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Role of *Crotalaria verrucosa* L. extracts in synthesis of Zinc oxide nanoparticles

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ABSTRACT

Green synthesis of metal (zinc) oxide nanoparticles is a fascinating subject in the field of nanobiotechnology. Nanoparticles using green plants are suitable for large scale biosynthesis at room temperature with affordable price and eco-friendly nature. The stable nanoparticles were synthesized using leaves, stem, roots, flowers, immature and mature seeds extracts of *Crotalaria verrucosa*. Zinc Nitrate hexahydrate [$Zn(NO_3)_2 \cdot 6H_2O$] was used as precursor material in this experiment. The formation of nanoparticles was monitored by visual color changes and UV-Visible spectrophotometric analysis. UV-Visible absorption peaks between 299-317 nm confirmed the presence of ZnO at nano-scale. The present technique confirmed that this plant species could be explored in the field of nanotechnology for human welfare.

***Keywords*:** *Crotalaria verrucosa*; Zinc nitrate; Broth solutions; Surface Plasmon Resonance

1. INTRODUCTION

Crotalaria verrucosa L. (family Papilionaceae) is popularly known as Blue rattle, Kilukiluppai and Ghelegherinta. It is native to India and widely distributed in the tropical regions of Burma, Malaya and China. The plant is cultivated as a green manure crop to improve the quality of the soil (Kumar and Sane, 2003).

It is a much branched annual herb reaches the height of one meter, with simple, elliptic and entire leaves, zigzag and angled stem, racemose inflorescence consists of terminal or lateral bluish-purple flowers. *C. verrucosa* represent the transition gynoecium between the primitive and advance types of the angiosperms. It possess multicrpellate gynoecium consisted of two or three free carpels and stamens are monadelphous with dimorphic anthers. Fruits (pods) are oblong, inflated and measuring 2.5-3 cm long (Nagar and Albert, 2013).

Preparation of materials at nano scale is one of the emerging and growing fields with novel approaches and its applications in science and technology for the rationale of industrialized nanoparticles. It is apparent that the green synthesis of ZnO nanoparticle is eco-friendly, uncomplicated and resourceful than the conventional chemical and physical methods.

Green synthesis of nanoparticles can be a reliable method and performed at room temperature from various bio-resources (Firdhouse *et al.*, 2015). The flexible properties of zinc oxide nanoparticles (ZnO NPs) such as molecular UV-absorbers, nano-fertilizers, photocatalytic property and nano-pesticides (Devalapally *et al.,* 2007; Kulkarni and Muddapur, 2014; Sabir *et al.*, 2014) have attracted many research groups to pursue research for the production zinc oxide nanoparticles using plant extracts. It is believed that the plant extracts contain various phytochemicals which used work as reducing agents in the synthesis of nanoparticles in the reaction mixtures.

The plant gains prime importance due to its expectorant, emetic and diuretic medicinal properties. Its leaves are used to cure rheumatism, skin allergies, tetanus, salviations, biliousness, dyspepsia, fever, scabies, impetigo, jaundice, throat and mouth diseases and heart complaints (Asolkar *et al.,* 1992; Senthilkumar *et al.*, 2006; Rahman *et al.*, 2007). The curing properties of this plant are due to presence of some special types of bioactive compounds in different parts of *C. verrucosa*.

The phytochemical characterization of *C. verrucosa* reveals that the leaves and stem (aerial parts) contains tropane alkaloids, fixed oils, taraxerol, ß-sitosterol and a large number of saturated and unsaturated fatty acids. The seeds were reported for the presence of pyrrolizidine alkaloids including crotalaburnine, crotaverrine, iso-senkirkine, O-acetylcrotaverrine, isosenkirkine acetate, sitosterol, isovitexin, vitexin, flavonoids, vitexin-O-glycoside and apigenin-O-glycoside (Suri *et al.*, 1976; Rastogi and Mehrotra, 1993).

Literature reveals that there are limited reports on silver nanoparticles from *C. verrucosa* (Murugan *et al.*, 2016) and no reports available for the synthesis of zinc oxide nanoparticles using the aqueous extract of *C. verrucosa*. Therefore, the objective of the present study was to synthesize zinc oxide nanoparticles using different organs extract of *C. verrucosa* and characterize them.

2. MATERIALS AND METHODS

2. 1. Collection of plant material

C. verrucosa has been cultivated as a green manure crop to improve the soil quality. The plant material was collected from the coromandel coastal areas south India and identified using the Flora of Presidency of Madras (Gamble, 1921).

The whole plant was harvested and fresh green leaves, stem, root, flowers, immature and mature seeds were collected (Figs. 1-6A) during the months of January to August, 2015.

All the parts were washed several times with water to remove the dust particles and then shade dried to evaporate the residual moisture. Finally the materials were rinsed with double distilled water and finely chopped into small pieces (Figs. 1-6B).

