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Biosynthesis of Zinc Oxide Nanoparticles from the Aerial Parts of *Hibiscus rosa-sinensis* L.

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Abstract

Plant extracts mediated synthesis of metal oxide nanoparticles has been developed to minimize the toxic and harmful impacts of chemical and physical methods. The present study is a green approach to synthesize zinc oxide nanoparticles (ZnO NPs) from the aqueous extracts of aerial parts of *Hibiscus rosa-sinensis*. Zinc nitrate hexahydrate ($Zn(NO_3)_2.6H_2O$) was used as a precursor and the extracts of leaves, stem segments and flower petals were used for the reduction of zinc oxide nanoparticles. The resulted reaction mixture with zinc colloids were characterized using UV-Visible spectrophotometric analysis. The absorbance peak was obtained at 300 nm, 291 nm and 293 nm for leaf, stem and flower petals respectively. This is an eco-friendly approach for quick scale up of ZnO nanoparticles from *H. rosa-sinensis* and it can be performed at room temperature.

Keywords: *Hibiscus rosa-sinensis,* Zinc nitrate hexahydrate, Reaction mixtures, UV-Visible spectrophotometer

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1. INTRODUCTION

The enormous applications of nanoparticles created the technological hot spot in bio-material research (1) and provides innovative and novel applications in various fields such as food processing, agriculture and formulation of medicines due to their peculiar spatial dimension (less than 100 nm) (2, 3). Application of zinc oxide nanoparticles (ZnO NPs) in the field of medicine is increasing day by day. Zinc oxide is transparent to visible light, so it can absorb UV radiation effectively (4). It is widely used as main ingredients in ointments, lotions, sunscreens, paints and coatings, ceramics and deodorants (5). ZnO nanoparticles are reported to exhibit antibacterial, antifungal, anti-diabetic and larvicidal properties (6-8).

Hibiscus rosa-sinensis (Malvaceae family) plant is an important ingredient of various Ayurvedic medicines, and cultivated extensively in tropics for its various medicinal and ornamental values. It is native to Tropical Asia (China). The perennial shrub bears simple, ovate-lanceolate leaves with terrete margins. The stem is green, erect, cylindrical and branched. Large attractive pedicellate flowers arise at the axils; corolla consists of 5 petals which are red in color (9).

The plant is used in traditional systems of medicine to alleviate menstrual cramps, induce abortion, assist in childbirth and relieve headache, fever, inflammation, respiratory problems, skin infections, digestive disorders etc. (10). Leaves and flowers are used as an antiseptic for boils and ulcers (11). The essential oil from the seeds is reported to cure kidney colic. The flower extract is used to treat veneral diseases and to promote hair growth. The anthocyanin pigments from the flowers are reported to possess cytotoxic activities against hepatoma and breast cancer cell lines (12).

Aerial parts are reported to contain alkaloids, flavonoids, saponins, phenols, tannins, terpenoids, steroids and coumarins. Leaves and stems possess β-sitosterol, stigmasterol, taraxeryl acetate and three cyclo propane compounds. Flowers contain cyanidine diglucoside, flavonoids and vitamins such as thiamine, riboflavin, niacin and ascorbic acid (13, 14).

The whole plant exhibits various pharmacological activities. Leaves and stems are reported to possess antioxidant and anticancer properties (11, 14). Flower exhibits antioxidant, antibacterial, anticonvulsant, antispermatogenic, androgenic, antitumor, antidiarrheric, antiphlogistic, antioestrogenic, analgesic, abortifacient, antipyretic, antifungal, antispasmodic, hypotensive, embryotoxic, larvicidal and anti-inflammatory properties (14-21).

The present study aimed in deriving protocol for the eco-friendly synthesis of zinc oxide nanoparticles from aerial parts of *H. rosa-sinensis*.

2. MATERIALS AND METHODS

2.1. Collection of plant materials and preparation of plant extracts

The plant was marked from the coastal regions of Puducherry (India), and identified with the help of Gamble flora (22). The various parts like leaves, stem segments and flower petals were procured from the disease free healthy plants (Fig. 1) during June-December, 2016. The plant parts were separated with fine razor blade and washed under constantly running tap water to remove the foreign materials such as soil, dusts and fungal particles. All the plant materials were washed thoroughly with double distilled water and finely cut into small pieces (Figs. 2A, 2B, 3A, 3B, 4A, 4B). Five grams of finely cut plant materials were boiled in clean and sterilized glass vessels with 50 ml of double distilled water for 5 min for the preparation of broth solutions. After boiling, the plant extracts were filtered through Whatman filter paper (90 mm). The extraction procedure was repeated three times and stored in refrigerator for further study.

Fig. 1. Plant *Hibiscus rosa-sinensis* with flower.

