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Original Article

Green synthesis of silver nanoparticles using *Delonix regia* leaf extract and evaluation of their antimicrobial efficacy

S. Devi Bala[1*], M. Francis Sathiyaseelan[2] and T. Dons[3]

[1]*M.Sc. Student, Department of Botany, St. Joseph College, Trichirappalli-2.
[2]Assistant Professor, Department of Botany, St. Joseph College, Trichirappalli-2.
[3]Department of Botany, St. Joseph College, Trichirappalli-2.

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ABSTRACT

The green synthesis of nanoparticles is eco-friendly. Nanoparticles are of great scientific interest as they are, in effect, a bridge between bulk materials and atomic or molecular structures. The synthesised nanoparticles are playing an important role in the field of nanotechnology. Synthesized nanoparticles were characterized using UV-Visible Spectrophotometer used to predict that where the synthesis has been done or not. The absorbance of UV- Vis analysis is 455 nm. FTIR- Fourier Transform Infrared Spectroscopy with different wave number and it has determined by the functional group data analysis. SEM- Scanning Electron Microscopy, Particle size analyser shows that the particles are in 62 nm. The Photoluminescence spectrum showed the excitation peak at 650 nm**.** The antibacterial activities are carried out against bacteria and fungi by using disc diffusion method. The present study states that the green synthesis of silver nanoparticles of *Delonix regia* is having many advantageous properties and antimicrobial activity. The synthesized nanoparticles can be used in the field of pharmaceutical industry to produce medicine.

Keywords: Silver nanoparticles, Antimicrobial activity, *Delonix regia*, Antioxidant activity.

INTRODUCTION

Nanoparticles (NPs) are defined as having one dimension 100nm or less in size and due to their large surface area they tend to react differently than larger particles of the same composition, allowing them to be utilized in novel, antibacterial, electrochemical and catalytic properties[1,2]. Unfortunately chemical synthesis of silver NPs utilizes environmentally toxic or biologically hazardous reducing agents [3]. Silver nanoparticles are important that have been studied extensively. They can be synthesized by several physical, chemical and biological methods [4-6]. The biogenic synthesis for nanoparticle production is easy, trouble-free, environment friendly, low cost and employed to get optimized parameters for controlled synthesis of nanoparticles which can be applied with particular ingredients as catalysis, while traditional methods cannot exhibited these properties [7]. Due to attractive physiochemical properties of silver nanoparticles, it has a great potential in the field of medicine and biology. Silver based products have been recognized as potent antibacterial and antifungal activities, which has been used from prehistory times against for the treatment and prevention of different disease especially infections [8]. The present study is to synthesis of nanoparticles by green method and we were characterized the nanoparticles by UV, FTIR, PL, Particle size analyser and SEM. Then we have evaluated the antimicrobial activity against human pathogens by disc diffusion method.

MATERIALS AND METHODS

Chemicals required: Silver nitrate used as a substrate for the synthesis of silver nanoparticles. $AgNO_3$ was purchased from Himedia laboratories Pvt. Ltd., Mumbai, India. The water has been obtained through glass double distillation [9].

Preparation of plant sample: The plant materials were collected and washed with tap water and then again washed with distilled water and the leaves were separated from the plant and the leaves were shad dried and powdered.

Preparation of plant extract: 2g of powdered leaf sample were boiled for 15 min in 100ml sterile

***Corresponding Author Address: S. Devi Bala,** M.Sc. Student, Department of Botany, St. Joseph College, Trichirappalli-2, Tamil Nadu, India*

distilled water and filtered through the Whatman filter paper (pore size 25µm). The filtrate was used for the further study.

Synthesis of nanoparticles: 1mM of $AgNO_3$were reduced using 100ml of 5% of leaf extract at under the sun light for 1 hour; it resulting in the was dark brown solutions indicating the formation of silver nanoparticles. Silver nitrate aqueous solution (1 mM) was prepared and used for the bio inspired synthesis of silver nanoparticles. Sea buckthorn leaves extract 5 ml was added into 95 ml of aqueous solution of 1mM $AgNO_3$ and heated on horizontal shaking water bath at 75^0 C for 60 min in a dark room. Reduction of $AgNO_3$ to silver ions was confirmed by change the colour from colourless to brown [10].

