



Production of Zinc Oxide Nanoparticles using aqueous extracts of a medicinal plant *Micrococca mercurialis* (L.) Benth.

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ABSTRACT

The synthesis of Zinc oxide (ZnO) nanoparticles using plant parts is always an attractive and eco-friendly method. The present study focuses on the production of ZnO nanoparticles from Zinc Nitrate hexahydrate ($Zn(NO_3)_2 \cdot 6H_2O$) using the aqueous extracts of a medicinal plant *Micrococca mercurialis*. The plant is found to be rich in primary and secondary metabolites such as proteins, steroids and alkaloids, which are responsible and enhancing the biogenic synthesis of ZnO nanoparticles. Leaves, stems, roots and fruits of *M. mercurialis* were used to prepare the aqueous extracts. The characterization and confirmation of synthesized ZnO nanoparticles was performed by UV-Visible spectrophotometer. The reaction mixtures showed significant and sharp UV absorbance peaks at 305 nm, 299 nm, 311 nm and 302 nm corresponding to the aqueous extracts of leaf, stem, root and fruits reacted with zinc nitrate respectively. It has been proved that the medicinal plant *M. mercurialis* can be used to synthesize ZnO nanoparticles and the biological method of synthesis of ZnO nanoparticles are safe and good alternative to the chemical and physical methods.

Keywords: *Micrococca mercurialis*; Bioreduction; Zinc oxide nanoparticles; UV-Visible spectrophotometer

1. INTRODUCTION

Micrococca mercurialis (L.) Benth. belongs to the family Euphorbiaceae (Synonyms *Tragia mercurialis* L, *Mercurialis alternifolia* Lam. and *Claoxylon mercurial* Thwaites), growing naturally in various tropical regions of the world, and distributed throughout India, Sri Lanka, tropical Africa, Madagascar, Malaysia and the north Australia (Sutapa *et al.*, 2014). It is an erect annual herb reaches up to the height of 50 cm, leaves are alternate with crenate margins and crisped stems (Fig. 1). Flowers are arranged in interrupted clusters of axillary racemes. The fruits are glabrescent capsules and the seeds are globose, flowering and fruiting observed during August to October months. The plant habitats in seasonally waterlogged alluvium soil, and grow at the altitude ranging from 365 to 300 m (Almeida *et al.*, 1985).



Fig. 1. Plants growing in the natural habitat.

The use of plants in the synthesis of nanoparticles has drawn immense interest of researchers due to the simplicity of the method and single step biosynthetic process. Plants become the desirable and superior option for the safe, non toxic method of nanoparticle synthesis because the natural capping agents are readily taken from the plants (Ingale and Chaudhari, 2013).

Zinc oxide nanoparticles (ZnO NPs) are extensively studied material due to their low toxicity, high electron mobility, wide band gap, strong room-temperature luminescence, good transparency and photochemical stability (Zelechowska, 2014). The attractive properties of zinc oxide nanoparticles at room temperature and pressure make them enormous use in electronics, optoelectronics and laser technology (Bacaksiz *et al.*, 2008). At present, zinc oxide nanoparticles are used in new light-emitting devices, solar cells, biosensors, and photocatalysts (Fan and Lu, 2006).

The inexpensive bioreduction of zinc oxide nanoparticles is an upsurge of research at present. In recent years, plant-mediated biosynthesis of zinc oxide nanoparticles has been achieved in *Parthenium hysterophorus* (Rajiv *et al.*, 2013), *Sapindus rarak* (Maryanti *et al.*, 2014), *Acalypha indica* (Gnanasageetha *et al.*, 2014), *Passiflora foetida*, *Ficus benghalensis* (Shekhawat *et al.*, 2014, 2015), *Zingiber officinale* (Anand Raj and Jayalakshmy, 2015) etc.

The phytochemical constituents of plant parts are the basis for their therapeutic effects. Generally, different solvent extracts of *M. mercurialis* leaves, stem, root and fruits indicated the presence of different phytochemicals. The qualitative and quantitative analysis of phytoconstituents from *M. mercurialis* has been reported using petroleum ether, benzene, chloroform and methanolic solvent extracts. It yields reducing sugars, proteins, anthocyanins, alkaloids, steroids, flavonoids, triterpenoids, gums, saponins etc. (Sutapa *et al.*, 2014; Raj *et al.*, 2015a; Prasanna *et al.*, 2015).

