



Green Synthesis of Zinc Oxide Nanoparticles Using *Colocasia esculenta* Tuber Peel Extract and Antimicrobial Studies of White Yam Pathogens

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Authors' contributions

This work was carried out in collaboration among all authors. Author UPT designed the study, performed the phytochemical analysis, wrote the protocol and the first draft of the manuscript. Authors ABA and OFG managed the analyses of the study. DS managed the literature searches and antimicrobial analyses. All authors read and approved the final manuscript.

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ABSTRACT

Aims: To biosynthesize and characterize Zinc Oxide NPs using *Colocasia esculenta* (Cocoyam) tuber peel extract as well as explores its antimicrobial potential against white yam pathogens.

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Methodology: The method described by Nakade [1] was used for phytochemical analysis (Tanins, Saponins, Flavonoids, Alkaloids, Steroids, Quinones, Starch, Terpenoids and Glycosides). The biosynthesized ZnO NPs were characterized by UV-Visible, XRD, SEM, EDX and FTIR.

Antimicrobial sensitivity test was by the method described by Shiriki et al [2] with slight modification.

Results: Optimization studies revealed that the maximum rate of synthesis could be achieved with 0.50 M ZnO solution at 90 °C in 5 hours. The study revealed ZnO NPs that are crystalline with hexagonal shapes. The average crystallite size was 10 nm with a range of 7.81 nm- 9.23 nm. FTIR spectra of the tuber peel extract and the synthesized ZnO NPs revealed reducing, capping and stabilizing agents such as amines, peptides, amides and phenolic groups. The biosynthesized ZnO NPs exhibited antimicrobial action in a dose-dependent manner against five white yam pathogenic fungi: *Aspergillus niger*, *Botryodiplodia theobromae*, *Zygosaccharomyces bailli*, *Zygosaccharomyces rouxii*, and *Myrothecium verrucaria* as well as three bacteria: *Klebsiella oxytoca*, *Serratia marcescens*, and *Pseudomonas aeruginosa*.

Conclusion: The biosynthesized ZnO NPs exhibited slightly to moderate and effective inhibition ranging from 15.54%- 98.52% on the test organisms when compared with standard antifungal (Fluconazole) and antibacterial (Ciprofloxacin) agents.

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

1. INTRODUCTION

The goal of nanotechnology is to make it easier to produce nanomaterials or nanoparticles by modifying matter through physical, chemical, or biological processes. This allows for the creation of materials with particular properties that can be applied to a variety of situations [3]. The focus of green nanotechnology is on straightforward work-up processes for the production of recyclable, economical, and environmentally benign nanoparticles with long-term commercial viability. The term "nanomaterials" is defined by the European Commission (EC) and the International Organization Standards (ISO) as a subgroup of nano-objects, and their agglomerates (weakly bonded, embedded) and aggregates (strongly bonded, fixed). They are the conventional materials which are consciously and deliberately engineered to nanostructure of modern application of nanotechnology. Nanomaterials are the forms of matter with at least one of their dimensions ranging from 1 to 100 nm [4]. These are the traditional materials that have been purposefully and carefully developed to match the nanostructure of contemporary nanotechnology applications in numerous industries, including agriculture, pharmaceuticals, electronics, electrical and mechanical, textiles, paint, ceramics, rubber, water purification, and environmental remediation, have discovered extensive uses for nanomaterials.

The preparation of nanoparticles can be done in two general ways: top-down and bottom-up. NPs

can be created in top-down processes by splitting a huge solid into smaller pieces, or nanosize. According to [4], the bottom-up approach to the creation of nanoparticles entails building up from atoms or molecular entities to nanometric scale. Better nanoparticles with few or no flaws are produced via bottom-up methods [3].

To synthesize NPs, physical, chemical, and biological techniques are used. But chemical and physical processes need a lot of energy, and they can occasionally produce hazardous wastes that have negative effects on human health and the environment [5]. Biological methods for the synthesis of nanoparticles are simpler, more cost-effective, environmentally friendly, and non-toxic than physical or chemical routes. Biological route can also produce large quantities of stable, purer nanoparticles under relatively mild operating conditions [6,7]. Biological or green synthesis of nanoparticles which, represents a connection between biotechnology and nanotechnology has received increasing consideration due to the growing need to develop environmentally friendly technologies for material synthesis. Recently, considerable efforts are directed at exploring the potentials of plant extracts for the synthesis of NPs as alternative to synthetic chemicals for various applications. These plant extracts are rich in phytochemicals such as alkaloids, flavonoids, terpenoids, phenols, glycosides, tannins, phytates, saponins, steroids etc with proven antimicrobial actions; serve as reducing, capping and stabilizing agents during synthesis [1].

Many metals and metal oxides, such as iron, nickel, silver, gold, aluminum, copper, iron oxide, copper oxide, zinc oxide, titanium dioxide, and silicon oxide, are known to yield nanomaterials. Zinc oxide (ZnO) is one of the most widely used metal oxide nanoparticles (NPs) due to its excellent antibacterial properties, non-toxicity, good ultraviolet (UV) absorbance, high stability (breaking down only into zinc vapour and oxygen at around 1975 °C), and photocatalytic qualities [8]. Zinc oxide (ZnO), a food supplement, is considered safe for human use by the US Food and Drug Administration (US FDA) [8, 9].

The herbaceous tropical perennial starchy plant known as cocoyam, or taro (*Colecosia esculenta* L.), is rich in phytochemicals with antifungal, antibacterial, and antiviral qualities, including alkaloids, flavonoids, phenols, glycosides, tannins, phytates, saponins, and steroids [10]. The crop is of great nutritional and economic importance to humankind. The leaves are used as vegetables and are a rich source of proteins, ascorbic acid, dietary fibre, minerals and vitamins such as calcium, phosphorus, iron, magnesium, potassium, vitamin C, thianine, ribboflavin and niacin. The juice from the leaves is utilised to cure snakebite or scorpion stings [11]. According to Eleazu [10], the corns have anthocyanins, cyanidin, glucoside, pelargradin, 3-glycoside, and 3-rhamnoside, while the tubers are high in starch.

