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Synthesis of Zinc oxide nanoparticles from *Clitoria ternatea* L. extracts: a green approach

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ABSTRACT

We report the development of green methods for synthesis of zinc oxide at nano scale. Biological systems are being explored for the synthesis of nanoparticles. The plant *Clitoria ternatea* contains alkaloids, tannins, glycosides, resins, steroids, saponins, flavonoids, phenols, kaempferol, clitorin, taraxerol, aparajitin, sistosterol, hexacosanal and anthoxanthin. The whole plant is traditionally used to treat leprosy, bronchitis, inflammation, leucoderma and tuberculosis. The present report describes the biosynthesis and characterization of zinc oxide nanoparticles using aqueous extracts of leaves, stem, root, flowers and fruits of *Clitoria ternatea*. The aqueous reaction mixture of leaf, flower petals and roots showed strong broad UV spectral peak at 296 nm, stem at 293 nm and roots at 311 nm.

***Keywords*:** Biogenesis; Zinc oxide nanoparticles; *Clitoria ternatea*; UV-Visible characterization

1. INTRODUCTION

The nanoparticles synthesized through biological methods have been exploited for human healthcare systems such as nanomedicine, diagnosis, manufacture of commercial products etc. (Bar et al., 2009; Cruz et al., 2010). The unique physical and chemical properties of nanoparticles have been reported to exhibit various biological activities (Haverkamp and Marshall, 2009). Recently, plant extracts were used to synthesize number of eco-friendly metal nanoparticles such as silver (Ag), zinc oxide (ZnO), gold (Au), magnesium oxide (MgO), titanium oxide (TiO_2), platinum (Pt) and iron oxide nanoparticles (Fe_3O_4) (Zhan et al., 2011; Masarovicova and Kralova, 2013). The primary and secondary metabolites present in the plant crude extracts were employed in redox reaction for the reduction of ionic form into metallic eco-friendly nanoparticles (Kim et al., 2007; Aromal and Philip, 2012).

Zinc oxide (ZnO) is an inorganic compound and widely used in many products such as ceramics, glass, cement, car tyres, lubricants, paints, ointments, adhesives, plastics, sealants, pigments, batteries fire retardants, Zn added food materials etc. due to its physical, optical and antimicrobial properties (Sabir et al., 2014). Zinc oxide nanoparticles are reported to be non-toxic and used as an alternative to molecular UV-absorbers and protects from broader UV ranges. The broad spectrum coverage nature of zinc oxide nanoparticles either absorb or transmit solar radiations (Firdhouse et al., 2015).

Clitoria ternatea L. (Fabaceae) is an ornamental climber with immense medicinal properties. It is popularly known as Butterfly pea, Shankhapuspi and Blue pea etc. and native to the south-east Asia and distributed in India, Philippines and Madagascar (Anonymous, 1998). The perennial vine possesses elliptic and obtuse leaves, slender stem, and vivid deep blue or white flowers with yellow striations (Fig. 1). The fruits are flat and long pods with 8-10 seeds which are edible. It prefers moist and neutral soil to grow. The roots exhibit symbiotic association with *Rhizobium*, and possess cathartic, biter, laxative, diuretic and refrigerant properties (Nadkarni, 1976).

