Open Access Contents
Int. J. Drug Dev. & Res.
| January - March 2014 | Vol. 6 | Issue 1 | ISSN 0975-9344 |
www.ijddr.in

Full Length Original Research Paper

Biological Synthesis of Zinc oxide Nanoparticles from C*atharanthus roseus* (l.) G. Don. Leaf extract and validation for antibacterial activity

G. Bhumi

N. Savithramma

Department of Botany,
Sri Venkateswara University,
Tirupati-517502,
Andhra Pradesh, India.

Corresponding Authors:
G. Bhumi
bhumi.gaddala10@gmail.com

Abstract:
Biologically synthesized nanoparticles have been widely using in the field of medicine. Research in nanotechnology highlights the possibility of green chemistry pathways to produce technologically important nanomaterials. Present study focuses on the Biological synthesis of Zinc oxide nanoparticles (ZnO-NPs) by Zinc acetate and sodium hydroxide utilizing the biocomponents of leaves of *Catharanthus roseus*. The samples were characterized by x-ray diffraction (XRD), Scanning Electron Microscopy (SEM), Energy Dispersive Spectroscopy (EDAX) and FT-Raman Spectroscopy. The synthesized ZnO-NPs were found to be spherical in shape with an average size of 23 to 57 nm. These ZnO-NPs were evaluated for antibacterial activity. The maximum diameter of inhibition zones around the ZnO-NPs disk used for *Bacillus thuringiensis* indicates the resistance to ZnO NPs followed by *Escherichia coli*. Among the four bacterial species tested, the *Pseudomonas aeuroginosa* is more susceptible when compared with other three species. It is concluded that the biological synthesis of ZnO NPs is very fast, easy, cost effective and eco-friendly and without any side effects and ZnO Nps may be used for the preparation of antibacterial formulations against *Pseudomonas aeuroginosa*.

Keyword: *Catharanthus roseus*, Zinc oxide nanoparticles, Zinc acetate, Biological synthesis, Raman FT-IR.

Introduction

Nanotechnology is an important branch in the major fields of biology, chemistry, physics and material sciences. Nanoparticles possess a wide array of application in the different fields' viz., medicine, electronics, and therapeutics and as diagnostic agents. The nanomaterials can be synthesized by different methods including chemical, physical, irradiation and biological methods. The development of new chemical or physical methods has resulted in environmental contaminations, since the chemical procedures involved in the synthesis of nanomaterials generate a large amount of hazardous byproducts [1]. Thus, there is a need of "green synthesis" that includes a clean, safe, eco-friendly and environmentally nontoxic method of nanoparticle synthesis. Moreover in this method there is no need to use high pressure, energy, temperature and toxic chemicals [2, 3].

ZnO-NPs have found fabulous applications in biomolecular, diagnostics and microelectronics [4]. ZnO-NPs have been used to remove arsenic and sulphur from water even through bulk zinc oxide cannot absorb arsenic. It is because nanoparticles have much larger surface areas than bulk particles [5]. ZnO-NPs are always in the centre of attention due to their fascinating properties and extensive applications. Bio-inspired synthesis of ZnO-NPs has been achieved using environmentally and eco-friendly accepted systems. Several studies have been investigated the use of natural materials for ZnO-NP synthesis

such as DNA [6], albumen [7], orange juice [8], Pea starch [9] and peptide structures [10] etc.

Zinc oxide nanomaterials are used in the preparation of substances processing medicinally as well as cosmetically useful properties. Due to its antibacterial properties, zinc oxide is applied on the skin, in the form of powders, antiseptic creams, surgical tapes and shampoos, to relieve skin irritation, diaper rash, dry skin and blisters. Zinc oxide is used along with iron oxide to prepare calamine lotion and with eugenol to prepare zinc oxide eugenol which is used for dental applications [11, 12].