M. mercurialis is eaten as leafy vegetable and used to treat fever in children (Rao and Satyanarayanaraju, 1975). The plant sap instilled into nose, eyes or ears to treat headache and filariasis of the eye (Tiwari *et al.*, 2011). This plant is reported to possess purgative and anticancer activities (Jeyachandran and Bastin, 2013). Raj *et al.*, (2015b) explored the fresh and powdered leaf of *M. mercurialis* for the synthesis of stable metallic silver nanoparticle, these were further evaluated for antibacterial activity against *Bacillus subtilis* and *Aeromonas sobria*.

To accomplish the demand of agricultural needs for ever-increasing population in current world, the green revolution technology using biosource in nanotechnology could bring significant contribution than the existing technologies. This is the first attempt to produce the zinc oxide nanoparticles using the different aqueous extracts of *M. mercurialis*.

2. MATERIALS AND METHODS

2.1. Chemicals used in the study

All the chemicals (analytical grade) used in the present investigation were purchased from HiMedia (Mumbai, India), and deionised water was used throughout the study.

2.2. Collection of plant material

The experimental material selected for the present study was *Micrococca mercurialis* (L.) Benth. an important medicinal plant. The plant material was collected from the Institute campus (Pondicherry, India), during the months of July to November 2014, and identified with the help of the French Institute of Pondicherry. The different parts of plant material such as leaf, stem, root and fruits were used for determination and characterization of zinc oxide nanoparticles analysis.

2. 3. Preparation of the plant extracts

Plant extracts were prepared by conventional extraction method. Fresh and healthy plants were collected, washed well and soaked in water in order to remove the contaminants present on the surface. After 30 minutes the plants were air dried completely to remove the moisture contents of the plant materials. Dried plant materials like leaf, stem, root and fruits were separated using sterile scissors (Fig. 2A, 2B to 5A, 5B). These parts were chopped into small pieces and then weighed (5 g) and boiled using 50 ml of deionised water in water bath at 50 °C for about 30 minutes. The extracts were filtered using Whatman[®] No.1 Filter paper. The extract was stored at 4 °C for further use.

