Available online at www.worldscientificnews.com

World Scientific News

WSN 47(2) (2016) 267-278 EISSN 2392-2192

Production of Zinc oxide nanoparticles using extracts of *Passiflora edulis* Sims. f. *flavicarpa* Deg.

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ABSTRACT

The synthesis of zinc oxide nanoparticles (ZnO) using herbal extract is always environmentally benign. The present investigation focuses on the synthesis of ZnO nanoparticles using aqueous extracts of various parts of *Passiflora edulis* Sims. f. *flavicarpa* Deg. (Passion fruit). The plant is rich in alkaloids, glycosides, flavonoids and triterpenes. UV-Visible double beam spectrophotometric analysis was used for characterization of ZnO nanoparticles with different time intervals which were synthesized by plant extracts. The bio-reduction completes in two hr incubation of reaction mixtures with strong broad peaks at 332 nm from leaves, 296 nm from stem and 326 nm from flower extracts. Hence the biosynthesis of ZnO nanoparticles using aqueous extracts of *P. edulis* could be an alternative to chemical methods and exploited in various biofields.

***Keywords*:** *Passiflora edulis* Sims. f. *flavicarpa* Deg.; UV-Visible double beam spectrophotometry; aqueous extracts; ZnO nanoparticles

1. INTRODUCTION

Nanotechnology gained attraction mainly due to the controlled synthesis of the material which is less than 100 nm in size. This ultra-small size material is comparable to naturally occurring proteins and biomolecules in the cell (McNeil, 2005). The unique physical and chemical properties of ZnO nanoparticles played crucial role in biomedical and cancer applications, which attracted the scientific and medical communities to exploit this field

(Rasmussen *et al.,* 2010). The unique properties such as, high chemical stability, high electrochemical coupling coefficient, broad range of radiation absorption and high photostability, make ZnO a multifunctional material (Segets *et al.,* 2009; Lou, 1991). ZnO metal oxide has been used in electronics, optoelectronics and laser technology mainly based on its broad energy band (3.37 eV), high bond energy (60 meV) and high thermal and mechanical stability at room temperature (Bacaksiz *et al.,* 2008; Wang *et al.,* 2005). In addition, the piezo- and pyroelectric properties of ZnO attracted to be used in hydrogen production as a sensor, converter, energy generator and photocatalyst (Wang, 2008; Chaari and Matoussi, 2012).

The rigidity, low toxicity, hardness, biocompatibility and biodegradable properties of zinc oxide make it a material of interest in the field of biomedicine (Ozgur et al., 2005; Ludi and Niederberger, 2013). Zinc oxide effectively absorbs UV-A and UV-B radiation and has been exploited in number of medicine formulations due to its antibacterial, antifungal, disinfecting and drying properties (Liu *et al.,* 2013). At present ZnO has been used in ointments, creams, sunscreen lotions, dental pastes, dusting and liquid powders and medicines to cure epilepsy and diarrhea. The wound healing properties of ZnO has been used in the formulation of dermatological substances against inflammation and itching (Mason, 2006; Newman *et al.,* 2009). Wang *et al.* (2009) reported that ZnO nanoparticles have been preferred in diagnostics of cancer due to its inherent preferential cytotoxicity against malignant cells.

At present, biological source such as different parts of plants, fungi, algae and microbes are used to synthesize valuable eco-friendly nanoparticles such as Pelladium (Petla *et al.,* 2012), Silver (Shekhawat *et al.,* 2012), Gold (Ankamwar, 2010), Iron (Herrera-Becerra *et al.,* 2008), and zinc oxide nanoparticles (Sangeetha *et al.,* 2011). The various parts of plants such as leaves, stem, root, tubers, fruits etc. have been reported for the synthesis of nanoparticles as a source material (Li *et al.,* 2007; Satishkumar *et al.,* 2009; Shekhawat *et al.,* 2012).