2.2. Preparation of precursor and synthesis of ZnO nanoparticles

Zinc nitrate hexahydrate ($Zn(NO_3)_2.6H_2O$) (Merck, Mumbai) was used as a precursor to synthesize ZnO NPs from *H. rosa-sinensis.* One milli molar (1mM) Zinc nitrate solution was prepared using Zinc nitrate hexahydrate with double distilled water and stored in refrigerator at 4ºC for further use. For the synthesis of Zinc oxide nanoparticles, three boiling tubes were taken, one containing 10 ml of 1 mM Zinc nitrate solution as control and the second one containing 10 ml of broth solution from appropriate part the plant to observe the color change and the rest one containing 9 ml of 1 mM Zinc nitrate solution and 1 ml of plant extracts as test solution (Figs 2C, 3C and 4C).

2.3. Characterization of ZnO NPs

The synthesized zinc oxide nanoparticles from the plant extracts were centrifuged at 10000 rpm for 10 min to obtain the pellet and it is used for further study. Supernatant is discarded and the pellet is dissolved in deionized water. The synthesis of zinc oxide nanoparticles were confirmed and characterized by UV-Visible spectrophotometer (Systronics Double Beam Spectrophotometer, (Model 2202, Systronics Ltd.). The UV-Visible absorption spectra of the zinc colloids from various parts of the plants were confirmed by using wave length scan between 200 nm and 700 nm.

3. RESULTS AND DISCUSSIONS

The present study was intended to synthesize zinc oxide nanoparticles (ZnO NPs) using aqueous extracts of aerial parts (leaf, stem and flower petals) of *H. rosa-sinensis* as reducing and stabilizing agents.

Biosynthesis of eco-friendly zinc oxide nanoparticles were reported to be non-toxic and cost effective (23). Attempts have already been taken to synthesize various metal nanoparticles from the selected species with astonishing biological properties. Shabana et al. (24) synthesized silver nanoparticles from the flower extracts of *H. rosa-sinensis* using 1 mM silver nitrate ($AgNO_3$) as precursor and the silver nanoparticles exhibits potential antimicrobial activity against *Aeromonas hydrophila*, which is a pathogen to the fishes (25). Bala et al. (26) synthesized zinc oxide nanoparticles from the leaves of *Hibiscus subdariffa* which were showing potential anti-diabetic and antibacterial activities against *Escherichia coli* and *Staphylococcus aureus*.

3.1. Synthesis of zinc oxide nanoparticles

Aqueous extract of leaf, stem and flower petals of *H. rosa-sinensis* is used to produce zinc oxide nanoparticles in this study. The zinc ions from zinc nitrate were reduced to zinc oxide (ZnO) nanoparticles when 1 ml of plant extract was mixed with 9 ml of zinc nitrate solution. At the ratio of 1:9 the reaction mixtures exhibits color change from colorless to yellow in the aqueous precursor and plant extracts. The color change in aqueous solution of reaction mixture was mediated through the excitation of surface plasmon vibration in zinc oxide nanoparticles (27).

3.2. UV-Visible spectral analysis

The reduction of zinc ions were confirmed in the samples by visual observation of color change to yellow in reaction mixtures. The leaf sample with precursor exhibited dark yellow but leaf and flower petals reaction mixtures were presented with pale yellowish solutions. This color variation in different samples may be attributed to the surface plasmon vibration of zinc oxide nanoparticles synthesized by various phytochemicals present in respective plant parts. The complete reduction of ZnO nanoparticles was observed by 24 hours of reaction at room temperature. The gradual formation of ZnO nanoparticles was investigated by UV-Visible spectrophotometer. UV-Visible spectral analysis of leaf reaction mixture produced a peak at 300 nm, stem at 291 nm and flower petals at 293 nm (Figs. 2D, 3D and 4D). It was reported that the synthesis of nanoparticles depended on the rate of the reaction and the stability of the nanoparticles on the reaction time. Similar reports on the utilization of whole plants or parts for the production of zinc oxide nanoparticles reported recently in *Hybanthus enneaspermus, Lawsonia inermis* (28, 29), *Peperomia pellucida* and *Celosia argentea* (30).

