Research Journal of Chemical Sciences ____ISSN 2231-606X
Vol. **13(3),** 16-22, October **(2023)** *Res. J. Chem. Sci.*

Green Synthesis, Characterization, and the Antibacterial Activity Study of Zinc Oxide Nanoparticles Using Lemon Peel

Kamaluddeen S.K. and Ismail A.*[*]
Department of Applied Chemistry Federal University Dutsin-Ma, Katsina State, Nigeria
aismail@fudutsinma.edu.ng

Available online at: www.isca.in, www.isca.me
Received 15th May 2023, revised 16th June 2023, accepted 1st August 2023

Abstract

The widespread use of synthetic materials for the synthesis of nanoparticles has led to an increase in environmental pollution. Hence, green synthesis has recently emerged as a sustainable alternative as it utilizes biodegradable materials like lemon peels. The present study aimed to examine the green synthesis, characterization, and antibacterial activity of zinc oxide nanoparticles using lemon peels as a natural precursor. The nanoparticles were synthesized using a simple, cost-effective, and environmentally friendly method. The synthesized nanoparticles were characterized using various techniques, such as UV-Vis spectroscopy, FTIR, and Particles Size Analysis using Zetasizer Nano series. The results indicated that zinc oxide nanoparticles with a size average of 48.77nm were synthesized using the method employed. Antibacterial studies of the synthesized zinc oxide nanoparticles revealed excellent antibacterial activity against the gram-positive and gram-negative bacteria tested, Staphylococcus aureus *and* Escherichia coli*, respectively. The zones of inhibition for the gram-positive bacteria were 13mm at 20mg/ml and 15mm at 40mg/ml, while those for the gram-negative were 10mm at 20mg/ml and 12 mm at 40mg/ml. The study demonstrated the potential of lemon peel extract as a green, sustainable, and effective source for the synthesis of zinc oxide nanoparticles with excellent antimicrobial activity. The results of this study contribute to the development of eco-friendly and sustainable nanotechnology with broad biomedical applications.*

Keywords: Nanoparticles, Nanowires, Nanorods, Nanoscience, Nano-materials, top-down, and bottom-up.

Introduction

Nanotechnology has the promise of several advancements and serves as a pillar for continuing research in a variety of sectors. It is defined as the process utilized in the creation of applications using engineered materials with at least one nanometer-scale dimension[1]. Nano-materials are structural components with a size range of 1-1000 nanometers and are made up of subgroups of particles with a size range of 1-100 nanometers, which are referred to as nanoparticles (NP)[1]. Nanoparticles come in a variety of shapes and configurations, including conical, spiral, flat, hollow, and more, and they have physical attributes that are superior to those of their bulk counterparts, including enhanced stability, higher mechanical strength, and more.

They are frequently employed, demonstrating that they possess characteristics that are helpful in a number of different contexts[2].

Types of NPs: NPs are categorized in accordance with their size, activity, shape, and material composition. This is a brief description of how NPs are grouped according to the substances they are made of. You can categorize nanoparticles as organic or inorganic. Although organic nanoparticles (NPs) are divided into two groups: polymeric NPs and biomolecule-derived Nanoparticles[3].

General Attributes: These are the characteristics that practically all types of nanomaterials share with nanoparticles. In fact, many applications of NPs are built on these features.

Nanoparticles' Sizes: The characteristics of the NPs and the applications for which they are designed depend critically on their size. The synthesis technique and reaction parameters have a significant impact on the size of the NPs, which in turn influences their function in many applications. The iron oxide nanoparticles (iron oxide NPs), which were created hydrothermally, showed high crystallinity and a combination of the crystalline phases magnetite and maghemite. The proportion of the maghemite phase to magnetite, however, increased as the NP size grew until it was entirely pure for the 123.44nm sized particles[4].

The NPs' Shape: In addition to NP size, in many technical applications, shape and structure are essential. Controlling the synthesis settings is a challenge to regulate the shape of the NPs has been partially and largely solved. As a result, the NP of the same material in various forms may be created to handle particular applications. The nanoscience have a well-established research area known as shape-controlled synthesis of NPs[5].