Passiflora edulis Sims. f. *flavicarpa* Deg. (Passifloraceae) is a woody climber, which is native of Brazil. The genus occupies large number of species. The plant is commonly called as yellow passion fruit, maracuja and yellow granadilia etc. (Beninca *et al.,* 2007). *P. edulis* is widely cultivated around the tropical countries of the world for its edible fruits and exported to the several countries due to its delicious fruit juice (Souza *et al.,* 2004; Machado *et al.,* 2008). The species is included in pharmacopoeias of several countries (Rudnicki *et al.,* 2007). Its flowers are attractive violet or blue to pale violet colored, in axillary solitary cymes (Fig.1) and are hermaphrodites (Sridhar, 2011).

Traditionally this plant is used as sedative, antiasthmatic and emetic (Anonymous, 2003). Its leaves are used in the treatment of epilepsy, ulcers, haemorrhoids and insomnia (Relw and Espig, 1991). Leaf and stem contains cyanogenic glycosides (2R)–â-Dallopyranosyloxy- 2-phenylacetonitrile, (2S)–â-D-allopyranosyloxy-2-phenylacetonitrile, (2R)–prunasin, (2S)-sambunigrin, luteolin-6-Cchinovoside, luteolin-6-C-fucoside, passicapsin, passibiflorin, passicoriacin, epitetraphyllin B and amygdalin (Chassagne and Crouzet, 1998; Seigler *et al.,* 2002). Leaves are reported to possess a cyclopropane triterpine glycoside Passiflorine, maracugine, resins, acids and tannins (Bombardelli *et al.,* 1975). Passion fruit is rich in saponins, alkaloids, tannins, flavonoids, vitamins, and free amino acids, namely arginine, aspartic acid, glycine, leucine, lysine, proline, threonine, tyrosine, valine and many alkaloids (Dhawan *et al.,* 2004).

Fig. 1. Plant growing under natural conditions with flowers.

The species is exploited for its antibacterial, antiseptic, astringent, antiulcer, spermicidal anti-inflammatory (Vargas *et al.*, 2007), antihypertensive (Benson *et al.*, 2008), anti anxiety (Reginatto *et al.*, 2006), antioxidant (Rudnicki *et al.*, 2007), anti-tumour (Puricelli *et al.*, 2003) and antifungal (Pelegrini *et al.*, 2006) activities. Pelegrini *et al.* (2006) reported the presence of antifungal peptide (Pe-AFP-1) from the seeds of *P. edulis* which inhibits the development of filamentous fungi *Trichoderma harzianum*, *Fusarium oxysporum* and *Aspergillus fumigatus*.

Recently we have reported ZnO nanoparticles using various extracts of *Passiflora foetida* using Zinc Nitrate hexahydrate (Shekhawat et al., 2014). And the present study aims to synthesize zinc oxide nanoparticles using different extracts of *Passiflora edulis*.

2. MATERIALS AND METHODS

2. 1. Collection of plant materials and preparation of broth solutions

Passiflora edulis (Passion fruit) is cultivated in South India for its edible fruits and ornamental flowers. The plant material was collected from the coastal area of Pondicherry, India. The plants were identified with the help of 'The Flora of Presidency of Madras' (Gamble, 1921). Fresh green leaves, stem and flowers were harvested during the months of September 2014 to January, 2015. The plant parts were separated with fine razor blade and immersed in 40% ethanol to remove foreign materials such as soil, dust particles and fungal spores.

The materials were thoroughly washed with distilled water and finely cut in small pieces (Fig.2-4A and B). Five grams of finely chopped plant materials were boiled in a cleaned and sterilized 250 ml Erlenmeyer flask with 50 ml of sterile distilled water and then boiling the mixture for 5 min in water bath 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.

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 for the synthesize of ZnO nanoparticles using various extracts. One mM Zinc nitrate solution was prepared using Zinc Nitrate hexahydrate with double distilled water and stored in refrigerator at 4 °C for further use.

For the synthesis of Zinc oxide nanoparticles, three boiling tubes were used, 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 1mM Zinc nitrate solution and 1 ml of plant extracts as test solution (Fig. 2-4C).

Fig. 2A-C. Leaves and reaction mixtures.

