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Green Synthesis And Characterization of Zinc Oxide Nanoparticles Using Jatropha Curcas for Enhanced Antibacterial Potential

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Abstract

Green-synthesized nanoparticles offer various advantages over conventionally physico-chemically synthesized nanoparticles. These synthesized nanoparticles have various biological and medicinal applications. In this study, zinc oxide nanoparticles were synthesized using the leaf extract of Jatropha curcas and zinc acetate dihydrate (as a precursor) for nanoparticle synthesis. The optical, functional group, morphological, and structural properties of the synthesized nanoparticles were investigated using ultraviolet-visible spectrophotometers (UV-Vis), Fourier transform infrared (FTIR), and scanning electron microscopy (SEM), and the antibacterial analysis was done using the agar dilution method against some Gram-positive, Staphylococcus aureus, and Gram-negative E. coli, Klebsiella pneumonia, and Pseudomonas aeruginosa. The formation of ZnO NPs was confirmed by a change in the color of the reaction mixture. UV peaks at 290 nm confirm the presence of ZnO NPs. In contrast, the presence of various bioactive functional groups responsible for reducing the bulk zinc acetate dihydrate to ZnO NPs was confirmed using FTIR. SEM analysis showed that the nanoparticles are spherical. Green-synthesized JC-ZnO NPs demonstrated important antibacterial activities when tested against certain bacteria strains; this implies that plant-synthesized nanoparticles can be used to develop many critical biomedical products.

Keywords: Secondary Metabolites, Capping Agent, Jatropha Curcas, Green Synthesis, Nanotechnology

Introduction

Phytochemical analyses have shown that *J. curcas* plants contain phenolic (Fu, R., et al., 2014), flavonoid (Rahu, M. I., et al., 2021), saponin (Lokanata, S., Molek, and Siregar. M. A. 2023). and alkaloid compounds. One of the reported traditional uses of jatropha is that the bleeding soon stops if the crushed leaf of this plant is applied directly to cuts and bleeding wounds (Osoniyi, O. and Onajobi, F., 2003). These properties have been attributed to various bioactive compounds in the plant. Jatropha species are excellent sources of secondary metabolites and phytochemicals (Rahu, M. I., et al., 2021).

These various phytochemicals and secondary metabolites, such as alkaloids, terpenoids (Ge, J., et al., 2022), flavonoids, compounds, cyclic peptides. eudemonic acids, and lignans, are known to be responsible for the various therapeutic properties (Anjum, S., et al, 2021), antiinflammatory (Bastos, E. M. S., et al, 2021), analgesic (Irmaleny, 2022), anti-cancer, antimicrobial (Enggrianti, E. et al, 2023), antifungal (Haq, A., et al, 2021), cytotoxic, larvicidal, antioxidant (Mohamed, A., et al, insecticidal, and anti-diabetic characteristics (Rahu, M. I., et al., 2021) exhibited by the plant.

