International Journal of Botany Studies
www.botanyjournals.com
ISSN: 2455-541X
Received: 13-11-2021, Accepted: 28-11-2021, Published: 15-12-2021
Volume 6, Issue 6, 2021, Page No. 1597-1600

Synthesis, characterization and antimicrobial activity of zinc oxide nanoparticles from *Acacia farnesiana* (L.) leaf extract

Dwarkanath V, Yathisha NS, Santhosh DB, Sharathcnadra RG
Department of Studies and Research in Biotechnology, Tumkur University, Tumakuru, Karnataka, India

Abstract
Metal and semiconductor nanoparticles synthesis is an growing field of interest, owing to possible applications in the development of new technologies. Biologically synthesized nanomaterial has recently evolved into an significant branch of nanotechnology. The present work identified the synthesis of Zinc Oxide Nanoparticles (ZnO NPs) using *Acacia farnesiana* (L.) leaf aqueous extract, antifungal and its antimicrobial activities. The nanoparticles were characterized by the study of UV-visible spectroscopy, Scanning electron microscope (SEM), and X-ray diffraction (XRD). Furthermore, ZnO NPs showed significant bactericidal effect against both Gram+ve and Gram-ve bacterial pathogen strains in an agar well diffusion method. These findings emphasize that such biocompatible ZnONPs with multifunctional activities may find their application in the field of nanomedicine.

Keywords: nanoparticles, zinc oxide nanoparticles, antimicrobial activity, SEM, XRD

Introduction

Development of efficient remediation processes of water borne pathogens, such as Shiga toxin producing *Escherichia coli* has generated significant scientific interest to design novel biocidal or disinfecting agents to complement conventional antibiotics (Dutta *et al*., 2013) [3]. Antibacterial agents can be broadly classified into two types, organic and inorganic. Organic antibacterial materials are often less stable particularly at high temperatures and/ or pressures compare to inorganic antibacterial agents (Sawai, 2003) [13]. Inorganic materials such as metal and metal oxides have attracted more attention over the past decade due to their ability to withstand harsh process conditions and are generally regarded as safe materials to human beings and animals (Jacob *et al*., 2013) [4]. The inorganic NPs such as silver, gold, copper, CuO, TiO2 and ZnO have profound antibacterial activities. Among the inorganic NPs, ZnO NPs are of particular interest because they can be prepared easily inexpensive and safe material for human beings and animals. They are extensively used in the formulation of health care products (Manna *et al*., 2013) [8]. ZnONPs has entered the scientific spotlight for its semiconducting properties, unique antibacterial, anti-fungal, wound healing and UV filtering properties, high catalytic and photochemical activity (Nagajyothi *et al*., 2013) [9].

There are several methods available for synthesis of ZnO NPs such as Wet chemical, chemical Micro emulsion, Hydrothermalmal, Vapor phase process, Solvothermal, Microwave- assisted combustion, chemical, Direct precipitation and Sono-chemical (Sangeetha &Thambavani, 2013) [12]. Recently increasing awareness towards green chemistry and other biological processes have led to the development of an eco-friendly approach for the synthesis of nanoparticles (Rosi&Mirkin, 2005) [11]. This biological approach appears to be a cost effective alternative to Conventional physical and chemical methods of synthesis.

Biosynthesis of ZnO NPs was recorded from plants like *Aspergillus, Citrus aurantifolia, Ocimum sanctum, Solanum nigrum, Ocimumbasilicum*, etc. Biosynthetic and eco friendly technology is believed to be non-toxic, bio-safe and bio-compatible for the synthesis of ZnONPs. This is especially suitable for bio-medical applications such as drug distributors, cosmetics and fillings in surgical materials (Lakshmeesha *et al*., 2014) [6]. Methods for green synthesis of ZnO NPs are so many. These methods have advantages in the present work such as consuming time much less and not using any intermediate substance (any base groups), low cost precursor, high purity and quantity product, handling procedure is uncomplicated and does not require expensive equipment and environment.

