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GREEN SYNTHESIS, CHARACTERISATION AND ANTIBACTERIAL ACTIVITY OF ZINC OXIDE NANOPARTICLES USING *Persea americana* Mill. AQUEOUS SEED EXTRACT

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ABSTRACT. The avocado seed is a non-edible part of the fruit that is typically discarded as waste; yet it has valuable phytochemicals. A phytochemical screening of the aqueous extract from avocado seeds was conducted under controlled experimental conditions using standard methods. The results indicated the presence of flavonoids, saponins, terpenoids, eugenols, tannins, phenolics, glycosides, and alkaloids while steroids were absent. Zinc oxide nanoparticles (ZnO NPs) were synthesized from the avocado seed aqueous extract using zinc acetate as a precursor. The biosynthesized NPs were characterized using X-ray diffraction (XRD), which confirmed their hexagonal wurtzite crystalline structure. The XRD analysis revealed an average crystallite size of 29.876 nm for the ZnO NPs. These ZnO NPs were subsequently tested as antibacterial agents against Gram-positive bacteria (*Staphylococcus aureus* and *Bacillus subtilis*) and Gram-negative bacteria (*Escherichia coli, Klebsiella pneumoniae* and *Pseudomonas aeruginosa*). The biogenic ZnO NPs exhibited an inhibition zone of 20 mm against *S. aureus;* therefore, indicating its usefulness in the pharmaceutical sector.

Keywords: ZnO NP, *Persea americana*, *Staphylococcus aureus,* maceration.

INTRODUCTION

Plants are rich sources of secondary metabolites, which include flavonoids, alkaloids, phenolic compounds, tannins, saponins, *etc.* (ADEYEMI *et al.,* 2022). Avocado by-products, mainly seeds and peels, which are usually referred to as fruit waste, are sources of important phytochemicals. These phytochemicals can be extracted using different solvents such as water, ethanol, *n*-hexane, ethyl acetate, *etc.* The resulting phytochemical extracts possess significant antibacterial, antioxidant, anti-inflammatory, and anticancer activities and are suitable for the synthesis of nanoparticles (HAIDER *et al*., 2019; MOHAMED *et al.,* 2023; OVONRAMWEN *et al*., 2024).

Avocado (*Persea americana* Mill.), a member of the Lauraceae family, is a tropical plant mostly found in Central and South America (SEGOVIA *et al*., 2018). While numerous

varieties exist, the Hass variety is the most widely known (ORHEVBA and JINADU, 2011). The avocado seed, which constitutes 13-18% of the fruit's total mass, is an inedible by-product typically discarded as waste (ADARAMOLA *et al.,* 2016). Improper disposal of this waste can potentially increase the number of insects and rodents in the environment. However, the avocado seed is a valuable source of a range of useful chemicals (IFESAN *et al*., 2015), including important phytochemicals (SETYAWAN *et al.,* 2021). Notably, the seed possesses a higher total phenolic content and antioxidant capacity than the fruit's pulp or peel (WANG *et al*., 2010).

Nanotechnology is a branch of science focused on designing, producing, and utilizing structures, devices and systems by manipulating the molecular structure at the nanoscale level. Nanomaterials offer numerous applications thanks to their unique size and morphology (KARNWAL *et al*., 2024; OVONRAMWEN *et al.,* 2025). However, chemical synthesis of nanoparticles can result in some toxic chemicals adsorbed onto their surface, which can pose hazards when they are used. The use of biological methods has provided a more eco-friendly, low-cost, and non-toxic method for the synthesis of nanoparticles (HAIDER *et al*., 2019; MOHAMED *et al.,* 2023; KARNWAL *et al*., 2024; OVONRAMWEN *et al*., 2024). Several types of inorganic metal oxides have been synthesized and remained in recent studies, like TiO_2, CuO, and ZnO. Of all these metal oxides, ZnO NPs are of maximum interest because they are inexpensive to produce, safe and can be prepared easily (JAYASEELAN *et al.,* 2012; OVONRAMWEN *et al*., 2024). ZnO nanoparticles offer several advantages, including strong antibacterial activity at low concentrations (0.16-5.00 mmol/L), activity against a broad spectrum of microbial strains, and relatively low production costs (HOUSKOVA *et al*., 2007; MANZOOR *et al*., 2016).

