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Biogenesis of Zinc Oxide Nanoparticles using Aqueous Extracts of *Hemidesmus indicus* (L.) R. Br.

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Abstract: *This study reports the biosynthesis of Zinc oxide nanoparticles from the leaves, stem and root extracts of Hemidesmus indicus and their characterization. The development of eco-friendly, nontoxic and green methods for the synthesis of Zinc oxide nanoparticles (ZnO-NPs) has attracted increasing attention of the researchers. H. indicus is known as Indian Sarasaparilla, Nannari and Sariva in India and widely used in the Indian traditional systems of medicines. It is useful in the treatment of blood diseases, respiratory disorders, syphilis, eye diseases, kidney and urinary disorders etc. Leaves stem and root aqueous extracts contain various primary and secondary metabolites are responsible for the synthesis of nanoparticles. Zinc Nitrate hexahydrate ($Zn(NO_3)_2.6H_2O$) solution was used as precursor solution to synthesize the nanoparticles. The synthesis of nanoparticles was confirmed by changes in the color to pale green after heat treatment to the reaction solution using oven. The Zinc oxide nanoparticles were characterized by UV-Visible spectrophotometer and the absorption peaks were reported in between 29 nm to 310 nm proved the formation of Zinc oxide nanoparticles in the reaction mixture.*

Keywords: *Zinc oxide nanoparticles, green-synthesis, Hemidesmus indicus, characterization.*

1. Introduction

Recently nanoparticles gained immense scientific interest due to their specific intrinsic properties, determined by size, morphology and distribution [1, 2], they represent a bridge between the bulk materials and the molecules. The commercial exploration of nanoparticles paved the way with huge applications to humankind [3]. Metal oxide nanoparticles have been exploited enormously by their ability to withstand under harsh conditions and harmless to the environment [4].

The interaction of nanoparticles with biological materials establishes a series of bio-nanoparticle interfaces due to colloidal forces and biophysicochemical interactions [5]. The inorganic nanoparticles are very immense material because of their high surface area, easy to enter in cells through plasmodesmatal connections at nanoscale size, and they have potential properties for sensing and detection of various biological analytes. It has been reported that the semiconductor metal zinc oxide nanoparticles (< 30 nm) have the ability to alter the biological properties.

Zinc oxide nanoparticles (ZnO-NPs) have significant applications in various areas including optical piezoelectric, magnetic and gas sensing, high catalytic efficiency, strong adsorption ability, high isoelectric point, biocompatibility, and fast electron transfer kinetics for biosensing purposes. ZnO is extensively used as an alternative to TiO_2 for photodegradation mechanism. It has highest photocatalytic activity because of its more active sites, higher reaction rates and is more effective in generating hydrogen peroxide [6]. These have extensive applications in water purification [7] to remove arsenic, sulphur etc. from the polluted water [8]. ZnO-NPs are versatile elements with various biomedical properties like, antimicrobial [9], antibacterial activities [10] etc. These are also used in the preparation of cosmetics as well as eugenol for dental applications [11, 12], in biomolecular detection, diagnostics, antimicrobial textile industries and micro-electronics [9].

Biogenesis of ZnO-NPs using plant parts has already attracted researchers towards this field, and it has been achieved in *Acalypa indica* [13], *Aloe barbadensis* [14], *Morinda pubescens*, *Passiflora foetida*, *Hybanthus enneaspermus*, *Ficus benghalensis* and *Lawsonia inermis* [15-19].

Hemidesmus indicus (L.) R. Br. belongs to the family Asclepiadaceae, and commercially known as Indian Sarasaparilla, Nannari and Sariva, which is popularly exploited by the Indian traditional systems of medicines. It is a slender, laticiferous, twining shrub and has wide spectrum application in treating biliousness, blood diseases, diarrhea, respiratory disorders, skin diseases, syphilis, fever, bronchitis, asthma, and eye diseases, epileptic fits in children, kidney and urinary disorders, loss of appetite, burning sensation and rheumatism [20, 21]. The phytochemistry reveals the presence of saponins, tannins, hemidesmine, hemidesmol, hemidesterol, stearoptin, pregnane glycosides, β-sitosterol, indicusin, coumarin, volatile oils, triterpines, flavonoids in *H. indicus* [21, 22].