Catharanthus roseus is an important medicinal plant belongs to the family Apocynaceae, the other common names are periwinkle, Madagascar periwinkle, sadabhar. Traditionally *Catharanthus roseus* has been used in folk medication to take care of diabetes, high blood pressure and diarrhoea [13, 14, 15]. Though, in modern medicine alkaloids and chemotherapeutic agents from *C. roseus* known for pain relieving property in cancer treatment. The plant is recognized to control major disease such as leukemia and diabetes [16, 17, 18]. It is cultivated mainly for its alkaloids, which are having anticancer activities [19]. To the best of our knowledge, biological approach of using leaf extract of *C. roseus* is the first time as a reducing material as well as surface stabilizing agent for the synthesis of ZnO-NPs. Biosynthesis of ZnO-NPs by using number of plants such as *Calotropis gigantean* [20], *Aleo barbadensis* [21]. Lot of work had been carried out on *C. roseus* for isolation of secondary metabolites but synthesis of nanoparticles particularly ZnO-NPs are scanty. Hence in the present study, we have explored the biological synthesis of ZnO-NPs by using the leaves of *C. roseus* and characterized these NPs with SEM, EDAX, XRD and Raman FT-IR. Furthermore, the synthesized ZnO-NPs were evaluated for the antibacterial activity against *Bacillus thuringiensis, Escherichia coli, Staphylococcus aureus* and *Pseudomonas aeuroginosa.*

MATERIAL AND METHODS

Zinc acetate dehydrate (99% purity) and sodium hydroxide (Pellet 99%) was used as the introductory materials supplied by Sigma-Aldrich chemicals. Fresh and healthy leaves of *C. roseus* were collected from S.V.U. Botanical Garden; Taxonomic identification of the plant was carried out with the help of Gamble [22] and also compared with the herbarium present in Department of Botany, Sri Venkateswara University, Tirupati, and Andhra Pradesh, India. Primarily the leaves were washed with distilled water, cleaned and pressed with blotted paper. Then the leaves were shade dried and ground to make a fine powder and used for experimental studies.

Biological synthesis of ZnO nanoparticles

The aqueous leaf extract of *C. roseus* was added to 0.025 M aqueous Zinc acetate and adjusted the pH 12. The resulted solution was pale white in color. After stirring, the precipitate was washed against with distilled water followed by ethanol to get free of impurities. The solution was vaccum dried and used for characterization of ZnO NPs.

SEM analysis of ZnO-NPs

Scanning electron microscope (SEM) analysis was carried out by using HitachiS-4500 SEM machine. Thin film of the sample was 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 was allowed to dry.

Covered in Scopus & Embase, Elsevier Int. J. Drug Dev. & Res., January - March 2014, 6 (1): 208-214

EDAX measurements

In order to carry out the EDAX analysis, the drop of leaf extract with reduced ZnO nanoparticles was dried on coated with carbon film and performed on Hitachi-S-3400 N SEM instrument equipped with thermo EDAX attachments.

X-Ray Diffraction (XRD) analysis

The particle size and nature of the ZnO-NPs were determined using XRD. This was carried out with Shimadzu XRD-6000/6100 model with CuK α radians at 2θ angle. X-ray powder diffraction is rapid analytical technique primarily used for phase identification of a crystalline material and can provide information on unit cell dimensions. The size of the ZnO-NPs was determined by Debye Sherrer's equation.

$$D = 0.94\lambda/\beta \cos \theta$$

FT-Raman Spectroscopy analysis

FT-Raman Spectra were obtained on a Bruker RFS-100 Instrument. It is equipped with an ND: YAG laser (1064 nm line) and the laser power can be controlled using the OPUS software. The main Instrument is connected to a microscope using which small areas of samples can be analyzed. In the analysis of ZnO-NPs, the powder is placed under the microscope the energy of the laser photons being shifted up or down. The shift energy gives information about the vibration modes in the system.

