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Characterization and antimicrobial analysis of biogenically synthesized zinc oxide nanoparticles from *Datura stramonium* leaf extract

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Abstract

Green synthesis of metal oxide nanoparticles has emerged as an eco-friendly and sustainable alternative to conventional chemical and physical synthesis methods. In the present study, zinc oxide nanoparticles (ZnONPs) were synthesized using aqueous leaf extract of *Datura stramonium* as a reducing and stabilizing agent. The synthesized nanoparticles were characterized using UV-Visible spectroscopy, Fourier Transform Infrared Spectroscopy (FTIR), Scanning Electron Microscopy (SEM), Transmission Electron Microscopy (TEM), and X-Ray Diffraction (XRD). The antimicrobial activity of the synthesized ZnONPs was evaluated against selected bacterial pathogens using the agar well diffusion method. The UV-Visible spectrum confirmed nanoparticle formation with characteristic absorption in the UV region. FTIR analysis indicated the presence of phytochemicals such as alkaloids, flavonoids, phenolics, and terpenoids involved in reduction and stabilization. SEM and TEM studies revealed predominantly spherical nanoparticles with nanoscale dimensions. The synthesized ZnONPs exhibited significant antimicrobial activity against both Gram-positive and Gram-negative bacterial strains. The findings suggest that *Datura stramonium*-mediated ZnONPs possess promising antimicrobial potential and can be utilized in biomedical and pharmaceutical applications.

Keywords: Zinc oxide nanoparticles, *Datura stramonium*, Green synthesis, Antimicrobial activity, Characterization, Nanotechnology

Introduction

Nanotechnology is one of the rapidly expanding fields due to its extensive applications in medicine, catalysis, agriculture, biotechnology, and environmental sciences. Nanoparticles are particles having dimensions below 100 nm and exhibit unique physical, chemical, and biological properties due to their high surface area to volume ratio (Rai *et al.*, 2008) [14]. Metal oxide nanoparticles, particularly zinc oxide nanoparticles (ZnONPs), have attracted significant attention because of their remarkable antimicrobial, photocatalytic, and biocompatible properties.

Zinc oxide nanoparticles are considered promising inorganic *nanomaterials* due to their high chemical stability, broad radiation absorption spectrum, electrochemical coupling coefficient, and photostability (Agnieszka and Jesionowski, 2014) [1]. ZnONPs have been widely employed in cosmetics, food additives, pharmaceuticals, ceramics, textiles, and biomedical applications (Sirelkhatim *et al.*, 2015; Eixenberger *et al.*, 2017) [18, 6]. Their antimicrobial properties arise from the generation of reactive oxygen species (ROS), release of Zn²⁺ ions, and interaction with microbial cell membranes.

Conventional physical and chemical methods for nanoparticle synthesis often involve hazardous chemicals, high energy requirements, and toxic by-products. Green synthesis using plant extracts offers a sustainable and eco-friendly alternative by utilizing naturally occurring phytochemicals as reducing and stabilizing agents (Raveendran *et al.*, 2003) [16]. Plant-mediated synthesis is advantageous because plant extracts are readily available, safe to handle, economical, and rich in bioactive compounds.

Datura stramonium, commonly known as Jimson weed or Thorn apple, belongs to the family Solanaceae and contains diverse phytochemicals including alkaloids, flavonoids, tannins, terpenoids, phenolics, glycosides, and steroids (Muller and Arfmann, 1987; Evan and

Lampard, 1972) [8, 13]. These bioactive compounds play a crucial role in the reduction and stabilization of nanoparticles.

The present study focuses on the green synthesis, characterization, and antimicrobial evaluation of ZnO nanoparticles synthesized using *Datura stramonium* leaf extract. The work highlights the role of plant phytochemicals in nanoparticle synthesis and explores the biomedical significance of the synthesized nanoparticles.

2. Materials and Methods

2.1 Collection of Plant Material

Fresh leaves of *Datura stramonium* were collected from healthy plants and thoroughly washed with distilled water to remove dust and impurities. The leaves were shade dried and powdered using a sterile grinder.



Fig 1: Representing *Datura stramonium* plant.

