

Biosynthesis of Zinc oxide Nanoparticles using *Melia azedarach* L. extracts and their Characterization

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Abstract

The present study focuses on the bioproduction of Zinc oxide nanoparticles (ZnO NPs) from the aqueous extracts of leaves, stem, roots, flowers and fruits of *Melia azedarach* L. The plant is enriched with alkaloids, sterols, glycosides, phenolic compounds, tannins, flavonoids, saponins and other biologically active phytochemicals. Zinc Nitrate hexahydrate [Zn (NO₃)₂.6H₂O] was used as precursor to synthesize ZnO nanoparticles. UV- Visible spectral studies were conducted for the characterization and confirmation of the synthesized ZnO nanoparticles. It showed that the test solutions had strong absorbance in the range of 290-330 nm. Hence the biogenic synthesis of ZnO nanoparticles using *M. azedarach* could be explored in various fields.

Keywords: *Melia azedarach*, Zinc oxide nanoparticles, UV-Visible spectral analysis, characterization.

1. Introduction

Nanotechnology has a great potential to modify conventional agricultural practices [1]. It could have a dominant position in transforming agriculture and food production. The bioprocessing mediated nanotechnology converts the agricultural and food wastes into energy and useful by-products [2]. Metal oxide nanoparticles are reported to play important role to promote the growth and yield of plants [3]. Biosynthesized metallic nanoparticles from plant derivatives are used in medical and commercial sectors including waste water treatment, cosmetics and food industry. Recently, the plant mediated nanoparticles synthesis has drawn more attention due to its vast application in various fields due to their physico-chemical properties. Zinc oxide nanoparticles (ZnO NPs) were synthesized from natural resources and have been studied exclusively. Zinc oxides are multifunctional material with its unique physical and chemical properties. ZnO nanoparticles were more abrasive than bulk ZnO (particle sizes in the range of 0.1-1 μm) [4].

ZnO nanoparticles are used in medicine field because of their site specific mechanical damage to the bacterial cell membrane and the enhanced bactericidal effect [5]. ZnO semiconductors have been extensively studied due to their photocatalytic activity under UV light [6]. These nano antimicrobial substances based on phytochemicals have been found to be effective for safe therapeutic properties [7]. Many researchers have attempted to prove the biological properties of ZnO nanoparticles. Their targeting potential has utility in the treatment of cancer and autoimmunity [8]. The colloidal solution of Zinc oxide nanoparticles are used as nano fertilizer and play an important role in agriculture [9-11]. Nano fertilizers can be absorbed rapidly by plants. Nano encapsulated slow release fertilizers can save fertilizer consumption and minimize environmental pollution. Particularly, Zinc oxide nanoparticles have remarkable optical, physical and antimicrobial properties and therefore have great potential to enhance agriculture production.

Several approaches have been employed to synthesize Zinc oxide nanoparticles such as physical, chemical, enzymatic and

biological methods [12]. Recently, synthesis of metallic nanoparticles using plant extracts is proved to be non-hazardous and getting more popular [13]. Green synthesis of nanoparticles includes the use of ecofriendly plant materials and safe solvents like water and natural extracts [14]. Plants are the largest source of herbal medicine since the ancient ages as traditional health care system. The bioactive phytochemical constituents of plants are the base of modern drugs as well as conventional medicinal systems that are being still in use.

Melia azedarach L. is popularly known as Indian lilac or Persian lilac. It belongs to the family Meliaceae and native to India, Indochina, Southeast Asia and Australia. This plant is naturalized in most of the tropics and subtropical countries. In India it is widely distributed in Himalayan region between the altitudes of 700 to 1000 m. The plant is characterized by the presence of dense and dark green leaves. Its bark is dark brown in color and fissured. The leaves are alternate, leaflets are short stalked. Flowers are white with purple stripes and characterized by the presence of a typical fragrance. Fruits are yellow berries, smooth and fleshy [15].

The phytochemical composition of *M. azedarach* is relatively complete. It is endowed with alkaloids, sterols, glycosides, fixed oil and fats, phenolic compounds, tannins, flavonoids, saponins, gum and mucilages, triterpenes, limonoids, azadirachtin, nimbin, nimbidin, gedunin, melianoninol, melianol, melianone, meliandiol, vanillin, nimbinene, meliacin, quercetin and rutin [15-20].

