

Original Research Article

Preparation of *Tradescantia pallida*-mediated zinc oxide nanoparticles and their activity against cervical cancer cell lines

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Abstract

Purpose: To synthesize zinc oxide nanoparticles (ZnO NPs) using *Tradescantia pallida* (Commelinaceae) and determine their fluorescent and cytotoxic properties.

Methods: ZnO NPs were synthesized according to a simple protocol using *T. pallida* aqueous leaf extract (TPALE). Scanning electron microscopy (SEM) and transmission electron microscopy (TEM) were used to analyze the morphology of the ZnO NPs. X-ray diffraction (XRD) and Fourier transform-infrared spectroscopy (FTIR) measurements were performed to determine their crystalline nature and functional groups, respectively. Fluorescence spectroscopy was used to assess the photoluminescence properties of ZnO NPs. Upon confirmation of ZnO NP synthesis, cytotoxicity tests were carried out against HeLa cell line by 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide (MTT) assay.

Results: The agglomerated ZnO NPs were rod-shaped and had a mean particle size of 25 ± 2 nm. Further, they exhibited good photoluminescence with correlation to ZnO crystals. MTT assay results indicated significant cytotoxicity against HeLa cervical cancer cell line.

Conclusion: A simple approach for ZnO NP synthesis based on TPALE has been developed successfully. The synthesized ZnO NPs demonstrate good luminescence properties and cytotoxicity against cervical cancer line.

Keywords: Commelinaceae, Cytotoxicity, Photoluminescence, *Setcreasea pallida*, *Setcreasea purpurea*, *Tradescantia pallida*, ZnO nanoparticles

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INTRODUCTION

Nanotechnology is one of the most versatile areas of current research and has wide-ranging applications. A number of toxic physio-chemical techniques, such as spray pyrolysis, gas-phase methods, chemical vapor deposition, electrochemical methods, and laser ablation techniques, have been introduced to synthesize nanoparticles (NPs) [1]. In this study, we focused

on non-toxic-mediated synthesis of NPs based on plant extracts. *Tradescantia pallida* plants are mostly used as ornamental plants in Romania [2], but they are highly adaptable, particularly to shaded environments. Most researchers have used *T. pallida* root for histopathological studies [3]. Recently, a research group reported histopathological studies indicating that *T. pallida* can adapt to shade environments when calcium crystals are present [4]. Various researchers

have focused on metal NP synthesis, but there are no results concerning *T. pallida*-mediated synthesis of metal NPs. In this study, we synthesized zinc oxide nanoparticles (ZnO NPs) using *T. pallida* aqueous leaf extract (TPALE). These ZnO NPs have a wide variety of applications, e.g., anti-microbial [5], cosmetics [6], anti-cancer [7], agriculture [8], optical [9], and electrical [10]. Currently, a number of researchers are interested in using ZnO NPs to diagnose medical disorders [11]. There have been a number of studies concerning photoluminescence and cytotoxicity studies of synthetic ZnO NPs [12-14].

EXPERIMENTAL

Materials

Zinc acetate was procured from Avra Laboratories, Hyderabad, India. HeLa cell lines were collected from the King Institute, Guindy, Chennai, Tamil Nadu, India. Methyl thiazolyldiphenyl-tetrazolium bromide (MTT, 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide) was obtained from AVRA Laboratories. Double-distilled water was used in all experiments with no purification.

Preparation of plant extract

T. pallida leaves were collected from the gardens of the 117th Hospital of the Chinese People's Liberation Army, Hang Zhou City. The leaves were dried in the shade for 4 days. Once dried, the leaf material was ground to a powder. Then, 30 g of the leaf powder material was immersed in 100 mL distilled H₂O and placed in a water bath for 1 h at 60 °C. The solution was filtered, then the filtrate was stored in the refrigerator until needed.

ZnO NP synthesis

TPALE (20 mg) was mixed with 1-mM zinc acetate (80 mL) and placed in a water bath at 60 °C for 4 h. The resultant solution was centrifuged for 30 min at 4,500 rpm. After centrifugation, a pellet was obtained and heated in a furnace at 350 °C for 5 h. Once the calcination process was completed, the powder samples were characterized as described below.

