



## Phyto-assisted synthesis of zinc oxide nanoparticles using *Cassia alata* and its antibacterial activity against *Escherichia coli*



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### ABSTRACT

Zinc oxide, an established inorganic metal oxide in nanoparticles form exhibits tremendous anti-bacterial activity. The present study focuses on determining the anti-bacterial activity of green synthesized zinc oxide nanoparticles (ZnO NPs). Results clearly validate the effective synthesis of spherical shaped nanoparticles with average size range of 60–80 nm. SEM and EDAX data buttresses the results obtained by XRD pattern in terms of size and purity. ZnO NPs exhibited dose-dependent anti-bacterial activity against *Escherichia coli* (*E. coli*) and the IC<sub>50</sub> value was calculated to be around 20 µg/mL. Growth kinetics study was conducted in the presence of nanoparticles which demonstrated the bacteriostatic effect of ZnO NPs. The study recommends the potential use of ZnO NPs in industries like food, pharmaceutical, agriculture, cosmetic industries for its anti-bacterial activity.

### 1. Introduction

Nanobiotechnology is an interdisciplinary field which involves the application of nanotechnology for biotechnological purposes [1]. This emerging field has vast applications in food, cosmetics, and pharmaceutical industries. Use of Nanotechnology in these fields can be conducted in several forms like nanoparticles, nanocomposites. Use of nanoparticles in science and medicinal field has been an important area of research in the past decade [2]. Nanoparticles have unique beneficial electrical, optical, magnetic, anti-inflammatory, wound-healing, antimicrobial properties, which enables them to target the disease most effectively unlike their bulk form [3].

ZnO NP is a type of metal oxide nanoparticles with a band gap of 3.3 eV and excitation binding energy of 60 meV at room temperature [4]. It has gained considerable attention due to its unique catalytic, antibacterial, antifungal, photochemical, UV-filtering, anti-inflammatory properties owing to its large surface area to volume ratio [5]. Synthesis of metal and metal oxide nanoparticles through green routes and environmentally benign method has been focused by researchers to avoid the use of toxic chemicals making the process and the final product eco-friendly and non-toxic in nature. Use of plants as a green source for nanoparticles synthesis is most commonly practiced as plants are the hub of a wide range of phytochemicals which can act as

reducing and stabilizing agent for nanoparticles synthesis. Different phytochemicals like flavonoids, ketones, aldehydes, amine, amide, organic acid are responsible for the reduction of Zinc ion to nanoparticles form and different proteins aid in the stabilization of synthesized nanoparticles [6].

Plant-mediated ZnO NP using an extract of *Ixora coccinea* [7], *Carica papaya* [8], *Pongamia pinnata* [9], *Solanum nigrum* [10], *Azadirachta indica* (L.) [11], *P. caerulea* L. [12], *Cassia fistula* [13] have been recently reported. Metal oxide nanoparticles are considered as promising nano antibiotics as they show remarkable antibacterial and antifungal activity [14]. ZnO NP is a multifunctional inorganic nanoparticles which is utilized in antiseptic creams, cosmetic products, calamine lotion, shampoos and surgical tapes because of the potential anti-microbial property that it possesses [15].

*Cassia alata* is an annual medicinal herb with laxative leaves having excellent antibacterial and antifungal properties [16]. The important phytochemicals already reported in *Cassia alata* responsible for the immediate reduction of Zinc ion into Zinc oxide nanoparticles are flavonoids, tannins, terpenoids, steroids, cardiac glycosides [17]. Flavone components of the plant are generally responsible for its antimicrobial activity [18].

The present work focusses on the biosynthesis of ZnO NP using medicinal herb *Cassia alata*. Phytochemicals serve as a reducing and

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stabilization agent through reduction and oxidation reaction. Mechanistic pathway involved in ZnO NP has been proposed based on previous studies. Anti-bacterial activity of ZnO NP has been studied and its mechanistic approach has been evidenced.

## 2. Materials and methods

### 2.1. Synthesis of zinc oxide nanoparticles

Plant extract was prepared by mixing 10 g of *Cassia alata* fresh leaves with 100 mL of Milli-Q water. The mixture was boiled for 30 min at 60 °C. The mixture was cooled down to room temperature and double filtered using Whatman filter paper no. 1. Filtered mixture was used as the extract and used further for the synthesis of ZnO NP nanoparticles.

