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Ecofriendly synthesis of Zinc oxide nanoparticles from asthma plant - *Euphorbia hirta* L.

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ABSTRACT

The present study states about the biosynthesis and UV-Visible characterization of Zinc oxide (ZnO) nanoparticles using leaf, stem, roots and inflorescence of the medicinal herb *Euphorbia hirta*. The aqueous extracts of plant parts were acted as capping and reducing agent while Zinc Nitrate Hexahydrate used as precursor. One milliliter of plant extracts mediated the formation of ZnO nanoparticles in solution which were visually confirmed by the development of yellowish color in the reaction mixtures. The optical properties of the reaction mixtures were analyzed by the UV-Visible double beam spectrophotometer. The leaf reaction mixture showed strong absorption band at 302nm, stem reaction mixture at 299nm, and the root and inflorescence reaction mixtures at 310nm. The results indicate that whole *Euphorbia hirta* plant can be used for the biosynthesis of Zinc oxide nanoparticles.

***Keywords*:** *Euphorbia hirta*, Zinc oxide nanoparticles, Reaction mixtures, Absorption spectrum

1. INTRODUCTION

Plants are better suited for the synthesis of metal oxide nanoparticles than any other sources due to the presence of abundant secondary metabolites and easy reduction of their salts. The present scenario of the nanotechnology prefers use of nontoxic phytochemicals as capping and reducing agents. The use of plant extracts in synthesis of nanoparticles could

overcome the drawbacks rendered by the toxic physical, chemical and cost enhanced microbial synthesis (Sharma *et al.*, 2009; Singhal *et al.*, 2011; Prasad, 2014)

Biosynthesis of zinc oxide (ZnO) nanoparticles using plant extracts is receiving more attention due to their strong applications in medicine, agriculture, cosmetics, bio-fertilizers etc. (Sabir *et al.*, 2014). Recently number of medicinal plants was explored to synthesize ZnO nanoparticles (NPs). Biosynthesis of zinc oxide nanoparticles were achieved in *Passiflora foetida* (Shekhawat *et al.*, 2014a), *Acalypha indica* (Gnanasangeetha and Thambavani, 2013), *Hybanthus enneaspermus* (Shekhawat *et al.*, 2014b), *Punica granatum* (Mishra and Sharma, 2015), *Camellia sinesis* (Shah *et al.*, 2015), *Micrococca mercurialis* (Manokari *et al.*, 2016) etc.

Euphorbia hirta L. belongs to the family Euphorbiaceae, native of the Central America but distributed in the temperate, sub-tropical, and tropical regions of the world. It is commonly known as snakeweed, hairy spurge, common spurge, Australian asthma herb, asthma plant, bara dudhi, amman paccharisi and chara. It is also known as *E. pulifera*, *E. capitata*, *Chamaesyce hirta* (Bhagwat *et al.*, 2008; Abubakar, 2009).

Euphorbia hirta L. is a small, erect annual herb reaching up to the height of 50 cm (Fig 1). The leaves are oppositely arranged, lanceolate and are usually greenish or reddish underneath and darker on the upper surface. The stem is slender and often reddish in color, covered with yellowish bristly hairs. Aerial parts produce a milky juice when cut or on injured. The flowers are small, numerous and crowded together in dense cymes. The fruits are yellow, hairy, keeled capsules containing wrinkled seeds (Lind and Tallantire, 1971).

Phytochemical characterization of *E. hirta* reveals the presence coumarins, flavonoids, tannins, sugars, mucilage, cardiac glycosides, diterpenes, aromatic acids, alkaloids, anthocyanins, campesterol and stigmasterol (Johnson *et al.*, 1999; Kumar *et al.*, 2010; Chitra and Muga, 2011). Thin Layer Chromatography (TLC) and Gas Chromatography – Mass Spectrometry (GC-MS) analysis reported the presence of afzelin, quercitrin, myricitrin, rutin, quercitin, euphorbin-A-D, kaempferol, gallic acid, protocatechuic acid, β-amyrin, 24-methylenecycloartenol, β-sitosterol, heptacosane, nonacosane, shikmic acid, tinyatoxin, choline, camphol, rhamnose, chtolphenolic acid etc. (Williamson, 2002; Sood *et al.*, 2005; Rastogi and Mehrotra, 2002; Liu *et al.*, 2007).