XRD analysis

X-ray diffraction (XRD) analysis (Model D8, Advance Powder X-ray Diffractometer, Bruker, Germany) and Fourier transform infrared (FT-IR) spectrum analysis (Jasco 6600, Oklahoma City, OK, USA) were used to identify the crystalline

nature and functional groups of the synthesized ZnO NPs, respectively. The crystalline size of the ZnO NPs was calculated using Scherrer's relationship (Eq 1).

$$D = k\lambda / \beta \cos \theta \dots\dots\dots (1)$$

where D is the particle size, k is Scherrer's constant (0.94), λ is the wavelength, derived from Bragg's equation ($2d\sin\theta = n\lambda$), β is the half-width full-maximum, and θ is the diffraction angle.

Morphological analysis

Scanning electron microscopy (SEM, Hitachi 7100, Tokyo, Japan) was used to determine the structure of the sample at a voltage of 100 kV. A Bruker 501 transmission electron microscopy (TEM) system, equipped with X-ray microanalysis capabilities, was used to perform microstructural analysis of the powder. Energy dispersive X-ray spectroscopy (EDAX) was carried out with an Oxford Instruments X-act 10-mm SDD (Philips, CM 200) (operating voltage range: 20–200 kV; resolution: 2.4 Å).

Fluorescence studies

Fluorescence spectroscopy was used to investigate the photoluminescence properties of the synthesized ZnO NPs. Fluorescence spectra were obtained using an F-7000 FL spectrophotometer (Hitachi Perkin-Elmer, Bengaluru, Karnataka, India). Cell viability was calculated as in Eq 2.

$$\text{Cell viability (\%)} = (A_t/A_n)100 \dots\dots\dots (2)$$

where A_t and A_n are the absorbance of samples for treated and normal cells, respectively.

Assessment of photoluminescence

The fluorescence spectrum of the ZnO NPs was analyzed to identify the emission bands and the transition between oxygen vacancies and interstitial oxygen [15,16].

Cytotoxicity assay

MTT assays were performed using ZnO NPs on a HeLa cell line [17]. The HeLa cells were cultured in Eagle's minimum essential medium (MEM) containing 1 % phosphate-buffered saline (PBS), 100 μ g/mL penicillin, and 100 μ g/mL streptomycin at 37 °C. 3-(4,5-dimethyl-2-thiazoyl)-2,5-diphenyltetrazolium bromide (MTT) assay was used to determine the cytotoxicity of ZnO NPs against cancer cell lines. The cancer

cells were maintained in a humidified atmosphere of 50 mg/mL CO₂ at 37 °C. The cells were placed in 24-well plates. After an incubation period of 48 h, the cells reached confluence. They were then incubated in various concentrations of ZnO NPs in 0.1 % dimethyl sulfoxide (DMSO). The sample solution was removed and washed with PBS (pH 7.4) 200 IL/well (5 mg/mL) followed by addition of 0.5 % MTT. After an incubation period of 48 h, 0.04 M HCl/isopropanol was added. The proportion of remaining viable cells was determined using an ultraviolet-visible (UV-Vis) spectrophotometer to measure the absorbance at 570 nm.

RESULTS

XRD spectrum of ZnO NPs

XRD results showed that ZnO NPs were present in wurtzite form, in agreement with the Joint

Committee Powder Diffraction Standards (JCPDS) 89-7102 illustrated in Figure 1 [18-20]. The average size of the crystals was 25 nm.

FT-IR spectrum of the ZnO NPs

The functional groups responsible for the conversion of metal precursors into metal NPs were identified using FT-IR analysis. The ZnO NPs were compared to TPALE. The peak at 1450–1500 cm⁻¹ corresponds to N–H stretching vibrations. ZnO NP stretching was identified at 400–800 cm⁻¹, O–H stretching at 3433 cm⁻¹, and aldehyde C–H stretching at 2934 cm⁻¹. A protein peak was observed at 1250 – 1270 cm⁻¹; thus, the ZnO NPs were covered with a layer of primary and secondary metabolites, i.e., proteins and functional groups, as illustrated in Figure 2 [21].

