



Green Synthesis, Characterization and Medicinal Applications of Zinc Oxide Nanoparticles

M. N. Jasim^{1*}, H. G. Ismail², M. D. Abdulla³ and G. A. Naeem⁴

¹College of Basic Education, Haditha, University of Anbar, Iraq

²College of Education, Hasaheisa, Gezira University, Wad Madani, Sudan

³College of Science, Sudan University of Science and Technology, Khartoum, Sudan

⁴College of Applied Science, Hit, University of Anbar, Anbar, Iraq

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*mhamad87@uoanbar.edu.iq



ABSTRACT

In this study, zinc oxide nanoparticles (ZnO NPs) were green synthesized using the leaves of *Cardaria draba*, their properties have been thoroughly investigated and their antibacterial and anticancer activities were assessed. X-ray diffraction (XRD) revealed the crystalline structure and the phase purity of ZnO nanocrystals with an average crystal size of 30 nm. UV-visible spectroscopic analysis indicated the occurrence of an absorption band at 310 nm attributed to the phenomenon of Surface Plasmon Resonance (SPR). FTIR spectroscopic analysis was employed to study the functional groups present in the nanoparticles. Additionally, microscopic characterization of ZnO NPs was performed using AFM, DLS and SEM with EDEX. The ZnO NPs showed an inhibition zone of 26 mm against *S. aureus* and 19 mm against *E. coli* at 100 µg/mL. The green synthesized zinc oxide nanoparticles exhibited good anti-cancer activity against MCF-7 breast cancer cells.

Keywords: ZnO nanoparticles; *Cardaria draba*; Antibacterial activity; Green synthesis; Anticancer activity.

1. INTRODUCTION

Nanotechnology improves the properties of materials by manipulating their size and shape to be within the nanoscale. This has been achieved in the past decades through chemical and physical techniques. However, nanomaterials will be of greater importance when prepared in green or environmentally friendly ways (Rabeca *et al.* 2021). The superiority of these green techniques lies in their cost-effectiveness, non-toxicity, speed, and high crystallinity in forming nanoparticles of various sizes and shapes (Nikita *et al.* 2019; Mohammadi and Ghasemi, 2018). Environmentally safe techniques are used for development, most of which involve plant extracts (Ghodake *et al.* 2010). Plant extracts contain a high concentration of polyphenols that convert metal salts into highly pure nanoparticles (Zayed and Eisa, 2014). Thanks to these properties, nanoparticles are produced and widely used in biomedical applications due to their good biocompatibility (Gajanan *et al.* 2011; Carvajal, 1993; Nhiem *et al.* 2010).

In particular, zinc oxide nanoparticles have attracted significant interest from scientific and medical communities and are one of the most commonly utilized nanomaterials (Hadi *et al.* 2017). The applications of ZnO NPs are not limited only to a specific field. They find use in cosmetics and pharmaceutical industries. ZnO NPs are known for their non-toxic nature. Their antimicrobial properties make them valuable in medical

applications (Basher *et al.* 2023; Mirzaei and Darroudi, 2017). In medicine, ZnO nanoparticles can be designed to target specific diseases, offering new possibilities for treating conditions that are difficult to treat with antibiotics (Moezzi *et al.* 2012). They have been shown to be endowed with antimicrobial and anticancer activities. Moreover, ZnO NPs find use in bio-sensing devices for gene transfer (Mohamed *et al.* 2022). This study aims to investigate the effectiveness of green synthesized zinc oxide nanoparticles against two bacteria (*S. aureus* and *E. Coli*) and human breast cancer cell line (MCF-7).

2. EXPERIMENTAL DETAILS

2.1 Materials

The raw materials employed in preparing zinc oxide nanoparticles are the leaves of *Cardaria draba* and zinc nitrate ($\text{Zn}(\text{NO}_3)_2 \cdot 6\text{H}_2\text{O}$). The plant material was collected from a local farm in the city of Haditha, located in Anbar Governorate, western Iraq. Hydrated zinc nitrate with 99.9% purity was purchased from Alpha Chemica, India. *Cardaria draba* (Fig. 1), known as hoary cress, is a perennial plant that propagates through seeds and has branched roots. The plant, belonging to Cruciferae family (mustard family), contains flavonoids, alkaloids, saponins, and dozens of glucosinolate compounds (Naser *et al.* 2019).