Fig. 3A-C. Stems and reaction mixtures.

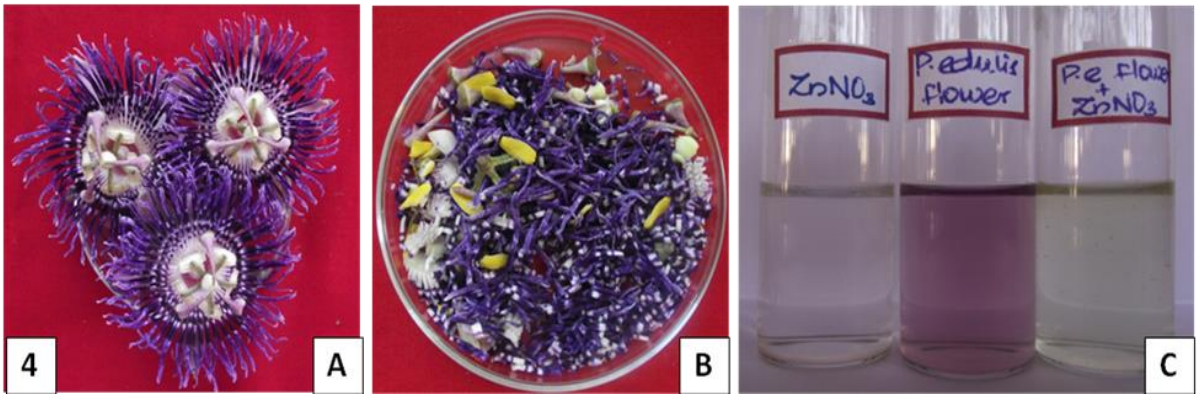

Fig. 4A-C. Flowers and reaction mixtures.

2. 3. Characterization of ZnO nanoparticles

The synthesized zinc oxide nanoparticles from the plant extracts were centrifuged at 10000 rpm for 10 min to obtain the pellet which is used for further studies. 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-Vis 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 DISCUSSION

The green synthesis of ZnO nanoparticles have been achieved using *P. edulis* plant extracts with Zinc Nitrate in this investigation. ZnO nanoparticles have been synthesized using plants with different precursors. Gnanasangeetha and Thambavani (2013) used Zinc acetate dihydrate and sodium hydroxide as a precursor for the synthesis of ZnO nanoparticles from *Corriandrum sativum* leaves. Zinc chloride has been used as zinc source for synthesis and characterization of ZnO nanoparticles by Oladiran and Olabisi (2013). Singh *et al.* (2011) used Zinc acetate and Sodium chloride with milky latex of *Calotropis procera* for the synthesis and characterization of ZnO nanoparticles.

The nanoparticles from bio-sources have been utilized effectively in various applications of pharmaceutical industries and biomedical fields. Cost effective and non-toxic approaches have proved the potential benefit of biogenesis of nanoparticles, which attracted large scale research in this field. The whole plant mediated synthesis of ZnO nanoparticles could reveal the better understanding of production of nanoparticles of interest. Biogenesis of zinc oxide nanoparticles using whole plant parts have also been achieved in *Passiflora foetida* (Shekhawat *et al.*, 2014), *Morinda pubescens* (Shekhawat and Manokari, 2014), *Lawsonia inermis* (Shekhawat *et al.*, 2015) and *Duranta erecta* (Ravindran *et al.*, 2016).

The aqueous media/extracts of *P. edulis* leaf, stem and flower extracts as reducing and stabilizing agents. The reduction of zinc nitrate into zinc nanoparticles during exposure to the plant extracts is followed by the color change from colorless to pale yellow. The reaction

mixture made of leaf and flower extracts were readily turned into pale yellow with ultimate addition of Zinc nitrate solution, but there was no color change observed in cell free extracts of stem reaction mixture (Fig. 2-4C). Color change was observed, when the stem reaction mixture was heated in water bath for 15 min at 60 °C.

