

Antimicrobial Studies of Biosynthesized Zinc Oxide Nanoparticles

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ARTICLE INFO

Citation: Ugosor, P.T and Wada, S. T (2025). Antimicrobial Studies of Biosynthesized Zinc Oxide Nanoparticles.

Microbiology Archives, an International Journal.

DOI: <https://doi.org/10.51470/MA.2025.7.1.46>

Received 28 February 2025

Revised 28 March 2025

Accepted 20 April 2025

Available Online 16 May 2025

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ABSTRACT

Nanoparticles of zinc oxide (ZnO NPs) were synthesized through a sustainable chemistry approach using aqueous extracts from *Colocasia esculenta* leaves. Characterization was conducted using UV-Visible spectroscopy, X-ray diffraction (XRD), scanning electron microscopy (SEM), energy-dispersive X-ray spectroscopy (EDX), and Fourier-transform infrared spectroscopy (FTIR). Statistical analysis of antimicrobial activity data was carried out using Social Package for Social Sciences (SPSS) version 20, with results reported as mean \pm standard deviation (Mean \pm SD). One-way Analysis of Variance (ANOVA) with Least Significance Difference (LSD) post hoc tests were performed at significance level ($p < 0.05$). Optimal nanoparticle formation occurred at 0.50 mol/dm³ ZnO solution at 80 °C over a 4-hour reaction period. The synthesized ZnO NPs exhibited a hexagonal wurtzite crystal lattice structure with an mean particle size of approximately 12 nm. FTIR analysis showed phenolic compounds, amines, peptides, and amides, which facilitated reduction of zinc ions,

capping, and stabilization of the ZnO NPs. The ZnO NPs demonstrated concentration-dependent antimicrobial effects against five fungal pathogens (*Aspergillus niger*, *Rhizopus stolonifer*, *Aspergillus flavus*, *Fusarium oxysporum*, and *Botryodiplodia theobromae*.) and three bacterial strains (*Klebsiella oxytoca*, *Serratia marcescens*, and *Pseudomonas aeruginosa*). Their inhibitory efficiency ranged from 32.22% to 89.95%, showing comparable performance to standard antimicrobial agents such as clotrimazole and ciprofloxacin. These findings suggest that ZnO NPs synthesized from *Colocasia esculenta* have promising applications in postharvest disease management and pharmaceutical development.

Keywords: Phytochemicals, nanotechnology, biosynthesis, pathogens, antimicrobial activity.

1.0 Introduction

Postharvest deterioration of agricultural produce remains a critical issue in developing nations, primarily due to inadequate storage infrastructure, limited access to technology, and microbial infestations. Fungal and bacterial pathogens, which produce various enzymes and mycotoxins [30,34,37], are among the leading causes of spoilage, especially in crops like white yam (*Dioscorea spp.*) [23,32,33].

Globally, it is estimated that over a quarter of yam harvests are lost annually due to microbial infections and pest attacks [27,29]. In Nigeria, postharvest losses can exceed 50%, with significant deterioration observed within three to six months of storage [27,30,32]. This high rate of spoilage poses a serious threat to food availability and economic sustainability. Conventional control strategies have included cultural practices and the use of synthetic chemicals such as borax, captan, benomyl, and sodium hypochlorite [1,5,9]. While effective, these chemical treatments often come with drawbacks such as environmental toxicity, non-biodegradability, pesticide resistance, and potential health hazards due to residual accumulation in the food chain [22,28,30]. Moreover, their high costs and improper usage further limit their effectiveness, particularly among small-scale farmers.

In response to these concerns, there is increasing interest in developing environmentally friendly alternatives.

One promising approach is the use of plant-based materials in combination with metal or metal oxide nanoparticles [3,4,5]. These biosynthesized nanoparticles offer broad-spectrum antimicrobial activity and can function as preservatives by inhibiting microbial growth, prolonging shelf life, and protecting nutrient integrity [22,19,35].

