

Green Synthesis of Zinc Oxide Nanoparticles Using Leaf Extract of *Bacopa Monnieri*: Characterization and Assessment of its Antibacterial Activity Properties (*Staphylococcus Aureus*, *Escherichia Coli*)

Nawar Jaber Hussein Al-Asadi ¹, Dhulfiqar S. Mutashar ²

¹ College of Science, Wasit University, Iraq

² Department of Applied Science, University of Technology, Baghdad, Iraq

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Annotation: This study looks into the environmentally friendly manufacture of zinc oxide nanoparticles (ZnONPs) with *Bacopa monnieri* leaf extract, as well as their use as an antibacterial agent. (UV-Vis), (XRD), (FTIR), (FESEM), (EDX) and zeta potential examination all confirmed the development of hexagonal ZnONPs with an average dimension of 31 nm. The antibacterial activity was notable against both Gram-negative and Gram-positive bacterial strains. The effectiveness is attributed to interactions between nanoparticles and bacterial cell membranes, as well as the synergistic effects of the phytochemicals that surround the nanoparticles. The sustainable synthesis process employing *Bacopa monnieri* is consistent with the general trend of using plant extracts to improve the bioactivity and sustainability of nanoparticles.

Keywords: Green Synthesis, Zinc Oxide Nps, Antibacterial Activity, *Bacopa Monnieri*.

1. Introduction

Nanotechnology is the scientific discipline of designing, synthesizing, characterizing, and advancing nanoparticles [1, 2]. Nanoparticles (NPs) are defined as objects with dimensions ranging from 1 - 100 nanometers [3, 4]. Drug delivery technology is used to target areas within the body using active targeting methods, reducing the side effects associated with drug therapy. [5]. Currently, the process of preparing metal oxide nanoparticles (MONPs) for biological purposes is underway [6,7]. MONPs include iron oxide FeONPs (Fe_2O_3 and Fe_3O_4), MgONPs, ZnONPs, TiO_2 NPs, and CuNPs [8-10]. Nanometallic oxides are increasingly being used in a wide range of industries, including medicinal treatments, solar cells, batteries for energy storage; they are also used in cosmetics, sunscreen, textiles, clothing, and health care [11- 13].

ZnONPs have garnered significant interest because of their distinctive physical and chemical features. They possess a broad spectrum of prospective biomedical uses that will significantly impact the medical field, including antifungal, antiviral, antiparasitic, and antibacterial properties. They can also combat cancer, diabetes, and are anti-inflammatory, in addition to contributing to wound healing [14-18]. Biological applications hold promising potential for ZnONPs, due to their unique properties, including size, structure, shape, and chemical composition [19,20]. Nanoparticle sizes have diverse properties, and their properties may differ due to size, for example, morphological, optical, structural, and others [20].

Recently, the environmentally friendly synthesis process has been used to create biodegradable and biocompatible ZnONPs, making them ideal for various biomedical applications [16, 21]. This method is eco-friendly and produces no toxic byproducts or unwanted waste, resulting in a trustworthy, sustainable, and ecologically responsible synthesis alternative [22, 23]. Furthermore, this reduces the need for hazardous solvents or chemicals commonly used in green synthesis, resulting in the production of advanced nanoparticles that are biocompatible and environmentally friendly [24]. This strategy is marketed as an economical and simple strategy that doesn't require specialized equipment [25]. Ultimately, reducing and capping agents, which are required for the green synthesis of ZnONPs, are produced from natural chemicals present in plant extracts, decreasing the reliance on hazardous and expensive reagents [26, 27].

These environmentally produced nanomaterials have special properties that make them widely used in medical and pharmaceutical applications. Recently, there has been a significant increase in interest in the antibacterial efficiency of metal oxide nanoparticles as part of attempts to produce effective antibacterial agents. The addition of phytochemical substances that enclose green produced nanoparticles improves their interaction with receptors on the bacterial cell wall, hence increasing their antibacterial activity. Green synthesis ZnONPs exhibit activity against microbes due to their biocompatibility, high specific surface area, and low toxicity. Therefore, it is used in food packaging, cosmetics, and agricultural disease control [28, 29].

This research aimed to synthesis ZnONPs through an environmentally friendly method utilizing the natural extract from *Bacopa monnieri* leaves, analyze their characteristics employing diverse techniques, and investigate their antibacterial efficacy.

