ORIGINAL ARTICLE

Antibacterial Activity of Zinc Oxide Nanoparticles Synthesized by Green Eichhornia Crassipes Extract Method

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Abstract

Purpose: Zinc Oxide Nanoparticle (ZnO NPs) production has become more common because of the benefits of the green strategy, which include its ease of use, environmental friendliness, and cheap operating costs. In order to accomplish the goal of green chemistry, the green synthesis process also uses safe solvents like water and ethanol. ZnO NPs are among the metal oxide-NPs used in antibacterial and bioremediation applications.

Materials and Methods: Energy Dispersive X-ray spectroscopy (EDX), Fourier Transform Infrared spectroscopy (FT-IR), X-Ray Diffractometer (XRD), and Field Emission Scanning Electron Microscopy (FESEM) were used to analyze the green-produced ZnO NPs.

Results: ZnO nanoparticles have an average size of 22.89 nm, which is corroborated by FESEM pictures, indicating that the particles are tiny. The excellent purity of ZnO NPs has been confirmed by EDX data. The antibacterial activity of ZnO NPs was assessed against a few dangerous pathogens. Zinc oxide nanoparticles were shown to have an interesting antibacterial action against both gram-positive and gram-negative bacteria at micromolar concentrations because they exhibit the maximum diameter of inhibition zone at concentrations 100 mg/ml of S. aureus, E. coli, K. pneumonia, Acintobacret spp, S. fecalis reaching (27,19,18,17, and 14) mm, respectively while S. pneumonia were resistant.The ZnO NPs recorded at a concentration of 12.5 mg/ml lowest areas of the inhibition zone against the same isolates reaching (16, 11, 11, 12, and 10) mm while S. pneumonia were resistant, respectively, as well.

Conclusion: ZnO NPs, since they have excellent antibacterial properties, and are biocompatible, they will open up a new line of inquiry for antibacterial agent research because they are stable, nontoxic, and harmless.

Keywords: Antibacterial Activity; Zinc Oxide Nanoparticles; Green Eichhornia Crassipes.

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1. Introduction

Concerns about issues like water pollution, climate change, depletion of natural resources, human health, and other concerns have led to an increased focus on developing environmentally friendly products and processes in recent years [1]. Nanotechnology has several uses in the medical and pharmaceutical industries. Additionally, it is utilized in information technology and civil engineering, where it is employed in the development of nanoscale additives for oil well cementation and drilling muds to increase the withdrawal coefficient from reservoirs, in the manufacturing of nanocatalysts for the petrochemical, oil, and gas industries, in the production of nanocoatings that are resistant to heat, corrosion, eclipse, abrasion, and friction, as nanolubricants to improve energy efficiency, in the creation of nanosensors and nano biosensors, to reduce pollutants, and to develop green technologies [2]. Highly valued, metal oxide nanoparticles are used in sunscreens, cosmetics, optical, electrical, mechanical, and gas sensors [3]. Zinc oxide is the most commonly utilized metal because of its numerous positive properties, such as its large surface area, ease of sorption removal, strong contamination selectivity at detectable concentrations, nontoxicity, and ease of production [4]. Increasing the material's surface area, decreasing its particle size, and altering its shape can all help to improve these properties since the advent of nanotechnology [5, 6]. Zinc oxide is an extremely valuable material for many different industries because of its many properties, which are further enhanced by its nanoscale production [7]. Numerous physical and chemical processes have been used to produce nanoparticles. Since many of the current methods have disadvantages, like the use of dangerous chemicals, toxic solvents, high energy consumption, etc., the synthesis of metal nanoparticles needs to be done in an environmentally friendly manner. Environmentally acceptable material synthesis procedures must be developed in order to increase the biological applications of materials [8]. Since the procedure uses mild reaction conditions, an environmentally friendly aqueous medium, and renewable plant extracts, it is safer than other dangerous approaches [9]. Eichhornia crassipes is a freely growing aquatic perennial plant. It lowers the oxygen level of the water, reduces water circulation, creates obstructions, removes fish, and causes problems in many countries. This floating macrophyte is loaded with toxic metals, some of which are essential to plant growth. Water hyacinth grows quickly in areas with high nutrient levels, such as nitrogen and phosphorus [10]. It's a well-known, eye-catching water plant. It features lovely lilac to blue flowers as well as oval to round leaves with flexible coiled petioles. A mature plant has rhizomes, stolons, long, hanging roots, leaves, inflorescences, and fruit. Because of the air-filled sacs inside of them, the leaves and stems float on the water's surface. The plant can grow up to a maximum height of 1 m, with an average height of 40 to 60 cm. The plant's inflorescence can support six to ten lily-like flowers, each with a diameter of four to seven centimeters. Because it can float, the plant can grow for extended periods in unfavorable conditions like moist sediments [11].

