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Biological Approach for the Synthesis and Characterization of Zinc Oxide Nanopaticles from *Croton bonplandianum* Baill. Extracts

M. Manokari, Mahipal S. Shekhawat

Department of Botany, Kanchi Mamunivar Centre for Postgraduate Studies, Pondicherry - 605 008, India

E-mail address: manokari01@gmail.com

ABSTRACT

The biogenesis of nanoparticles recently gained more attention. Here, we report the biogenesis of zinc oxide (ZnO) nanoparticles using aqueous extracts of the leaves, stem, root, flowers and fruits of *Croton bonplandianum* Baill. at room temperature. *Croton bonplandianum* is an important medicinal plant used to cure many pathological conditions in the traditional systems of Indian medicines due to the presence of important and specific bioactive compounds in the plant parts of this plant. Aqueous solution of Zinc Nitrate hexahydrate [$Zn(NO_3)_2 \cdot 6H_2O$] was used as a precursor and the various plant extracts played as reducing agents. The formation of ZnO nanoparticles was monitored by UV-Visible spectrophotometric analysis. The leaf extract showed strong absorbance peak at 302 nm, stem and fruit at 293 nm, root at 290 nm and flowers at 305 nm.

***Keywords*: *Croton bonplandianum*, Zinc oxide nanoparticles, Plant extracts, UV-Visible spectrophotometry**

1. INTRODUCTION

The interaction between nanoparticles and biological materials paved the way in the formation of new materials with controlled size and shape [1]. Nanoparticles from metals like gold nanoparticles (Au NPs), nanosilver (Ag NPs), nanocopper (Cu NPs) and zinc oxide nanopaticles (ZnO NPs) are main field of research now a day due to their significant applications in science and technology [2]. Zinc oxide has its significant application on solar

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cells, gas sensors, ceramics, catalysts and cosmetics. Zinc oxide nanoparticles possess antimicrobial, antibacterial activity against *Bacillus subtilis* and *Escherichia coli* [3]. Biological method of preparation of zinc oxide nanoparticles have significant attention owing to its eco-friendly method of synthesis and making pollution free effects (Tundo et al., 2000). There is an intense research interest on the application of biogenic of zinc oxide nanoparticles in the field of medicines due to their pharmacological activities. The use of zinc oxide nanoparticles in drug research, antimicrobial formulations, sunscreen lotions have been well acknowledged [4].

Plants are exploited for the synthesis of Pelladium [5], Silver [6], Gold [7], Iron [8], and zinc oxide nanoparticles [9]. Researchers consider the biological synthesis of nanoparticles is extracellular and convenient method for environmentally safe nanoparticles production [10]. So far, different plant parts like fruits [11], stems [12], bark [13], seeds, latex [14], callus [15] and whole plants have been employed for the synthesis of nanoparticles.

Fig. 1. *Croton bonplandianum* Baill, plant in natural habitat.

Croton bonplandianum Baill. is a perennial herb belongs to the family Euphorbiaceae (Castor family) and subfamily Crotonoideae. The herb is locally known as Ban Tulsi, Jungle tulasi, Eliamanakku, Kukka mirapa, Alpabedhi soppu, three-leaved caper in English and Kalabhangre in Hindi. This plant grows up to 60 cm in height. Its leaves are simple, ovate-lanceolate in shape with serrate margin and two glands present at the base. This wild herb exhibits flowering and fruiting during the months of September to December. Flowers are small and white, which occurs at long racemes at the end of the branches (Fig. 1). Flowers are subsessile with 5 petals and sepals. Fruit is capsule with warty surface [16].

It is a medicinally important plant and used to treat high blood pressure, skin diseases, cut wounds, cholera, head ache etc. [17]. The latex shows antifungal activity against *Microsporium gypsum* and *Trichophyton* sp. [18], and has healing effect in curing of cuts and wounds [19]. Seed oil gains considerable attention medicinally. The seeds are used in the treatment of jaundice, acute constipation, abdominal dropsy and internal abscesses [16]. The whole plant is acknowledged for the curing of liver diseases, ring worms and body aches [20].

