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Original Research Article

Biogenic synthesis of iron nanoparticles from *Catharanthus roseus*

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Abstract

Recently the biosynthesis of nanoparticles using plant extracts has drawn the attention of researchers. The aim of the present study is to investigate the potential of *Catharanthus roseus* for the biological synthesis of iron nanoparticles and to evaluate the antifungal efficacy of the synthesized particles against the selected fungal strains. Biosynthesis of iron nanoparticles was performed through the n-hexane leaf extract of *Catharanthus roseus*. The structural properties of the synthesized nanoparticles (concentrations, 10%, 30%) were further investigated through UV-visible spectroscopy; particle size analyzer (PSA), scanning electron microscopy (SEM) and fourier transform infrared spectroscopy (FTIR). Results of the UV-Vis spectroscopy of the synthesized iron nanoparticles showed the absorption spectra of iron nanoparticles (FeNPs), prominent peak at 260 nm corresponding to the absorption of iron nanoparticles was obtained. Particles size analyzer revealed the average size of the iron nanoparticles that was calculated as 108 and 266 nm. Phenolics (OH group at 3350 cm^{-1}) were observed as main bioactive phytochemical of the plant extract that acted as capping agent in iron nanoparticles synthesis. Surface morphology using SEM revealed the aggregates of irregular shaped iron nanoparticles. The antifungal activity of these biosynthesized FeNPs against *Aspergillus nidulans* and *Aspergillus terreus* was also evaluated, nanoparticles showed high activity against *A. terreus*. This study concludes that the biosynthesis of iron nanoparticles is a safe and economical approach.

Keywords: Nanoparticles, Green synthesis, *Catharanthus roseus*, n-Hexane, capping agent, Economical approach.

Introduction

Nanotechnology is multifaceted area, being considered as a novel and potential field of 21st century that deals with fabrication of materials at nano scale level. At present many ongoing advancements at nano scale level involves that nanotechnology will definitely have a very fascinating role in numerous main technologies, reorganization and exploitation of the materials composition in the range of 1 nm to 100 nm (Bar et al., 2009). Recently, a number of methods (chemical, biological and physical) are employing for the synthesis of nanoparticles. Though, the physical and chemical methods are most well acknowledged for the fabrication of nanoparticles, but the use of harmful chemicals limits their value (Liu et al., 2011). The advancements in the synthesis of nanoparticles through green route synthesis are growing into a main field of nanotechnology (Raveendran et al., 2006).

Use of plants and plant parts for the synthesis of nanoparticles is referred as green nanotechnology. Nanobiotechnology has appeared as incorporation between nanotechnology and biotechnology for the synthesis of biosynthesized nanoparticles. The use of

green nanotechnology approaches eliminates or lowers the use and production of lethal chemicals during the synthesis of nanomaterials. Metal nanoparticles such as gold, zinc, iron and silver are most commonly used for biomedical applications (Cai et al., 2008). However, nano sized iron has enormous significance due to its different uses in various fields of science (Huber, 2005). Biological synthesis of FeNPs through plants is a significant technique as it is free from the requirements of unsafe chemicals, pressure and temperature requirements (Pattanayak and Nayak, 2013).

There are various studies that describe the synthesis of nanoparticles using plants. The biosynthesis of iron nanoparticles from *Amaranthus dubius* was reported. Results showed the spherical surface structure of nanoparticles, size ranged from 43-220 nm. Due to the diverse applications in different areas green synthesized nanoparticles are receiving much interest (Harshiny et al., 2015). Leaf extract of *Gardenia jasminoides* and *Lawsonia inermis* were used to synthesize iron nanoparticles. Resulted nanoparticles were characterized through different characterization techniques (Naseem and Farrukh, 2014).