Area of the NPs' surfaces: The high surface areas of NPs, which are important for catalysis, adsorption, electrochemical reactions, reactivity, and other processes, are the reason why they are used in so many different applications[6].

Characteristics Unique to Particular NP kinds: Not all NP kinds may exhibit these characteristics since several properties, including optical, magnetic, and antibacterial capabilities, are connected with individual NPs. certain specific NP traits will be briefly covered.

Light Attributes: The scientific community is very interested in the metal NPs' optical characteristics, and their application dates back to the middle of the 1800s[7].

Magnetic Qualities: A unique class of NPs called magnetic NPs can be controlled by magnetic fields[7].

Anti-microbial characteristics: Due to their remarkable antibacterial qualities, many NPs have been employed as antibacterial agents. Its great biocompatibility is the reason for, the cytotoxicity and interactions of ZnO nanoparticles and materials with cells, tissues, and biomolecules have been studied. They interact with bacterial cells by chemical and physical mechanisms and have high antibacterial potential. Nanoparticles interact physically to create antimicrobial effects by rupturing cell envelopes, internalizing cells, or causing mechanical damage while also producing H_2O_2, a chemically released Zn^{2+} ion and a photo-induced reactive oxygen species. According to a review, the impact of various ZnO nanomaterials morphologies is also discussed[8].

For the correct activation and distribution of NPs, numerous polymeric materials-aside from metal and metal oxide Nanoparticles are used in conjunction[9]. According to reports, a significant portion of hospital acquired illnesses are transferred by contaminated surfaces or catheters, which are frequently constructed of plastic. Applications in biomedicine and healthcare involve a range of polymers. These polymer matrices may benefit from the addition of anti-microbial Nanoparticles to slow the transmission of pathogens through the polymers[10].

Green Nanoscience and Nanotechnology: Today, green nanoscience and nanotechnology are exploding. This is where scientists and chemists are concentrating their efforts, as well as their applications. Nanoparticles are particles with a size between one and one hundred nanometers[11]. Constructing nanoparticles (NPs) with varied chemical compositions using experimental approaches, dimensions, as well as form, and characteristics are essential components of nanoscience. Researchers have recently attempted to develop biological nanoparticles production methods as an alternative to chemical or physical approaches. Biological approaches for producing NPs are believed to be safe and environmentally friendly; they are also cost effective and ensure that harmful substances are completely eliminated[12].

Syntheses of nanoparticles: Physical, chemical and biological processes are used to synthesize NPs, and there are two categories for these methods: top-down and bottom-up techniques[5].