2. Methodology

a. Preparation of Leaf Extract of *Bacopa monnieri*

Bacopa monnieri plants were obtained from the University of Technology's garden in Baghdad, Iraq. All dirt, debris, and foreign substances were methodically removed from the plant leaves by washing them with pure water 5 g of *Bacopa monnieri* leaves were coarsely chopped and boiled in 100 mL DW at 55°C for 20 min. The extract was filtered using Whatman filter paper Grade-1 to

remove contaminants and solid plant residues and kept in a sterile container at 4°C.

b. Synthesis of ZnONPs from *Bacopa monnieri*

A 0.05 M solution of zinc acetate dehydrate (Qualigens) was added to 50 mL of DW and mixed with a magnetic stirrer. After 15 min, 1ml of leaf extract was added to the mixture and left to react for a variable amount of time while stirring. Subsequently, 15 ml of 0.1M NaOH (Qualigens) was added to the reaction mixture dropwise. After 30 min of constant agitation, a white-to-light-yellow precipitate began to form. The reaction mixture was then heated in an oven at 100°C for two hours before being allowed to cool to room temperature; the solution was gently agitated to help the precipitate settle. The precipitate was carefully collected using a glass pipette and then centrifuged for 30 min at 3000 rpm. The resultant pellet was rinsed four times with distilled water and centrifuged at 4000 rpm for 10 min each. The precipitate was methodically collected using a glass pipette and then centrifuged for 30 min at 3000 rpm. The resulting pellet was washed three times with distilled water and centrifuged at 4000 rpm for 10 min each time. The precipitate was dried in an oven at 50°C overnight and pounded into powder. The fine powder was then calcined in a muffle furnace at 450°C for two hours. The powder was kept in airtight jars for future use.

2.3. Antibacterial tests for ZnONPs

The bacteria's sensitivity to the nanoparticles was tested using Mueller-Hinton agar. Mueller-Hinton agar was prepared by sterilizing agar in Petri dishes at 121°C for 15 minutes, followed by cooling and solidification. The agar surface was wiped with a cotton ball soaked in bacterial growth and left at 25°C for 30 minutes. 6 mm diameter holes were created in the agar surface, and these holes were filled with 100 ml of zinc oxide nanoparticles at concentrations of 10, 20, and 30 mg/ml. All petri dishes were transferred to an incubator at 37°C for 24–72 hours. After the incubation period, we measured the areas on the agar surface where bacteria were prevented from growing to assess the effectiveness of the nanoparticles in combating bacteria. [30].

3. Results and discussion

a. UV-visible absorption spectrum

The most important method for studying the optical properties of ZnONPs nanoparticles prepared by green synthesis is UV-Vis spectroscopy, which allows us to understand the structural analysis of the produced nanoparticles and the energy band gap. Figure 1 shows the absorption spectrum of ZnONPs nanoparticles, showing a significant absorption peak at a wavelength of 337 nm. The appearance of this value may indicate the biogenic formation of ZnONPs nanoparticles, and it is consistent with these studies [29, 31].

The (E_g) value of the ZnO nanoparticles using the Tauc plot equation (1) for allowed direct transition, plotting $(\alpha h\nu)^2$ against the photon energy. The best-fit line intersects the energy photon axis at $(h\nu)$ equal to zero, which represents the value of the optical energy gap was calculated using the following formula [32]:

$$\alpha h\nu = A(h\nu - E_g)^n \dots\dots 1$$

Figure 1 displays the absorption peak in the UV spectrum of the ZnO nanoparticles at a wavelength of 337 nm, which corresponds to a band gap of 3.3 eV.

