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RESEARCH ARTICLE

Phytosynthesis of zinc oxide nanoparticles from *Caesalpinia pulcherrima* (L.) Sw.

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Abstract

The zinc oxide nanoparticles (ZnO NPs) were synthesized using whole plant extracts of *Caesalpinia pulcherrima* in this study. Five grams of cleaned plant materials were boiled in 50 mL distilled water at 60°C for 10 min. The plant extracts were filtered through Whatman number-1 filter paper. One mM solution of Zinc nitrate hexahydrate [$Zn(NO_3)_2.6H_2O$] was prepared using distilled water and used as a precursor for the synthesis of ZnO nanoparticles. Initial visual color change (yellow) was observed as a preliminary confirmation of reaction mixtures with varied time intervals. The synthesis of ZnO NPs was further confirmed using the UV- Visible absorption spectroscopy. Leaf and flower reaction mixtures exhibited strong peaks at 314 nm, stem at 305 nm, root at 299 nm and seeds at 296 nm. This green approach is safe, eco-friendly, cost-effective and the prepared nanoparticles can be used in agriculture, cosmetics and pharmaceutical industries.

Keywords: *Caesalpinia pulcherrima*, reaction mixtures, UV-Visible spectroscopy, absorption spectra

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1. INTRODUCTION

The bottom up and top down approaches of nanoparticles synthesis is costly and slow therefore cannot be applied for the large scale production of nanoparticles (1). Physical methods require costly equipments, large space, high temperature and pressure (2), whereas the chemical methods need various chemicals which are ultimately harmful to the environment and people involved in the process. The search of eco-friendly, non-toxic protocols of nanoparticles synthesis paved the way for biological synthesis of nanoparticles (3).

Recently, various types of inorganic metal oxide nanoparticles have been synthesized such as Tittanium oxide (TiO_2), Copper oxide (CuO) and Zinc oxide (ZnO). Zinc oxide is a semiconducting metal oxide, used in the fields of electronics, optics, biomedical systems, and its production is simple, less expensive and safe (4-7). It has been reported that biosynthesis of ZnO nanoparticles (ZnO NPs) using plants, bacteria, fungi and algae are cheaper and permit bulk production without any impurities (8, 9). Zinc oxide NPs have been explored enormously for their anti-inflammatory, wound healing, anticancer, antidiabetic, antifungal, high catalytic, optic and UV filtering property (10-12). Large scale synthesis of ZnO NPs are in process for manufacturing of rubber, paints, to remove sulfur and arsenic pollutants from water bodies, bioremediation of weeds and in dental ailments (13)

Caesalpinia pulcherrima (L.) Sw. (syn. *Caesalpinia lutea*, *Poinciana pulcherrima* L.) belongs to the family Caesalpiniaceae, distributed in the Indo-Pakistan subcontinent, Taiwan, South East Asia, Africa and tropical America. It is commonly known as Patag, Brazil wood, Dwarf gulmohor, Pride of Barbados and Peacock flower (14, 15).

It is a small tree grows up to 6-10 m height and bears bipinnately compound leaves with 8-12 pairs of leaflets. Stem often bears thorns, roots woody; flowers are red and yellow, arranged in terminal as well as axillary panicles. Fruits are woody pods (16). The plant is reported to be drought tolerant (17) and cultivated for red dye (Brazilian dye) from its heartwood (18, 19), as an ornamental plant for flowers, and the leaves and pods used as fodder (20).

Traditionally the plant is used to cure various illnesses such as diarrhea, dysentery, inflammation, hemorrhages, fever, strangury, bronchitis, malaria, wheezing, pyrexia, muscular and rheumatic pain, cardiovascular diseases and leucorrhea (21-22). *C. pulcherrima* exhibits various biological activities (23-26). Phytochemical characterization of *C. pulcherrima* reveals the presence of numerous compounds such as carbohydrates, tannins, flavonoids, betacyanins, diterepenoids, phenols, glycosides and essential amino acids (27, 28). Its wood is reported to contain various aromatic compounds like brazilin, caesalpin, pulcherrimain, quercetic, rhamnetin, essential oils, saponin and gallic acid (29, 30).

The primary and secondary metabolites present in the plant parts help in the reduction of metal ions to nano particles. Till now, this plant has not been explored in synthesis of ZnO NPs. Therefore, the present study aimed to synthesize and standardize the protocol for production of ZnO nanoparticles from the aqueous extracts of various parts of the medicinal plant *Caesalpinia pulcherrima*.