Nanoparticles derived from metals and their oxides have attracted significant interest. Among these, zinc (Zn) and its oxide (ZnO) are the most extensively studied. Zinc is a highly reactive element with strong reduction properties and readily oxidizes to form zinc oxide. As one of the essential trace elements, zinc plays an important physiological role in the human body (MARET, 2011). It is present in all tissues, with the highest levels found in myocytes, which contains 85% of the body's total zinc content (KRÓL *et al.,* 2017). Zinc is crucial for the proper function of numerous macromolecules and enzymes, where it exhibits both catalytic (as a coenzyme) and structural roles.

The rise of antibiotic resistance in bacteria represents one of the most critical problems facing global health care. Antibiotic resistance was reported by the World Health Organization (WHO) in 2019. to be responsible for the deaths of 1.27 million globally and contributed to 4.95 million deaths with an estimate of 20 million by 2050, which is equivalent to a cost of $2.9 trillion. Antibiotic resistance poses a threat to medical care like surgeries, cancer treatments and organ transplants, that require the use of antibiotics for the prevention of infections (ALQURASHI *et al.,* 2025). The continuous threat of resistant infections increases the risk associated with medical procedures and may even limit their feasibility. One promising strategy in combating microbial resistance is the application of the metal nanoparticles and their oxides (GUDKOV *et al*., 2021). They have shown promising activities in medical-related fields due to their intrinsic antimicrobial activity that enhanced their functionalization (SOLANKI *et al*., 2024; PARVIN *et al.,* 2025).

The review of previous and recent research work shows that the synthesis of metal nanoparticles through green synthesis is a more environmentally friendly, non-toxic, and low-cost method for synthesizing nanoparticles. ZnO NPs have high antibacterial potential in relation to Gram-positive and Gram-negative bacteria. In this vein, we report the secondary metabolites in avocado seed used as a capping agent in the green synthesis of ZnO NPs and their activities against urinary tract microorganisms. In the course of this study, *P. americana* aqueous seed extract was used to synthesise ZnO NPs utilizing zinc acetate dihydrate as a precursor.

MATERIALS AND METHODS

Materials

Analytical grade reagents were used. All reagents and chemicals were used as received, with no further purifications.

The avocado fruits were obtained from the New Benin Market in Benin City, Edo State, South-South, Nigeria (6.3995485: 5.6019037). The fruits were assigned the voucher number UBH-P408 by Prof. H.N. Akinnibosun of the Department of Plant Biology and Biotechnology, University of Benin, Benin City.

Methodology

The *P. americana* ripe fruits (2,800 g) were washed with distilled water and cut into two halves, de-capped, and the seeds (500 g) were washed with water, sliced into pieces, and sun-dried for 4 days (68.0 g). The moisture-free samples were ground with a mechanical grinder, stored in an airtight plastic container (63.68 g), and finally taken to the laboratory for analysis (Fig. 1).

(a)  (b)  (c) 

Figure 1. *P. americana* (a) fresh fruits (b) dried seeds (c) ground seed powder.

Plant extract preparation

Solvent extraction was used to separate bioactive compounds from the avocado seed. 20 g of the avocado seed powder was added to 200 mL of water (1:10 w/v), marcerized for 72 h with occasional shaking, and filtered with a clean white cloth three times to yield a residue of 7.45 g (Fig. 2). The filtrate was concentrated at 70 °C in the water bath (12.55 g).

(a)  (b) 

Figure 2. Macerated seed extract (a) before and (b) after concentration.

Analysis of phytochemicals

The following qualitative examination of phytochemical content was carried out according to standard methods (TREASE and EVANS, 1989; SOFOWORA, 1993):

Detection of Alkaloids

Hager's Test: The aqueous extract was filtered after being diluted in a few mL of diluted hydrochloric acid. A few drops of Hager's reagent (picric acid in a saturated aqueous solution) were added to 2 mL of filtrate. The presence of a bright yellow precipitate shows that the test for alkaloids is positive (ALHAITHLOUL, 2023).