Antibacterial assay

The following bacterial strains were used in this study viz., *Bacillus thuringiensis* (ATCC 10792), *Escherichia coli* (ATCC 25922), *Staphylococcus aureus* (ATCC 6538) and *Pseudomonas aeuroginosa* (ATCC 15442). Disc diffusion method was carried out by using standard protocol[23] and overnight bacterial cultures (100 µL) was spread over Muller Hinton Agar (Hi Media Laboratories Private Limited, Mumbai, India) plates with a sterile glass L-rod. 100 µL of the each extracts were applied to each filter paper disc Whatmann No.1 (5 mm dia) and allowed to dry before being placed on the agar. Each extract was tested to triplicate and the plates were inoculated at 37^0C for 24 hours after incubation. The diameter of inhibition zones was measured and tabulated.

Results and Discussion

Biological synthesis of ZnO-NPs by using *C. roseus* revealed that the pale white precipitate was appeared (**Fig-1**). *C. roseus* leaf has been used for the reducing material as well as surface stabilizing agent for the synthesis of spherical shaped ZnO-NPs. SEM image showed that the individual ZnO-NPs as well as the number of aggregates relatively spherical shape nanoparticles formed with diameter range 23 to 57 nm. Aggregate molecules where formed in the range of 20 nm, show in **Fig-2**. EDAX spectrum (**Table 1 & Fig-3)** revealed that the other elements along with Zn are identified.

XRD spectra showed strong diffraction peaks at 20, 32, 35 and 40 degrees of 2θ which corresponds to 111, 200, 220 and 311 crystal planes, which were significant agreement with the JCPDS file 36145 (a = b = 3.249A^0, C = 5.206 A^0) and indexed as the hexagonal wurtezite structure of ZnO (**Fig-4**). High purity and crystallinity of the prepared ZnO-NPs confirmed the sturdy and clear peak. For other impurities no characteristic peaks were accessible.

In order to find out the possible functional groups of capping agents associated in the stabilization of ZnO-NPs, Raman spectrum of the nanoparticles was recorded. **Fig-5** gives the selective enhancement of Raman bands of the organic capping agents bound to the nanoparticles. The

Covered in Scopus & Embase, Elsevier Int. J. Drug Dev. & Res., January - March 2014, 6 (1): 208-214

spectrum shows a strong and sharp band at 1340 cm^{-1} which can be attributed to the stretching vibration of Zn-N and Zn-O bonds. This peak indicates the formation of chemical bond between zinc and amino nitrogen and, zinc and carboxylate groups of *Catharanthus roseus* molecules. It confirms that the organic molecules of *C. roseus* is bound to the Zn either through amino (or) carboxylate group (or) both the broad ones at 1351 and 1523 cm^{-1} correspond to symmetric and asymmetric C = 0 stretching vibrations of carboxylate group respectively. FT-Raman spectroscopic study confirmed that the carboxyl group of amino acid residues of *C.roseus* has a strong binding ability with Zinc Suggesting the formation of ZnO-NPs.

Biosynthesized ZnO-NPs were analyzed for their antibacterial activity against two gram negative (*Escherichia coli* – ATCC 25922, *Pseudomonas aeuroginosa* – ATCC 15442) bacteria and two gram positive bacteria (*Staphylococcus aureus* – ATCC 6538, *Bacillus thuringiensis* – ATCC 10792) by disk diffusion method. The highest antimicrobial activity was observed against *Pseudomonas aeuroginosa* followed by *Staphylococcus aureus* (**Table 2 &Fig.6**). The results were compared with the ciprofloxacin as a positive control, Zn acetate as a negative control and plant extract as a control.

Conclusion

The Biosynthesized ZnO-NPs were great interest due to their eco-friendliness, economic prospects, and feasibility and Short time for synthesis may be wide range of applications in nanomedicine, catalysis medicine mainly for the pharmaceutical industry for development of new formulations against the microbial strains which are developing resistance to traditional antibiotics.

Acknowledgement

The first Author is highly grateful to DST for sanctioning of Inspire Fellowship.

