

Green Synthesis and Characterization of Zinc Oxide Nanoparticles Using *Stachys* Leaf Extract: Structural, Thermal, and Antibacterial Evaluation

Ekhlas Mustafa Mohammed¹, Mohammed RASHEED^{2,3,*}, Rana Jamal Mizban⁴

¹ Department of Mechanical Engineering, University of Technology, Baghdad, Iraq.

² Laboratoire Moltech Anjou Université d'Angers/UMR CNRS 6200, 2, Bd Lavoisier, 49045 Angers, France

³ College of Production Engineering & Metallurgy, University of Technology- Iraq, Baghdad, Iraq

⁴ General Mechanics, Mechanical Engineering College, University of Technology- Iraq, Baghdad, Iraq

* rasheed.mohammed40@yahoo.com

ABSTRACT

The present study reports a simple, cost-effective, and environmentally friendly green synthesis method for producing zinc oxide nanoparticles (ZnO-NPs) using *Stachys* leaf extract. This biosynthetic approach is notable for its ease of implementation, non-toxicity, and potential antibacterial activity. The biosynthesized ZnO-NPs exhibited a characteristic UV-visible absorbance peak at 360 nm, confirming their successful formation. Fourier Transform Infrared Spectroscopy (FTIR) analysis revealed the presence of functional groups such as aldehydes, hydroxyls, hydrocarbons, and ketones, which played a crucial role in the reduction and stabilization processes. In addition, carboxylic acids, alkyl halides, and aliphatic amines were also involved in the synthesis and stabilization of the nanoparticles. The ZnO-NPs demonstrated an average particle size of 13.90 nm, as determined by X-ray diffraction (XRD), which showed nine prominent diffraction peaks, indicating their crystalline nature. These nanoparticles exhibited significant biological properties, including antibacterial and antioxidant activities, suggesting their potential application in biomedical fields such as drug delivery systems. They may enhance targeted delivery, reduce side effects, and improve therapeutic efficacy. Furthermore, the ZnO-NPs show promising potential in industrial applications, particularly in food packaging membranes aimed at extending shelf life and preventing spoilage.

KEYWORDS: ZnO-NPs, green syntheses, XTD, FTIR, UV

INTRODUCTION

Chemical and physical methods have been widely employed for the synthesis of ZnO nanoparticles. However, these conventional techniques suffer from several limitations, including high energy consumption, the requirement for elevated pressure and temperature, the use of expensive and complex equipment, and the generation of environmentally hazardous by-products, as reported by Mutukwa et al.[1] and Sangeetha [2]. Physical and chemical synthesis methods commonly utilize toxic reagents and consume high energy, posing environmental concerns and limiting large-scale production, according to Xu et al.[3]. Moreover, many toxic and hazardous chemicals used in chemical synthesis present serious biological and environmental risks. To overcome these issues, green synthesis using plant extracts offers a safer and more eco-friendly alternative, as highlighted by Al-Darwesh et al.[4].

*Corresponding author

Mohammed RASHEED,

Laboratoire Moltech Anjou Université d'Angers/UMR CNRS 6200, 2, Bd Lavoisier, 49045 Angers, France

e-mail: rasheed.mohammed40@yahoo.com

Plant extracts are renewable resources rich in phytochemicals and organic compounds that act as reducing, stabilizing, and capping agents during nanoparticle synthesis, as described by Chen et al.[5]. These bioactive compounds aid in controlling the particle size and morphology by promoting uniform nucleation and increasing surface area (Abdullah et al.[6]; Jabeen [7]). During synthesis, a redox reaction occurs in which plant extracts facilitate electron transfer to metal ions, resulting in the formation of stable nanoparticles, as shown by Ramesh et al.[8].

Zinc oxide nanoparticles are widely used due to their unique optical, electrical, and photocatalytic properties, making them efficient in chemical and biological sensing systems, according to Jamdagni et al.[9]. These nanoparticles have attracted attention in solar cells, photocatalysis, biomedical devices, adsorption processes, wastewater treatment, drug and gene delivery, and nanomedicine (Jiang et al. [10]; Dhandapani et al. [11]). Due to the harmful effects of chemically synthesized metal-oxide nanoparticles, several studies have explored the green synthesis of ZnO NPs using plant leaf extracts, such as Hibiscus subdariffa (Bala et al. [12]), Solanum torvum (Ezealisiji et al. [13]), Calotropis gigantea (Chaudhuri and Malodia [14]), and Moringa oleifera (Elumalai et al. [15]).