2. Material and Methods

2.1 Collection of plant materials

Disease free healthy plants of *Caesalpinia pulcherrima* were marked in the institute campus (Puducherry, India) (Fig.1). Fresh green leaves, stem segments, root segments, flowers and mature seeds were harvested (Figs. 2A and C, 3A and C, 4A) during January-June 2017. Plant parts were cleaned properly and shade dried for 2 hrs. The materials were cut into small pieces (Figs. 2A,B,D,E; 3A,B,D,E; 4A,B).

Fig. 1. *Caesalpinia pulcherrima* plant under natural habitat.

2.2 Preparation of the plant extract

Five grams of cleaned plant materials were soaked in 250 mL Erlenmeyer flask containing 50 mL distilled water. The mixtures were boiled at 60°C for 10 min. The plant extracts were allowed to cool at room temperature, filtered through Whatman number-1 filter paper, and the filtrate was stored for further experimental use.

2.3 Preparation of precursor

One millimolar (mM) solution of Zinc nitrate hexahydrate [$Zn(NO_3)_2.6H_2O$] (Merck, Mumbai) was prepared using distilled water and used as a precursor for the synthesis of ZnO nanoparticles from *C. pulcherrima*.

2.4 Synthesis of ZnO nanoparticles

Three boiling tubes were taken, one containing 10 ml of 1 mM Zinc nitrate solution as reference, and the second one containing 10 ml of aqueous plant extract and the third tube containing 5 ml of 1 mM Zinc Nitrate solution and 5 ml of leaf, stem, root, flower and fruit extracts (reaction mixtures) and incubated at room temperature. To observe the visual color change in to yellow, the reaction mixtures were incubated in the room temperature for one hour. Appearance of yellow color from various reaction mixtures was noted. The reaction mixtures were centrifuged at 8000 rpm for 15 min, pellets were dissolved in double distilled water and used for characterization.

2.5 Characterization of biosynthesized ZnO NPs

The formation of ZnO nanoparticles were confirmed by measuring the UV-Visible spectroscopy. The UV–Visible absorption spectra of the reaction media were recorded at room temperature in a quartz cuvette (1 cm path length) and at the wavelength ranging from 200 to 700 nm using a Systronics Double Beam Spectrophotometer (Model 2202, Systronics Ltd.) in diffuse reflectance mode using Zinc Nitrate solution as reference.

3. Results and discussion

The present study involves the synthesis of ZnO nanoparticles using various parts of the medicinal plant *Caesalpinia pulcherrima.* The plant parts (flowers) were already utilized for the synthesis of gold (31) and silver nanoparticles (32).

3.1 Visual observation/changes

Plant extracts were turned into yellow when challenged with equal volume of the precursor (Zinc nitrate), but incubation time varied with different extracts. Stable increase in the concentration of color was observed in leaf and flower extracts than the remaining reaction mixtures. Color change was observed in leaf reaction mixture after 30 min, stem reaction mixture after 64 min, root reaction mixture after 81 min, flower reaction mixture after 37 min and seeds reaction mixture after 49 min after addition of precursor. Color changes in the reaction mixtures are preliminary test and indicate the reduction of zinc ions into zinc metal oxide (7, 33, 34).

3.2 UV-Visible analysis of ZnO NPs

UV-Visible spectroscopic analysis was conducted to authenticate the synthesis of ZnO NPs from different parts of the medicinal plant *C. pulcherrima*. Optical properties of ZnO NPs from the reaction mixtures were characterized based on UV absorption spectra with the wavelength range between 296-314 nm. UV-Visible spectra of leaf reaction mixtures revealed the peak at 314 nm. Stem reaction mixture initially exhibited peak at 290 nm but slowly rose and stable peak was observed at 305 nm. Root reaction mixture exhibited strong peak at 299 nm, flower and seeds reaction mixtures presented peak at 314 nm and 296 nm respectively (**Figs.** 2C and F, 3C and F, 4C).