Table 1: EDAX of synthesized elements during the formation of ZnO-NPs through the leaves of *C. roseus*

Element	Weight	Atomic (%)
OK	07.62	25.21
ZnK	92.38	74.79
Matrix	Correction	ZAF

Table 2: *In vitro* antibacterial activity of ZnO-NPs of leaf extract of *C. roseus*

S. No.	Name of the tested bacteria		Zone of inhibition (mm)		
		Ciprofloxacin	Zinc acetate	plant extract	ZnO-NPs
1.	*Pseudomonas*	27 ± 0.7	13 ± 0.2	5 ± 0.0	13 ± 0.4
2.	*Staphylococcus*	12 ± 0.22	12 ± 0.15	5 ± 0.0	12 ± 0.1
3.	*E. coli*	19 ± 0.1	18 ± 0.1	5 ± 0.0	10 ± 0.1
4.	*Bacillus*	24 ± 0.4	9 ± 0.4	5 ± 0.0	12 ± 0.02

± indicate the S.E average of triplicates

(a) **(b)**

Fig 1: Synthesis of ZnO-NPs (Colour change) by using leaf extract of *C. roseus*
(a) Leaf extract, (b) Treated with Zinc acetate

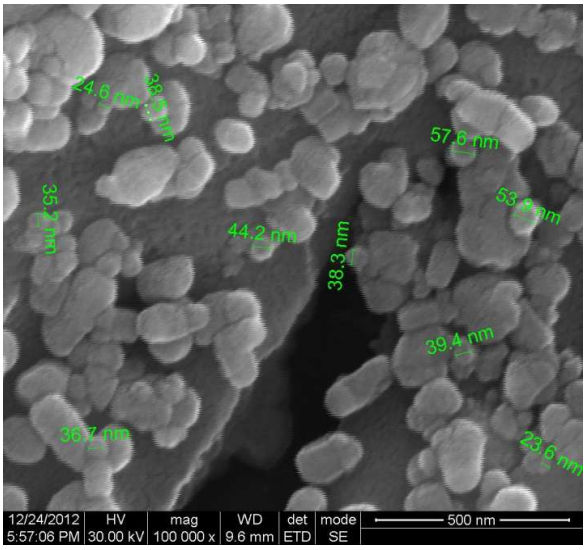

Fig 2: SEM images of ZnO-NPs synthesized by using leaf extract of *C. roseus*