Traditionally it has been used in the treatment of leprosy, inflammations, cardiac disorders, malaria, diabetes, cough, skin diseases, strangury, amenorrhoea, bronchitis, eczema, asthma, fever, gonorrhoea, piles, gingivitis, tuberculosis and rheumatism [21-25]. The various parts of *M. azedarach* are explored for its immense biological activities, such as anthelmintic [23], antiviral [26], antifertility activity [27], ovicidal [28], larvicidal [29], antioxidant [19], antibacterial [30, 31], antiplasmodial [32], antiparasitic [33], antihyperglycemic, anticancer [34] and cytotoxic activities [35].

Biogenic synthesis of ZnO nanoparticles using whole plant extract has been successfully achieved in *Hybanthus*

enneaspermus, *Passiflora foetida* [36-37], *Hemidesmus indicus*, *Couroupita guianensis* [38-39], *Duranta erecta* [40], *Coriandrum sativum*, *Acalypha indica* [41, 42], *Calotropis procera*, *Calotropis gigantea* [43, 44] etc. The green methods were developed using aqueous extracts of various parts of *Melia azedarach* as reducing material and surface stabilizing agent for the first time in the synthesis of zinc oxide nanoparticles.

2. Materials and Methods

2.1. Collection of Plant material

Different parts of *Melia azedarach* were used to make the aqueous extracts. The young, disease free and healthy plant materials were collected from the east-coast of the south India (Puducherry, Cuddalore, Nagapattinam and Karaikal districts) after conducting the field surveys. Plant specimens were identified by The French Institute, Puducherry. The fresh leaves, stems, roots, flowers and fruits were collected and washed with running tap water. The surface water was dried and the plant parts were weighed about 5gm.

2.2. Preparation of the extracts (broth solutions)

The plant parts were thoroughly washed in distilled water and chopped into small pieces (Fig. 1-5A and B). 5 gm of chopped plant parts were boiled in a clean and sterilized conical flask of desired size with 50 ml of double distilled water for 5 min to prepare broth solution. The extracts were filtered with Whatman filter paper No.1 after boiling and stored in refrigerator. The filtrate aqueous extract was used as reducing agent.

2.3. Preparation of precursors and synthesis of Zinc oxide nanoparticles

Zinc Nitrate hexahydrate [$Zn(NO_3)_2 \cdot 6H_2O$] (Merck, Mumbai, India) was used as precursor to synthesize ZnO nanoparticles using *M. azedarach*. 1 mM Zinc nitrate solution was prepared using Zinc Nitrate hexahydrate with double distilled water and stored in refrigerator at 4 °C for further use. Three boiling tubes were taken for the synthesis process; one containing 10 ml of 1 mM Zinc nitrate solution as control, the second tube containing 10 ml of broth solution from appropriate part of the plant to observe the color change and the third tube containing 9 ml of 1 mM Zinc nitrate solution and 1 ml of plant extracts as test solution (reaction mixture) and incubated at room temperature (Fig. 1-5C).



Fig 1: A. Leaves, B. Chopped leaves and C. Reactions solutions.



Fig. 2. A. Stems, B. Stem cuttings and C. Reactions solutions.



Fig 3: A. Roots, B. Pieces of roots and C. Reactions solutions.

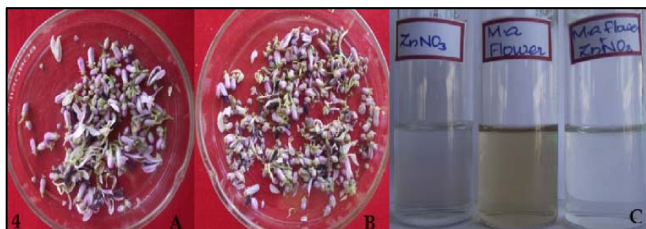


Fig 4: A. Flower petals, B. Chopped flower petals and C. Reactions solutions.



Fig 5: A. Fruits, B. Pieces of fruits and C. Reactions solutions.

2.4. Characterization of nanoparticles using UV-Vis spectroscopy analysis

The synthesized Zinc oxide nanoparticles using the plant extracts were centrifuged at 10000 rpm for 10 min in order to obtain the pellet which was used for further study. Supernatant was discarded and the pellet dissolved in deionized water. The reduction of pure zinc ions and the synthesis of Zinc oxide nanoparticles were confirmed and characterized by using UV-Visible spectrophotometer (Model 2202, Systronics Ltd. India). The UV-Vis absorption spectra of the zinc colloids from various parts of the *M. azedarach* were confirmed by using wave length scan between 200 nm and 700 nm.