Characterization of synthesized AgNPs: The synthesized nanoparticles are characterized by UV-Visible Spectrophotometer is used to identify the reduction of silver ions. According to these studies, reduction of pure Ag^+ ions was usually monitored after 3 to 5 hours of diluting the small aliquot of the sample into distilled water [11, 12]. The measurements were taken for the AgNPs synthesized after 24 hrs of reaction and were done using a FTIR SHIMADZU 8400S with a wavelength range of 400 ~ 400 nm where the samples were incorporated with KBr pellets to acquire the spectra. The results were compared for shift in functional peaks of critical value [13]. FTIR-Fourier Transform Infrared Spectroscopy spectrum is the instrument to found out the presence of the functional group in the AgNPs which were synthesized chemically.The synthesized silver nanoparticles analysis was done using Hitachi S-4500 SEM machine. The thin coated of the silver nanoparticles synthesized powder were prepared on a carbon coated copper grid by just dropping a very small amount of the sample on the grid, extra solution was removed using a blotting paper and then the film on the SEM grid were allowed to dry putting it under a mercury lamp for 5 minute [14]. Particle size analyser was used to calculate the size of the NPs. The formations of the AgNPs were also confirmed by PL spectroscopy. The PL spectra were recorded in a Hitachi (F 2700) spectrophotometer. PL- Photoluminescence was used the study of absorption of the photon emission [15].

Antimicrobial assay: The antimicrobial activity of *Delonix regia* extract and its AgNPs was carried out by disc diffusion method. The AgNPs and $AgNO_3$ were loaded onto different filter paper discs and the antibiotics. The discs were placed on the nutrient agar medium containing bacterial cultures and incubated for 12 hours at 35^0C. The zone of inhibition was recorded. Antimicrobial capability of SNPs allows them to be suitably employed in numerous household products such as textiles, food storage containers, home appliances and in medicinal devices. The most important application of silver and SNPs is in medical industry such as tropical ointments to prevent infection against burn and open wounds. Silver nanoparticles are reported to have many therapeutic uses. There are reported to possess anti-viral properties [16].

Authentication of the plant material: The plant *Delonix regia* was authenticated by Dr. S. Soosai Raj, PhD. St.Joseph's College, Trichirappalli-2. The Authentication number is SJCBOT 2219.

RESULTS

UV-Visible Spectrophotometer: The UV–Vis absorption spectra showed after 24 h period of observation and are shown in Fig. 1. The peak of absorbance, found near the wavelength 455 nm, can be attributed to the plasmonic peak of AgNPs formed in the solution. The UV- Vis spectra were recorded at different temperature such as 25^0 C and 60^0C along with peel extract and $AgNO_3$solution. The intensity in the range of 400-500 nm continued to increase with increasing temperature [17].

Fourier Transform Infrared Spectroscopy: Infra-red spectroscopy was used to determine and predict possible physiochemical interactions between the components in a formulation using FT-IR. The Fig: 2 show that the spectrum of *Delonix regia* extract shows the peaks at 3438.02cm-1, 2074.04cm-1, 674.54cm-1. This image shows the peak values which indicate the chemical compounds are present.

SEM- Scanning Electron Microscope: The shape and size of the prepared samples were determined by SEM. This Fig: 3 give an idea of the shape and structure of the NPs. The SEM image showing the high density silver nanoparticles synthesized by the leaf extract further confirmed the development of silver nanostructures. SEM image show that SNPs are relatively uniform in diameter [18].

PL- Photoluminescence: The Photoluminescence is light emission from away from of matter after the absorption of photons. It one of many forms of luminescence and is initiated by photo-excitation. The Fig: 4 is the Photoluminescence spectra in which the excitation peak was found at 650 nm, while emission peak was observed at 644 nm. It shows the emission of photons from the extract of AgNPs.

