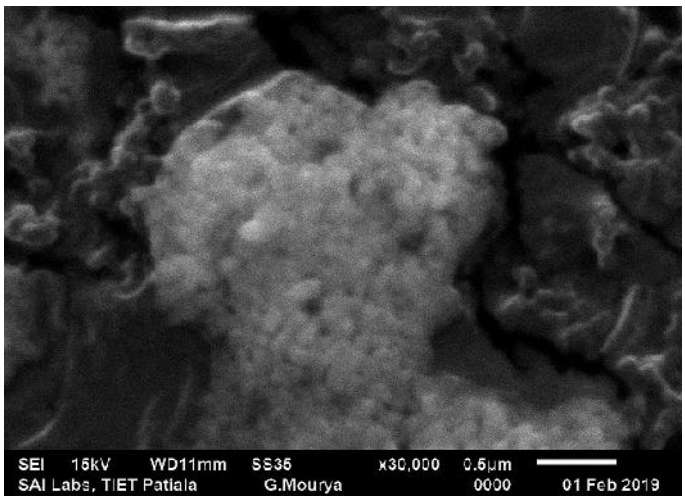


Figure 1: SEM Analysis of AgNPs from *Syzygium cumini* leaf extracts

TEM Analysis

The size of nanoparticles was 50 nm as observed by TEM analysis (Fig 2).

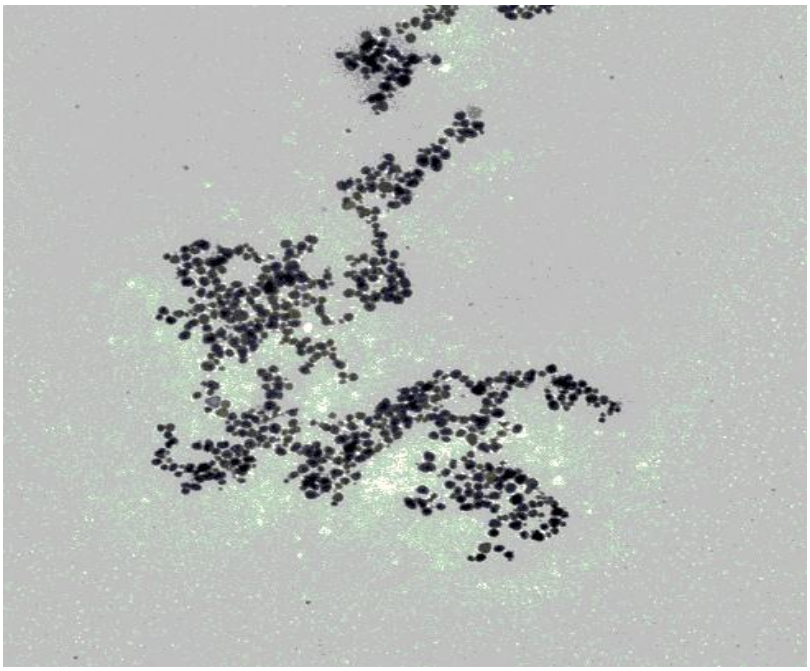


Figure 2: TEM Analysis of AgNPs synthesized from *Syzygium cumini* leaf extracts.

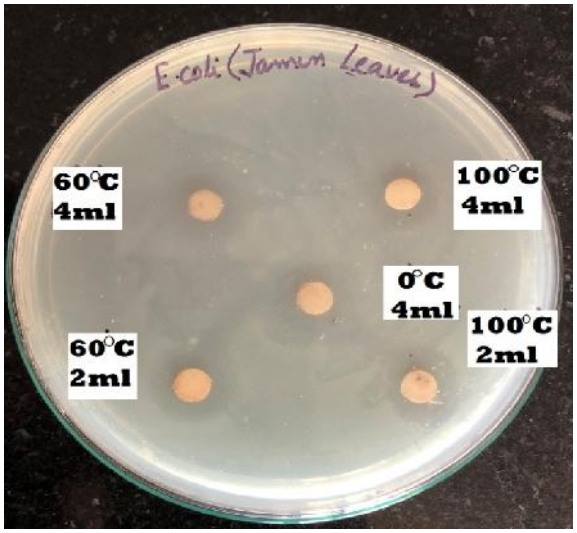
Antibacterial Efficacy of Silver Nanoparticles

The pH of the extract was found to decrease when Silver nitrate solution was mixed with leaf extract. The acidic conditions inhibit the growth of bacteria (Chatli et al., 2015). The isolated strains *Escherichia coli* and *Pseudomonas* sp. represented different sensitivity profiles to $AgNP_S$ (Graph 2, Fig 3).