2.2 Preparation of Plant Extract

Approximately 20 g of powdered leaf material was mixed with 100 mL distilled water and heated at 60 - 70°C for 30 minutes. The extract was filtered using Whatman No.1 filter paper and stored at 4°C for further use.

2.3 Green Synthesis of ZnO Nanoparticles

DZn ONPs (Zn ONPs synthesized using *Datura stramonium* leaf extract) were synthesized by addition of 10 ml aqueous DLE (*Datura stramonium* leaf extract) to 40 ml aqueous 1 mM zinc nitrate solution by constant stirring at 75°C for 40 minutes at neutral pH (Figure 2C). The synthesized DZn ONPs were purified by centrifugation at 17000 rpm for 20 minutes.

3. Characterization of ZnO Nanoparticles

3.1 UV-Visible Spectroscopy

UV-Visible spectroscopy was performed to confirm nanoparticle formation. The absorption spectrum of ZnO nanoparticles showed a characteristic absorption peak in the range of 350- 380 nm, indicating successful synthesis of ZnO nanoparticles.

3.2 Fourier Transform Infrared Spectroscopy (FTIR)

FTIR analysis was conducted to identify the functional groups involved in nanoparticle synthesis and stabilization. The spectrum revealed peaks corresponding to hydroxyl

groups, amines, phenolic compounds, and alkaloids present in the plant extract. The Zn-O stretching vibration confirmed the formation of ZnO nanoparticles.

3.3 Transmission Electron Microscopy (TEM)

TEM analysis confirmed the nanoscale size distribution of the synthesized ZnO nanoparticles.

3.4 X-Ray Diffraction (XRD)

XRD analysis confirmed the crystalline nature of the synthesized ZnO nanoparticles.

4. Antimicrobial Activity

4.1 Test Microorganisms

The antimicrobial activity of synthesized ZnO nanoparticles was evaluated against selected Gram-positive and Gram-negative bacterial strains including:

- *Staphylococcus aureus*
- *Bacillus subtilis*
- *Escherichia coli*
- *Pseudomonas aeruginosa*

4.2 Agar Well Diffusion Method

The antimicrobial activity was assessed using the agar well diffusion method. Nutrient agar plates were inoculated with bacterial cultures. Wells were prepared and loaded with different concentrations of ZnO nanoparticle suspension. Plates were incubated at 37°C for 24 hours.

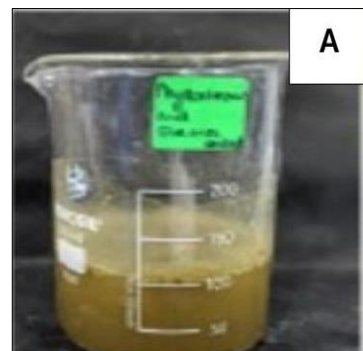
5. Results and Discussion

5.1 Preliminary Confirmation of Nanoparticle Formation

The formation of zinc oxide nanoparticles was initially confirmed by visual observation of color change during the synthesis process. Upon addition of *Datura stramonium* leaf extract to the zinc nitrate solution, the reaction mixture gradually changed from pale yellow to whitish cream color, indicating the reduction of zinc ions and formation of ZnO nanoparticles.

The color change occurs due to excitation of surface electrons and formation of nanosized zinc oxide particles. The phytochemicals present in the plant extract such as flavonoids, phenolics, alkaloids, and terpenoids acted as reducing and stabilizing agents during nanoparticle synthesis.

Similar observations were reported by Mohanpuria *et al.* (2008) [12] and Dhillon *et al.* (2012) [5], who described visible color change as a preliminary indication of successful biosynthesis of metal nanoparticles using plant extracts. The visual change therefore served as an initial confirmation for ZnO nanoparticle formation prior to spectroscopic and microscopic characterization.