The active principles isolated from the plant are rutin, crotsearinine, crotasparine, phlobatannin, terpenoid, glycoside, phenolics, flavonoids, steroids, resins, saponin, alkaloid, cholesterol, etc. [21]. The seeds are reported to possess diterpenes, phorbol ester, including 12-orthotrideconeoly-phorbol-13-acetate and myristoyl phorbol acetate [22].

The phytochemical constituents from the plant exhibits antibacterial, antifungal [23], wound healing, antioxidant and antitumor activities [24].

Plant mediated zinc oxide nanoparticles have been reported in *Calotropis procera* [25], *Corriandrum sativum* [26], *Calotropis gigantea* [27], *Curcuma rubescens* [2] etc. Concentrating the broad spectrum usage of zinc oxide nanoparticles, the present investigation was conducted for the synthesis and characterization of zinc oxide nanoparticles using aqueous extracts from different parts of the medicinal plant *C. bonplandianum*.

2. MATERIALS AND METHODS

2. 1. Collection of Plant Materials and Preparation of Broth solution

The whole plant was collected from the regions of South India, and identified with the help of Gamble flora [28]. All the plant parts like leaf, stem, root, flowers and mature fruits were collected from the disease free healthy plants during 2014-2015. The plant materials used in the study were washed thoroughly with double distilled water and finely cut into small pieces (Figs. 2-6A and B).

The plant parts were separated with fine razor blade and immersed in 40% ethanol to remove foreign materials such as soil, dusts and fungal particles. Five grams of finely chopped plant materials were boiled in the clean and sterilized conical flasks of desired size with 50 ml of double distilled water for 5 min 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 extracts stored in refrigerator for further study.

2. 2. Preparation of Precursor and Synthesis of ZnO NPs

Zinc Nitrate hexahydrate [$Zn(NO_3)_2 \cdot 6H_2O$] (Merck, Mumbai) was used as a precursor for the synthesis of ZnO NPs from the extracts of *C. bonpandianum.*