Catharanthus roseus is an important therapeutic plant of family Apocynaceae that has been used in traditional medicines. Plant is an evergreen herb that grows up to 1 m in height. *C. roseus* is commonly found in tropical and subtropical areas of the world. Plant contains a wide range of bioactive compounds of medicinal importance. Two of the most vital alkaloids vincristine and vinblastine are used for cancer treatment. *C. roseus* has significant biological activities including, anticancerous, antibacterial, antioxidant, antifungal, antiviral activities ((Jaleel et al., 2009; Nayak and Pereira, 2006). In this study the effect of iron nanoparticles on the growth of two fungal species was observed. *Aspergillus nidulans* and *Aspergillus terreus* both are the members of phylum ascomycota. These are filamentous pathogenic fungi that cause severe diseases. *Aspergillus terreus* is a soil born saprotrophic fungi that causes infectious diseases in plants and animals (Arabatzis and Velegraki, 2012; Osmani and Mirabito, 2004). This study was designed using a simple, cost-effective and ecofriendly synthesis method at ambient conditions using *C. roseus* leaf extract as reducing agent. Aim of the present study is to synthesize iron nanoparticles from the leaves extract of *Catharanthus roseus* and to assess the fungicidal potential of synthesized iron nanoparticles.

Materials and Methods

Reagents and Chemicals

Malt extract and ferric sulfate were obtained from the local Merck.

Preparation of Plant Extract

Fresh leaves of the *Catharanthus roseus* were collected from the university. Leaves were washed with tap water and dried properly under shade then squashed and ground to powder. Plant extract was obtained through microwave assisted extraction. For this 20 gm of plant material was added into 100 ml of hexane (solvent), beaker was wrapped with polythene sheet. During heating to avoid the developed pressure small holes were made in sheet using injecting needle. Plant material was heated at 800 Watt microwave irradiation for 120 seconds, then the extract was strained using Whatman's filter paper No 1 and stored at 4ºC for further use.

Synthesis of Iron Nanoparticles

Two concentrations of iron nanoparticles were prepared (10 %, 30 %). For the synthesis of 10 % nanoparticles both the plant extract and metal salt solution were mixed in 1:1 proportion while for the synthesis of 30 % iron nanoparticles 3:1 proportions of plant extract and salt solution were mixed to each other in a clean beaker. Plant extract that acted as a reducing agent was gradually added to the metal salt solution. Bioreduction of Fe ions occurred upon the gradual addition of plant extract in the ferric sulfate solution. A shift in color of solution from green to dark brown upon mixing indicated the reduction of metal ions and synthesis of iron nanoparticles.

Antifungal Activity of Synthesized Iron Nanoparticles

Antifungal activity of Fe-NPs was evaluated against two species of Aspergillus. In this assay, fungicidal potential of the 30% Fe-NPs was assessed. Different concentrations of synthesized nanoparticles (0, 1, 2, 3 and 4%) were prepared by adding (10mL, 8.5mL, 7mL, 5.5mL, 4mL) of the media to the (0mL, 1.5mL, 3mL, 4.5mL and 6mL) of stock solution. Agar dilution method was employed to determine the antifungal activity.

Reduction in fungal biomass was calculated using formula:

% reduction in fungal biomass = biomass of control – biomass of treatment / biomass of control x 100

Characterization techniques
Biosynthesized iron nanoparticles were characterized through different techniques.

1. UV-Vis Spectroscopy: The absorption spectroscopy in the ultra violet visible region of spectrum is referred as UV-Visible spectroscopy. It uses light in the visible and light of nearby ranges. The absorption in the visible region directly influences the apparent color of the chemicals used. Electronic transitions of molecules occurred in this region of electromagnetic spectrum (Pattanayak and Nayak, 2013).

2. Particle Size Analyzer: The average diameter of synthesized iron nanoparticles was evaluated through BT-90 nano laser particle size analyzer.

3. Fourier Transform Infrared Spectroscopy: It is usually exercised to observe the surface capping materials of synthesized nanomaterials, since nanoparticles have great surface area to volume ratio so the variation in surface structure by a suitable adsorbent makes nanoparticles very valuable. This characterization method is employed to determine the bioactive molecules that act as capping materials in the fabrication of iron nanoparticles. Although all the biological molecules absorbs light in the infra-red part at different wavelengths because of light absorption the bonds within the molecules shudder that can be sensed by FTIR (Kumar, 2014).