0.01 M Zinc acetate was used as precursor and 5 mL of leaf extract was used as a reducing agent. The mixture was stirred on a magnetic plate at 80 °C for 20 min. pH of reaction mixture was adjusted to 12 using 2 M NaOH. The mixture was stirred for 2 h and UV–Visible readings were recorded, wherein a strong peak was observed at the end of 3 h. The mixture was centrifuged at 5000 rpm for 10 min. The sedimented pellet was double washed with Milli-Q water and dried overnight in a hot air oven operating at 80 °C. The white colored powder was obtained and used for characterization.

### 2.2. Characterization of ZnO NP synthesis

Synthesis of nanoparticles was confirmed through UV–Visible readings after the visual color change. Shape and size were determined using a Scanning electron microscope (SEM). Fourier Transform Infrared Spectroscopy (FTIR) analysis was used to identify functional groups of plant extract responsible for the reduction of zinc ion to zinc nanoparticles form. X-ray diffraction analysis (XRD) was used to identify the lattice plane, crystal structure of ZnO NP. Elemental composition of synthesized nanoparticles was calculated using energy dispersive X-ray analysis (EDAX). Atomic Force Microscopy (AFM) measurements were done to estimate the surface roughness and topography.

### 2.3. Bactericidal activity of ZnO NP

Bactericidal effect of synthesized ZnO NP was calculated using the method by Luria-Bertini (LB) agar plates were prepared and ZnO NP was added in different concentrations (10–100 µg/mL). *E. coli* ( $10^5$  CFU) was inoculated on the plates and incubated at 37 °C for a day. Colonies were counted using a colony counter after the incubation period. Control plates did not contain any ZnO NP. The experiment was conducted in triplicate.

The bacterial growth curve was constructed by inoculating bacteria ( $10^5$  CFU) to 100 mL of LB broth medium containing ZnO NP at a concentration of 50 µg/mL. Optical density (O.D) value was recorded at 600 nm every half an hour for growth curve generation. Control was maintained without the addition of nanoparticles.

## 3. Results and discussion

### 3.1. ZnO NP synthesis and its characterization

Nanoparticles synthesis was predicted through visual observation of solution color change from colorless to off-white. Synthesis of nanoparticles was later confirmed using UV–Visible spectroscopy. UV–Visible readings were recorded in the wavelength range of 250–500 nm. Maximum absorption was found in the range of 300–350 nm and a strong peak was found at 320 nm (Fig. 1). This was in agreement with previous studies where ZnO NP was synthesized using *Coptidis rhizoma* [19].

Broad peaks observed in the XRD pattern depict enhanced

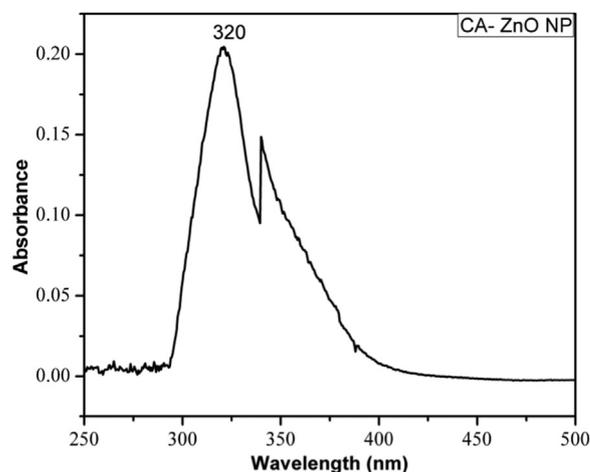


Fig. 1. UV–Visible absorption spectra of ZnO NP synthesized using *Cassia alata*.

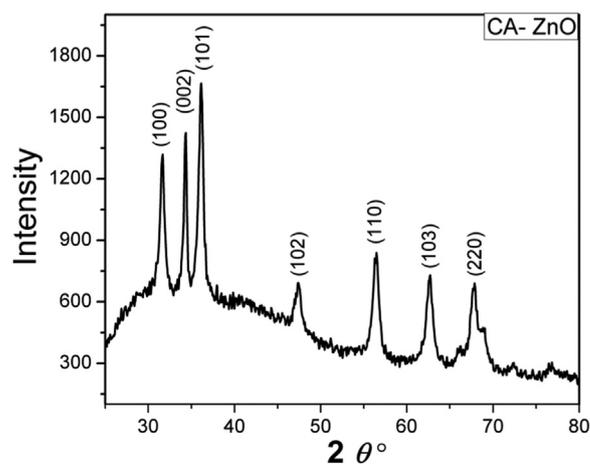


Fig. 2. XRD pattern of ZnO NPs.

crystallinity as an effect of annealing temperature [20]. The crystallite size of the nanoparticles was determined using the Scherrer equation which was found to be in the range of 60–80 nm (Fig. 2).