Euphorbia hirta is widely used as a traditional medicinal herb to treat asthma in all the tropical countries (Loh *et al.*, 2009). It exhibits number of actions in the biological system such as antioxidant, anticancer (Mothana *et al.*, 2009), antibacterial (Sudhakar *et al.*, 2006), antifungal (Masood and Rajan, 1991), diuretic, antihypertensive, anthelmintic (Adedapo *et al.*, 2005), anxiolytic, antidiarrhoeial (Galvez *et al.*, 1993), antimalarial (Tona *et al.*, 1999) and anti-inflammatory activities (Singh *et al.*, 2006).

Gold nanoparticles (Au NPs) synthesized using aqueous leaf extracts of *E. hirta* were reported to possess antimicrobial activity against *Escherichia coli*, *Pseudomonas aeroginosa* and *Klebsiella pneumonia* (Annamalai *et al.*, 2013). Leaf extracts of *E. hirta* were resulted in the formation 31nm sized silver nanoparticles using Silver nitrate as precursor and these exhibited potential antibacterial activity (Manopriya *et al.*, 2011). Silver nanoparticles from the leaves were reported to be active against the cotton bollworm *Helicoverpa armigera* (Durga devi *et al.*, 2014). Poovizhi and Krishnaveni (2015) synthesized zinc oxide nanoparticles from the leaves of *E. hirta* and these ZnO nanoparticles were active against human bacterial and fungal pathogens.

The present study reports an ecofriendly and rapid synthesis method of zinc oxide nanoparticles using aqueous extracts of leaf, stem, root and inflorescence of *E. hirta* and their UV-Visible characterization.

Fig. 1. Morphology of the plant *Euphorbia hirta*.

2. MATERIALS AND METHODS

2. 1. Plant Collection

Disease free herbs of *Euphorbia hirta* were collected from the West Coast region of Puducherry (Mahe, U.T. of Puducherry, India). Different parts of the plants such as leaves, stem segments, root segments and inflorescence were collected (Fig. 2A-5A) during June-December 2016. These were initially washed with running tap water to remove the dirt and

other pathogenic spores, further rinsed in double distilled water and shade dried for 30 min. These plant materials were cut into small pieces (Fig. 2B-5B).

2. 2. Preparation of aqueous plant extracts

Five grams of chopped leaves, stem branches, roots and inflorescence of *E. hirta* were mixed with 50 ml of double distilled water and boiled for 20 min. After boiling all the samples were filtered using whatman filter paper (No. 1) and the aqueous extracts were used for the synthesis of ZnO nanoparticles.

2. 3. Preparation of precursor

Zinc nitrate hexahydrate [Zn $(NO_3)_2.6H_2O$] (Merck, Mumbai, India) was used as a precursor for the synthesis of ZnO nanoparticles from the various extracts of *E. hirta*. One mM solution of Zinc nitrate was prepared using double distilled water and stored in refrigerator at 4^oC for further use.

2. 4. Synthesis of ZnO nanoparticles

The plant extracts were used to reduce the zinc metal ions in to metallic zinc oxide nanoparticles. Three boiling tubes were used to synthesize ZnO nanoparticles, one containing 10 ml of 1mM Zinc nitrate solution as reference, and the second one containing 10 ml of aqueous plant extract and the third one containing 9 ml of 1 mM Zinc Nitrate solution and 1 ml of leaf, stem, root and inflorescence extracts as reaction medium and incubated at room temperature (2C-5C). To observe the visual color change in to yellow, the reaction medium was boiled for 20 min at 60 °C temperature. The test solution from the third tube was centrifuged at 5000 rpm for 20 min to obtain the pellet. The supernatant was discarded; the pellet dissolved in double distilled water and used for further experimentation.

