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Biogenesis of Zinc Oxide Nanoparticles using *Couroupita guianensis* Aubl. Extracts - A Green Approach

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ABSTRACT

Zinc oxide nanoparticles (ZnO NPs) were synthesized by the green synthesis methods using aqueous extracts of *Couroupita guianensis* Aubl. This is an eco-friendly, time conservative and cost effective way of nanoparticle production. The aqueous extracts of leaves, stem, flower petals and bark were used to synthesize the nanoparticles. Zinc Nitrate hexahydrate ($Zn\ (NO_3)_2 \cdot 6H_2O$) was used as a precursor material. The prepared Zinc oxide nanoparticles were characterized using UV-Vis spectrophotometric analysis. The wavelength specific UV-Vis absorption peaks for Zinc oxide nanoparticles were recorded between 290 nm to 302 nm which confirmed the presence of Zinc oxide in nano scale. The reaction mixture with aqueous extract of leaf showed maximum absorbance spectral peak at 302 nm, stem at 294 nm, flower petals at 292 nm and bark at 288 nm. This is the first report to explore the use of *Couroupita guianensis* for the biogenic production of Zinc oxide nanoparticles to meet the demand of the pharmaceutical, medicinal and environmental industries.

***Keywords*:** Zinc oxide nanoparticles; *Couroupita guianensis*; aqueous extracts; characterization

1. INTRODUCTION

Zinc oxide nanoparticles (ZnO NPs) are reported to have extensive applications in biological and pharmacological areas of research. The non toxic and low cost production properties make these suitable in the drug research and in the field of agriculture. Zinc oxide metal nanoparticles efficiently protect a broader Ultra Violet (UV) range than any of the molecular UV absorbers. It either absorbs or transmits radiations from the sun; therefore, it is extensively used in cosmetics, sunscreen lotions etc. (Firdhouse *et al.*, 2015). The physical and chemical properties of ZnO and other metal oxides are enormously applied in biomedical and cancer applications, therefor, the ecofriendly green synthesis methods to synthesize nanoparticles are gaining interest in scientific community (Rasmussen *et al.*, 2010).

Among different metal oxide nanoparticles, ZnO nanoparticles have attained its own importance due to vast area of applications, such as optical devices, solar cells, gas sensor, biosensor, storage, window materials for displays, solar cells and drug-delivery. ZnO is presently listed as “generally recognized as safe” (GRAS) material by the Food and Drug Administration and also used as food additive.

The synthesis of nanoparticles received prime preference in the field of nanotechnology due to the various properties of particle size (Prakasham *et al.*, 2014). Nowadays, the green synthesis of metal oxide nanoparticles is emerged as an interesting section of nanoscience. Also, there is growing attention to the large-scale biosynthesis of metal nanoparticles using plants, which are more stable with various shapes and sizes (Ramesh *et al.*, 2014). Currently bioactive principles of plants and their nanoproducts have been enormously studied in various bio-fields. The biological method of the synthesizing ZnO nanoparticles is gaining importance due to its simplicity and extensive antimicrobial activity (Gunalan *et al.*, 2012). The eco-friendly biogenesis of nanoparticles is an alternative to chemical synthesis to avoid chemo-toxicity in the environment (Mahanty *et al.*, 2013).

Plant extracts mediated synthesis of ZnO nanoparticles has been carried out recently in many plant species like *Camellia sinensis* (Shah *et al.*, 2015), *Ficus benghalensis* (Shekhawat *et al.*, 2015), *Punica granatum* (Mishra and Sharma, 2015), *Trifolium pratense* (Dobrucka and Dlugaszewska, 2015), *Hibiscus subdariffa* (Bala *et al.*, 2015) and *Aloe vera* (Varghese and George, 2015) etc.

Couroupita guianensis Aubl. is an important medicinal tree species with valuable socio-economic heritage and commonly known as Cannon ball or naglingam tree (Morankar *et al.*, 2013). It grows up to the height of 30-35 m with peculiar cauliflorous inflorescence (Fig. 1). The phytochemical screening of *C. guianensis* revealed the presence alkaloids, phenolics, flavonoids, saponins, rutin, quercetin, kaempherol, lutolin, ursolic acid, hopanes, indirubin, isatin, sterols, fernesol in this plant parts (Rajamanickam *et al.*, 2009; Manimegalai and Rakkimuthu, 2012). These phytochemicals have antioxidant, antifungal, antibacterial, anticancer, antioxidant and antimalarial activities, and also active against several human pathogens such as *Staphylococcus aureus, E. coli, Micrococcus spp., Corynebacterium diphtheria, Candida albicans* etc. (Regina and Rajan, 2012).