In Ayurveda, *C. ternatea* roots and leaves are used to treat neurological disorders, sterility in females, leucorrhea, insomnia, piles, bronchitis, leucoderma, tuberculosis, psychosis and stomatitis (Sharma and Dash 1988; Yoganarasimhan 2000). The stem and flowers are aided in treatment of scorpion sting and snake bite (Morris, 1999). The plant exhibits significant sedative (Kulkarni et al. 1988), anxiolytic, antidepressant, anticonvulsant, antistress (Jain et al. 2003), antipyretic, anti-inflammatory, analgesic (Devi et al. 2003, Gomez and Kalamani 2003), antimicrobial (Kamilla et al. 2009) and anthelmintic activities (Salhan et al. 2011). Phytochemical screening of *Clitoria ternatea* revealed the presence alkaloids, tannins, glycosides, resins, steroids, saponins, flavonoids and phenols (Neda et al., 2013; Manjula et al., 2013). At present, ZnO nanoparticles occupied dominant position in agriculture and food production. Food and Drug Administration recognized ZnO as safe and it can be used as food additive (Wang, 2004; Song et al., 2006). It has been reported to potentially enhance the yield and growth of food crops. Prasad et al. (2012) reported that peanut seeds treated with Zn at nano level improves seed germination, seedling vigor and plant growth. Green approaches of nanoparticles synthesis is ecofriendly, alternative to physical and chemical methods and reduce the risk of pollution (Reed and Hutchison, 2000). Biogenic production of zinc oxide nanoparticles has been reported from the aqueous extracts of *Passiflora foetida* (Shekhawat et al., 2014), *Plectranthus amboinicus* (Vijayakumar et al., 2015), *Punica granatum* (Mishra and Sharma, 2015), *Pongamia pinnata* (Ambika and Sundrarajan, 2015), *Aloe vera* (Varghese and George, 2015) and *Camellia sinensis* (Shah et al., 2015). This plant has already been explored for the synthesis of silver nanoparticles with antibacterial properties (Malabadi et al., 2012a, b) magnesium oxide nanoparticles with antioxidant properties (Sushma et al., 2016). Considering the medicinal properties of *C. ternatea* and ecofriendly nature of ZnO nanoparticles, the present study focused on the production and characterization of zinc oxide nanoparticles from different parts of *C. ternatea*.

Fig. 1. *Clitoria ternatea* with flower and fruits growing in the field.

2. MATERIALS AND METHODS

2. 1. Collection of plant material

Clitoria ternatea has been cultivated for horticultural and medicinal purposes. The plant material was collected from the coromandel coastal areas of the south India and identified using the Flora of Presidency of Madras (Gamble, 1921). The whole plants were harvested and fresh green leaves, stem, root, flowers and fruits were collected (Figs. 2-6A) during the months of January to June, 2015. All the parts were washed with double distilled water and finely cut into tiny pieces (Figs. 2-6B).

2. 2. Preparation of plant extracts

The plant extracts were prepared by boiling 5 grams of plant materials such as chopped leaves, stem, root, flowers and fruits in a 250 ml Erlenmeyer flask with 50 ml of Milli-Q water for 15 min. The aqueous herbal extracts were collected in separate conical flasks by standard filtration method and stored at 4 °C in a refrigerator for further experiments.

2. 3. Preparation of precursor

A volume of 1mM Zinc nitrate solution was prepared using Zinc Nitrate hexahydrate [$Zn(NO_3)_2 \cdot 6H_2O$] (Merck, Mumbai) to synthesize ZnO nanoparticles from *C. ternatea*. The solution was prepared using Milli-Q water and stored in refrigerator at 4 °C for further use.

2. 4. Synthesis of ZnO nanoparticles

The plant extracts were used to reduce the metal ions to metallic oxide nanoparticles. Three boiling tubes were used to synthesize ZnO nanoparticles, one containing 10 ml of 1mM Zinc nitrate solution 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 plant extracts as test solution. The reaction mixture from the third tube was centrifuged at 5000 rpm for 15 min to obtain the pellet after 2 to 3 hrs. Supernatant is discarded and the pellet is dissolved in Milli-Q water.