2. 2. Preparation of plant extracts

The plant extracts used to reduce the zinc ions to zinc nanoparticles were prepared by boiling 5 grams of plant materials such as chopped leaves, stem, root, flowers, immature and mature seeds in a 250 ml Erlenmeyer flask with 50 ml of double distilled water for 15 min separately. The aqueous herbal extracts were cooled to room temperature and filtered by standard filtration method. The extracts were stored in separate conical flasks at 4 °C in a refrigerator.

2. 3. Preparation of precursor

A volume of 1 mM Zinc nitrate solution was prepared using Zinc Nitrate hexahydrate [$Zn(NO_3)_2 \cdot 6H_2O$] (Merck, Mumbai) to synthesize ZnO nanoparticles from various organ extracts of *C. verrucosa*. The solution was prepared using double distilled water and stored in refrigerator at 4 °C in order to carry further experiments.

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 5 ml of 1 mM Zinc nitrate solution and 5 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 dispersed in double distilled water.

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

The absorption spectra of ZnO nanoparticles synthesized by reducing the metal ions solutions with different extracts were monitored by measuring the UV-Visible spectrophotometer. The UV-Visible 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

Many plant species of the family Papilionaceae have been used in the biosynthesis of nanoparticles by many researchers, like *Cassia densistipulata* (Kooluru and Sharada, 2014), *Vigna radiata* and *Cicer arietinum* (Banerjee *et al.*, 2014), *Acacia leucophloea* (Murugan et al., 2014), *Clitoria ternatea* (Manokari and Shekhawat, 2016) with potential applications in human welfare.

3. 1. Synthesis of ZnO nanoparticles

The leaves extract were reacted with equal volume of zinc nitrate solution for 12 min and turned the solution into deep yellow at room temperature. The extracts derived from stem and seeds responded next to leaves at 17th min. Flowers and root reaction mixtures exhibited yellow color when boiled for 15 min. The intensity of the color increased with increase in temperature till 40 °C.

The change in color of the reaction mixture indicates the formation of zinc oxide nanoparticles as reported in *Passiflora foetida* (Shekhawat *et al.*, 2014), *Ficus benghalensis* (Shekhawat *et al.*, 2015) and *Camellia sinesis* (Shah *et al.*, 2015). The precursor solution Zinc nitrate, source of Zn ions has been transformed into ZnO during the biological synthesis of plant extracts.

Plant extracts have been explored successfully to synthesize biocompatible nanoparticles due to their diverse properties (Vidya *et al.*, 2013)

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Fig. 1. A- Leaves, **B-** Finely chopped leaves, **C-** Leaf extract with reaction mixture.
Fig. 2. A- Stem segments, **B-** Stem pieces, **C-** Stem extract with reaction mixture.