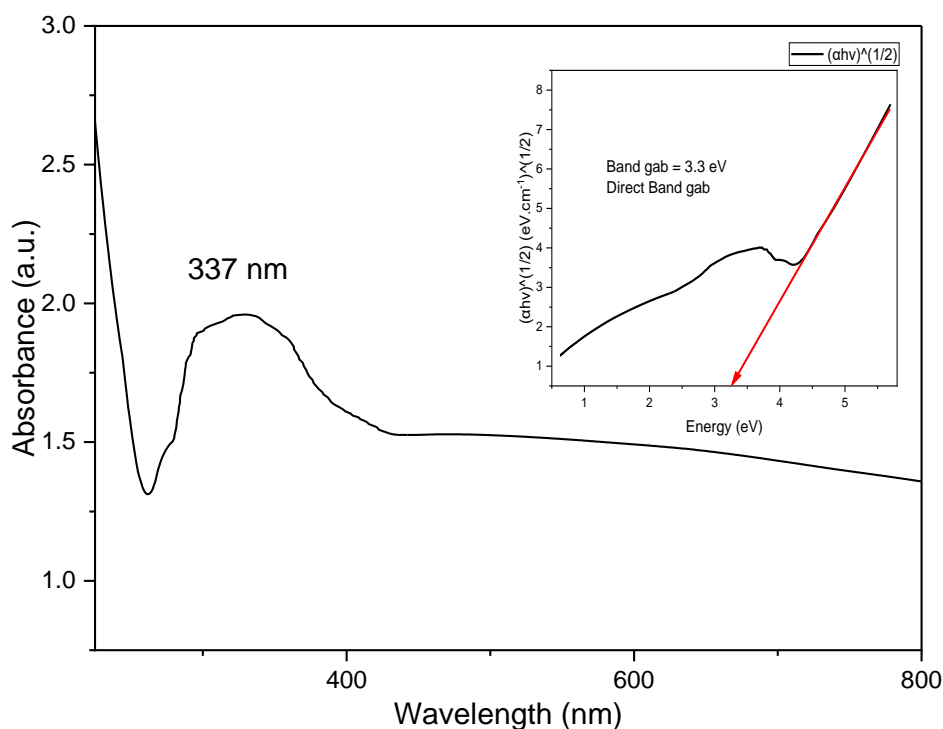


Fig 1. UV-vis absorption spectrum and E_g of ZnONPs.

b. FTIR analysis

The FTIR spectrum of a substance is often used to determine the functional groups that exist within it. Figure 2 shows the FTIR spectrum of the green synthesized ZnONPs. Appear a strong absorption band at 3445 cm^{-1} in the extract, indicating the presence of O-H stretching vibrations and hydrogen-bonded groups from phenolic compounds, alcohol, or water molecules [33]. Alkynes exhibit C≡C stretching vibrations, resulting in spectral peaks at 2927 cm^{-1} and 2314 cm^{-1} . The prominent absorption peaks at 1532 cm^{-1} indicate stretching vibrations of C=O carboxyl or hydroxyl groups on the sample surface. The signal at 1329 cm^{-1} represents the asymmetric stretching vibrations of nitrate ions (NO_3^-) [34]. Stretching vibrations of ZnONPs were discovered at 470 cm^{-1} . Zn-O vibrations are specifically targeted in the $400\text{--}800\text{ cm}^{-1}$ spectral range. The stabilizing and capping processes of the produced ZnONPs may be caused by coordination interactions between ZnONPs and O-H or C=O functional groups. Furthermore, the presence of phenolic and flavonoid chemical groups plays an important role in the reduction process [35].

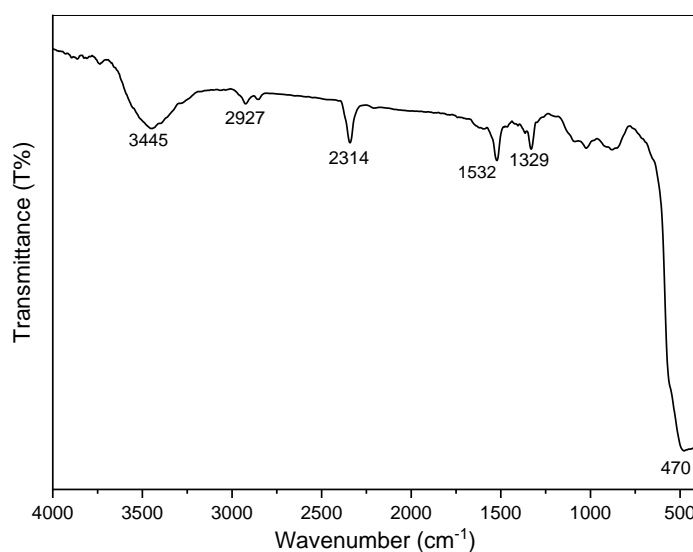


Fig 2. FT-IR spectra of green synthesized ZnONPs.

c. Results and Discussion

The XRD pattern of the ZnONPs particles is used to determine the structure and Miller parameters. Diffraction peaks were seen at 2θ values of 31.89, 34.49, 36.26, 47.68, 56.68, 66.55, 68.15, and 69.28, correspond to the lattice planes (1 0 0), (0 0 2), (1 0 1), (1 0 2), (1 1 0), (1 0 3), (2 0 0), (1 1 2), and (2 0 1). These peaks were identified as the hexagonal phase of ZnONPs particles [36-38].

