



Green synthesis of zinc oxide nanoparticles using *Atalantia monophylla* leaf extracts: Characterization and antimicrobial analysis

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ABSTRACT

In the recent decades, nanotechnology has become an important research field of modern material science. Green synthesized nanoparticles have garnered wide interest due to its inherent features like rapidity, eco-friendly and cost-effectiveness. For the first time, Zinc Oxide nanoparticles were successfully synthesized using *Atalantia monophylla* leaf extract in the present's investigation. The biosynthesized Zinc Oxide nanoparticles were characterized by UV-Vis spectrophotometer, Fluorescence spectrometer (PL) and their peaks were identified to be at 352 and 410 nm respectively. The morphology of the Zinc Oxide nanoparticles was characterized by TEM with EDAX. The X-ray diffraction (XRD) studies showed the crystalline nature and revealed the purity of Zinc Oxide nanoparticles. FTIR spectroscopy was used to analyze the specific functional groups responsible for reduction, stabilization and capping agents present in the nanoparticles. The success-ability of bacteria and fungi with Synthesized ZnO nanoparticles tested using agar well diffusion method were discussed. The bacterial and fungal destruction was better for ZnO nanoparticles than reported for plant extracts and standard drugs. Thus this study proves that Zinc Oxide nanoparticles would contain natural anti-microbial agents through green synthesis which may serve for the production of drugs for antimicrobial therapeutics.

1. Introduction

In the past decade, metal oxide nanoparticles have been found to be promising in the field of nanotechnology because of their extremely small size and large surface area. The metal oxides have a high fraction of atoms and they are accountable for their captivating properties like antimicrobial, magnetic and catalysis [1]. The various methods adopted for synthesizing Zinc Oxide nanoparticles are viz., Sol-gel processing, homogeneous precipitation, organo-metallic synthesis, spray pyrolysis, thermal evaporation, microwave methods, mechanical milling and mechano-chemical synthesis [2]. However, these methods are usually expensive, labour-intensive and unsafe to the environment. The presence of some toxic chemicals involved in the chemical methods, may have hazardous effects in medical applications [3]. Hence, there is a need for green chemistry routes which has emerged as sources of an alternate, low cost and eco-friendly nanoparticle production. Zinc Oxide nanoparticles are attracting as a prodigious material for its wide range of applications in electronics, optics, opto-electronics, biomedical and antimicrobial therapies [4–13]. More over Zinc oxide has strongly attracted the attention of the scientific community as a future candidate

for pharmaceutical sector due to its unique properties [14] (Vijaykumar et al., 2018). Owing to their unique advantage, these ZnO have been found in a wide range of applications and hence they received much more recognition in the biomedical field.

Previously, many studies have been reported for the green synthesis of Zinc Oxide nanoparticles using various leaf extracts such as *Anisochilus carnosus* [15], *Solanum nigrum* [16], *Tamarindus indica* [17], *Plectranthus amboinicus*, *Vitex negundo* [18], *Azadirachta indica* [19], *Plectranthus amboinicus* [20], *Hibiscus rosa-sinensis* [21], *Lantana aculeate* [22] and *Passiflora caerulea* [23]. Hence, in the present study *Atalantia monophylla* was considered due to its novelty and known medicinal properties for the synthesis of Zinc Oxide nanoparticles using methanolic leaf extracts.

A. monophylla (L.) DC is a small tree belonging to the family Rutaceae. It is commonly known as “Kattunaregam”, Nimbu, Aranyanimbuka, Banjamir nimbu, Bannimbu, and “Kattu-elumichai” and is rare and endemic to southern peninsula. Traditionally, various parts have been used for several purposes like the treatment of chronic rheumatism, paralysis and inflammation. The leaves and bark of this plant are used in the treatment of diabetes [24], antioxidant activities

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[25], immuno-modulatory [26], and antibacterial activities [27].

Antimicrobial resistance has spread globally as a major threat and hence it is essential that we need novel therapeutic agents to replace antibiotics in curing bacterial infections. Green synthesized nanoparticles from antimicrobial sources do have an additional property of self functionalization of molecular drugs on the nanoparticles as indicated by their enhanced anti bacterial effect. Hence this method has been employed in the current study which has revealed the production of better antimicrobial nanoparticles and be further extended for antimicrobial therapies.

Therefore, the present study has utilized *A. monophylla*, a common medicinal plant, to synthesize and characterize the Zinc Oxide nanoparticles from the methanolic leaf extracts. Furthermore, purified, characterized Zinc Oxide nanoparticles were evaluated by agar well diffusion method against the pathogenic organisms.

2. Materials and methods

2.1. Collection and preparation of plant extract

Atalantia monophylla leaves were collected from the campus of A.V.V.M Sri Pushpam College, Poondi Tamil Nadu, and India. The collected plant was identified by Rapinat herbarium, ST.Joseph College, Tiruchirappalli, India and voucher number (PHC169) was deposited at A.V.V.M Sri Pushpam College Poondi for future reference. The leaves were rinsed with purified Milli- Q water to eliminate the unwanted impurities, particulate suspensions and then shade dried for 10 days to be made into a good powder using the electronic blender.

Ten grams of leaf powder was mixed with 100 ml of Milli- Q water and kept in water bath at 60 °C for 20 mins. Then the extracts were cooled to room temperature and filtered using Whatmann No. 1 filter paper. The extract was stored in a refrigerator for further studies [1].