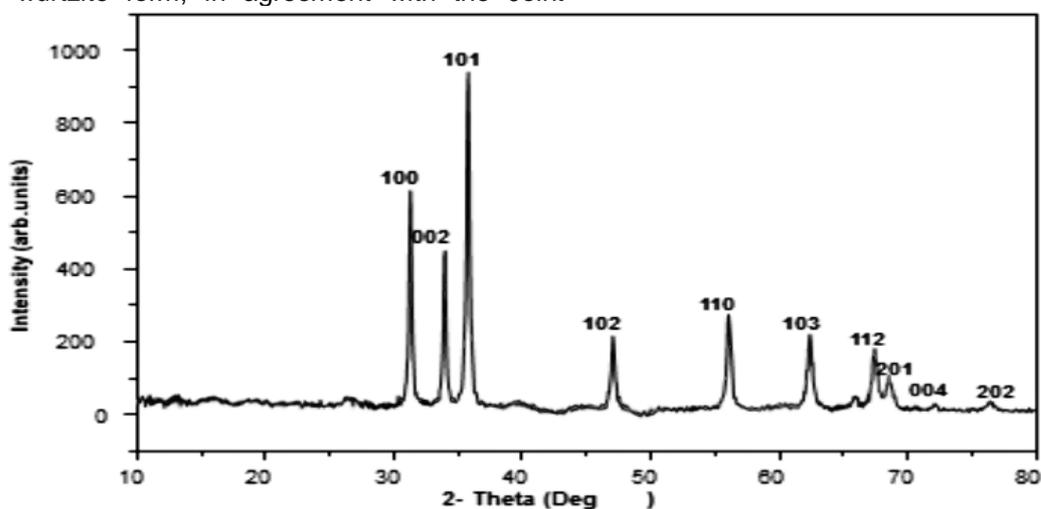


Figure 1: Zinc oxide nanoparticles (ZnO NPs) X-ray diffraction spectrum

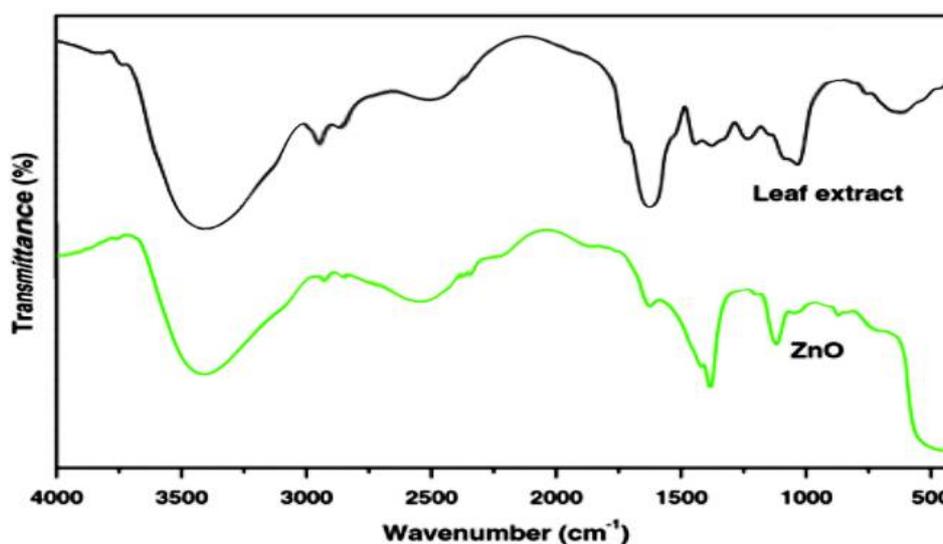


Figure 2: Fourier transform infrared spectra of *Tradescantia pallida* aqueous leaf extract and ZnO NPs

Morphological and EDAX analysis

Morphological analyses were performed using SEM and TEM to identify the size and structure of the TPALE-mediated synthesized ZnO NPs. The resulting agglomerated particles were rod-shaped and had a size of 25 ± 2 nm, as illustrated in (Figure 3A–D). The EDAX spectrum shows that Zn and O were present in proportions of 83 % and 17 % respectively, as shown in Figure 4.

Photoluminescence of ZnO NPs

Fluorescence spectroscopy results of the ZnO NPs showed three color bands, namely, red, blue and green. Absorbance occurred at 447, 402, and 469 nm, corresponding to the blue bands, and 483 nm corresponding to the green band. The blue bands were caused by defects in the ZnO crystals, and the green band was caused by the oxygen transition vacancy, as shown in Figure 5. [22,23].