This work uses an eco-friendly, straightforward, and cost-effective technique to synthesize ZnO NPs using plant extract from Eichhornia crassipes. Additionally, ZnO NPs' antibacterial efficacy against a few harmful microorganisms was evaluated.

2. Materials and Methods

2.1. Preparation of ZnO Nanoparticles

Zinc oxide nanoparticles were prepared using (Eichhornia water hyacinth) leaves extract denoted as: (ZnO NPs).

2.2. Preparation of Eichhornia Aqueous Extract

Eichhornia leaves are collected from the location at Tigress riverside and then washed using tap water. they are devoid of any dirt that is suspended after which it was dried and repeatedly cleaned with distilled water. It is ground and kept out of the moisture with an electric grinder. In a flask, 400 ml of deionized water was added to 5 grams of Eichhornia powder, and the mixture was boiled for 30 minutes at 70 degrees Celsius. After cooling to ambient temperature, the extract was filtered, centrifuged for four minutes at 1600 rpm to remove any remaining biomaterials, and then kept at room temperature until needed.

2.3. Preparation of Zinc Oxide Nanoparticle

After dissolving (0.4) gm of zinc chloride in 400 ml of deionized water and stirring continuously, (10) ml of the plant extract was gradually added, stirring constantly at room temperature until the color changed to light green.

The solution was then heated to 70 °C, and the mixture's pH was adjusted by adding a few drops of sodium hydroxide solution. The mixture was then filtered, and repeatedly cleaned with deionized water, and added 100% ethanol to eliminate any remaining impurities. It was then dried in an oven set to 80°C for two hours in order to produce white particles known as zinc oxide nanoparticles. The processes and flow diagram for making zinc oxide nanoparticles with an extract from Eichhornia leaves are shown in Figure 1.

Figure 1. The steps of the preparation of zinc oxide nanoparticles using Eichhornia aqueous extract

2.4. Preparation of Different Concentrations of Prepared Zinc Oxide Nanoparticles

. In one milliliter of deionized water, dissolve 100 mg of (ZnO NP) to get a concentration of 100 mg /ml, then dilute it to a concentration of (50, 25, and 12.5) mg/ml as given below:

. Take half a milliliter of the concentration of 100 mg/ml with half a milliliter of deionized water, to get the concentration (50 mg\ml).

. (25 mg\ml) was obtained by adding half a milliliter of deionized water to half a milliliter of a solution of concentration 50 mg\ml.

. The (12.5 mg\ml) was prepared by adding half a milliliter of deionized water to half a milliliter of a concentrated solution of 25mg\ml.

2.5. ZnO.NPs' Antibacterial Activity was Assessed Using the Agar Well Diffusion Method

1- In order to prepare the suspended bacteria and place them in tubes containing brain heart infusion broth to activate the bacteria, many bacterial colonies were carried via a loop. The tubes were incubated at (37°C) for eighteen to twenty-four hours. The conventional MacFarland solution (1.5 x 108 CFU/mL) was contrasted with the suspended bacteria. Following that, the suspended bacteria were distributed using a sterile brush over Muller Hinton agar plates, and the plates were then allowed to air dry for a while.

2- Using a sterile corn borer, holes with a diameter of 5 mm were created in the culture media.

3- Using a micropipette, 100 µl of a zinc oxide nanoparticle solution was applied to each hole separately. Next, incubate the dishes for twenty-four hours at 37 °C.

4- The diameter of the inhibition zone surrounding each hole was measured to assess the efficacy of each concentration.

3. Results & Discussion

3.1. Characterization of the Adsorbents X-Ray Diffraction

The crystalline phase of the nanoparticles was verified and determined using X-ray diffraction. The X-ray

diffraction patterns of the nanoparticles are displayed in Figure 2, and the data of the strongest three peaks for zinc oxide synthesized using extract from Eichhornia leaves are displayed in Table 1. The Deby-Sherrer formula, shown below, was used to compute the particle sizes [12] (Equation 1).

$$D = \frac{0.9\,\lambda}{\beta \cos\theta} \qquad (1)$$

In which: **D**: is the crystallite size. **λ**: is the wavelength of radiation. **θ**: is the Bragg's angle. **β**: is the Full Width at Half Maximum (FWHM).

