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Biosynthesis of ZnO Based Nanoparticles Using *Calotropis Gigantea* Leaf Extract: Characterization and Its Evaluation of Antibacterial Activity

A. Ubaithulla Baig[1*], R. Vadamalar[1], N. Jabena Begum[2], R. Mohan[3], T.A. Vijayan[3], K. Srinivasan[4], S. Fairose[5]
[1]PG & Research Department of Physics, Muthurangam Government Arts College (Autonomous), Vellore -632 002.
[2]Department of Physics, DKM College for Women (Autonomous), Vellore-632 001.
[3]PG & Research Department of Physics, Sree Sevugan Annamalai College, Devakkottai -630 303.
[4]PG & Research Department of Physics, Sree Sevugan Annamalai College, Devakkottai -630 303.
[5]PG & Research Department of Physics, Urumu Dhanalakshmi College, Trichy-620 019.

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Corresponding Author Email: baig.ubaidulla@gmail.com

Abstract
The concept of green chemistry and engineering has provided a new platform for the environmentally propitious synthesis, non-hazardous to the environment and human health. Now-a-days synthesis of nano-materials with plant extracts have been a source of brainwave in designing commercial products for promising applications like biosensors, photo catalysis, antimicrobial and antioxidant technologies, etc. In the present study, we reported for the synthesis of *Calotropis gigantea* (*C. gigantea*) leaf extract capped ZnO based nanoparticles (NPs) by a simple soft chemical route via green approach. The resultant products were thoroughly analyzed using different analytical techniques such as UV-Vis NIR spectrophotometer, photoluminescence spectrometer, Fourier Transform Infrared spectrophotometer and XRD technique. The observed properties were compared with uncapped ZnO based NPs counterparts and reported. The XRD patterns reveal that the synthesized NPs crystallized with (101) growth direction. The optical studies indicate that the *C. gigantea* capped ZnO NPs show higher band gap when compared to the uncapped NPs. In PL spectra, both uncapped and *C. gigantea* leaf extract capped ZnO NPs showed two peaks at 395 nm and 426 nm corresponding to band edge and interstitial impurities emission, respectively. The synthesized NPs are evaluated using its antimicrobial efficacy for three different human pathogenic organisms such as *Escherichia Coli, Klebsiella pneumonia* and *Staphylococcus aureus*. It is shown that the *C. gigantea* leaf extract capped ZnO based NPs exhibit better antibacterial activity against human pathogens when compared to uncapped one. These capped ZnO NPs exhibit enhanced antibacterial efficacy towards *Escherichia Coli,* among the other tested pathogens.

Keywords
Green synthesis, ZnO nanoparticles, Calotropis gigantean, antibacterial activity, soft chemical route

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1. INTRODUCTION

Recently, the development of biosynthetic and environmental friendly technology for synthesis of nanomaterials, gaining attention among the research community. The chemical synthesis methods lead to adsorbance of certain toxic chemical species on the surface which causes adverse effects in health care applications. Hence, biological method using plant extracts is one of the valuable alternatives to chemical synthesis [1]. The plants are having the capability to adsorb, hyper-accumulate and degrade certain inorganic metallic ions from their surroundings [2, 3]. It is noteworthy to mention here that the phytochemicals of plant tissues capable of significantly reducing environmental contamination [4].

Zinc oxide (ZnO) is considered to be a technologically prodigious material having wide range of applications in the field of sensors, antimicrobial agents, photo-catalyst, etc., [5-9] due to its wide band gap (3.37 eV), large exciton binding energy (60 meV), high refractive index, high thermal conductivity, etc., [7-9]. In recent decades, ZnO nanoparticles (NPs) have fascinated more research interest and a lot of work has been done on its synthesis using various plant extracts [10]. In the present study, leaf extract of *Calotropis gigantea (C. gigantea)* plant was used as a capping agent for the synthesis of ZnO based nanoparticles.