Wagner Test: To aqueous extract, 2 mL of Wagner's reagent was added with appearance of a reddish-brown precipitate (AKPOMIE *et al., 2*021).

Dragenoff's test: To 2 mL of the extract, 2 mL Dragenoff's reagent was added, and reddish-brown precipitate was an indication of a positive test.

Detection of Tannin

Two to three mL of the boiling extract was filtered and 2 drops $FeCl_3$ (10%) solution was added to the filtrate. There was a greenish grey or dark blue precipitate an indication of the presence of tannin.

Detection of Saponin

With vigorous shaking, 5 mL of distilled water was added to the filtrate of the 5 mL aqueous extract. The formation of froth indicated the presence of saponins.

Detection of Terpenoid (Salkowski test).

To 5 mL of the extract, 2 mL of chloroform and 3 mL of concentrated H_2SO_4 were added along the side of the inner test tube. A reddish-brown colouration at the interface confirmed the presence of terpenoids (AKPOMIE *et al*., 2021).

Detection of Glycoside (Keller-Killani test)

To 1 mL of the aqueous extract, 1 mL of glacial acetic acid and a drop of ferric chloride solution were added, followed by the addition of 1 mL of concentrated H_2SO_4. The formation of a brown ring at the interface indicates the presence of a deoxysugar.

Detection of Flavonoid

To 2 mL of pre-boiled aqueous extract filtrate, a few drops of 10% lead acetate solution were added. The formation of a yellow precipitate indicates the presence of flavonoids.

Detection of steroids

Two mL of $(CH_3CO)_2O$ were added to 1 mL of plant extract in 2 mL of dilute H_2SO_4 in a test tube. The solutions turned reddish, indicating the presence of steroids in the extracts.

Detection of phenols

To 5 mL of 90% ethanol, approximately 1 mL of *P. americana* aqueous seed extract was added. Two drops of 10% ferric chloride were then introduced, resulting in a brownish or blue-black-green colour indicating the presence of phenols (AKPOMIE *et al*., 2021).

Synthesis of ZnO nanoparticles from Zinc acetate

1.77 g of zinc acetate dihydrate ($Zn(CH_3COO)_2.2\ H_2O$) was dissolved in 80 mL of H_2O (0.01 mol). 20 mL of the aqueous extract was added and stirred using a magnetic stirrer. 4 g of NaOH were dissolved in 50 mL of water (0.02 M) and added dropwise until the pH reached 12.5. The mixture was continuously stirred for 3 h. Subsequently, a colour change was observed, resulting in the formation of a white precipitate in the container. The precipitate was then centrifuged and washed three times at 3500 rpm for 30 min, air-dried in an oven at 70–80 °C for 2 h, and calcinated in a furnace at 400 °C. The resulting particles, weighing 1.047 g, were placed in an airtight container for further analysis (Fig. 3).

Characterisation of ZnO NPs

Several analytical techniques, including Fourier Transform InfraRed (FT-IR) spectroscopy, Scanning Electron Microscopy (SEM), and UV-Visible (UV-Vis) analysis, were employed to determine the morphology, chemical composition, and specific phenolic compounds present in the samples.

The UV-Visible (UV-Vis) analysis of the biosynthesized ZnO NPs was conducted using a GENESYS 10Sv1.200 spectrophotometer (2 LJ35002). Fourier Transform Infrared (FTIR) spectroscopy was employed to identify the functional groups present on the synthesised ZnO nanoparticles derived from the *P. americana* seed sample, utilizing a Cary 630 from Agilent Technologies. Scanning Electron Microscopy (SEM) was utilized to assess the topography and composition of the *P. americana* seed extract, utilizing an Agilent Microlab PC located in Zaria, Nigeria. X-ray Diffraction (XRD) analysis was performed to evaluate the crystalline structure and phase purity of the biosynthesized ZnO nanoparticle powder, using a Bruker D8 Advance X-ray diffractometer over a 2θ range of 20–80º.