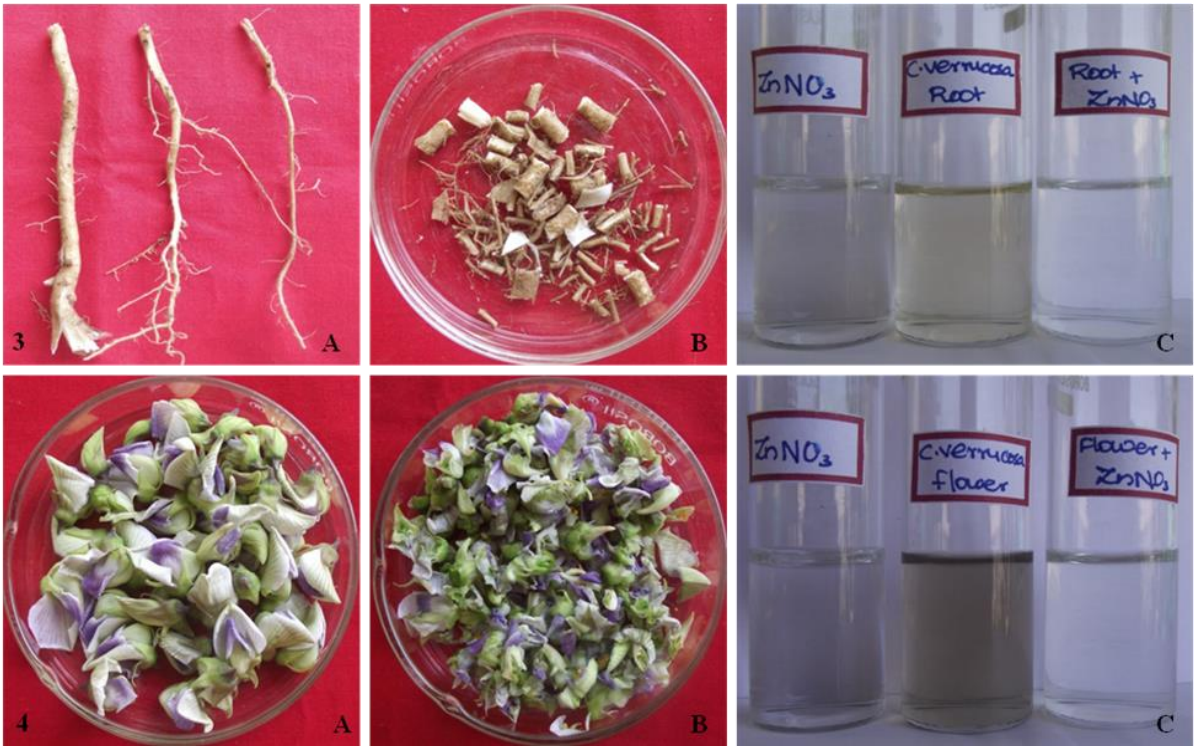


Fig. 3. A- Roots, **B**- Root pieces, **C**- Root extract with reaction mixture.
Fig. 4. A- Flowers, **B**- Chopped petals and sepals, **C**- Flower extract with reaction mixture.