The aqueous extract of *H. indicus* was used for the biosynthesis of ZnO-NPs. As per the literature survey no data were available regarding the biogenesis of Zinc oxide nanoparticles using *H. indicus*. The present study intended to synthesize and characterize ZnO nanoparticles from various extracts of this valuable medicinal plant for the first time.

2. Materials and Methods

Preparation of broth solutions: The broth solutions (plant extracts) were prepared by using 5gm of washed and chopped fresh leaves, stem segments and root parts in a 250ml Erlenmeyer flask with 50ml of sterile double distilled water and then boiling the mixture for 5min. The herbal aqueous extract was collected in separate conical flasks by standard filtration method and stored at 4^oC in a refrigerator to carry further experiments.

Preparation of precursor solution: Zinc Nitrate hexahydrate [$Zn(NO_3)_2.6H_2O$] (Merck, Mumbai) was used as a precursor to synthesize ZnO nanoparticles from *H. indicus* in the present study. 1mM Zinc nitrate solution was prepared using sterile double distilled water and stored in brown bottle at 4^oC for further use to synthesize ZnO nanoparticles from leaf, stem, and root, immature and mature fruits of *H. indicus.*

Reaction mixture preparation: Three boiling tubes were used to synthesize ZnO nanoparticles, one containing 10ml of 1mM Zinc nitrate solution and the second one containing 10ml of aqueous plant extract and the third one containing 9ml of 1mM Zinc nitrate solution and 1ml of plant extracts as test solution/ reaction mixture.

Synthesis and characterization of Nanoparticles: The reaction mixture from the third tube was centrifuged at 8000 rpm for 15 min to obtain ultra fine pellet after 2 to 3 hrs. Supernatant is discarded and the pellet is dissolved in deionized water. The zinc nanoparticles were confirmed and characterized by UV-Visible spectrophotometer (wave length scan between 200nm and 700nm) (Systronics Double Beam Spectrophotometer, Model 2202, Systronics Ltd. India).

3. Results and Discussion

The use of plant or whole plant parts for the synthesis of nanoparticles is a quite novel method leading to truly green chemistry compare to other methods like chemical and physical methods. This is cost effective, environment friendly and easily scaled up process for large scale synthesis of important nanoparticles [23, 24]. The application of whole plant extracts in the genesis of metallic nanoparticles can overcome the synthesis of nanoparticles using fungi and bacteria (microbes) by reducing the cost and time and it do not require any special culture preparation and isolation techniques.

The experiments for biogenesis of zinc oxide nanoparticles using leaf, stem and root extracts of *Hemidesmus indicus* was carried out in this study. The color in the reaction mixture was unchanged for about two hrs; hence the precursor (reaction mixture) was subjected to heat. Color change from colorless to pale yellow was observed after 60 minutes of continuous heating with the aid of hot air oven at 60^oC. In case of *Morinda pubescens* plant extract for zinc oxide nanoparticles synthesis, color change was observed after 10 minutes of heating [15]. In contrast the bio-synthesis of silver nanoparticles from plant extract the color change was reported within 5 minutes [16]. But crystalline zinc oxide is thermochromic, which changes the color of reaction mixture from white to yellow when heated and reverting to white on cooling [14].

It was reported that the biosynthesis of zinc oxide nanoparticles is an enzyme-mediated process, where the plant extracts play a role as reducing and capping agents and Zinc Nitrate hexahydrate is a precursor for ZnO-NP's synthesis. The protein molecules in the extracellular extracts from *H. indicus* encapsulate the ZnO nanoparticles and become stable [13].