According to Wang [11] and Nakade et al. [1], the related anthocynin with flavonoids improves blood circulation by reducing capillary fragility, improves vision, and functions as a strong antioxidant, anti-inflammatory, and anti-cancer agent. Additionally, the corns have calcium oxalate, an irritant that makes them unfit for

consumption when raw or only partially cooked [1]. According to reports from Pritha et al. [12] and Nakade et al. [1], *C. esculenta* tuber is used in ethnomedicine to treat wounds, ringworm, cough, sore throats, and diabetes mellitus. It also reportedly contains antihelminthic and anticancer qualities. Its phytochemicals are the cause of these biological characteristics.

The synthesis of ZnO NPs from a variety of plant components, including leaf, flower, seed, fruit, root, rhizome, stem, bark, and peel extracts, has been validated by a number of studies [9,13]. For example, studies by many researchers utilised leaf *Ocimum basillium* [14], *Aleo barbadensis* [15], *Plectranthus amboinicus* [16], *Azadiracha indica* L [17], *Couroupita guinensis* [18], *Hibiscus rosa-sineensis* [19] etc; flower extracts of *Cassia auriculata*, seed extracts of *Cuminum cyminum* *Pongamia pinnata* [9], fruit extracts of *Embilca officinalis*, *Borassus flabellifer*, *Artocarpus gomezianus* and orange juice [20], root extracts of *Rubus fairholmianus*, *Withania somnifera* [9], rhizome extracts of *Zingiber officinale*, *Bergenia ciliate* [9,21], stem extracts of *Phyllanthus embilica*, bark extracts of *Cinnamomum verum*, *Albizia lebbeck* [12], *Gum kayara* [22] as well as peel extracts of *Rubantan* [23], *Punica granatum*, *Musa sapientum* [12,9].

Numerous variables, including pH, temperature, precursor concentration, and extract volume, can affect the production of nanoparticles and the properties that arise [24]. Precursor concentration and the volume ratio of the precursor-extract utilized determine the morphology and particle size [25, 26]. ZnO In this study, NPs are synthesized and characterized using *C. esculenta* tuber peel extract, and their antibacterial activity against white yam pathogens is investigated.

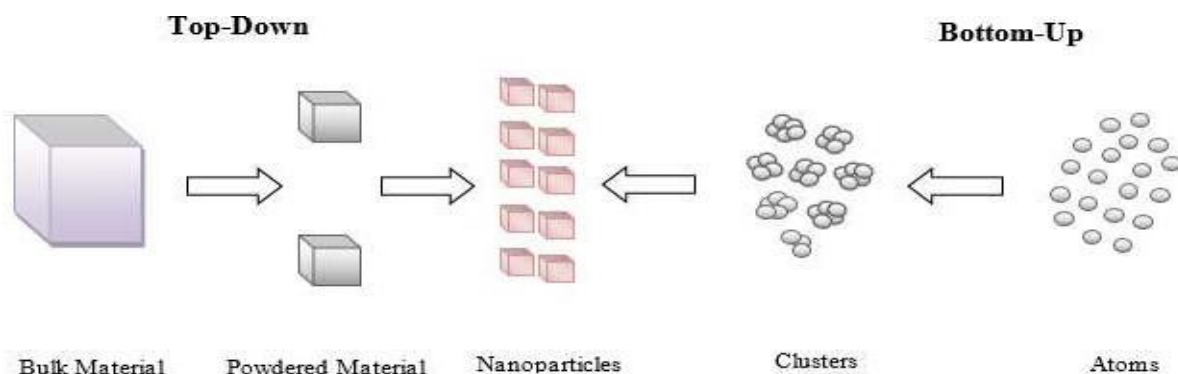


Fig. 1. Approaches for the synthesis of nanoparticles [4]

2. MATERIALS AND METHODS

Previously isolated and identified white yam rot pathogens comprising of five fungi: *Aspergillus niger*, *Botryodiplodia theorome*, *Zygosaccharomyces bailli* and *Zygosaccharomyces rouxii* and *Myrothecium verrucaria* and three bacteria: *Klebsiella oxytoca*, *Serratia marcescens* and *Pseudomonas aeruginosa* of 2022 harvest year were obtained from the Laboratory, Department of Biological Sciences, Benue State University, Makurdi where they were preserved and used for the antimicrobial study. *C. esculenta* tubers were purchased from Railway market, Makurdi, Benue State, properly labeled, packed in clean cellophane bags and transported to the Department of Botany, Benue State University, Makurdi for authentication by a plant taxonomist. ZnO was purchased from Agbe Sciences, Makurdi, Benue State, Nigeria. All reagents used were analytical grade and used as received without further purification. All solutions were freshly prepared using double-distilled water and kept in the dark to avoid photochemical reactions. All glassware used in the experimental procedures were sterilized in 10 % sodium hypochlorite solution, rinsed thoroughly in double-distilled water and dried before use. Aseptic condition was maintained throughout the experiments.

2.1 Plant Extract Preparation

The *C. esculenta* tubers were thoroughly washed with sterile water, peeled and dried in the shade for two weeks to avoid chemical decomposition.

Upon drying, the peels were made into fine powder using a wooden mortar and pestle.

2.2 Extraction Procedure

The method described by Shiriki et al., [27] was employed with little modification. The sample (500 g) was packed into the thimble and placed inside the extractor. 800 mL methanol was put in the round bottom flask of the extractor and heated on a heating mantle for 8 hours. After extraction, the methanol was recovered and the extract evaporated in a beaker to a constant weight over an evaporation bath for 24 hours. The sample was then weighed and the yield calculated in percentage. The extract was kept in the refrigerator for further analysis.

2.3 Phytochemical Analysis

The method described by Nakade [1] was used for phytochemical analysis.

i. Test for tannins

Ferric Chloride Test: 4 mL of the extract was treated with 4 mL of FeCl₃ in a test tube. Formation of a bluish green precipitates indicated the presence of tannins.

ii. Test for Saponins

Froth Test : 5 mL of the extract was diluted to 20 mL with distilled water and shaken in a graduated cylinder for 15 minutes. Formation of 1 cm layer of foam indicated the presence of saponins.