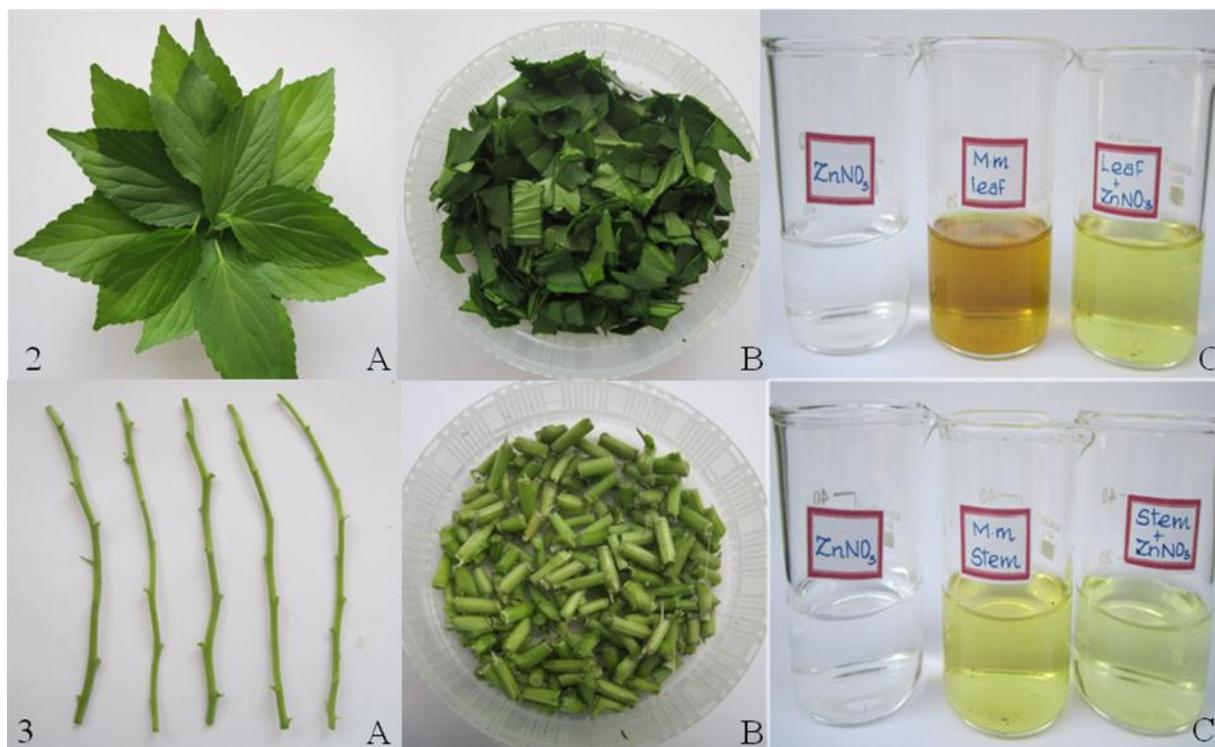


Fig. 2. A. Fresh leaves, B. Chopped leaves, C. Extract and reaction mixtures.

Fig. 3. A. Stems, B. Small pieces of stem segments, C. Extract and reaction mixtures.

2. 4. Preparation of precursor and reaction mixtures

Zinc Nitrate hexahydrate ($Zn(NO_3)_2 \cdot 6H_2O$) was used as precursor to synthesize nano zinc oxide from the various extracts of *M. mercurialis*. 1mM 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 1 ml $Zn(NO_3)_2$ and 9 ml of an appropriate plant part extract was mixed at room temperature to obtain reaction mixture. Figures 2C to 5C shows the plant extracts, precursor and reaction mixtures of leaf, stem, root and fruits. The reaction mixtures 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.

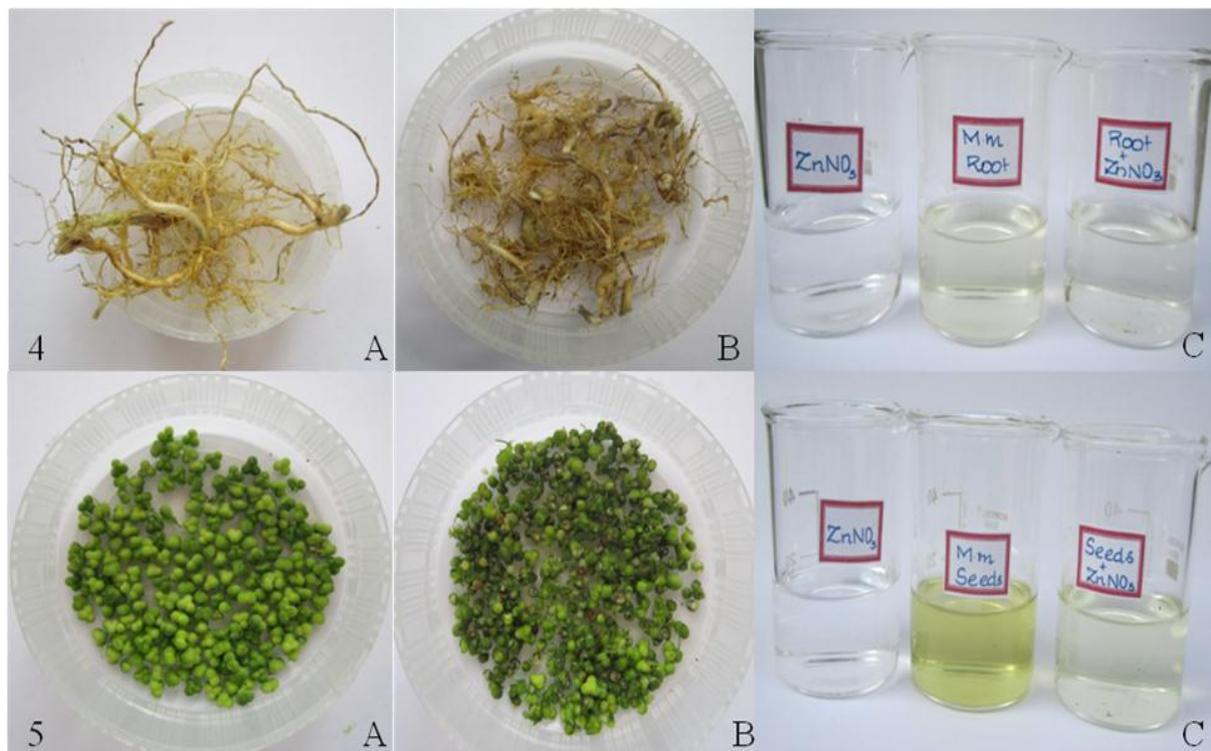


Fig. 4. A. Roots, B. Small pieces of root cuttings, C. Extract and reaction mixtures.