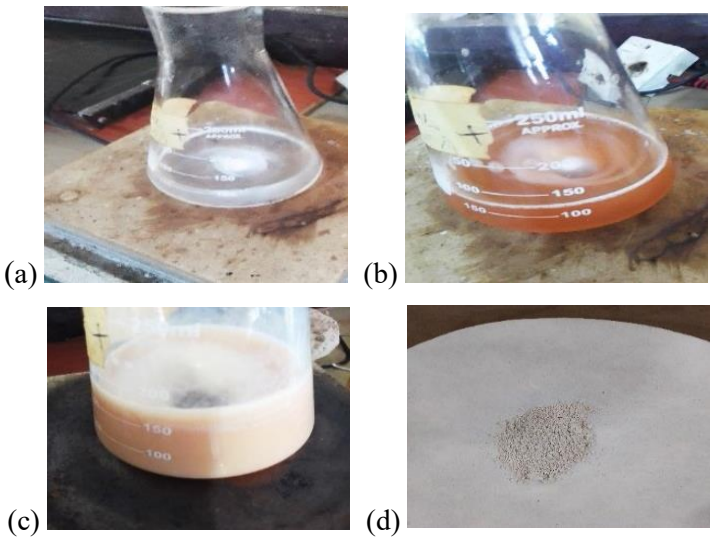


Figure 3. (a) 0.01 M $Zn(CH_3COO)_2.2\ H_2O$ solution (b) $Zn(CH_3COO)_2.2\ H_2O$ and the extract (c) synthesised ZnO NP (d) calcinated ZnO NP.

Antibacterial activity

The antibacterial activity of the synthesized ZnO NPs was examined against a variety of clinical pathogenic bacteria, including Gram-positive (*Staphylococcus aureus* and *Bacillus subtilis*) and Gram-negative bacteria (*Escherichia coli*, *Klebsiella pneumoniae,* and *Pseudomonas aeruginosa*) which were collected from University of Benin Teaching Hospital and the analysis were carried out in Pharmaceutical Microbiology Laboratory, University of Benin.

The test was conducted using the standard agar well diffusion technique in sterile dishes. Nutrient agar was prepared by dissolving 7.5 g of nutrient agar powder in 150 mL of distilled water. The medium was then sterilized in an autoclave at 121 °C for approximately 15 min at a pressure of 0.165 MPa. A 0.5 Marfarland standard solution was prepared for the inoculum of microorganisms.

80 mg of the sample and 100 mg ciprofloxacin were weighed and dissolved in their respective 2 mL of the 25% Tween 80 solvent. A bacterial suspension was created by dissolving bacterial strains in 1 mL of sterilized water, which was then used to streak freshly prepared Mueller-Hinton agar to promote bacterial growth. Wells were drilled into the bacterial media in each dish using a sterile gel drill or cork-borer, proportional to the number of samples. The wells were filled with 1 mL of the synthesized ZnO NP. The plates were allowed to sit on the bench for 1 h for ZnO NP to diffuse into the agar. The Mueller-Hinton agar plates were incubated at 37 °C for 24 h. After the incubation period, all plates were examined for any signs of inhibition, which appeared as clear zones completely devoid of growth around the wells (zones of inhibition). The diameters of these zones were measured using a transparent ruler calibrated in millimeters (mm).

Determination of Minimum Inhibitory Concentration (MIC)

The MIC of the NPs was determined using an agar dilution method. A total of 0.1 g, 0.2 g, and 0.4 g of the ZnO NPs were diluted in 1 mL of water to achieve concentrations of 100 mg/mL, 200 mg/mL, and 400 mg/mL, respectively (1:10 w/v). Each sample was then mixed with 9 mL of Mueller Hinton agar, inoculated with *S. aureus,* and incubated at 37 °C for 24 h. After the incubation period, the tubes were examined for the presence or absence of growth, using turbidity as a criterion. The lowest concentration in the series that showed no visible signs of growth was considered the MIC.

Determination of Minimum Bactericidal Concentration (MBC)

The MBC was established based on the results of the MIC. A sterile wire loop was dipped into the tube that did not exhibit turbidity during the MIC test. The contents were then streaked onto freshly prepared sterile nutrient agar plates. The plates were incubated at 37 °C for 24 h. After the incubation period, the plates were examined for the presence or absence of bacterial growth.

