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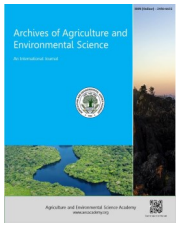

ORIGINAL RESEARCH ARTICLE

Biosynthesis and characterization of zinc oxide nanoparticles using *Thunbergia erecta* (Benth.) T. Anders plant extracts

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ABSTRACT

The present study focuses on the green synthesis of zinc oxide nanoparticles using leaf, stem, root and flower extracts of *Thunbergia erecta*. Zinc nitrate hexahydrate (Zn $(NO_3)_2.6H_2O$) solution was used as precursor to synthesize the nanoparticles. Five grams of plant materials (leaf, stem, root and flowers) were weighed and cut in to small pieces and boiled with deionised water in water bath at 50°C for about 30 min. The extracts were filtered and mixed with Zinc nitrate hexahydrate solution for the preparation of nanoparticles. Synthesis of nanoparticles were monitored by visual color change from colorless to yellow and characterized by UV-Visible double beam spectrophotometric analysis. The absorption peaks of leaf reaction medium was at 308 nm, stem and root reaction media were at 296 nm and flower reaction medium was at 302 nm as shown by the UV-Visible spectrophotometer. The results conclude that *T. erecta* could be exploited for green synthesis of zinc oxide nanoparticles which can be used in the development of pharmaceutical products beneficial to the mankind.

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INTRODUCTION

The nanoparticles (NPs) (ranging from 1-100 nm) are considered as extremely useful owing to their extensive applications in human welfare. So far, metal nanoparticles includes aluminium, cobalt, cesium, copper, gold, silver, zinc, magnetite, nickel, palladium, platinum, silicon nanoparticles were used in the fields of agriculture, phytoremediation, biomedical, cosmetics, drug delivery, pesticides, fertilizers etc. (Haleemkhan *et al.,* 2015). In general nanoparticles could be synthesized through physical, chemical and biological approaches. But biological synthesis of nanoparticles using bacteria, algae, fungi, actinomycetes and plants has gained attention from scientists. Among which plant extract based synthesis has been greatly acknowledged and explored with many plant species (Mittal *et al.,* 2014). Literature survey reveals that the leaves were primarily used for the synthesis of various metallic nanoparticles (Annamalai *et al.,* 2011; Shekhawat *et al.,* 2013). Recently various plant parts such as stem bark, latex, root, flower, fruit, seed extracts and whole plants were also used to synthesize nanoparticles (Mondal *et al.,* 2011; Jayaseelan *et al.,* 2013; Shekhawat and Manokari, 2014).

Genus *Thunbergia* belongs to the family Acanthaceae consists of 100 species of annuals, perennials and shrubs (Sultana *et al.,* 2015). The genus has ornamental value and is native to tropical regions of Africa, Madagascar, Australia and South Asia (Retief and Reyneke, 1984). *Tunbergia erecta* (Syn: *Meyenia erecta*) is commonly known as Blue Bell, Bush Clock Vine or King's Mantle and native to Western Africa (Mathew and Benny, 2016). It grows up to the height of 4-6 feet. The leaves are heart-shaped with acute apex, green, pubescent, opposite, entire margin, flowers are purple with yellow throat (corolla tube), angular stem, tuberous roots, and prefer full sun and well-drained soil to grow (Sultana *et al.,* 2015).

Traditionally the leaves, stems and roots of *Thunbergia* species have been used as anti-inflammatory agents and antipyretics for centuries (Tejasen and Thongthapp, 1980). *Thunbergia* species are also reported to possess antibacterial activities against gram positive as well as gram negative bacteria such as *Escherichia coli*, *Klebsiella pneumonia*, *Staphylococcus aureus*, *Bacillus cereus*, *Proteus mirabilis* and *Streptococcus pyogenes* (Jeeva *et al.,* 2011). *Thunbergia* species exhibits anti-inflammatory (Boonyarikpunchai *et al.,* 2014), carminative (Inta *et al.,*

2013), antipyretic, analgesic, antidiabetic, anthelmintic, antioxidant, cytotoxic, hepatoprotective (Oonsivilai *et al.,* 2008; Wonkchalee *et al.,* 2012), antinociceptive and anti-tumor activities (Jetawattana *et al.,* 2015). The members of *Thunbergia* genus are reported to contain glucosides, alkaloids and phenolic compounds such as flavonoids, tannin, phenolic acids, rosmarinic acid, feruloylmalic and coumaroylmalic acid, naphthalene, iridoid glucosides, benzyl beta glucopyranoside, grandifloric acid, delphinidin and apigenin (Kanchanapoom *et al.,* 2002; Areekul *et al.,* 2009).