This plant is grown as an ornamental tree around the temples due to its pleasant aroma, and the magnificent Nagalingam flowers have attracted the people religiously in India because the flower resembles the Linga of Lord Shiva (reduced stigma) protected by the holy King cobra (staminal sheath) (Santosh 2011; Santosh et al. 2013).

Fig. 1. *Couroupita guianensis* tree with flowers in natural habitat.

The Government of Puducherry (India) has declared *C. guianensis* flower (Nagalingam flower) as the Official State Flower to conserve this valuable tree under natural habitats in South India (Deepa 2007).

Traditionally, *C. guianensis* fruit pulp and leaves are rubbed externally to treat skin diseases, seeds are used to treat reproductive disorders and infertility (Cheryl *et al.*, 2001; Morankar *et al.*, 2013). The whole plant is used to diagnose cold, stomach ache, tooth ache, throat infection, tumor etc. (Biset *et al.*, 2009; Pradhan *et al.*, 2009). In the present study, aqueous extracts of *Couroupita guianensis* has been implemented for the first time as a reducing material as well as surface stabilizing agent of zinc oxide nanoparticles.

2. MATERIALS AND METHODS

Zinc Nitrate hexahydrate ($Zn\ (NO_3)_2 \cdot 6H_2O$) and distilled water were used to synthesize ZnO nanoparticles from the aqueous plant extracts of *C. guianensis*. The leaves, stem, flower petals and bark of *C. guianensis* were collected from East Coast region of Puducherry (India) (Fig. 2A-5A). The chemicals used in the present study were procured from Himedia (Mumbai) Laboratories Pvt. Ltd.

2. 1. Collection of plant materials and preparation of extracts

The plant materials were thoroughly washed in continuously running water and dried in hot air oven for 10 minutes. The aqueous broth solution of samples was prepared by boiling 5 gm of plant materials (finely cut into small pieces of leaves, stem, flower petals and bark) (Fig. 2B-5B) in 250 ml Erlenmeyer flask using 50 ml of double distilled water at $60^{o}C$ for ten minutes. The resulting extracts were cooled to room temperature, filtered and used as the bio-reducing solution for the synthesis of ZnO NPs.

2. 2. Preparation of Zinc Nitrate solution

To synthesize ZnO nanoparticles, Zinc Nitrate hexahydrate ($Zn\ (NO_3)_2 \cdot 6H_2O$) was used as precursor. 1 mM Zinc Nitrate solution was prepared using double distilled water, filtered by Whatman® no.1 filter paper and stored at 4 °C for further experiments.

2. 3. Synthesis of ZnO nanoparticles

The aqueous extracts of leaf, stem, flower petals and bark were used in this study. Three containers measuring 50 ml were used for the experimentation. One container is filled with 10 ml of 1 mM Zinc Nitrate solution and another with 10 ml of plant extract to compare with the reaction mixture. The third container was filled with the reaction mixture (test solution), which is having mixture of 1ml plant extract and 9 ml precursor solution (Fig. 2C-5C). The reaction mixture was centrifuged at 5000 rpm for 15 min to separate pellet and supernatant; pellet is diluted with double distilled water and used for further study.

$$\text{Plant aqueous extracts + Zinc nitrate} \longrightarrow \text{ZnO nanoparticles + byproducts}$$