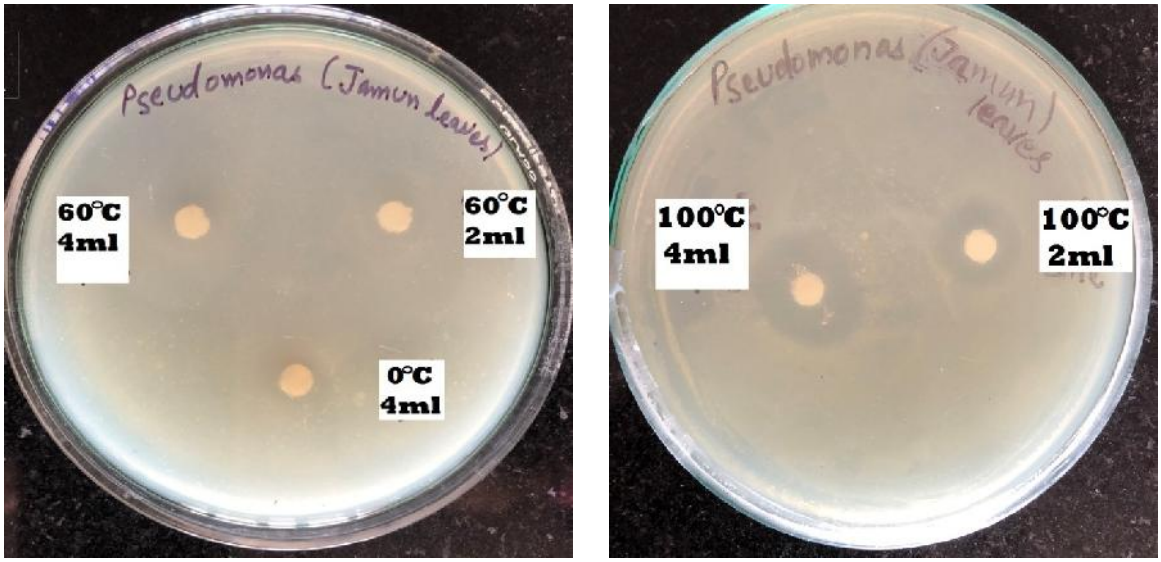
The maximum antibacterial activity was observed at 4 ml concentration at 60^0C temperature. The zone of inhibition at this temperature was bigger (11mm) than that at 0^0C and 100^0C (10 mm and 8 mm, respectively) in case of *E.coli*. In contrary to this, the zone of inhibition at 100^0C at 4 ml concentration was larger (12 mm) than at 0^0C and 60^0C temperatures in case of *Pseudomonas* sp. (Table 2). This indicated that in the extracts prepared at 100^0C, the nanoparticles persist and show their antimicrobial activity.

Table 1. Halo zones (mm) against *E. coli* and *Pseudomonas* sp.

Temperatures	**0°C**	**60°C**		**100°C**	
Concentrations (ml)	**4**	**2**	**4**	**2**	**4**
Bacterial Isolates	Zone of inhibition (mm)				
E.coli	10	8	11	7	8
Pseudomonas sp.	6	6	7	10	12

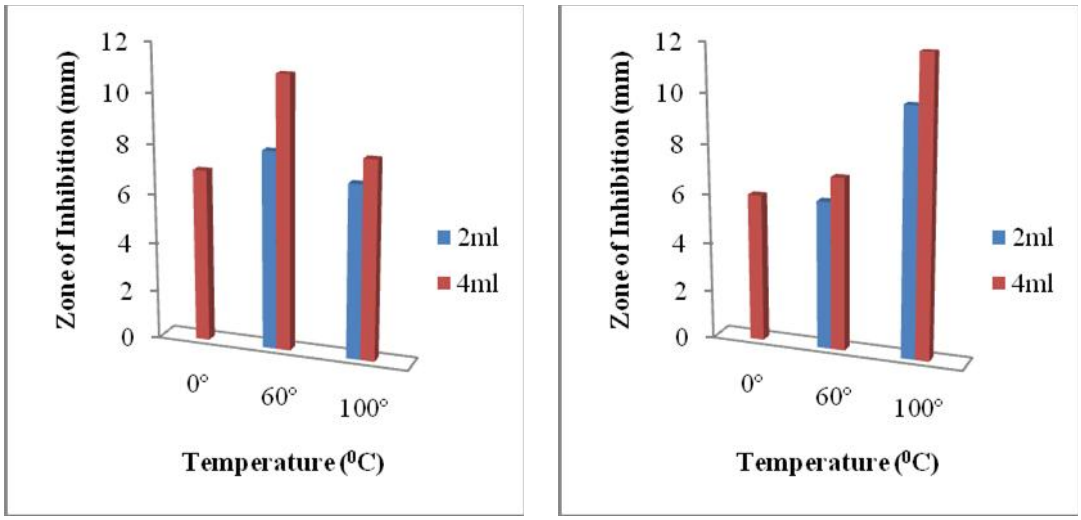


a)



b)

Figure 3: Antibacterial efficacy of AgNPs against a) *E. coli* and b) *Pseudomonas* sp.



a) b)

Graph 2: Antibacterial Effect of AgNPs against a) *E. coli* and b) *Pseudomonas* sp.

Conclusions

Syzygium cumini leaf extracts can be used for biosynthesis of Silver nanoparticles. Preliminary characterization was performed on the basis of color changes and Calorimeteric readings and further confirmed by SEM and TEM analysis. To test the applicative potential of Jamun leaf extracts, their antibacterial activity was tested. These metallo nanoparticles prepared in extracts at 100^0C represented maximum antimicrobial activity in *Pseudomonas* sp.

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	Website: www.ijarbs.com
Quick Response Code	Subject: Biotechnology
DOI:10.22192/ijarbs.2019.06.03.013	

How to cite this article:
Manpreet Kaur, Neha Gupta, Jaspreet Kaur and Anshu Sibbal Chatli. (2019). Biosynthesis and characterization of AgNPs and their antibacterial effects . Int. J. Adv. Res. Biol. Sci. 6(3): 226-231.
DOI: http://dx.doi.org/10.22192/ijarbs.2019.06.03.013