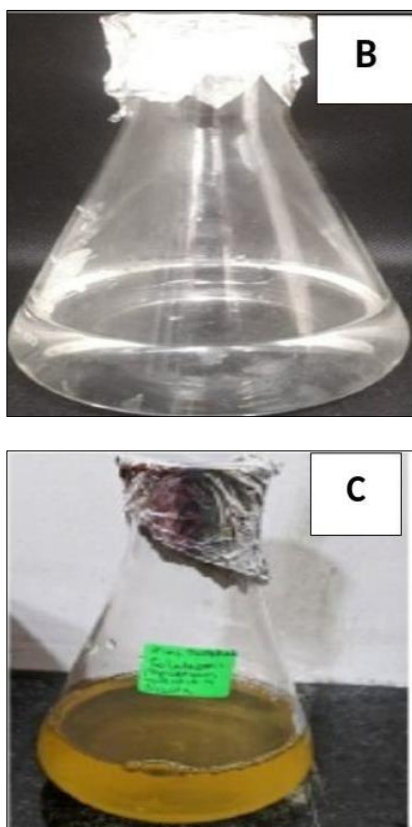


Fig 2: A) *Datura stramonium* leaf extract B) Zinc nitrate solution C) *Datura stramonium* ZnONPs.

5.2 UV-Visible Spectroscopic Analysis

The synthesis of zinc oxide nanoparticles (Zn ONPs) was initially confirmed by UV-Visible spectroscopic analysis. The absorption spectrum exhibited a characteristic peak in the range of 350-380 nm (Figure 3), which is typical for Zn O nanoparticles and confirms the successful formation of Zn ONPs from zinc ions. Similar absorption peaks have been reported by Song and Kim (2009) ^[19] and Sabir *et al.* (2014) ^[17]. Likewise, Medina *et al.* (2018) ^[11] and Kumar *et al.* (2021) observed blue-shifted absorption bands attributed to quantum confinement effects in nanosized Zn O particles.

The observed absorption band is associated with the surface plasmon resonance (SPR) of ZnO nanoparticles. The sharp and intense peak indicated the formation of stable and uniformly dispersed nanoparticles. Minor shifts in the absorption peak may result from variations in particle size, morphology, agglomeration state, and dielectric properties of the surrounding medium.

The reduction and stabilization of ZnO nanoparticles were facilitated by phytochemicals present in the *Datura stramonium* leaf extract, including alkaloids, flavonoids, phenolics, tannins, and terpenoids, which acted as reducing and capping agents.

The UV-Visible analysis further suggested the nanoscale nature of the synthesized particles, as bulk ZnO generally exhibits absorption at longer wavelengths. The observed blue shift in the absorption region may be attributed to quantum confinement effects. Overall, the results confirmed the successful green synthesis of ZnO nanoparticles using *Datura stramonium* leaf extract.

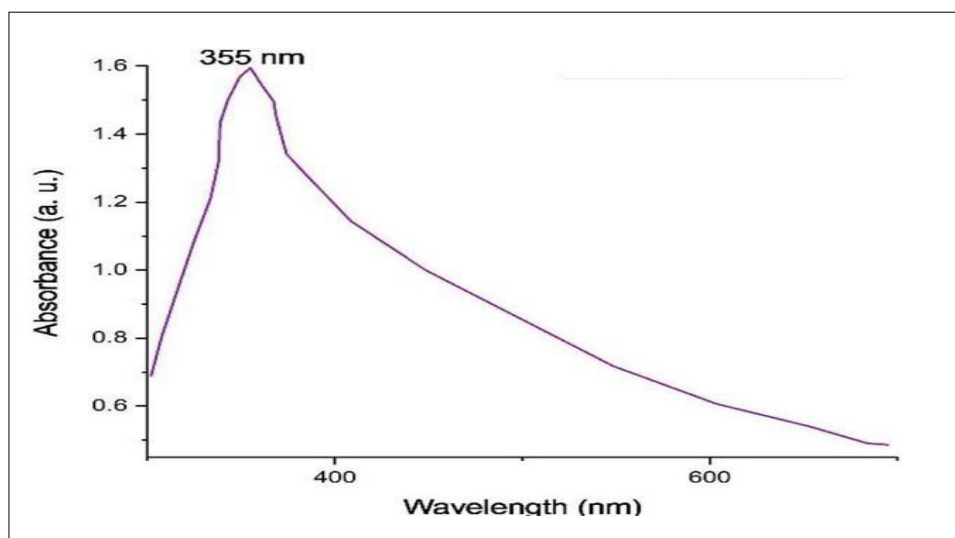


Fig 3: UV-Vis Spectroscopic analysis of ZnONPs synthesized using *Datura stramonium* leaf extract.