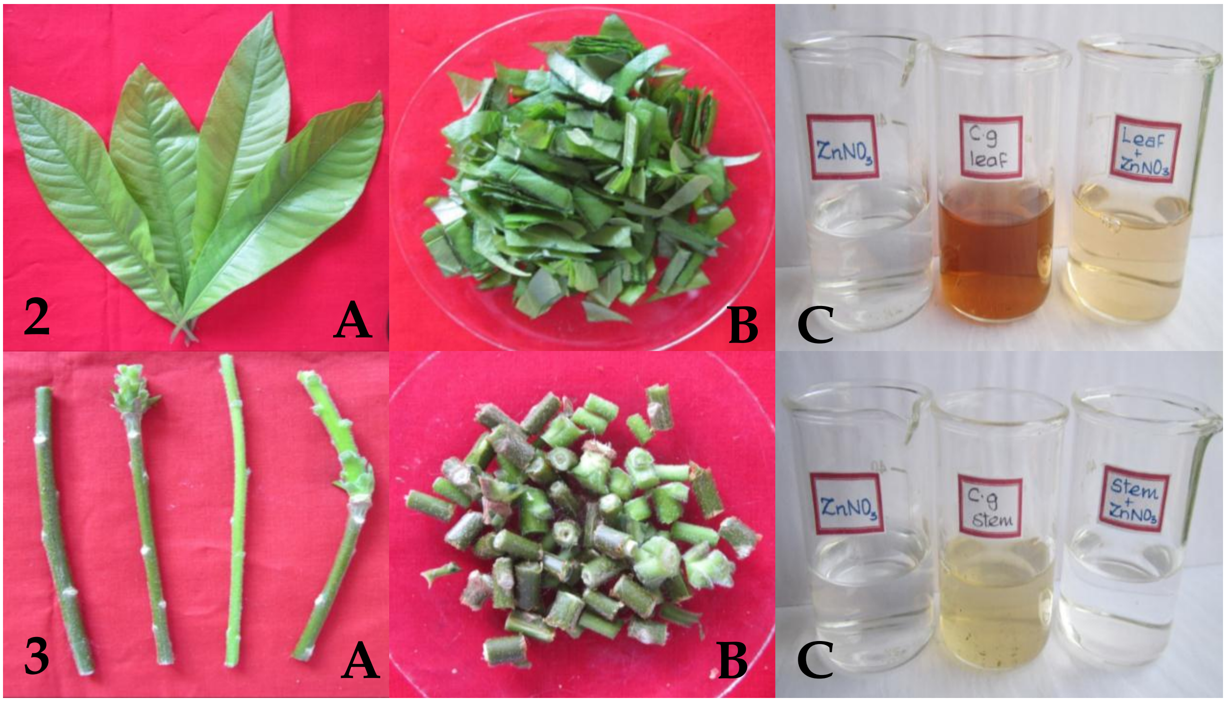

Fig. 2. A. Leaves, B. Chopped leaves and C. Reactions solutions.
Fig. 3. A. Stems, B. Stem cuttings and C. Reactions solutions.