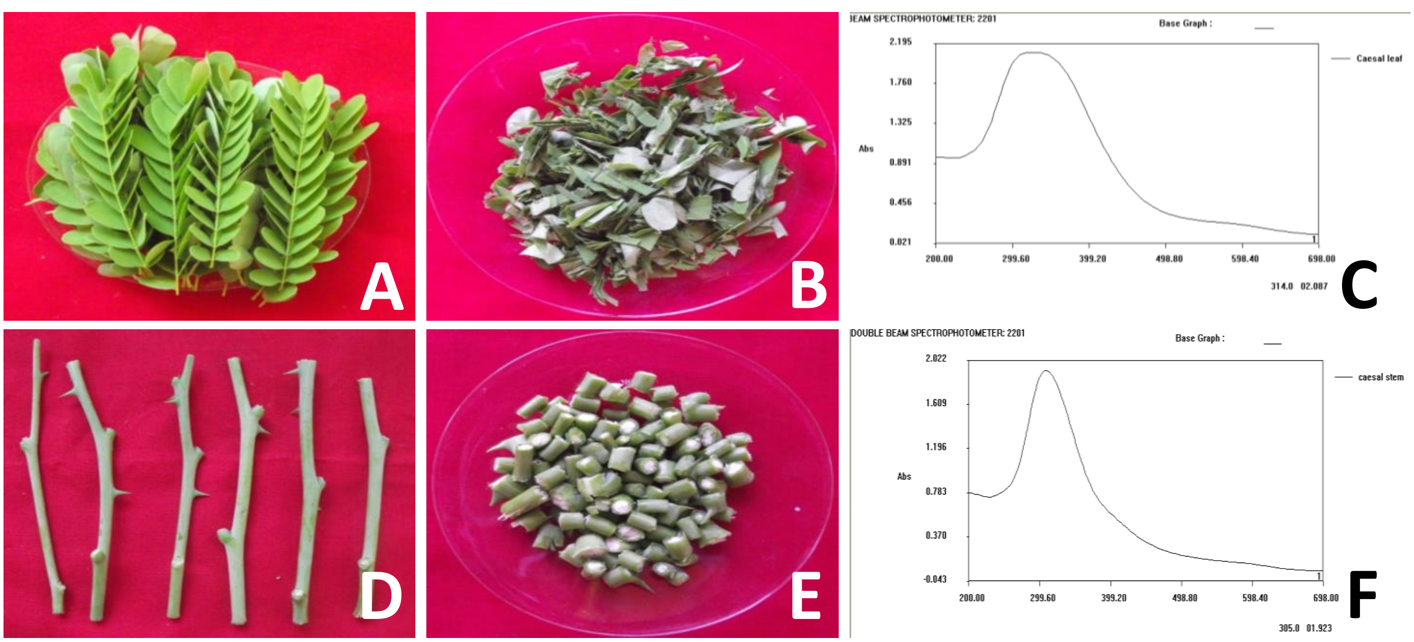

Fig. 2. A. Fresh leaves, **B.** Chopped leaves, **C.** Absorption spectra of the leaf reaction mixture, **D.** Stem segments, **E.** Small pieces of stem and **F.** Absorption spectra of the stem reaction mixture.