A. farnesiana (L.) is an evergreen tropical-American tree. Acacia is the generic name of the family Mimosaceae species in the genus Acacia. A's snap, and leaves. *A. farnesiana*is crushed and boiled and inhaled by malaria patient (Jain *et al*., 2009) [5]. In India, *A. farnesiana* is known in local area as Mulla tumma, Kamputumma and it is also widely known as Fragrance and Sweet Acacia. Grown in all of India, and often planted in gardens. If we see its yield, it produces much for local use in India and other Eastern countries and from the third year Trees start to bloom, mainly from November to March. *A. farnesiana* L. grown in all of India and often planted in gardens.If we see its yield, it produces much for local use in India and other Eastern countries and from the third year Trees start to bloom, mainly from November to March. This plant's bark is utilized as astringent and demulcent. The leaves and roots are for medicinal use (Lewis, 2005) [7].

Material and methods

Preparation and extraction of plant extract

Fresh and healthy *A. farnesiana* leaves were collected and cleaned twice with tap water to remove dust particles, then washed twice with distilled water. Washed leaves were dried in shade,away from direct sunlight for 20 days, dried leaves were ground to a fine powder using a pestle and mortar. 50g of *A. farnesiana* leafextract was subjected to

Soxhlet extraction using ethanol for two hours. After filtering, the extract was concentrated using a rotary flash evaporator at 45°C. According to previously published standards, the extract was kept at 4ºc in airtight bottles for further studies.

Synthesis of ZnO nanoparticles

For the synthesis of zinc oxide nanoparticles, different concentrations of ethanol extract were used. The aqueous mixture of 1.4878 g of zinc nitrate ($Zn(NO_3)2.6H2O$), 15ml of distil water, and different concentrations of *A. farnesiana* leaf extract (Table-01) were separately stirred for 2 hours at 90°C. Then the mixtures were transferred to four silica crucibles, which were placed inside a preheated muffle furnace at 900 °C for 30 min. The reaction proceeds and finally forms a nanocrystalline ZnO. The products were calcined at 500°C for 3 hours and grinded to fine powder were used for further characterization.

Table 1: Different concentrations of Zinc Nitrate

SL NO	$Zn(NO_3)_2.6H_2O$ (in g)	Ethanol Extract (in g)	Distilled Water (in ml)	Sample Names
1	1.4878	0.25	15	ZN1
2	1.4878	0.50	15	ZN2

Characterization of ZnOnanoparticles

The phase identity and crystalline size of ZnONps were characterized by Shimadzu X-ray diffractometer (PXRD-7000) using Cu-K radiation of wavelength λ = 1.541 A. ˚ The absorption spectrum of the sample was measured on a Shimadzu UV-1800 UV–visspectrophotometer. Morphologicalfeatures were studied by using Hitachi-7000 Scanning Electron Microscopy (SEM).

Antibacterial activity

Antibacterial activities of the ZnOnanoparticles were investigated using pathogenic bacterial strains namely Gram+ ve bacteria *Staphylococcus aureus* [NCIM-5022], Gram-ve bacteria *Pseudomonas aeruginosa* [NCIM-2242] and *Pseudomonas desmolyticum* [NCIM-2028], by agar well diffusion method (Suresh *et al*., 2015). Nutrient Agar plates were prepared and swabbed using Sterile L-shaped glass rod with 100 µl of 24 h mature broth cultures of individual bacterial strains. The wells were made by using a sterile cork borer (6mm) wells are created into the each Petri-plates. Varied concentrations of ZnONPs (50 and 100 µg/well) were used to assess the activity of the nanoparticles. The compounds were dispersed in sterile water and it is as a negative control and simultaneously the standard antibiotics Ciprofloxacin (3µg/30µl) (Hi Media, Mumbai, India) as positive control was tested against the bacterial pathogens. Then the plates were incubated at 37 C for 36 h. After the incubation period. The zone inhibition of each well was measured in mm and values were noted. Triplicates was maintained in each of the compound and the average values are calculated for the ultimate antibacterial activity

Results and Discussions

UV-Visible Spectrophotometer Analysis

The reduction of Zinc nitrate to zinc oxide is analyzed by UV-Visible Spectrometer. In case ZnO nanoparticles are synthesized in green way the band is observed around 300-400nm. This is the 'Surface Plasmon Resonance' band and this band is ascribed to the valence electrons of ZnO arranged in Nanoparticles. The metal nanoparticles have free electrons that give the absorption band SPR (Surface Plasmon Resonance), due to the combined vibration of metal nanoparticles electron in resonance with light wave. From our studies we found the SPR peak for *Acacia farnesiana* peak at range between 300-550 nm (Fig. 01). The reduction of the metal ions may occur fairly rapidly, after addition of metal ions to the plant extract. The metal particles were observed to be stable in solution.

