Biogenic Zinc Oxide Nanoparticles Synthesis Using *Tabernaemontana Divaricate* Leaf Extract and Its Anticancer Activity against MCF-7 Breast Cancer Cell Lines

Rajeshwari Sivaraj*, Pattanathu KSM Rahman, P. Rajiv, and Rajendran Venckatesh

Abstract**—**This investigation explains the biosynthesis and characterization of zinc oxide nanoparticles from an Indian medicinal plant by an eco-friendly method. The main objective of this study is to synthesize zinc oxide nanoparticles from *Tabernaemontana divaricate* leaves through a green chemistry approach. Highly stable, spherical zinc oxide nanoparticles were synthesized by using 50% concentration of *Tabernaemontana* leaf extract. Formation of zinc oxide nanoparticles have been characterized by X-ray diffraction (XRD), Raman spectroscopy and transmission electron microscopy (TEM) analysis. All the analysis reveals that zinc oxide nanoparticles were 36 ± 5 nm in size. Functional groups and chemical composition of zinc oxide were also confirmed. *Tabernaemontana* mediated zinc oxide nanoparticles showed effective cytotoxic effect against MCF-7 breast cancer cell lines with an IC_{50} value of 30.65 μg/ml/24 h by the MTT assay. These results clearly support the benefits of using biological method for synthesizing zinc oxide nanoparticles with anticancer activities.

Keywords**—***Tabernaemontana divaricate,* zinc oxide, anticancer, MTT assay

I. Introduction

Plants and/or their extracts provide a biological synthesis route of several metallic nanoparticles which are more eco-friendly and allow controlled synthesis with well-defined size and shape [1]. The enzymes [2], plant leaf extract [3] and bacteria [4] play vital role in green synthesis of zinc oxide nanoparticles. *T. divaricata* belongs to *Apocynaceae* family. Crude extract of this plant has anti-infection, anti-parasitic, antibacterial, antifungal and anti-inflammation activity [5, 6]. Aqueous extract of *T. divaricata* leaf has rich phytochemicals such as alkaloids, tannins, Flavonoids, phytosterols, phenols, terpenes and carbohydrates. Flavonoids are known to be synthesised by plants in response to microbial infections [7].

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Zinc oxide nanoparticles are an important semiconductor material due to unique optical and electrical properties [8, 9]. It has application on solar cells, gas sensors, ceramics, catalysts and cosmetics [10]. Nanotechnology has vital role in advances in medicinal research, reproductive science and technology, transfer of agricultural and food wastes to energy and other useful by-products through enzymatic nanobioprocessing, disease prevention, and treatment in plants using various nanocides [11]. The wide application of nanomaterials has caused toxicity to concerned mammals (including mammalian cell cultures) and aquatic species, soil invertebrates, soil microorganisms, or plants [12-14]. Engineered nanoparticles could sequester nutrients on their surfaces and thus serve as a nutrient stock to the organisms, particularly those engineered nanoparticles having high specific surface area. These positive effects could be probably due to the antimicrobial properties of engineered nanoparticles, which can enhance strength and resistance of plants and animals to stress [15]. In this paper, we adopt a green chemistry approach for the synthesis of zinc oxide nanoparticles from plant extract and particle's shape and size were detected by the influence of various standard techniques. Anticancer activity of green synthesized zinc oxide nanoparticles was determined against MCF-7 breast cancer cell lines. Based on our observation and analysis, zinc oxide nanoparticles have effective anticancer activity.

II. Materials And Methods

A. Synthesis and Characterization of Tabernaemontana mediated zinc oxide nanoparticles

Tabernaemontana divaricate leaves were collected from in and around Karpagam University, Eachanari, Coimbatore, India. All the chemicals and solvents used in this experiment were of analytical grade and were obtained from Sigma-Aldrich Chemicals, India.

B. Synthesis and Characterization of Tabernaemontana mediated zinc oxide nanoparticles

Zinc oxide nanoparticles were synthesized via biological reduction of zinc nitrate by 50% plant extract of *Tabernaemontana* as described by Sangeetha et al. [16] with small modification. Synthesized nanoparticle's purity and

grain size were analysed using X-ray diffraction (Perkin-Elmer spectrum one instrument). Phyto mediated zinc oxide nanoparticles were characterized by Raman spectroscopy (Perkin-Elmer 1725x). The nanoparticles average size and shape were determined by Transmission electron microscopy (TEM) (Model JSM 6390LV, JOEL, USA).

C. Anticancer activity of Tabernaemontana mediated zinc oxide nanoparticles

Human breast MCF-7 cell lines (cell culture) were obtained from the National Centre for Cell Science (NCCS), Pune, India. The cells were cultured in Eagles Minimum Essential Medium (EMEM) added with FBS (10%, v/v) at 37 ˚C in a CO_2 incubator (95% air, 5% CO_2 and 100% relative humidity). In order to evaluate the cytotoxic effect of the green synthesised copper oxide nanoparticles against MCF-7 cells, the cells were collected in the exponential stage of growth, seeded into 96-well plates (15,000 per well) and permitted to adhere for 48 h. Then, Different concentrations (6.5, 12.5, 25, 50, 100 µg/ml) of *Tabernaemontana* mediated copper oxide nanoparticles were added to the desired wells and incubated for 48 h. A 20 µl of EMEM medium having MTT (5 mg/mL) was added to each well and incubated for 4 h at 37 ˚C. Later, the medium was altered with 100 µL of DMSO, and optical densities were measured at 570 nm. All studies were performed in triplicates and expressed as the mean ± standard error.