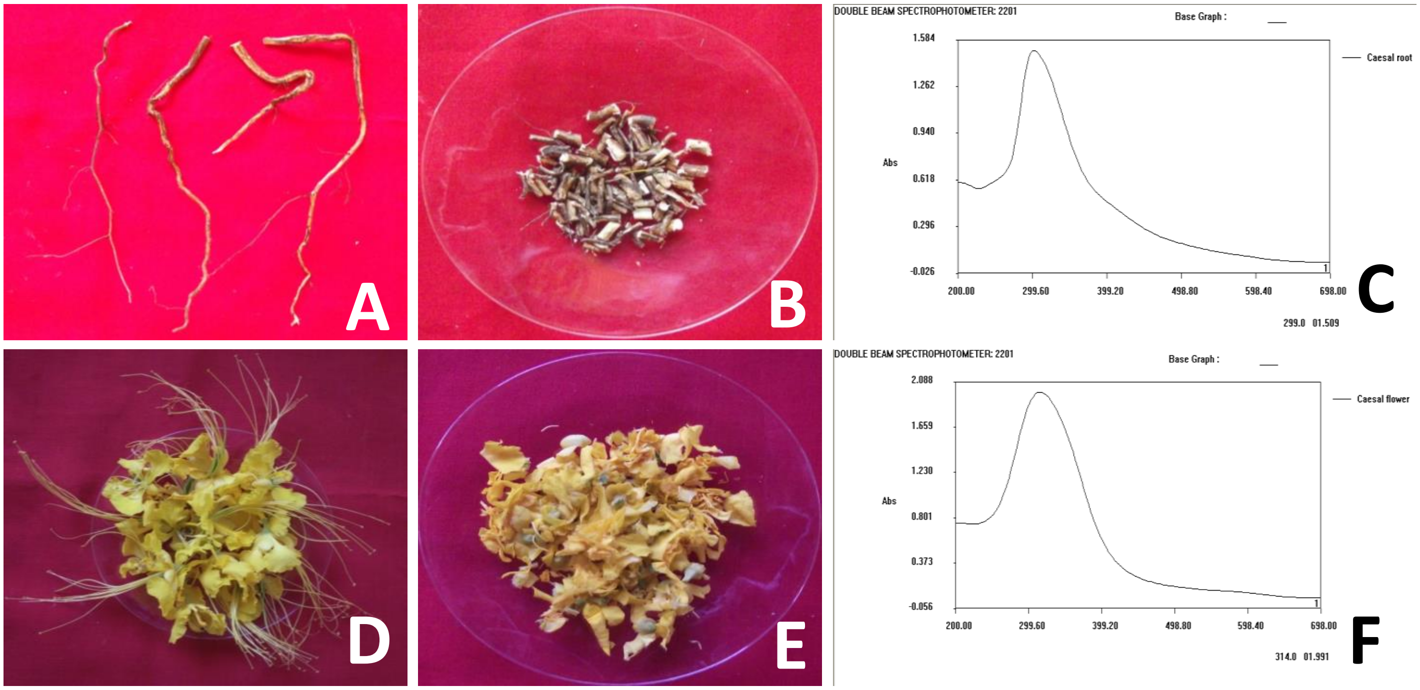

Fig. 3. A. Roots, **B.** Pieces of roots, **C.** Absorption spectra of the roots reaction mixture, **D.** Flowers, **E.** Small pieces of flowers and **F.** Absorption spectra of the flower reaction mixture.

Fig. 4. A. Fruits and seeds, **B.** Seeds for grinding, **C.** Absorption spectra of the seed extract reaction mixture.

Nagaraj et al. (31) synthesized gold nanoparticles using the aqueous flower extracts of *C. pulcherrima* with chloroauric acid ($AuCl_4$) solution. The synthesized gold nanoparticles were spherical, 10-50 nm in dimension and presented UV-Visible spectra at ~450 nm with strong antimicrobial activities. In a more recent report, spherical silver nanoparticles (Ag NPs) with the range of 12 nm were synthesized from the flowers of *C. pulcherrima*, which exhibited antimicrobial, antioxidant, cytotoxic, and genotoxic activities (32).

World Health Organization supports continuous research into new drugs to combat the increasing threat of global antibiotic resistance (35). Zinc oxide was reported as safe metal oxide by US FDA (36). *Caesalpinia* species are exploited for the synthesis, characterization and biological activities of various metal nanoparticles. Rao et al. (37) reported that *C. bonduc* stem bark extract mediated silver nanoparticles showed UV-Visible absorption peak at 470 nm. The synthesized nanoparticles were spherical (5-50 nm), and effective against *Staphylococcus aureus*, *Bacillus subtilis* and *Escherichia coli*. Leaf extracts of *C. sappan* is used to synthesize silver nanoparticles (38). Synthesized Ag nanoparticles showed strong surface plasmon peak at 440 nm, spherical in shape and were active against *S. aureus, B. subtilis, E. coli* and *Pseudomonas aeruginosa*. Jun et al. (39) reported that the cubic silver nanoparticles of 30.2 – 47.5 nm diameters from *C. sappan* act as an effective antibacterial agent against methicillin-resistant *S. aureus*. The leaf extract of *C. coriaria* were used to synthesize triangle, spherical and hexagonal silver nanoparticles, which showed strong inhibition of *E. coli*, *P. aeruginosa*, *Klebsiella pneumoniae* and *S. aureus* (40).

This is the first report on synthesis of ZnO NPs using aqueous extract of *C. pulcherrima* parts. Therefore, further studies in this direction may have potential benefits in human welfare using this medicinal plant.

4. Conclusion

Green synthesis of ZnO NPs reported in this study is eco-friendly, non-toxic, time and cost effective. The presence of similar phytochemicals in the leaf and flower extract helps in the synthesis of metal oxide nanoparticle by inducing UV-Visible absorption spectra at 314 nm. Clear absorption peak between 296-314 nm revealed that analogous functional groups of phytochemicals from the plant have mediated the oxidation and reduction reactions in ZnO NPs synthesis. Further research is required to explore the complete plant potential in human welfare.

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