Fig.1: *Cardaria draba* plant

2.2 Preparation of Plant Extract

The collected leaves of *Cardaria draba* plant were washed with deionized water (D.W) and dried in a hot air oven. Then the dry leaves were crushed using an electric blender. About 5 g of the powder of the dried leaves was placed in 500 mL of D.W. The mixture was stirred using a magnetic stirrer at 100 °C for 180 minutes. The extract was then filtered using a filter paper, and large particles were separated using a centrifuge. After obtaining the pure extract, it was stored in a refrigerator until further use.

2.3 Preparation of Zinc Oxide Nanoparticles

About 8.5 g of $\text{Zn}(\text{NO}_3)_2 \cdot 6\text{H}_2\text{O}$ was dissolved in 200 mL of D.W. and stirred for 45 minutes at room temperature. After obtaining a clear solution, 35 mL of *Cardaria draba* leaves extract was added and the mixture was stirred continuously for 60 minutes. The solution was left overnight, then washed with alcohol and water. The particles were separated by centrifugation at 3000 rpm for 15 minutes. The precipitate was dried in an air oven at 100 °C for 2 hours. The zinc oxide particles were calcined at 600 °C for 2 h to obtain nano-sized zinc oxide particles.

The analytical techniques used to characterize ZnO NPs nanoparticles include a UV-Vis spectrophotometer (Shimadzu, UV-2550, Japan) for optical property analysis and Fourier Transform Infrared (FTIR) spectrometry (Thermo Fisher Scientific, USA) for identifying functional groups. X-ray diffraction (XRD) analysis was performed using Panalytical X' Pert Pro (United Kingdom) to determine the crystalline structure. Scanning Electron Microscopy (SEM) with Energy Dispersive X-ray (EDX) analysis was conducted using the ZEISS SIGMA VP (Germany) to examine morphology and elemental composition. Atomic Force Microscopy (AFM) analysis was carried out using the Dual Scope TMDS (Germany) for surface topology

evaluation. Dynamic Light Scattering (DLS) measurements were performed using the Brookhaven Instruments Model (BI-ZTU, USA) to determine particle size distribution.

2.4 Characterization of manufactured ZnO NPs

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3. RESULTS AND DISCUSSION

3.1 UV-visible Spectroscopy Analysis

The UV-Vis spectrum of the ZnO NPs (Fig. 2) shows an absorption maximum at 310 nm resulting from surface plasmon resonance phenomenon (Manjunatha *et al.* 2019).

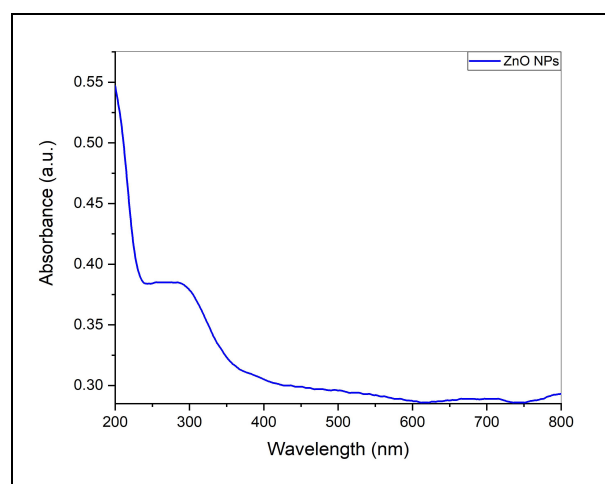


Fig. (2): UV-absorption spectrum of ZnO nanoparticles

3.2 XRD Analysis

The X-ray diffraction pattern of the prepared ZnO NPs (Fig. 3) exhibits seven diffraction peaks at 2θ : 31.84°, 34.51°, 36.35°, 47.62°, 56.68°, 62.96° and 67.9° corresponding to the planes (100), (200), (101), (102),

(110), (103) and (112), respectively, attributed to the standard hexagonal wurtzite phase of zinc oxide ZnO according to JCPDS card No. (36-1451) (Sangeetha *et al.* 2011). The peak at (101) crystal plane confirms the high phase purity of the zinc oxide NPs prepared by the green method.