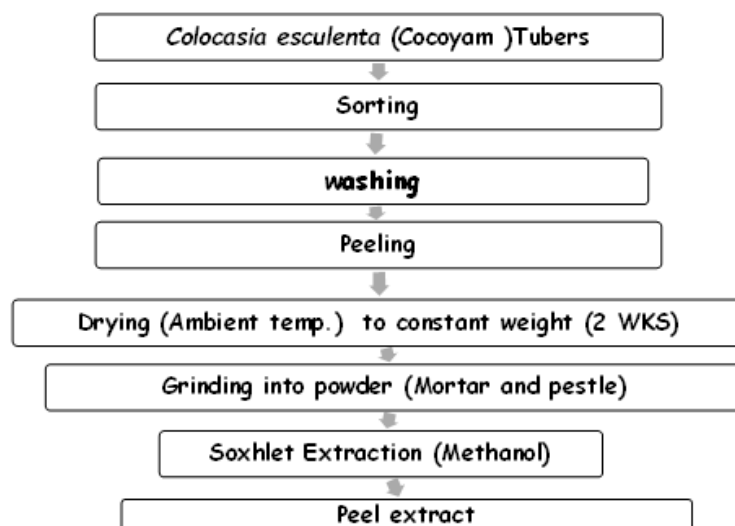


Fig. 2. Flow chart of the preparation of the cocoyam sample

iii. Test for flavonoids

Lead Acetate Test: 1 mL of 5 % lead acetate solution was added to 1 mL of the extract solution in a test tube and the mixture was allowed to stand for five minutes. The formation of precipitate in the mixture confirmed the presence of flavonoids.

iv. Test for Phenols

Ferric Chloride Test: 3 drops of ferric chloride solution was added to 1 mL of the extract in a test tube. The appearance of bluish-black colour indicates the presence of phenols.

v. Test for alkaloids

Hager's Test: 5 mg of the extract was dissolved in 3 mL of with dilute Hydrochloric acid and filtered. 2 mL of the filtrate was treated with Hager's reagent (saturated picric acid solution) in a test tube. The formation of yellow precipitates confirmed the presence of alkaloids.

vi. Test for steroids

Libermann Burchard's Test: 2 mL of the extract was treated with 2 mL of acetic anhydride and a drop of acetic acid, heated for 5 minutes and cooled in ice followed by addition of 1 mL of concentrated tetraoxosulphate (vi) acid carefully by the sides of the test tube. An array of colours changes from violet to blue or green indicated the presence of steroids.

Vii. Test for quinones

Hydrochloric Acid Test: 1 mL of the extract was treated with 3 drops of concentrated hydrochloric acid. A green colour indicated the presence of quinones.

Viii. Test for starch

Iodine Test: 1 mL of the extract was treated with 3 drops of iodine solution. A blue-black colour or dark blue colour indicated the presence of starch.

ix. Test for terpenoids

Salkocoski Test: 5 mL of the extract was treated with 2 mL chloroform, followed by 3 mL of concentrated tetraoxosulphate (vi) acid to form a layer. A reddish brown interface indicated the presence of terpenoids.

x. Test for glycosides

Keller- Killani Test: 5 mL of the extract was treated with 2 mL glacial acetic acid, followed by a drop of FeCl_3 solution and then 1 mL of concentrated tetraoxosulphate (vi) acid. Violet green rings appearing below the brown ring in the acetic acid layer indicated a positive test for glycosides.

2.4 Synthesis of Zinc Oxide Nanoparticles (ZnO NPs)

The method described by *Farjana et al*, [28] was used with slight modification. 10 mL aqueous $0.50 \text{ mol dm}^{-3} \text{ ZnO}$ was mixed with 5 mL of the extract in a 250 mL beaker. Then, the pH of the solution was adjusted to 12 by a drop-wise addition of 0.02 mol dm^{-3} aqueous solution of NaOH. The influence of temperature on ZnO NPs formation was studied by heating the solution on a water bath from 4°C - 90°C with constant stirring using a magnetic stirrer for 5 hours. The colour changed from light orange to white, indicating the formation of ZnO NPs. The solution was cooled to 30°C , purified by centrifugation at 1200 rpm for 6 minutes to obtain white precipitates. The precipitates were then washed four times with deionized water, dried for 24 hours at 90°C and stored in a desiccator for further analysis.

2.5 Characterization of ZnO NPs

The biosynthesized ZnO NPs were characterized by UV-visible spectroscopy, X-Ray Diffraction (XRD) analysis, Scanning Electron Microscopy (SEM), Energy Dispersive X-ray (EDX) and Fourier Transform Infrared (FTIR) spectroscopy. UV-Visible spectrophotometer (Uv-3600 Plus, Shimadzu, Japan) in the range of 350-700 nm was used to confirm the formation of the ZnO NPs. Phase and unit cell dimension information was determined with the use of XRD-6000, Shimadzu, Japan with monochromatic Cu K α radiation (1.5419 \AA), operated at 40 kV and 30 mA at 2θ (25° - 75°) and speed of 4° per minutes. SEM equipped with EDX (Philips XL-30, Eindhoven, Netherlands) was used to study the surface morphology and elemental composition of the ZnO NPs. FTIR analysis of the ZnO NPs was performed with Perkin Elmer FTIR Spectrophotometer-100 with the KBr pellet method in the range of $500 - 4000 \text{ cm}^{-1}$ to determine the functional groups responsible for the reduction of the Zn^{2+} as well as capping and stabilizing agents of the ZnO NPs.