4. Scanning Electron Microscopy: This technique is used to reveal the surface shape and structure of the sample material (Prashanth et al., 2011).

Results

UV-Visible Analysis of Iron Nanoparticles
Iron nanoparticles were synthesized when metal salt (iron sulfate) solution reacted with the hexane fraction, a change in solution color (light green to dark green) was observed indicated the synthesis of nanoparticles. The biosynthesis of nanoparticles was confirmed through UV- visible spectroscopy using wavelength range from 190 to 700 nm. Absorption spectrum of hexane nanoparticles was observed to examine the λ (max) that shifted from 199 to 260 nm during 24 hours. Maximum wavelength with respective absorbance at different times illustrated the fabrication of nanoparticles (Table: 1).

Table - 1: Effect of Hexane Fractions on the Synthesis of Fe-NPs

Hexane NPs Solution(mL)	At start $\lambda_{(max)}$	After 3 hours $\lambda_{(max)}$	After 6 hours $\lambda_{(max)}$	After 24 hours $\lambda_{(max)}$
Hexane NPs 10 mL	199±2.80	200±2.89	200±2.90	258±3.01
Hexane NPs 30 mL	199±2.57	200±2.97	210±3.00	260±3.00

Particle Size Analyzer
The average particle diameter of the biosynthesized iron nanoparticles was calculated through nano laser particle size analyzer as shown (Table: 2).

Table - 2: Particle Size of Hexane NPs

Particle name and concentrations (mL)	Average size (nm)
Hexane-NPs 10	266
Hexane-NPs 30	108

Fourier Transform Infrared Spectroscopy (FTIR)
FTIR spectrum of the biosynthesized hexane Fe-NPs illustrated the considerable absorption band at 1625 cm^{-1} , 2150 cm^{-1} and 3360 cm^{-1} due to COOH, C=C and O-H bonds which corresponds to carboxylic group, alkenes and phenolics, while the peak at 670 cm-1 corresponds to the Fe vibrations as shown in Figure (3).

Scanning Electron Microscopic analysis (SEM)
Scanning electron microscopic characterization of iron biosynthesized iron nanoparticles was performed through JSM- 6480 machine to determine the surface morphology of biosynthesized iron nanoparticles. Iron nanoparticles were examined by using 10, 20, 50 and 100 µm micron markers. SEM examination was exercised to examine the 30% hexane iron nanoparticles. SEM micrographs showed that iron nanoparticles were in the form of groups of irregular shape (Figure 4: a & b).

Antifungal activity of Hexane Fe-NPs of *Catharanthus roseus*
The biosynthesized iron nanoparticles of *C. roseus* were found to be effective against the *Aspergillus*

nidulans and *Aspergillus terreus*. Iron nanoparticles caused the inhibition in fungus growth. All the five used concentrations of iron nanoparticles showed considerable reduction in growth but the 4% concentration caused the more inhibition in fungal mycelium growths. All the concentrations of Fe-NPs proved to be significantly efficient against the both tested species of Aspergillus as compared to control (Figure 5 & 6).