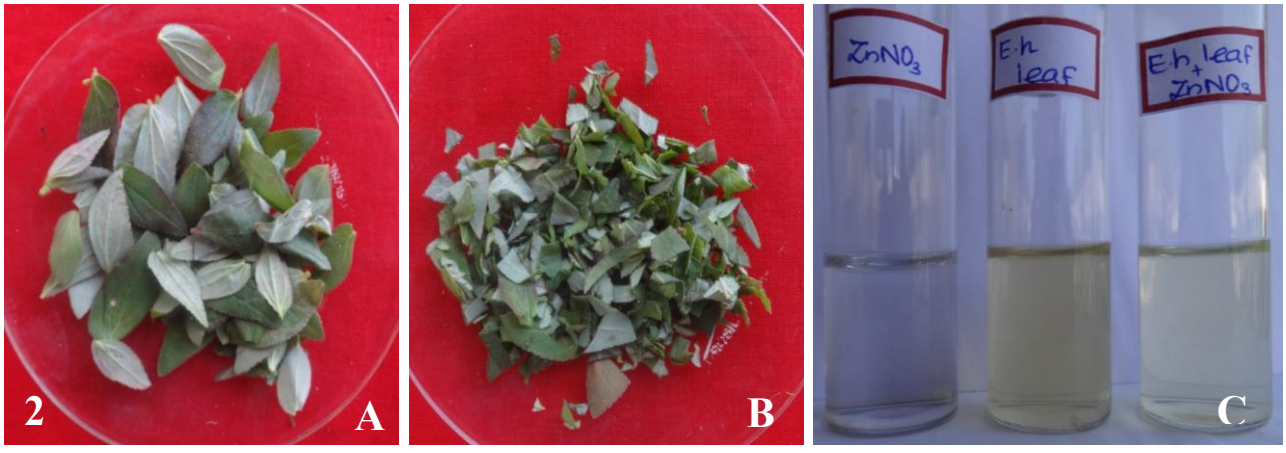

Fig. 2. Leaf mediated synthesis of ZnO nanoparticles
A- Leaves, **B-** 5 grams of finely chopped leaves, **C-** Precursor, leaf extract and leaf reaction mixture.

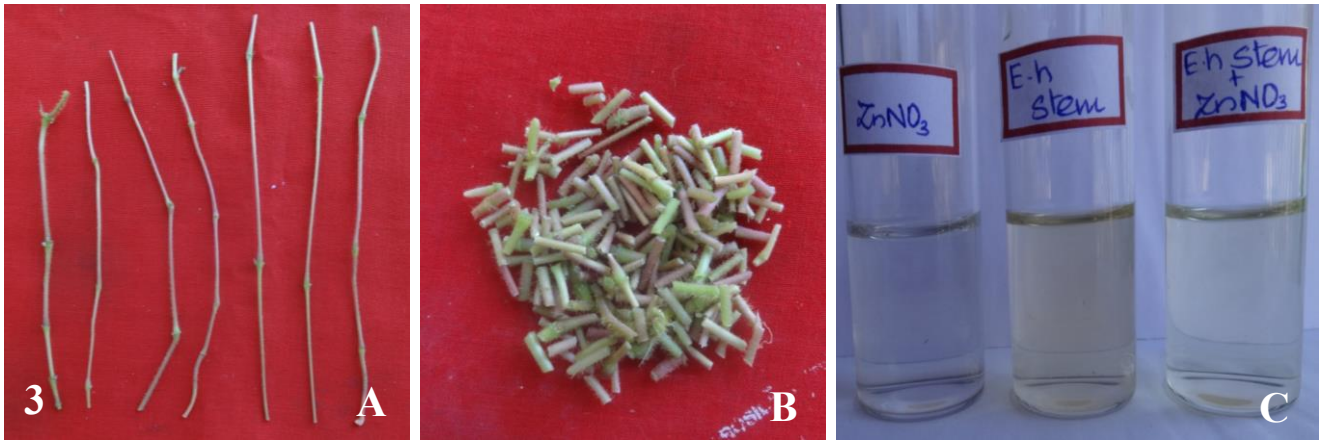

Fig. 3. Stem mediated synthesis of ZnO nanoparticles
A- Stem segments, **B-** 5 grams of finely chopped stem segments,
C- Precursor, stem extract and stem reaction mixture.