Nanoparticles size obtained using XRD pattern was further validated using SEM micrograph which demonstrated similar results. SEM spectra revealed the spherical shape of nanoparticles with aggregation. EDAX spectra clearly revealed the presence of separate peaks for zinc and oxygen which established the purity of the synthesized nanoparticles (Fig. 3).

FTIR spectroscopic analysis revealed the phytochemicals groups that acted as a capping agent for the synthesis and stabilization of nanoparticles like phenolic groups, amines, ether, carboxylic acid and a hydroxyl group. Strong band obtained at 3377.36, 1587.42, 1419.61, 1004.91, 476.42  $\text{cm}^{-1}$  corresponds to O-H stretching vibration of phenol group, C=C stretching of alkyl ethers, N = O bend of secondary amines, a C-O stretch of ethers, carboxylic acid and Zn-O-Zn stretching vibrations respectively (Fig. 4).

ZnO NPs at a concentration of 50 µg/mL demonstrated potential inhibition of bacterial growth after 10 h of incubation. Growth resumed in *E. coli* after 20 h of incubation suggesting the coagulation and removal of nanoparticles from LB broth medium after interaction with intracellular components of the bacteria (Fig. 5). Positively charged ZnO NPs interact with the negatively charged bacterial membrane and thus, leads to membrane integrity loss and intracellular protein leakage resulting in growth delay [21–24].

Nanoparticles solutions were prepared at different concentrations using serial dilution and inoculated with bacteria. Formed colony

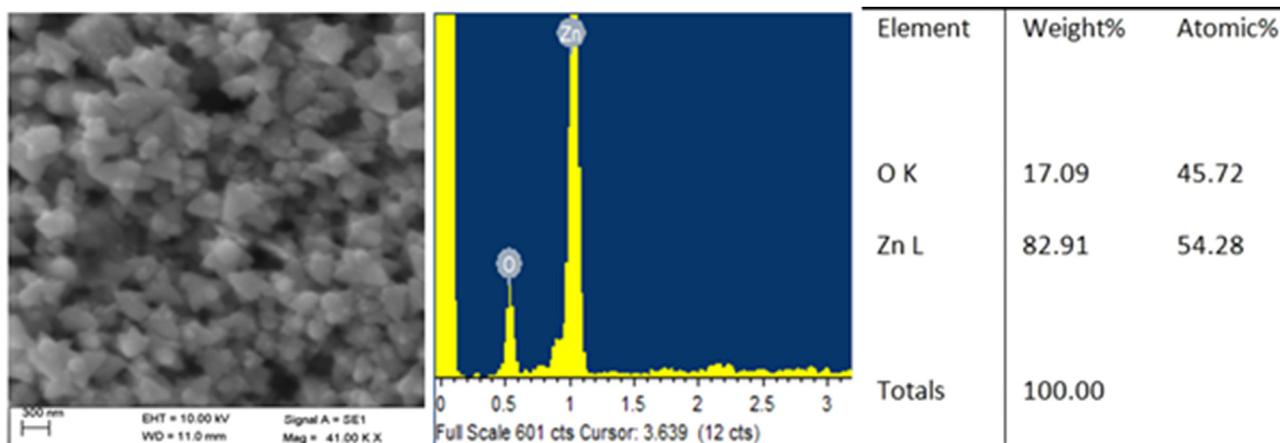


Fig. 3. SEM and EDAX spectra of ZnO NPs.