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

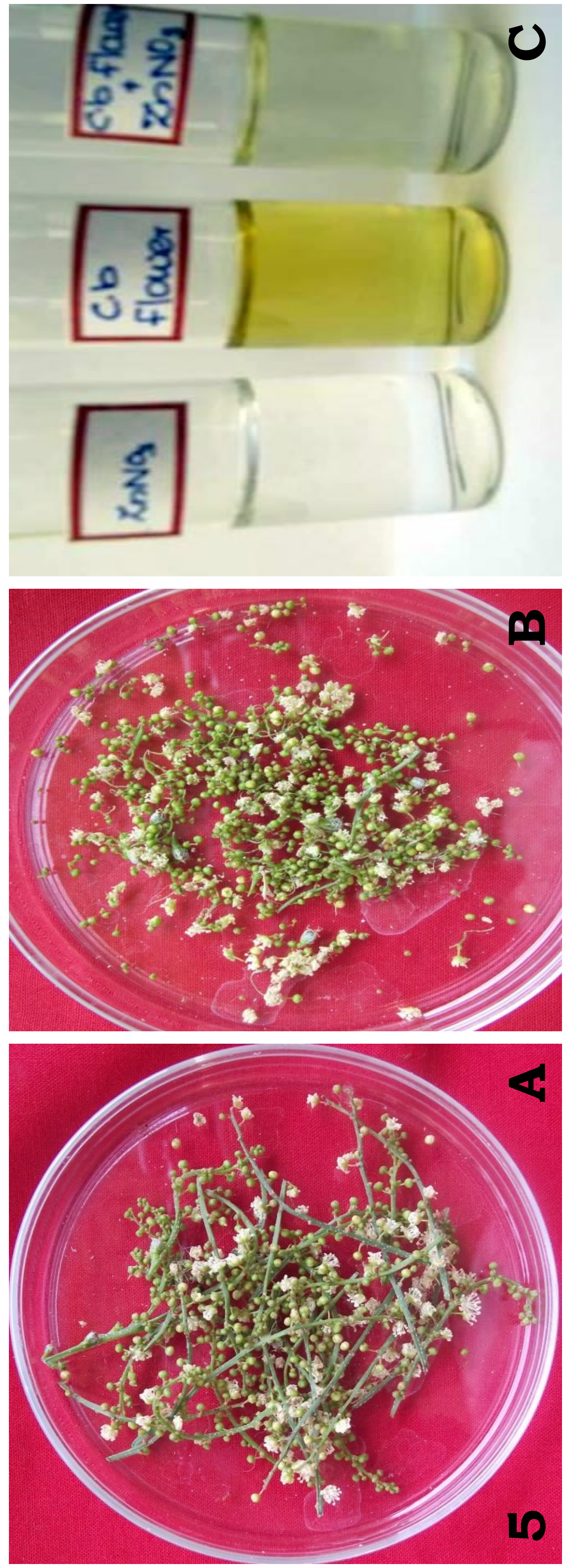


Fig. 4. A. Roots, **B**. Root cuttings and **C**. Reactions solutions.
Fig. 5. A. Flowers, **B**. Flower pieces and **C**. Reactions solutions.