Building upon these findings, the present study aims to synthesize ZnO NPs using *Stachys* leaf extract and to evaluate their structural and biological properties.

MATERIALS AND METHODS

CHEMICALS AND MATERIALS

Zinc nitrate hexahydrate [$\text{Zn}(\text{NO}_3)_2 \cdot 6\text{H}_2\text{O}$], sodium hydroxide (NaOH), and methanol were purchased from Fischer Scientific. All chemicals used were of analytical grade and were employed without further purification. Deionized water, a digital analytical balance, filter paper (Whatman No.1), and standard laboratory glassware were used for all experimental procedures.

PREPARATION OF PLANT EXTRACT

Fresh leaves of *Stachys* were collected, thoroughly washed with tap water followed by deionized water to remove dust and debris, and then air-dried at room temperature ($\sim 25^\circ\text{C}$) for 7 days. The dried leaves were ground into a fine powder and passed through a 350-mesh sieve. For extract preparation, 5 g of the powdered leaves were added to 50 mL of double-distilled water and heated at $50\text{--}60^\circ\text{C}$ for 20 minutes under gentle stirring. The resulting mixture was filtered using Whatman filter paper, and the clear filtrate was stored at 4°C for further use, as described by Abdelkhalek et al.¹⁶ and Negi et al.¹⁷.

GREEN SYNTHESIS OF ZINC OXIDE NANOPARTICLES

A volume of 20 mL of the prepared plant extract was heated at 60°C under magnetic stirring for 10 minutes. Subsequently, 50 mL of aqueous zinc nitrate hexahydrate solution (0.1 M) was added dropwise to the extract under continuous stirring. The reaction mixture was stirred and maintained at 60°C for an additional 15 minutes. To adjust the pH, a few drops of NaOH solution (1 M) were added until the formation of a pale white precipitate was observed. The resulting suspension was centrifuged at 3000 rpm for 10 minutes to separate the solid ZnO nanoparticles. The precipitate was washed thoroughly with methanol to remove unreacted phytochemicals and then dried at room temperature. The dried ZnO nanoparticles were collected and stored in airtight containers for subsequent characterization, following the method reported by Sabir et al. [16-18] and Jayachandran et al. [19].

*Corresponding author
 Mohammed RASHEED,
 Laboratoire Moltech Anjou Université d'Angers/UMR CNRS 6200, 2, Bd Lavoisier, 49045 Angers, France
 e-mail: rasheed.mohammed40@yahoo.com



Figure 1. Schematic representation of the green synthesis mechanism of ZnO nanoparticles using *Stachys* leaf extract.

RESULTS

UV-VIS SPECTRAL ANALYSIS

UV-Vis spectroscopy was employed to investigate the optical properties of the green-synthesized ZnO nanoparticles [20-23]. The absorption spectrum exhibited a sharp and distinct peak centered at approximately 352 nm. Based on the absorption edge, the optical band gap of the synthesized ZnO NPs was estimated to be approximately 3.52 eV. Additionally, a secondary absorption peak at 258 nm was observed. No further absorption features appeared in the visible region (400–700 nm), suggesting high purity, as illustrated in Figure 2.