The reaction mixtures were kept under constant rotation of 10000 rpm for 10 min in a centrifuge. After achieving complete dissolution of aqueous zinc nitrate and plant extracts, the pellets were dissolved in double distilled water and incubated at room temperature. The supernatant with plant byproducts and biomolecules were discarded. The reaction mixture was subjected to spectrophotometric analysis with different time intervals for the determination of ZnO nanoparticles synthesis.

The UV-Visible spectrophotometric analysis of the zinc oxide nanoparticles at different time intervals indicated the time taken for completion of reaction using different extracts. The strong peaks were observed between 290-332 nm, characteristics of ZnO nanoparticles in a suspension. The time taken for color change and completion of reaction was varied with the different plant extracts. The organ specific secondary metabolites present in the plants could catalyze the specific reactions at different time intervals. Sabir *et al.* (2014) acknowledged the time taken for the reaction mixture to change color was varied with plant extracts.

Initially, clear peaks were not observed in spectrophotometric analysis after challenging with precursor and broth solutions. The aqueous reaction mixture of leaves showed broad peak at 317.6 nm with one hour incubation. Stem and flower reaction mixture showed peaks at 290 and 291 nm respectively with one hour incubation (Fig. 5A-C).

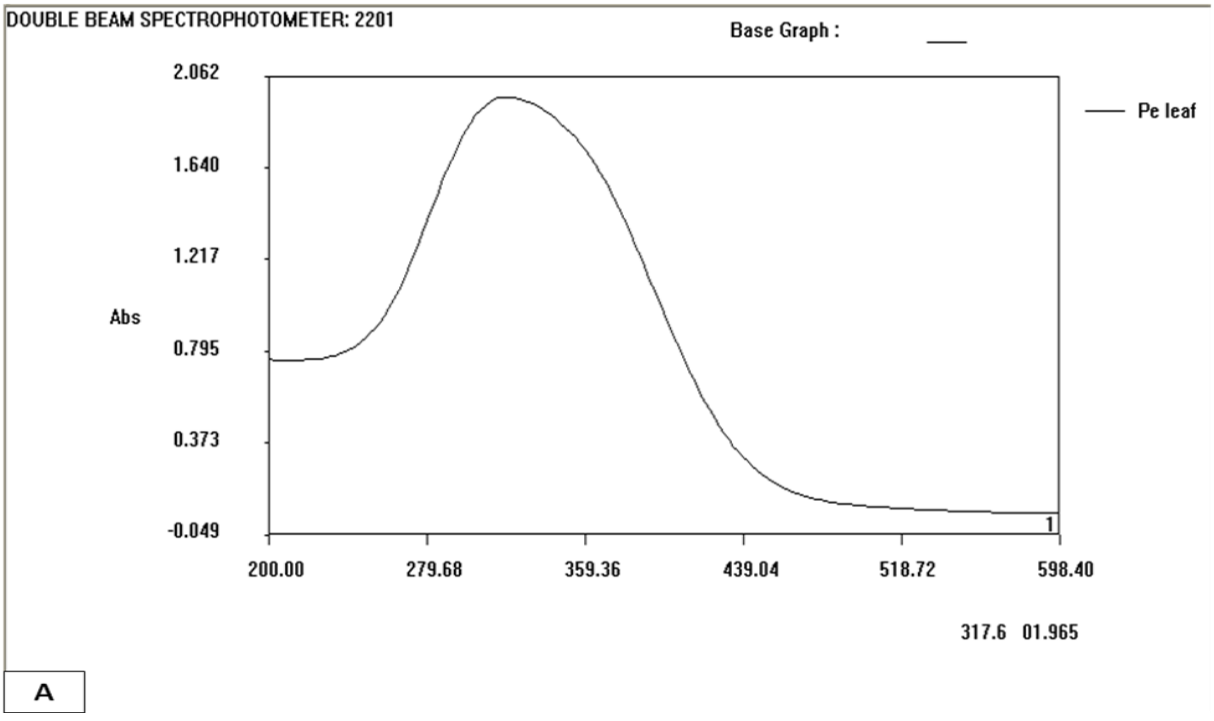

Fig. 5A. Spectral absorbance peak of reaction mixture of leaves extract after one hr.