Particle size analyser: Particle size and size distribution are the most important characteristics of NPs systems. The obtained results indicate the diameter of the particle is 62 nm. The particle size analyser is the technical procedures, or laboratory techniques which determine the size range, and the average, or mean size of the particles in a powder or liquid sample.

Anti-Bacterial Activity: The anti-bacterial activity was carried out by *Staphylococcus aureus, Bacillus subtilis, Escherichia coli, Vibrio cholera*, and *Klebsiella pneumonia*. The activity was carried out by disc diffusion method shown in Fig: 5. In this method, we made a conclusion that the bacterial pathogen ***Staphylococcus aureus*** shows the maximum zone of inhibition it is shown in Table: 1. Due to the growing need to develop environmentally benign technologies in material synthesis, biosynthesis of AgNPs has received considerable attention. Also environment friendly NPs have gained insight as an excellent antimicrobial agent due to their non-toxic effect on human cells at low concentrations and weaker ability to develop resistance towards silver ions [19]. With respect to the microbes, the silver nanoparticles get attached to the cell wall, thereby disturbing the permeability of cell wall and cellular respiration. The nanoparticles may also penetrate deep inside the cell wall, thus causing cellular damage by interacting with phosphorus and sulphur containing compounds, such as DNA and protein, present inside the cell. The bacteriocidal properties of silver nanoparticles are due to the release of silver ions from the particles, which confers the antimicrobial activity [20].

Antifungal activity: The antifungal property is presented tremendous in the synthesized nanoparticles. The Silver nanoparticles were also has antifungal activity. This study was carried out by dis diffusion method. Silver NPs contains high Antifungal property against ***Aspergillus niger*** it is shown in the Table: 2. Nanoparticles tend to adsorb on the bacterial cell and undergo dehydrogenation due to respiration process which occurs at the cell membrane of bacteria. The bacteria had inactivated their enzymes, generating hydrogen peroxide that causes bacterial cell death [21]. In the recent days, silver nanoparticles have been synthesized from the naturally occurring sources and their products like green tea (*Commellia sinensis*), Neem (*Azadirachta indica*), leguminous shrub (*Sesbaniadrum mondii*), various leaf broth, natural rubber, starch, *Aloe vera* plant extract, lemongrass leaves extract, etc…[22].

DISCUSSION

In this present study is we have concluded that the UV–Vis absorption spectra found near the wavelength 455 nm. Infra-red spectroscopy of Delonix *regia* extract shows the peaks at 3438.02cm-1, 2074.04cm-1, 674.54cm-1. SEM image show that the shape and structure of the NPs. The particle size analyser indicates the diameter of the particle is 62 nm. Photoluminescence spectra were observed at 644 nm. The anti-microbial activity state that the maximum zone of inhibition is absorbed against the bacteria ***Staphylococcus aureus*** and the fungi is ***Aspergillus niger.*** The nanoparticles having many applications in medicinal field.

CONCLUSION

Silver nanoparticles are having a lot of applications in various fields like antimicrobials, preservatives, paints, biosensors and cosmetics. So improving of nanoparticles synthesis is the major object in the field of synthesis of silver nanoparticles. The usage of bacteria is the good approach to the production of Eco-friendly and costs effectual silver nanoparticles [23].From this summary, it was concluded that plant mediated synthesis of silver nanoparticles possess potential antimicrobial applications. The characterization analysis proved that the particle so produced in nanodimensions would be equally effective as that of antibiotics and other drugs in pharmaceutical applications. The use of silver nanoparticles in drug delivery systems might be the future thrust in the field of medicine [24].