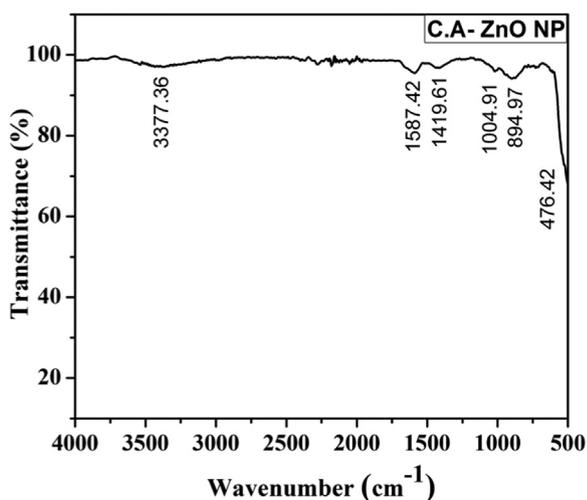


Fig. 4. FT-IR Spectra of ZnO NP.

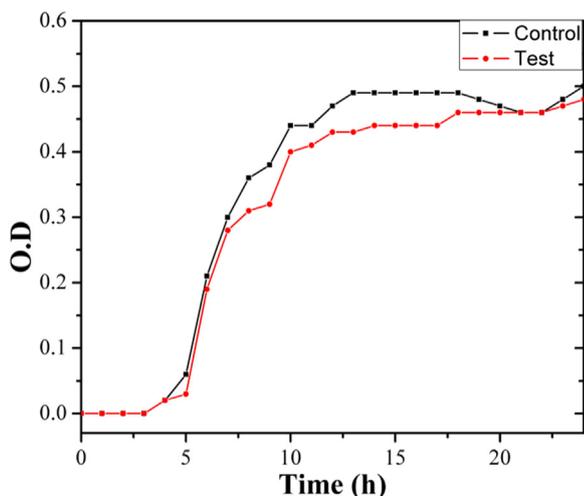


Fig. 5. Growth curve of control (native *E. coli*) and test (ZnO NP treated *E. coli*). Change OD 600 nm.

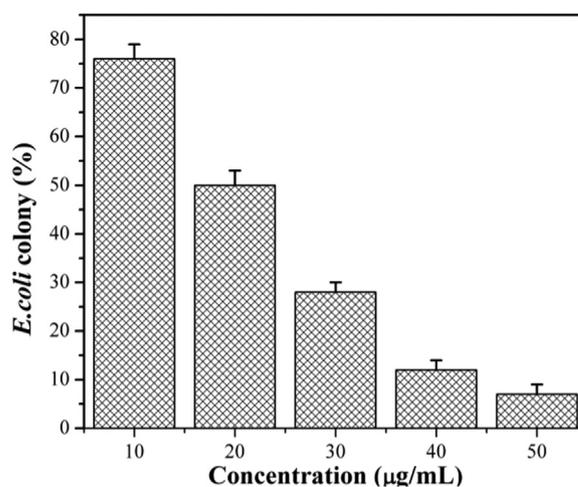


Fig. 6. *E. coli* colony percentage as a function of ZnO NP treatment in different concentrations.

number decreased in a concentration-dependent manner (Fig. 6). The IC<sub>50</sub> value was found to be 20 µg/mL.

#### 4. Conclusion

Green synthesis of zinc oxide nanoparticles using fresh leaf extract of *Cassia alata* provides an eco-friendly, rapid, simple, non-toxic and efficient means for the synthesis of nanoparticles. Synthesized ZnO NPs were further characterized using UV-Vis absorption spectroscopy, XRD, SEM and EDAX, FTIR spectroscopy. UV-Visible spectra suggested the presence of a strong peak at 320 nm confirming the nanoparticles synthesis. SEM micrograph demonstrated the presence of spherical nanoparticles with a size range of 60–80 nm. The crystallite size was further confirmed using XRD pattern analysis. EDAX and XRD evidenced the pure nature of formed nanocrystals. FTIR spectra depicted the peak at 476.42 cm<sup>-1</sup> corresponding to stretching vibration of Zn-O-Zn which is the characteristic peak for zinc oxide nanoparticles synthesis. This study also revealed the excellent anti-bacterial potential of ZnO NPs by growth curve analysis and bactericidal activity testing of nanoparticles. The ZnO NPs treatment with bacteria demonstrated the IC<sub>50</sub> value to be 20 µg/mL. The ZnO NPs have potential in pharmaceuticals, cosmeceuticals and agricultural industries.

#### Appendix A. Supporting information

Supplementary data associated with this article can be found in the

online version at doi:10.1016/j.bbrep.2019.01.002.

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