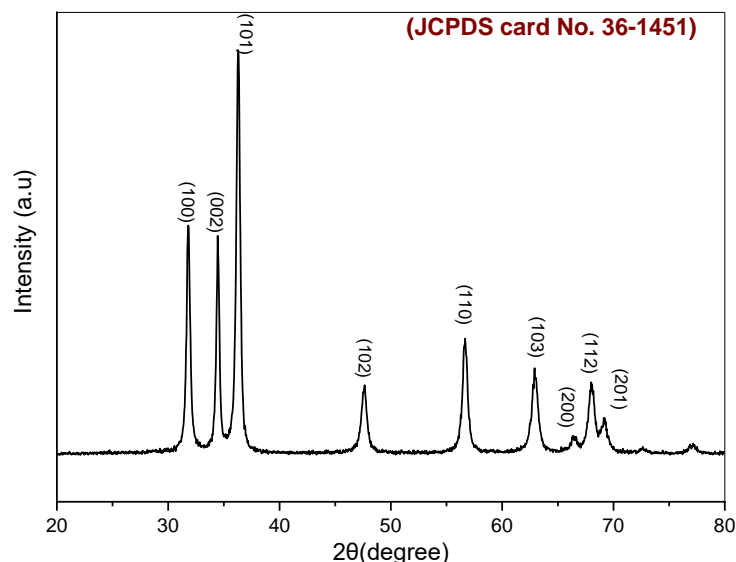


Fig 3. XRD spectra of green synthesized ZnO NPs.

The morphology of ZnONPs was meticulously examined using field emission scanning electron microscopy. This FE-SEM picture illustrates the surface morphology of ZnO nanoparticles. The FE-SEM picture displays individual ZnO nanoparticles amongst various aggregates. Figure 4 (a) illustrates that the particles mostly exhibit a star-like morphology and coalesce into larger aggregates without a distinct structure. The FE-SEM picture shows that the diameters of the ZnONPs range from 31.64 nm. Figure 4(b) shows a strong peak from energy-dispersive X-ray spectroscopy (EDX), which shows the existence of ZnONPs. Energy dispersive X-ray analysis confirmed the elemental composition of zinc and oxygen, resulting from the green manufacturing of ZnONPs. The study produced weight percentages of 14.99% for oxygen and 85.01% for zinc. This spectrum clearly shows the production of ZnONPs.

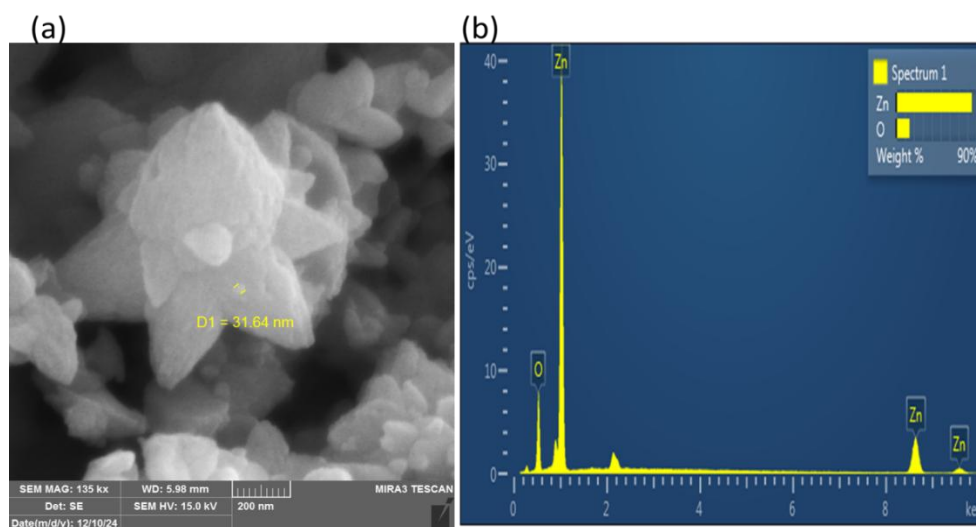


Fig.4: (a) FESEM image of green synthesized ZnONPs.

(b) EDX spectra of green synthesized ZnONPs.

Green synthesis ZnONPs has a star-shaped morphology, which affects their zeta potential largely through surface chemistry and stabilizing processes. Star-shaped nanoparticles have longer edges and facets than spherical or rod-like structures, resulting in more active sites for interactions with stabilizing biomolecules found in plant or microbial extracts. The uneven surface of star-shaped particles enables deeper packing of biomolecules (e.g., proteins, polysaccharides) derived from green synthesis agents such as *Nostoc* sp. cyanobacteria or plant extracts.