The crystalline size (D) of the ZnO nanoparticles was estimated by the (Scherer-Debye) relationship (Alghafour *et al.* 2020):

$$D = \frac{K\lambda}{\beta \cos \theta} \quad \dots (1)$$

where D = crystallite size, K = constant (0.9), λ = wavelength, θ = diffraction angle and β = full-width at half maximum (FWHM).

The micro strain (ϵ) of the crystal lattice was determined by the following formula (Naeem *et al.* 2024):

$$\epsilon = \frac{\beta}{4 \tan \theta} \quad \dots (2)$$

The dislocation density (δ) was determined by the following relationship:

$$\delta = \frac{1}{D^2} \quad \dots (3)$$

The surface area (S.A.) of ZnO lattice was determined by the equation:

$$S.A = \frac{k_{sv}}{\rho D_{av}} \quad \dots (4)$$

where ρ represents the density of zinc oxide nanoparticles.

3.3 FTIR Spectroscopy Analysis

FTIR is an essential technique for characterizing materials by analyzing their unique vibration patterns. Previous studies have shown that metal oxides give absorption bands below 1000 cm^{-1} attributed to interatomic vibrations, while other peaks are attributed to the functional groups of compounds present in the plant used in the green synthesis of nanoparticles (Senthilkumar and Sivakumar, 2014). Chemicals found in plants are responsible for the oxidation of nanoparticles. Fig. 4 shows the FTIR spectra of the pure plant extract and the green synthesized ZnO NPs. The spectrum of the plant extract (black) shows prominent peaks at 3308 cm^{-1} , 1590 cm^{-1} , 1417 cm^{-1} , 1272 cm^{-1} , and 1090 cm^{-1} . The peak at 3308 cm^{-1} is the result of the O-H stretching vibration of the phenolic OH- group, while the peaks at 1643 cm^{-1} and 1508.38 cm^{-1} indicate the presence of phenolic rings. The peaks at 1417 cm^{-1} and 1590 cm^{-1} indicate the presence of polyamines in the plant extract. The spectrum of ZnONPs (red) shows an absorption band at 596 cm^{-1} .

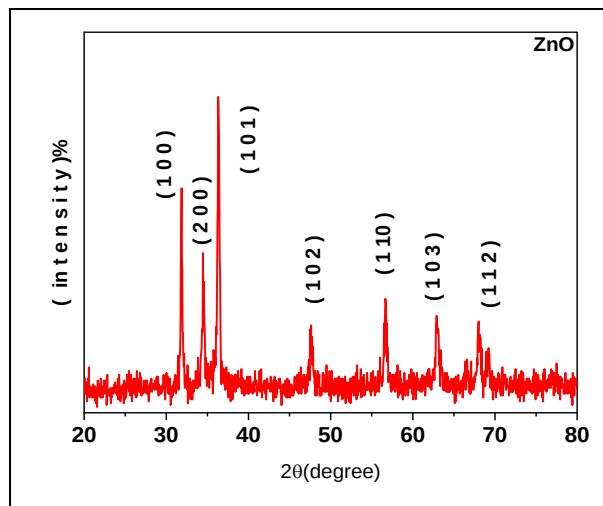


Fig. (3): XRD pattern of green synthesized ZnO NP

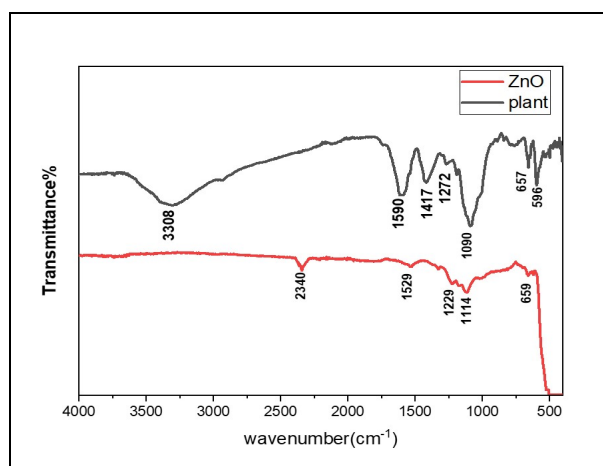


Fig. (4): FTIR spectrum of plant extract and ZnO NPs

3.4 Topographical Analyses

Atomic force microscopy was used to study the topography of the prepared ZnONPs. Fig. 5 shows atomic force microscopy images to study the surface structure of the nanoparticles. The images show that the zinc nanoparticles are highly uniform and distributed regularly with an average grain size of 56.66 nm. The average surface roughness was 3.95 nm.