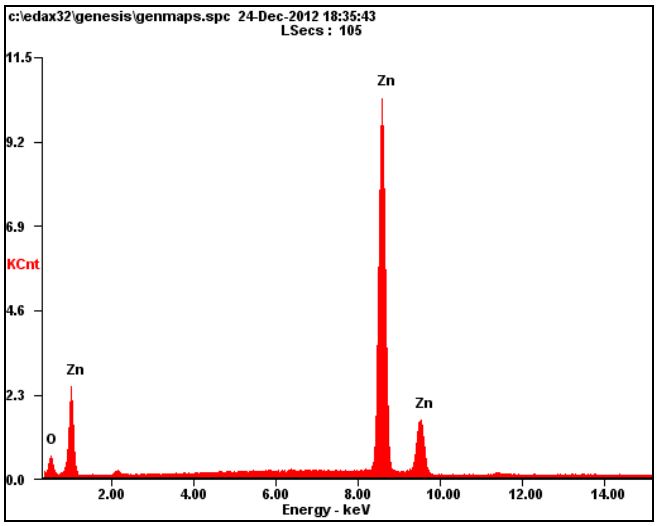

Fig 3: EDAX images of ZnO-NPs synthesized by using leaf extract of *C. roseus*

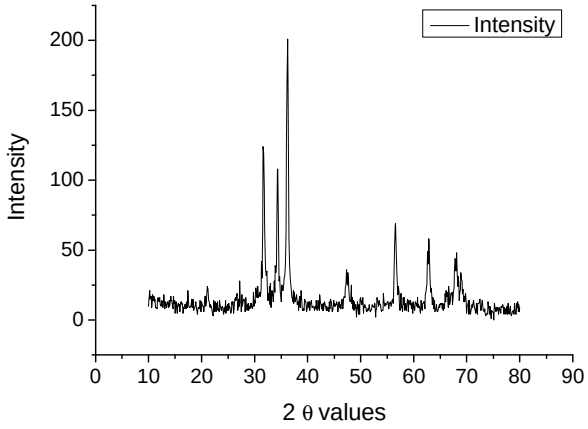

Fig 4: XRD pattern of ZnO-NPs of *C. roseus*

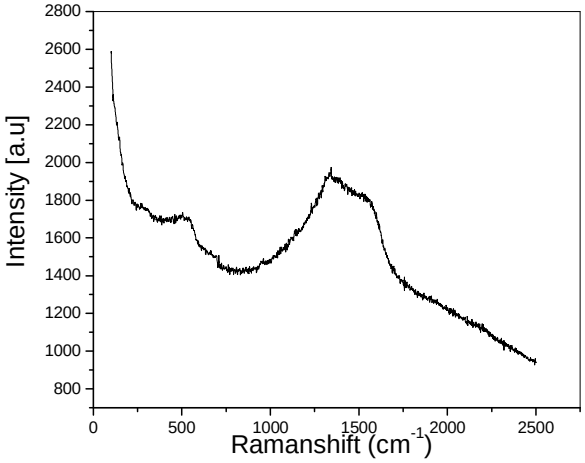

Fig 5: FT-IR spectra of ZnO-NPs synthesized by using the leaf extract of *C.roseus*

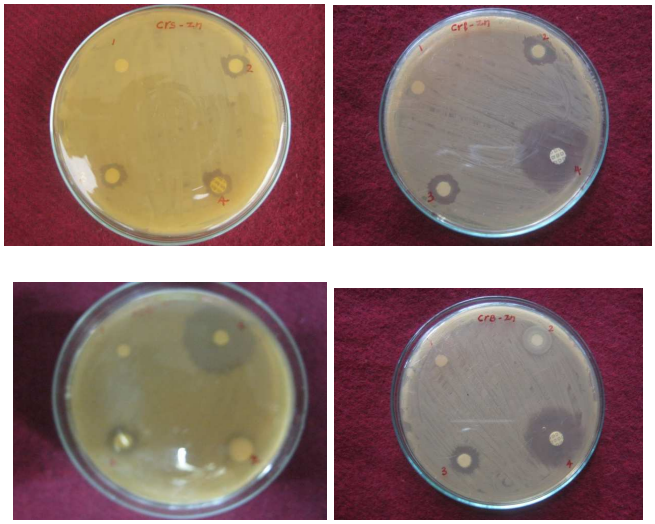

Fig 6: Antibacterial activity of leaf extract of *C. roseus*

a) *Staphylococcus* and b) *Pseudomonas* d) *Bacillus*, c) *E. coli*
1) Plant extract, 2) Zinc acetate, 3) ZnO-NPs, 4) Ciprofloxacin

References

1) Zhang M, Liu M, Prest H and Fischer's. Nanoparticles secreted from iry rootlets for surface climbing. Nanoletters, 2008; 8: 1277-1280.

2) Jeong S, Yeo S and Yis. Antibacterial characterization of silver nanoparticles

against *E. coli* ATCC-15224. Journal of Material Science, 2005; 40: 5407.