5.2 FTIR Analysis

Fourier Transform Infrared Spectroscopy (FTIR) analysis was performed to identify the functional groups present in *Datura stramonium* leaf extract responsible for the reduction, capping, and stabilization of zinc oxide nanoparticles (ZnONPs). The FTIR spectrum exhibited several characteristic peaks corresponding to various phytochemical constituents (Table 1 & Table 2).

A broad absorption peak around 3200-3400 cm^{-1} was attributed to O-H stretching vibrations of alcohols and phenolic compounds, indicating the involvement of hydroxyl groups in the reduction of zinc ions. Peaks

observed near 2900 cm^{-1} corresponded to C-H stretching vibrations of alkanes and aromatic compounds.

The peaks in the region of 1600-1650 cm^{-1} were assigned to C=C stretching of aromatic rings and N-H bending vibrations of amine groups, suggesting the participation of proteins, alkaloids, and flavonoids in nanoparticle stabilization. Peaks between 1000-1400 cm^{-1} represented C-O stretching vibrations of alcohols, ethers, and phenolic compounds.

A characteristic absorption peak below 600 cm^{-1} confirmed Zn-O stretching vibrations, indicating the successful formation of ZnO nanoparticles (Figure 4). The presence of phytochemical functional groups on the nanoparticle surface

demonstrated that bioactive compounds from *Datura stramonium* acted as natural capping and stabilizing agents. The FTIR findings are consistent with reports by Kulkarni and Muddapur (2014) ^[10], who demonstrated the role of flavonoids, phenolics, alkaloids, and terpenoids in nanoparticle synthesis and stabilization. Jha and Prasad (2009) ^[9] also reported the involvement of phenolic compounds in nanoparticle reduction, while the Zn-O

stretching peak below 600 cm^{-1} agrees with the findings of Agnieszka and Jesionowski (2014) ^[11]. Overall, the FTIR analysis confirmed that biomolecules present in the plant extract not only facilitated the biosynthesis of ZnO nanoparticles but also contributed to their stabilization and prevention of agglomeration, thereby enhancing their biocompatibility and biological activity.

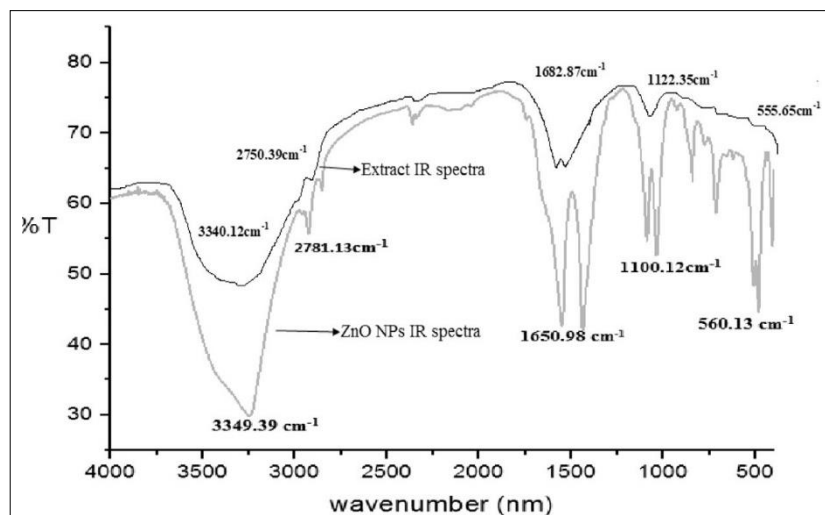


Fig 4: FTIR Spectra of *Datura stramonium* plant extract and ZnONPs synthesized using it.

Table 1: Representing FTIR Analysis of plant extract of *Datura stramonium*.