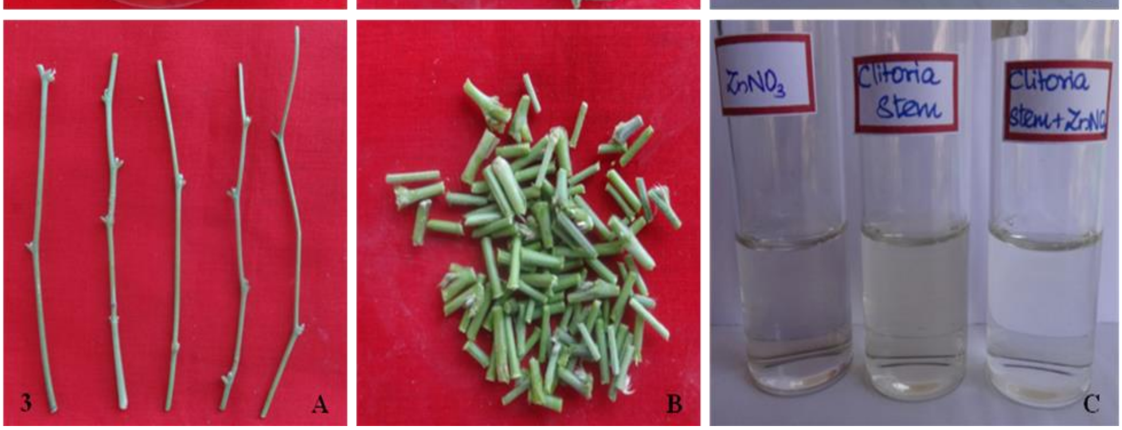


Fig. 2. A- Leaves selected for the synthesis of ZnO NPs, **B-** 5 gm of finely cut leaves, **C-** Leaf reaction mixture.

Fig. 3. A- Stem segments, **B-** 5 gm of finely cut stem, **C-** Stem reaction mixture.

Fig. 4. A- Roots of *C. ternata*, **B-** 5 gm of finely cut roots, **C-** Root reaction mixture.

Fig. 5. A- Flowers, **B-** 5 gm of finely cut flower petals, **C-** flower petals reaction mixture.

Fig. 6. A- Fruits, **B-** 5 gm of finely cut fruits, **C-** Fruit reaction mixture.

2. 5. Characterization of ZnO nanoparticles (UV-VIS spectra analysis)

The absorption spectra of ZnO nanoparticles synthesized by reducing the metal ions solutions with different extracts were monitored by measuring the UV-VIS spectrophotometer. The UV-VIS spectral analysis of the sample was done by using Systronics Double Beam spectrophotometer (Model 2202, Systronics Ltd.) at room temperature operated at a resolution between 200 nm and 700 nm ranges.

3. RESULTS AND DISCUSSION

The present investigation deals with the bio-reduction of zinc oxide nanoparticles using leaf, stem, root, flowers and fruit extracts of *C. ternatea*. The broth solutions prepared using various extracts with 1mM Zinc Nitrate were incubated at room temperature to synthesize metal ZnO nanoparticles from cell free extracts of *C. ternatea*. The color was remaining unchanged till 20 min. The reaction mixtures revolutionized into pale yellow with different time duration corresponding to the different part's extracts of the plant. The cell free extract of leaf and fruits with precursor were turned yellow after 25 min incubation at room temperature, followed by root and stem aftere 28^{th} min. The color of the reaction mixture from flower source was unchanged till 45 min at room temperature, but turned yellow within 20 min by heating the reaction mixture in water-bath (Fig. 2-6C). The excitation of electrons of the zinc oxide nanoparticles mediates the color change of the reaction mixtures, which confirms the synthesis of ZnO NPs begins at the room temperature. The biosynthesis of ZnO nanoparticles were reported to be mediated by the enzymes presented in the source material (Raliya and Tarafdar, 2013).

The visual color change in the reaction mixtures indicates the synthesis of nanopaticles, but the color varies with the respective metal nanoparticles. The reaction mixture color change into brown color indicates the synthesis of silver nanoparticles from various plants extracts (Suvidya et al., 2012; Shekhawat et al., 2013). The formation of gold nanoparticles indicated by the color change from yellowish solution to pink, grey-blue and pale violet at ambient temperature. Platinum nanoparticles in the reaction mixture change from yellow brown to dark brown (Sanchez-Mendieta and Vilchis-Nestor, 2012). ZnO nanoparticles develop yellowish–green and deep yellow in the reaction mixtures (Apurba et al. 2006; Vidya et al., 2013).

The reduction of zinc metals with different extracts of *C. ternatea* were further confirmed and characterized by UV-Visible spectrophotometeric analysis at regular intervals. It has been reported that the UV-Visible spectroscopy efficiently analyze the formation and stability of metal nanoparticles in the reaction mixtures (Shekhawat and Manokari, 2014). The primary and secondary metabolites present in the cell free extract of plants produced nanoparticles (Mittal et al., 2013). The synthesis of zinc oxide nanoparticles from bio-source have been explored enormously in revolutionizing agriculture, development of antimicrobials in food packaging and crop production (Nair et al., 2010; Khot et al., 2012).