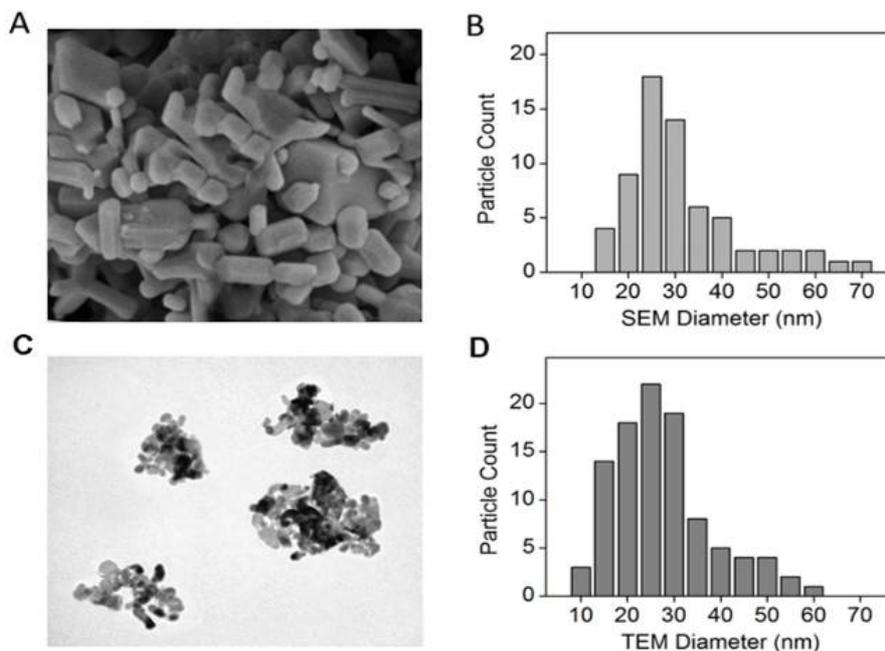


Figure 3: (A) ZnO NP scanning electron microscopy (SEM) image, (B) ZnO (SEM) particle size histogram, (C) ZnO NP transmission electron microscopy (TEM) image, and (D) ZnO NP (TEM) particle size histogram