3.5 Morphological Analyses

Fig. 6 shows the uniform distribution of zinc oxide nanoparticles with particle sizes ranging from 20 to 38 nm. The average particle size is 25.36 nm. The images show that the particles are spherical in shape. The shape of the nanoparticle depends on the type of oxidizing agent it contains. Homogeneous distribution of metal oxide anions in the 3D network structure may lead to agglomeration through condensation, resulting in narrowing of the particles (Nemati *et al.* 2019). The particles aggregate to form clusters of sponge-like

particles. Agglomerated particles are larger in size and exhibit a lower surface-to-volume ratio. This agglomeration may be due to condensation of nanoscale particles due to polarity and electrostatic attraction (Pillai *et al.* 2020).

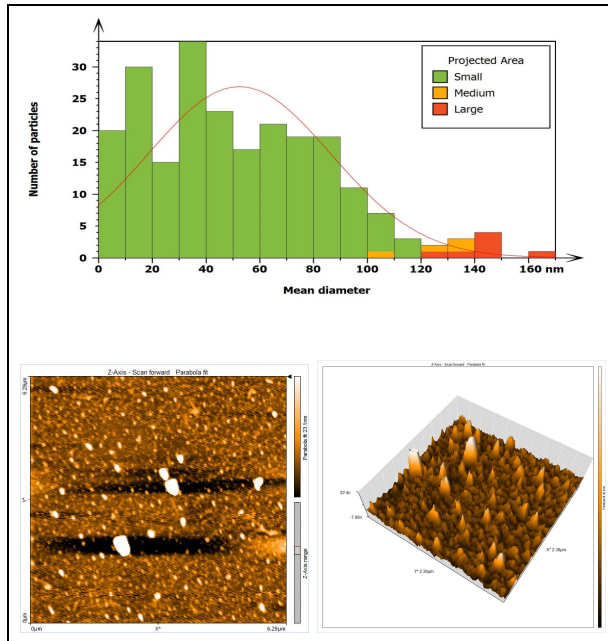


Fig. (5): AFM images of zinc oxide nanoparticles

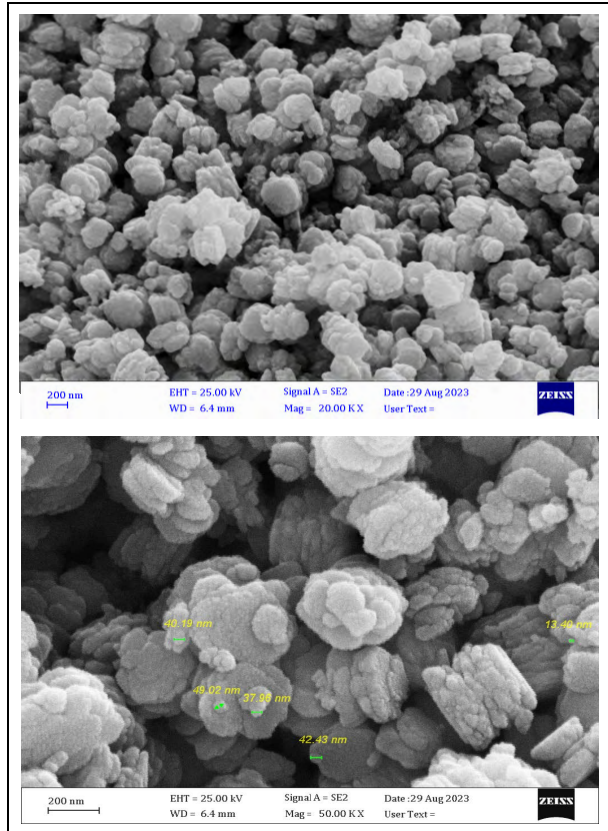


Fig. (6): SEM images of zinc oxide nanoparticles

3.6 Dynamic Light Scattering Analytics

Dynamic light scattering (DLS) is widely used to calculate the hydrodynamic diameter of suspended nanoparticles based on the Brownian motion exhibited by the particles. It is observed from Fig. 8 that the average hydrodynamic diameter calculated by DLS is 274.36 nm, which is much larger than the reported size. This difference in nanoparticle size can be attributed to the polydispersity of the nanoparticles (Jamdagni *et al.* 2018).