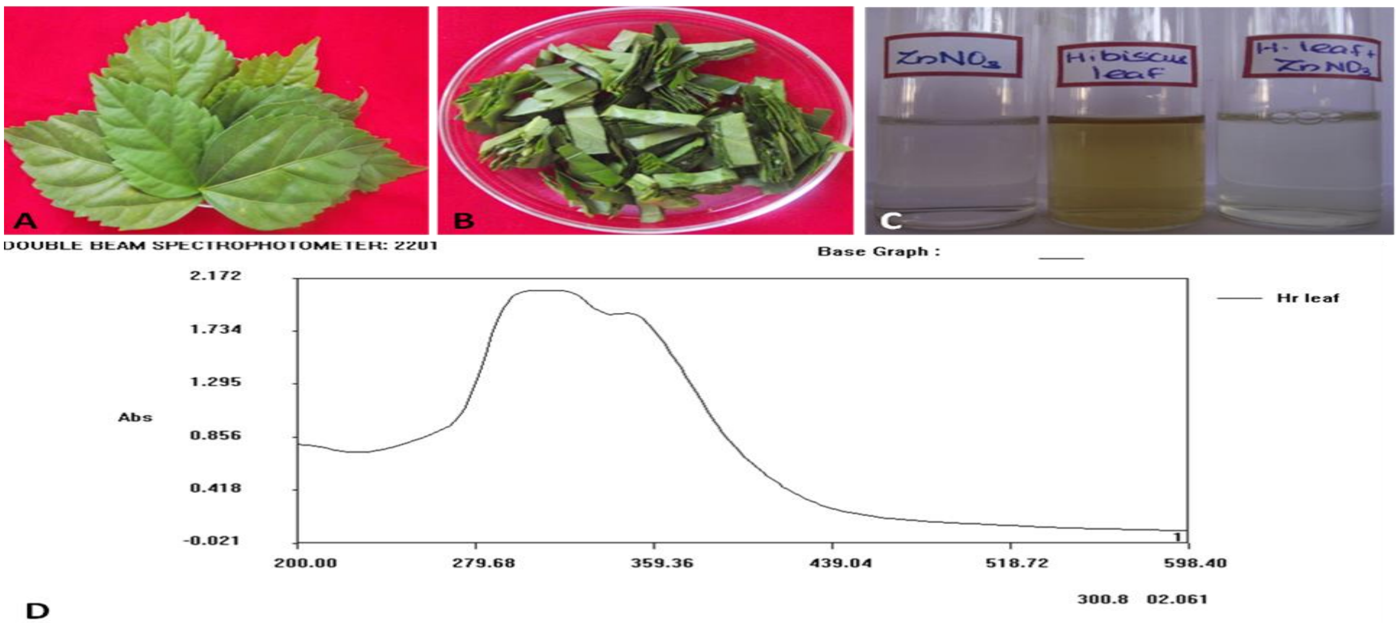

Fig. 2. A. Leaves used in the synthesis of ZnO nanoparticles, **B.** Chopped leaves, **C.** Reaction mixtures and **D.** Absorbance spectra of the reaction mixture.