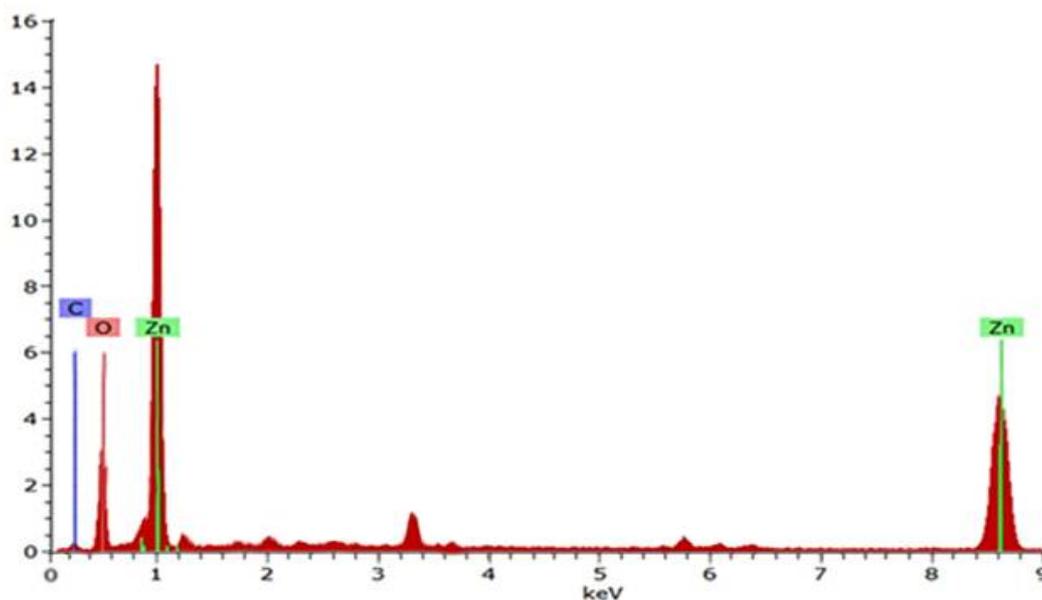


Figure 4: Energy dispersive X-ray analysis of ZnO NPs

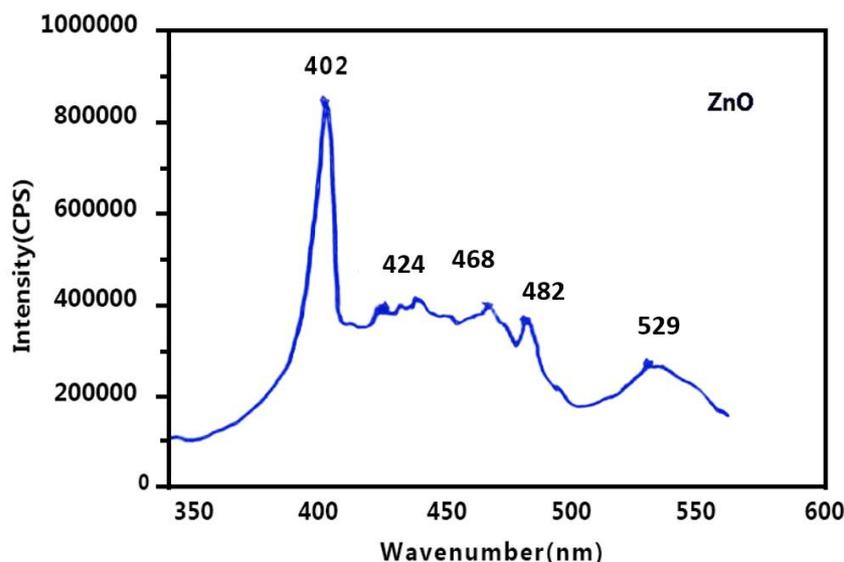


Figure 5: Photoluminescence spectrum of ZnO NPs

Cytotoxicity activity

The cytotoxicity assay results showed that 98.9 % of the cancer cells died after being subjected to a high dose 1000 $\mu\text{g/mL}$ of ZnO NPs. We determined the concentration required for 50 % inhibition of viability (IC_{50}) graphically. The IC_{50} of ZnO NPs was 62.5 $\mu\text{g/mL}$ for the HeLa cervical cancer cell line. Cells from the HeLa cell line were treated with various concentrations of ZnO NPs for 48 h. UV-visible spectroscopy was used to determine the proportion of live cells. The absorbance peak at 570 nm is clearly shown in Figure 6 [18].

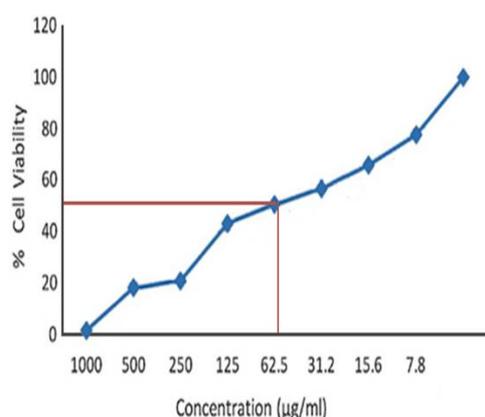


Figure 6: Methyl thiazolyldiphenyl-tetrazolium bromide assay against HeLa cell line

DISCUSSION

There has been great interest in NP synthesis based on plants, as there are several drawbacks to physical- and chemical-mediated NP synthesis. TPALE has not been used previously

to synthesize NPs. Here, we synthesized ZnO NPs using TPALE and conducted cytotoxicity tests against HeLa cells. The ZnO NPs had a size of 25 nm and an agglomerated rod shape.

The present results indicate that TPALE-mediated ZnO NPs are toxic to HeLa cells, killing 98.9 % of the cancer cells when a high dose of 1000 $\mu\text{g/mL}$ was used. The concentration required for IC_{50} , determined via graphic interpolation, was 62.5 $\mu\text{g/mL}$ for the HeLa cervical cancer cell line. There are several existing reports detailing the green synthesis of ZnO NPs from plant sources. These sources include *Citrus aurantifolia* peel, which resulted in prism-shaped particles with an average size of 35 nm [24], *Vitex negundo* L. extract, *Caralluma fimbriata*, and *Euphorbia Jatropa* latex [25,26]; however, few reports detail cytotoxicity assay and photoluminescence investigations of green-synthesized ZnO NPs. In future studies, it is intended that a simpler method to synthesize ZnO NPs will be developed. Such a method would serve as a reference for researchers to develop other methods to ecologically synthesize metal NPs for potential application as treatments for various medical disorders.

CONCLUSION

A simple approach for ZnO NP synthesis based on TPALE has been developed successfully. The mean particle size of the ZnO NPs was 25 nm, and the final product consisted of agglomerated rod-shaped particles. The synthesized nanoparticles demonstrate good luminescence properties. Cytotoxicity results against cervical cancer lines indicate 98.9 % of cancer cells died

after exposure to a dose of 1000 µg/mL ZnO NPs.

DECLARATIONS

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Conflict of Interest

No conflict of interest associated with this work.

Contribution of Authors

The authors declare that this work was done by the authors named in this article and all liabilities pertaining to claims relating to the content of this article will be borne by them.

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