RESULTS AND DISCUSSION

The biosynthesis of ZnO nanoparticles using *P. americana* as a reducing and stabilizing agent yielded percentage results for both the extract and the synthesized compound, as presented in Table 1. The avocado seed exhibited a yield of 17.86% of the avocado fruit, which is consistent with literature reports (COSTAGLI and BETTI, 2015). The extraction yield of *P. americana* was 62.76%. The presence of phenolic compounds (secondary metabolites) may be responsible for the reduction and stabilization effects (WANG *et al*., 2010; SETYAWAN *et al*., 2021), as well as the antibacterial activity, as shown in Table 2. The colour change observed in the reaction mixtures provided clear evidence of ZnO nanoparticle formation. This observation is consistent with previous studies that also reported colour changes during the plant-mediated synthesis of ZnO nanoparticles (OVONRAMWEN *et al*., 2024; TAKCI *et al*., 2025).

Table 1. Percentage yield of extract and synthesised ZnO NP.

Sample	Yield (%)
Seeds	17.86
Ground sample	93.64
Extraction	62.76
Synthesised ZnO NP	59.15

Table 2. Secondary metabolites of qualitative phytochemical screening.

Secondary metabolites	Results
Glycosides	+
Saponins	+
Alkaloids	+
Tannins	+
Steroids	-
Terpenoids	+
Eugenols	+
Flavonoids	+
Phenolics	+

+ = Present; - = Absent

The synthesis of ZnO NPs based on aqueous seed extract of *P. americana* was further analyzed through UV spectrophotometry. The maximum absorption peak observed was at 371 nm, confirming the formation of ZnO NPs (Fig. 4). These results align with the standard absorption pattern of ZnO, as all oxide materials possess wide band gaps and typically exhibit shorter wavelengths. Furthermore, nanoscale materials tend to display shorter wavelengths. This is further supported by the results obtained for ZnO NPs. The measured wavelength is consistent with the reported value (MANIMEGALAI *et al*., 2023; TAKCI *et al*., 2025).

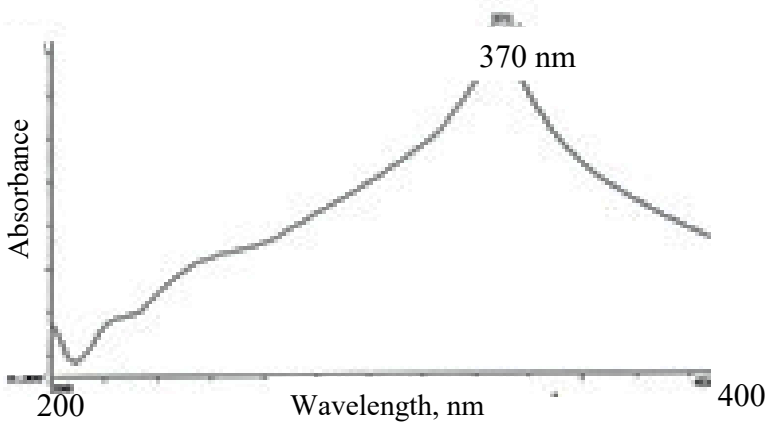


Figure 4. UV peaks recorded by the spectrophotometer.

Identification of Functional groups

To determine the functional groups related to the formation of ZnO NPs. The FTIR analysis was conducted, as illustrated (Fig. 5). The figure shows a functional group region at 3384.4 cm^{-1} which corresponds to O-H stretching of the alcohols and polyphenols. Peaks at 2384.2 cm^{-1} and 2113.4 cm^{-1} which correspond to the C-H stretching of alkenes and alkynes, respectively. The peak at 1640.0 cm^{-1} indicates a C=O stretching vibration of the carboxylic group. In the fingerprint region, there is a variable intensity at the wavenumber 1468.6 cm^{-1} indicates the presence of the C-H deformation. Additionally, the band at 1401.5 cm^{-1} corresponds to aromatic C-H bending, while the bands at 868.5 cm^{-1} (aromatic C-H symmetric stretching) and 705.5 cm^{-1} (aromatic C-H asymmetric bending). These observed peaks similar to the results obtained for ZnO NPs from the aqueous peel extract of *Aloe vera* (CHAUDHARY *et al*., 2019).

