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Research article

Green Synthesis of ZnO Nanoparticles Using *Delonix elata* & *Gynura cusimbua* Leaf Extracts and Evaluating Their *In-vitro* Antibacterial Properties

Hiranmayi Devadula Sai Rama*, Anzila Yumlembam, and Suba Gnana Abraham Manuel

Mount Carmel College, Autonomous, #58 palace road, Bengaluru, Karnataka, India

*Corresponding author: hiranmayispare@gmail.com

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ABSTRACT

With an increasing focus on the application of nanoparticles in the field of research, this study aims to evaluate the *in-vitro* antibacterial properties of chemically and green synthesized zinc oxide nanoparticles (ZnO NPs) from two plant sources *Delonix elata* and *Gynura cusimbua*. The bioactive compounds present in the leaf extracts were utilized to stabilize the nanoparticles. UV-visible spectrophotometry (UV-vis), x-ray diffraction (XRD), and scanning electron microscopy (SEM) were used to elucidate the optical and structural properties of the synthesized ZnO NPs. The in-vitro antibacterial potential of ZnO NPs was evaluated by agar disc diffusion assay against two pathogenic bacterial strains: *Bacillus cereus,* a gram-positive animal pathogen and *Pseudomonas syringae,* a gram-negative plant pathogen, making it a well-rounded approach. The UV-visible spectrum was measured in the 250 to 400 nm range, and the crystallite structure was analyzed via XRD. Energy-dispersive x-ray spectroscopy (SEM-EDS) analysis confirmed the nanostructures with partial nanoflakes and aggregates for all three samples of the synthesized ZnO NPs. The *D. elata* ZnO NPs showed relatively greater antimicrobial activity against both bacterial strains than that of *G. cusimbua* ZnO NPs. Consequently, plant-based NPs may be an excellent strategy for developing versatile and environmentally friendly biomedical products. They have an added advantage due to their pre-existing medicinal properties, which make them a more suitable alternative to the broadly used chemically synthesized nanoparticles.

Keywords: *Delonix elata, Gynura cusimbua,* Zinc oxide nanoparticles, Antibacterial activity, *Bacillus cereus, Pseudomonas syringae.*

INTRODUCTION

Green chemistry, using environmentally friendly agents like plant parts, phytoplankton, yeasts, bacteria, and fungi to synthesize nanoparticles, has piqued researchers' interest in recent years[1]. Due to its ease of use, diversity and presence of a wide range of phytoconstituents, plants extract a natural nanoparticle stabilizing and reducing agent.[2] Lately, antibiotic resistance has become one of the greatest pressing problems in the medical field. Nanoparticles and other derivates have been combined with many biomolecules to improve antimicrobial responses.[3] Various NPs synthesized using plant extracts have shown promising antibacterial effects towards a diversity of pathogens.[4-6]

Delonix elata (D. elata) has been widely reported to have strong anti-inflammatory, antinociceptive and antimicrobial properties.[7-9] Its root decoction is used to treat abdominal pain and leaf extracts are also used for scorpion bite treatment.[10] The presence of quercetin and rutin enables *D. elata* extracts to create a stable structure with nanoparticles.[11] *Gynura cusimbua (G. cusimbua)* is a succulent herb that has previously been studied for various anti-ulcer effects.[12] Numerous studies have been conducted on the potential health benefits of *Gynura* species.[13,14]

The current study aims to broaden the applications of ZnO NPs (Zinc oxide nanoparticles) by chemically and green synthesizing them with leaf extracts of *D. elata* and *G. cusimbua* and characterizing their reduction by UV-visible spectrophotometry, X-ray diffraction (XRD) & scanning electron microscopy/energy-dispersive x-ray spectrography (SEM/EDS). By also evaluating their antibacterial property against a plant and a human pathogen, it is intended to provide an integrated preliminary outcome for further studies on ZnO NPs.