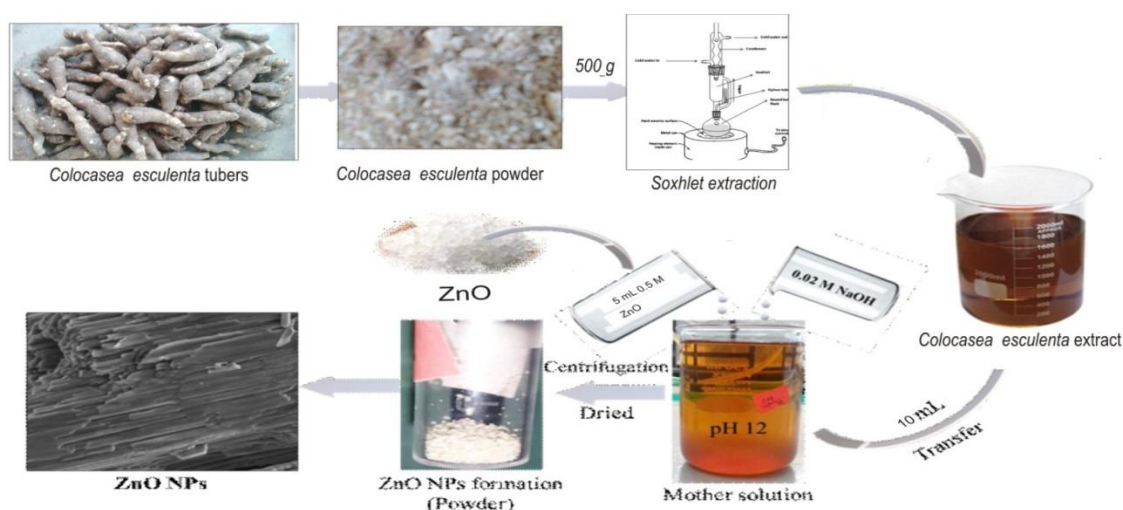


Fig. 3.A modified schematic diagram for the preparation of ZnO NPs using *C. esculenta* tuber peel extract [28]

2.6 Antimicrobial Sensitivity Test

The method of Shiriki et al [2] was employed with slight modification. The biosynthesized ZnO NPs was tested against five previously isolated and identified white yam pathogenic fungi: *Aspergillus niger*, *Botryodiplodia theobromae*, *Zygosaccharomyces bailli*, *Zygosaccharomyces rouxii*, and *Myrothecium verrucaria* as well as three bacteria: *Klebsiella oxytoca*, *Serratia marcescens*, and *Pseudomonas aeruginosa*. The pure isolates were individually cultured on ZnO NPs incorporated Potato Dextrose Agar (PDA) and Nutrient Agar (NA) plates for fungi and bacteria respectively and incubated at 37 °C for 7 days (fungi) and 24 hours (bacteria). Triplicates samples were prepared. The controls consisted of 1 mL 100 % fluconazol (200 mg) and 100 % of 1 mL ciprofloxacin (500 mg) tablets for fungi and bacteria respectively. Zone of inhibition (mm) where present was recorded with a transparent plastic ruler after the incubation period and the percentage inhibition zones calculated as follows:

$$\% \text{ Inhibition Zone (\% IZ)} = \frac{\text{Average diameter of pathogen colony}}{\text{Average diameter of pathogen control}} \times 100\% \quad (5)$$

The percentage inhibition was rated on the scale described by Pritha et al [12] as follows:

100 % inhibition (highly effective); 50 – 99 % inhibition (effective); 20 – 49 % inhibition (moderately effective); 0 – 19 % inhibition (slightly effective) and ≤ 0 % inhibition (not effective).

2.7 Statistical Analysis

The data obtained from the zone of inhibition (mm) was analyzed (descriptive statistics and inferential statistics to report the findings and to test hypothesis at 0.05 level of significance respectively) using statistical package for social science, SPSS Version 21. Results were reported as Mean \pm SD. The statistical difference between more than 2 groups of data was evaluated using ANOVA with LSD post hoc test. Differences between means were considered significant at $p < 0.05$.

3. RESULTS AND DISCUSSION

3.1 Phytochemical Screening

Table 1 presents the result of the phytochemical analysis of *C. esculenta* tuber peel extract. The result indicated the presence of tannins, saponins, flavonoids, phenolics, alkaloids, steroids, starch, terpenoids and glycosides.

3.2 UV-Visible Spectroscopy Analysis

The progress of the formation of ZnO NPs was followed by recording the absorption spectra as a function of time. The interaction between Zn^{2+} containing solution (ZnO) and the *C. esculenta* tuber peel extract shows λ_{max} at 365 nm with the absorption steadily building up with time and increasing temperature, reaching a maximum at 90 °C in five hours with a colour change from light orange to white, indicating the formation of ZnO NPs.

Table1. Phytochemical Analysis of *Colocasia esculenta* tuber peel extract

Secondary Metabolite	Test	Result
Tannins	FeCl ₃ Test	+
Saponins	Froth test	+
Flavoniods	Lead Acetate Test	+
Phenols	Ferric Chloride Test	+
Alkaloid	Hager's Test	+
Steroids	Libbermann Burchard's Test	+
Quinones	Hydrochloric Acid Test	-
Starch	Iodine Test	+
Terpenoids	Sakocoski's Test	+
Glycosides	Keller-Kallani's Test	+

Key: + = positive, - = negative.