Discussion

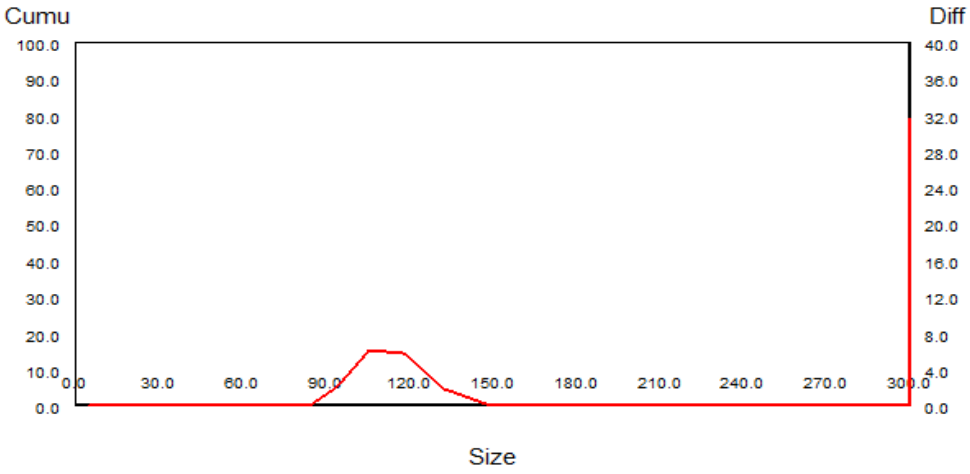
Synthesis of nanoparticles through plants seems to be an easiest, simple, cost effective and environmental friendly technique. In this study the biosynthesis of iron nanoparticles from *C. roseus* leaf extract was carried out. n- Hexane was used as a solvent for the extraction of plant material. The formation of iron nanoparticles was checked through ultra violet -visible spectrophotometric analysis. An alteration in solution color upon mixing of plant extract into the iron sulfate solution indicated the formation of iron nanoparticles. UV-visible spectroscopy analysis of FeNPs showed the absorption peaks from 199-260 nm. In fact the alterations in solution color occur due to the excitations of surface plasmon resonance that are examined by ultra violet spectrophotometer signifying the synthesis of FeNPs (Song and Kim, 2009). Similar results have been reported from the *Azadirachta indica* biosynthesized FeNPs (Pattanayak and Nayak, 2013). UV-Visible spectrum of nanoparticles aggregate is different from the individual nanoparticle. The SPR of a group of NPs is changed to a longer wavelength than the SPR of a single particle (Masarovičová and Kráľová, 2013). In the present study the absorption peaks of 10 and 30% FeNPs were seen at 258 and 260 nm after 24 hours respectively. Shorter wavelengths at the time of mixing and after 4 hours showed that aggregates of NPs did not form. Somewhat similar results were seen by iron nanoparticles (Alqudami and Annapoorni, 2007). Particle size results of iron nanoparticles showed the synthesis of smaller sized nanoparticles from 30 % concentration of Fe-NPs while 10 % concentration of Fe-NPs give nanoparticles of large diameter. The average size of iron nanoparticles was 266 nm (10 % hexane Fe-NPs) and 108 nm was observed (30% hexane Fe-NPs). Results demonstrated that the plant extract concentrations greatly affect the diameter of synthesized nanoparticles, as the concentration of plant extracts increased nanoparticles with more reduced significant sizes can be synthesized. This indicates the size of NPs directly depends on the concentration of plant extracts.

FTIR spectroscopy of iron nanoparticles showed bands and stretches at different wavelengths due to the vibrations of different compounds (functional groups). Similar peaks were observed from the FTIR analysis of the iron nanoparticles synthesized from *Gardenia jasminoides* and *Lawsonia inermis* (Naseem and Farrukh, 2015). SEM micrographs of synthesized Fe-NPs showed irregular shaped clusters of iron NPs. The nature of solvent used greatly affects the morphology of synthesized nanoparticles. As the different solvents have different phytochemicals extracted in them. These plant biochemicals act as reducing agents and capping agents for nanoparticles. The biosynthesis of iron nanoparticles from *Gardenia jasminoides* and *Lawsonia inermis* leaves extract was reported. Biosynthesized iron nanoparticles were characterized through SEM analysis showed that iron nanoparticles were grouped due to the adhesive nature having shape of vague hexagonal like structure (Naseem and Farrukh, 2015).