MATERIALS AND METHODS

Preparation of leaf extract

The collected leaves were washed, dried at room temperature and powdered. In 10 g of leaf powder was mixed in 100 mL of deionized water and heated at 60°C on a magnetic stirrer, with continuous stirring, for 3 hours. The leaf extract was cooled to room temperature and filtered using Whatmann No. 1 filter paper. The final filtrate was stored at 4°C.[11,15]

Chemical Synthesis of ZnO NPs

An aqueous solution of zinc nitrate (Zn (NO3)2·6H2O) (50 mL, 0.1 M) and an aqueous solution of sodium hydroxide (NaOH) (50 mL, 0.8 M) were prepared by constant stirring for one hour. NaOH was added dropwise to the zinc nitrate solution through the vessel's walls

under slow and constant stirring for 45 minutes and an additional stirring of 2 hours under sealed conditions. The stirred solution was allowed to settle overnight, and the supernatant solution was carefully discarded. The precipitate from the residual solution was extracted by centrifugation (10 minutes). The precipitated ZnO NPs were washed three times with deionized water followed by one ethanol wash and then dried at 60°C overnight. The nanoparticles were stored at 4°C. [16] The particles are referred to as Chemical ZnO NPs.

Green synthesis of ZnO NPs using the leaf extracts

D. elata and *G. Cusimbua* leaf extracts were used for green synthesis. About 50 mL of each leaf extract was mixed with 0.2M 50 ml Zinc acetate (Zn (CH3COO)2) solution under constant stirring at room temperature. 0.2M NaOH was then added dropwise to the solution until it reached pH 12. The mixture was stirred for another 1 hour and centrifuged for 30 min at 8000 rpm and 4°C. The precipitate was dried at 80°C, overnight. The powder obtained was stored at 4°C.[17] The particles are referred to as *D. elata* ZnO NPs & *G. cusimbua* ZnO NPs.

UV-visible spectroscopy of the synthesized ZnO NPs

The reduction capacity of leaf extracts in the synthesized ZnO NPs was examined by UV–visible spectroscopy.[18] The nanoparticles were suspended in distilled water and measured for their λmax absorbance in the wavelength range of 250 to 400 nm.[19]

XRD Analysis of the synthesized ZnO NPs

The crystallite size and formation of the synthesized ZnO NP powder were examined using X-ray diffraction by Rigaku Smartlab XRD at CeNSE, IISc, Bengaluru. Cu Kβ radiation was used to record the X-ray diffraction pattern of the synthesized ZnO NPs in the scan range of $2\theta = 10^{\circ} - 90^{\circ}$[16]. Using Scherrer's formula, the crystallite size of the ZnO NPs synthesized was determined as follows.[18]

$$D = \frac{k\,\lambda}{\beta \cos\theta}$$

where D is the crystallite size; k is the particle shape factor, 0.9; β is the half-width of (hkl) reflection; λ is the X-ray wavelength (1.5406); ø = 2ø/2, is Bragg's angle corresponding to (hkl) reflection [20].

Scanning Electron Microscopy (SEM) and Energy-dispersive X-ray spectroscopy (EDS) analysis of the synthesized ZnONPs

Scanning electron microscopy was coupled with energy-dispersive X-ray spectroscopy (SEM-EDS) to observe and analyze the surface topology and elemental composition of the synthesized ZnO NPs. To improve contrast in SEM pictures, the nanoparticle powder was placed on a carbon strip and coated with gold.[21-23]

Disc diffusion assay of the synthesized ZnO NPs to assess antibacterial activity.