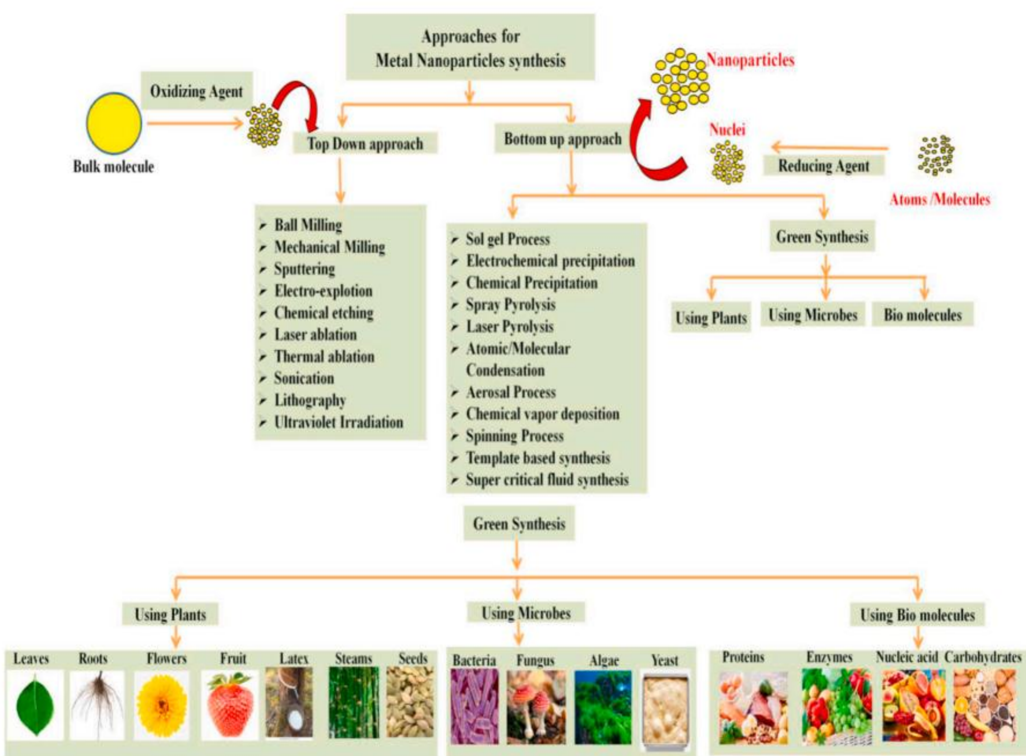

Figure-1: Flow chart showing metal oxides nanoparticles synthesis using various source[13].

ZnO Nanoparticles: Zinc oxide has the formula ZnO and is an inorganic compound. This is a white powder that is water insoluble. Using novel atomistic potentials, the structure of ZnO nanoparticles was explored computationally. For many nanoparticle applications, mechanical features such as internal stress and adhesion characteristics are necessary to preserve patterning accuracy and durability. ZnO is a hexagonal wurtzite with a hexagonal structure. ZnO crystallizes into hexagonal wurzite and Zinc blend in most cases[14].

ZnO nanoparticles have been discovered to be skin-friendly, with antimicrobial and dermatological properties[15]. Sunscreen contains ZnO nanoparticles, which act as UV blockers. Due to the generation of reactive oxygen species on the surface of nanoparticles, ZnO nanoparticles have a high resistance to microorganisms[15].

ZnO Nanoparticle Applications: Recently, zinc oxide has gained recognition as an important semiconductor with a wide band gap (3.37eV) and a high exciton-binding energy (60 meV)[14,15]. Because of its unique optical and electrical properties, ZnO has been chosen as a multitasking metal oxide[15]. ZnO nanoparticles have been widely used in a variety of industries, including solar cells, UV light-emitting devices, photocatalysts, gas sensors, pharmaceuticals, and cosmetics[16]. Furthermore, metal nanoparticles have nontoxic, self-cleansing, surface Plasmon resonance properties in the UV-visible region[17]. ZnO nanoparticles are also skin-friendly, with antimicrobial and dermatological properties[15]. Sunscreen contains ZnO nanoparticles, which act as UV blockers. Due to the generation of reactive oxygen species on the surface of nanoparticles, ZnO nanoparticles have a high resistance to microorganisms[15].

Synthesis of Zinc Oxide Nanoparticle: There are several physical and chemical procedures for synthesizing large quantities of ZnO nanoparticles in a short period of time. It has been reported, that chemical precipitation, sol gel, solvothermal/ hydrothermal, electrochemical, and photochemical reduction methods are among the simple-solution based methods that are preferred. Green synthesis methods are also used to make ZnO nanoparticles from plant leaf extract, bacteria, fungi, and enzymes. Because no toxic chemicals are used in green synthesis methods, they are environmentally friendly and suitable for pharmaceutical and other biomedical applications[17].

Materials and methods

Materials: i. Lemon Peel, ii. zinc nitrate [Zn $(NO_3)_2 6H_2O$], and iii. De-ionized water were used.

Methods: Preparation of extract: A number of lemons were collected and washed, and the peels were removed using a clean knife. The removed peels were air dried and then converted into powder form by crushing them using a mortar and pestle. 2-5g of the lemon peel powder and 100 ml of deionized water were taken into a 250-ml beaker and stirred for 2 hours. For 60 minutes, the mixture was heated to 90 degrees Celsius in a water bath. After filtering the combination with Whatman filter paper, it was placed in a glass container with an inert environment for storage.