Tables

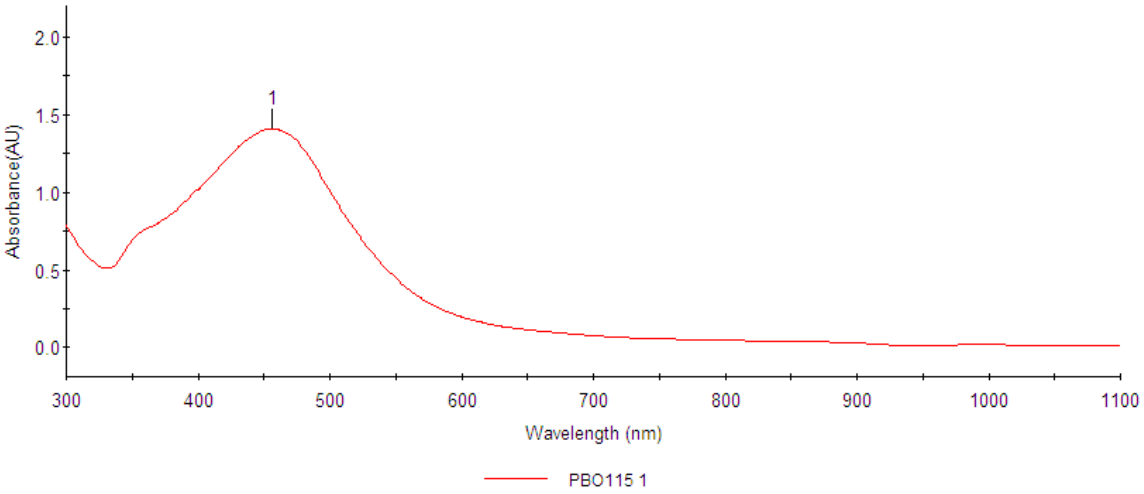
Table: 1. Zone of inhibition for AgNPs (*Delonix regia)* Leaf Extract

Test Bacterial species	Zone of inhibition(mm)		
	AgNPs	**Chloramphenicol**	**$AgNO_3$**
Staphylococcus aureus	14mm	25mm	10mm
Bacillus subtilis	13mm	27mm	13mm
Escherichia coli	12mm	24mm	10mm
Vibrio cholera	13mm	21mm	14mm
Klebsiella pneumonia	13mm	24mm	10mm

Table: 2. Zone of inhibition for AgNPs (*Delonix regia)*Leaf Extract

Test Fungal species	Zone of inhibition(mm)		
	AgNPs	**Nystatin**	**$AgNO_3$**
Candida albicans	10mm	24mm	10mm
Aspergillus fumigatus	11mm	20mm	13mm
Candida dubliniensis	12mm	19mm	10mm
Aspergillus niger	14mm	25mm	13mm
Aspergillus flavus	11mm	23mm	10mm

Fig: 1 UV–Vis absorption spectra



Name	No.	Peak (nm)	Peak (AU)	No.	Valley (nm)	Valley (AU)
PBO115	1	455.8	1.409685459			