The prepared zinc oxide has an estimated particle size of (17.36) nm—the produced ZnO NPs are nanocrystalline, as evidenced by the presence of strong peaks in the XRD sample and particle sizes smaller than 100 nm.

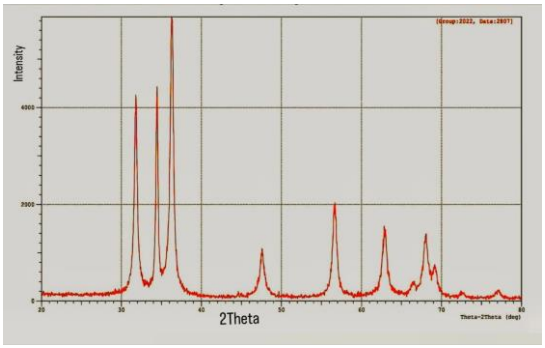

Figure 2. XRD pattern of prepared Zinc oxide nanoparticles

Table 1. The strongest three peaks in XRD of ZnO NPs

No.	2θ/(deg)	d /(Å)	FWHM/ (deg)	Intensity/ (counts)
1	36.3029	2.47264	0.48170	919
2	31.8061	2.81121	0.45560	594
3	34.4487	2.60136	0.35920	584

3.2. Field Emission Scanning Electron Microscopy

The morphology of ZnO NPs was assessed by FESEM measurement. Figure 3 depicts pictures of the ZnO nanoparticle sample made with extract from Eichhornia leaves. Images a, b, c, and d show the ZnO NPs at 1 µm, 2 µm, 500 nm, and 200 nm, respectively. The Figure demonstrates the presence of several nanotubes together with the spherical shape of the nanoparticles. Since the distribution of nanoparticles is visible throughout the whole surface, the high-resolution photographs vividly

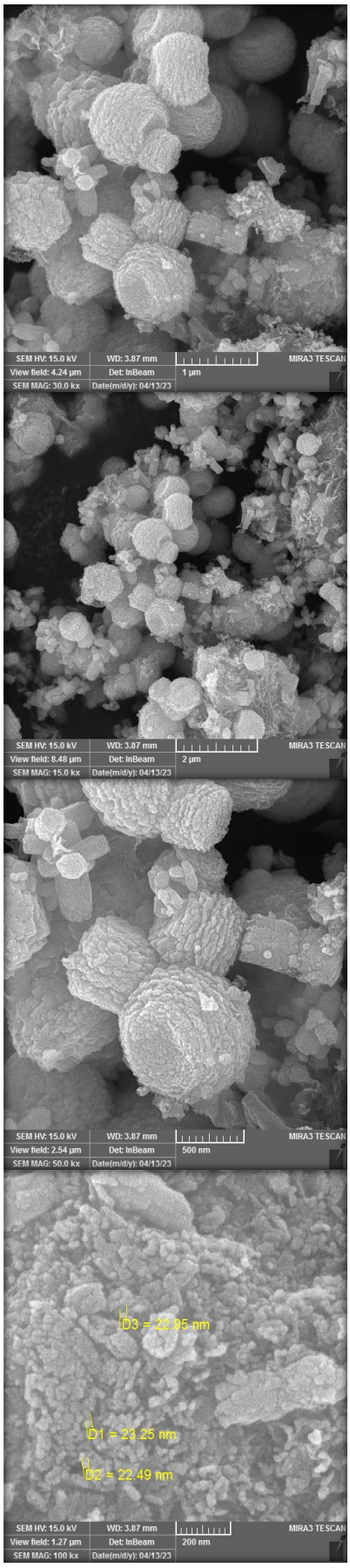

Figure 3. FESEM images a, b, c, and d show the prepared zinc oxide nanoparticles at 1 µm, 2 µm, 500 nm, and 200 nm, respectively

display the surface features. It is verified by FESEM pictures that the particles are tiny; ZnO nanoparticles have an average size of 22.89 nm.

3.3. Energy Dispersive Spectroscopy (EDX)

The elements contained in the ZnO NPS samples were identified using EDX. The results showed that the sample was made up of Zn (50.25%) and O (49.75%), as shown in Figure 4 and Table 2. This result has validated the excellent purity of ZnO-NPs. Similar results were also obtained in earlier research by Bandar A. Al-Mur (2023) [13], who showed that the prominent peaks associated with Zn and O in zinc oxide nanoparticles, as well as the ratio of zinc (49.25%) to oxygen (50.75%), validate the high level of purity of the ZnO NPs that were generated.