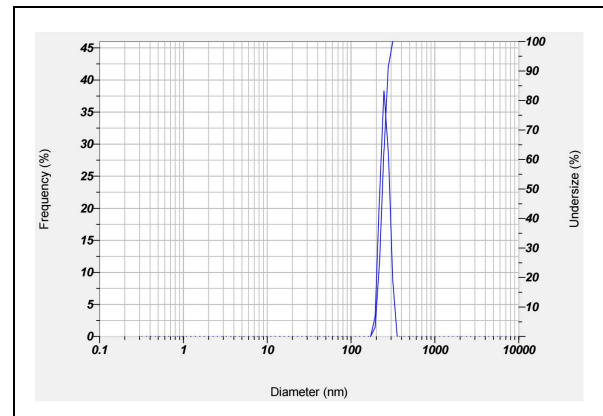


Fig. (8): Size distribution of zinc oxide nanoparticles

Table 3. Shows the antibacterial activity of nanoparticles

Type of Bacteria	Zone of inhibition(mm)				
	ZnO NPs				
	A	B	C	D	E
<i>S. aureus</i>	6	7	10	16	19
<i>E. coli</i>	6	23	24	25	26

4. APPLICATIONS IN MEDICINE

4.1 Antibacterial Activity

The activity of ZnO NPs against Gram-positive *Staphylococcus aureus* and Gram-negative *Escherichia coli* was investigated employing agar diffusion assay (Bahjat *et al.* 2021; Khashan *et al.* 2020). Gentamicin was used as the standard. About 20 mL of Mueller-Hinton agar (MH) was poured into clean Petri dishes. Bacterial strains were collected from their cultures employing a sterile wire loop subsequent to inoculation. Using a sterile pipette tip on the agar plates, 6-mm wells were made. Different concentrations of ZnO NPs samples were introduced in the wells. The test organisms were cultured overnight at 37°C before measuring and recording the average inhibition zone diameter. It was observed that the inhibitory effect of ZnO NPs increased with increasing concentration. The highest inhibitory zone of 26 mm was observed against *E. coli* bacteria and 19 mm against *S. aureus* bacteria at 100 micrograms/mL. The effectiveness of the nanoparticles against bacteria is

attributed to their interaction with the bacterial membrane, resulting in membrane collapse and rupture, leading to bacterial cytoplasm leakage (Zhong *et al.* 2018). Oxidative stress is responsible for the antimicrobial activity of ZnONPs caused by the generation of reactive oxygen species (ROS) from surfaces (Krishnamoorthy *et al.* 2012). Previous studies have reported differences in the severity of Gram-negative and Gram-positive bacteria when exposed to ZnONPs. Also, the antimicrobial mechanism in zinc oxide nanoparticles is attributed to the fact that when zinc reaches toxic concentrations, it strongly binds to thiol groups on cell surface proteins and enzymes, leading to bacterial death (Agarwal *et al.* 2018) in Table 1. shows the antibacterial activity

4.2 Anticancer Activity

The anticancer assay of the ZnO nanoparticles was performed using a 96-well plate with the cell line seeded at 10^4 cells/well. After 24 hours. The monolayers of cells were obtained and treated with different concentrations of ZnONPs. After 48 hours of treatment, cell viability was evaluated by MTT assay (Al-Ziaiydi, A *et al.* 2020). The absorbance was measured at 492 nm on a microplate reader. The cell growth inhibition rate (cell toxicity percentage) was calculated using the following equation:

$$\text{Inhibition Rate} = (A - B) \times 100\% \quad \dots (5)$$

where A is the optical density of the control, and B is the optical density of the samples. The findings of this study are shown in Fig. 9. It is observed that cytotoxicity increases proportionally with the increase in the percentage of zinc oxide nanoparticles (ZnONPs), which stimulate the death of cancer cells without any toxic effects on normal cells, and this is consistent with the results of a previous study (Sarala *et al.* 2020).