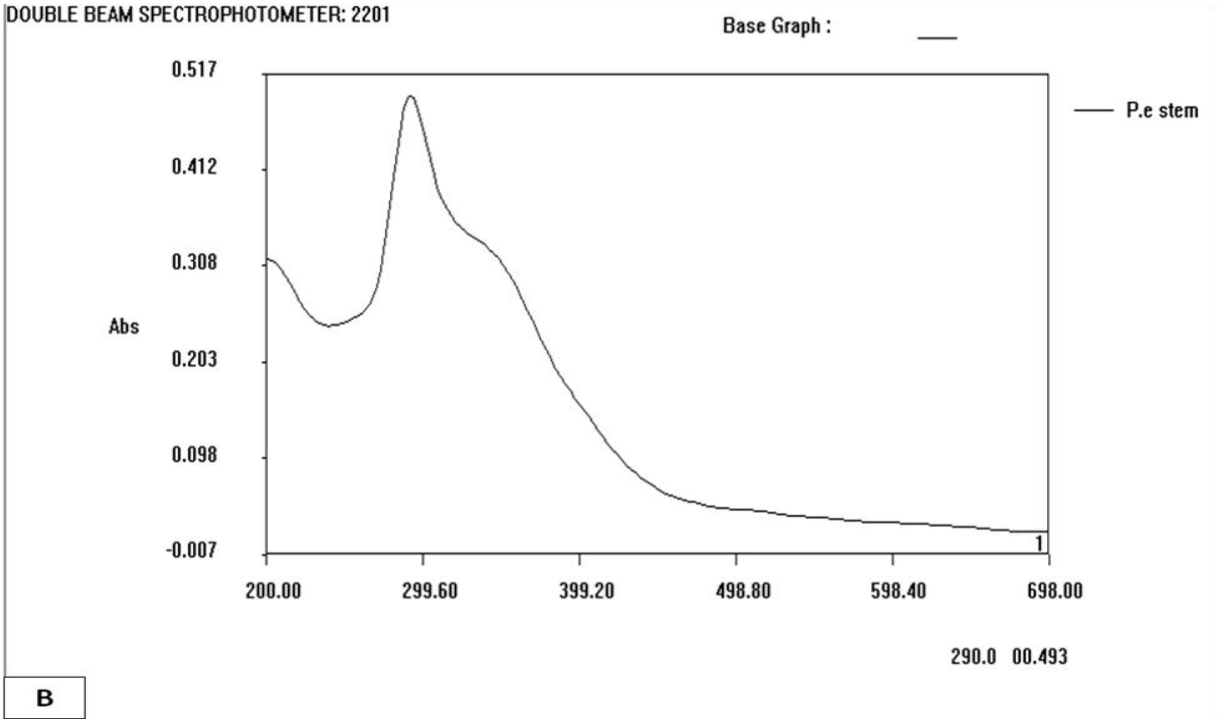

Fig. 5B. Spectral absorbance peak of reaction mixture of stems extract after one hr.