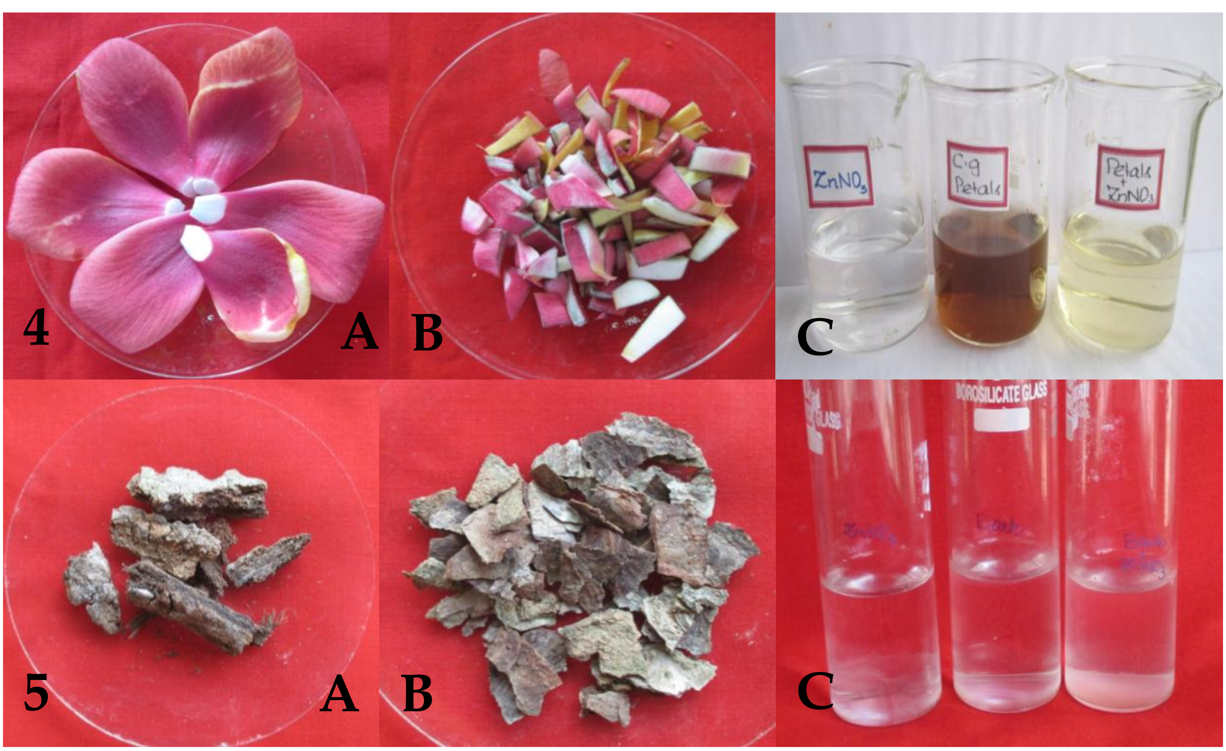

Fig. 4. A. Flower petals, B. Chopped flower petals and C. Reactions solutions.
Fig. 5. A. Bark pieces, B. Bark small pieces and C. Reactions solutions.

2. 4. UV–Vis spectral analysis

The reaction mixtures of leaf, stem, flower petals and bark were measured for its maximum absorbance using UV-Vis spectrophotometry. The optical property of ZnO nanoparticles was analyzed via ultraviolet and visible absorption spectroscopy (Systronics Double Beam spectrophotometer, Model 2202) in the range of 250-700 nm. UV-Vis spectra were measured at room temperature in a quartz cuvette with the path length of 1 cm in the present study.

3. RESULTS AND DISCUSSION

Plants are endowed with broad variety of metabolites, which are explored to reduce into metallic ions through biogenesis. The plant based synthesis of nanoparticles was reported as stable than other approaches (Rout *et al.*, 2012). UV–Vis spectral analysis is the widely accepted technique for the structural characterization of nanoparticles (Kulkarni and Muddapur, 2014).

The optical absorption of UV-Vis spectrum recorded in the present investigation revealed the ZnO nanoparticles dispersed in the aqueous reaction mixtures carried out by Double-Beam Spectrophotometer.