Scanning Electron Microscopy (SEM)

SEM was employed to analyze the surface morphology of the ZnO nanoparticles at magnifications of × 26,000, × 28,000, and × 30,000 (Fig. 6). The majority of the nanoparticles appear spherical, with an average size of approximately 23.43 nm. Furthermore, the images indicate that the ZnO nanoparticles exhibit a narrow spacing between particles, which may enhance their antibacterial activity.

NPs synthesis revealed several broad diffraction peaks at 28.595°, 31.768°, 32.654°, 34.474°, 36.267°, 47.521°, 56.640°, 62.893°, and 68.042° (Fig. 7). These peaks correspond to the hexagonal wurtzite structure of ZnO, as documented by the Joint Committee on Powder Diffraction Standards (JCPDS-36-1451) (SELIM *et al.,* 2020). The prominent peak observed at 36.267° indicates favoured development plane, underscoring the exceptional purity of the ZnO NP. This finding aligns with previous studies involving the aqueous bark extract of *Acacia nilotica* (JANANI *et al.,* 2020).

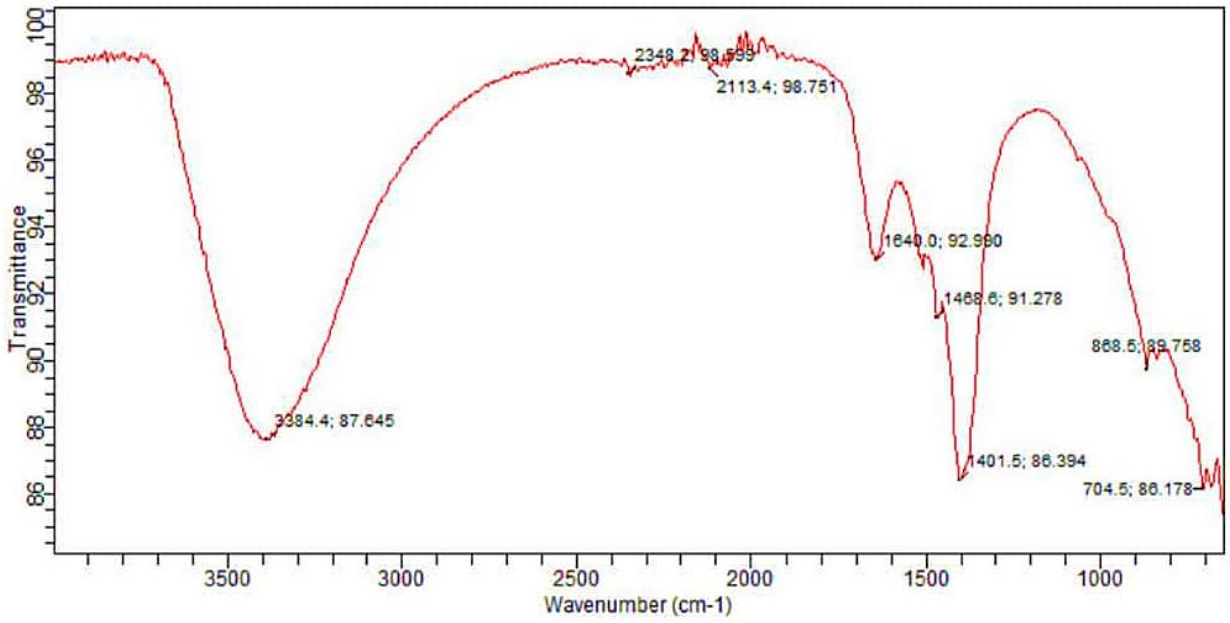


Figure 5. FTIR image of ZnO NPs synthesized.