Colocasia esculenta (commonly known as cocoyam) has been shown to possess numerous bioactive phytochemicals such as flavonoids, saponins, phenolics, alkaloids, and glycosides, which are associated with antibacterial, antifungal, antiviral, and antioxidant properties [20,22,36]. Meanwhile, zinc oxide (ZnO), a compound recognized as safe by regulatory authorities, is widely used in pharmaceutical and cosmetic products due to its antimicrobial and protective qualities [1,2,18]. When synthesized at the nanoscale, ZnO exhibits enhanced biological activity because of its large surface area and characteristic physicochemical qualities [6,11,13,38].

This research aims to prepare ZnO nanoparticles using *Colocasia esculenta* leaf extract and determine their antimicrobial efficacy against pathogens responsible for white yam rot. The goal is to develop an accessible, sustainable, and cost-effective strategy for disease management in agricultural systems, particularly for farmers in Benue State, Nigeria.

2.0 Materials and Methods (Rewritten)

2.1 Plant Material and Authentication

2.1.1 Collection

Fresh leaves of *Colocasia esculenta* were gathered from the Fr. Adasu University campus, Makurdi, Benue State, Nigeria, during January 2025. The leaves were labelled appropriately and stored in clean polyethylene bags for transport and analysis.

2.1.2 Identification

Botanical authentication of the plant material was conducted at the Department of Biological Sciences, Fr. Adasu University, Benue State, Nigeria.

2.2 Processing and Extraction

2.2.1 Drying and Pulverization

The *Colocasia esculenta* leaves were carefully washed with distilled water and ambient-dried (to preserve phytochemical integrity) for two weeks. Dried samples pulverized using an electric blender pestle and kept in airtight containers [6,9,10].

2.2.2 Aqueous Extraction

Aqueous extract of *Colocasia esculenta* leaves was obtained by boiling 200 grammes of the powdered leaves in 1000 mL of demineralized water at 80 °C for one hour. The mixture was cooled, then filtered and the extract refrigerated at 4 °C until further use [15,16].

2.3 Microbial Culture Preparation

2.3.1 Source of Test Organisms

Microbial strains, comprising five fungal pathogens (*Aspergillus niger*, *Aspergillus flavus*, *Botryodiplodia theobromae*, *Rhizopus stolonifer*, *Fusarium oxysporum*) and three bacterial species (*Klebsiella oxytoca*, *Serratia marcescens*, and *Pseudomonas aeruginosa*), were sourced from the Department of Biological Sciences, Fr. Adasu University, Benue State, Nigeria.

2.3.2 Culture and Maintenance

Microbial isolates were revived in sterile distilled water and cultured on Potato Dextrose Agar, PDA (for fungi) and Nutrient Agar, NA (for bacteria). After incubation at 37 °C (24 hours for bacteria, 7 days for fungi), pure colonies were maintained on fresh media and preserved in 20% glycerol at -70 °C [17,33]. Microbial suspensions were standardized to an optical density of 0.144 at 550 nm (approximately 1.0×10^6 CFU/mL). All glassware and reagents used were sterilized or analytical grade, and aseptic conditions were maintained throughout the procedures [32,33].

2.4 Phytochemical Screening

Qualitative tests for phytochemicals (flavonoids, phenols, saponins, alkaloids, tannins, terpenoids, cardiac glycosides, and steroids) were performed using standard procedures of [16].