Cultures of human pathogenic gram-positive bacteria, *Bacillus cereus (MTCC 1307)* and plant pathogenic Gram-negative bacteria, *Pseudomonas syringae (MTCC 1604),* were used for the assay. Pure isolates of the test organism were inoculated and incubated in Mueller-Hinton broth for 16 hours at 37°C[9] and swabbed on sterile Mueller-Hinton agar plates. Sterile Whatman No.1 filter paper discs of 6 mm diameter were saturated with different concentrations (100, 200, and 300 mg/mL) of the synthesized NPs dissolved in distilled water. The discs were placed at equidistance over the culture plates. Distilled water as negative control and tetracycline (100 µg/mL) as positive control were employed. The inoculated plates were incubated at 37°C for 24 hours. The inhibition zones around the discs were measured in mm.[24,25]

Statistical Analysis

One-way ANOVA test and Tukey's honestly significant difference (HSD) test were conducted to determine the significant differences in mean inhibition zones and were performed using Python (version 3.12.0) with `stats models` library [44]. A significance level of $p < 0.05$ was employed.

RESULTS AND DISCUSSION

Characterization of ZnO NPs

UV-Visible spectroscopy

Chemical ZnO NPs and *G. cusimbua* ZnO NPs showed maximum absorbance at 250 nm and *D. elata* NPs at 266 nm (Fig. 1), which correlate to the similar findings of chemically synthesized ZnO NPs (258 nm),[16] and in *S. grandiflora* NPs (~250 nm).[26] Previously green synthesized ZnO NPs have also shown peaks ranging between 300 and 400 nm.[18,27-29]

XRD Analysis

XRD analysis of the synthesized ZnO NPs revealed peaks (Fig 2) that were matched with the ICDD card number 01-079-0207, and the shape of the nanoparticles was found to be hexagonal in nature, similar to the previous studies done on ZnO NPs.[27] Sharp peaks in Chemical ZnO NPs (Fig 2A) indicate the good crystallite of ZnO NPs [30]. The multiple peaks at different angles in *D. elata* & *G. cusimbua* ZnO NPs (Fig 2B & 2C) indicate the polycrystalline nature of ZnO NPs.[30] The average crystallite size *D. elata* ZnO NPs, *G. cusimbua* ZnO NPs and the Chemical ZnO NPs were calculated to be 76.273 nm, 77.164 nm, and 89.897 nm, respectively, which were found to be significantly larger than most findings.[16,27,31]

SEM-EDS analysis

Scanning electron microscopy revealed the spherical structure of the Chemical ZnO NPs with partial nanoflakes, partial macroflakes and aggregates, while *D. elata* and *G.cusimbua* ZnO NPs were observed to have nanoflakes along with aggregates (Fig. 3) similar to the reports of previously synthesized ZnO NPs.[26,28,32,33] The SEM images show that the NPs are within the 200 nm range (Fig. 3 A1–A3) and support the crystallite size findings measured in XRD analysis. ZnO NPs in the range of 50 to 500 nm have also been previously observed in other findings utilizing XRD pattern matching backed by SEM.[30,34]

The elemental details of the synthesized NPs and their composition were given by Energy dispersive spectrometry (EDS) (Table 1). The weight% of element Zn was observed to be greater than that of element O, similar to the reports in several studies. [27,35-37] The EDS spectrum shows that elements other than Zn and O were also present in the samples (Fig. 3, B1–B3). The presence of element C in the samples may be related to the carbon strip used

Table 1: EDS analysis of the synthesized ZnO NPs

Element	*Sample*					
	Chemical ZnO NP		***D. elata*ZnO NP**		***G.cusimbua*ZnO NP**	
	Weight %	Atomic %	Weight %	Atomic %	Weight %	Atomic %
Zn	42.12	13.76	61.10	26.67	33.96	9.90
O	34.93	46.61	32.22	57.46	33.73	40.17