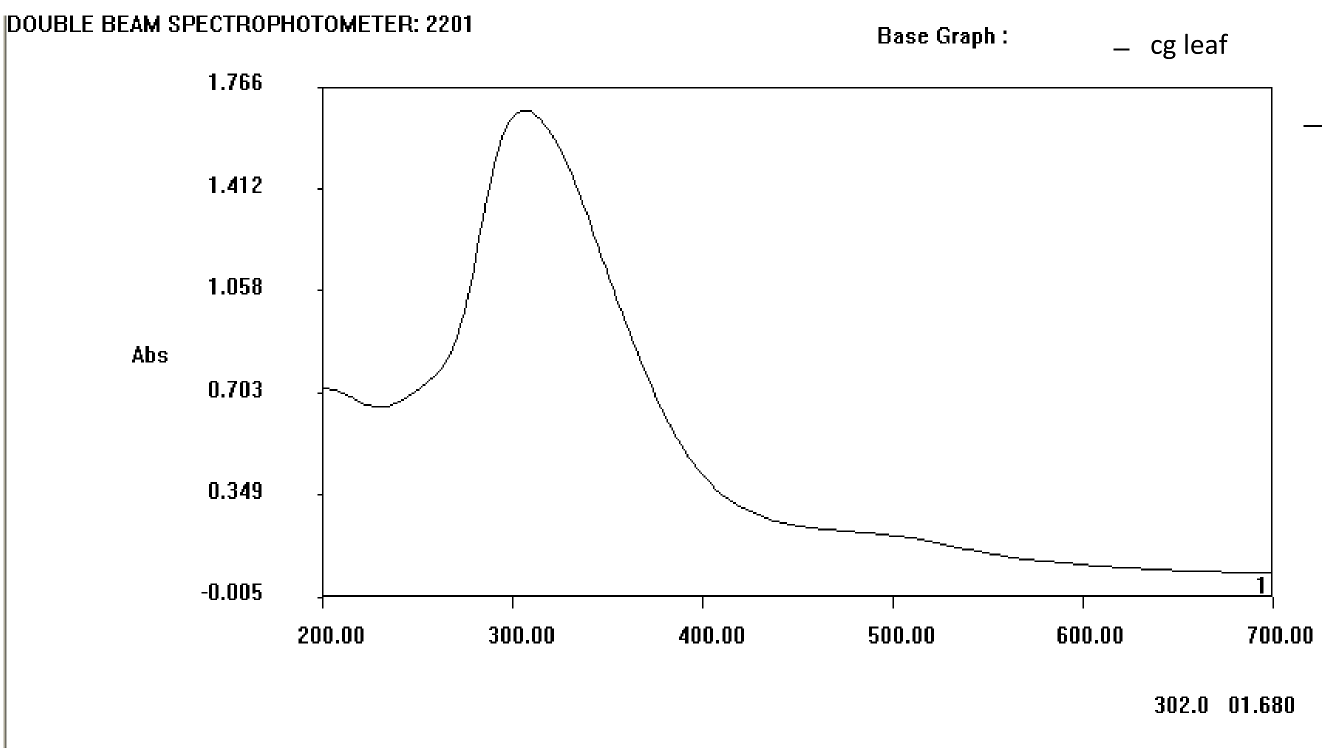

Fig. 6. Spectral absorbance peak of reaction mixture of leaf extract.

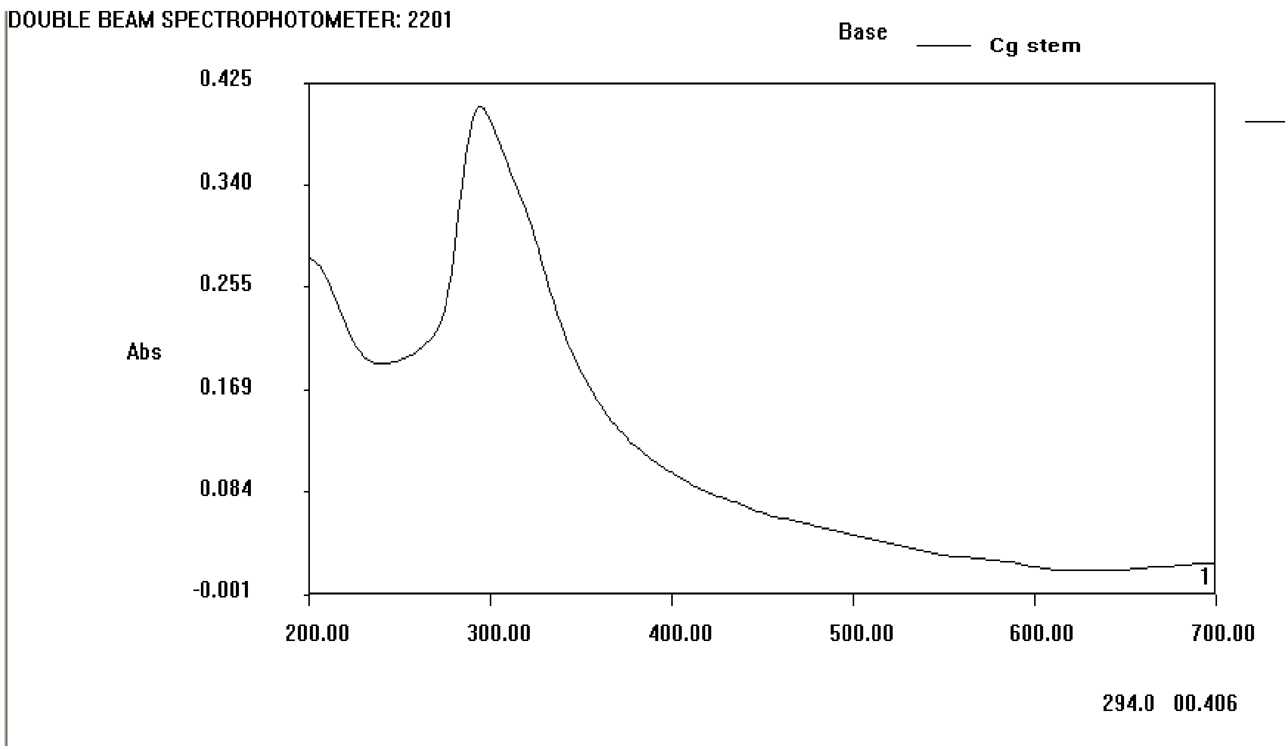

Fig. 7. Spectral absorbance peak of reaction mixture of stem extract.