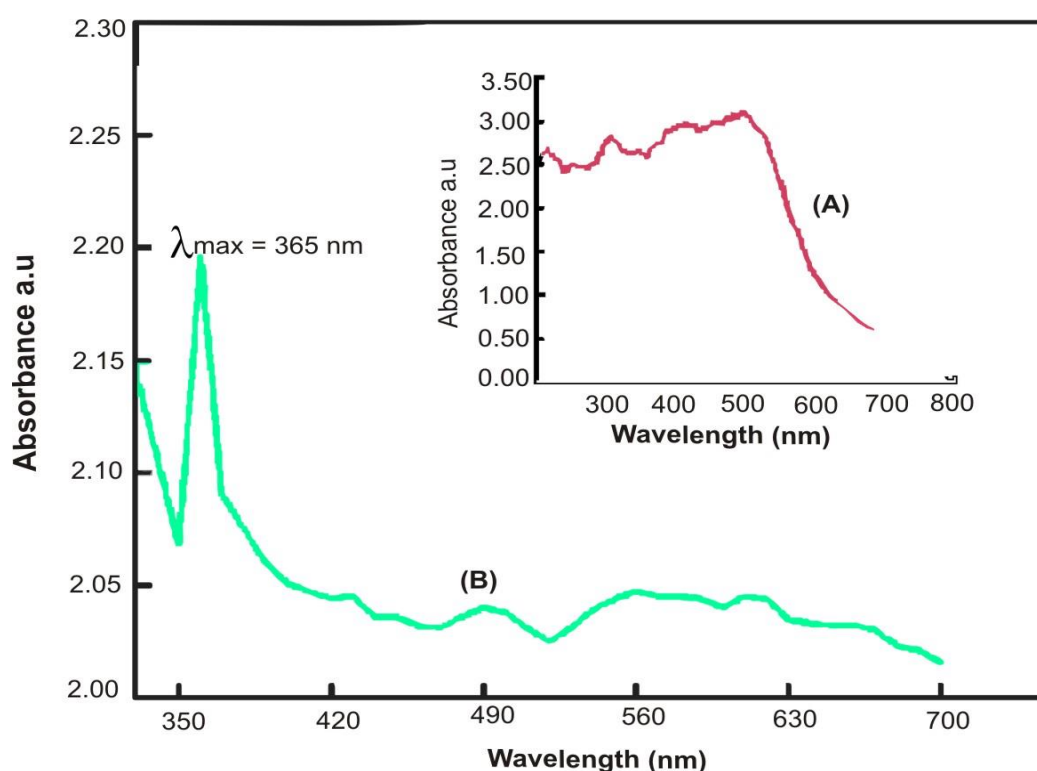


Fig. 4. UV-Vis spectra of (A) *Colocasia esculenta* tuber peel extract and (B) the biosynthesized ZnO NPs recorded as a function of reaction time

3.3 X-Ray Diffraction Analysis

X-Ray diffraction is a non-destructive analytical method for the identification and quantitative determination of various crystalline forms (phases) and crystallite sizes of powder or solid samples. Diffraction occurs as the light waves interact with the regular structure whose repeated distance (d) is about the same as the wavelength (λ) according to the Bragg's equation:

$$n\lambda = 2d\sin\theta \quad (6)$$

Where λ = wavelength, d = interplaner spacing, θ = diffracted angle and n = ,1,2,3...

The biosynthesized ZnO NPs was subjected to XRD analysis.

Fig. 4 represents the XRD spectra of the ZnO NPs. The XRD spectra revealed ten distinctive diffraction peaks at 2θ angles of 31.84°, 34.50°, 36.26°, 47.57°, 56.56°, 62.90°, 66.42°, 67.92°, 69.19° and 77.02°. Diffraction peaks are converted to d-spacing which is characteristic for each material that allow for the identification of

the material. The diffracted peaks above can be assigned to miller indices of 100, 002, 101, 102, 110, 103, 200, 112, 201 and 202 respectively, corresponding to the reflection lines of hexagonal wurtzite structure according to the Joint Committee on Powder Diffraction Standards (JCPDS) card no: 36-1451 [29].

The average crystallite size of the ZnO NPs was estimated by the Debye-Scherrer's equation

$$d = \frac{k\lambda}{\beta \cos \theta} \quad (7)$$

Where d = crystallite size (nm), k = correction/shape factor (0.9), β = full width at half maximum (FWHM) and θ = Bragg's angle (rad). The average crystallite size of the biosynthesized Zn NPs was calculated to be 10 nm with the range of 7.81 nm – 9.23 nm.. A decreasing

crystallite size leads to the broadening of the peak as peak width is inversely proportional to the crystallite size. The smaller the size, the narrower is the peak and vice versa.

3.4 Scanning Electron Microscopy (SEM) With Energy Dispersive X-Ray (EDX)

The scanning electron microscope (SEM) is a non-destructive method that uses accelerated and focused high-energy electrons to produce a variety of signals at the surface of solid samples because of electron-sample interactions. The size and surface morphology of the biosynthesized ZnO NPs was analyzed using SEM, while the elemental determination was carried out using energy dispersive X-ray (EDX). The SEM image of the ZnO is presented in Fig. 5 which shows hexagonal shapes with good crystallinity.