Fig. 5. A. Fruits, B. Small pieces of fruits, C. Extract and reaction mixtures.

2. 5. Characterization of ZnO nanoparticles through UV- Visible spectral analysis

The aqueous microemulsion (reaction mixtures) was starting solution for the production of nano zinc oxide. The confirmation of ZnO nanoparticles synthesis was obtained by measuring the optical property of reaction mixture using UV- Visible absorption spectroscopy analysis (Systronics Double Beam Spectrophotometer, Model 2202) between 250-800 nm. All the processes were carried out in a laboratory at 25 °C temperature.

3. RESULTS AND DISCUSSION

The aqueous solutions of *M. mercurialis* plant parts exhibited many phytochemicals which were utilized in the reduction of metal particles and in the synthesis of zinc oxide nano particles. Successful determination of biologically active compounds from plant material is largely depends on the type of solvent used in the extraction procedure (Zelechowska, 2014). Usually the primary metabolites are essential for normal growth and development of plants. Many primary metabolites play significant impact as precursors or pharmacologically active metabolites in drug research. With this background the present study framed to investigate the aqueous plant part extracts to synthesize environmentally benign zinc oxide nanoparticles.

The aqueous extracts of root, stem, leaves and fruits of *M. mercurialis* were screened for the synthesis of metallic zinc oxide nanoparticles for the first time.