Synthesis of ZnO NPs: The synthesis of ZnO-NPs began by placing 2.50g of zinc nitrate in 50ml of lemon peel extract. The mixture was stirred for 60 minutes and then placed in a water bath at 60°C until it presented a thick consistency due to the evaporation of water. For 60 minutes, the mixture was heated to 400°C. The mixture was then ground in a mortar to create a white-cream powder.

Characterization: The synthesized nanoparticles (ZnO) were characterized by the following analytical techniques: FTIR, UV-Vis, and particle size analysis using the ZetasizerNano series.

Antibacterial Activity: The created substance (ZnO nanoparticles) was dissolved in DMF and put through the paper disc diffusion procedure for in vitro testing. All items were disinfected using this method in a hot oven. *Staphylococcus aureus*, a gram-positive bacteria, and *Escherichia coli*, gram-negative bacteria were sub-cultured and incubated in nutrients for 6 to 8 hours. The Nutrient Agar plates were equally swabbed with the viable bacterial cells. Using sterile forceps, the 9.0 mm-diameter paper discs were submerged in the various test samples (concentrations of 20 and 40mg/ml), drained, and then put in the agar plates. The plates were incubated for 48 hours at 37 degrees Celsius. After the incubation period, the zones of inhibition were measured in mm.

Results and discussion

Results of Characterizations: Fourier Transform Infrared spectroscopy (FTIR): The outcome (FTIR spectrum) of ZnO nanoparticles is shown below.

UV-Vis of ZnO NPs: The result (UV-Vis spectrum) of ZnO nanoparticles is shown below.

Particle Size Analyses Results of ZnO NPs: The results of the particle size analyses of ZnO nanoparticles are shown below.

Table-1: Results of ZnO NPs Size Distribution by Intensity.

Z-Average (d.nm)	48.77
Pdl	0.399
Intercept	0.908

Table-2: Data/ Results of ZnO NPs Size Distribution by Intensity.

Peak	Size (d.nm)	% Intensity	St Dev (d.nm)
Peak 1	97.61	76.4	107.0
Peak 2	12.19	11.6	3.466
Peak 3	2.148	6.3	1.392

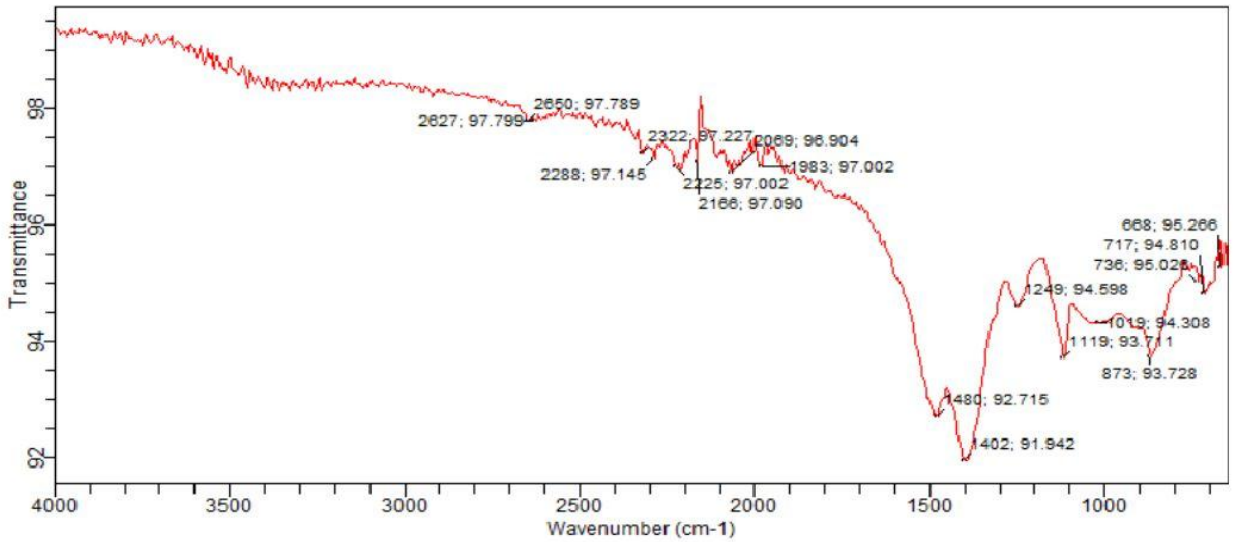

Figure-2: FTIR spectrum of ZnO Nanoparticles.