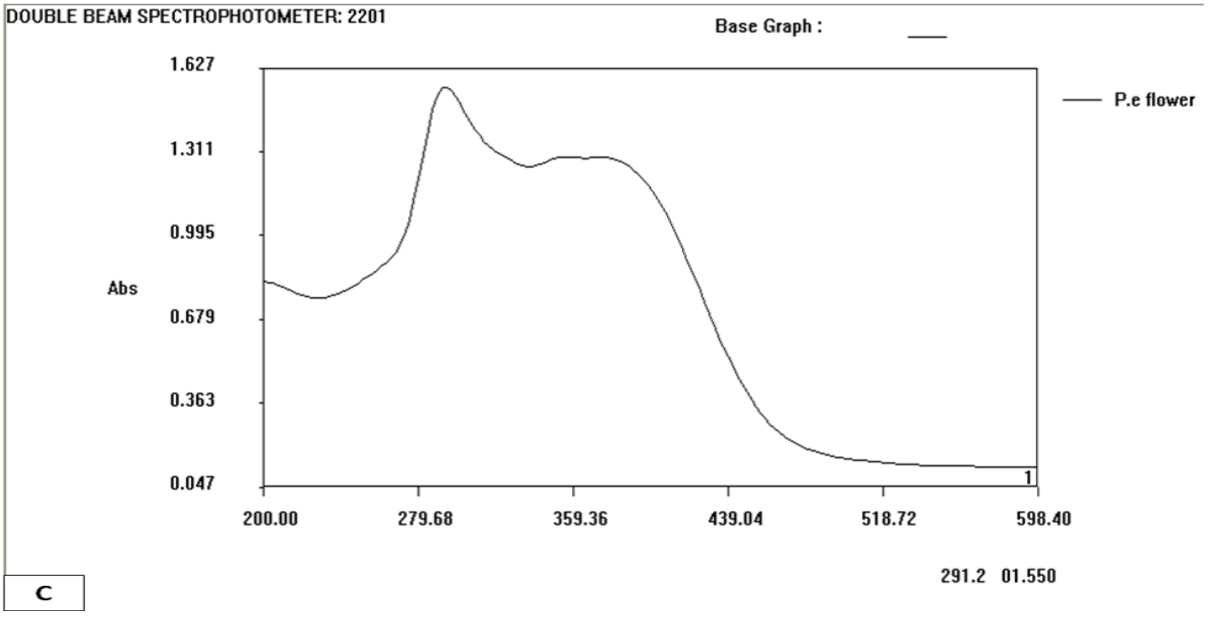

Fig. 5C. Spectral absorbance peak of reaction mixture of flowers extract after one hr.

After two hrs of incubation of at room temperature, there were strong broad peaks observed at 332 nm, 296 nm and 326 nm using leaf, stem and flower reaction mixtures (Table 1, Fig. 6A-C).

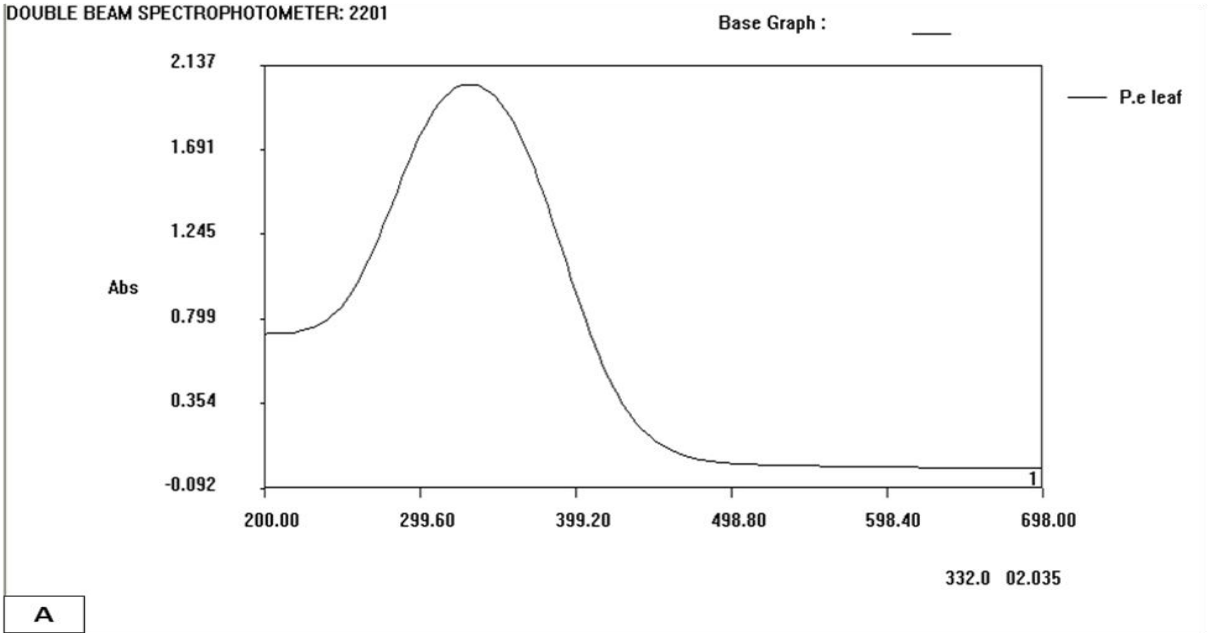

Fig. 6A. Spectral absorbance peak of reaction mixture of leaves extract after two hrs.