Bio-fabrication of nanoparticles with the help of plant extracts is a fascinating subject in the study of nanotechnology due to its eco-friendliness (Haider, A., et al, 2020). cost-efficiency, potential biocompatibility, and low toxicity. For example, ZnO NPs have been reported to have UV-blocking properties (Fouda, A., et al, 2018), catalysts (Lavate, D. A., Sawant, V. and Khomane, A., 2019), gas-sensing capabilities (Wang, S., et al., 2020), excellent semiconducting properties (Luque, P., et al., 2022), biomedical applications (Anjum, S., et 2021), environmental remediation purposes (Doria-Manzur, A., Sharifan, H. and Benítez, L. P. T., 2022), and positive effects on agricultural practices (Zhou, X., et al., 2023), Plant-derived bioactive compounds and ZnO materials in health sectors have received considerable attention due to their therapeutic potential (Anandalakshmi, K., 2021; Tade, R. S., et al., 2020) as antimicrobial (Gudkov, S. V., et al., 2021; Rohani, R., et al., 2022), anti-inflammatory (Chinnathambi, A. and Alahmadi, T. A., 2021; López-Miranda, J. L., et al., 2023), anticancer, and antioxidant activities. Many reports have been published on preparing ZnO NPs with the help of chemical and physical techniques. In the biological fabrication process, microbes, fungi, and extracts of plants, vegetables, and fruits were used to fabricate metal oxide nanoparticles. In the case of green-synthesized ZnO NPs, plant extract or a component derived from plants is used as a stabilizing, capping, and reducing agent to convert zinc ions into zinc oxide nanoparticles. The plant extract contains bioactive compounds such as polyphenols, flavonoids, terpenoids, or enzymes that facilitate the reduction of metal ions and the subsequent formation of nanoparticles (Adeyemi, J. O., et al., 2022). Several recent studies have revealed that NPs have efficient antimicrobial potential against different pathogenic Grampositive bacterial strains, including Bacillus subtilis, Staphylococcus aureus, and one Gram-negative bacterial strain, E. coli, Klebsiella pneumonia, and Pseudomonas aeruginosa, responsible for urinary tract infection. The result of this work would give more insight into the potential pharmacological properties of green synthesized ZnO NPs utilizing J. curcas leaf extract, and this would aid in the creation of fresh strategies for administering medications using plant-based nanoparticles. specifically ZnONPs produced from *I. curcas* leaves.

Materials and Methods

Analytical reagents such as zinc acetate dihydrate, sodium hydroxide pellets, and tween 80 were used.

Sample Collection and Preparation

The plant's (I. curcas Linn) leaves were collected from the Ekosoden (300213) axis, located just opposite Uniben, Ovia-North East local government area, Edo State, Nigeria (Figure 1). The plant was identified as J. curcas Linn by Dr. Akinnibosun Henry Adewale in the Department of Plant Biology and Biotechnology of the Faculty of Life Sciences, University of Benin, Benin City, Nigeria, with voucher No. UBH-J404. The sample was washed thoroughly with water to remove any impurities, dried at ambient temperature for 14 days (in a shady environment in the absence of sunlight to avoid the loss of important phytochemicals), and pulverized into small, fine particles with the aid of a mechanical grinder at the laboratory of the Faculty of Pharmacy of Benin (postal code 300212), Ovia-North East Local Government, Benin City, Nigeria, and packaged into a plastic container.

Extraction of Phytochemicals from samples

The sample was extracted using water as a solvent. Twenty grams of dry leaf were soaked in 200 mL of distilled water (1:10 w/v) in a reagent bottle covered with foil paper for 24 hours. At intervals of every 3 hours, the bottle was shaken and uncapped to allow for the escape of gas. This was also repeated for 72 h (Figure 2). The extracts were filtered three times separately using a clean white cloth.

Production of zinc oxide nanoparticles

1.77 g of zinc acetate dihydrate was dissolved in a flask containing 80 mL of distilled water (0.01 M). The flask was then placed on a magnetic stirrer. 85 mL of the extract was then added to the solution. NaOH solution (0.02 M) was added dropwise to basify to pH 12.5. Upon adding the extract to the zinc acetate solution, an orange-colored cloudy precipitate, which later became deep upon adding NaOH, was formed. The temperature of the system was maintained at room temperature throughout the experiment. The synthesized product was centrifuged and dried in the oven (Figure 3).

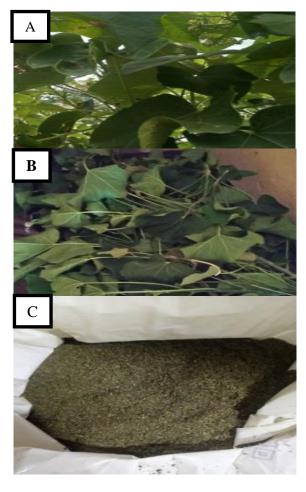


Figure 1: Fresh healthy (A), Dried (B), and Pulverized (C) leaves of *I. curcas* plant

Characterization

The UV analysis was carried out with the aid of a spectrophotometer to ascertain the existence of aromatic rings

and chromophoric groups present in the plant samples based on the detection of the electronic transition of lone pairs, σ -bonds, and also π -bonds of electrons. The FT-IR spectrum of *J. curcas* was obtained using the FT-IR (Cary 630 Agilent Technologist) spectrometer from Zaria to identify the bioactive compounds in samples and also detect their functional groups. The actual composition and topography of JC-ZnO NPs were obtained using SEM (Agilent Microlab PC, Zaria, Nigeria).