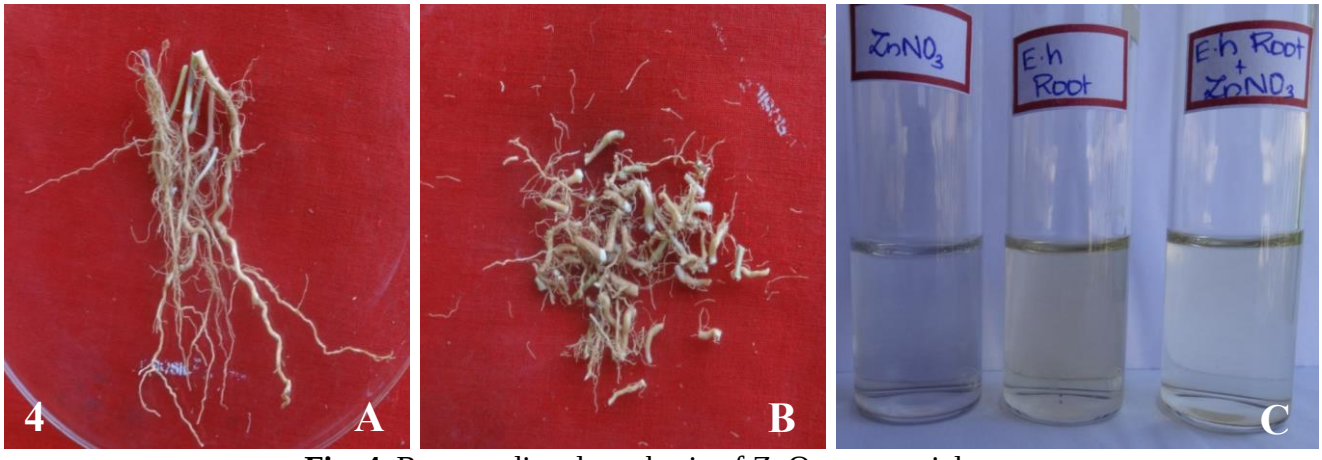

Fig. 4. Root mediated synthesis of ZnO nanoparticles
A- Root segment, **B-** 5 grams of finely chopped roots,
C- Precursor, root extract and root reaction mixture.

Fig. 5. Inflorescence mediated synthesis of ZnO nanoparticles
A- Inflorescences, **B-** 5 grams of finely chopped inflorescence,
C- Precursor, inflorescence extract and inflorescence reaction mixture.

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

The bioreduction of ZnO nanoparticles using *E. hirta* aqueous extracts were monitored 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 700nm using a Systronics Double Beam Spectrophotometer (Model 2202, Systronics Ltd.) in diffuse reflectance mode using Zinc Nitrate as reference.

3. RESULTS AND DISCUSSION

The present study involves the synthesis of ZnO nanoparticles using various parts of asthma weed *Euphorbia hirta.* The phytochemicals present in this plant has already been explored for the formulation of various medicines and nanoparticles. Till now, its leaf extracts were utilized for the bioproduction of gold (Au), silver (Ag) and zinc oxide (ZnO) nanoparticles (Annamalai *et al.*, 2013; Manopriya *et al.*, 2011; Durga devi *et al.*, 2014; Poovizhi and Krishnaveni, 2015).

3. 1. Visual confirmation of ZnO NPs synthesis

The various aqueous extracts changed the color into pale yellow when subjected to 9 ml of Zinc nitrate solution. Intensity in color was observed in leaf, root and inflorescence extracts after addition of precursor but stem extract changed its color after 30 min. Various reports state that the change in color of the reaction mixtures indicates the reduction reaction of zinc ions into zinc metal oxide (Vidya *et al.*, 2013; Manokari and Shekhawat, 2016; Talm *et al.*, 2012). Colorless precursor solution turns into yellow with the addition of 1ml of plant extracts proves that the synthesis of ZnO nanoparticles are mediated by enzymes/ secondary metabolites which are present in the aqueous extracts of *E. hirta*. The plant is endowed with coumarins, flavonoids, tannins, sugars, mucilage, reduced compounds, cardiac glycosides, diterpenes, aromatic acids, alkaloids, anthocyanins, campesterol, stigmasterol and quercitin (Johnson *et al.*, 1999; Kumar *et al.*, 2010; Chitra and Muga, 2011; Williamson, 2002). Possibly these chemicals in various concentrations participate in the formation of nanoparticles.

3. 2. UV-Visible characterization of ZnO nanoparticles

The absorption spectra have been acquired after every 30 min after mixing the precursor with plant extracts. UV-Visible absorption spectroscopy is used to inspect the optical properties of nanoparticles (Talam *et al.*, 2012). The absorption spectrum of ZnO nanoparticles synthesized using various extracts of *E. hirta* is shown in Figure 6, and the measurements showed the fall of absorbance peak between 299 nm to 310 nm over the two hr time period. The reaction mixtures of leaf, stem, root and inflorescence were analyzed using UV-Vis Spectrophotometer between the wavelengths from 200 nm to 700 nm. The UV-Visible spectroscopic analysis of ZnO nanoparticles from leaf reaction mixtures was confirmed by the strong absorption peak at 302 nm, stem reaction mixture at 299 nm, and the root and inflorescence reaction mixtures exhibited strong absorption spectra at 310 nm (Fig. 6A and 6D). The maximum absorption wavelengths were observed in root and inflorescence

reaction mixture but it was quick in leaf reaction mixture. The one milliliter extracts of various parts of plant mediated and stabilized the ZnO nanoparticles within two hr in the present study.