The members of the genus are traditionally used in detoxification of insecticides, ethyl alcohol, arsenic and strychnine (Thongsaard and Marsden, 2002). The phenolic compounds of *T. laurifolia* have been shown to possess antioxidant activity and used to treat gastric ulcer, diarrhea and jaundice (Suwanchaikasem *et al.,* 2012). Toxic effects recorded after administrating *T. laurifolia* in the blood cells and the circulatory system (Chivapat *et al.,* 2009; Tangjitman *et al.,* 2015).

Biogenic production of zinc oxide nanoparticles has been reported from the aqueous extracts of many plants (Manokari and Shekhawat, 2014) but this plant is not exploited for production of zinc oxide nanoparticles. Therefore, the objective of the present study is to demonstrate the eco-friendly synthesis of zinc oxide nanoparticles using aqueous extracts of parts of *Tunbergia erecta*.

MATERIALS AND METHODS

Chemicals: Zinc Nitrate hexahydrate (Zn $(NO_3)_2.6H_2O$) used in the present investigation was purchased from Himedia (Mumbai, India). The glassware was washed with sterilized distilled water before use. Deionized water was used for the synthesis and for the preparation of plant extracts.

Collection of plant material for the study: The experimental material selected for the present study was *Thunbergia erecta* (Benth.) T. Anders, an ornamental plant (Figure 1). The plant material was collected from the Institute campus (Pondicherry, India), during the months of July-November 2016, and identified with the help of monographs of French Institute, Pondicherry. Apparently healthy plants were marked and leaves, stem, root and flowers were collected, washed thoroughly with tap water and dried at room temperature for 60 min and used for determination and characterization of zinc oxide nanoparticles.

Figure 1. *Morphology of Thunbergia erecta plants with flowers in the natural habitat.*

Preparation of the plant extracts: Plant extracts were prepared by standard extraction methods (Shekhawat and Manokari, 2014). The plant parts such as leaf, stem, root and flowers were separated using sterile scissors (Figures 2A-5A). Five grams of plant materials were weighed and cut in to small pieces (Figures 2B-5B) and boiled with 50 ml of deionized water in water bath at 50°C for about 30 min. The extracts were filtered through cheesecloth and the extracts were maintained at its original volume and used for further processes.

Preparation of precursor and reaction media: Zinc Nitrate hexahydrate (Zn $(NO_3)_2 \cdot 6H_2O$) was used as precursor to synthesize zinc oxide nanoparticles (ZnO NPs) from *T. erecta*. One mM Zinc Nitrate solution was prepared using deionized water, filtered by Whatman® No.1 filter paper and stored at 4°C for further experiments. Ten ml of aqueous suspension consisted of zinc nitrate and appropriate plant extracts at various ratios (1:9, 2:8, 3:7, 4:6 and 5:5) were mixed at room temperature to obtain reaction mixture. Figures 2C–5C shows the plant extracts, precursor and reaction media of leaf, stem, root and flowers. The solutions were further kept in rotary shaker for 2 hours at 100 rpm. Supernatant was discarded and suspension at the bottom of glass vessel was diluted using deionised water and used for the spectrophotometric analysis.

Characterization of ZnO nanoparticles through UV-Visible spectral analysis: The aqueous microemulsions (secondary metabolites + precursor) were starting solutions for the production of metallic zinc oxide nanoparticles. The confirmation of ZnO nanoparticles synthesis was obtained by measuring the optical property of reaction mixture using UV-Visible absorption spectroscopic analysis (Systronics Double Beam Spectrophotometer, Model 2202) between 250-800 nm wavelengths. All the processes were carried out in the laboratory at room temperature (25°C).