Evaluation of biosynthesized nanoparticles under UV- Visible spectrophotometry at room temperature reveals their optical properties. Intensive ultraviolet absorption was observed at 290-310 nm. The falling of absorption peak at 293 nm reveals the confirmation of zinc oxide nanoparticles from leaf reaction mixture (Fig. 1) and 300 nm by stem (Fig. 2) and 305 nm by root reaction mixture (Fig. 3). It is concluded that the presence of phytochemicals in the whole plants with slight variation could be responsible for the synthesis of zinc oxide nanoparticles, which aided in the reduction of metallic zinc from the plant tissue in to nano zinc as reported by Sangeetha et al. [14] and Shekhawat and Manokari [15]. The absorption wavelength at about 300 nm of ZnO suggested the excitonic character at room temperature. The biologically synthesized zinc oxide nanoparticles promote growth parameters in plants [25]. Zinc nanoparticles are used in cosmetics as the ingredients for age spot remover, and it is evident that from ancient times, *Hemidesmus* has been used to treat skin diseases in Indian Systems of Medicines [20]. So the nano source of this plant could be better exploited in medicine as well as pharmaceutical industry.

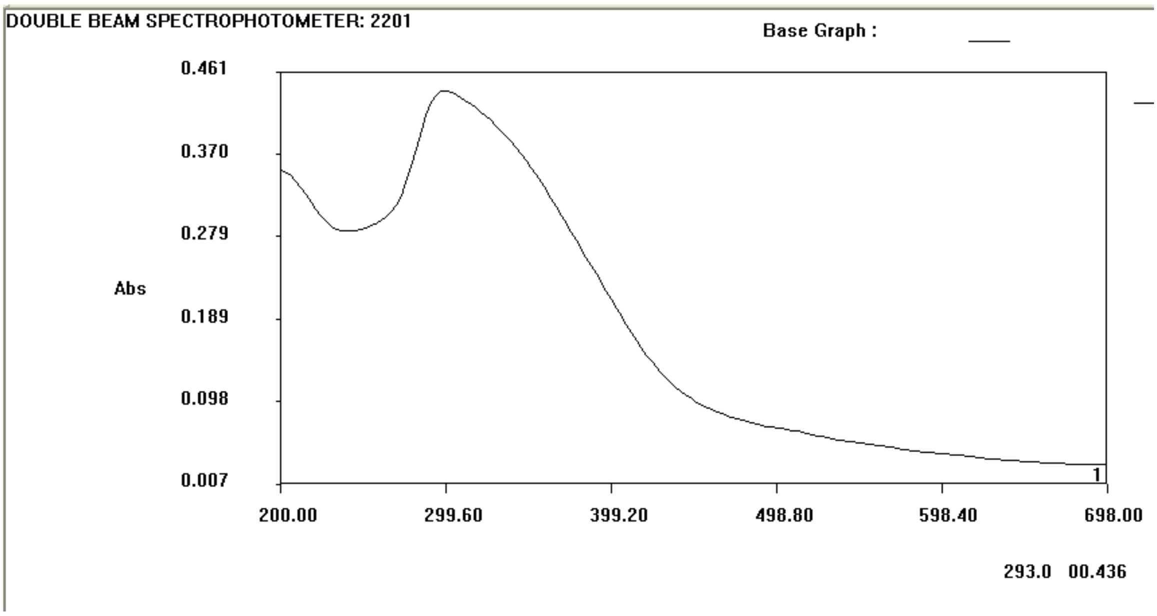

Fig. 1. *Spectrophotometric absorbance peak of leaf extract reaction mixture of H. indicus.*