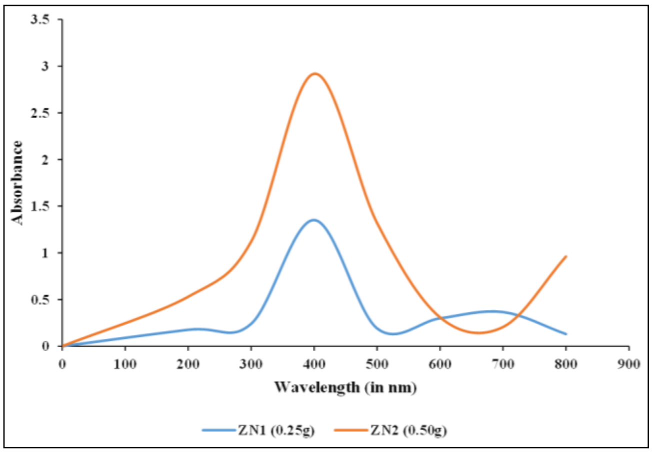

Fig 1: UV–vis spectra of ZnO nanoparticles solution. with different concentration of plant extract (ZN1) 0.25g, (ZN2) 0.50g.

Scanning Electron Microscopy

The synthesizedZnO nanoparticles were subjected to SEM to get a profound insight of the shape and size of the compound formed. The size of the particles ranges from 90-110nm. The morphology of the particles obtained by annealing at 120ºC, where studied by using SEM. SEM images of ZnOnanoparticlesclearly shows that the particles are almost spherical in nature which is free from agglomeration.Further it is observed that micro structure is independent concentration of plant extract.

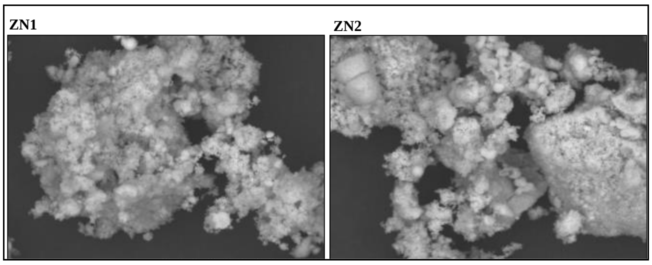

Fig 2: SEM images of ZnO nanoparticles show spherical in shape with different concentration of plant extract (ZN1) 0.25g, (ZN2) 0.50g.

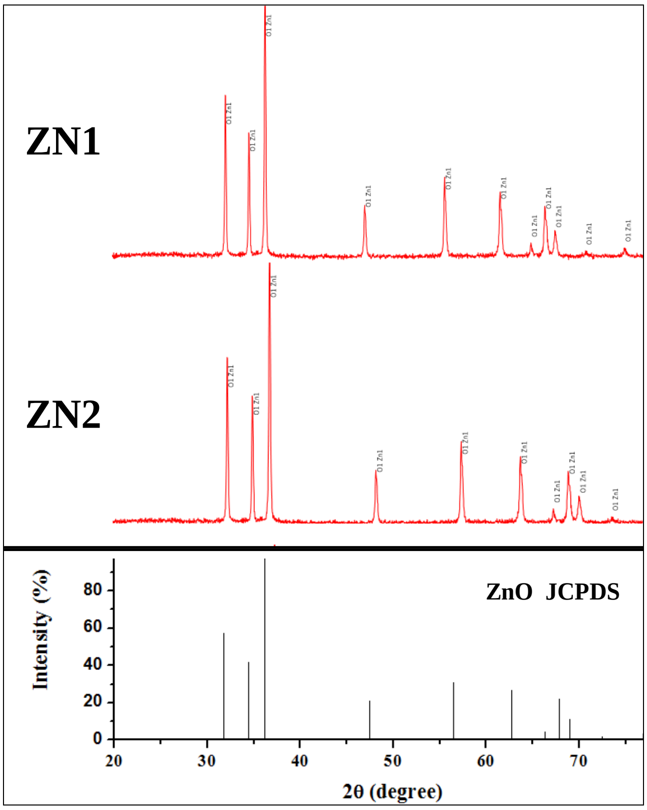

Fig 3: XRD pattern of prepared ZnO nanoparticles with different concentration of plant extract (ZN1) 0.25g, (ZN2) 0.50g.