D. Statistical analysis

Experiments were carried out in a randomized block design with three replications and repeated twice. The data were analysed using one way analysis of variance (ANOVA) by software SPS version 16.

III. Results And Discussion

X-ray diffraction was taken for confirmation of zinc oxide phase of the nanoparticles. The XRD pattern of zinc oxide nanoparticles is shown in Fig. 1. The XRD peaks were identified as (100), (002), (101), (102), (110), (112) and (202) reflections, respectively. The narrow and strong diffraction peaks indicate spherical and well crystalline nature of zinc oxide is comparison with the data from JCPDS card No. 89-7102. The Scherrer formula was used for calculate the particles sizes and was found to be in the range of 30–38 nm [17].

The Raman spectra of zinc oxide nanoparticles are shown in Fig. 2. The strong peak of 437 cm^{-1} denotes the zinc oxide nanoparticles phase. The peak 437 cm^{-1} is due to the Raman active E_2 optical phonon mode from the zinc oxide nanoparticles. This peak confirmed that zinc oxide nanoparticles have the crystal structure. Other peak at 580 cm^{-1} characterizes the EIL mode and it attributed to zinc interstitials or oxygen vacancies. High intensity E_2 mode was present and conforms to pure crystalline zinc oxide nanoparticles.

Fig. 3 shows the transmission electron micrograph of the zinc oxide sample. These Figures undoubtedly indicate the morphology of the particles to be spherical. Some of the particles are agglomerates. TEM image confirms the formation of zinc oxide nanoparticles and it has an average size about 36 ± 5 nm, which is very similar to previous studies [16, 18].

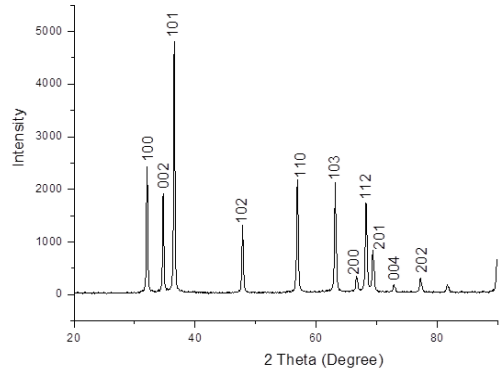

Fig. 1 X-ray diffraction patterns of *Tabernaemontana* mediated zinc oxide nanoparticles

The cytotoxicity of the zinc oxide nanoparticles was evaluated against MCF-7 breast cancer cell lines at various concentrations (6.5- 100 µg/ml). The IC_{50} value for zincoxide nanoparticles was found to be 30.65 µg/ml. Maximum concentration of copper oxide nanoparticles (100 µg/ml) effectively inhibited the growth of cell by more than 97%. Sankar et al. [21] reported the anticancer activity of *Origanum vulgare* mediated silver nanoparticles and cytotoxic effects of green synthesized *O. vulgare* mediated silver nanoparticles against human lung cancer A549 cells.

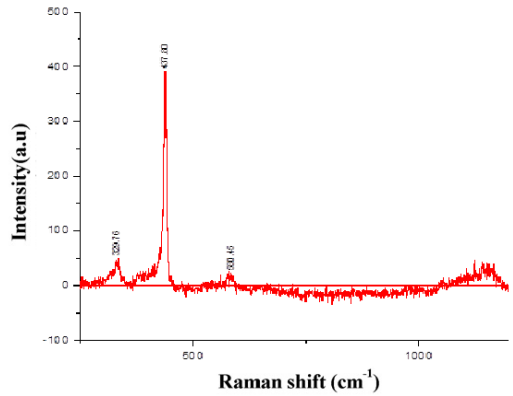

Fig. 2 Raman spectra of *Tabernaemontana* mediated zinc oxide nanoparticles

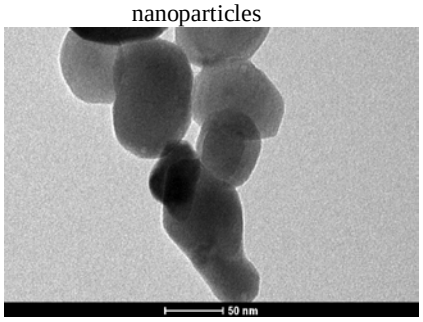

Fig. 3 TEM images of *Tabernaemontana* mediated zinc oxide nanoparticles

IV. Conclusion

The present study reported that copper oxide nanoparticles can be synthesized in a simple method using *Tabernaemontana* leaf extract. The TEM analysis showed that the sizes of the synthesized zinc oxide nanoparticles ranged from 36 ± 5 nm. Plant mediated zinc oxide nanoparticles showed best anticancer activity.

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