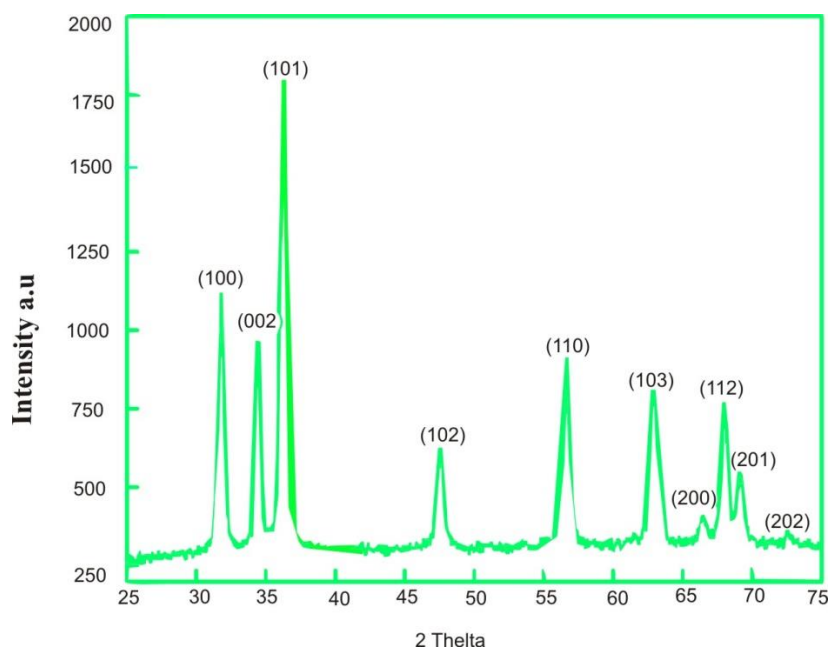


Fig. 5. XRD pattern of the biosynthesized ZnO NPs

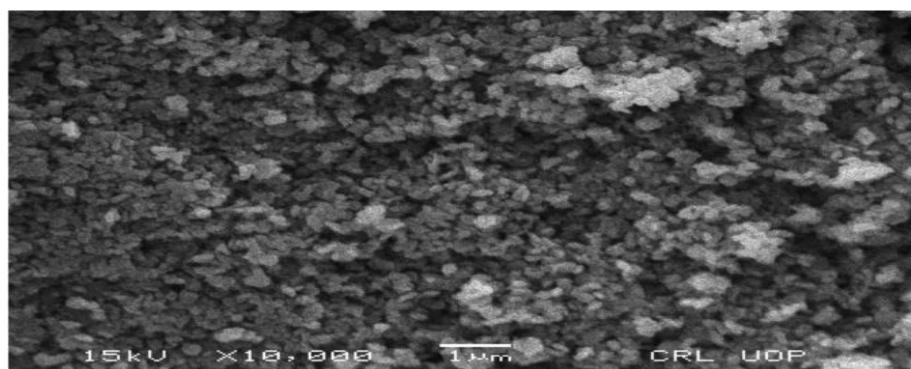


Fig. 6. SEM image of biosynthesized ZnO NPs

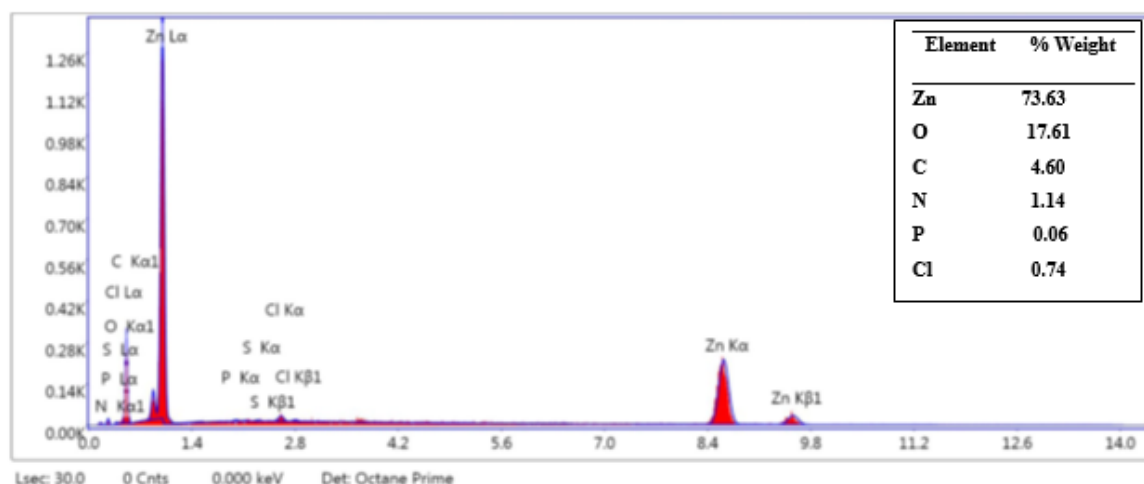


Fig. 7.EDX spectrum of the biosynthesized ZnO

“pH strongly influences the degree of agglomeration, particle setting and size due to the change of surface charge and zeta potential of particles which allow for a greater degree of particle interaction. Generally, metal and metal oxide nanoparticles synthesis under acidic or neutral conditions tends to cause particle agglomeration. The optimal pH for the synthesis of ZnO NPs with lower agglomeration is 8” [28]. “At low pH, the adsorption of metallic particles that were positively charged to negatively charged organic matter from capping agents is enhanced, resulting in higher degree of aggregation” [25]. The acidic process would also change chemical structures and activities of the reductant to form alkoxide ions. Other studies [25, 28] confirmed that “the size and density of the nanoparticles increase as a response to a decrease in the acidity of the medium”. “When the pH is decreased continuously to a certain limits, it causes the re-dissolution of Zn(OH)₂, resulting in increased size” [28].

“The calculation of the crystallite size of the ZnO NPs using ImageJ software gives the size of about 9.87 nm, which is in agreement with the calculated crystallite size of 10 nm from XRD data using the Debye-Scherrer equation. Hydrogen bonding and electrostatic interactions between biogenic capping molecules, and Nps cause agglomeration” [25]. The SEM image shows that the ZnO NPs are not in direct contact with each other, signifying little agglomeration and the stabilization of ZnO NPs.

“The elemental composition and purity of the ZnO NPs were determined by EDX analysis. Energy dispersive X-ray spectroscopy is an

analytical technique used for the elemental analysis or chemical characterization and purity of a sample. The characterization capabilities are due in large part to the fundamental principle that each element has a unique atomic structure, allowing x-rays that are characteristic of an element’s atomic structure to be identified uniquely from each other” [28].

Fig. 6 shows the chemical composition of the ZnO NPs with their respective percentages. The EDX spectrum shows characteristic peaks and elemental composition of Zn and Oxygen (73.63%) and O (17.61%) respectively, but low percentage of C, N, P, S and Cl which indicate high purity of the biosynthesized ZnO NPs.

3.5 Fourier Transform Infra-Red Spectroscopy (FTIR) Analysis

The small peak at 662 cm⁻¹ is allotted to the hexagonal wurtzite phase Zn-O stretching vibration. The broad peak at 3382 cm⁻¹ is characteristic of stretching vibration of hydroxyl group (O-H) from polyphenols. The peak at 2374 cm⁻¹ is attributed to the H-O-H vibrations of water molecules of crystallization. The peaks at 1656 cm⁻¹, 1524 cm⁻¹, 1380 cm⁻¹, 1014 cm⁻¹ and 883 cm⁻¹ are assigned to bending vibrations of C=C stretching of alkene, aromatic ring and polyphenols (C=O), C-H bending vibrations of alkane groups, stretching of C≡N, and bending vibrations of C-H respectively.