Figure 2: Extraction of sample by Maceration

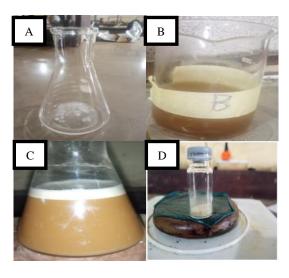


Figure 3: Beaker containing zinc acetate solution (A), plant extract and zinc acetate solution (B), formation of ZnO NP precipitate(C), and green synthesized ZnO NPs of *J. curcas* (D).

Antibacterial Analysis of ZnO NPs

The antimicrobial activity of greensynthesized ZnO NPs of J. curcas was carried out at the Pharmaceutical Microbiology Laboratory, University of Benin. The antibacterial analysis of the greensynthesized JC-ZnO NPS was carried out against various bacteria (Gram-positive, S. aureus, and Gram-negative E. coli, K. pneumonia, and P. aeruginosa) responsible for urinary tract infection using the agar extract dilution method.

Antibacterial activity

0.2 g of JC-ZnO NPs was dissolved in 0.25 g of Tween 80 as a diluent. The particles were dissolved in 0.75 g of sterile water to create a 2 mL, 25% Tween 80water solution. The stock solution was further diluted to the desired concentration. A sterile container dissolved 7.5 g of Mueller-Hinton agar in 150 mL of distilled water. The agar solution was autoclaved at 121 °C for 15 min to sterilize. The sterilized agar was cooled and allowed to be set on four different petri plates labeled with the names of each bacteria. Labeled wells were created in the solidified agar plates using a sterile cork borer, which was later used to apply the antimicrobial solution. The various bacteria were then streaked on the surface of the prepared agar plates so that each was streaked on the plate bearing its name. About 10 µL of the stock solution was then applied to each agar plate's wells with a micropipette. The plates were then incubated at 37 °C for 24 h. This allowed the bacteria to grow and interact with the antimicrobial solution. After incubation, inhibition zones were measured millimeters using a transparent ruler.

A MIC test was then conducted to determine the minimum inhibitory concentration of the JC-ZnO NPs using a series of dilutions (a two-fold dilution scheme) to create stocks with concentrations of 12.5, 25, and 50 μ L. The observations were also recorded.

Results and Discussion

The phytochemicals from the leaf of *J. curcas* linn were extracted by maceration at room temperature, a procedure similar to that of Sangeetha *et al.* (2010) (Sangeetha, J., et al., 2010). The formation of ZnO NPs using the extract, zinc acetate dihydrate, and sodium hydroxide as raw materials was also carried out at 35 °C, a procedure similar

to that of Zhou *et al.* (2023) (Zhou, X., et al., 2023), The change in color of the zinc acetate extract solution upon the addition of NaOH indicates the formation of brownish-white particles in the extract. The new product was centrifuged at a revolution of 3500 rpm and washed thoroughly using distilled water. The particles formed were oven-dried at a controlled temperature.

The UV analysis of the green synthesized ZnO NPs of *J. curcas* was carried out in the range of 200–400 nm, and the result showed a maximum wavelength of 290 nm and an absorbance of 0.389 (Figure 4). This absorption band shows a chemical shift, which may have occurred due to the quantum confinement of excitation in the sample.

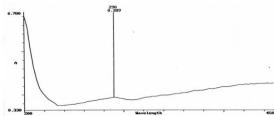


Figure 4: UV analysis of green synthesized JC-ZnO

The FT-IR spectrum of JC-ZnO NPs is depicted in Figure 5. Results from the FT-IR analysis of green synthesized JC-ZnO NPs were detected at 3753.4, 3220.4, 2922.2, 2344.5, 2102.2, 1908.4, 1397.8, 1088.4, 1028.7, 931.8, 846.1, 771.6, and 715.6 cm⁻¹ respectively. This spectrum is closely related to that of Rahman *et al.* (2022) (Rahman, F., et al., 2022).