Figure 03 represents the XRD pattern of ZnO nanoparticles. The fact that the XRD peaks have a distinct line broadening suggests that the prepared NPs [(ZN1) 0.25 g, (ZN2) 0.50 g] contains nanoscale particles. We determined intensity of peak, location, and width using XRD patterns analysis, as well as full-width at half-maximum (FWHM) data. The diffraction peaks located at 31.84°, 34.52°, 36.33°, 47.63°, 56.71°, 62.96°, 68.13°, and 69.18° have been keenly indexed as spherical ZnO-NPs (Jain *et al*., 2009; Ramasami *et al*., 2015) [5, 10] with lattice constants a=b=0.324 nm and c=0.521 nm (JPCDS card number: 36-1451) (Zargar *et al*., 2011) [16], Furthermore, the absence of any identifiable XRD peaks other than ZnO peaks suggests that the produced NPs was devoid of contaminants. The diameter of the produced ZnO nanoparticles was determined using the Debye-Scherrer formula. (Sondi&Salopek-Sondi, 2004) [14] d=0.89$\lambda\beta$cosθ,(1)where 0.89 is Scherrer's constant, λ is the wavelength of X-rays, θ is the Bragg diffraction angle, and β is the full width at half-maximum (FWHM) of the diffraction peak corresponding to plane ⟨101⟩. The average particle size of the sample was determined to be 16.21 nm, which was calculated using Scherrer's formula as from FWHM of the more significant peak corresponding to the 101 plane at 36.33°.

Antibacterial Studies

The antibacterial activity of the biosynthesized ZnO nanoparticles are evaluated against four pathogenic bacterial strains namely Gram+ ve bacteria *Staphylococcus aureus*, Gram-ve bacteria *Pseudomaonas aeruginosa* and *Pseudomonas desmolyticum* using agar well diffusion method. In this method, ZnONPs significant antibacterial activity on all the bacterial strains. Zone of inhibition with the concentration 50 and 100 µg per well is shown in (Figure.4). As can be estimated from Table-1 that ZnONPs prepared from the *Mimosapudica*was found to possess very significant antibacterial activity towards the different pathogenic bacterial strains. However, higher concentration of ZnONPs were significant in bactericidal effect. In a previous study, similar antibacterial activity was exhibited by green ZnONPs synthesized from *Vitex negundo* extract against gram negative E. coli and gram positive*S. Aureus* (Azizi *et al*., 2014) [2]. In another report, papaya fruit derived green ZnONPs have been shown to display effective antibacterial activity against *E. coli* and *Pseudomonas aeruginosa* and the activity was comparable to that of standard antibiotics (Jain *et al*., 2009) [5]. The ZnONPs exhibit antibacterial activity by attaching to the bacterial cell membrane (Sondi&Salopek-Sondi, 2004) [14]. Since the bacterial plasma membrane is the site of respiratory chain components, energy transducing systems and for active transport of molecules and ions (Abdul Salam *et al*., 2014) [1]. any modification in the structure of the membrane would eventually result in inhibition of bacterial growth.

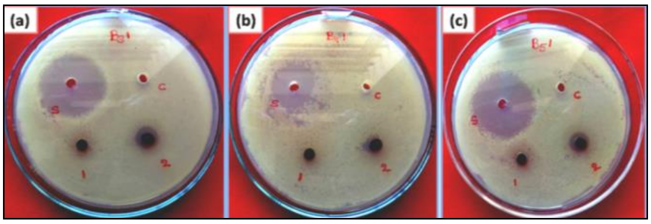

Fig 4: Shows the Zone of inhibition of biosynthesized ZnO NPs against bacterial strains such as (a*) P. aeruginosa* (b) *S. aureus* (c) *P. desmolyticum.*

Acknowledgement

Authors thank to Tumkur University for providing infrastructure facilities.