The free electrons of ZnO NPs, confer a surface plasmon resonance absorption band appropriate to the vibration of electrons of metal nanoparticles in resonance with the light wave (Maye et al., 2002; Shankar et al., 2004). Surface plasmon resonance spectra for ZnO nanoparticles from leaves, flowers and fruits are obtained at 296 nm (Fig. 7A, D and E), stem at 293 nm (Fig. 7B) and root at 311 nm (Fig. 7C) (Table. 1).

Reaction time and concentration of reaction mixture plays major role in the synthesis of nanoparticles (Annamalai et al., 2011; Ravindran et al., 2016).

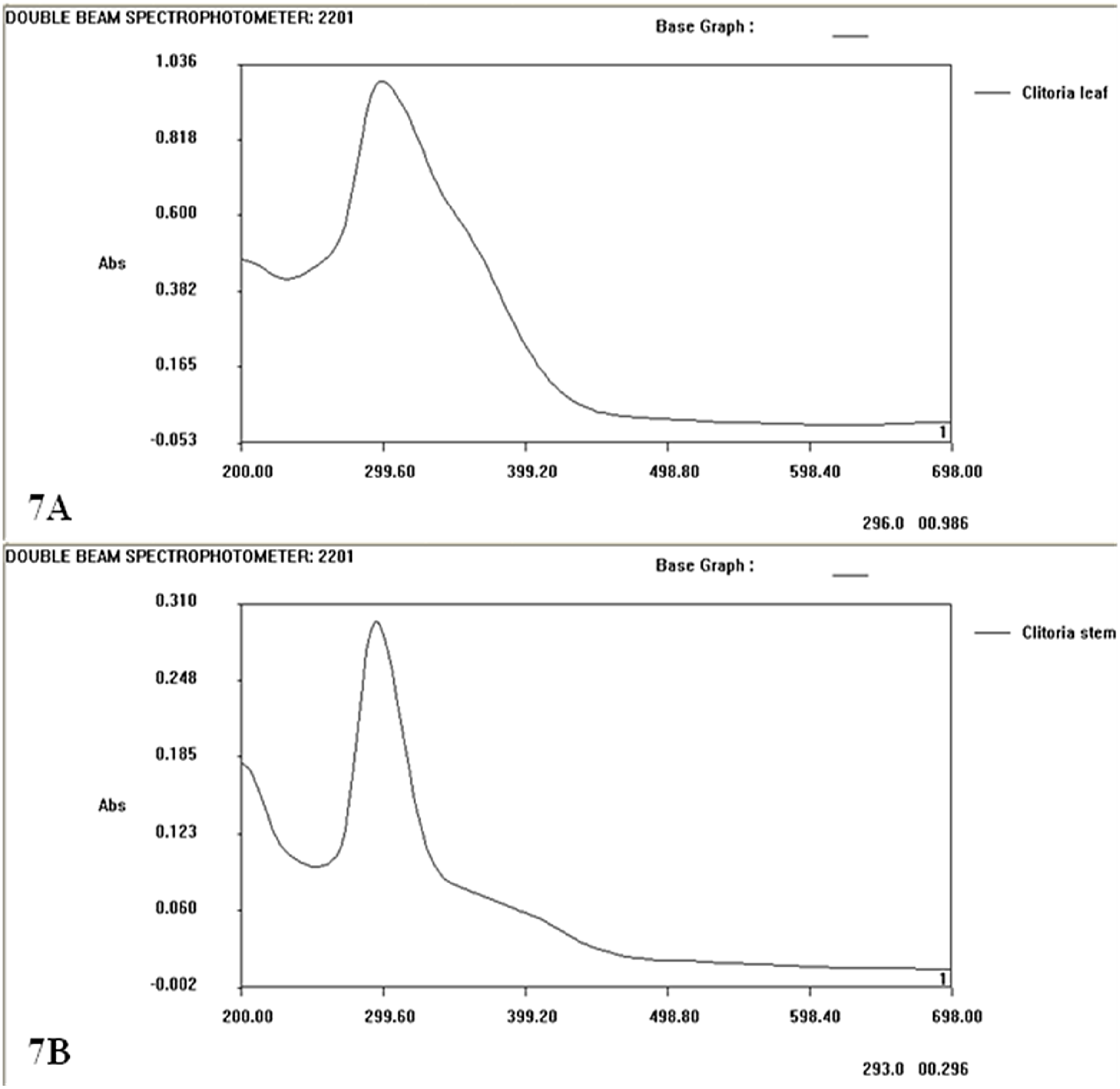
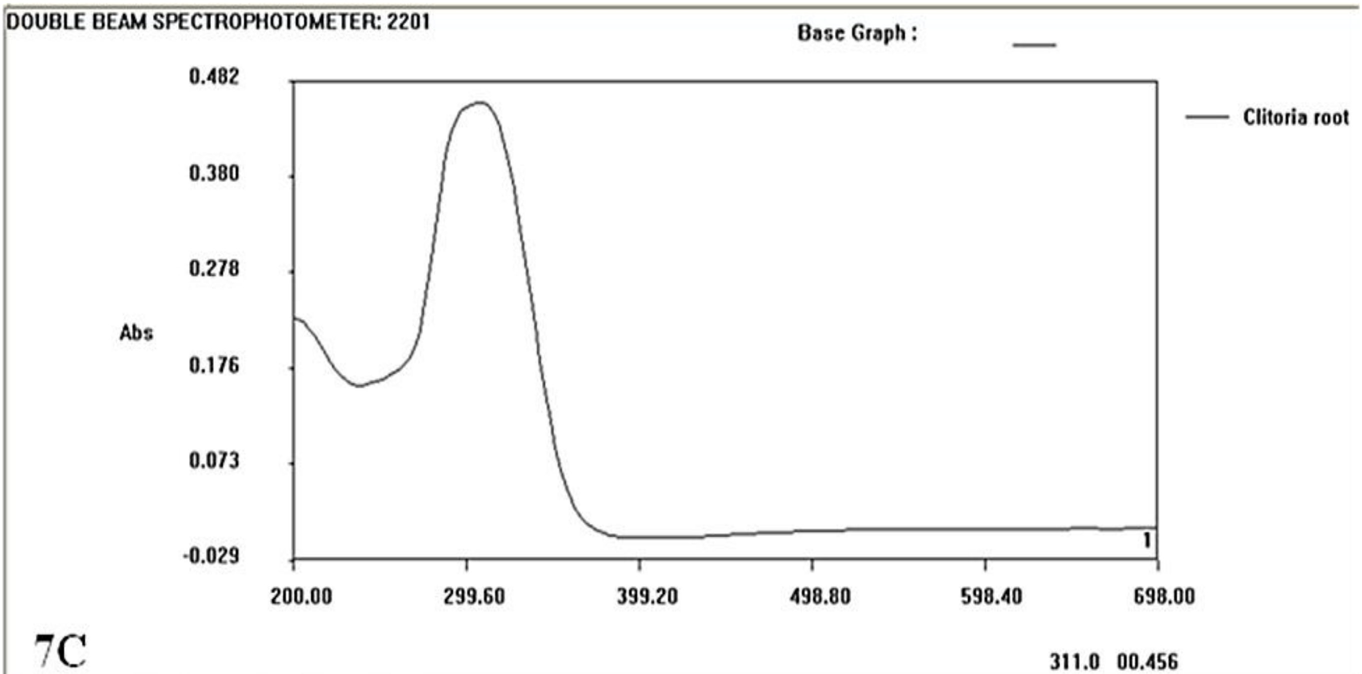