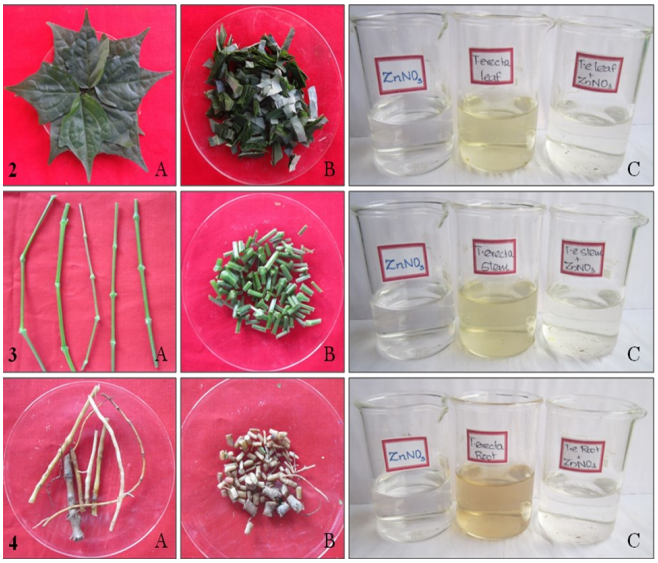


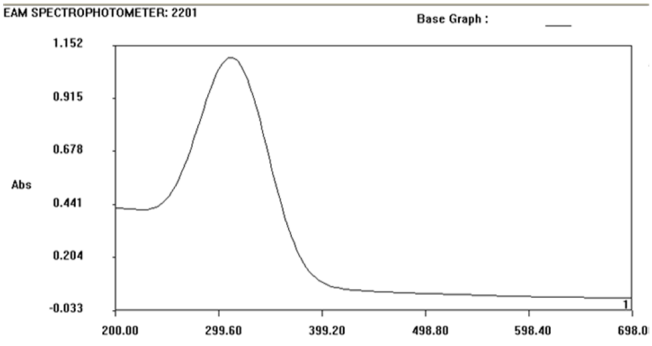
Figure 2. A- *Leaves procured for the synthesis of ZnO NPs,* **B-** *5 g of finely cut leaves,* **C-** *Leaf reaction mixture.* **Figure 3. A-** *Stem segments of T. erecta,* ***B-*** *5 g of finely cut stem,* **C-** *Stem reaction mixture.* **Figure 4. A-** *Roots segments,* **B-** *5 g of finely cut roots,* **C-** *Root reaction mixture.* **Figure 5. A-** *Flowers,* **B-** *5 g of finely cut flowers,* **C-** *flower reaction mixture.*