References

1. Abdul Salam H, Sivaraj R, Venckatesh R. Green synthesis and characterization of zinc oxide nanoparticles from Ocimumbasilicum L. var. Purpurascens Benth.-Lamiaceae leaf extract. Materials Letters,2014:131:16-18. https://doi.org/10.1016/j.matlet.2014.05.033
2. Azizi S, Ahmad M B, Namvar F, Mohamad R. Green biosynthesis and characterization of zinc oxide nanoparticles using brown marine macroalga Sargassum muticum aqueous extract. Materials Letters,2014:116:275-277. https://doi.org/10.1016/j.matlet.2013.11.038
3. Dutta RK, Nenavathu BP, Gangishetty MK. Correlation between defects in capped ZnO nanoparticles and their antibacterial activity. Journal of Photochemistry and Photobiology B, Biology,2013:126:105-111. https://doi.org/10.1016/j.jphotobiol.2013.07.010
4. Jacob J, Raghuraman B, Gopish AR. Aspergillus niger mediated synthesis of ZnO nanoparticles and their antimicrobial and invitro anticancerous activity. World Journal of Pharmaceutical Research,2013:3:3044-3054.
5. Jain D, Daima H, Kachhwala S, Kothari S. Synthesis of Plant-Mediated Silver Nanoparticles using Papaya Fruit Extract and Evaluation of their Anti Microbial Activities. Digest Journal of Nanomaterials and Biostructures,2009:4,557-563.
6. Lakshmeesha TR, Sateesh MK, Prasad BD, Sharma SC, Kavyashree D, Chandrasekhar M *et al*. Reactivity of

Crystalline ZnO Superstructures against Fungi and Bacterial Pathogens: Synthesized Using Nerium oleander Leaf Extract. Crystal Growth & Design,2014:14(8):4068-4079. https://doi.org/10.1021/cg500699z
7. Lewis GP. Legumes of the world. Royal Botanic Gardens, Kew, 2005.
8. Manna J, Begum G, Kumar KP, Misra S, Rana RK. Enabling Antibacterial Coating via Bioinspired Mineralization of Nanostructured ZnO on Fabrics under Mild Conditions. ACS Applied Materials & Interfaces,2013:5(10):4457-4463. https://doi.org/10.1021/am400933n
9. Nagajyothi P, An TN, Tvm S, Lee J, Lee D, Lee K. Green route biosynthesis: Characterization and catalytic activity of ZnO nanoparticles. Materials Letters,2013:108:160-163. https://doi.org/10.1016/j.matlet.2013.06.095
10. Ramasami AK, Raja Naika H, Nagabhushana H, Ramakrishnappa T, Balakrishna GR. Tapioca starch: An efficient fuel in gel-combustion synthesis of photocatalytically and anti-microbially active ZnO nanoparticles. Materials Characterization, 2015, 99. https://doi.org/10.1016/J.MATCHAR.2014.11.017
11. Rosi NL, Mirkin CA. Nanostructures in Biodiagnostics. Chemical Reviews,2005:105(4):1547-1562. https://doi.org/10.1021/cr030067f
12. Sangeetha DG, Thambavani D. NOVEL SYNTHESIS AND CHARACTERIZATION OF ZNO NANOPARTICLES BY OCIMUM SANCTUM. Asian Academic Research Journal of Multidisciplinary,2013:1:164-180.
13. Sawai J. Quantitative evaluation of antibacterial activities of metallic oxide powders (ZnO, MgO and CaO) by conductimetric assay. Journal of Microbiological Methods,2003:54(2):177-182. https://doi.org/10.1016/S0167-7012(03)00037-X
14. Sondi I, Salopek-Sondi B. Silver nanoparticles as antimicrobial agent: A case study on E. coli as a model for Gram-negative bacteria. Journal of Colloid and Interface Science,2004:275(1):177-182. https://doi.org/10.1016/j.jcis.2004.02.012
15. Suresh D, Nethravathi PC, Udayabhanu Rajanaika H, Nagabhushana H, Sharma SC. Green synthesis of multifunctional zinc oxide (ZnO) nanoparticles using Cassia fistula plant extract and their photodegradative, antioxidant and antibacterial activities. Materials Science in Semiconductor Processing,2015:31:446-454. https://doi.org/10.1016/j.mssp.2014.12.023
16. Zargar M, Hamid AA, Bakar FA, Shamsudin MN, Shameli K, Jahanshiri F *et al*. Green Synthesis and Antibacterial Effect of Silver Nanoparticles Using Vitex Negundo L. Molecules,2011:16(8):6667-6676. https://doi.org/10.3390/molecules16086667