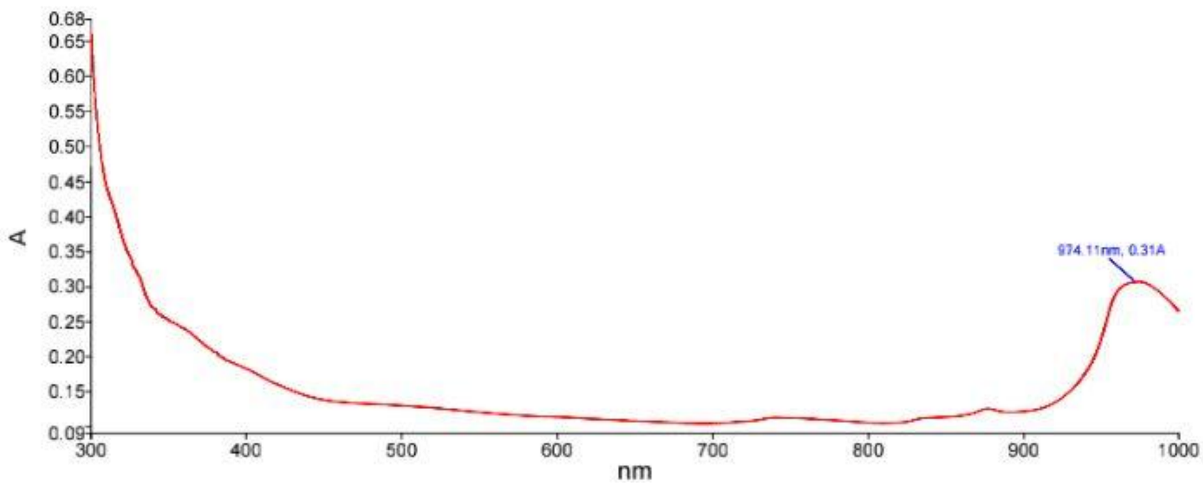

Figure-3: UV-Vis spectrum of ZnO NPs.

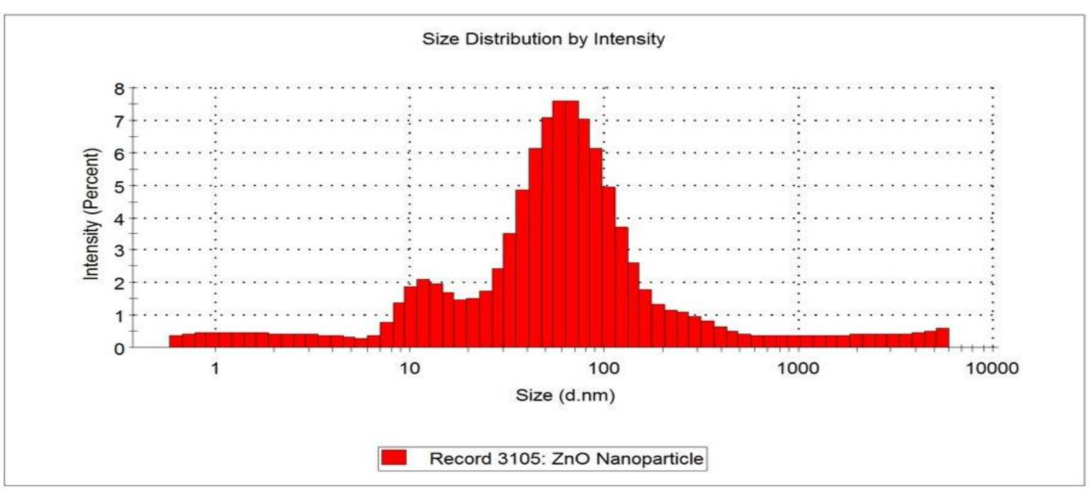

Figure-4: ZnO NPs Size Distribution by Intensity.

Table-3: Results of ZnO NPs Size Distribution by Volume.