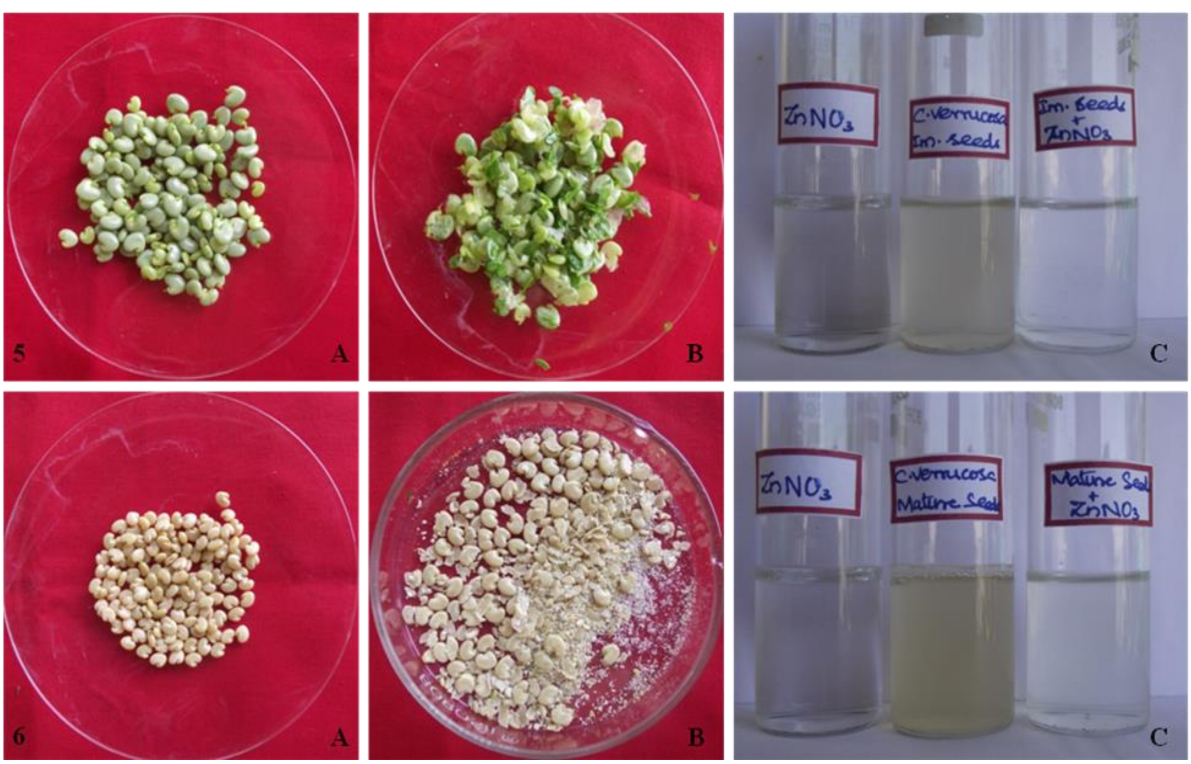


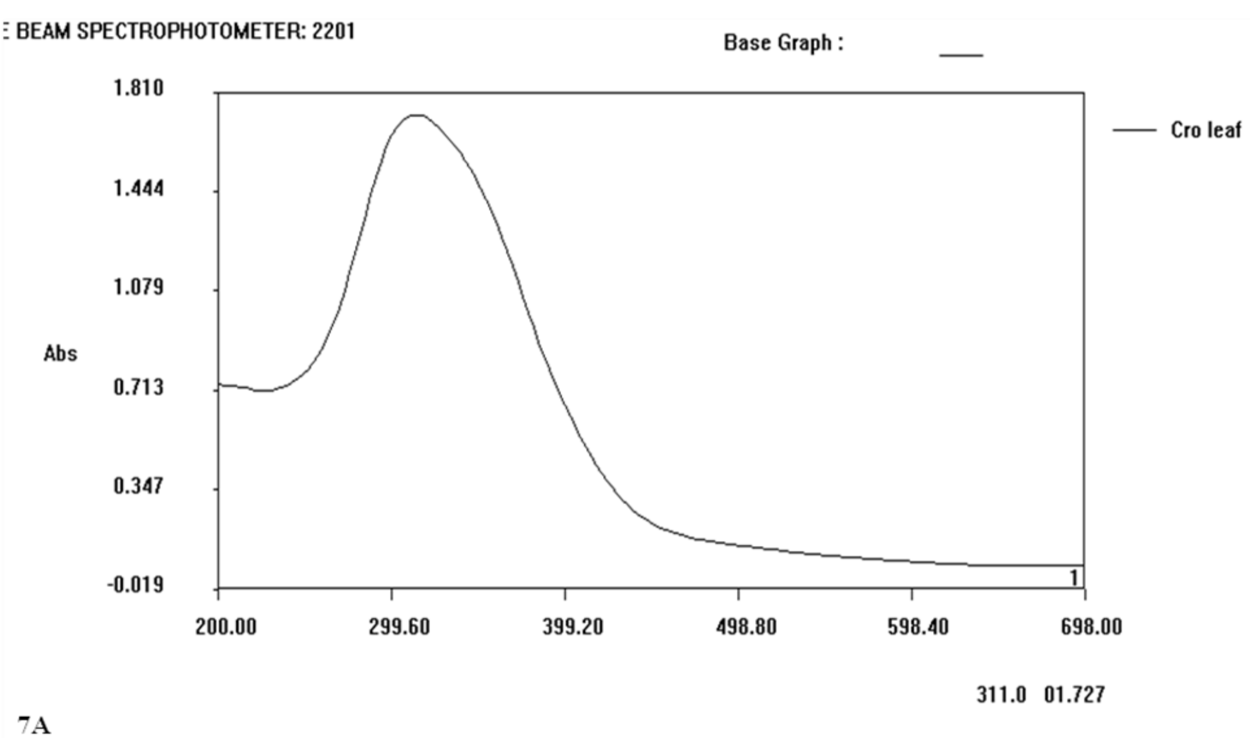
Fig. 5. A- Immature seeds, **B**- Crushed pieces, **C**- Seed extract with reaction mixture.
Fig. 6. A- Mature seeds, **B**- Crushed seed pieces, **C**- Extract with reaction mixture.

3. 2. Characterization of ZnO nanoparticle

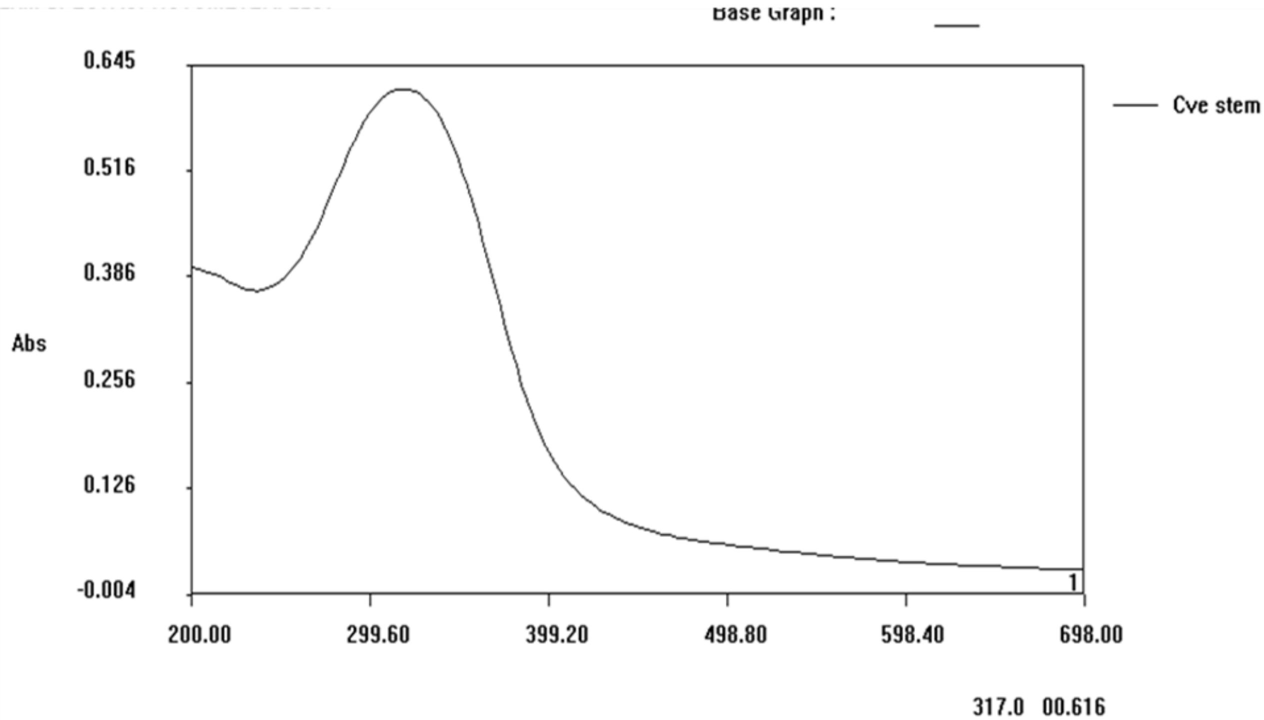
The optical properties of *C. verrucosa* mediated ZnO nanoparticles were characterized by UV-Visible spectroscopy using zinc nitrate as reference at room temperature. The UV-Visible absorption spectra of the leaf reaction mixture were recorded at the wavelength of 311 nm. There was concentrated absorption in the ultraviolet band of stem and flower reaction mixtures observed at 317 nm. The absorption wavelength of root extract and zinc nitrate was recorded at 299nm, mature and immature seeds were resulted at 302 nm and 305 nm respectively (Figs. 7A-7F, Table 1).