3) Savithramma N, Lingarao M, Rukmini K and Suvarnalatha DP. Antimicrobial activity of silver nanoparticles synthesized by using medicinal plants. International Journal of Chem Tech Research, 2011; 3: 1394-1402.
4) Singhal M., Chhabra V., Kang P and Shah DO. Materials Research Bulletin 1997; 32: 239-247.
5) Dhermendre K, Tiwari and Behari J, Prasenjit sen. Application of nanoparticles in waste water treatment. World Applied Science Journal, 2008; 3: 417-433.
6) Cai A-J, Wang Y-L, Xing S-T, Du L-Q, Maz-C. Tuned morphologies of DNA-assisted ZnO struggling against pH. Ceran Int 2013; 39: 605-609.
7) Nouroozi F, Farzaneh F. Synthesis and characterization of brush-like ZnO nanorods using albumin as biotemplate. J Braz Chem Soc 2011; 22: 484-488.
8) Jha AK, Kumar V, Prasad K. Bio synthesis of metal and oxide nanoparticles using Orange juice. Journal of Bionanoscience 2011; 5(2): 162-166.
9) Yu J, Yang J, Liu B, Ma X. Preparation and characterization of glycerol plasticized-pea starch/ ZnO – carboxy methyl cellulose sodium nano composites. Bio Resour Technol 2009; 100: 2832-41.
10) Tomczak MM, Gupta MK, Drummy LF, Rozenbak SM, Naik RR. Morphological control and assembly of zinc oxide using biotemplate. Acta Biomater 2009; 5: 876-82 Pubmed Abstract.
11) Ferracane, Jack L. Materials in Density: Principles and Applications. Lippincott Williams & Willkins, 2001.
12) Van Noort Richard Introduction to Dental Materials. 2nd Edition. Elsevier Health Science, 2002.
13) Adeyemi O, Okpo S, Adesanya A. Gastrointestinal activity of the aqueous extract of a Nigerian herbal preparation. West Afr J Pharmacol 2003; 19: 22-27.
14) Abdulkarim A, Sadiq Y, Gabriel OA, Abdukadir UZ, Ezzeldin MA. Evaluation of five medicinal plants used in diarrhea treatment in Nigeria. J Ethno Pharmacol 2005; 101: 27-30.
15) Appidi JR, Grierson DS, Afolayan. Ethnobotanical study of plants used for the treatment of diarrhea in the Eastern cape, South Africa Pakistan J Bio Sci 2008; 11: 1961-1963.
16) Chatopadhyay RR, Banerjee RN, Sarkar SK, Ganguly S, Basu TK. Antiinflammatory and acute toxicity studies with the leaves of *Vinca rosea* Linn in experimental animals. Ind J Physiol Pharmacol 1992; 36: 291-292.
17) Singh SN, Vats P, Suri, Shyam R, Kunria MM, Ranganathan S, *et al.* Effect on antidiabetic extract of *Catharanthus roseus* on enzymic activities in Streptozotocin induced diabetic rats. J Ethno Pharmacol 2001; 76: 269-77.
18) Ei-Sayed M, Verpoorte R, and Methyl jasmonate accelerates catabolism of nanoterpenoid indole alkaloids in *Catharanthus roseus* during leaf processing. Fitoterpia 2005; 76: 83-90.
19) Jaleel CA, Gopi R, Paneerselvam R, Alterations in non-enzymatic antioxidant components of *Catharanthus roseus* exposed to paclobutrazol, gibberellic acid and *Pseudomonas fluorescens*. Plant Omics J 2009; 2: 30-40.

20) Vidya C, Shilpa Hiremath, MN Chandra Prabha, M A Lourdu Antony raj, Indu VenuGopal, Aayushi Jain and Kokil Bansali, Green synthesis of Zinc Oxide Nanoparticles by *Calotropis gigantea*, 2013.

21) Gunalan Sangeetha, Sivaraj Rajeshwaria, Rajendran Venckatesh. Green synthesis of zinc oxide nanoparticles by aloe barbadensis Miller leaf extract: Structure and optical properties, *Materials Research Bulletin*, 2011; 46: 2560-2566.

22) Gamble JS, Flora of the presidency of Madras, printed by S.N.Guha Ray at Sree Saraswaty Press Ltd., Achargy prafulla Chandra Road, Calcutta, 1957.

23) Anonymous, Pharmacopiea of India (The Indian Pharmacopiea) 3rd ed. Delhi: Ministry of Health and Family Welfare, 1996.

Article History: ----------

Date of Submission: 17-01-2014

Date of Acceptance: 13-02-2014

Conflict of Interest: NIL

Source of Support: NONE