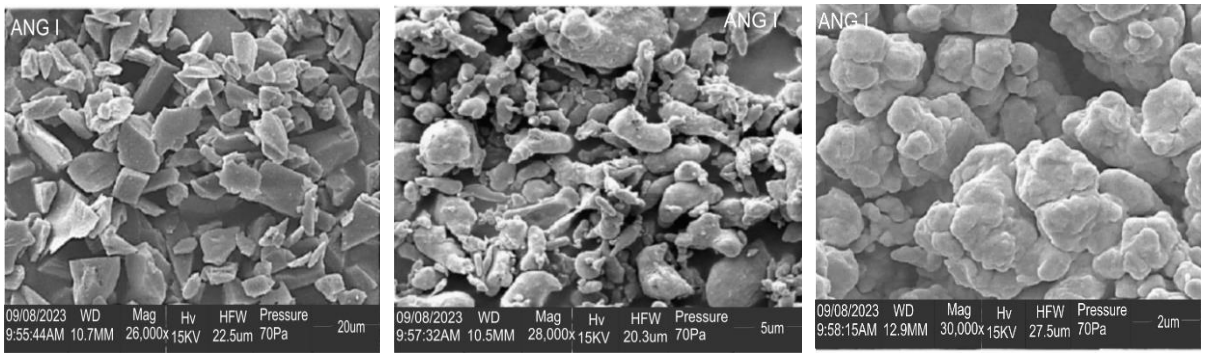


Figure 6. SEM images of ZnO NPs of *P. americana* at different magnification.

Using Scherrer's equation (1), the NP diameters were determined as follows

$$D = \frac{K\lambda}{\beta \cos\theta} \quad (1)$$

Where D is the grain or crystal size of the nanoparticles, λ is the wavelength of radiation, β indicates the full width at half maximum measured in radians, and θ is the angle of diffraction.

From Tab. 3, the average crystal size of the synthesised ZnO NPs is 29.876 nm. This small size is attributed to a narrow peak width; and an increase in peak width typically results in smaller crystal sizes. All ZnO nanoparticle samples exhibited strong, narrow peaks during diffraction, indicating that the particles are well-crystallised (DOBRUCKA and DŁUGASZEWSKA, 2016). The nanoparticle size falls within the reported range (FAISAL *et al*., 2021; MURALI *et al.,* 2023).

The XRD-EDX analysis elucidated the elemental composition of the biosynthesized ZnO NP (Figure 6). The qualitative elemental analysis revealed that zinc constituted 88.5% (Fig. 8). This percentage of zinc also supports the high level of purity.

The results of the antimicrobial activity, as shown in Tab. 4, reveal that the ZnO NPs synthesized from avocado seeds do not exhibit any inhibition zones against *Escherichia coli, Bacillus subtilis, Klebsiella pneumoniae,* and *Pseudomonas aeruginosa*. A similar report has been reported by some researchers (OVONRAMWEN *et al*., 2024). It has been reported the dosage effect of the nanoparticles on bacteria, with different inhibition effects on both Gram-positive and Gram-negative organisms (CHAUDHARY *et al*., 2019; TAKCI *et al*., 2025).