S.No.	Peak values	Bond	Functional group	Group frequency
1.	3340.12	O-H stretching	Hydroxyl (-OH)	3200-3600
2.	2750.39	C-H stretching	Aldehyde (-CHO)	2700-2850
3.	1682.87	C=O stretching	Carbonyl (ketones, amides)	1650-1750
4.	1122.35	C-O stretching	Alcohols / Ethers / Esters	1000-1300
5.	555.65	Aromatic C-H bending	Aromatics	400-600

Table 2: Representing FTIR Analysis of ZnONPs synthesized using *Datura stramonium* leaf extract.

S.No.	Peak values	Bond	Functional group	Group frequency
1.	3340.12	O-H stretching	Hydroxyl (-OH)	3200-3600
2.	2781.13	C-H stretching	Aldehyde (-CHO)	2700-2850
3.	1650.98	C=C / C=O stretching	Alkenes / Carbonyl (Amide I)	1600-1680
4.	1100.12	C-O stretching	Alcohols / Ethers	1000-1300
5.	560.13	Zn-O stretching	Metal-Oxygen (Zn-O)	400-600

5.3 TEM Analysis

Transmission Electron Microscopy (TEM) analysis was performed to determine the size, shape, and distribution of the synthesized ZnO nanoparticles. TEM images revealed predominantly spherical nanoparticles with sizes ranging from 20-80 nm (Figure 5). The nanoparticles were well dispersed with minimal agglomeration, indicating effective capping and stabilization by phytochemicals present in the *Datura stramonium* extract.

The nanoscale particle size is significant as smaller nanoparticles possess a higher surface area-to-volume ratio, enhancing interaction with microbial cell membranes and promoting the generation of reactive oxygen species (ROS), thereby improving antimicrobial activity. TEM analysis also confirmed the crystalline nature of the nanoparticles, with clearly defined particle boundaries. The controlled particle size distribution demonstrates the effectiveness of the green synthesis approach in producing stable ZnO nanoparticles without the use of toxic chemical stabilizers. The present findings are in agreement with studies by Rasmussen *et al.*

^[15], Espitia *et al.* ^[7], and Medina *et al.* ^[11], who reported spherical ZnO nanoparticles within the 10-100 nm range synthesized using plant extracts. Similar stabilization effects of plant phytochemicals were also reported by Kulkarni and Muddapur ^[10].

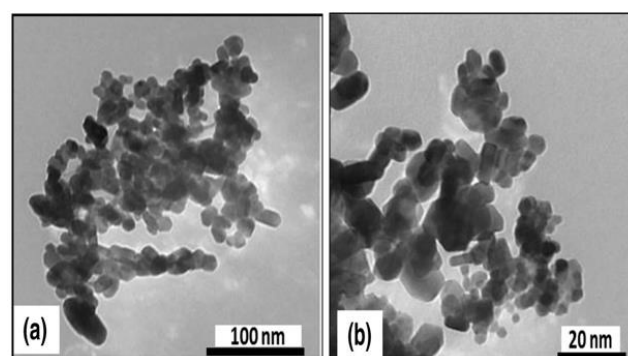


Fig 5: TEM images of ZnONPs synthesized with *Datura stramonium* extract (a) 100 nm scale and (b) 20 nm scale

5.5 XRD Analysis

X-Ray Diffraction (XRD) analysis was carried out to determine the crystalline structure and phase purity of the synthesized ZnO nanoparticles. The XRD pattern showed distinct diffraction peaks corresponding to the characteristic crystalline planes of ZnO nanoparticles. The observed peaks at specific 2θ values confirmed the hexagonal wurtzite crystalline structure of ZnO (Figure 6).

The sharp and intense diffraction peaks indicated high crystallinity and purity of the synthesized nanoparticles. No additional impurity peaks were observed, confirming the absence of contaminating phases. The average crystallite size calculated using the Debye-Scherrer equation revealed nanoscale dimensions.

The nanosized crystallites contribute to enhanced antimicrobial and physicochemical properties due to increased surface area and improved interaction with microbial cells. High crystallinity also enhances the stability and photocatalytic activity of ZnO nanoparticles.

The XRD results confirm the successful synthesis of highly crystalline ZnO nanoparticles using *Datura stramonium* extract. The present findings are consistent with reports by Chen *et al.* [4], Dutta *et al.*, and Agnieszka and Jesionowski [1], who described the characteristic hexagonal wurtzite crystalline structure of ZnO nanoparticles. Similar nanoscale crystalline ZnO synthesized through biological methods was also reported by Rasmussen *et al.* [15].