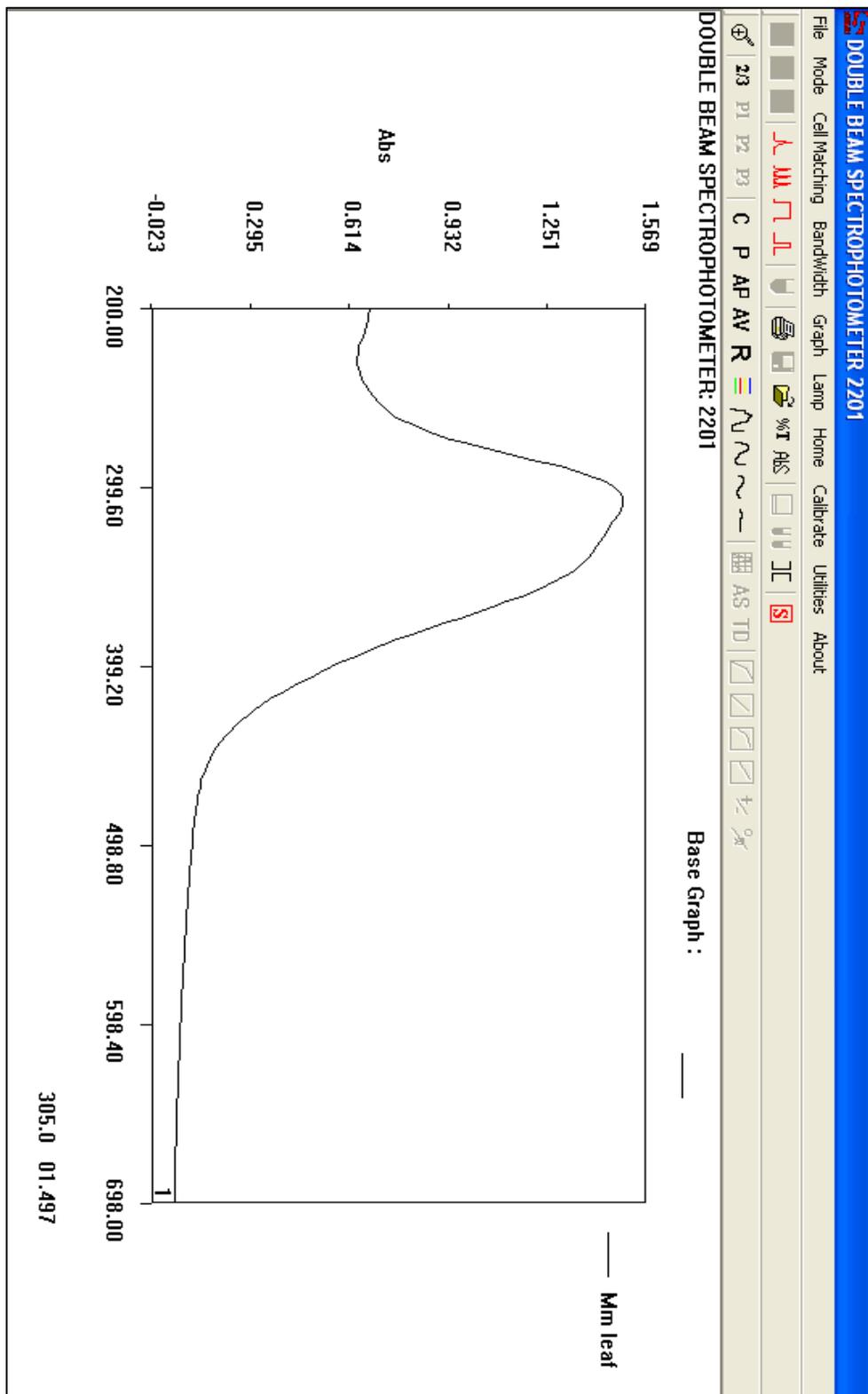


Fig. 6. Spectral absorbance peak of reaction mixture of leaf extract.