Antifungal capacity of zinc oxide nanoparticle against *Erythricium salmonicolor* was assessed; synthesized nanoparticles significantly reduced the fungal growth (Arciniegas et al. 2017). Biosynthesized iron nanoparticles were found to be potent in reducing the fungal biomass. From all the applied concentrations of NPs, 3 and 4% concentration effectively reduced the fungal growth. Greater quantities of the phytochemicals including alcohols, acids, alkaloids, alkanes, esters, alkenes were present in 3, 4 % concentrations and reaction of hexane solvent with iron might be accountable for the antifungal activity of synthesized iron nanoparticles.

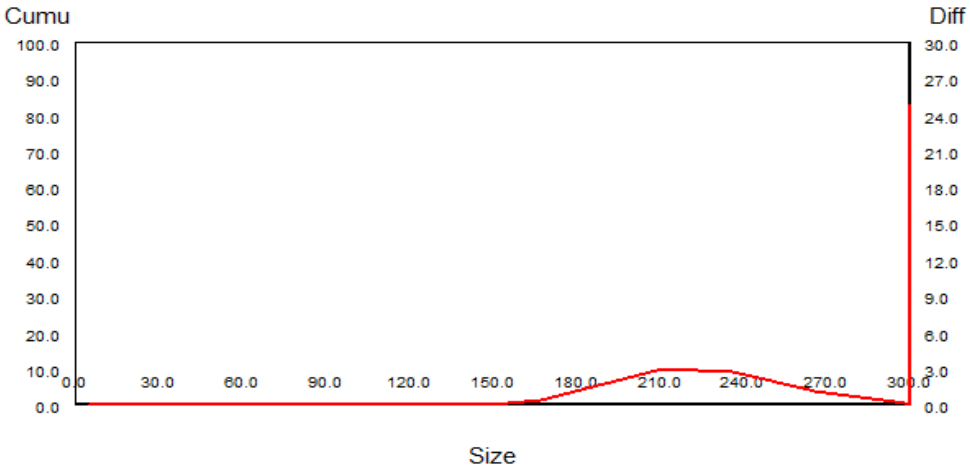
Table - 3: Effect of Hexane FeNPs on Fungus Growth

Sr. No	Concentration of NPs	0 % (Control)	1%	2%	3%	4%
1	*Aspergillus terreus*	0	81	83	88	97
2	*Aspergillus nidulans*	0	42	51	71	82



Diam nm	Percent
88.9	0.00
140	16.02
223	16.02
353	16.02
560	16.02
889	16.02
1409	16.02
2233	16.02
3539	16.02
5610	100.00

Figure - 1: Particle size (nm) of hexane-NPs 30 %



Diam nm	Percent
158	0.00
217	4.17
299	8.97
412	9.06
567	9.06
782	9.06
1076	9.06
1482	29.57
2041	88.40
2811	100.00

