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Bio-Fabrication of zinc oxide nanoparticles using leaf extract of *Parthenium hysterophorus* L. and its size-dependent antifungal activity against plant fungal pathogens

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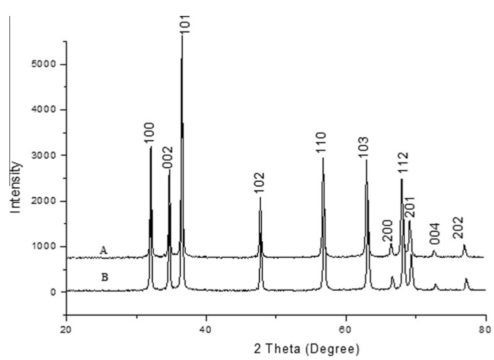
HIGHLIGHTS

- This paper reports on the synthesis of zinc oxide nanoparticles using weed plant extract by novel method.
- Highly stable, spherical and hexagonal zinc oxide nanoparticles are synthesized.
- The particle sizes and shapes are controlled by varying the *Parthenium* leaf extract concentrations.
- *Parthenium* mediated zinc oxide nanoparticles are proved to be good antifungal agents.
- Green synthesized nanoparticles are eco-friendly and have wide applications in biomedical and cosmetic industries.

GRAPHICAL ABSTRACT



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ABSTRACT

The study reports the synthesis and characterization of zinc oxide nanoparticles from weed plant by a novel method. The aim of this work is to synthesize zinc oxide nanoparticles from *Parthenium hysterophorus* L. by inexpensive, ecofriendly and simple method. Highly stable, spherical and hexagonal zinc oxide nanoparticles were synthesized by using different concentrations of 50% and 25% parthenium leaf extracts. Both the concentrations of the leaf extract act as reducing and capping agent for conversion of nanoparticles. Formation of zinc oxide nanoparticles have been confirmed by UV–Vis absorption spectroscopy, X-ray diffraction (XRD), Fourier trans-form infrared spectroscopy (FT-IR), scanning electron microscopy (SEM) and transmission electron microscopy (TEM) analysis with energy dispersive X-ray analysis (EDX). SEM, TEM and EDX analysis reveals that spherical and hexagonal zinc oxide nanoparticle sizes were 27 ± 5 nm and 84 ± 2 nm respectively and chemical composition of zinc oxide were present. We synthesized different sized zinc oxide nanoparticles and explored the size-dependent antifungal activity against plant fungal pathogens. Highest zone of inhibition was observed in 25 µg/ml of 27 ± 5 nm size zinc oxide nanoparticles against *Aspergillus flavus* and *Aspergillus niger*. *Parthenium* mediated zinc oxide nanoparticles were synthesized and proved to be good antifungal agents and environment friendly.

Introduction

Particles smaller than tens of nanometers in primary particle diameter (nanoparticles) are of interest for the synthesis of new materials because of their low melting point, special optical properties, high catalytic activity, and unusual mechanical properties compared with their bulk material counterpart [1]. Zinc oxide is attracting tremendous attention due to its interesting properties like wide direct band gap of 3.3 eV at room temperature and high exciton binding energy of 60 meV [2]. Zinc oxide is a unique

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material that exhibits semiconducting, piezoelectric, and pyroelectric properties and has versatile applications in transparent electronics, ultraviolet (UV) light emitters, piezoelectric devices, chemical sensors, spin electronics, personal care products, and coating and paints [3–5]. In fact, zinc oxide is non-toxic and chemically stable under exposure to both high temperatures and UV [6].

Plants and/or their extracts provide a biological synthesis route of several metallic nanoparticles which are more eco-friendly and allows a controlled synthesis with well-defined size and shape [7]. Green synthesis of gold nanoparticles using plants such as neem [8], lemon grass [9], *alfalfa* [10], *Emblica officinalis* [11], and *Cinnamomum camphora* [12] have been reported. The use of environmentally benign materials like plant leaf extract [13], bacteria [14], fungi [15] and enzymes [16] for the synthesis of silver nanoparticles offers numerous benefits of eco-friendliness and compatibility for pharmaceutical and other biomedical applications as they do not use toxic chemicals for the synthesis protocol. The enzymes [17], plant leaf extract [18] and bacteria [19] were vital role in green synthesis of zinc oxide nanoparticles.

Plants are often affected by plant pathogens such as fungi, bacteria and viruses which results in great agricultural loss [20]. Several conventional methods have been used for the control or reduce of these pathogens and each of these methods has one or more limitations (pesticides cause dangerous effect on the environment and human health). Thus, use of nanoparticles has been considered as an alternate and effective approach which is eco-friendly and inexpensive for the control of pathogenic microbes [21]. Nanoparticles have a vital role in the management of plant diseases as compared to synthetic fungicides [22]. Zinc oxide nanoparticles have also been proposed as an anti-microbial preservative for wood or food products [23–25]. The size dependent activity was studied in the range of 100 nm to 0.8 μm in *Staphylococcus aureus* and *Escherichia coli* and 12, 45, and 200 nm range in *E. coli*, however little is known about the activity of zinc oxide nanoparticles in the 10–300 nm range toward *S. aureus* [26,27].