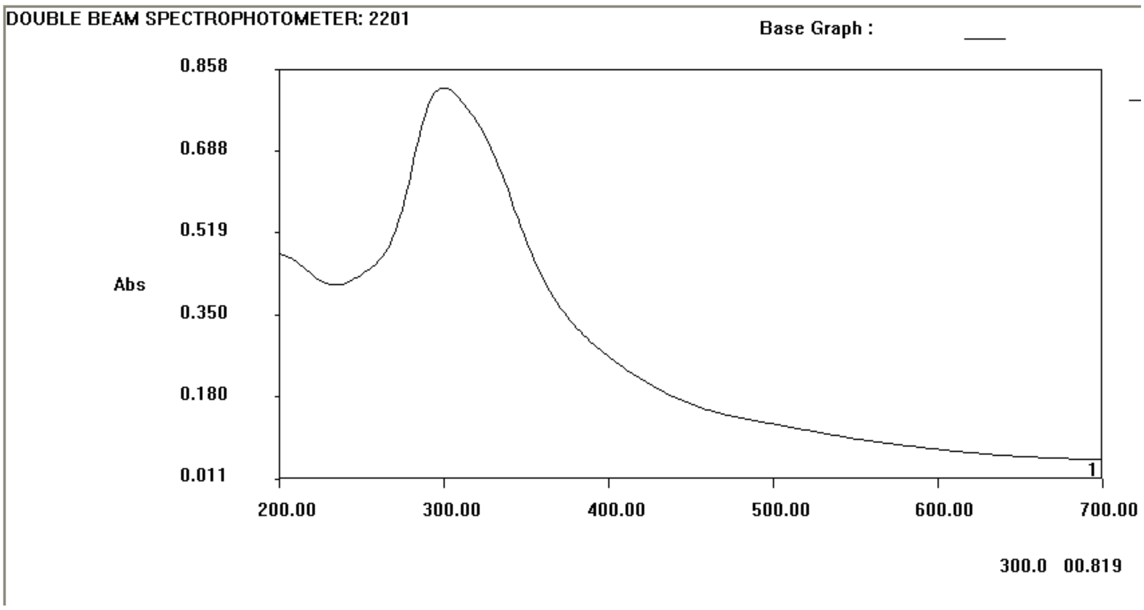

Fig. 2. *Spectrophotometric absorbance peak of stem reaction mixture.*

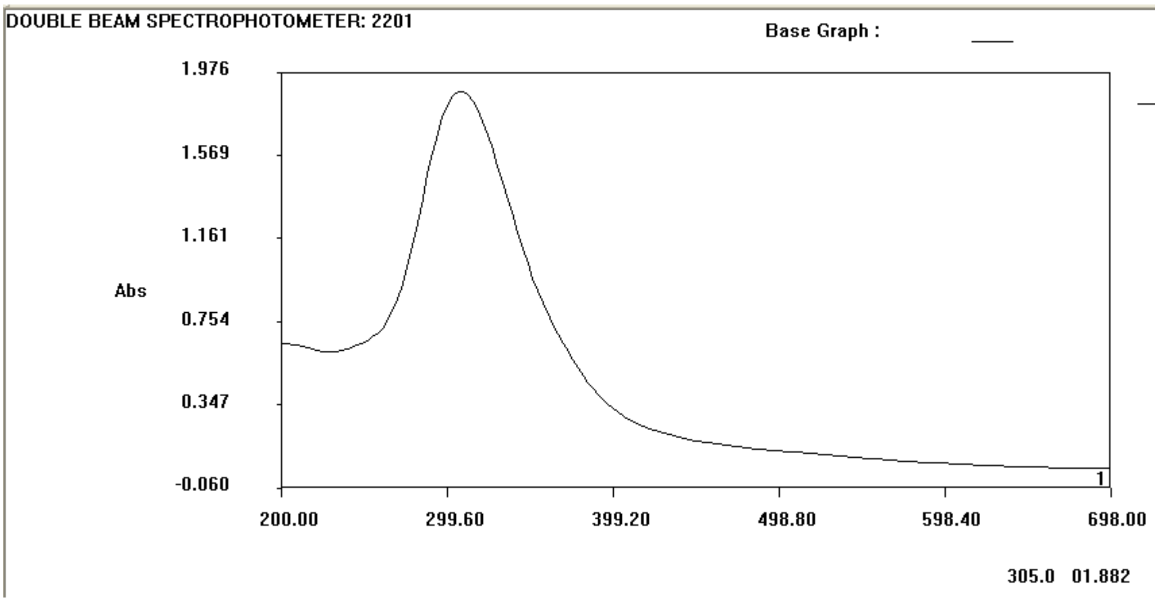

Fig. 3. *Spectrophotometric absorbance peak of root extract reaction mixture.*

4. CONCLUSION

This is the first report on eco-friendly biosynthesis protocol for the production of ZnO nanoparticles with *Hemidesmus indicus* plant extracts. The synthesis and characterization of ZnO nanoparticles using whole plant extracts play a vital role as preliminary key to identify the nano properties from different parts of the plant, and it could be exploited for further research in commercial application/ utilization of ZnO nanoparticles in various fields of medicine, agriculture, etc.

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