2.5 Preparation of ZnO Nanoparticles

Green route method was carried out by mixing 40 mL of 0.50 mol/dm³ zinc ethanoate, Zn(CH₃COO)₂ solution with 20 mL of the *Colocasia esculenta* leaves extract. The pH was regulated to 12 by dropwise addition of 0.02 mol/dm³ sodium hydroxide, NaOH. The reaction mixture was heated between 30 °C and 90 °C for four hours with continuous stirring. The resulting white product was cooled, centrifuged at 1200 rpm for 5 minutes, rinsed severally with demineralized water, dried at 80 °C for 24 hours, and kept in a desiccator for further analysis [7,14]. A modified schematic diagram of the process is as shown below:

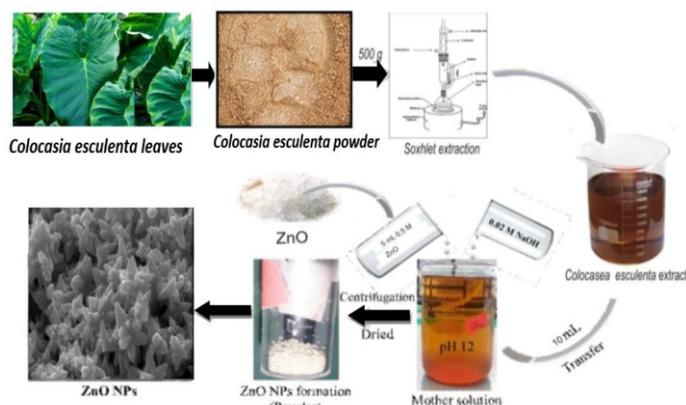


Fig. 1: A modified schematic diagram for the preparation of ZnO NPs using *C. esculenta* leaves extract [7].

2.6 Characterization of ZnO Nanoparticles

Uv-3600 Plus, Shimadzu, Japan in the range of 350–700 nm was employed to monitor ZnO NP synthesis. XRD Analysis (XRD-6000, Shimadzu, Japan) was performed using Cu K α radiation to determine crystal structure and average crystallite size. SEM hyphenated with Philips XL-30, Eindhoven, Netherlands was employed to observe nanoparticle shape, structure and component elements. Perkin Elmer FTIR Spectrophotometer-100 from of 500–4000 cm⁻¹. scale was carried out to identify the biomolecule which serve as reductant of zinc ions, capping, and stabilization of the zinc oxide nanoparticles [7].

2.7 Antimicrobial Activity Assay

A modified agar diffusion technique [29, 30, 32] was used to evaluate the antimicrobial efficacy of the nanoparticle against pure isolates. PDA (for fungi) and NA (for bacteria) were supplemented with different ZnO NP concentrations (25, 50, 75, and 100 mg/mL). Standard drugs (clotrimazole for fungi and ciprofloxacin for bacteria) served as positive controls. After incubation, inhibition zones were measured, and % inhibition zones were estimated as shown below:

% IZ = Mean diametre of pathogen colony / Mean diametre of pathogen in control x 100 % [19, 32, 33].

Percentage inhibition zones were graded as follows:

Highly effective = 100 %; effective = 50 - 99 %; moderately effective = 20 - 49 %; slightly effective = 0 - 19 %, and not effective (≤ 0 %) [32, 33].

2.8 Statistical Analysis

Experimental data were performed Version 20 of SPSS. Average values were reported with SD. Significance among groups was evaluated using ANOVA with LSD post hoc analysis. Results were significant at $p < 0.05$, and insignificant at $p > 0.05$.

3.0 Results and Discussion

3.1 Result of phytochemical Constituents

Table 1: Result of secondary metabolites screening

Secondary Metabolite	Test	Result
Tannins	Lead ethanoate Test	+
Alkaloid	Wagner's Test	+
Saponins	Foam Test	+
Quinone	Craven;s Test	-
Glycosides	Borntrager's Test	+
Steroids	Libbermann Burchard's Test	+
Flavoniods	Shinoda's Test	+
Starch	Lugol's iodine Test	+
Terpenoids	Libermann-Burchard's Test	+
Phenols	Bromine water Test	+

Key: + = Positive; - = Negative.