3. Results and Discussion

Plants mediated synthesis of nanoparticles is considered safe and nontoxic as this approach eliminates the challenges associated with chemical and physical methods. The plant metabolites (phytochemicals) mediated reduction of metallic ions is proved to be quicker than chemical and microbes mediated synthesis of nanoparticles. The biomimetic approach of synthesizing ZnO nanoparticles is single step bioreduction method and less energy is used to synthesize nanoparticles [45]. The different parts of various plants such as stem, root, fruit, seed, callus, peels, leaves and flower are used to syntheses of metallic nanoparticles in various shapes and sizes using biological approaches [46-48]. Biosynthesis reaction can be altered by wide range of metal concentration and amount of plant extract in the reaction medium. The production of zinc oxide nanoparticles from the aqueous extracts of leaf, stem, roots, flowers and fruits of *M. azedarach* was investigated in

the present study. Initially the color change was not observed in the cell free extracts when challenged with precursor (zinc nitrate). After incubation of 6 hrs at room temperature, the leaf reaction mixtures turned yellow. The color was not changed in stem, roots, flowers and fruit reaction mixtures at room temperature even after 24 hrs. Color changes observed within one hour by heating the reaction mixtures at 60 °C for 15 min. In the present study, 1 ml of plant extract scaled up the synthesis of ZnO nanoparticles within 1 hr. It is further characterized by spectroscopic analysis. The UV- Visible spectral analysis of the reaction mixtures confirmed the synthesis of ZnO nanoparticles (Table 1.). Leaf and root reaction mixtures showed strong broad peaks at 296

nm, stem extracts at 293 nm, flower extracts located at 305 nm and fruit extracts at 323 nm (Fig. 6A-6E).

Table 1: UV-Visible absorption spectra of zinc oxide nanoparticles synthesized by exposure of *Melia azedarach* aqueous extracts.

Sl. No	Reaction mixtures	UV- Vis absorption spectrum (nm)
1	Leaf extracts	296
2	Stem extracts	293
3	Root extracts	296
4	Flowers extracts	305
5	Fruits extracts	323

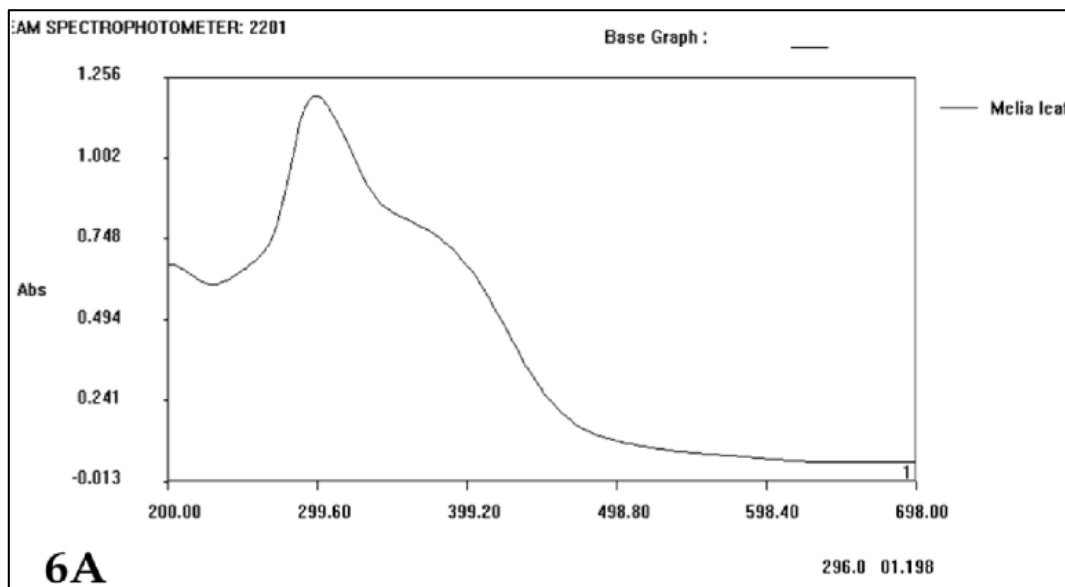


Fig 6A: Spectral absorbance peak of reaction mixture of leaf extract.