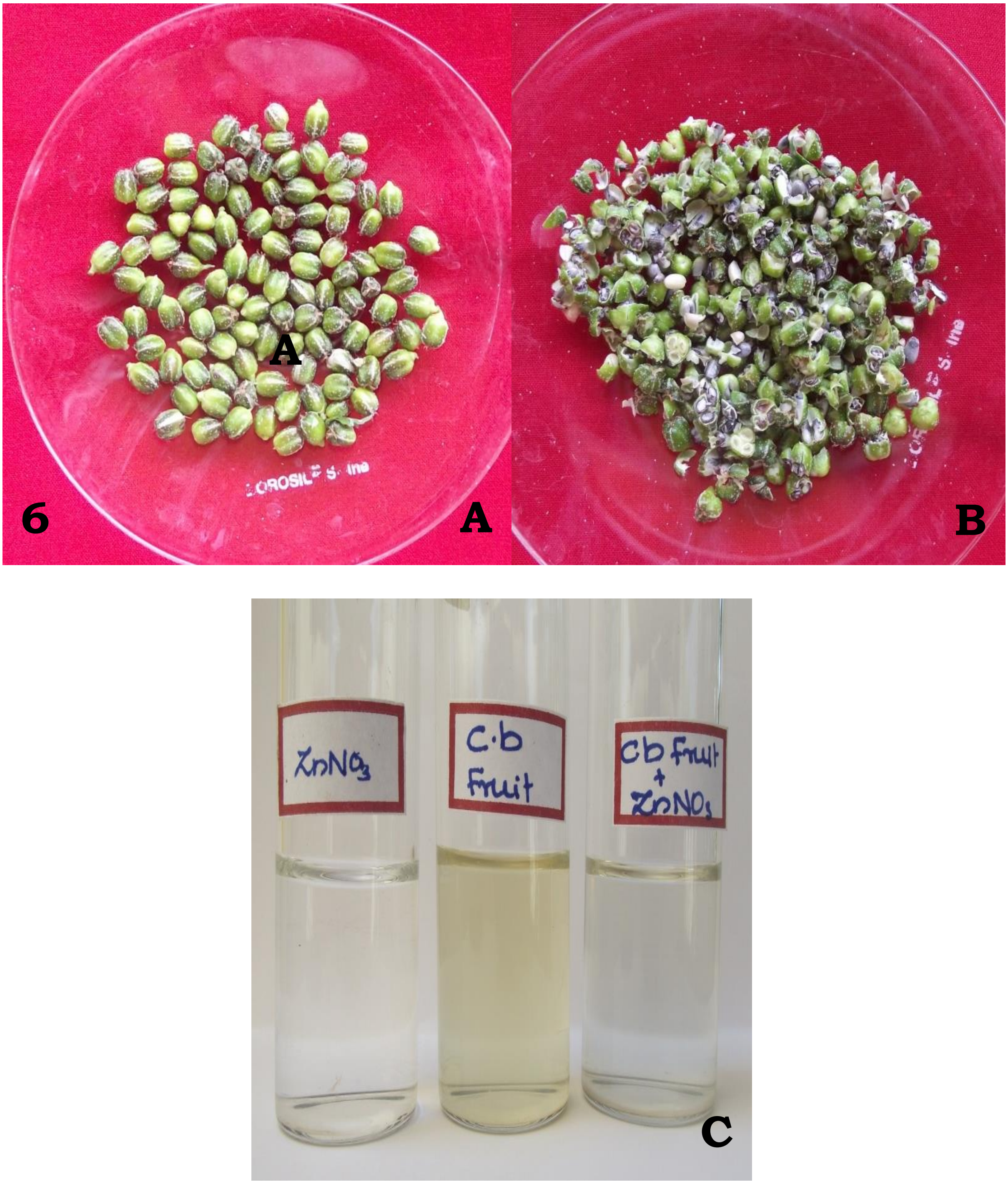


Fig. 6. A. Fruits, **B**. Fruit pieces and **C**. Reactions solutions.

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 taken, 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 1 mM Zinc nitrate solution and 1 ml of plant extracts as test solution (Figs. 2-6C).

2. 3. Characterization of ZnO NPs

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 analysis. 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. India). 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.