The FTIR analysis of the *C. esculenta* tuber peel extract shows peaks at 3281 cm⁻¹ which represents the symmetric O-H stretching, while that at 2918 cm⁻¹ is assigned to phospholipids,

cholesterol and creatine. The peak at 2831 cm^{-1} and 1734 cm^{-1} correspond to the C-H and C=O stretching respectively, while that at 1596 cm^{-1} represents C=N and NH_2 respectively. The peak at 1410 cm^{-1} is assigned to stretching C=N, N-H and C-H deformations. Peaks 1370 cm^{-1} corresponds to N-H deformation, while the peak at 1232 cm^{-1} is allocated to the overlapping of the protein amidelll and the nucleic acid phosphate vibrations. The peaks at 1022 cm^{-1} and 532 cm^{-1}

are assigned to the glycogen and sulphur compounds respectively.

The FTIR analysis confirmed the presence of functional groups from phytochemicals such as tannins, saponnins, alkaloids, terpenes, flavonoids, phenols, steroids, and aromatic hydrocarbon, amines, amides etc which are responsible for the reduction of the Zn ions as well as capping and stabilizing agents of the ZnO NPs.

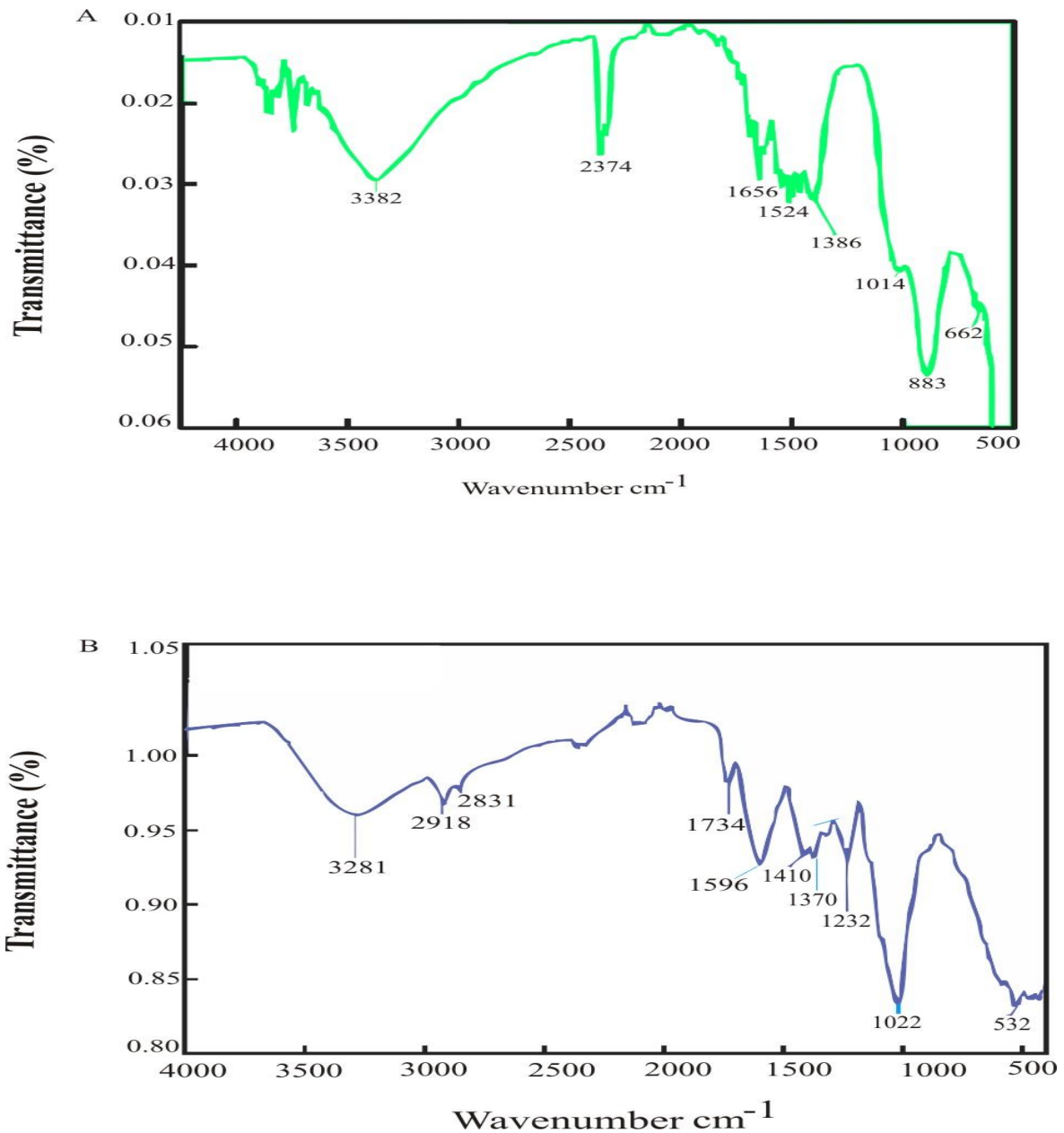


Fig. 8. FTIR spectra of (A) the biosynthesized ZnO NPs and (B) *Cocosia. Esculenta* tuber pee extract

Table 2: Antimicrobial Sensitivity Test/ Average Zone of Inhibition (mm) of Biosynthesized ZnO NPs