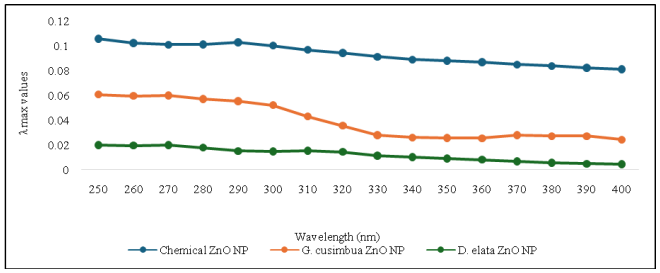

Fig. 1: UV-Visible absorption spectrum of the synthesized ZnO NPs

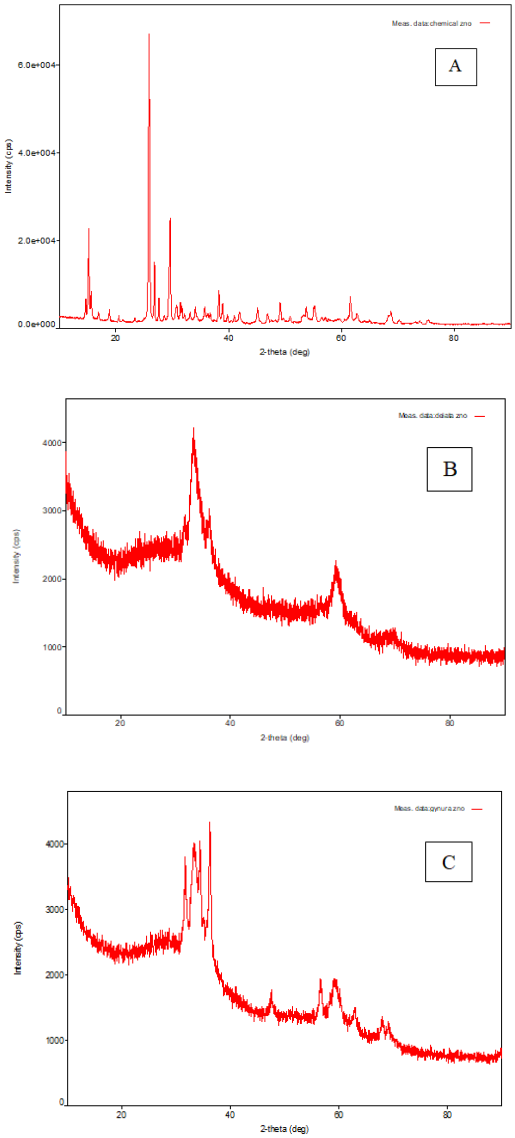

Fig. 2: X-ray diffraction spectrum of (A) Chemical ZnO NPs (B) D. elata ZnO NPs and (C) G. cusimbua ZnO NPs

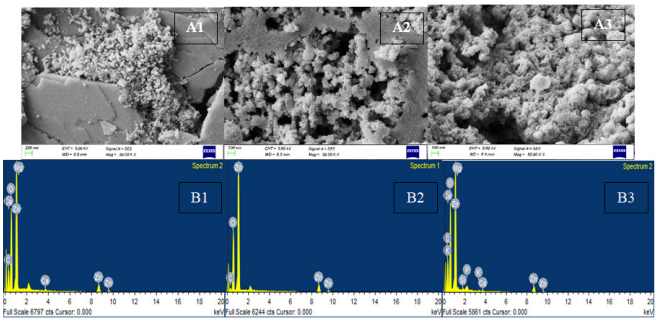

Fig. 3: SEM analysis of the synthesized ZnO NPs (A1) Chemical ZnO NPs, (A2) D. elata ZnO NPs, (A3) G.cusimbua ZnO NPs; EDS spectra - (B1) Chemical ZnO NPs, (B2) D.elata ZnO NPs, (B3) G.cusimbua ZnO NPs

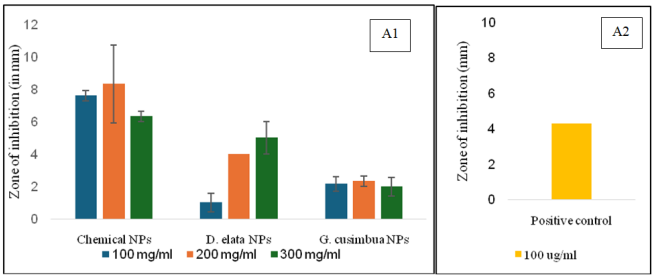

Fig. 4: Effect of varying concentrations of the (A1) synthesized ZnO NPs & (A2) Tetracycline against B. cereus growth.