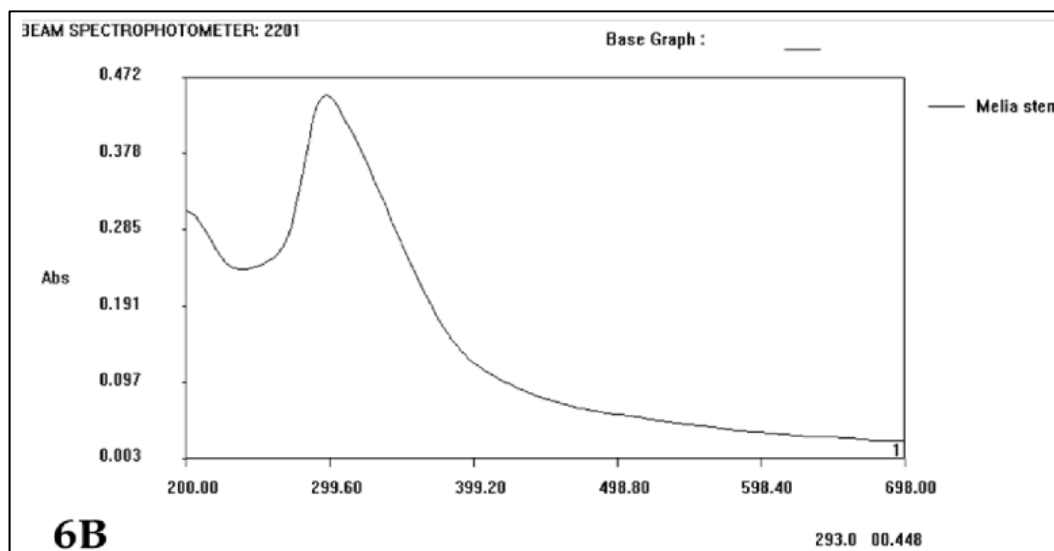


Fig 6B: Spectral absorbance peak of reaction mixture of stem extract.

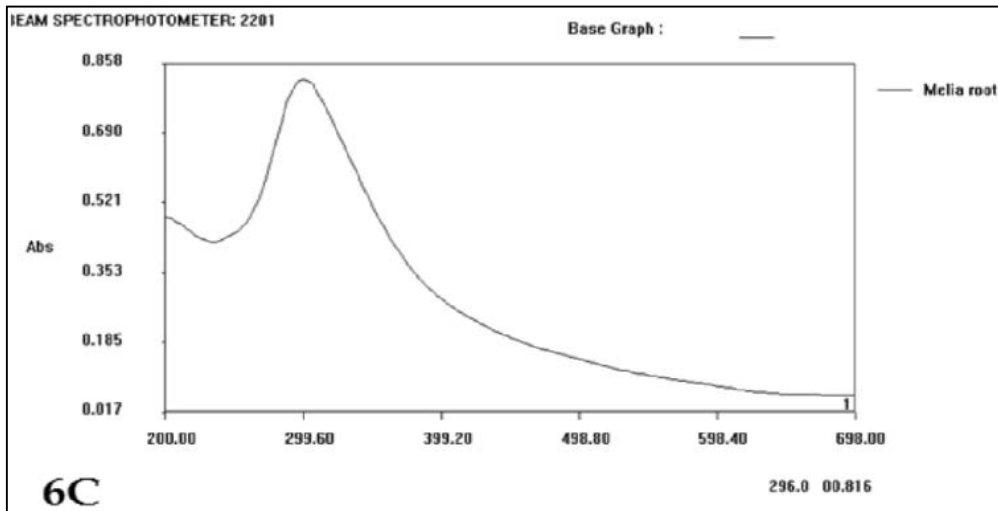


Fig 6C: Spectral absorbance peak of reaction mixture of roots extract.