The morphology of cells observed using an inverted microscope. The cells were inoculated in a 24-well plate at a density of 1×10^5 cells/mL and then grown at 37°C for 24 hours. The cells were then exposed to zinc nanoparticles for 24 hours. Subsequent to the exposure period, the slides were stained with the use of crystal violet and incubated at 37°C for 10-15 minutes. The spot was gently washed with running water until the stain is completely removed. The cells were observed under an inverted microscope at an amplification of $100\times$ and the images (Fig. 10) were taken with a digital camera connected to the microscope.

The primary explanation is that nanoparticles have optical, magnetic, or structural properties different from traditional solid materials. The interactions between positively charged zinc particles and cancer cells result from electrostatic interactions that enhance cell uptake,

endocytosis, and cellular toxicity (Rasmussen *et al.* 2010; Abercrombie *et al.* 1962).

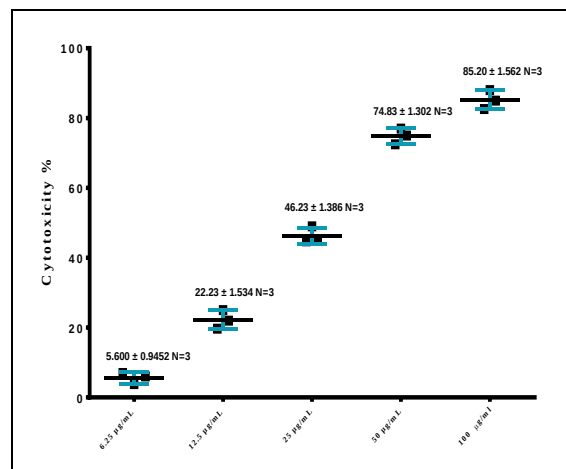


Fig. (9): Cytotoxicity effect of ZnO NPs in MCF-7 cells

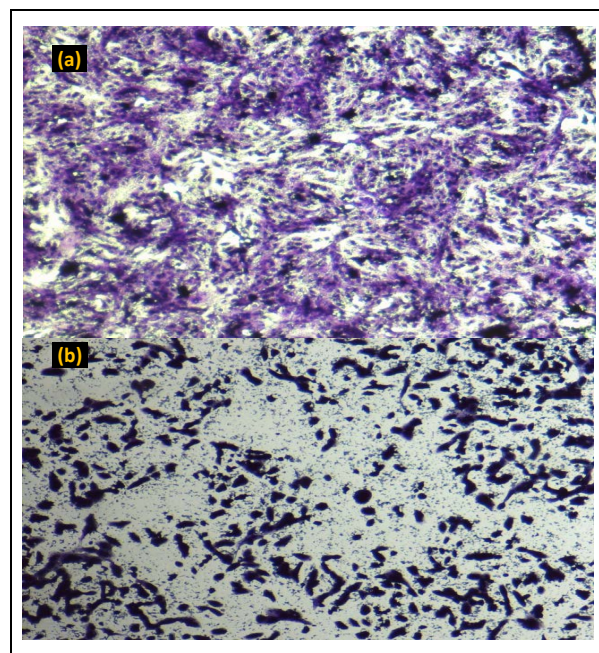


Fig. 10: (a) control untreated MCF-7 cells, and (b) morphological changes in MCF-7 cells after treated with ZnO NPs. Magnification power $\times 20$

5. CONCLUSION

The present study followed an environmentally safe approach to synthesize (ZnONPs) from *Cardaria draba* leaf extract. The zinc oxide nanoparticles were well characterized. The biological effectiveness of (ZnONPs) was studied in inhibiting bacteria and combating human breast cancer cells (MCF-7). Understanding the specific mechanism of action of these ZnONPs can also provide better insight into their application in various fields.

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CONFLICTS OF INTEREST

The authors declare that there is no conflict of interest.

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