Fig: 2.Fourier Transform Infrared Spectroscopy

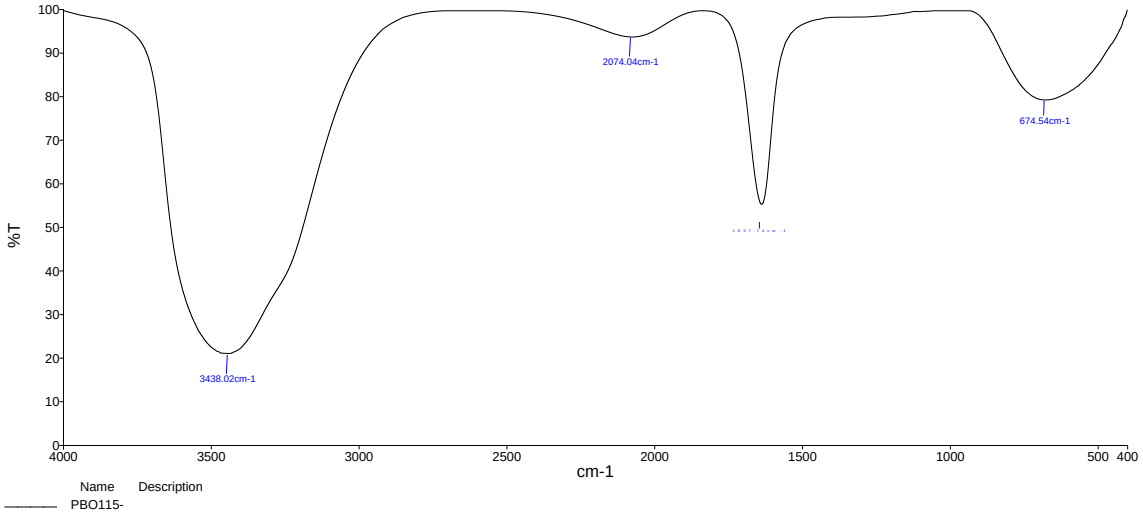


Fig: 3.Scanning Electron Microscope

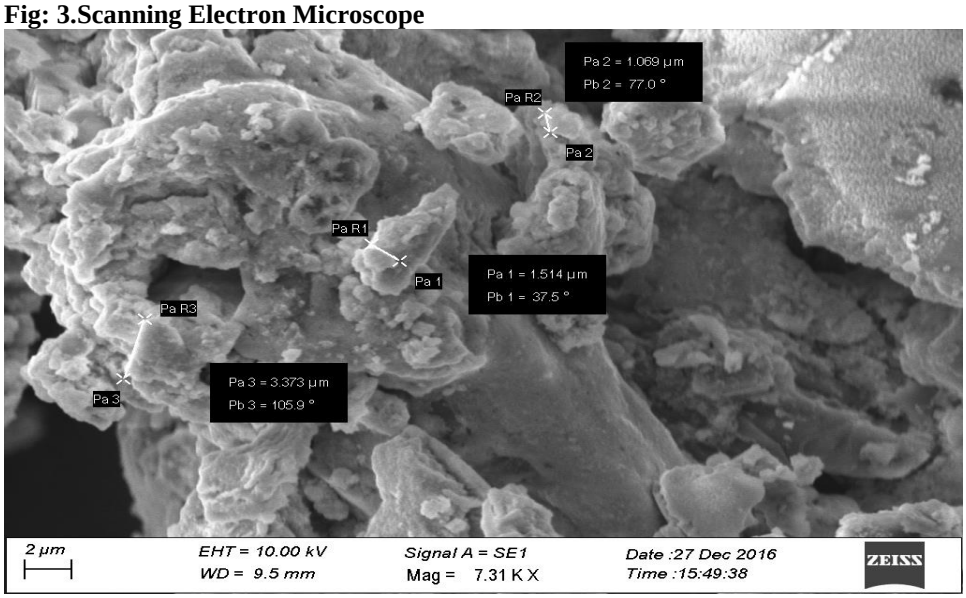


Fig: 4 Photoluminescence spectrums