The *C. gigantea* commonly called crown flower or giant milk weed used in traditional folk medicine for the treatment of bronchitis, dyspepsia, paralysis, swellings and intermittent fever [11]. Moreover, it exhibits antifungal, antiviral, antibacterial, anticarcinogenic and anticandidal activities. [12-20]. That's why, the study of *C. gigantean* plant has been the subject of interest among various research teams to use as a capping agent in nanoparticles synthesis.

Keeping the aforesaid points in mind, in the present study it is planned to synthesis*C. gigantea* leaf extract capped doped (Ni & Mg) ZnO nanoparticles and to compare their structural, optical, surface morphological and antibacterial properties with the uncapped ZnO NPs counterparts.

2. MATERIALS AND METHODS

2.1. Preparation of the leaf extract

Fresh leaves were collected from *Calotropis Gigantea* plants. The leaves were washed several times with water to remove the dust particles and then sun dried to remove the residual moisture. 50 g of washed dried fine cut leaves are added in 250 mL glass beaker along with 100 mL of sterile distilled water. The mixture was then boiled for 60 minutes until the colour of the aqueous solution changes from watery to light yellow. Then the extract was cooled to room temperature and filtered using filter paper. The filtered extract was stored in tied container in order to be used for further experiments.

2.2. Preparation of Calotropis Gigantea leaf extract capped ZnO based nanoparticles

C. gigantea leaf extract capped ZnO based (ZnO:Ni:Mg) nanoparticles (CDZO NPs) were synthesized using a facile and cost-effective soft chemical method. Zinc acetate dihydrate [Zn $(CH_3COO)_2.2H_2O$] (0.1M) was used as host precursor. Magnesium chloride hexahydrate ($MgCl_2.6H_2O$) and Nickel (II) sulfate hexahydrate ($NiSO_4.6H_2O$) were used as dopant precursors for Mg and Ni, respectively. The doping level was kept as 3 at. % for Mg and 3 at. % for Ni in the starting solution [21, 22]. The measured amount of host and dopant precursor salts were dissolved in 100 ml of solvent containing 80 ml of de-ionized water and 20 ml of prepared *C. gigantea* leaf extract. Required amount of NaOH solution was added with the precursor solution to maintain the pH value at 8. The resultant mixture was heated to 85˚C and magnetically stirred for 2 hrs. After the stirring process, it was kept undisturbed for 1 hr at room temperature. The precipitate obtained was separated carefully by filtration and washed several times with a mixture of water and ethanol kept in the ratio of 3:1. Finally the product was calcined at 550˚C for 3 hrs in a muffle furnace. The collected powder was mashed so as to get a finer nature for characterization. The uncapped ZnO based (ZnO:Ni:Mg) nanoparticles (DZO NPs) were also prepared using the same procedure, without adding the leaf extract in the starting solution, in order to compare its properties with the capped NPs counterparts. The schematic diagram for the synthesis of *C. gigantea* leaf extract capped ZnO based nanoparticles using simple soft chemical route is shown in Fig. 1.

2.3. Characterization of biosynthesized nanoparticles

The crystal structure of the bio-synthesized ZnO nanoparticles was obtained using an X-ray diffractometer (PANalytical-PW 340/60 X' pert PRO) with Cu-K_α radiation (λ= 0.15406 nm). Optical absorbance spectra were recorded using a Perkin-Elmer UV-vis-NIR spectrophotometer (Lambda 35 model). Photoluminescence (PL) spectra were recorded using a spectro-flurometer (JobinYvon_FLUROLOG-FL3-11) with xenon lamp (450 W) as the excitation source (λ=325 nm). Fourier transform infrared (FTIR) spectra were recorded using a Perkin Elmer RX-I FTIR spectrophotometer.

2.4. Evaluation of antibacterial activity of synthesized nanoparticles

The antibacterial activity of *C. gigantea capped* ZnO based NPs was tested against Escherichia coli *(E. coli)*, Staphylococcus aureus *(S. aureus)* and Klebsiella pneumonia (*K. pneumonia*) bacteria using Zone of inhibition (agar well diffusion) method. Nutrient agar medium was sterilized in an autoclave at 121 ºC for 15 minutes and then loaded into petri-dishes and allowed to solidify in a laminar air flow chamber. After solidification, fresh bacterial culture was spread over the plate using spread plate technique. Four wells (for control, standard and 2 samples) each of 5 mm in diameter were made in the agar plates with the help of sterile cork borer. The wells inoculated with 100 µg/mL of stock solution of the product were incubated at 37 ºC for 24 h. After incubation, the plates were observed for the formation of clear inhibition zone around the well and the diameter of zone of inhibition was measured in order to analyze the antibacterial efficacy of synthesized samples.