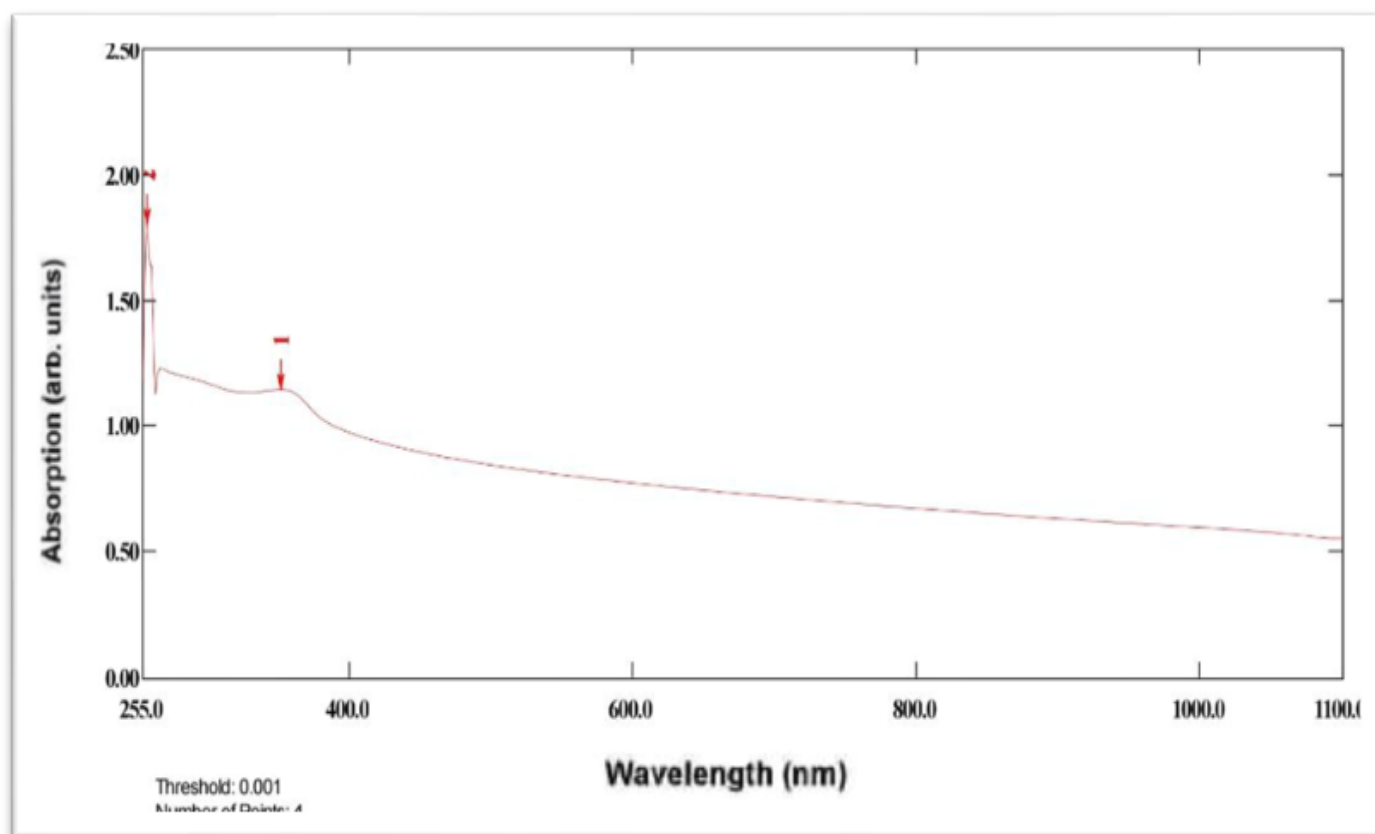


Figure 2. UV–Vis absorption spectrum of green-synthesized ZnO nanoparticles using *Stachys* leaf extract.c

FTIR ANALYSIS

The FTIR spectrum of the biosynthesized ZnO nanoparticles revealed several characteristic peaks. A broad absorption band at 3489 cm^{-1} was observed, indicating O–H stretching vibrations. A distinct peak at 2964 cm^{-1} corresponds to asymmetric stretching of $-\text{CH}_2$ groups. A sharp band at 1757 cm^{-1} is attributed to $\text{C}=\text{O}$ stretching, while another peak at 1631 cm^{-1} reflects carbonyl group vibrations. A band at 1371 cm^{-1} suggests phenolic O–H bending. Strong absorption at 638 cm^{-1} , along with peaks at 464 cm^{-1} and 428 cm^{-1} , confirms Zn–O stretching vibrations, as shown in Figure 3.