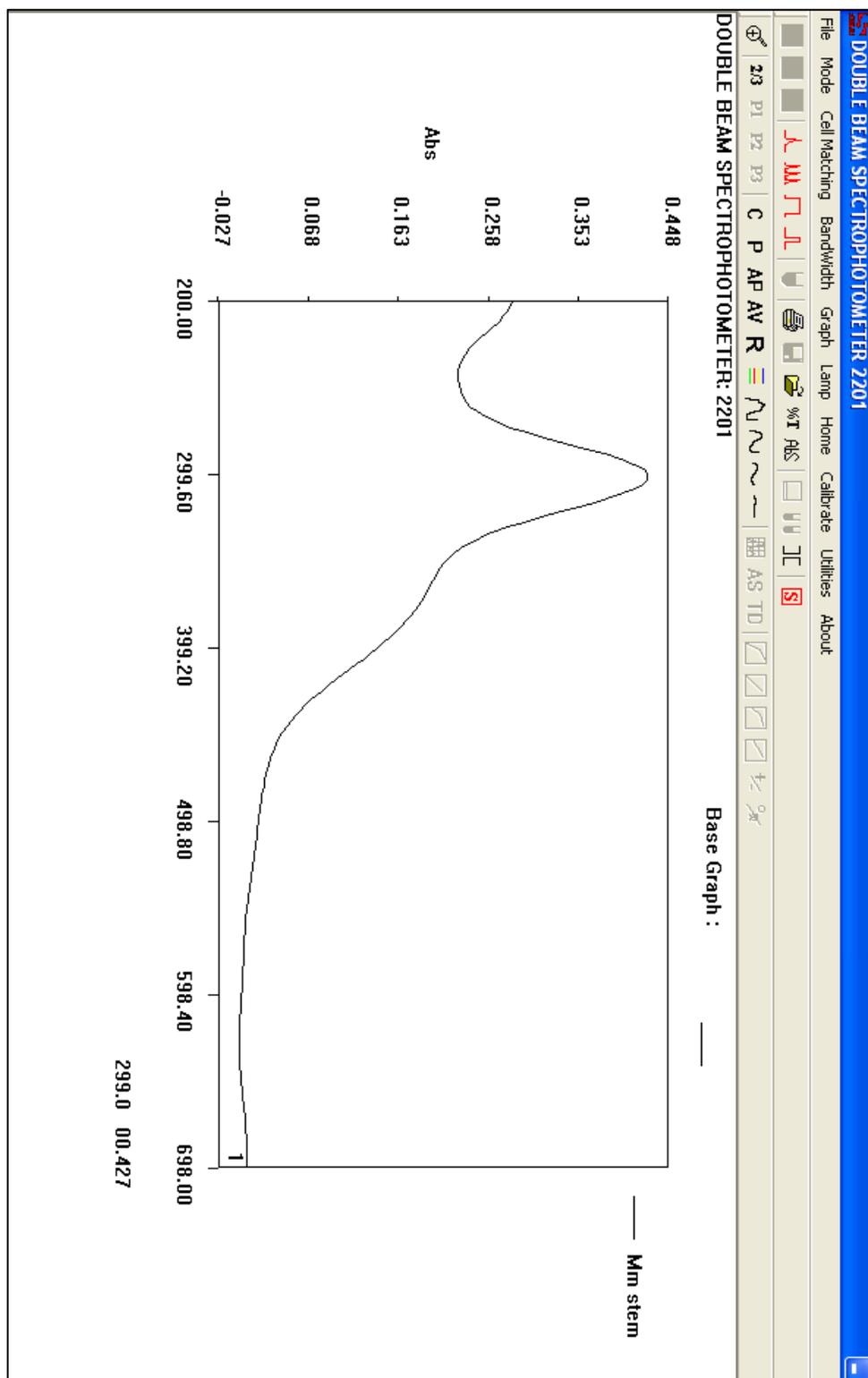


Fig. 7. Spectral absorbance peak of reaction mixture of stem extract.