2.2. Synthesis of zinc oxide nanoparticles

The Zinc Oxide nanoparticles were prepared by using a 0.1 M Zinc acetate dehydrates solution slowly mixed with 25 ml of the leaf extracts in a magnetic stirrer at 60 °C for 2 h. Then, the light yellow colour appeared and allowed to settle for 8 h. The particles were separated by centrifugation at 6000 rpm for 20 min, washed with distilled water followed by methanol to remove the unwanted impurities. Finally, powder of Zinc Oxide nanoparticles were acquired after overnight drying the purified precipitant of 80 °C in a hot air oven.

2.3. Characterization of zinc oxide nanoparticles

The absorption of UV-Vis spectrum was recorded in various wavelengths ranging from 300 to 700 nm using a UV-vis spectrometer (H U-2001) and the properties emission were analyzed by Fluorescence spectrometer (Jascov-650 spectrophotometer). The X-ray diffraction (XRD) of ZnO nanoparticles and leaf extracts were recorded by powder X-ray diffraction using K_{α} radiation with about $2\theta - 80^\circ$ (Model -D8 Advance, BRUKER, Germany). FT-IR (Fourier Transform Infra-Red Spectroscopy) was performed to analyze the functional groups of ZnO nanoparticles. The microstructure of the sample was analyzed by Scanning Electron Microscope (SEM, Hi-tech model s-3400n) with EDAX (Energy dispersive X-ray) and Transmission Electron Microscope (TEM, Hitachi H-7100) was exploited to prove the occurrence of pure elemental Zinc Oxide nanoparticles.

2.4. Antimicrobial assay

In the present study, Zinc Oxide nanoparticles synthesized using methanolic leaf extract of *A. monophylla* were assessed for their antimicrobial activities by agar well diffusion method [28]. Microorganisms like *Bacillus subtilis* MTCC 121, *Bacillus cereus* MTCC 430,

Staphylococcus aureus MTCC 737, *E. coli* MTCC 1303, *Pseudomonas aeruginosa* MTCC 429, *Klebsiella pneumoniae* MTCC 109, *Candida albicans* MTCC 227 and *Aspergillus niger* MTCC 281 were obtained from Microbial Type Culture Collection(MTCC), Chandigarh, India through Eumic Analytical Lab and Research Institute, Trichy, India. The pure culture of organisms was sub cultured on Muller-Hinton agar (MHA), Sabouraud Dextrose Agar (SDA) at 35 °C and 30 °C for bacteria and fungi respectively. Wells of 6 mm were punched in MHA and SDA, with the help of micropipette 20 μ l of the samples were poured in to wells of all plates. For comparison, plates of the same diameter with 20 μ l Ciprofloxacin (5 μ g/disc) for Gram positive bacteria, Gentamicin (10 μ g/disc) for Gram negative bacteria, Nystatin (50 μ g/disc) for *C. albicans* and Amphotericin B (20 μ g/disc) for *A. niger* was used as positive control. The incubation period was maintained in $30 \pm 2^\circ\text{C}$ at 24–48 h for bacteria and $37 \pm 2^\circ\text{C}$ at 72–96 h for fungi respectively. Further, the zones of inhibitions were measured as the diameter or radius in mms. The assays were carried out in triplicate [29].

3. Results and discussions

3.1. Structural analysis

X- ray diffraction (XRD) pattern of green synthesized Zinc Oxide nano particles was recorded in the range of $20^\circ < 2\theta < 80^\circ$. The XRD patterns of ZnO nanoparticles were matched with the standard JCPDS file as shown in Fig. 1. In the XRD spectra of, Zinc Oxide nano particles displayed strong diffraction peaks corresponding to 100, 002, 101, 102, 110, 103, 200, 112 and 201 reflection lines of Zinc Oxide nanoparticles with a hexagonal wurtzite structure matched with the JCPDS card number 008, 79–2205 and 05–0664. The peaks in the XRD spectrum have sharp and narrow diffraction peaks indicating that the synthesized nanoparticles are pure and crystalline in nature. The Debye Scherrer's formula can be used for calculating the crystalline size of the prepared nanoparticles. The scherrer's formula can be written as follows

$$D = \frac{0.9\lambda}{\beta \cos \theta}$$

Where D is the crystallite size, λ is the wavelength of X-ray used (1.5406 Å), β is the full width at half maximum (FWHM) θ is the Bragg's angle. The average particle size of the prepared ZnO nanoparticles is 33.01 nm and also matched with the TEM micrograph of ZnO nanoparticles

3.2. Optical characterization

3.2.1. UV- visible absorption and emission spectrum studies

UV-Vis absorption study is a simple and most prominent method to

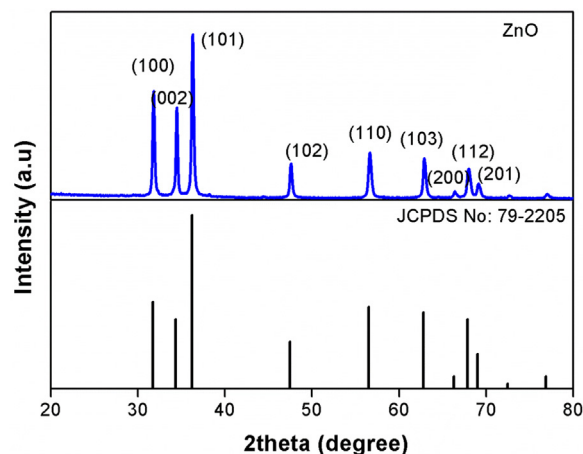


Fig. 1. XRD spectrum of *A. monophylla*/ZnO nano particles.

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