The phytochemical screening of *Colocasia esculenta* leaves extract revealed biocompounds, including tannins, alkaloids, saponins, glycoside, steroids, flavonoids, starch, terpenoids, and phenols. These bioactive compounds are known for their antimicrobial, antioxidant, and reducing properties, making them ideal for nanoparticle biosynthesis and stabilization. The presence of these metabolites supports the importance of the extract as reductants of zinc ions, and stabilizing agent of the nanoparticles, as confirmed by spectral analysis [7].

3.2 UV-Visible Spectrometry analysis

The synthesis of ZnO nanoparticles was affirmed by a distinct absorption intensity at 370 nm, which is characteristic of ZnO nanoparticles. The intensity and sharpness showed nanosized ZnO crystallite sizes. This peak intensified with increasing reaction time and temperature, suggesting enhanced nanoparticle formation as shown in spectrum below:

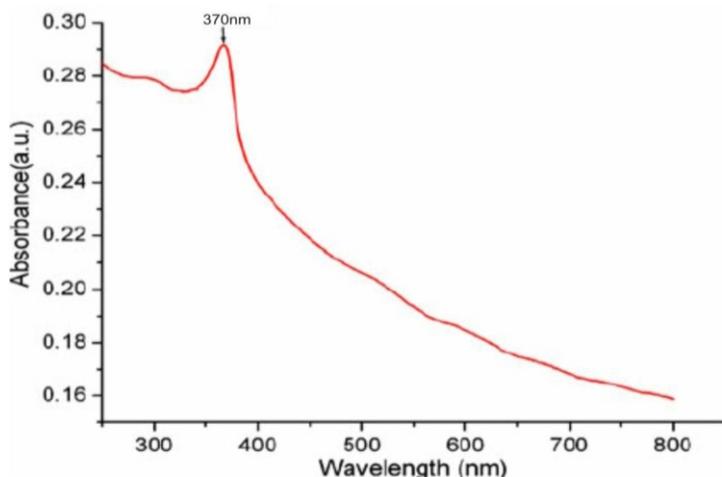


Fig. 2. UV-Vis spectra of the biosynthesized ZnO Nps.

3.3 XRD Analysis

X-ray diffraction pattern of the prepared nanoparticle displayed prominent intensities at 2θ values of 31.84° , 34.50° , 36.26° , 47.57° , and others relating to the (100), (002), (101), (102), and other crystal planes, matching hexagonal wurtzite structure (JCPDS Card No. 36-1451). The average crystallite size was calculated using the Debye-Scherrer equation:

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

Where d = crystallite size, K = Scherrer constants with values from 0.9 to 1 (shape factor), β = width of XRD peak at half height, λ = X-ray wavelength (1.5428 \AA) and θ = Diffraction angle.

Average crystallite size was estimated to be approximately 12 nm, with values ranging from 10.10 nm to 12.92 nm.

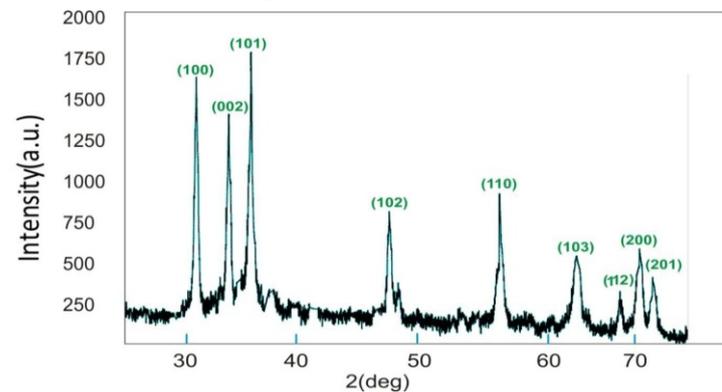


Fig. 3: XRD pattern of the biosynthesized ZnO Nps

3.4 Scanning Electron Microscope (SEM) and EDX Studies

SEM imaging revealed well-defined, segregated hexagonal nanoparticles with smooth surfaces, indicative of good crystallinity. Particle size analysis using ImageJ software estimated an average size of 11.99 nm, aligning well with XRD data. SEM image of the nanoparticles is presented in Figure 4.