3. RESULTS AND DISCUSSION

3.1. Structural properties

Fig. 1 depicts the XRD patterns of DZO and CDZO NPs. The obtained diffraction patterns reveal the hexagonal crystal structure of ZnO (JCPDS card no: 36-1451). The peak at (101) showed the highest relative intensity indicating that the synthesized ZnO NPs have a preferential orientation along (101) direction. Eventhough, both the samples exhibit reflection at same positions, the capped DZO NPs exhibit relatively lesser intensity of reflection and broad peak due to the incorporation of phyto-chemicals present in the extract of *C. gigantea* leaf. This relatively broader peak also designated the fine crystallite size of the NPs. The crystallite size was calculated using the Debye's-Scherer formula [23]. It is 32 nm and 26 nm for uncapped DZO and capped DZO NPs, respectively. In both the cases, the observed smaller size of NPs may be due to substitution of Ni and Mg on Zn sites. In general, the alkaline metal ions decrease the nucleation and subsequent growth rate of ZnO NPs [24]. In the case of CDZO NPs, the capping of *C. gigantea* leaf extract is considered additionally as a good source to tune the particle size of ZnO. It is also observed that there is no phase corresponding to Ni and Mg implying that there is a simple substitution of Ni and Mg on the Zn sites, rather than forming separate phase.

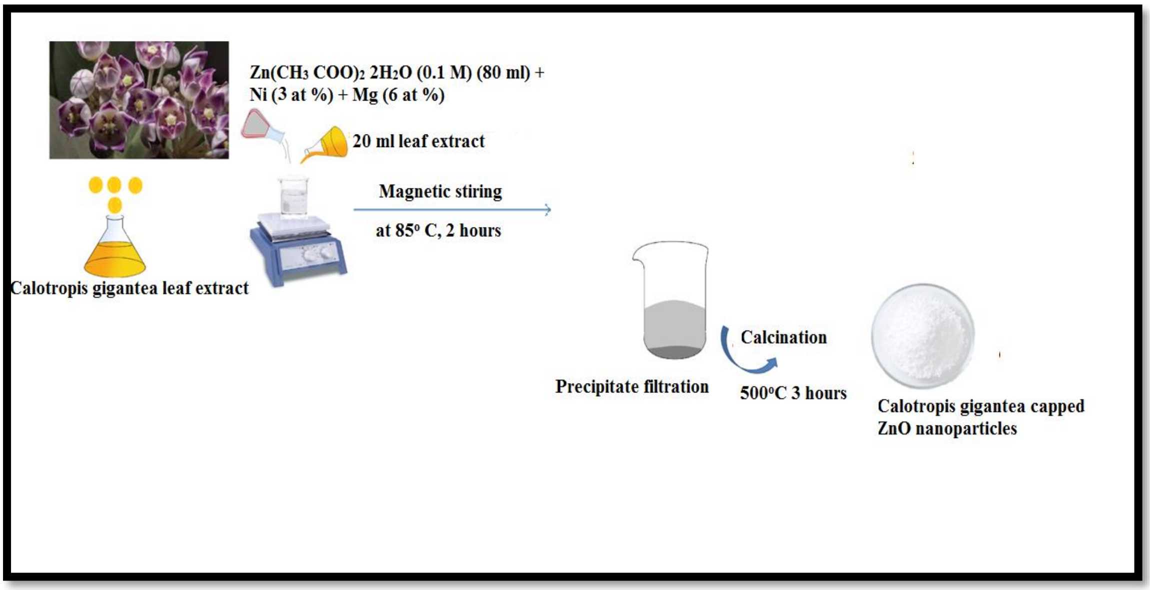

Fig 1: The schematic diagram of *C. gigantea* leaf extract capped ZnO based nanoparticles using simple soft chemical route