Figure - 2: Particle size (nm) of hexane-NPs 10 %

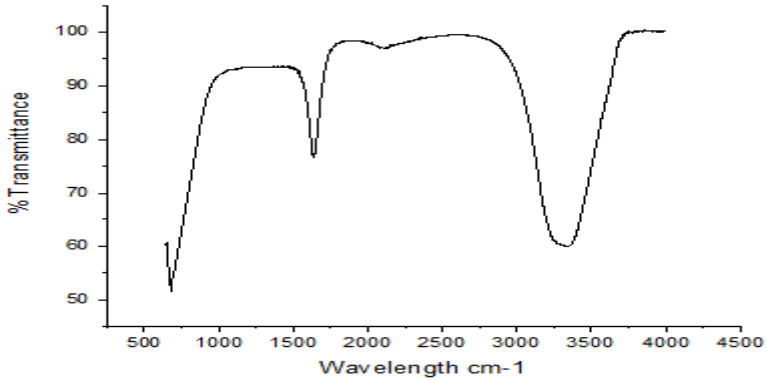


Figure - 3: FTIR spectrum of hexane FeNPs

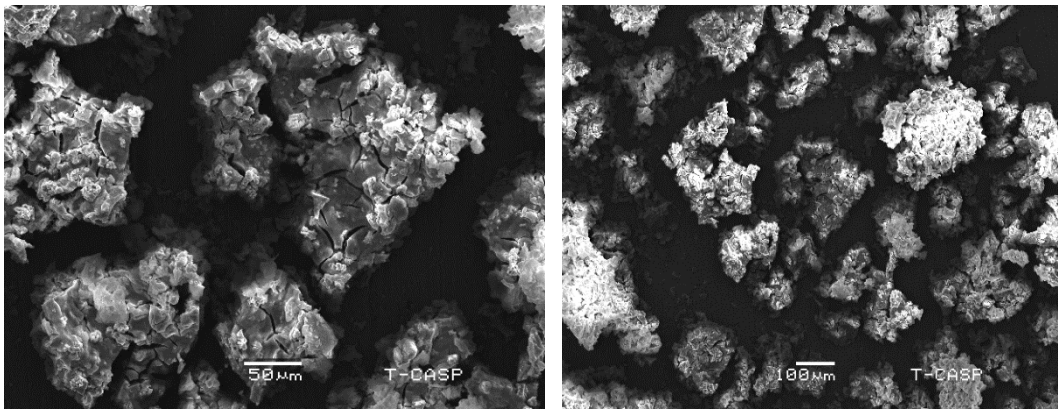


Figure - 4: (a & b): SEM micrographs of 30 mL concentration of hexane FeNPs of *Catharanthus roseus*

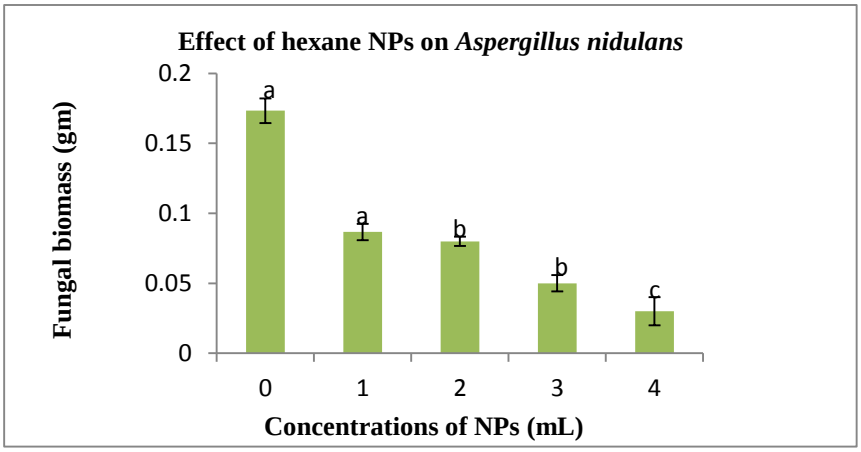


Figure - 5: Effect of hexane NPs on the growth of *Aspergillus nidulans*

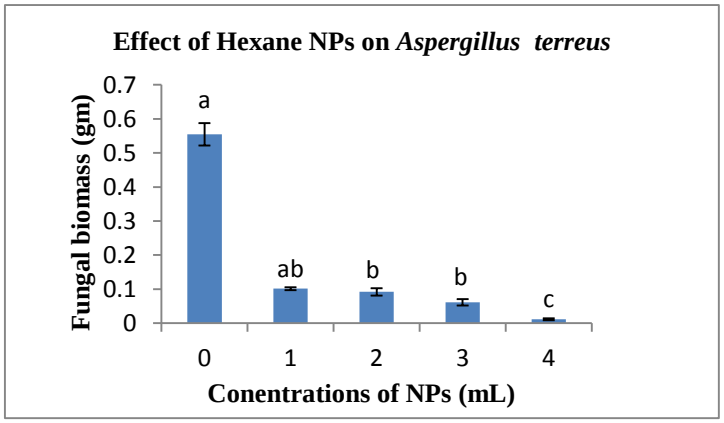


Figure - 6: Effect of hexane NPs on the growth of *Aspergillus terreus*

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