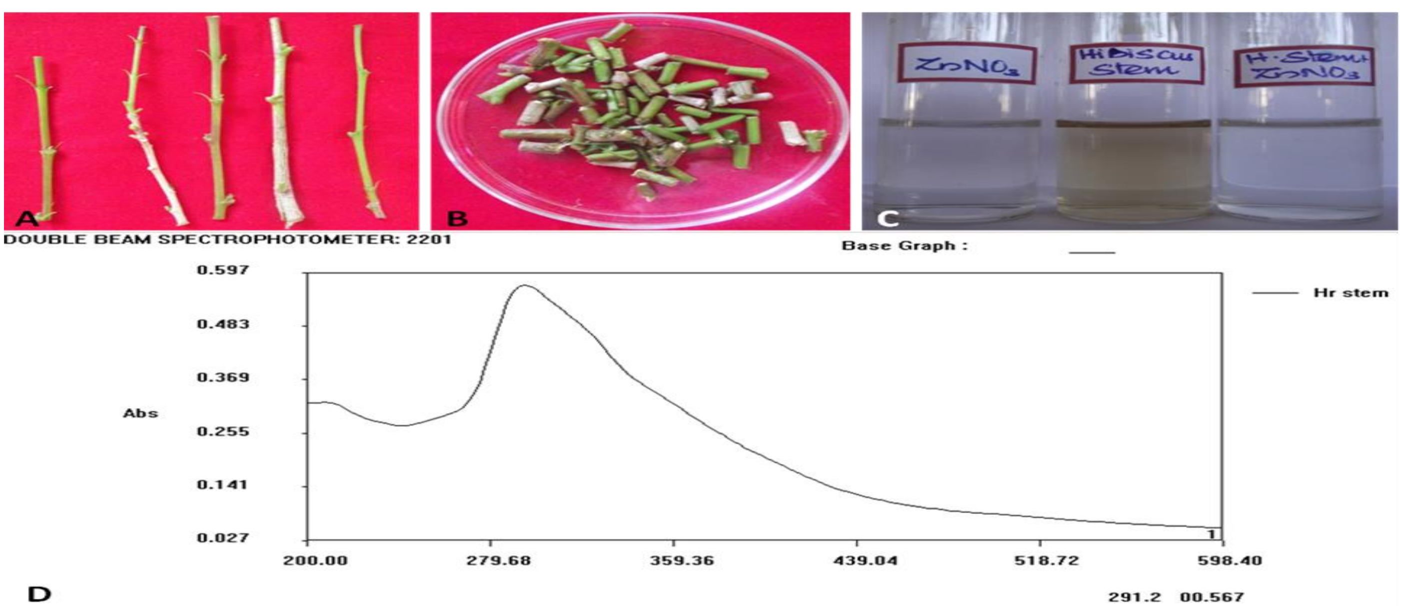

Fig. 3. A. Stem segments used in the synthesis of ZnO nanoparticles, **B.** Small cuttings of stems, **C.** Reaction mixtures and **D.** Absorbance spectra of the reaction mixture.

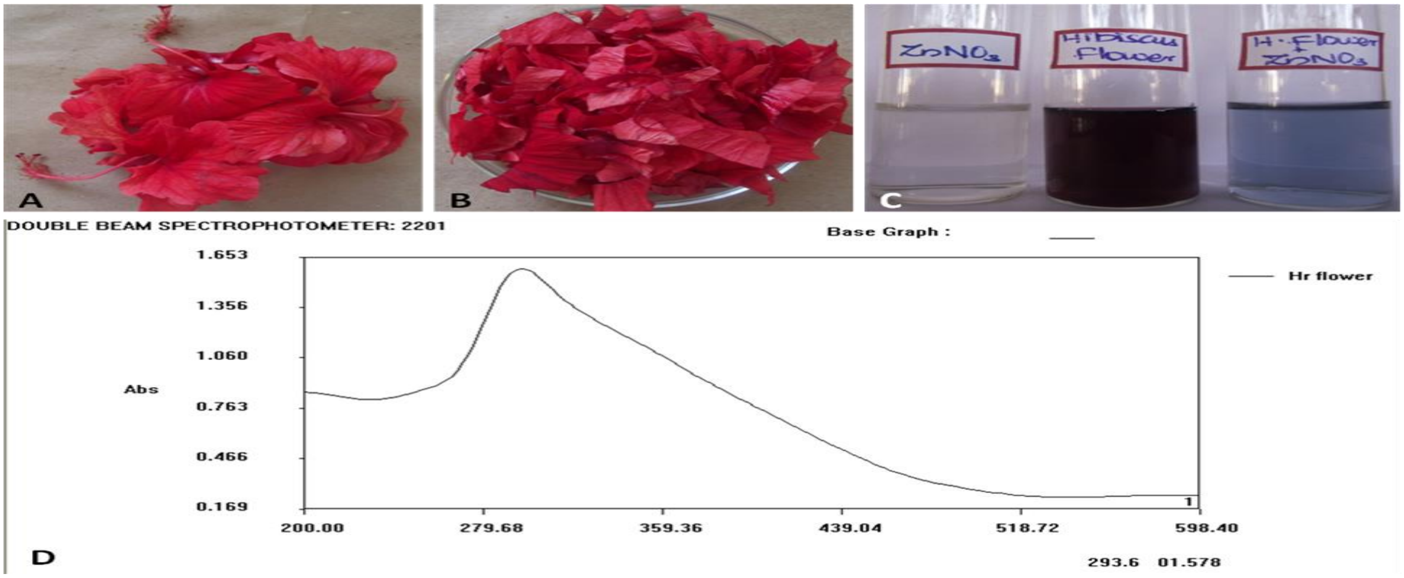

Fig. 4. A. Flower petals used in the synthesis of ZnO nanoparticles, **B.** Chopped petals, **C.** Reaction mixtures and **D.** Absorbance spectra of the reaction mixture.

Various reports revealed that the secondary metabolites such as flavonoids, phenolic compounds, reducing sugars, terpenoids, anthocyanins, some amino acids and proteins able to reduce zinc ions to zinc oxide (26, 31). Zinc oxide nanoparticles produced from the *Nyctanthus arbor-tristis* flowers exhibit potential antifungal activity against various phytopathogens such as *Alternaria alternata, Botrytis cinerea, Penicillium expansum, Aspergillus niger* and *Fusarium oxysporum* (32). Shabana et al. (24) reported that the phytochemicals present in the flower extracts of *H. rosa-sinensis* such as flavonoids, terpenoids, anthocyanins, amino acids and proteins may reduce the silver ions and strengthen the silver nanoparticles.

4. CONCLUSION

The present study concludes that the aerial parts of *H. rosa-sinensis* has the potential to reduce zinc ions and produce ZnO nanoparticles. The confirmatory graphs obtained by the UV-Visible spectral analysis indicate that the plant could be explored for the large scale production ZnO nanoparticles. Based on this report further studies can be carried out for the development of various medicines from *H. rosasinensis* sourced zinc oxide nanoparticles.

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