Table 1. UV-Visible absorption spectra of zinc oxide nanoparticles synthesized using various parts of *Crotalaria verrucosa*.

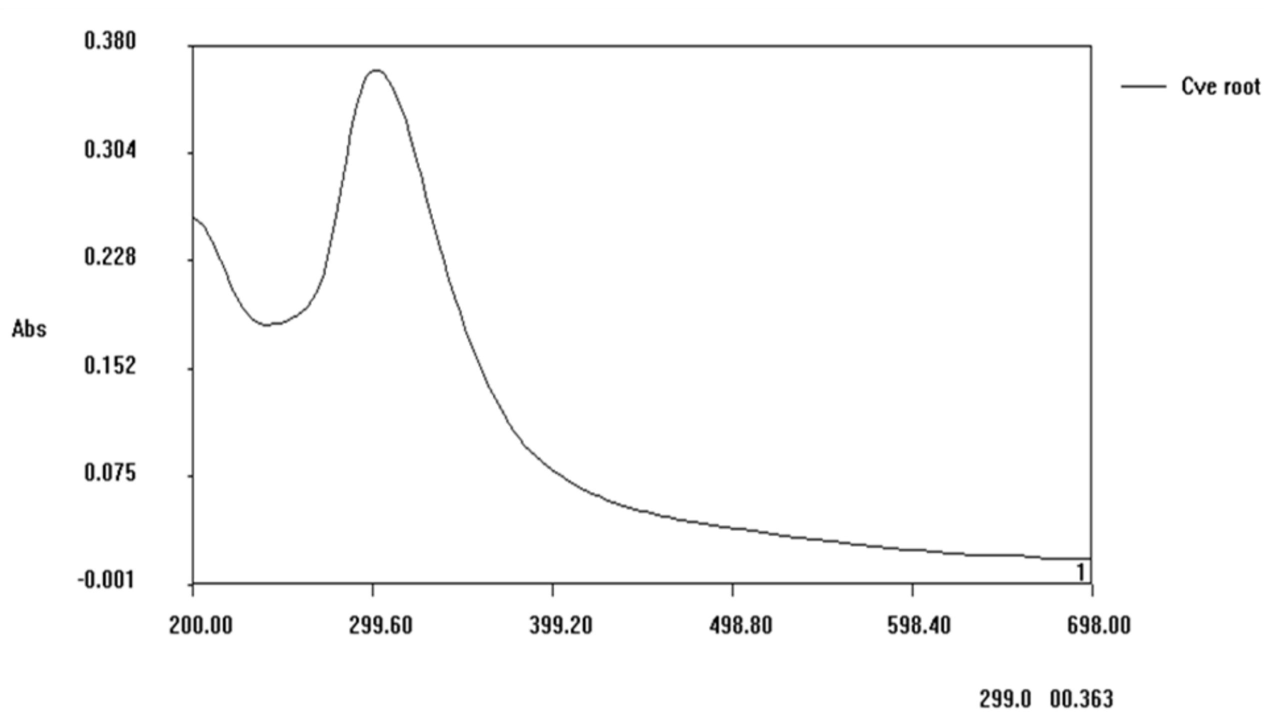
Sl. No	Source of reaction mixtures	UV-Vis absorption spectrum (nm)
1.	Leaves	311
2.	Stem	317
3.	Root	299
4.	Flowers	317
5.	Immature seeds	302
6.	Mature seeds	305