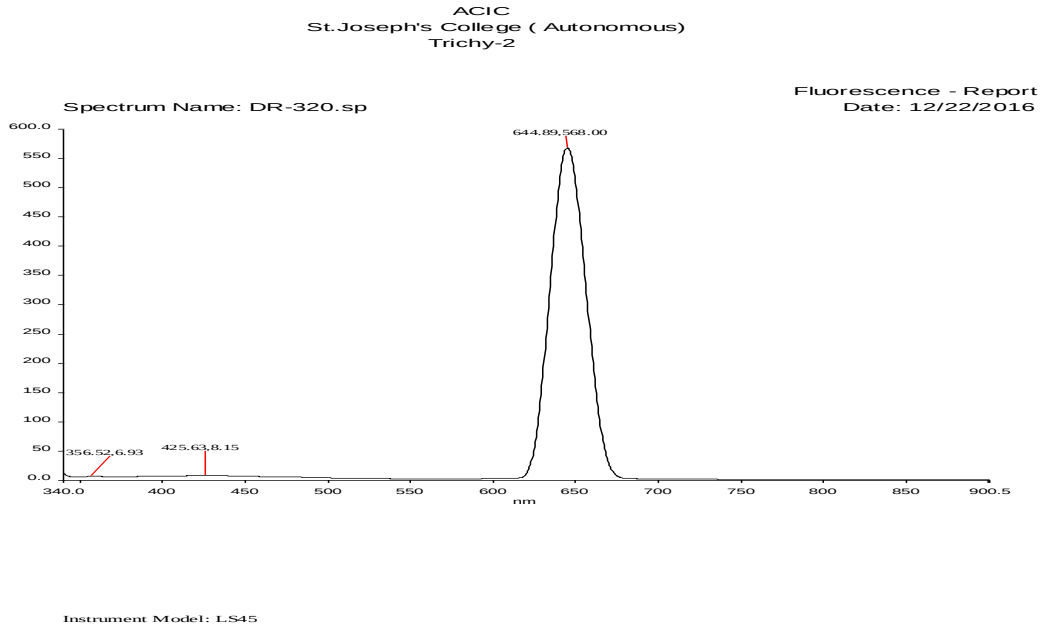


Fig: 5 Anti-Bacterial Activity

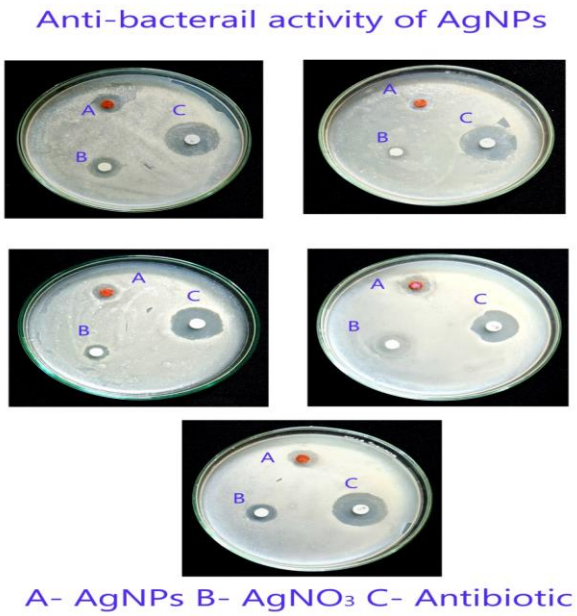
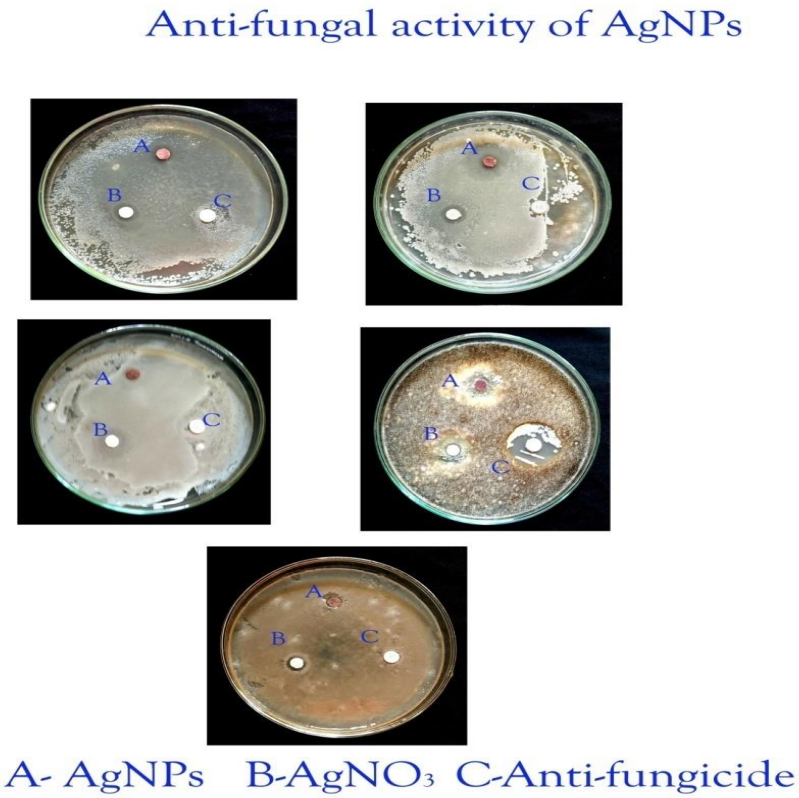


Fig: 6 Anti-fungal activity



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