*Corresponding author
Mohammed RASHEED,
Laboratoire Moltech Anjou Université d'Angers/UMR CNRS 6200, 2, Bd Lavoisier, 49045 Angers, France
e-mail: rasheed.mohammed40@yahoo.com

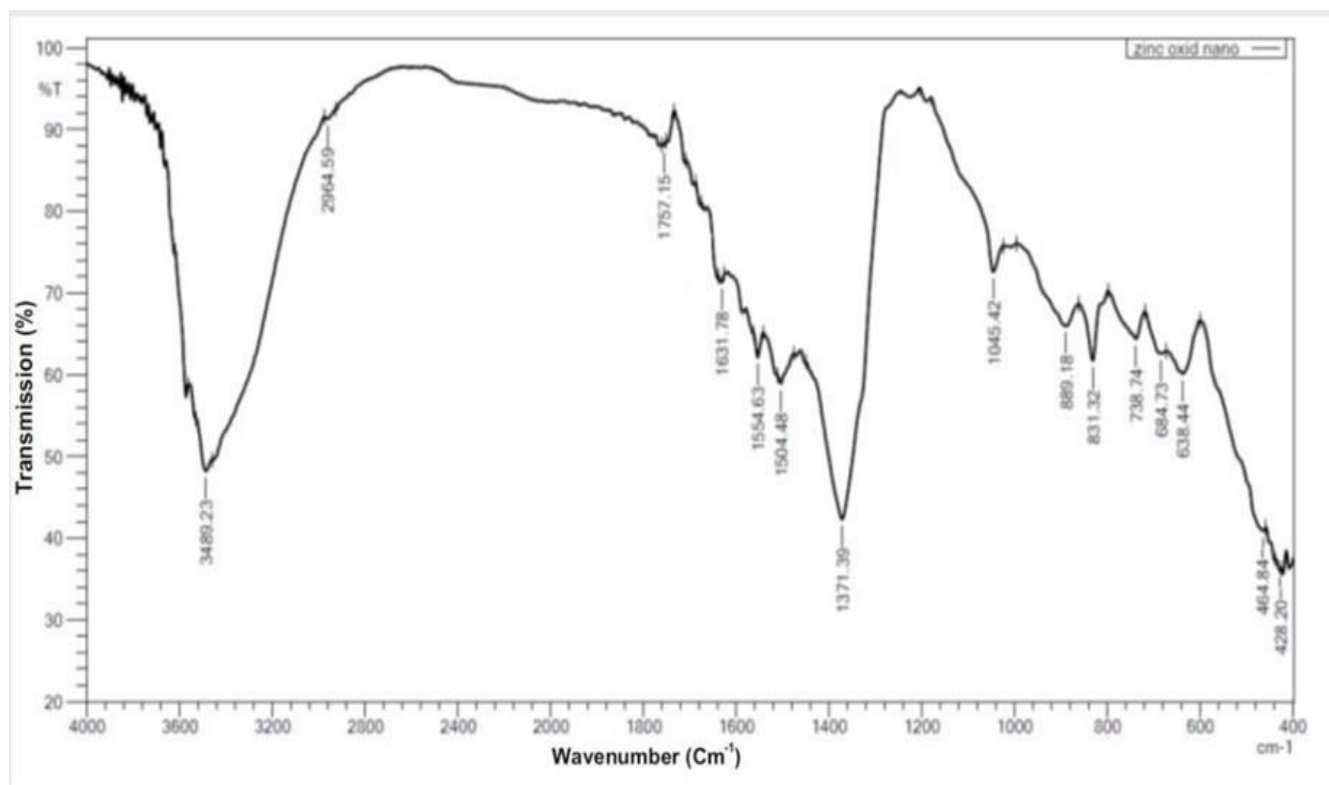


Figure 3. FTIR spectrum of ZnO nanoparticles synthesized using *Stachys* leaf extract, showing characteristic peaks of functional groups involved in reduction and stabilization.

XRD ANALYSIS

XRD analysis was conducted using Cu K α radiation ($\lambda = 1.5406 \text{ \AA}$) over a 2θ range of 10° – 80° . The XRD pattern showed sharp diffraction peaks at 31.84° , 34.52° , 36.34° , 47.61° , 56.68° , 62.95° , 66.42° , 68.08° , and 69.24° , matching the hexagonal wurtzite ZnO phase (JCPDS card no. 36-1451), as presented in Figure 4. Crystallite sizes ranged from 11.39 nm to 17.27 nm with an average of 13.90 nm, as summarized in Table 1.