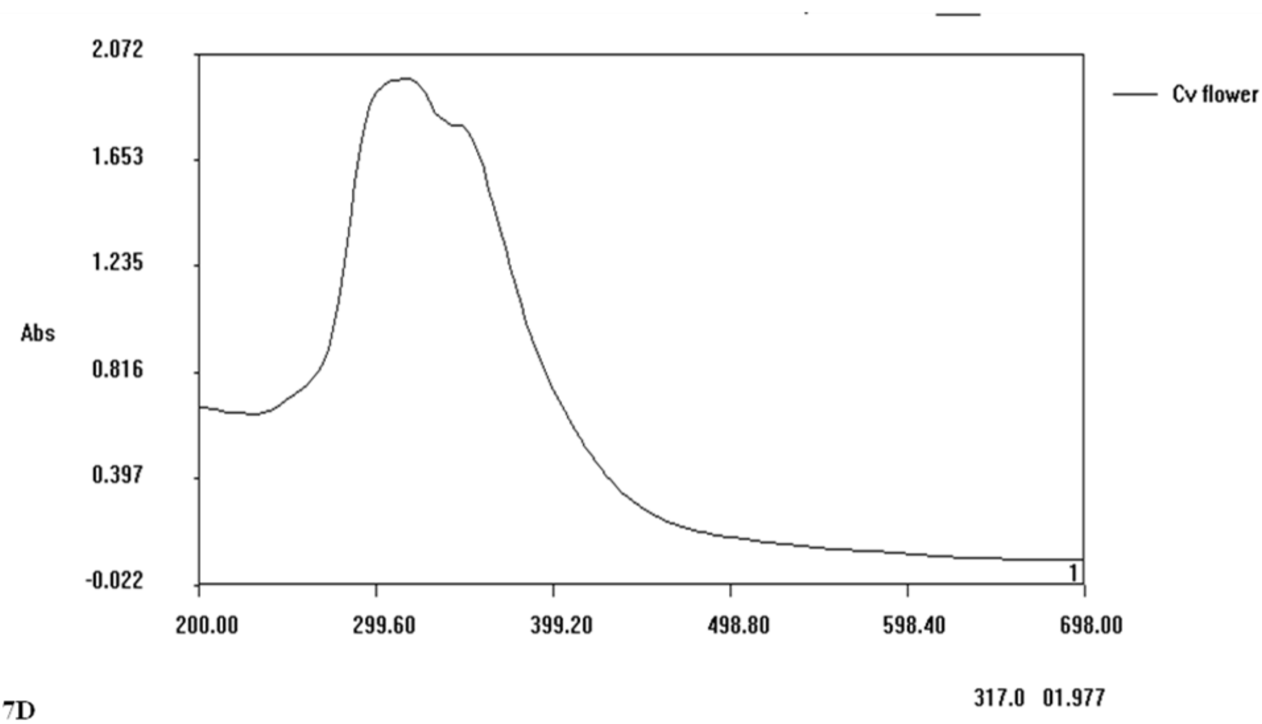
7A


Base Graph :
0.645
0.516
0.386
Abs
0.256
0.126
-0.004
200.00
299.60
399.20
498.80
598.40
698.00
Cve stem
1
317.0 00.616