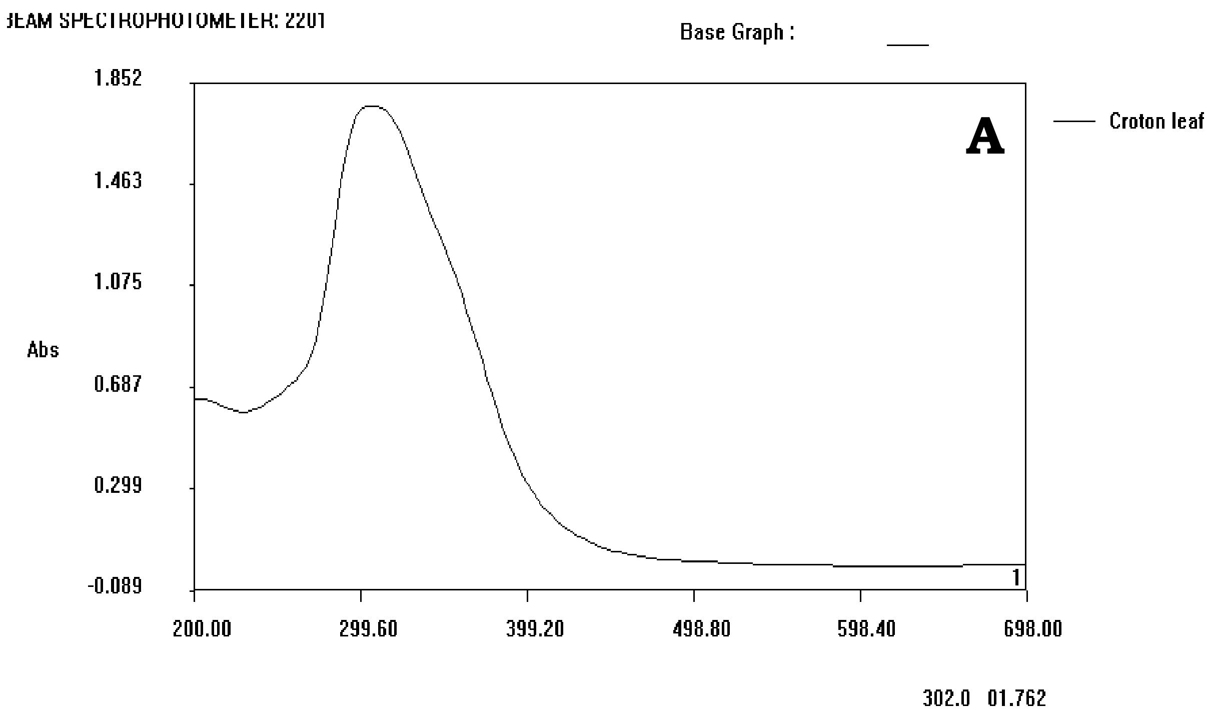
3. RESULTS AND DISCUSSION

The need of cost effective and non toxic method of nanoparticles synthesis could be convinced by the biogenic process of production of nanoparticles from bio-sources such as plants, fungi, bacteria, actinomycetes, algae etc. The biosynthesized nanoparticles proved thier potential application in several fields. Eco-friendly approaches, low cost of synthesis and non toxicity nature have attracted researchers in this field. In our earlier reports, biogenic synthesis of zinc oxide nanoparticles from aqueous plant extracts of *Passiflora foetida*, *Hybanthus enneaspermus* [9, 29] and *Ficus benghalensis* [30] have been demonstrated.

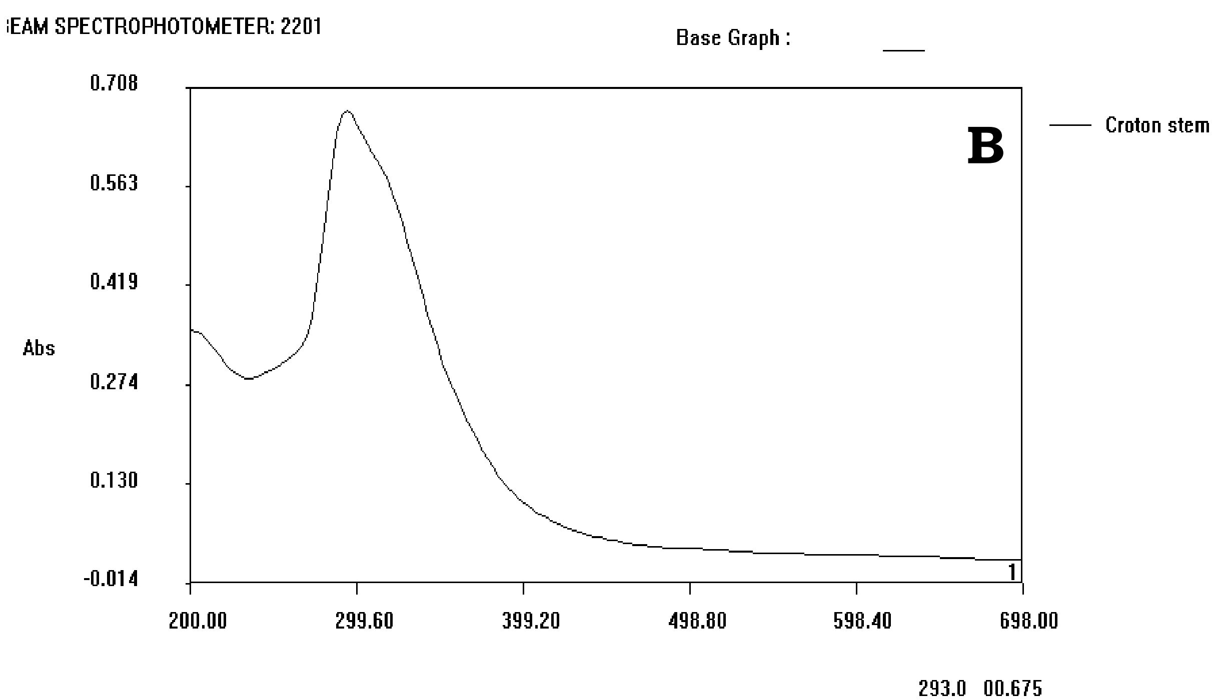
Here we report the synthesis of zinc oxide nanoparticles (ZnO NPs) in aqueous medium using *C. bonplandianum* leaf, stem, root, flower and fruit extracts as reducing and stabilizing agents. On treating zinc nitrate solution with the plant extracts, rapid reduction of zinc ions is reported leading to the formation of highly stable zinc oxide nanoparticles in the reaction solution. 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. UV-Visible spectrophotometric analysis of the reactions mixtures indicated the presence of zinc oxide nanoparticles with different plant extracts between 290-305 nm.