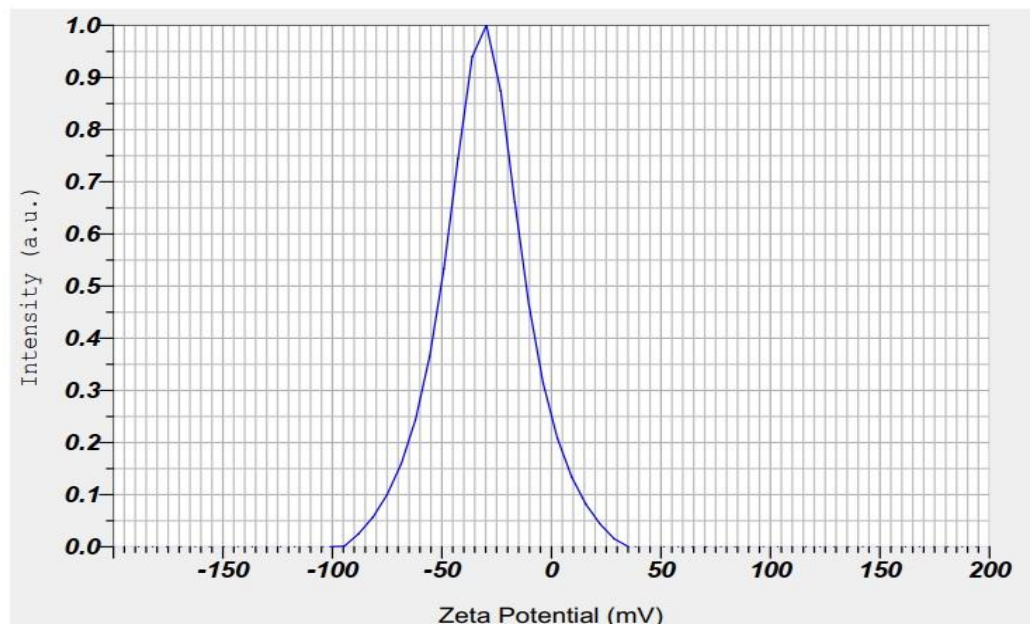


Fig 5: Zeta potential image for sample ZnONPs.

When a zeta potential test is performed, the surface charge of the ZnONPs sample and the stability of the nanoparticles can be determined. The charge value is (-31 mV), which plays a significant role in the distribution of the nanoparticles, as shown in Fig 4. The lack of aggregation may be due to the negative zeta potential between zinc oxide nanoparticles, which leads to interparticle repulsion [39, 40].

3.4 Antibacterial Activity of ZnONPs

ZnONPs reduce the activity of bacterial strains (Gram-negative and Gram-positive) without the use of antibiotics. The figure and table illustrate the effect of ZnONPs on *Escherichia coli* (*E. coli*), *Staphylococcus aureus* (*S. aureus*). The effect of ZnONPs on bacteria is shown in Figure 6. It was concluded that the concentration of the material has a significant effect on the inhibition process, as this can be observed through the concentrations used, and that the inhibition rate increases with increasing concentration of the nanomaterial, and this may be due to: This effect may be attributed to the small size of the nanoparticles, their star-shaped shape, and their possession of a surface charge that contributes to the destruction of the outer shell of the bacteria [41]. Or these reasons may be due to the reactive oxygen species (ROS) radicals that can eliminate bacteria [42]. An increase in H_2O_2 generated from the ZnONPs surface with increasing nanoparticle concentration [42]. These free radicals can cause oxidative damage and apoptosis by interacting with biological components such as proteins, lipids, and DNA. [43].



**Fig 6 : Images represent the inhibition ratio towards sample ZnONPs
For antimicrobial activity.**

Table 1: Bacteria used and the effect of zinc nanoparticles on each type of bacteria.

Bacterial- Gram negative	Concentration (ZnO NPs)	Inhibition zones
<i>E. coli</i>	10%	12
	20%	20
	30%	28
Bacterial- Gram-positive	Concentration (ZnO NPs)	Inhibition zones
<i>S. aureus</i>	10%	10
	20%	18
	30%	23

4. Conclusions

ZnONPs were successfully produced using an extract from *Bacopa monnieri* leaves. UV-Vis spectrum, XRD, FE-SEM, and zeta potential studies all corroborated the produced particles' crystal structure, stability, and nanoscale dimensions. The ZnONPs produced from *Bacopa monnieri* leaf have high antibacterial activity, which is related to bioactive chemicals that act as a covering agent. Furthermore, the tapered architecture of the produced nanoparticles enhances their antibacterial activity.

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Author Contributions

Each author made significant contributions to the study's concept and design. [Nawar Jaber Hussein Al-Asadi] and [Dhulfiqar S. Mutashar] prepared the materials, gathered the data, and assessed the results. All writers provided insights into prior versions of the article, with [Dhulfiqar S. Mutashar] in charge of drafting the first version. The final document was thoroughly examined and approved by [Nawar Jaber Hussein Al-Asadi].

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