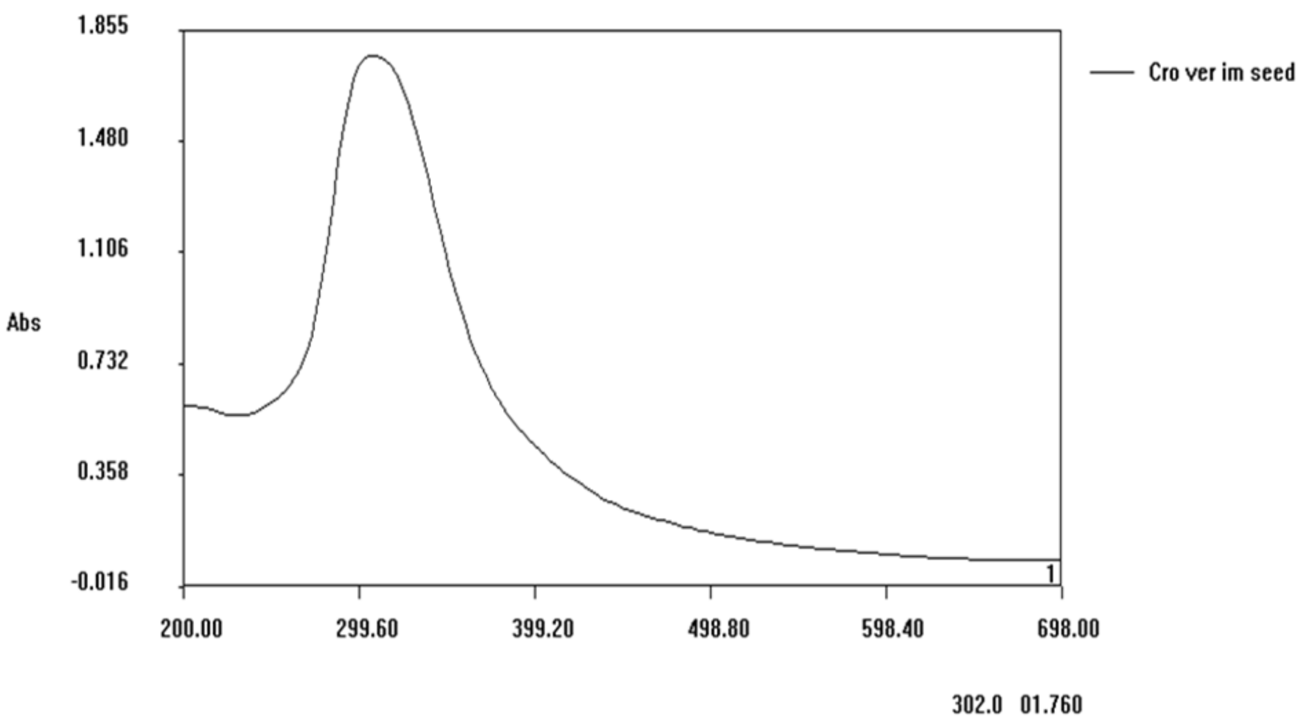
7B


0.380
0.304
0.228
Abs
0.152
0.075
-0.001
200.00
299.60
399.20
498.80
598.40
698.00
Cve root
1
299.0 00.363

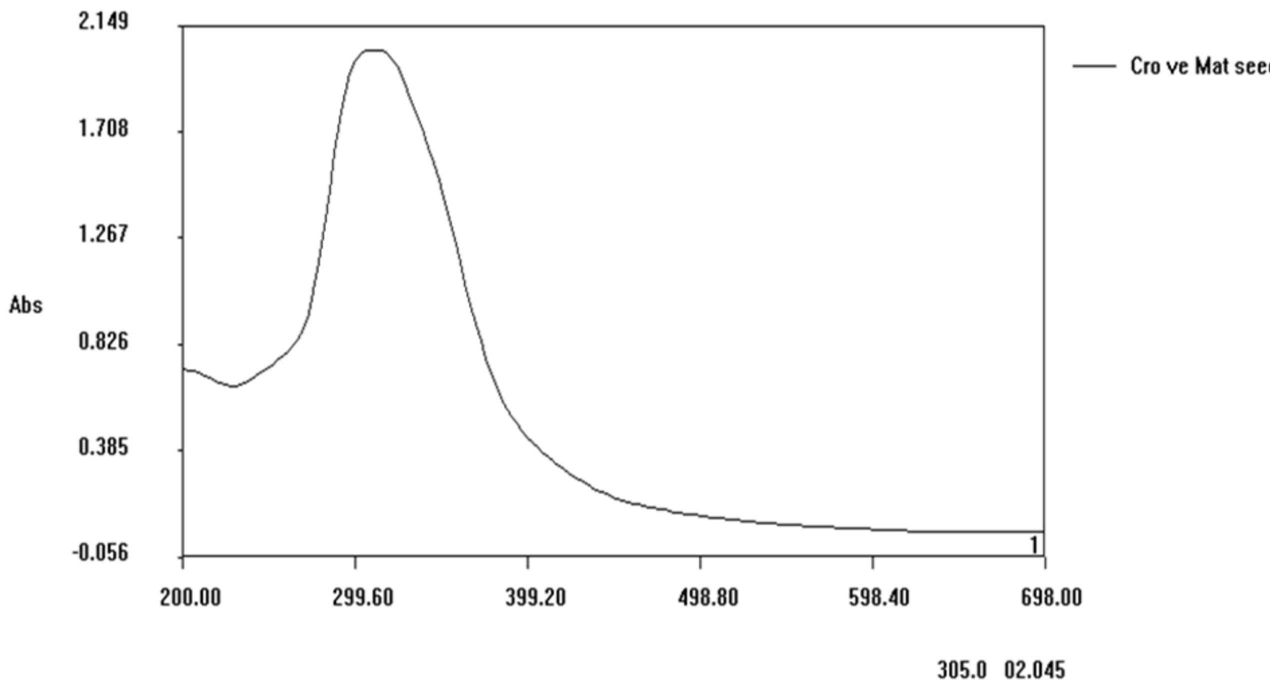
7C


2.072
1.653
1.235
Abs
0.816
0.397
-0.022
200.00
299.60
399.20
498.80
598.40
698.00
Cv flower
1
317.0 01.977

7D


1.855
1.480
1.106
Abs
0.732
0.358
-0.016
200.00
299.60
399.20
498.80
598.40
698.00
Cro ver im seed
1
302.0 01.760

7E



7F

Figs. 7. UV-Visible spectral absorbance peak of **A**- Leaves extract reaction mixture, **B**- Stem extract reaction mixture, **C**- Roots extract reaction mixture, **D**- Flowers extract reaction mixture, **E**- Immature seeds and **F**- Mature seeds extract reaction mixture.