Parthenium hysterophorus L. (family Asteraceae) is one of the 10 worst weeds in the world [28], which is a poisonous, pernicious and aggressive weed and is reported to have pharmacological properties against many diseases such as rheumatism, hepatic amoebiasis and tumours [29–31]. Parthenium is also known to cause allergy and toxicity in animals [32]. In this study, we synthesized two zinc oxide nanoparticles of varying sizes and tested their antifungal activity using well diffusion method.

Materials and methods

Materials

P. hysterophorus L. plants were collected from follow lands in and around Karpagam University, Eachanari, Coimbatore, India (11°16′N; 76°58′E) on 2011 before flowering stage. All the chemicals were obtained from sigma-aldrich chemicals, India. *Aspergillus flavus* (MTCC-7589), *Aspergillus niger* (MTCC-2587), *Aspergillus fumigatus* (MTCC-2550) *Fusarium culmorum* (MTCC-2090) and *Fusarium oxysporium* (MTCC-3379) were obtained from the Department of Microbiology, School of Life Sciences, Karpagam University, Coimbatore, India. The culture samples were maintained on potato dextrose broth at room temperature (aerobically) at 200 rpm shaking for 3 days.

Synthesis of zinc oxide nanoparticles using Parthenium leaf extract

Zinc nitrate was used as a precursor material for nanoparticles synthesis. The harvested plants were washed with tap water and whipped with tissue paper. The plant leaf samples were weighed carefully and finely cut. Samples were ground well by mortar and pistle using de-ionized water. The mixture of plant extract was heated with medium flame. After cooling this solution was filtered using filter paper (Whatman No. 42, Maidstone, England) and store in refrigerator for further studies.

Zinc nitrate was dissolved in de-ionized water under constant stirring. The 50% and 25% of plant extract was prepared with de-ionized water and the volume was made up to 250 ml. In order to, the zinc nitrate solution was added in plant extract under constant stirring. This mixture of the solution was kept under vigorous stirring at 90 °C for 4–5 h. After this process a green color precipitate was obtained. This mixture was centrifuged at 7000 rpm for 15 min and the green precipitate was discarded. The supernatant was stirred again at 150 °C for 1 h and a pale yellow color solid precipitate was obtained. The precipitate was washed with methanol and air dried. This product was annealed at 400 °C for 1 h. Finally, white color powder was obtained. The whole experiment was repeated three times. The methodology is given in the Supplementary information A1.

Characterization of zinc oxide nanoparticles

The aqueous zinc oxide nanoparticles were characterized by UV absorption spectra (UV-2450, shimadzu). The synthesized zinc oxide nanoparticles purity and grain size were identified by X-ray diffraction (Perkin–Elmer spectrum one instrument) Cu Kα radiations (λ = 0.15406 nm) in 2θ range from 20° to 80°. The powder sample of zinc oxide nanoparticles functional groups were analyzed by using Fourier trans-form infrared spectroscopy (Perkin–Elmer 1725×). The nanoparticles size and shape were characterized by using scanning electron microscopy (Model JSM 6390LV, JOEL, USA). The nanoparticles average size and size distribution were determined by transmission electron microscopy (JEOL JEM-3100F). The samples analyzed for energy dispersive X-ray analysis (RONTEC's EDX system, Model QuanTax 200, Germany).

Determination of size-dependent antifungal activity of zinc oxide nanoparticles by the well-diffusion method

Antifungal activities of the synthesized zinc oxide nanoparticles of different sizes were determined using plant fungal pathogens by a modified Kirby Bauer disc diffusion method [33]. The fungi were cultured in potato dextrose broth at room temperature on an orbital shaking incubator (Remi, India) at 200 rpm. A 100 μL of broth fungal culture was prepared and spread on potato dextrose agar plates. After that plates were allowed to stand for 10 min to allow for culture absorption. The 5 mm size wells were punched into the agar with help of sterile gel puncher. A 100 μL (25 μg/ml) of the nanoparticles solution sample and 100 μL (10 μg/ml) of positive control (amphotricin B) were poured into wells on all plates using micropipette. After incubation at room temperature for 48 h, the size of the zone of inhibition diameter in millimeter was measured. Each screening treatment was conducted with three replicates and the results are presented as mean ± SE (standard error of the mean).

Results and discussion

Characterization of zinc oxide nanoparticles

The UV–Visible absorption spectra of the as-grown a new technique for the preparation of the monodispersed semiconductor zinc oxide nanoparticles (50% and 25% plant extract) are shown in Fig. 1a and b. The room temperature spectra exhibit strong excitonic absorption peaks at 374.00 nm (3.32 eV) and 370.50 nm for samples 50% and 25% plant extract, respectively, which is in good agreement with the previous work [19,34,35].