3.2. Absorbance studies

The absorbance spectra of DZO and CDZO NPs are shown in Fig. 2. The sharp exciton absorption positioned at 385-390 nm confirms the synthesized NPs as ZnO. The sharpness of the peak suggested that the formed ZnO NPs are stable. Generallly, the non-uniform growth with different [25] crystallite shapes give broad absorption [26]. Hence, in the present study, the obtained sharp absorption peaks with absence of other peaks, indicating the uniform and controlled growth of the synthesized ZnO NPs. The band gap values were determined using Tauc's plot (not shown) for the DZO and *C. gigantea* capped DZO NPs and the values are found to be 3.20 eV and 3.24 eV, respectively. Compared to the DZO, *C. gigantea* capped DZO NPs show higher band gap because of its lesser particle size (as discussed in structural studies). It is well known that the decrease in particle size increases the energy separation between the ground and excited states resulting in blue shift in absorption [27].

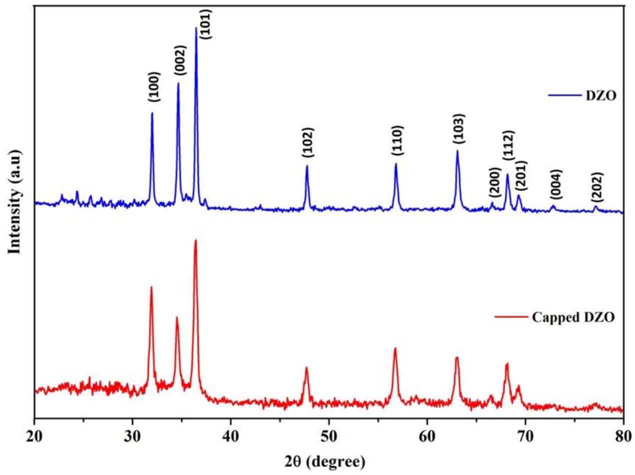

Fig 2: XRD patterns of DZO and CDZO nanoparticles

3.3. Photoluminescence studies

Fig. 3 shows the room temperature PL spectra of uncapped and C. gigantea capped DZO NPs. As shown in the fig. 3, near band edge (NBE) emission at ≈ 395 nm with certain surface defect level emissions were observed for both the samples. But, the NBE emission band was quenched and broadens in the case of CDZO NPs. These PL results suggested the development of extensive surface defects caused by the plant-derived chemicals and slight heterogeneity in particle size [28]. Eventhough, the NBE emission for CDZO is found to be supressed; the absence of appreciable emissions at longer wavelength (above 440 nm) indicated the absence of defect related emission. From this, it is inferred that the surface modification by functional groups of the leaf extract stabilizes the synthesized nanoparticles.