This data also shows that the phytochemicals in JC or their functional groups are solely responsible for reducing and stabilizing the IC-ZnO NPs. Peaks at 3220.4 (O-H stretching of flavonoids and polyphenols), 2922.2 (C-H stretching for aliphatic compounds), 1636.3 (C=0)stretching), 1088.4 (C-O), 1028.7 (C-N), 846.1 (C-H, bending), and 715.6 cm⁻¹ (C-Cl) might be responsible for the bioreduction of The secondary zinc to IC-ZnO NPs. metabolites can bind to nanoparticle surfaces, possibly through carbonyl groups pi-electron interactions. Moreover, reducing agents in the solution could contribute to creating ZnO NPs due to reducing the metal ion. The potential conversion of aldehydes to carboxylic acids by terpenoids could lead to the reduction of metallic ions.

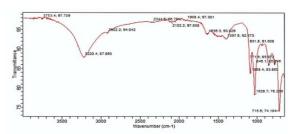


Figure 5: FT-IR spectra of JC-ZnO NPs from *J. curcas* linn

The surface morphology of the biosynthesised ZnO NPs was studied using SEM, and the results are similar to those of Tymoszuk and Wojnarowicz (2020) (Tymoszuk, A. and Wojnarowicz, J., 2020) (Figures 6 A–6 D). This depicts the SEM image of JC-generated ZnO NPs at magnifications of 1, 500, 1000, and 2000, respectively. The image shows spherical forms with particles clustered in a way that indicates the presence of a capping agent that stabilizes the nanoparticles.

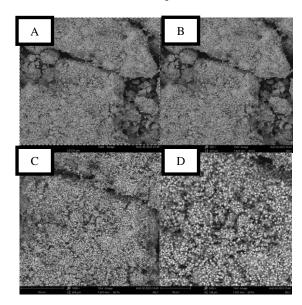


Figure 6: Scanning Electron Microscopy of green synthesised JC-ZnO NPs

The antibacterial properties of JC-ZnO NPs were tested against various causative organisms unitary tract infections, including the Gram-positive, S. aureus, and Gramnegative E. coli, K. pneumonia, and P. aeruginosa were investigated. The analysis noted that the Gram-positive S. aureus exhibited a significant inhibition zone of up to 18 mm. At the same time, the other Gramnegative E. coli (A), K. pneumonia (C), and P. aeruginosa (D) did not display such zones (Figure 7). The results showed that JC-ZnO NPs had a significant antibacterial effect on Gram-positive bacteria since no significant inhibition zones were observed for the other tested bacteria.

Inhibition The minimum Concentration (MIC) test was determined to indicate the minimum concentration (highest dilution) at which no bacterial growth would be observed. This was done by diluting the dissolved JC-ZnO NPs into the nutrient broth at 12.5, 25, and 50 g/mL, respectively, inoculating the bacteria, and incubating for 48 h. In this case, no growth was noted at 25 and 50 mg/mL, but bacterial growth at 12.5 mg/mL. The MIC results showed no bacterial growth occurred at 25 mg/mL and 50 mg/mL concentrations, indicating that IC-ZnO NPs are an effective bacteriostatic agent.



Figure 7: Antibacterial activities of JC-ZnO NPs in the petri dish: *E. coli* (A), *S. aureus* (B), *K. pneumonia* (C), *P. aeruginosa* (D)

Conclusion

Green synthesized nanoparticles offer advantages over several their conventionally synthesized counterparts because of their environmental friendliness, reduced toxicity as organic materials are used as raw materials, cost-efficiency, and functionality. This property makes JC-ZnO NPs contributor to sustainable development as organic raw materials are used.

JC-ZnO NPs also displayed notable inhibitory activity against Gram-positive (S. aureus) pathogenic urinary tract infection bacteria strains; this makes JC-ZnO NPs bioprospecting for developing antibacterial drugs.

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