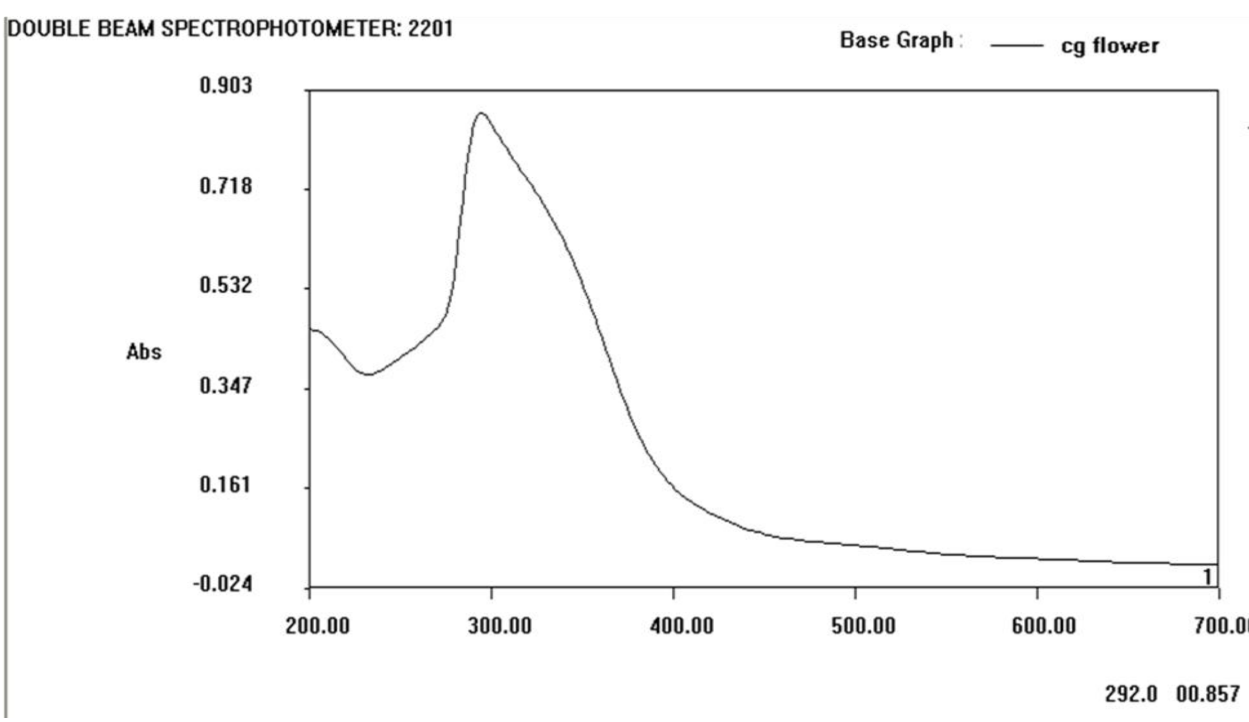

Fig. 8. Spectral absorbance peak of reaction mixture of flower petals extract.