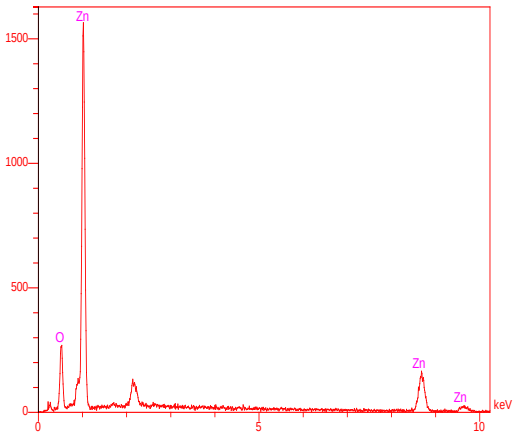

Figure 4. EDX spectrum of prepared zinc oxide nanoparticles

Table 2. Quantitative EDX results for elemental composition of ZnO-NPs

Element Name	Atomic Weight %
Zn	50.25
O	49.75
	100.00

3.4. Fourier Transform Infrared (FT-IR) Spectra Analysis

The ZnO NPs generated with the extract of Eichhornia leaves are shown in Figure 5 with their FT-IR spectra. Functional group bands were visible at 3431.61, 2925.85, 1509.06, 1384.66, 1047.12, 837.04, 709.77, and 479.00

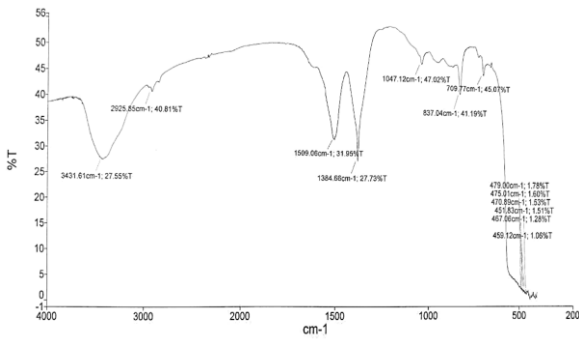

Figure 5. FTIR spectrum of prepared ZnO nanoparticles

cm^{-1} in the produced ZnO NPs. A strong wide band at 3431.61 cm^{-1} resulted from stretching (O–H) groups, while the absorption band at 2925.85 cm^{-1} was caused by vibrational stretching of the CH group. C = C stretching band vibration may be seen at 1509.06 cm^{-1}. 1384.66 cm^{-1} was the frequency of the -C-H-bending vibration band. It was determined that the =C-H groups' vibrational bending was responsible for the peaks at 1047.12 and 837.04 cm^{-1}. The band at 709.77 cm^{-1} was associated with the bending vibration of the O-H group. Lastly, the metal-oxide (ZnO stretching vibrations) absorption peak at 479.00 cm^{-1} is indicated. These findings were found to agree with previous studies of (Bandar A. Al-Mur,2023) [13].

3.5. Antibacterial Activity of ZnO NPs Against Pathogenic Bacteria

The results are displayed in Table 3 and Figure 6**.** Images a, b, c, d, e, and f show the antibacterial activity of ZnO NPs against gram-positive and gram-negative bacteria isolated from various sources. E. *coli*, K. *pneumonia*, P. *aeruginosa*, A*cintobacret spp*. S. *aureus*, S.*pneumonia*, and S. *fecalis* by agar well diffusion method at concentrations of (100, 50, 25, and 12.5) mg/ml, respectively. ZnO NPs showed the highest diameter of the inhibition zone at a concentration of 100 mg/ml of *S. aureus, E. coli, K. pneumonia, Acintobacret* spp, *S. fecalis* reached (27, 19, 18, 17, and 14) mm, respectively while *S.pneumonia* were resistance. The ZnO NPs recorded at a concentration of 12.5 mg/ml lowest areas inhibiting zone in comparison to the same isolates reaching (16, 11, 11, 12, and 10) mm while *S.pneumonia* were resistant, respectively, as well.