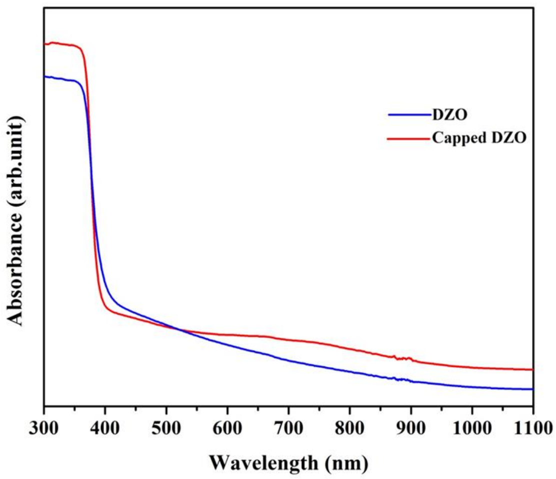

Fig 3: Absorbance spectra of DZO and CDZO nanoparticles

3.4. FTIR studies

The FTIR spectra recorded for DZO NPs and CDZO NPs are shown in Fig. 4. From this figure, one can clearly analyze the mode of bonding between the capped material (C. gigantea) with ZnO NPs. The band located near 621 to 692 cm^{-1} can be represented to the Zn-O stretching. The absorption peak at 952 cm^{-1} of CDZO NPs is assigned to the functional group like primary alcohol in the C. gigantea leaf extract [6]. In CDZO NPs, the observed strong absorption at 3500 cm^{-1}, 1100 cm^{-1} and 1580 cm^{-1} related to O-H alcohol, C-O stretching and C=O saturated aldehyde, respectively, corresponding to the functional groups present in the leaf extract. Ayodhya et al. [27] noticed the broadening of absorption while using the C. gigantea leaf extract as capping agent for CdS NPs and they interpreted that it might be due to the participation of phyto-chemicals like hydroxyl and carbonyl functional groups of polyphenols and terpenoids in a biochemical reaction. This discussion supplements the broadening of absorption spectrum of CDZO NPs noticed in the present study.

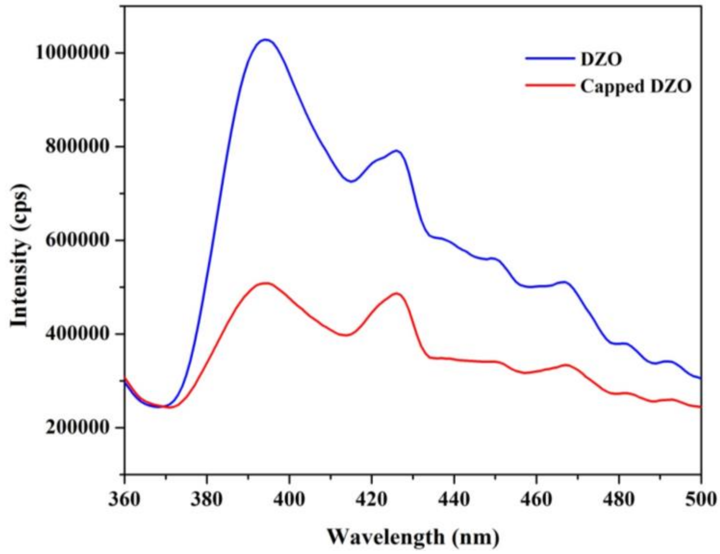

Fig 4: Photoluminescence spectra of DZO and Capped DZO nanoparticles

3.5 Antibacterial activity

The antibacterial activity of uncapped DZO NPs and C. gigantea capped DZO (CDZO) NPs was assessed by zone of inhibition method against three different bacterial strains. Fig. 5 shows the antibacterial activity of DZO NPs and CDZO NPs against *E.coli, S.aureus, K.pneumonia*. For the better understanding of the readers, the measured diameter of the zone of inhibition of synthesized NPs is compared as 3-D bar diagram (Fig. 6). From this bar diagram, it is obvious that C. gigantea leaf extract shows significant antibacterial activity on all the three strains.

Generally, the nanoparticle-bacterial interaction depends not only the type of physiological state of bacteria, it also depends on the physio-chemical properties of the NPs [29]. Here, the ZnO NPs can directly interact with the microbial cells and destroy them [30]. In addition, ZnO NPs can create reactive oxygen species (ROS) which in turn causes cell destruction. In the present study, the reason for exhibiting greater zone of inhibition in the case of CDZO NPs is not only the aforesaid causes and also due to the presence of phytochemicals of leaf extract which acts as stabilizing agents [4, 31-33]. In addition, the biological compounds present in the extract create more number of free radicals which in turn damage the cell membrane and consequently enhances the antibacterial activity [34, 35]. Moreover, the decreased particle size (reported in structural studies) is also a possible reason for the enhanced antibacterial efficacy [36] in the case of capped ZnO based NPs.