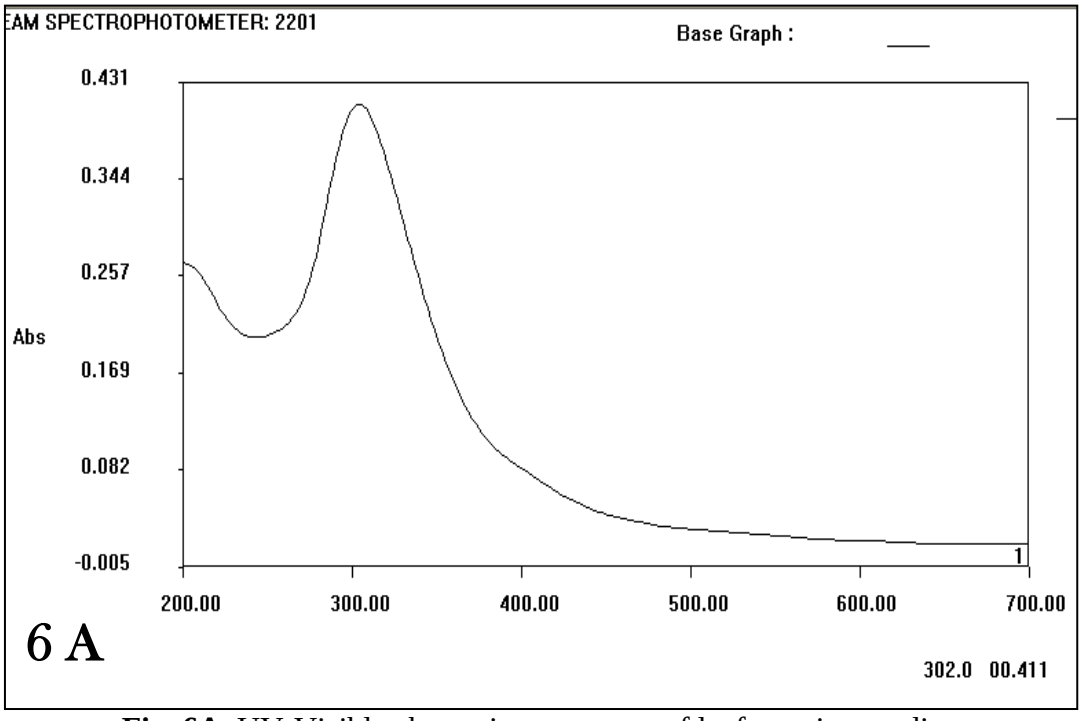

Fig. 6A. UV-Visible absorption spectrum of leaf reaction medium.