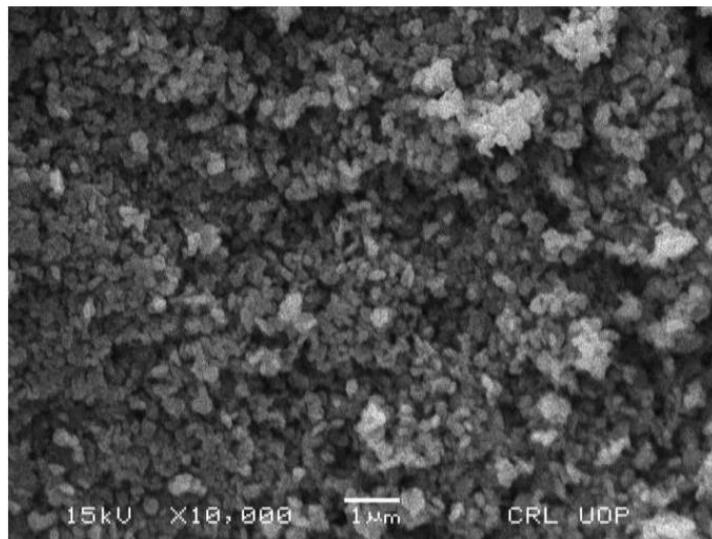


Fig. 4: SEM image of biosynthesized ZnO Nps

EDX spectrum affirmed the elemental makeup of the nanoparticles. Peaks corresponding to zinc (Zn) and oxygen (O) were dominant, with Zn constituting approximately 41.19 % and O about 51.58 %. with trace amounts of C (7.24%). This confirmed an approximate ratio of 1:1. The low levels C as impurity suggest high purity of the ZnO NPs as shown in Fig. 5.

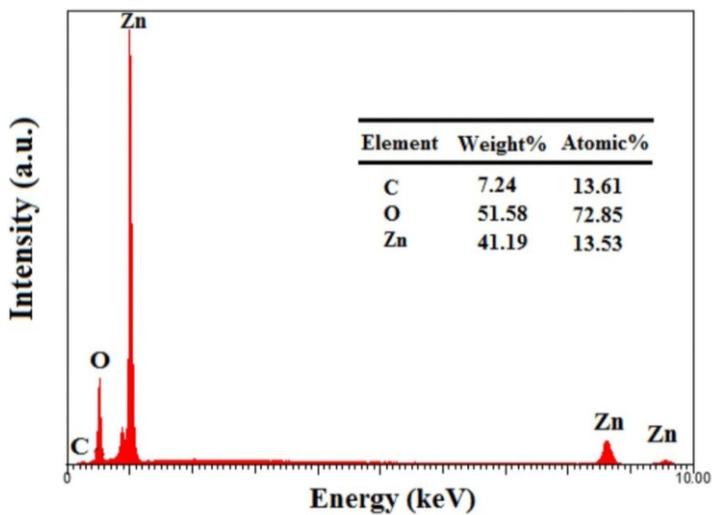


Fig. 5: EDX spectrum of the biosynthesized ZnO.

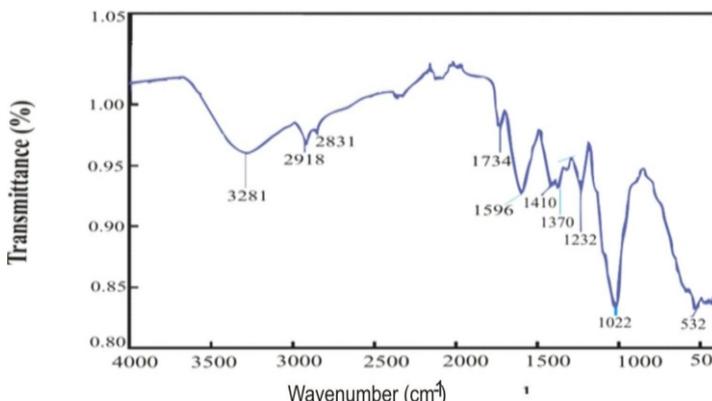


Figure 6: FTIR spectrum of ZnO NPS.