Table 3. Antibacterial activity of ZnO NPs on bacterial growth

Isolates	Average inhibition zone diameter (mm)			
	Concentration 12.5 mg/ml	Concentration 25 mg/ml	Concentration 50 mg/ml	Concentration 100 mg/ml
S. aureus	**16**	**18**	**23**	**27**
E. coli	**11**	**14**	**18**	**19**
K. pneumoniae	**11**	**14**	**16**	**18**
***Acintobacret* spp.**	**12**	**14**	**16**	**17**
S. fecalis	**10**	**11**	**12**	**14**
S.pneumonia	**R**	**R**	**R**	**R**

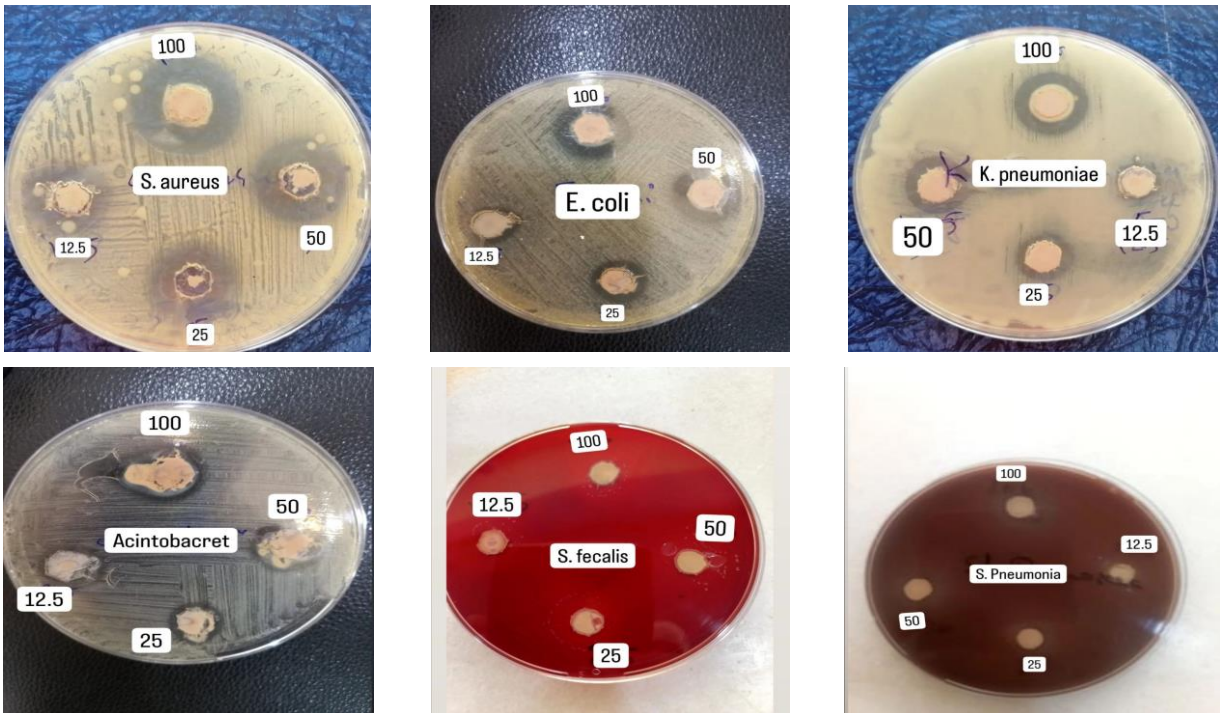

Figure 6. Images a, b, c, d, e, and f show Antibacterial activity of ZnO NPs against E. *coli*, K. *pneumonia*, P. *aeruginosa*, A*cintobacret spp*. S. *aureus*, S.*pneumonia*, and S. *fecalis* by agar well diffusion method at concentrations of (100, 50, 25, 12.5) mg/ml, respectively

4. Conclusion

The green synthesized ZnO NPs from Eichhornia crassipes leaves extract demonstrated bactericidal activity at micromolar concentrations against both gram-positive and gram-negative microorganisms. It shows the highest diameter of inhibition zone at a concentration of 100 mg/ml of S. aureus, E. coli, K. pneumonia, *Acintobacret spp*, S. fecalis reaching (27, 19, 18, 17, and 14) mm, respectively while *S.pneumonia* were resistant . The ZnO NPs recorded at a concentration of 12.5 mg/ml lowest areas of the inhibition zone against the same isolates reaching (16, 11, 11, 12, and 10) mm while *S.pneumonia* were resistant, respectively. Since they have excellent antibacterial properties, and are biocompatible, they will open up a new line of inquiry for antibacterial agent research because they are stable, nontoxic, and harmless.

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