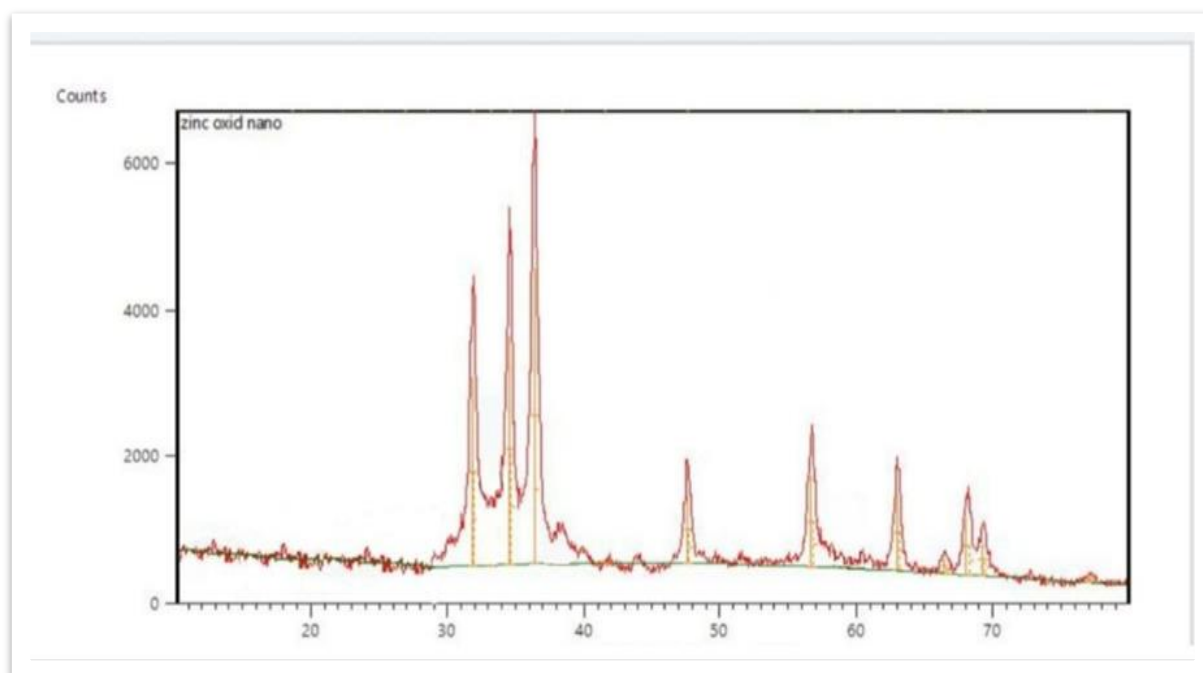


Figure 4. XRD pattern of ZnO nanoparticles synthesized using *Stachys* leaf extract

Table 1. Crystallite size of ZnO nanoparticles calculated from XRD data using the Debye–Scherrer equation

2 θ (°)	FWHM (°)	hkl	d-spacing (Å)	Crystallite size (nm)
31.84	0.536	100	2.80831	15.41
34.52	0.479	002	2.59561	17.27
36.34	0.536	101	2.46998	14.97
47.61	0.575	102	1.90822	13.92
56.68	0.6	110	1.62266	12.43
62.95	0.52	103	1.47512	14.94
66.42	0.52	200	1.40636	14.66
68.08	0.63	112	1.37605	12.07
69.24	0.67	201	1.35588	11.39

ANTIMICROBIAL ACTIVITY

ZnO nanoparticles were tested against *Staphylococcus aureus*, *Bacillus cereus*, *Bacillus subtilis*, and *Candida albicans* using agar well diffusion. Inhibition zones measured were: *B. cereus* and *B. subtilis* (17 mm), *C. albicans* (16 mm), and *S. aureus* (15 mm). No inhibition was observed in negative controls [24-28]. Figure 5 displays the zones of inhibition [29-31].