3.5 FTIR Spectroscopy

FTIR analysis presented in Fig. 6 reveals the chemical bonding between Zn and O. The spectrum identified a peak at 532 cm⁻¹ corresponding to Zn–O stretching, characteristic of wurtzite-phase ZnO. Additional bands at 3382 cm⁻¹, 2374 cm⁻¹, 1656 cm⁻¹, and 1380 cm⁻¹ were attributed to hydroxyl (–OH), water, C=C of aromatics, and alkane groups, respectively. These functional groups originate from the phytochemicals in the *Colocasia esculenta* extract and function as reductant of zinc ions, capping, stabilization and surface functionalization of the nanoparticles.

The plant extract spectrum reviewed peaks at 3281, 2918, 2831, and 1734 cm⁻¹, indicating O–H stretching, phospholipid content, aliphatic chains, and C=O stretching, respectively. These confirm the presence of bioactive molecules responsible for reducing and capping Zn²⁺ ions.

3.6 Antimicrobial Activity of ZnO Nanoparticles

Table 2: Mean zone of inhibition (mm) for fungal and bacterial strains

	Zinc Oxide Nanoparticles Concentration (mg/mL)				Co Control
	100	75	50	25	
Fungi					
<i>A. niger</i>	6.17 ± 0.01 ^b	5.17 ± 0.01 ^c	4.04 ± 0.01 ^d	2.86 ± 0.13 ^e	8.18 ± 0.01 ^a
<i>A. flavus</i>	5.57 ± 0.32 ^b	4.87 ± 0.07 ^c	3.96 ± 0.04 ^d	3.14 ± 0.01 ^e	9.71 ± 0.04 ^a
<i>B. theobromae</i>	6.78 ± 0.02 ^b	5.82 ± 0.06 ^c	4.99 ± 0.01 ^d	4.58 ± 0.01 ^e	10.99 ± 0.01 ^a
<i>R. stolonifera</i>	10.16 ± 0.01 ^b	9.27 ± 0.02 ^c	6.78 ± 0.01 ^d	5.03 ± 0.01 ^e	18.08 ± 0.08 ^a
<i>F. oxysporum</i>	19.73 ± 0.01 ^b	18.16 ± 0.09 ^c	17.52 ± 1.10 ^c	9.05 ± 0.01 ^d	29.67 ± 0.01 ^a
Bacteria					
<i>K. oxytoca</i>	18.14 ± 0.05 ^b	15.27 ± 0.40 ^c	12.08 ± 0.08 ^d	8.06 ± 0.01 ^e	37.25 ± 2.20 ^a
<i>S. marcescens</i>	17.24 ± 0.32 ^b	15.12 ± 0.09 ^c	12.82 ± 0.01 ^d	9.08 ± 0.01 ^e	40.32 ± 0.04 ^a
<i>P. aeruginosa</i>	21.04 ± 0.01 ^b	19.24 ± 0.11 ^c	16.25 ± 0.03 ^d	7.13 ± 0.01 ^e	38.07 ± 0.54 ^a

N= 5, values expressed as Mean ± SD. Values in the same row with different superscript (alphabetical letters) are significant at p <0.05.