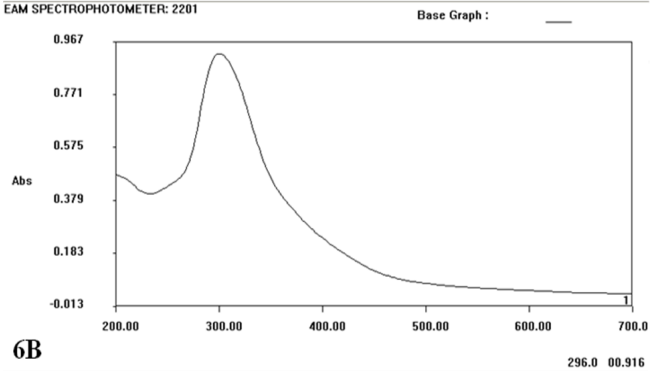
RESULTS AND DISCUSSION

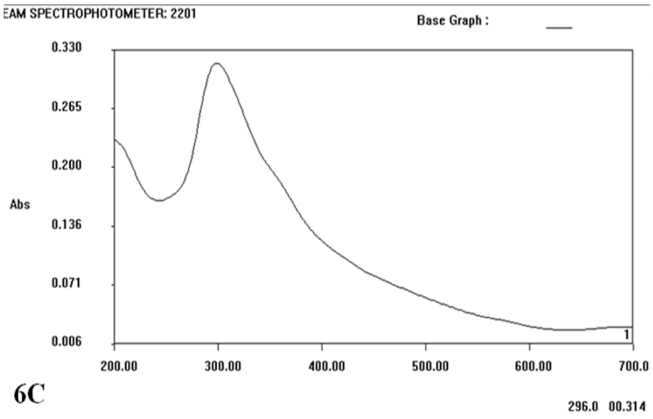
Thunbergia erecta is so far used as hedge and lawn plant (Fongod *et al.,* 2013). But the present report assures that the species could be greatly useful in various fields particularly in medical applications. *Cassia densistipulata* has successfully been utilized in the biosynthesis of nanoparticles by Kooluru and Sharada (2014). The present study involves whole plant parts including leaves, stem, root and flowers in the synthesis of ZnO nanoparticles. The color change of reaction media were recorded at different time intervals. The characteristic yellow color development was observed in leaf and stem reaction medium immediately. Color change was observed in root reaction medium after 2 hrs of incubation at room temperature. Deep yellow color formation was observed in the flower extract with zinc nitrate solution after 40 minutes (Figures 2C-5C). The change in color of the reaction mixture indicates the formation of zinc oxide nanoparticles as reported in *Passiflora foetida* (Shekhawat *et al.,* 2014) and *Camellia sinesis* (Shah *et al.,* 2015).

The reduction of zinc metal ions to zinc oxide nanoparticles in the reaction medium was preliminarily analyzed using UV-Visible Spectrophotometer between the wavelengths of 250-800 nm. The UV-Visible spectroscopic analysis of ZnO nanoparticles from leaf reaction medium was confirmed by the strong absorption spectra at 308 nm (Figure 6A). The maximum absorption of stem and root reaction media were observed at 296 nm (Figures 6B, 6C) with visual difference in the graphs. The flower reaction medium presented strong absorption spectrum at 302 nm (Figure 6D).

The UV-Visible spectrum of *T. erecta* plant extracts with zinc nitrate solution confirmed the synthesis of ZnO nanoparticles at different time periods. Reaction was completed







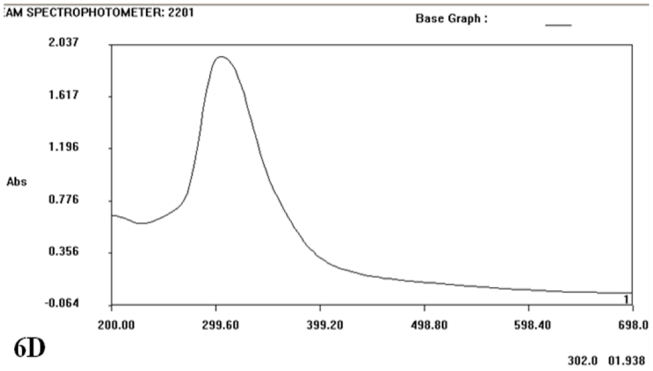


Figure 6. *UV-Visible spectral analysis of* (A) *– leaf reaction medium*, (B) *– Stem reaction medium*, (C) *– Root reaction medium and* (D) *– Flower reaction medium.*

within 60 min in leaf and stem reaction medium but root and flower reaction media took 2 hrs (120 min) to complete the reaction. Plant extracts have been explored successfully to synthesize biocompatible nanoparticles due to their diverse properties (Vidya *et al.,* 2013). Plant extracts at the concentration of 7 ml was found to mediate the ZnO nanoparticles synthesis within less time period, whereas the increased and decreased concentrations were less effective when challenged with the precursor. In our earlier report on *Moringa oleifera*, synthesis of ZnO nanoparticles was optimum at the volume of 5 ml of both plant extracts and the precursor (5:5) (Manokari and Shekhawat, 2016). Previous reports of ZnO nanoparticles synthesis from plant extracts revealed that the presence of phenolic compounds such as flavonoids arbitrate the conversion of zinc ions in to zinc oxide metallic nanoparticles (Raj and Jayalakshmy, 2015).

Conclusions

It is evident from the present study that the green synthesis of ZnO nanoparticle is eco friendly, simple and efficient than the conventional methods with the less usage of toxic chemicals. The characterization of ZnO nanoparticle with UV-Visible spectrophotometer confirmed the presence of ZnO nanoparticles from various extracts of *T. erecta*. It may be advantageous for pharmaceutical industry to explore *T. erecta* to develop effective drugs amended with ZnO nanoparticles. The nanoparticles formulated drugs could be used in diagnosis and treatment of various heath related issues.

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