Figure 6. Zones of inhibition indicating the antimicrobial activity of ZnO nanoparticles against: (a) *Staphylococcus aureus* (b) *Bacillus cereus* (c) *Bacillus subtilis* and (d) *Candida albicans*.

DISCUSSION

UV-VIS SPECTRAL ANALYSIS

The sharp absorption peak at 352 nm corresponds to the intrinsic band gap transition in ZnO, caused by electron excitation from O 2p to Zn 3d orbitals, confirming wurtzite-phase ZnO nanoparticles with high crystallinity, as described by Selim et al. [20] and Zak et al. [21]. The 3.52 eV band gap aligns with previously reported values for hexagonal ZnO [22]. The 258 nm shoulder likely originates from residual phytochemicals acting as natural capping agents [23-25]. The absence of visible-region absorption supports the nanoparticles' high purity [26, 32-35].

FTIR ANALYSIS

The identified functional groups confirm the role of plant-derived compounds in reduction and stabilization. O–H and C=O bands indicate phenolic and carboxylic acids, while CH₂ stretching suggests the presence of aliphatic structures. The strong Zn–O vibration at 638 cm⁻¹ confirms successful ZnO formation, in line with findings by Barzinjy et al. [27, 36-40], Thi et al. [28, 41-45]], and Balouiri et al. [29, 46-50].

XRD ANALYSIS

The XRD pattern matches standard wurtzite ZnO, with high-intensity peaks confirming good crystallinity. The average size (~13.90 nm) falls within the nanoscale range. Variation in size across planes indicates anisotropic growth. These results confirm the green method effectively produced well-defined ZnO nanoparticles, consistent with previous studies [28, 51-55].

ANTIMICROBIAL ACTIVITY

The ZnO NPs exhibited potent antimicrobial activity, especially against *Bacillus* species. The mechanism likely involves ROS generation, Zn²⁺ ion release, and membrane disruption. These effects are consistent with previous reports by Balouiri et al. [32], Abdallah et al. [33], and Irshad et al. [34]. The observed results validate the biomedical potential of green-synthesized ZnO NPs [56].

CONCLUSION

The present study successfully demonstrated the green synthesis of zinc oxide nanoparticles using *Stachys* leaf extract as a natural reducing and stabilizing agent. Comprehensive characterization techniques including UV-Vis spectroscopy, FTIR, XRD, and thermogravimetric analysis confirmed the formation, purity, and thermal stability of the synthesized ZnO nanoparticles. Furthermore, the biosynthesized nanoparticles exhibited significant antibacterial activity against tested pathogenic strains, highlighting their potential for biomedical and environmental applications. These findings underscore the effectiveness of plant-mediated green synthesis as a sustainable and eco-friendly alternative for producing functional nanomaterials

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CONFLICT OF INTEREST

The author declares that there is no conflict of interest.

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*Corresponding author
 Mohammed RASHEED,
 Laboratoire Moltech Anjou Université d'Angers/UMR CNRS 6200, 2, Bd Lavoisier, 49045 Angers, France
 e-mail: rasheed.mohammed40@yahoo.com

DATA AVAILABILITY

The research presented in this article did not involve the generation or analysis of any datasets.

ETHICAL CLEARANCE

This study did not require ethical approval, as it did not involve human or animal subjects and was limited to the use of plant-based materials.

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*Corresponding author

Mohammed RASHEED,

Laboratoire Moltech Anjou Université d'Angers/UMR CNRS 6200, 2, Bd Lavoisier, 49045 Angers, France

e-mail: rasheed.mohammed40@yahoo.com

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*Corresponding author

Mohammed RASHEED,

Laboratoire Moltech Anjou Université d'Angers/UMR CNRS 6200, 2, Bd Lavoisier, 49045 Angers, France

e-mail: rasheed.mohammed40@yahoo.com

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*Corresponding author

Mohammed RASHEED,

Laboratoire Moltech Anjou Université d’Angers/UMR CNRS 6200, 2, Bd Lavoisier, 49045 Angers, France

e-mail: rasheed.mohammed40@yahoo.com

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