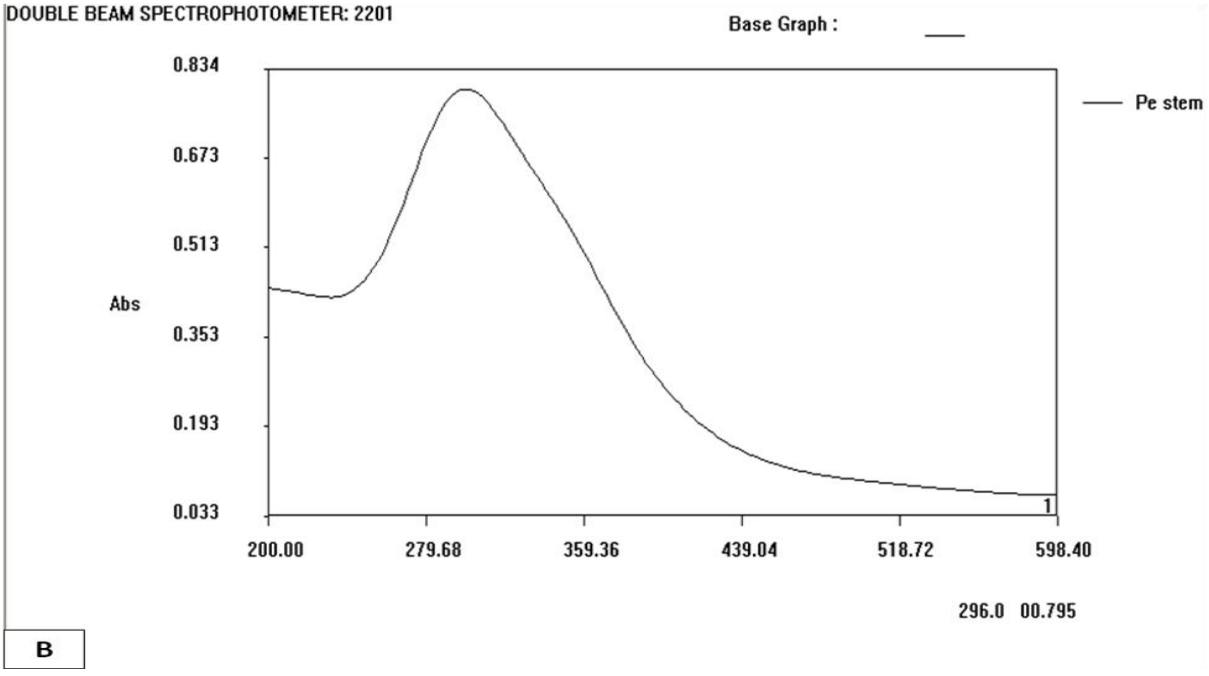

Fig. 6B. Spectral absorbance peak of reaction mixture of stems extract after two hrs.