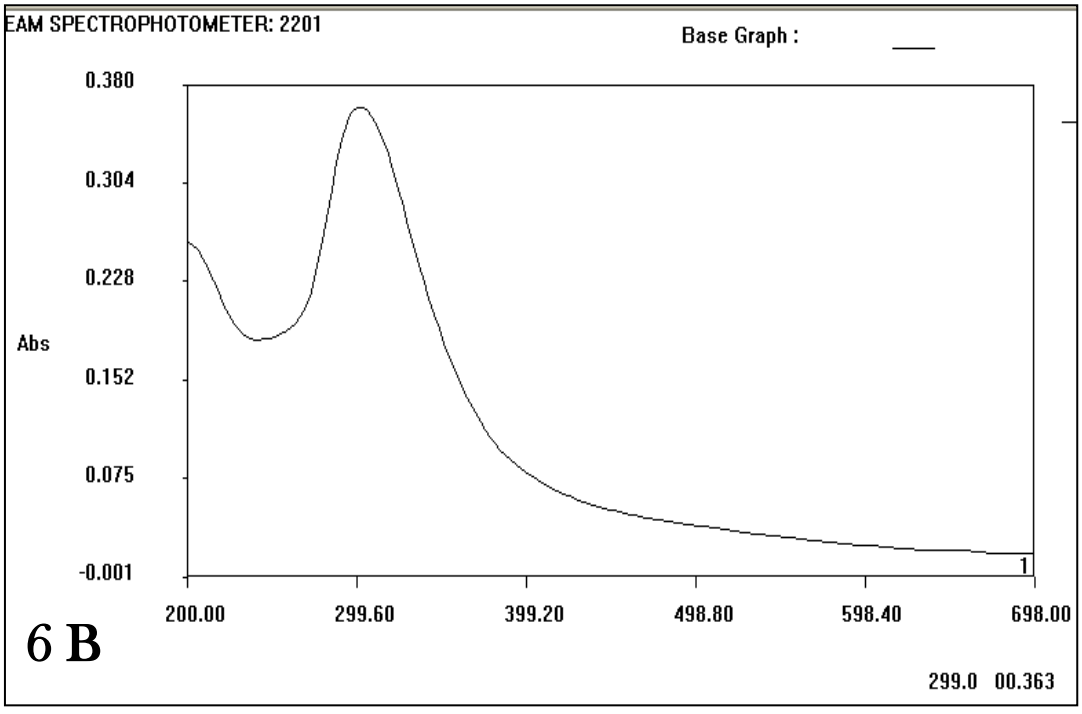

Fig. 6B. UV-Visible absorption spectrum of stem reaction medium.

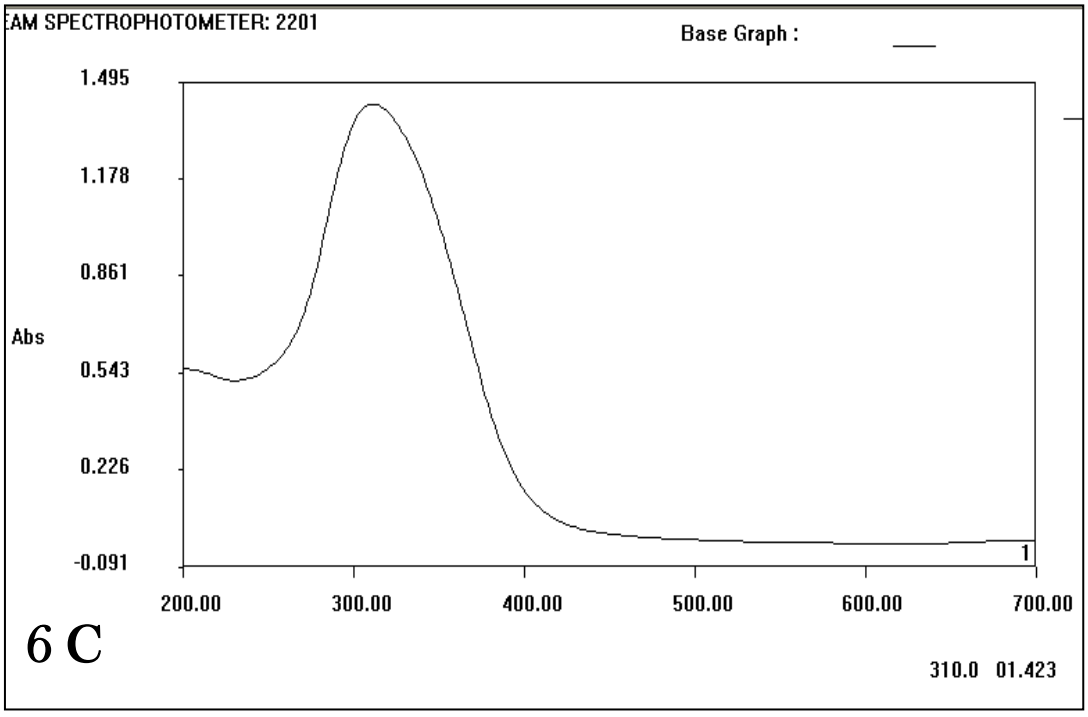

Fig. 6C. UV-Visible absorption spectrum of root reaction medium.