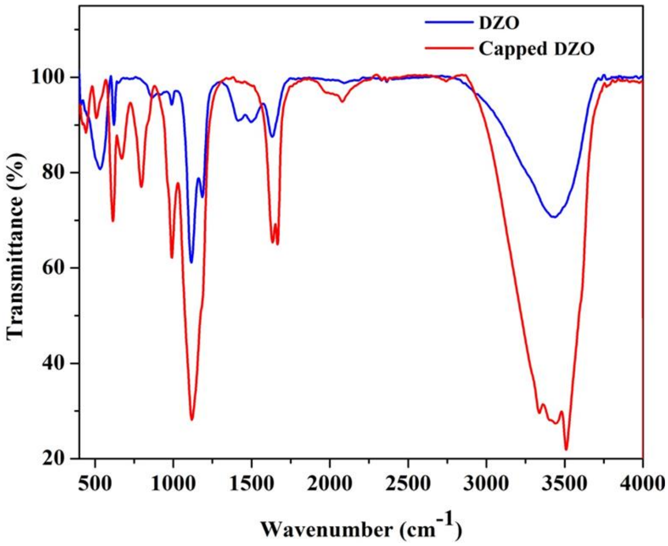

Fig 5: FTIR spectra of DZO and CDZO nanoparticles

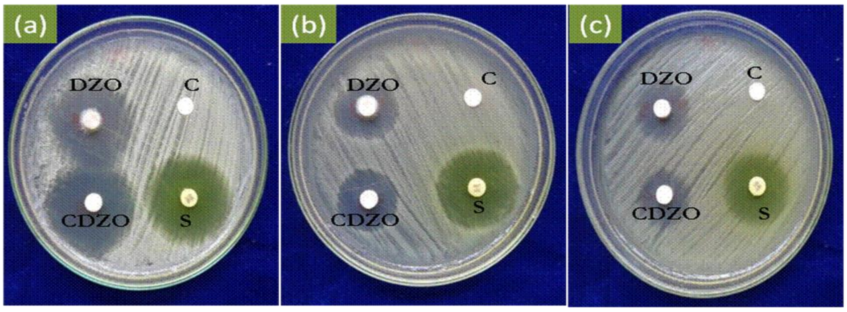

Fig 6: Antibacterial activity of DZO and CDZO nanoparticles against a) *E.coli* b) *S.aureus and* c) *K.pneumonia*

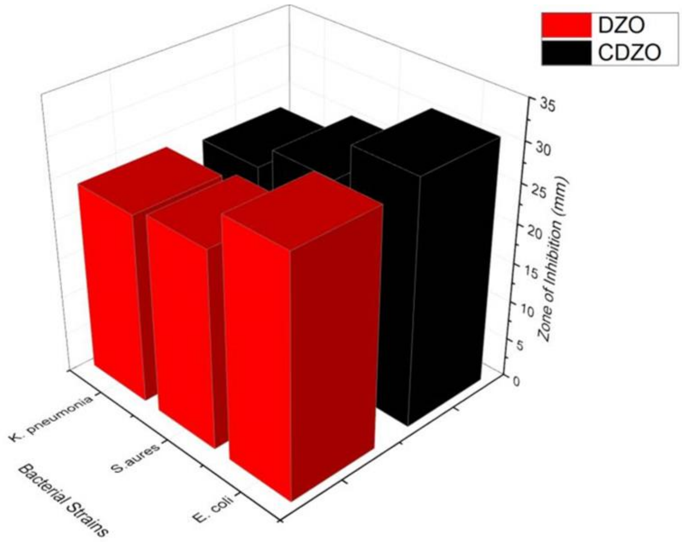

Fig 7: 3-D bar diagram comparing the antibacterial efficiency of DZO and CDZO nanoparticles against three different cultures

4. CONCLUSION

The ZnO based NPs were synthesized by green approach using C. gigantea leaf extract as a capping agent. XRD studies confirm the crystal structure of ZnO and also reveal particle size tuning nature of C. gigantea leaf extract. The absorbance spectra evidenced the uniform and controlled growth of biosynthesized NPs. The FTIR studies indicated the mode of bonding between the phyto-chemicals of C. gigantea leaf extract and ZnO NPs. The suppression of defects in the case of capped DZO NPs was confirmed using PL studies. From the observed antibacterial activity of CDZO NPs against *E.coli, S.aureus, K.pneumonia,* it can be inferred that these biosynthesized NPs can be used as efficient antibacterial agents in medical applications.

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