Z-Average (d.nm)	48.77
Pdl	0.399
Intercept	0.908

Table-4: Data/ Results of ZnO NPs Size Distribution by Volume.

Peak	Size (d.nm)	% Intensity	St Dev (d.nm)
Peak 1	0.8812	18.2	0.3996
Peak 2	10.52	40.8	3.072
Peak 3	45.28	40.9	37.14

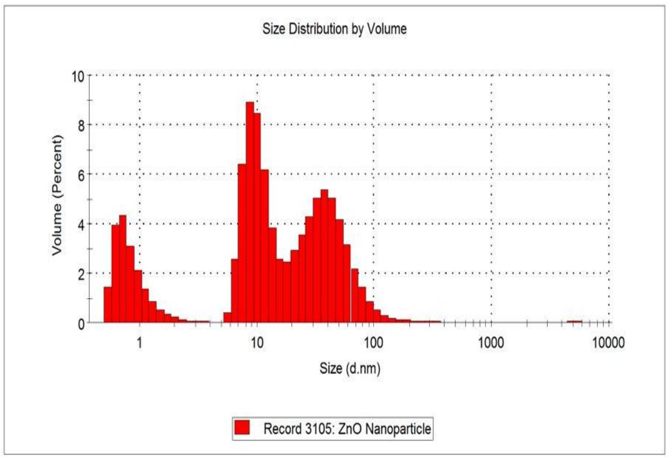

Fig-5: ZnO NPs: Size Distribution by Volume.

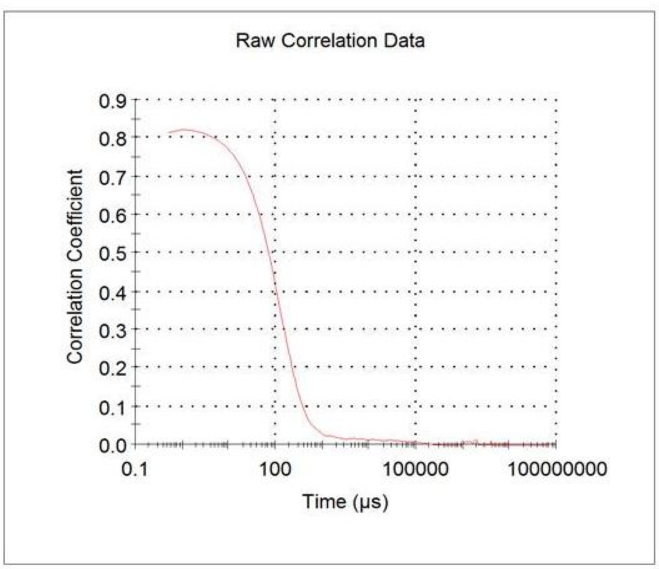

Figure-6: ZnO NPs: Result Quality Report (Raw Correlation Data).

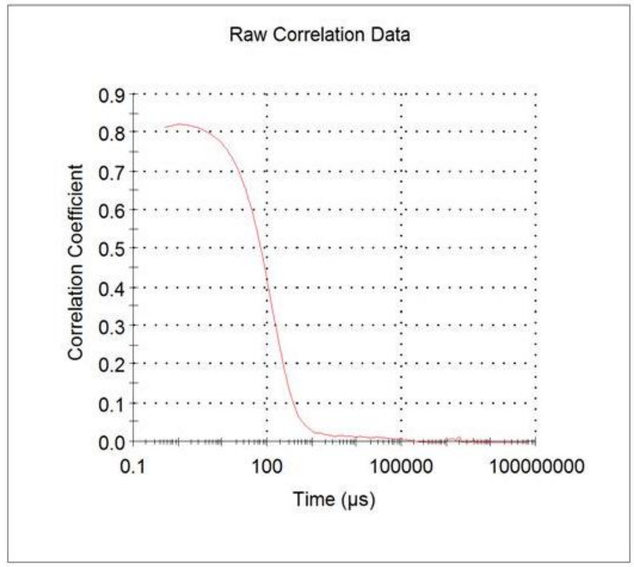

Figure-7: ZnO NPs: Result Quality Report (Size Distribution by Intensity).