After treating the extracts of *C. boplandianum* with zinc nitrate solution, the color change of the reaction mixture was visually observed in cell free extracts of leaf, stem and flower. The reduction of zinc nitrate ions into zinc nanoparticles during exposure to the plant extract is followed by color change from colorless to pale yellow. Initially the color of cell free extract of root and fruit with metallic precursor was unchangeable, and turned into lemon yellow when heated for 20 minutes (Fig. 2C-6C). The time taken for color change was varied with the different plant extracts. The secondary metabolites and coenzymes present in the living tissues of plants could catalyze the specific reactions. This bio-reduction strategy can be alternate to photochemical reduction techniques [31]. Excitation of surface plasmon vibrations turns the color, when the formation of zinc oxide nanoparticles occurs. The time taken for the reaction mixture to change color was varied with plant extracts [32].

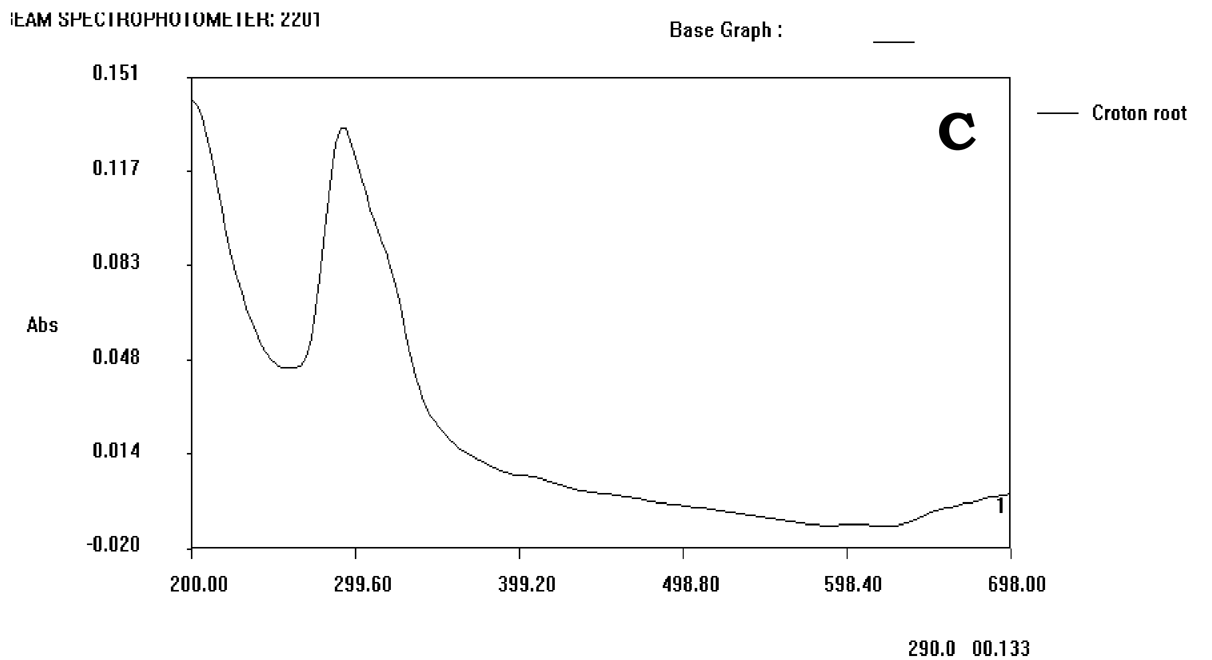
The aqueous reaction mixture of root showed strong and broad peak at 290 nm, stem and fruit peak located at 293 nm, leaf at 302 nm and flower peak observed at 305 nm (Fig. 7A-E, Table 1).