Fig. 7. UV-Visible spectral analysis of **(A) –** leaf extract reaction mixture and **(B)** – Stem extract reaction mixture.


DOUBLE BEAM SPECTROPHOTOMETER: 2201
Base Graph :
Clitoria root
Abs
0.482
0.380
0.278
0.176
0.073
-0.029
200.00
299.60
399.20
498.80
598.40
698.00
1
7C
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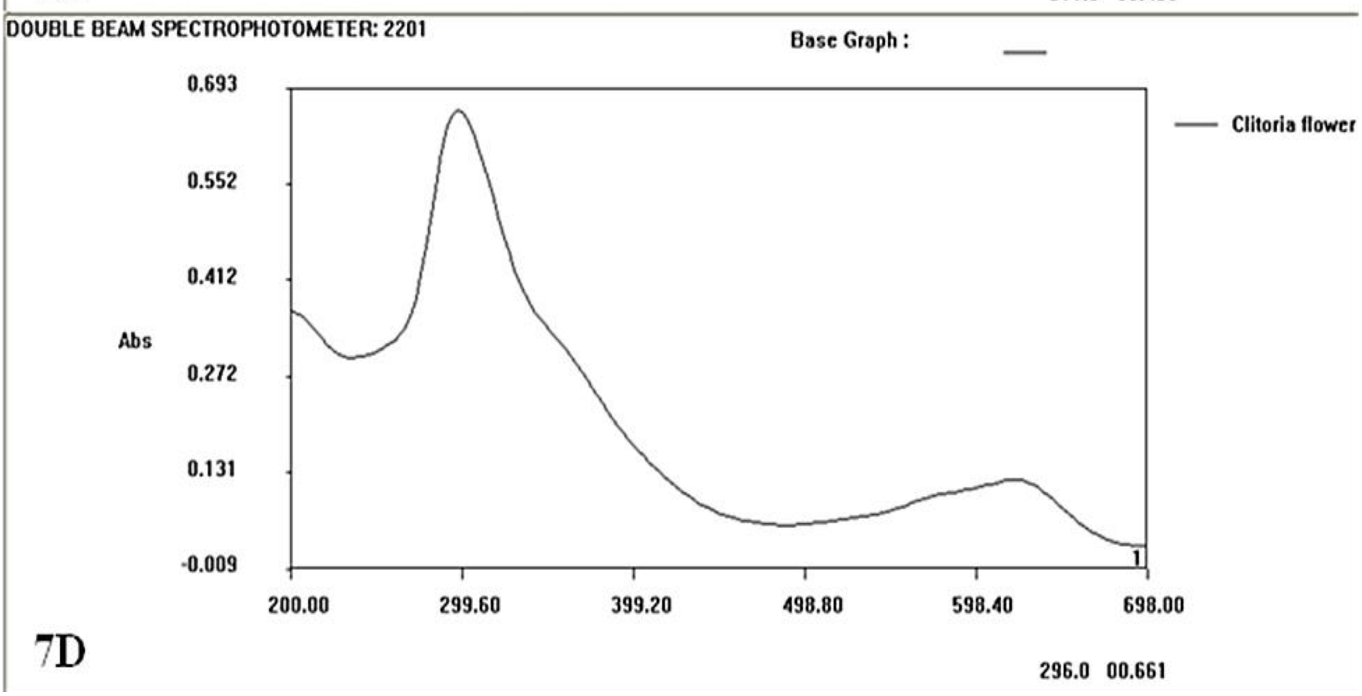
DOUBLE BEAM SPECTROPHOTOMETER: 2201
Base Graph :
Clitoria flower
Abs
0.693
0.552
0.412
0.272
0.131
-0.009
200.00
299.60
399.20
498.80
598.40
698.00
1
7D
296.0 00.661