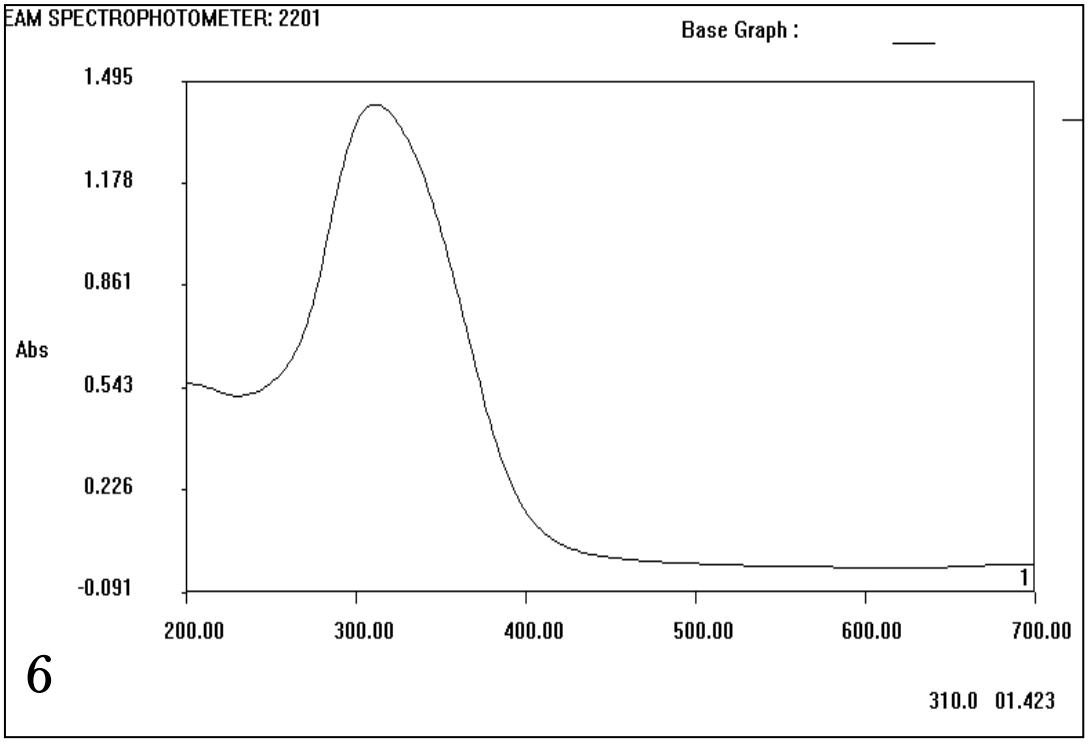

Fig. 6D. UV-Visible absorption spectrum of inflorescence reaction medium.

The aqueous extracts of stem bark and leaf of plant *E. hirta* have potent molluscicidal activities and also significantly alter the levels of total protein, free amino acid, nucleic acids,

activities of enzyme protease and alkaline phosphatase in various tissues of the vector snail *Lymnaea acuminata* in time and dose dependent manners (Singh *et al.*, 2005).

Zinc oxide is widely used in UV lasers, electrochemical nanodevices, sunscreen lotions, photo printing and gas sensors (Ravichandrika *et al.*, 2012). The biological synthesis of nanoparticles, particularly plant extracts could overcome the hazardous methods such as sputtering, spray pyrolysis, solvothermal, hydrothermal and wet chemical methods.

The plant exhibits antibacterial activity against Gram positive (*Staphylococcus aureus*, *Micrococcus* sp., *Bacillus subtilis* and *Bacillus thuringensis*), Gram negative (*Escherichia coli, Klebsiella pneumonia, Salmonella typhi* and *Pseudomonas mirabilis*) and antifungal activity against *Candida albicans* (Sudhakar *et al.*, 2006; Singh *et al.*, 2006; Rajeh *et al.*, 2010). Therefore, the ZnO nanoparticles from *E. hirta* could be exploited for the formulations of antibacterial and antifungal medicines. The present study reports a simple and easy method in production of ZnO nanoparticles at room temperature from various parts of *E. hirta*.

4. CONCLUSION

ZnO nanoparticles were synthesized from the aqueous extract of *E. hirta* using Zinc Nitrate as precursor. The optical properties of synthesized nanoparticles have been analyzed using UV-Visible spectroscopy, which reveal an excitonic absorption bands between 299nm-310 nm. This trouble-free, single step procedure of *E. hirta* extracts mediated synthesis appears to be suitable for large scale production of zinc oxide nanoparticles as it is cost effective, rapid, and environmentally benign.

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