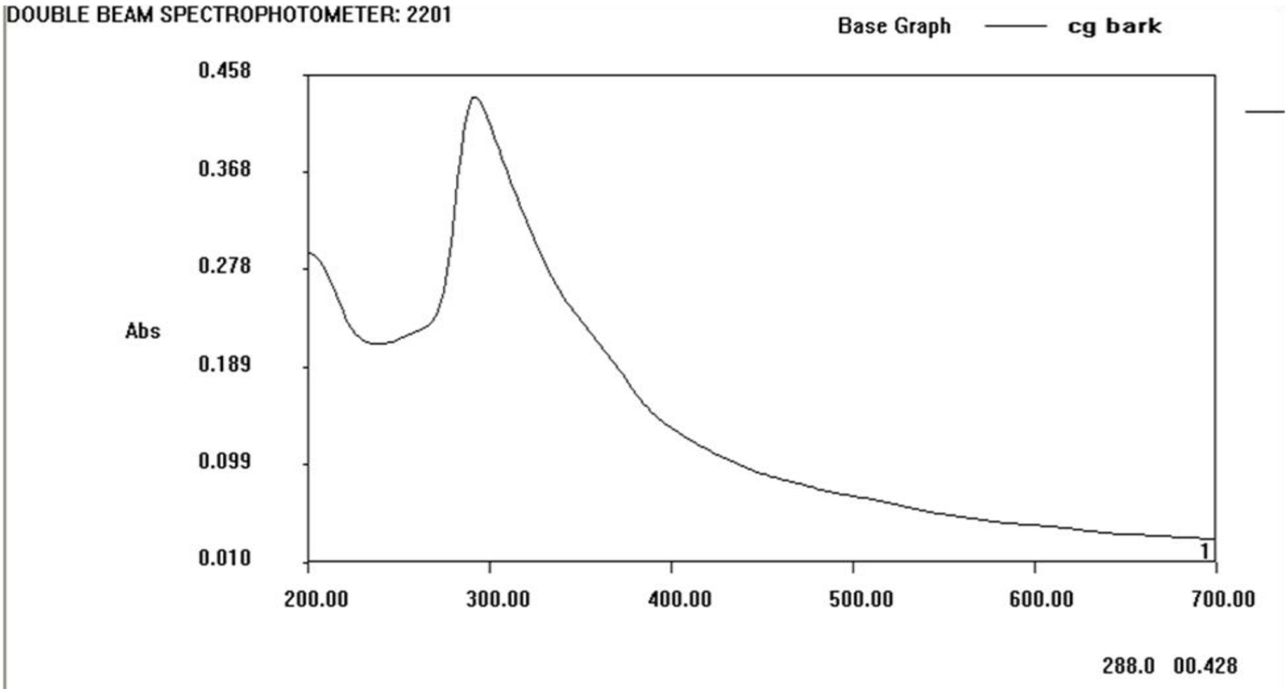

Fig. 9. Spectral absorbance peak of reaction mixture of bark extract.

The absorbance of the reaction mixtures was monitored after 24 h of reaction. Typical exciton absorption between 290 nm to 302 nm was observed at room temperature. The leaves and flower petal extract were turned into pale yellow when administered with 1mM zinc nitrate solution within 24 h, but stem and bark extracts were changed its color while heating the reaction mixture for ten to fifteen minutes. There was no color change observed in zinc nitrate solution. Figs. 6 to 9 showed the UV–Vis absorption spectrum of ZnO nanoparticles synthesized from the leaf, stem, flower petals and bark extracts of *C. guianensis* respectively.

The reaction mixture with aqueous extract of leaf showed maximum absorbance spectral peak at 302 nm (Fig. 6), stem at 294 nm (Fig. 7), flower petals at 292 nm (Fig. 8) and bark at 288 nm (Fig. 9). Gupta *et al.*, (2014) reported that the absorption edge systematically shifts to the lower wavelength or higher energy with the decreasing size of the nanoparticles. The results indicated that ZnO nanoparticles could be effectively synthesized using aqueous extracts of leaf, stem, flower petals and bark extracts of Cannon ball tree.

The phytochemical screening of various extracts of *C. guianensis* plant revealed the presence of alkaloids, phenolics, flavonoids, saponins, rutin, quercetin, kaempherol, lutolin, ursolic acid, hopanes, indirubin, isatin, sterols, fernesol. The presence of such metabolites played a key role in active reduction of Zinc Nitrate into ZnO nanoparticles (Shekhawat *et al.*, 2012).

The biomolecules from plant extracts can be used to reduce metal ions into nanoparticles in a single-step synthesis, which is quite rapid and effectively achieved at room temperature and pressure than any other approach (Aromal and Philip, 2012; Mittal *et al.*, 2013).

The use of plant extracts to synthesis metal oxide nanoparticles is inexpensive and quick process. Plant extracts play dual role as reducing as well as stabilizing agent in synthesizing nanoparticles (Kumar and Yadav, 2009). The synthesized Zinc oxide nanoparticles from plants sources are of controlled size and morphology and have been successfully employed in anti-bacterial activity and anti diabetic activities (Bala *et al.*, 2015). Plant extract strongly influences the characteristics of the nanoparticles (Mukunthan and Balaji, 2012). These were proved effective in inhibition of action of pathogenic microbes even at minimum concentrations (Applerot *et al.*, 2009).

It has reported that the plant extract based bio-reduction of using relevant metal salt for the synthesis of nanoparticles could be achieved at room temperature and relatively simpler than the harsh and complex methods of chemical bio-reduction.

4. CONCLUSION

The plant mediated synthesis of ZnO nanoparticles is an eco-friendly and cost effective approach. This assumption motivated to demonstrate the bio-reduction of ZnO nanoparticles using aqueous extracts of leaf, stem, flower petals and of barks *C. guianensis*. The synthesized ZnO nanoparticles were characterized by UV–Vis absorption spectroscopy and confirmed the presence of the ZnO nanoparticles in the reaction mixture. This green chemistry approach can be easily scaled up and quick process.

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