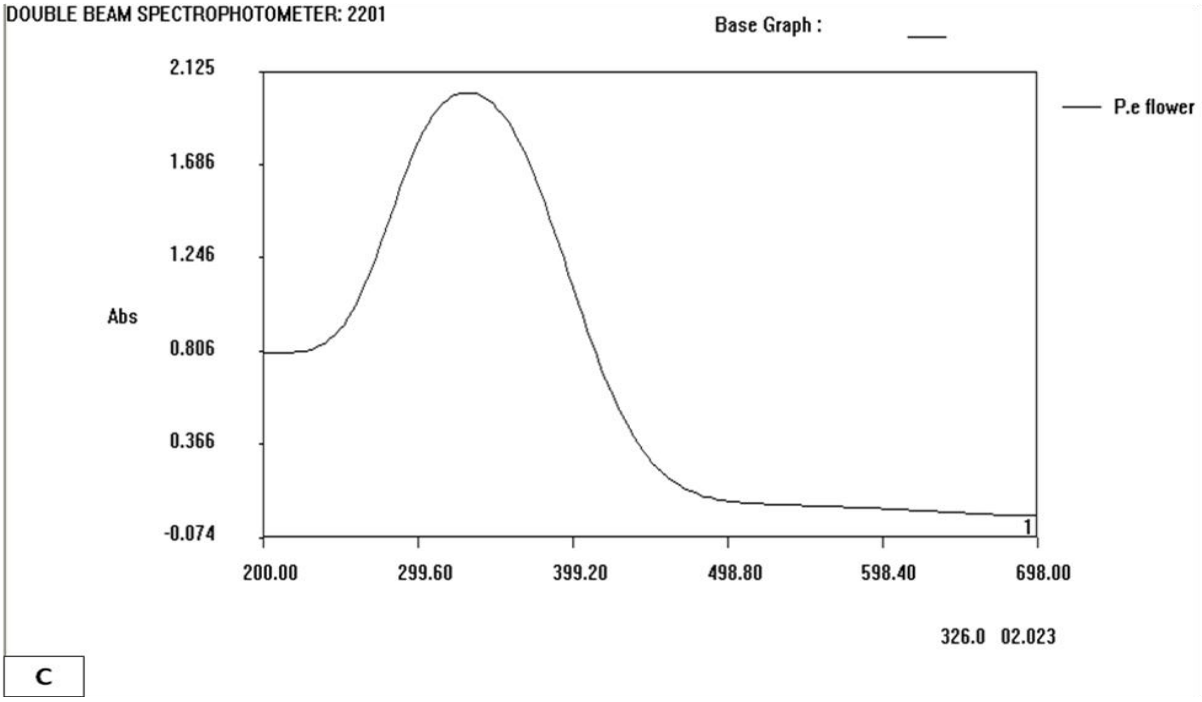

Fig. 6C. Spectral absorbance peak of reaction mixture of flowers extract after two hrs.

Table 1. UV-Visible absorption spectra of zinc oxide nanoparticles synthesized using *P. edulis* aqueous extracts.

Sl. No.	*P. edulis* Reaction mixtures	UV-Vis absorption spectrum (nm)	
		1 hr	2 hrs
1.	Leaf extracts	317.6	332.0
2.	Stem extracts	290.0	296.0
3.	Flower extracts	291.0	326.0

Mittal *et al.* (2013) reported the various biomolecules in the plant extracts which reduce metal ions and convert into nanoparticles. The spectrophotometric analysis beyond two hrs resulted in unclear peaks. Therefore, it can be concluded that the reaction completes in two hrs using *P. edulis* extracts mediated synthesis of ZnO nanoparticles. This process could be easily scaled up, readily conducted at room temperature and pressure. The secondary metabolites present in the aqueous extracts of *P. edulis* could reduce the zinc ion from Zinc Nitrate which leads to the synthesis of zinc oxide (ZnO) nanoparticles. At present researchers

are concentrating on greater efficiency in current manufacturing processes of nanoparticles to reduce pollution, conserve resources and elimination of the use of toxic materials.

4. CONCLUSION

The Zinc oxide nanoparticles were synthesized using Zinc Nitrate hexahidrate solution mixed with leaf, stem and flower extracts of *P. edulis* in this study. This is environmentally non-hazardous natural process of biosynthesis of nanoparticles. It was observed that the reaction could be completed after two hrs with different plant part extracts. The plant extracts containing various phytochemicals which work as reducing and stabilizing agents for the formation of zinc oxide at nano scale. These nano scale particles could be used in the development of drugs in medical field and fertilizers in various crops.

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(Received 18 April 2016; accepted 06 May 2016)