	Zinc Oxide Nanoparticles Concentration (%)				Control
	100	10 ⁻¹	10 ⁻²	10 ⁻³	
	IsolatesFungi				
<i>Aspergillus niger</i>	8.33 ± 0.82	7.83 ^a ± 0.99	7.00 ^{ab} ± 0.63	5.33 ^{abc} ± 0.52	9.67 ± 2.69abcd
<i>Botrodiphodia theoromae</i>	8.33 ± 0.82	7.50 ^a ± 0.54	6.33 ^{ab} ± 0.51	5.20 ^{abc} ± 0.20	10.33 ± 1.96abcd
<i>Zygosaccharomyces bailli</i>	8.33 ± 0.82	7.50 ^a ± 0.54	7.00 ^{ab} ± 0.63	6.67 ^{abc} ± 0.32	11.00 ± 0.89abcd
<i>Zygosaccharomyces rouxil</i>	10.67 ± 3.00	8.16 ^a ± 0.98	7.50 ^{ab} ± 0.55	5.80 ^{abc} ± 0.41	10.80 ± 0.98abcd
<i>Myrothecium verrucaria</i>	26.83 ± 12.98	21.0 ^a ± 4.05	13.00 ^{ab} ± 2.19	12.00 ^{abc} ± 2.19	30.00 ± 6.03abcd
	Bacteria				
<i>Klesiella oxytoca</i>	30.00 ± 4.60	15.67 ^a ± 5.61	9.00 ^{ab} ± 2.37	7.17 ^{abc} ± 1.17	34.83 ± 0.75abcd
<i>Serratia marcescens</i>	43.84 ± 1.72	26.00 ^a ± 0.89	9.00 ^{ab} ± 3.41	6.50 ^{abc} ± 1.05	41.83 ± 3.31abcd
<i>Pseudomonas aeruginosa</i>	37.16 ± 3.37	26.83 ^a ± 4.67	20.33 ^{ab} ± 1.63	9.83 ^{abc} ± 2.79	38.16 ± 3.87abcd

N= 6, values expressed as Mean ± SD. a = significant relative to 100 % at p < 0.05, b = significant compared with 10⁻¹ % at p < 0.05, c = significant compared with 10⁻² % at p < 0.05 [30-32]

Table 3. Percentage Zone of Inhibition of the Isolates to Different Concentrations of the Biosynthesized Zn NPs

Microorganism	Concentration (%)			
	100	10 ⁻¹	10 ⁻²	10 ⁻³
Fungi				
<i>Aspergillus niger</i>	86.14	80.97	72.39	55.12
<i>Botryodiplodia theobromae</i>	80.64	72.60	61.28	50.33
<i>Zygosaccharomyces bailli</i>	75.73	68.18	63.64	60.64
<i>Zygosaccharomyces rouxii</i>	98.52	75.35	69.25	53.55
<i>Myrothecium verrucaria</i>	89.43	70.00	43.33	40.00
Bacteria				
<i>Klebsiella oxytoca</i>	86.13	70.31	45.04	20.59
<i>Serratia marcescens</i>	83.27	62.16	21.52	15.54
<i>Pseudomonas aeruginosa</i>	97.37	70.31	53.27	25.76

3.6 Antimicrobial Study of the ZnO Nanoparticles

The antimicrobial study of the ZnO NPs was carried out against five previously isolated and identified white yam pathogenic fungi: *Aspergillus niger*, *Botryodiplodia theobromae*, *Zygosaccharomyces bailli*, *Zygosaccharomyces rouxii*, and *Myrothecium verrucaria* and three bacteria: *Klebsiella oxytoca*, *Serratia marcescens*, and *Pseudomonas aeruginosa*. Table 2 represents the average zone of inhibition (mm), while Table 3 presents the percentage zone of inhibition (% IZ) of ZnO against the isolates.

Generally, the results showed that the inhibitory effects of the biosynthesized ZnO NPs increased with increasing concentration ($p < 0.05$). The biosynthesized ZnO NPs showed effective to slightly effective inhibition against the test organisms, ranging from 98.52% - 15.54% (Table 3). Effective inhibition ($\geq 55.12\%$), ($\geq 60.64\%$) and ($\geq 53.55\%$) against *Aspergillus niger*, *Zygosaccharomyces bailli* and *Zygosaccharomyces rouxii* respectively at all concentrations. The ZnO NPs was able to inhibit effectively *Botryodiplodia theobromae* ($\geq 61.28\%$) at all concentrations. *Myrothecium verrucaria* showed effective inhibition ($\geq 70.00\%$) at concentrations of 100% and 10⁻¹%, while showing moderately effective inhibition ($\geq 40.00\%$) at 10⁻² % and 10⁻³ %. Effective inhibition was recorded against *Klebsiella oxytoca* ($\geq 70.31\%$) at 100% and 10⁻¹%, while moderately effective inhibition ($\geq 20.59\%$) was obtained at ZnO NPs concentration of 10⁻²%, 10⁻³%. and 10⁻³. Effective ($\geq 62.16\%$), moderately effective (21.52%) and slightly effective (15.54%) inhibitions were recorded at 100 %, 10⁻¹ %, 10⁻² % and 10⁻³ respectively against *Serratia*

marcescens. *Pseudomonas aeruginosa* was effectively inhibited ($\geq 53.27\%$) at 100%, 10⁻¹% and 10⁻² %, but moderately (25.76%) inhibited at 10⁻³. The results are effective against the test microorganisms when compared with standard antimicrobial agents: fluconazole and ciprofloxacin ($p > 0.05$).

4. CONCLUSION

ZnO NPs was effectively synthesized through the green route approach using *C. esculenta* tuber peel extract. Characterization of the biosynthesized ZnO NPs was done using FTIR, UV-Vis, XRD, EDX, and SEM. ZnO NPs with a hexagonal wurtzite structure and an average crystallite size of 10 nm, ranging from 7.81 nm to 9.23 nm, were proven to have formed at 365 nm by UV-Vis analysis. Higher percentage weights of Zn and O, but lower percentages of C, N, P, S, and Cl were confirmed by EDX, suggesting that the biosynthesized ZnO NPs were highly pure. The ZnO NPs' reduction, capping, and stabilization were all caused by organic functional groups, which the FTIR analysis verified were present.

The antimicrobial study of the ZnO NPs against white yam pathogens compared favourably with standard antifungal (fluconazole) and antibacterial (ciprofloxacin) agents. The biosynthesized ZnO NPs holds great potential in controlling and/or preventing white yam tuber rot and can provide an alternative to synthetic antimicrobial agents since it is less expensive, environmentally friendly, biocompatible and easy to prepare.

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COMPETING INTERESTS

Authors have declared that no competing interests exist.

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