Discussion: ZnO nanoparticles were synthesized using lemon peel extract through the green method of synthesis. White-cream colored powdered ZnO nanoparticles was obtained. However, it was characterized by the following analytical techniques: FTIR, UV-Vis, and particle size analysis using the Zetasizer Nano series. Afterwards, the antibacterial activity of the confirmed, synthesized nanoparticles (ZnO) was checked by the paper disk diffusion method.

Fourier Transform Infrared spectroscopy (FTIR): The FTIR spectroscopy was utilized to identify potential biomolecules that may be involved in the reduction and capping of MgO and ZnO NPs as well as the vibrational frequency of the molecules' stretching and bending modes. The figure of the FTIR depict ZnO nanoparticles' spectrum, and the analysis was done in the frequency range between 1000 and 4000cm^{-1} at ambient temperature (Figure-2). In the 1500-700cm^{-1} range, many bands could be identified; these correlate to the sample's organic components. The C-H group could be given credit for the band that was seen at 873cm^{-1}. The C-C stretching of aromatic rings was the cause of the absorption peaks at 1402cm^{-1}. The spectra showed a band at about 668cm^{-1}; this signal is the distinctive Zn-O bond signal, confirming that the substance is zinc oxide. This result is in line with what was obtained by the research study carried out by Luque[18].

UV-Visible Absorption Spectroscopy: The most popular analytical method for describing the electronic structure of the optical band gap of the nanomaterial is UV-Vis absorption spectroscopy. The analysis was performed between 300 and 1000 nm for the Zinc Oxide nanoparticles. The absorption spectrum of ZnO nanoparticles was examined (Figure-3). The spectrum of the produced 48.77 nm ZnO nanoparticles displays a peak at 974.11 nm, 0.31A.

Particle Size Analysis using the Zetasizer Nano series: Three properties of particles are measured using the Zetasizer Nano Series. These three fundamental parameters are particle size, zeta potential, and molecular weight. In this research, it was used to measure particle sizes. The produced nanoparticles' average size was discovered to be 48.77nm for ZnO nanoparticles. In Figure-4, 5, and, 6, there are more details about the results.

Antibacterial Activity: The antibacterial activity investigation of the synthesized nanoparticles (ZnO) against the gram-positive *Staphylococcus aureus* and gram-negative *Escherichia coli* bacteria was carried out by the paper disk diffusion method. The zones of inhibition of the synthesized nanoparticles were found and recorded in the table below.

Table-5: ZnO NPs: Zones of Inhibition.

Bacteria species	Concentration (mg/mL)	Zones of Inhibition (mm)
Staphylococcus Aureus	20	13
Staphylococcus Aureus	40	15
Escherichia coli	20	10
Escherichia coli	40	12

The gram-positive bacterium, *Staphylococcus Aureus* was more sensitive than the gram-negative bacterium, *Escherichia Coli*. The highest zone of inhibition found with *staphylococcus aureus* to be 15mm at 40mg/mL which is greater than the zone of inhibition found with *Escherichia Coli* to be 12mm at 40mg/mL.

Conclusion

In conclusion, the green method of synthesis was employed to produce Zinc oxide nanoparticles. The synthesized nanoparticles (ZnO) were characterized by various techniques including FTIR, Particle Size Analysis, and UV-Visible which confirmed their functional groups, sizes in nm, and electronic structure of the optical band gap. The antibacterial activity of the nanoparticles (ZnO) was evaluated against two bacterial strains, *E. coli* and *S. aureus*, and it was found that Zinc oxide nanoparticles exhibited a good antibacterial activity due to its large zones of inhibition against the studied bacteria. Therefore, the green method of synthesis can be considered an efficient and eco-friendly method for the production of Zinc oxide nanoparticles with potential applications in various fields including medicine and biotechnology. However, this research work provides new options for the synthesis of magnesium oxide nanoparticles using different methods and plant extracts via the green method of synthesis.

Recommendations

It is recommended that further research be carried out to synthesize as many different nanoparticles as possible using different plant extracts (natural products) via the green method of synthesis. It's also recommended that other applications of metal oxide nanoparticles be investigated.