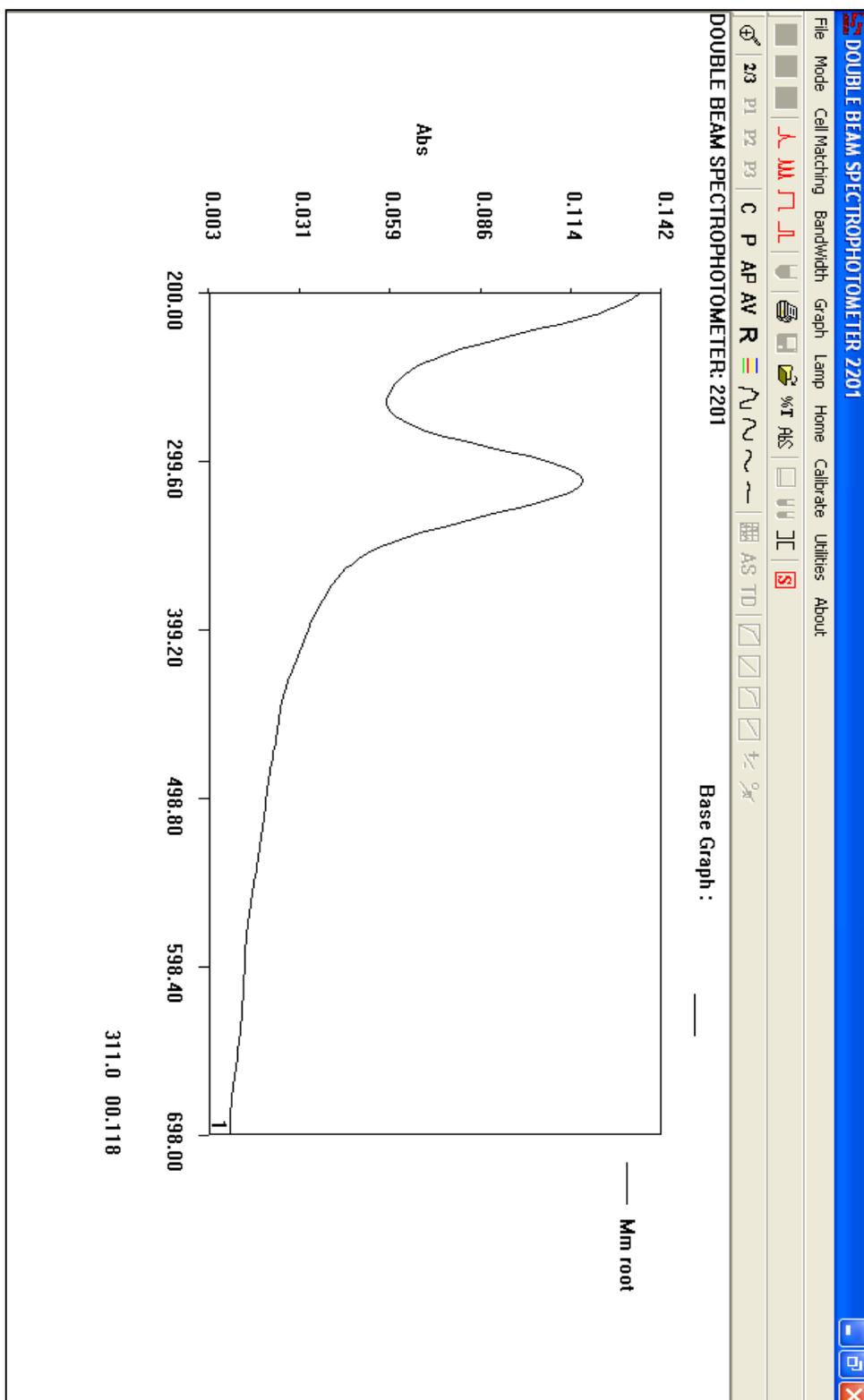


Fig. 8. Spectral absorbance peak of reaction mixture of root extract.