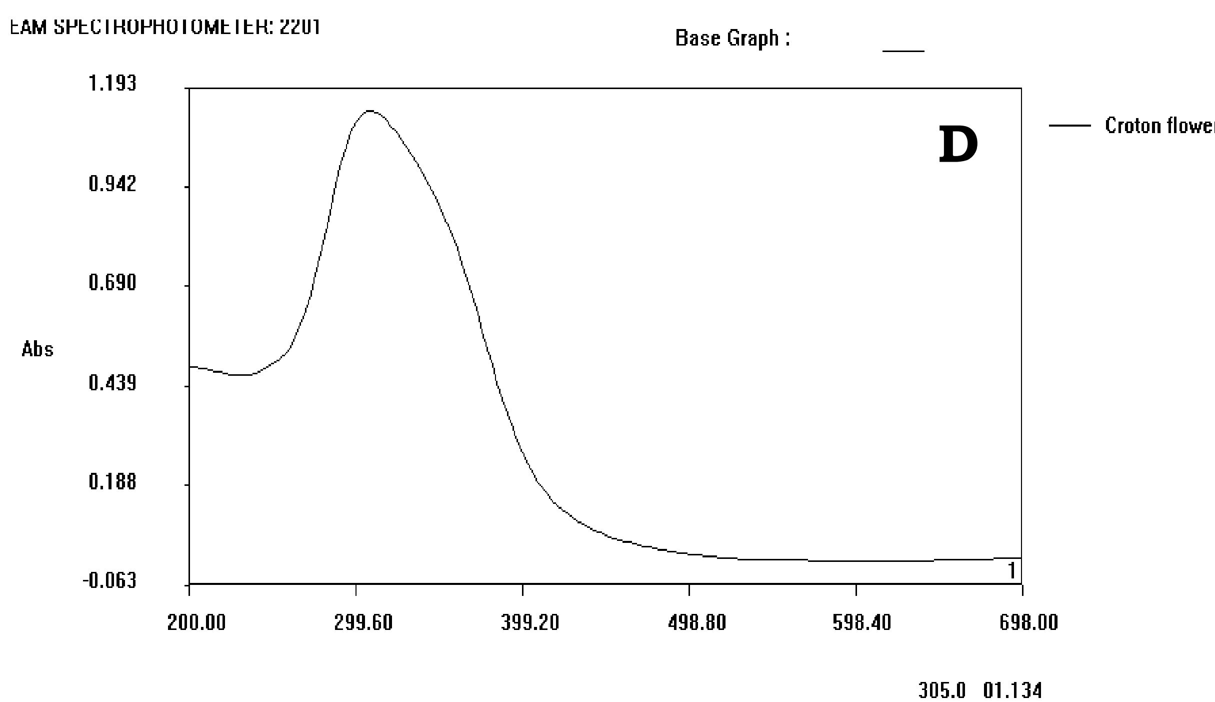


BEAM SPECTROPHOTOMETER: 2201
Base Graph :
1.852
1.463
1.075
Abs
0.687
0.299
-0.089
A
Croton leaf
1
200.00
299.60
399.20
498.80
598.40
698.00
302.0 01.762



BEAM SPECTROPHOTOMETER: 2201
Base Graph :
0.708
0.563
0.419
Abs
0.274
0.130
-0.014
B
Croton stem
1
200.00
299.60
399.20
498.80
598.40
698.00
293.0 00.675


EAM SPECTROPHOTOMETER: 2201
Base Graph :
Croton root
C
Abs
0.151
0.117
0.083
0.048
0.014
-0.020
200.00
299.60
399.20
498.80
598.40
698.00
1
290.0 00.133


EAM SPECTROPHOTOMETER: 2201
Base Graph :
Croton flower
D
Abs
1.193
0.942
0.690
0.439
0.188
-0.063
200.00
299.60
399.20
498.80
598.40
698.00
1
305.0 01.134