References

1. Yang, W., Peters, J. I., & Williams III, R. O. (2008). Inhaled nanoparticles—a current review. *International journal of pharmaceutics*, 356(1-2), 239-247.

2. Imani, M. M., & Safaei, M. (2019). Optimized synthesis of magnesium oxide nanoparticles as bactericidal agents. *Journal of Nanotechnology*, 2019.

3. Asadian, E., Ghalkhani, M. and Shahrokhian, S. (2019). Electrochemical sensing based on carbon nanoparticles: a review, Sens. *Actuators B Chem*., 293.183–209.

4. Ozel, F., Kockar, H. and Karaagac, O. (2015). Growth of iron oxide nanoparticles by hydrothermal process: Effect of Reaction Parameters on the Nanoparticle size. *Journal of Super conductivity and novel magnetism*, 28, 823-829. DOI: 10.1007/s10948-014-2707-9.

5. Muhammad, I., and Justina, P. (2020). Synthesis, characteristics, and applications in analytical and other sciences. *Microchemical Journal*, 154, 104623.

6. Feng, L., Cao, M., Ma, X., Zhu, Y., Hu, C. (2012). Superparamagnetic high-surface-area Fe_3O_4 nanoparticles as adsorbents for arsenic removal. *J. Hazard. Mater*., 217–218, 439–446.

7. Kelly, K.L, Coronado, E., Zhao, L.L. and Schatz, G.C. (2003). The optical properties of metal nanoparticles: the influence of size, shape, and dielectric environment. *J. Phys. Chem. B*., 107, 668–677.

8. Kumar, R., Umar, A., Kumar, G., Nalwa, H.S. (2017). Antimicrobial properties of ZnO nanomaterials: A review. *Ceram. Int*., 43, 3940–3961.

9. Hussain, I., Singh, N.B., Singh, A., Singh, H. and Singh, S.C. (2016). Green synthesis of nanoparticles and its potential application. *Biotechnol. Lett*., 38.545–560.

10. Palza, H. (2015). Antimicrobial polymers using metal nanoparticles. *International Journal of Molecular Sciences*, 15, 2099–2116.

11. Kannan, B.N., and Natarajan, S. (2010). Biological synthesis of metal nanoparticles by microbes. *Adv. Colloid Interface Sci*., 156(1-2), 1–13.

12. Daizy, P. (2010). Honey Mediated Green Synthesis of Silver Nanoparticles. *Spectrochimica Acta Part A: Molecular and Biomolecular Spectroscopy*, 75, 1078-1081.

13. Sajid, M., and Płotka-Wasylka J (2020). Nanoparticles: Synthesis, Charasteristics, and Applications in analytical and other Sciences. *Microchemical Journal.*, 154.1046233.

14. Manoranjan A. (2012). Investigation on the effect of zinc oxide nanoparticles in the aggregation of hen egg lysozyme. *A research thesis*, 13-20.

15. Deepti, K. and Pradeep, T. (2009). J. Crys, Precursor-controlled synthesis of hierarchical ZnO nanostructures, using oligoaniline-coated Au nanoparticle seeds. *J. of Crystal Growth Grow,* 311, 3889-3897.

16. Li, M., Bala, H., Lv, X., Ma, X., Sun, F., Tang, L., Wang, Z. (2007). Direct synthesis of monodispersed ZnO nanoparticles in an aqueous solution. *Mater. Lett*., 61, 690-693.

17. Yadav, A., Prasad, V., Kathe, A.A., Sheela, R., Deepti, Y., Sundaramoorthy, C. (2006). Vigneshwaran, functional finishing in cotton fabrics using zinc oxide nanoparticles. *Bull. Mater. Sci*., 29(6), 641-645.

18. Luque, P. A., Soto-Robles, C. A., Nava, O., Gomez-Gutierrez, C. M., Castro-Beltran, A., Garrafa-Galvez, H. E., ... & Olivas, A. (2018). Green synthesis of zinc oxide nanoparticles using Citrus sinensis extract. *Journal of Materials Science: Materials in Electronics*, 29, 9764-9770.