Table 3: Percentage zone of inhibition of the ZnO Nps

	Fungi Concentration (mg/mL) 100 75 50 25			
	<i>Aspergillus niger</i>	75.37	63.36	49.39
<i>Aspergillus flavus</i>	58.12	50.57	40.85	32.26
<i>Botrydiopodia theobromae</i>	61.55	53.45	45.36	41.45
<i>Rhizopus stolonifera</i>	56.39	51.39	37.67	27.89
<i>Fusarium oxysporum</i>	66.43	61.00	56.32	30.50
Bacteria				
<i>Klebsiella oxytoca</i>	51.97	43.30	34.74	23.11
<i>Serratia marcescens</i>	44.14	37.44	31.74	22.49
<i>Pseudomonas aeruginosa</i>	55.83	50.70	43.08	18.90

Key: a = 100 % inhibition (highly effective); b = 50 – 99 % inhibition (effective); c = 20 – 49 % inhibition (moderately effective); d = 0 - 19 % inhibition (slightly effective); e = ≤ 0 % inhibition (ineffective) [26].

The biosynthesized ZnO NPs displayed concentration-dependent antimicrobial effects against all tested pathogens. Inhibition zones increased with ZnO concentration, indicating enhanced antimicrobial potency at higher doses.

All the test microorganisms showed effective to moderately effective inhibition (75.37-22.11) % at 100 – 25 mg/mL, with the exception of *Pseudomonas aeruginosa* which indicated slightly effective inhibition (18.90%) at 25 mg/mL.

The ZnO NPs demonstrated broad-spectrum activity, particularly at 100 mg/mL, with inhibition percentages over 80 % for most pathogens. Their efficacy was statistically comparable (p < 0.05) to that of standard antimicrobial drugs, affirming their potential in disease management.

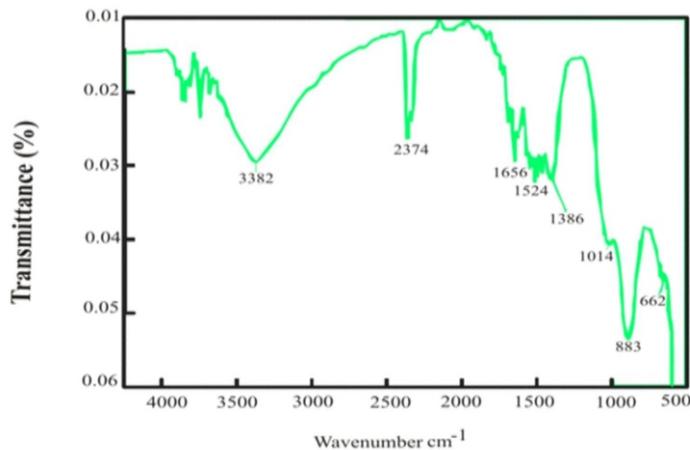


Figure 7: FTIR spectrum of *Colocasia esculenta* leaves.



Figure 8: Antimicrobial sensitivity test plates.

4.0 Conclusion

This study prepared zinc oxide nanoparticles using aqueous leaves extract of *Colocasia esculenta* through sustainable approach. Synthesized nanoparticles exhibited well-defined hexagonal wurtzite crystal lattice structure with mean crystallite size of approximately 12 nm. Spectroscopic and microscopic analyses confirmed the involvement of plant-derived phytochemicals as reducing agents of zinc ions, capping, and stabilizing of the nanoparticles.

The nanoparticles showed significant antimicrobial action against microorganisms associated with postharvest white yam rot. The inhibition was concentration-dependent and comparable to standard antimicrobial agents such as clotrimazole and ciprofloxacin.

The result highlight the prospects of *Colocasia esculenta*-facilitated ZnO nanomaterials as eco-friendly and cost-effective antimicrobial agents. Their application could serve as a sustainable substitute to harmful chemicals substances in postharvest disease control and development of new antimicrobial formulations. This approach aligns with the goals of promoting food security and environmental sustainability, especially for resource-limited farming communities.

Acknowledgement

The authors appreciate the staff of the Departments of Chemistry and Biological Sciences, as well as CEFTER, Fr. Adasu University, Makurdi, Benue State for their support and access to facilities for the successful execution of this research.

Competing Interests

Authors have declared that no competing interests exist.

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