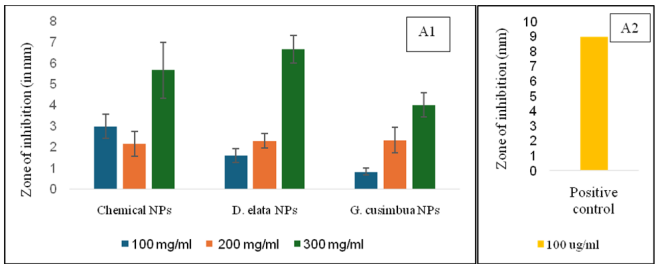

Fig. 5: Effect of varying concentrations of the (A1) synthesized ZnO NPs & (A2) Tetracycline against P. syringae growth.

to mount the samples, and the traces of element K in *G. cusimbua* ZnO NPs are due to the element's significant presence in the plant's chemical composition.[38]

Antibacterial activity - Agar Disc Diffusion Assay

The ANOVA results indicated that there were significant differences in the mean inhibition zones of *D. elata* ZnO NPs against *B. cereus* ($p = 0.013$) (Fig. 4) and *P. syringae* ($p = 0.0006$) (Fig. 5), while *G. cusimbua* ZnO NPs showed significant difference only against *P. syringae* ($p = 0.011$). Tukey's HSD test revealed that the inhibition zones for 300 mg/mL were significantly different from 100 mg/mL. This indicates that with the increase in concentration of NPs there is an increase in the zone of inhibition. ZnO NPs have antibacterial activity that is inversely related to their size and directly proportional to their concentration.[39] In line with this report, 300 mg/mL of *D. elata* ZnO NPs showed the highest inhibition against *P. syringae* with the smallest crystallite size. In previous studies, ZnO NPs with

a range of sizes (12–307 nm) were chosen, and their link between size and antibacterial activity was proven. They have minimal inhibitory effects as their size surpasses 100 nm.[40] The positively charged ZnO NPs are attracted to the negatively charged bacterial cell membrane, leading to membrane disruption and depolarization, which kills the bacteria.[41] Cell walls of Gram-positive bacteria have a thick peptidoglycan layer, while Gram-negative bacteria have a thin peptidoglycan layer with an outer lipid membrane.[41,42] The lower efficiency of *G. cusimbua* ZnO NPs against *B. cereus* could be attributed to gram-positive species' cell wall resistance.[43] These findings imply that using *D. elata* ZnO NPs against Gram-positive pathogens like *B. cereus* and gram-negative pathogens like *P. syringae* could be more effective than *G. cusimbua* ZnO NPs.

CONCLUSION

The ZnO NPs were successfully synthesized through chemical and green methods from *D. elata* and *G. cusimbua* leaf extracts. The hexagonal crystallite structure of the ZnO NPs synthesized was confirmed by XRD analysis, and their optical properties were verified by UV-visible spectrophotometry, with a peak at around 250 nm. The average crystallite size ranged from approximately 76 to 90 nm. The generated ZnO NPs' nanoflake surface, size, and elemental composition were disclosed by SEM-EDS analysis. Although all three synthesized ZnO NPs showed considerable inhibition against gram-positive bacteria, *B. cereus,* and gram-negative bacteria, *P. syringae*, among the green synthesized NPs, *D. elata* ZnO NPs showed better antibacterial activity than *G. cusimbua* ZnO NPs. These findings corroborate the utilization of these nanoparticles in the areas of nanomedicine, agriculture, and pharmaceuticals.

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