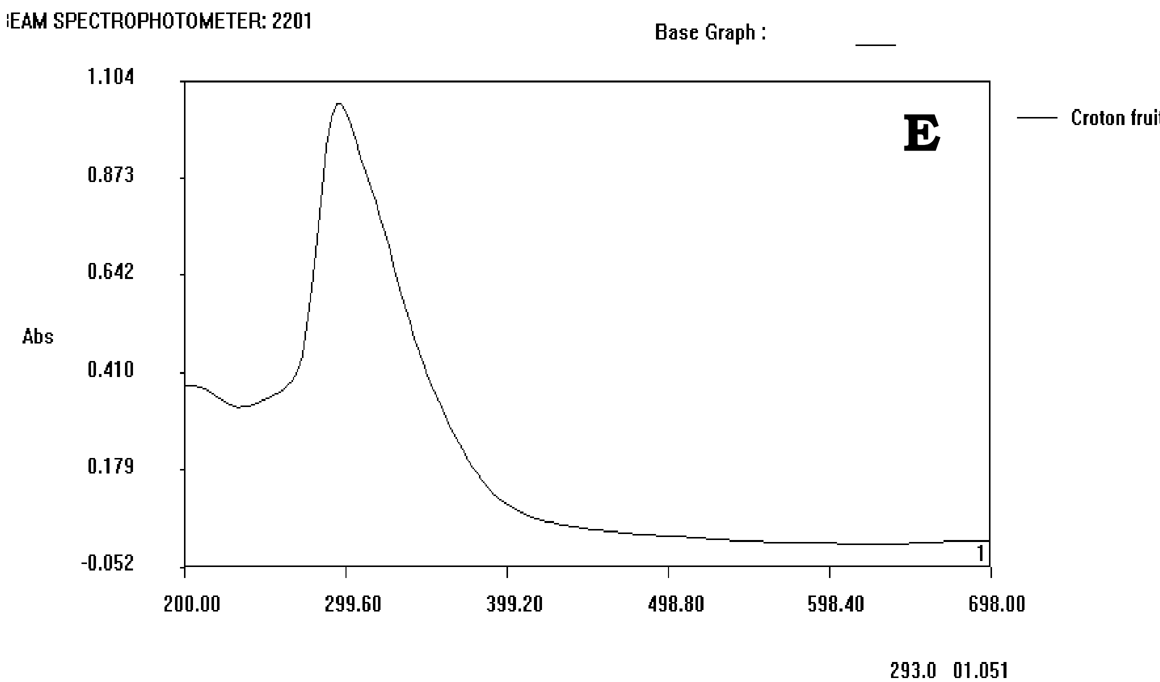


Fig. 7. A. Spectral absorbance peak of reaction mixture of leaf extract, **B.** Stem extract, **7C.** Root extract, **D.** Flower extract, **E.** Fruit extract.

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

Sl. No.	*C. bonplandianum* Reaction mixtures	UV-Vis absorption spectrum (nm)
1.	Leaf extracts	302
2.	Stem extracts	293
3.	Root extracts	290
4.	Flower extracts	305
5.	Fruit extracts	293

The biogenic reduction of zinc ion to base zinc oxide is easily scaled up, quite rapid, readily conducted at room temperature and pressure. The biosynthesis of zinc oxide nanoparticles using *C. bonplandianum* plant extracts is environmentally benign. The water soluble secondary metabolites and coenzymes present in the various parts of this plant were act as reducing agents. The biomolecules present in the crude plant extracts reduce metal ions and formation of nanoparticles in a single step biogenesis [33].

4. CONCLUSIONS

The biosynthesis of zinc oxide nanoparticles using plant parts of *C. bonplandianum* is eco-friendly, non-toxic, and cost effective. The zinc oxide nanoparticles were synthesized within few hours at room temperature. The phytochemicals present in the plant extracts worked as reducing and capping agents. The synthesized nanoparticles may be further analyzed to be used in the preparation of nano medicines and agricultural products.

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