The study focused on the production of ZnO nanoparticles using the aqueous extracts of various parts of *C. verrucosa*. The leaves and stem were found to be rich in tropane alkaloids, fixed oils, taraxerol, ß-sitosterol and the seeds were reported to contain pyrrolizidine alkaloids, isovitexin, vitexin, flavonoids, vitexin-O-glycoside and apigenin-O-glycoside. These elements in various concentrations in the different parts of the plant are responsible in the reduction of zinc nitrate into ZnO nanoparticles. It has been reported that aqueous plant extracts were used as a reducing as well as capping agents in the reaction mixtures (Firdhouse *et al.*, 2015).

4. CONCLUSION

The UV-Visible characterization of ZnO nanoparticles from various parts of *C. verrucosa* showed the characteristic absorbance between 299-317 nm, confirmed that the whole plant could be used to synthesize ZnO nanoparticle. The principal mechanism by which the plant extracts are responsible for the synthesis of nanoparticle from this species has to be studied. But the previous reports suggest that the synthesis is due to the presence of secondary metabolites which accelerate the synthesis of ZnO nanoparticles.

References

[1] S. Kumar, P.V. Sane, Legumes of South Asia: a checklist, 2003.

[2] P. Nagar, S. Albert. *Journal of Plant Taxonomy and Geography* 68 (2013) 187-190.

[3] O.P. Suri, R.S. Swhney, M.S. Bhatia, C.K. Atal. *Phytochemistry* 15 (1976) 1061-1063.

[4] R.P. Rastogi, B.N. Mehrotra BN, Compendium of Indian Medicinal Plants. Vol. V. PID New Delhi, 1993: 368.

[5] L.V. Asolkar, K.K. Kakkar, O.J. Charke, Part-I, India: Publications and Information Directorate (CSIR), New Delhi, 1992.

[6] M. Senthilkumar, P. Gurumoorthi, K. Janardhanan K. *Natural Product Radiance* 5 (2006) 382-388.

[7] M.A. Rahman, S.B. Uddin, C.C. Wilcock. *Indian Journal of Traditional Knowledge* 6 (2007) 508-517.

[8] J.M. Firdhouse, P. Lalitha, P. Vennila. *International Journal of Extensive Research* 2 (2015) 12-20.

[9] H. Devalapally, A. Chakilam, M. M. Amiji. *Journal of Pharmaceutical Sciences* 96 (2007) 2547-2565.

[10] N. Kulkarni, U. Muddapur. *Journal of Nanotechnology* 1 (2014) 1-8.

[11] [S. Sabir, M. Arshad, S. K. Chaudhari. The Scientific World Journal 10 (2014) 1-8

[12] K. Murugan, C.P. Sanoopa, P. Madhiyazhagan, D. Dinesh, J. Subramaniam, C. Paneerselam, M. Roni, U. Suresh, M. Nicoletti, A.A. Alarfaj, M.A. Munusamy, A. Higuchi, S. Kumar, H. Perumalsamy, Y.J. Ahn, G. Benelli, *Natural Product Research* 30 (2016) 826-833.

[13] J.S. Gamble. Flora of the Presidency of Madras. 1921, Vol.1. London (repr. Ed. 1995. Bishen Singh, Mahendra pal singh, Dehradun).

[14] N.R. Kooluru, S. Sharada. *International Journal of Engineering Research and Development* 10 (2014) 16-19.

[15] P. Banerjee, M. Satapathy, A. Mukhopahayay, P. Das. *Bioresources and Bioprocessing* 2014, DOI: 10.1186/s40643-014-0003-y.

[16] K. Murugan, B. Senthilkumar, D. Senbagam, S. Al-Sohaibani. *International Journal of Nanomedicine* 9 (2014) 2431-2438.

[17] M. Manokari, M.S. Shekhawat. *World Scientific News* 52 (2016) 216-227.

[18] M.S. Shekhawat, C.P. Ravindran, M. Manokari. *International Journal of Green and Herbal Chemistry* 3 (2014) 518-523.

[19] M.S. Shekhawat, C.P. Ravindran, M. Manokari. *International Journal of BioSciences, Agriculture and Technology* 6 (2015) 1-5.

[20] R.K. Shah, F. Boruah, N. Parween, *International Journal of Current Microbiology and Applied Sciences* 4 (2015) 444-450.

[21] C. Vidya, H. Shilpa, M. N. Chandraprabha, M. A. Lourdu Antonyrai, V. Indu, J. Aayushi, B. Kokil, *International Journal of Current Engineering and Technology* (2013) 118-120.

[22] M.S. Shekhawat. *International Journal of Biological Papers* 1 (2016) 9-15.

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