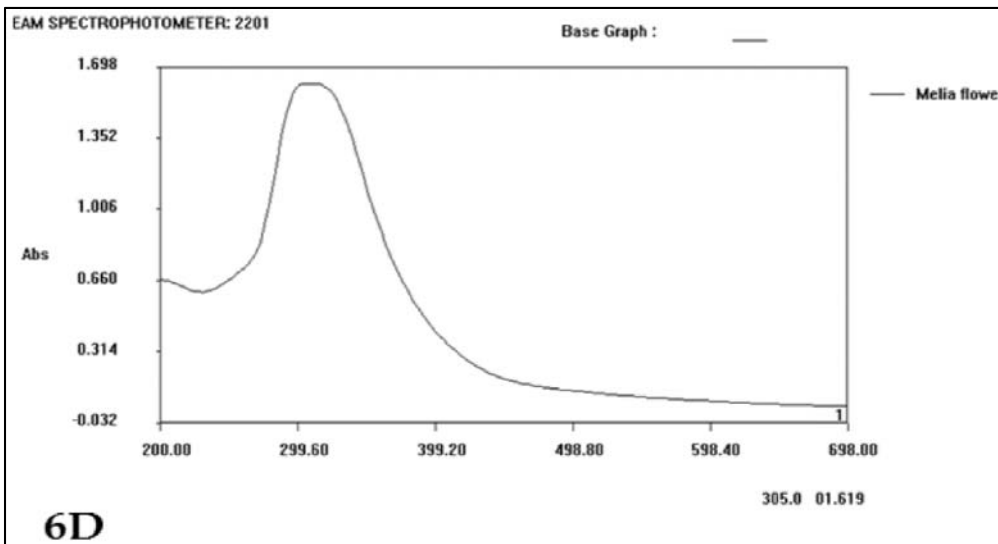


Fig 6D: Spectral absorbance peak of reaction mixture of flower extract.

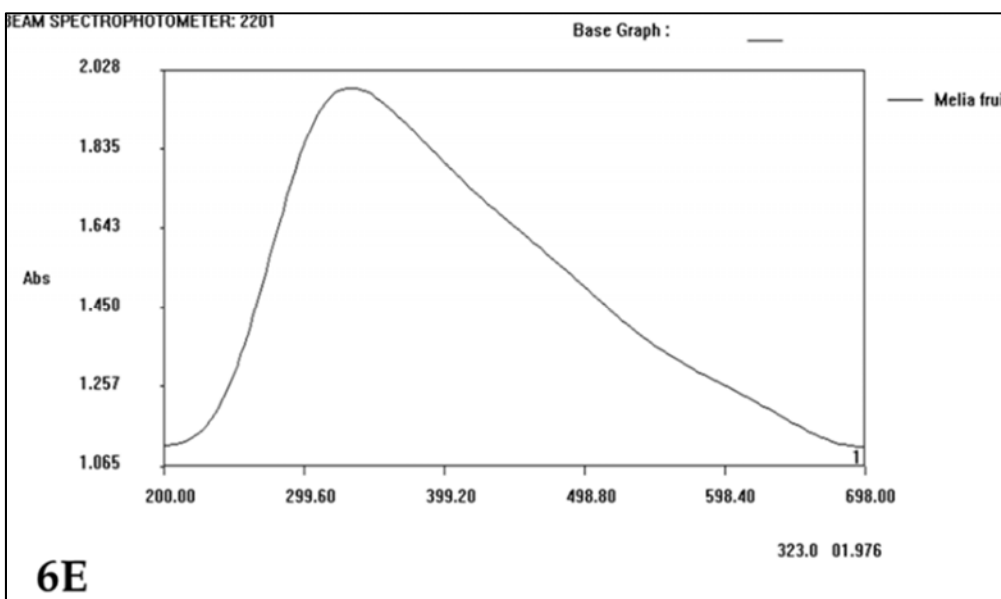


Fig 6E: Spectral absorbance peak of reaction mixture of fruits extract.

Zinc nitrate + plant extract \longrightarrow ZnO (nano size) + byproducts

The crude plant extracts of flowers and fruits contain secondary metabolites such as melianoninol, melianol, melianone, meliandiol, vanillin and vanillic acid [16]. Leaves have been reported to contain nimbinene, meliacin, quercetin and rutin [17, 18]. These compounds may act as reducing and stabilizing agents for the bioreduction reaction of ions into bulk metals and synthesis of metal nanoparticles [49, 50].

4. Conclusion

The bio reduction of aqueous zinc ions by the aqueous extracts of the medicinal plant *Melia azedarach* has been demonstrated. The whole plant *M. azedarach* can be a good source for the synthesis of zinc oxide nanoparticles. The biological synthesis of ZnO nanoparticles using aqueous extracts of medicinal plant *M. azedarach* is inexpensive, single step and eco-friendly method.

5. References

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