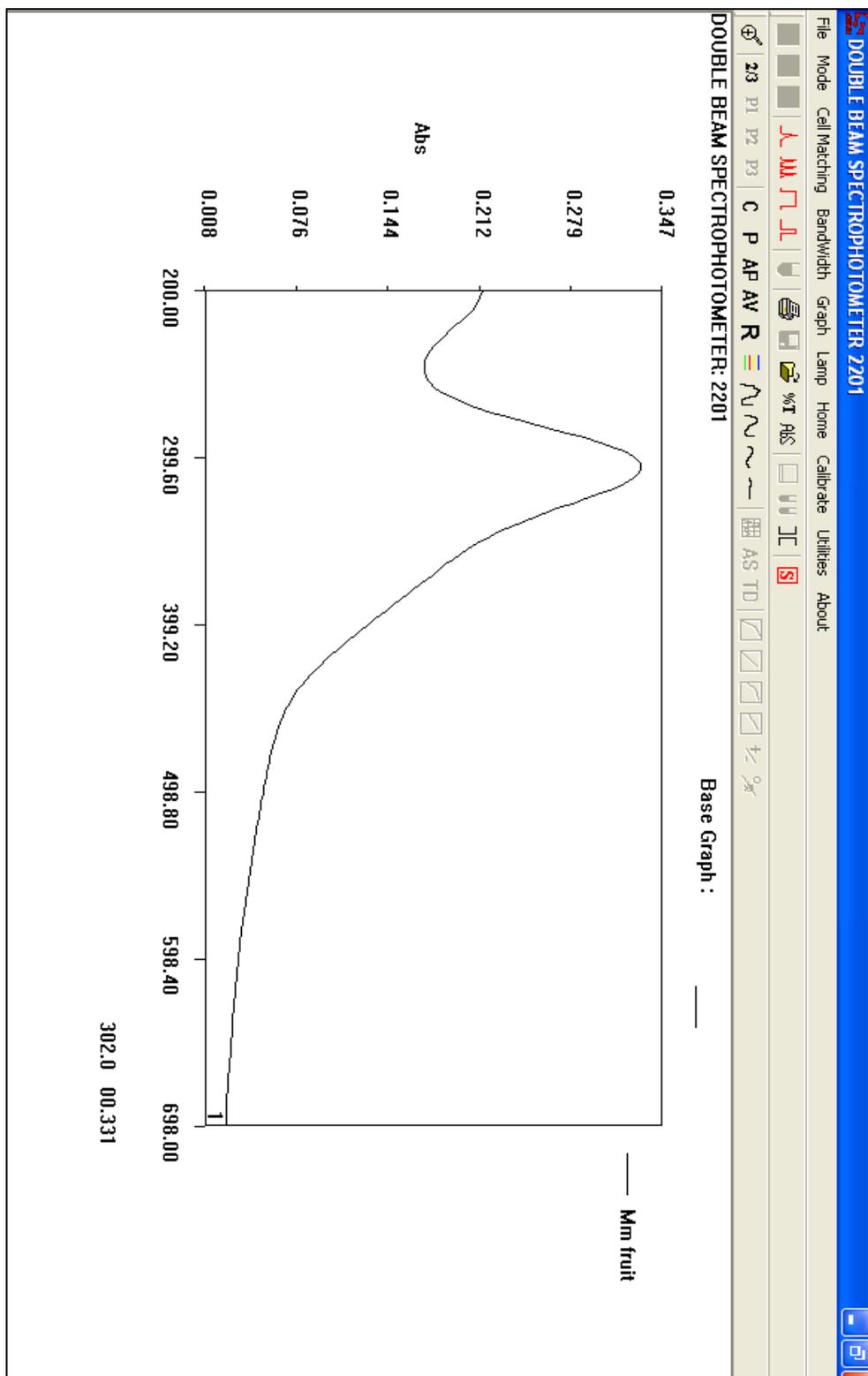


Fig. 9. Spectral absorbance peak of reaction mixture of fruits extract.

Zinc oxide nanoparticles were synthesized from Zinc Nitrate using the aqueous plant extracts of *M. mercurialis* as a capping agent in basic conditions in present study. On incubation at normal room temperature and pressure, yellow color solution (reaction mixture) was obtained. The biotransformed zinc oxide nanoparticles were confirmed through UV-Visible spectrophotometric analysis. These were further studied for the confirmation and characterization through UV-Visible spectral analysis.

The optical absorption of UV-Vis spectrum recorded in the present investigation revealed that the zinc oxide nanoparticles dispersed in the aqueous reaction mixtures of leaf, stem, root and fruits carried out by Double-Beam Spectrophotometer. The results demonstrated that the aqueous extracts showed a significant sharp UV absorbance bands at 305 nm with leaf extract (Fig. 6), 299 nm with stem extract (Fig. 7), 311 nm with root extract (Fig. 8), and 302 nm with fruits extract (Fig. 9) reacted with zinc nitrate solution.

Several reports stand on the synthesis of nanoparticles (Ag, Au, ZnO, Fe etc.) using aqueous extracts of various plant parts. An aqueous leaf extract of *Turnera ulmifolia* and *Couroupita guianensis* for the synthesis of Silver nanoparticles (Shekhawat *et al.*, 2012; 2013), *Allium cepa* extract for Gold nanoparticles (Parida *et al.*, 2011), *Azadirachta indica* leaf extracts for the production of Iron nanoparticles (Pattanayak and Nayak, 2013) and *Morinda pubescens* for zinc oxide nano particles (Shekhawat and Manokari, 2014) etc. were reported.

Zinc nitrate was used as precursor material to synthesis zinc oxide nanoparticles from various biological sources (Xu *et al.*, 2000; Shekhawat *et al.*, 2015). Raliya and Tarafdar, (2013) reported that the biosynthesis mechanism for ZnO nanoparticle using plants involves an enzyme-mediated process. The protein present in extracellular secrets, acts as capping protein, further encapsulates the ZnO nanoparticle and increases their stability.

Aqueous extracts of leaf and roots are reported to exhibit carbohydrate and proteins, amino acids at highest concentration (Prasanna *et al.*, 2015). The presences of alkaloids in plants are used in medicine as aesthetic agents and the steroids are studied to increase the nitrogen level in the body. Raj *et al.*, (2015a) reviewed the phyto-constituents in the plant parts which play key role in biosynthetic pathways and particular pharmacological activities. Therefore, the phytochemicals from *M. mercurialis* perhaps reduce the zinc nitrate into zinc oxide nanoparticles through the bioreductional process.

The biologically synthesized Zinc oxide nanoparticles using the aqueous extracts of *M. mercurialis* in present report can be directly used in agricultural, biomedical, engineering and other allied sectors of science and technology. The foliar spray of the ZnO nanoparticles showed better response in growth of the crop plants (Pandey *et al.*, 2010; Raliya and Tarafdar, 2013). This approach opens new path for fertilizer industries in the production of nanofertilizers for plant nutrition as growth supplement.

4. CONCLUSION

The present study demonstrates the low cost and eco-friendly approach in biogenic synthesis of ZnO nanoparticles using aqueous extracts of *M. mercurialis*. The presence of secondary metabolites in the plant extracts may probably be responsible for the reduction of Zinc nitrate to ZnO nanoparticles. The UV spectra of ZnO nanoparticles synthesized using leaf, stem, root and fruit extracts confirmed the presence of zinc oxide at nano scale. The

present research work can find significant applications in fields of agriculture science and technology.

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