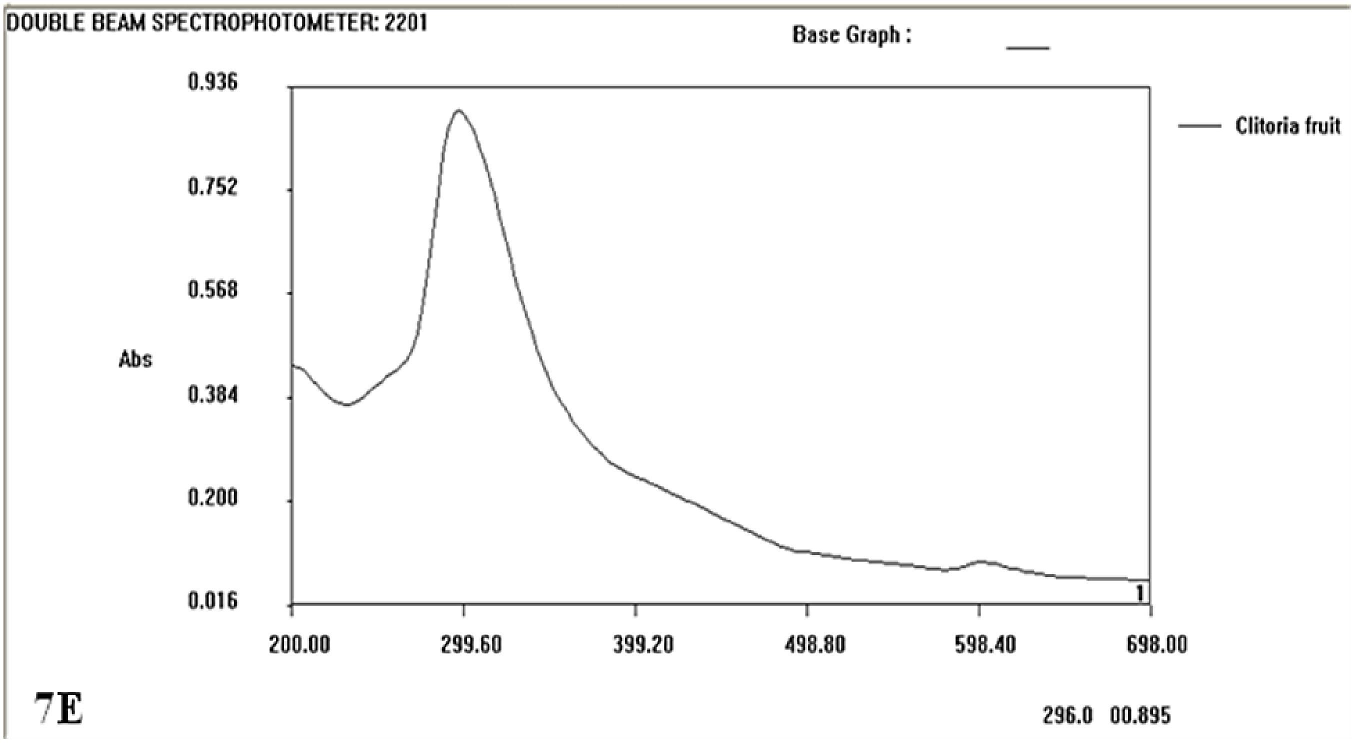

Fig. 7. UV-Visible spectral analysis of **(C)** – Root extract reaction mixture, **(D)** – Flower petal extract reaction mixture and **(E)** – Fruits extract reaction mixture.

Table 1. UV-Visible absorption spectra of zinc oxide nanoparticles synthesized using *Clitoria ternatea* plant extracts.

Sl. No	Source of reaction mixtures	UV-Vis absorption spectrum (nm)
1.	Leaves	296
2.	Stem	293
3.	Root	311
4.	Flowers	296
5.	Fruits	296

Malabadi et al. (2012a) synthesized silver nanoparticles from whole plant extracts of *C. ternatea* and tested antibacterial activity against *Bacillus subtilis*, *Staphylococcus aureus*, *Escherichia coli* and *Klebsiella pneumonia*. The silver nanoparticles from *in vitro* derived plants of *C. ternatea* exhibits efficient antimicrobial activity (Malabadi et al., 2012b).

UV-Visible recording for leaves, flowers and fruits falls at 296 nm, indicated that the secondary metabolites common to these parts may mediate the synthesis and stability of ZnO nanoparticles. Roots are reported to contain saponins, phenols, tannins and resins (Uma 2009, Manalisha and Chandra, 2011), these may accelerate the synthesis of nanoparticles. Manjula et al. (2013) reported that the shoots, stem, flowers and seeds contain flavonoids in common at various levels. The secondary metabolites particularly flavonoids, alkaloids and terpenoids were reported to be responsible for the synthesis of metallic nanoparticles (Aromal and Philip, 2012). The present study reveals that the presence of flavonoids in the various parts of *C. ternatea* was responsible for the reduction of ZnO metallic nanoparticles.

4. CONCLUSION

The present investigation describes the synthesis of zinc oxide nanoparticle from various sources of medicinal plant *C. ternatea*. It assures that the procedure is green, approach is ecofriendly and it can be easily scaled up for future needs. Since this plant has its important place in Ayurveda, the nano formulations could be effective in diagnosis and treatment of various illnesses.

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