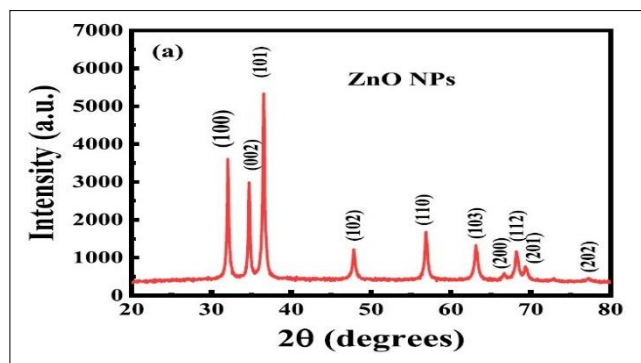


Fig 6: XRD pattern of ZnONPs using *Datura stramonium* leaf extract.

5.6 Antimicrobial Activity Analysis

The antimicrobial activity of the synthesized ZnO nanoparticles was evaluated against selected Gram-positive and Gram-negative bacterial pathogens using the agar well diffusion method. The nanoparticles exhibited significant antibacterial activity against all tested organisms. Among the bacterial strains, *Escherichia coli* showed the highest zone of inhibition, followed by *Staphylococcus aureus*, *Pseudomonas aeruginosa*, and *Bacillus subtilis* (Table 3).

The present findings are consistent with earlier reports by Bedi and Kaur [2], who demonstrated strong antibacterial activity of ZnO nanoparticles against *E. coli*, *S. aureus*, and *P. aeruginosa*. Similar antimicrobial mechanisms involving ROS generation and Zn^{2+} ion release were reported by Sirelkhatim *et al.* [18] and Sabir *et al.* [17]. Furthermore, Espitia *et al.* [7] observed greater susceptibility of Gram-negative bacteria toward ZnO nanoparticles, supporting the present results.

Table 3: Antimicrobial activity of ZnONPs using *Datura stramonium* leaf extract.

Microorganism	Zone of Inhibition (mm)
<i>Staphylococcus aureus</i>	18 ± 0.5
<i>Bacillus subtilis</i>	16 ± 0.4
<i>Escherichia coli</i>	20 ± 0.6
<i>Pseudomonas aeruginosa</i>	17 ± 0.5

The strong antimicrobial activity of ZnO nanoparticles may be attributed to several mechanisms

1. Generation of reactive oxygen species (ROS) such as hydroxyl radicals, superoxide radicals, and hydrogen peroxide.
2. Release of Zn^{2+} ions, which interfere with microbial metabolic pathways.
3. Direct interaction of nanoparticles with microbial cell membranes leading to membrane disruption.
4. Penetration of nanoparticles into bacterial cells causing protein denaturation, DNA damage, and oxidative stress.

The nanoscale size and increased surface area of ZnO nanoparticles enhance their interaction with bacterial cells and improve antibacterial efficiency. Gram-negative bacteria showed comparatively higher sensitivity due to differences in cell wall structure.

The phytochemicals adsorbed on nanoparticle surfaces may also contribute synergistically to antimicrobial activity. Naturally capped ZnO nanoparticles synthesized using *Datura stramonium* extract therefore possess improved biological effectiveness.

6. Conclusion

The present study successfully demonstrated the green synthesis of zinc oxide nanoparticles using *Datura stramonium* leaf extract. The synthesized nanoparticles were effectively characterized using UV-Visible spectroscopy, FTIR, TEM, and XRD techniques. The nanoparticles exhibited significant antimicrobial activity against selected bacterial pathogens.

The study confirms that *Datura stramonium* is a promising biological source for eco-friendly synthesis of ZnO nanoparticles. The synthesized nanoparticles possess potential applications in antimicrobial formulations, biomedical devices, pharmaceutical industries, and environmental remediation.

Further investigations on cytotoxicity, biocompatibility, and large-scale production may facilitate the development of novel nanoparticle-based therapeutic agents.

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