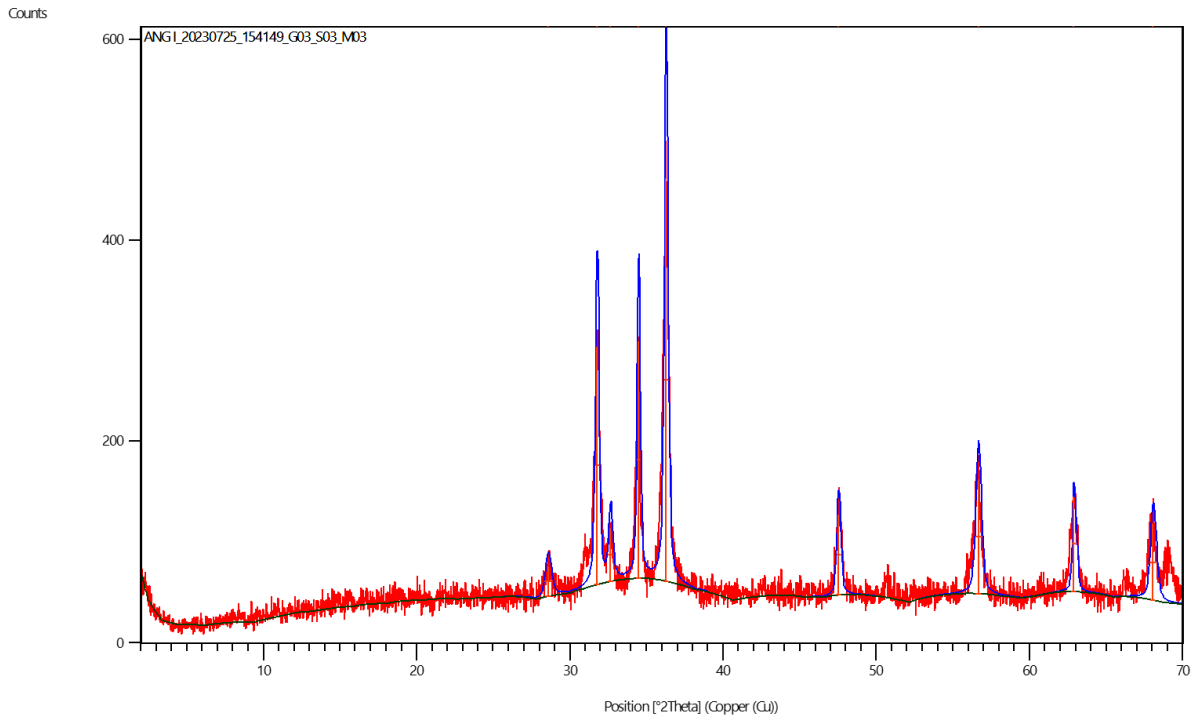
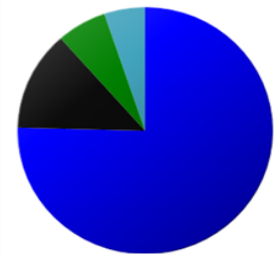


Figure 7. X-ray diffraction Examination (XRD) patterns of the ZnO NP.

Table 3. XRD data deduced from biogenic ZnO NPs.

FWHM(rad)	(°2TH)	Diameter (nm)	Average diameter(nm)
0.00842	28.5965	18.137	
0.00481	31.7680	31.303	
0.00549	32.6544	27.487	
0.00412	34.4736	36.802	
0.00481	36.2671	31.681	29.879
0.00481	47.5218	32.896	
0.00687	56.6403	23.946	
0.00412	62.8931	41.201	
0.00687	68.0423	25.433	



Quantitative Analysis Results

Colour	Phase name	Formula	%
	Zincite, syn	ZnO	75.4(11)
	Sphalerite	ZnS	13.1(7)
	Clinochlore	Al-Fe-SiO_2.OH	6.3(3)
	quartz HP, syn	SiO_2	5.2(5)

Figure 8. EDX analysis of biosynthesized ZnO NP.

However, they do demonstrate an inhibition zone for *Staphylococcus aureus* measured with a 20 mm diameter. From literature number of studies have shown excellent antibacterial activities of ZnO nanoparticles, (EL-FALLAL *et al*., 2023; TILAHUN *et al*., 2023; SREEKANTH *et al*., 2024) but the results of these experiments are similar to what was experienced from other researchers (GHARPURE *et al*., 2022).

Table 4. Zone of inhibition of ZnO NPs and standard against the pathogenic microbes.

Organisms	ZnO NP (mm)	Ciprofloxacin (mm)
S. aureus	20	35
B. subtilis	NZ	30
K. pneumonia	NZ	34
E. coli	NZ	NZ

NZ = No zone; control = ciprofloxacin

From Tab. 5 above, the minimum inhibitory concentration is observed at 20 mg/mL, while the minimum bacterial concentration is recorded at 40 mg/mL. At this concentration, the bacteria exhibited no signs of growth.

Table 5. MIC and MBC of green synthesized ZnO NPs of *S. aureus.*

Concentration (mg/mL)	***S. aureus***	
	MIC	MBC
10	G	G
20	NG	G
40	NG	NG

NG = No growth; G = growth

CONCLUSION

In this study, an aqueous extract of *Persea americana* seeds was used for the synthesis of ZnO nanoparticles, utilizing zinc acetate dihydrate as a precursor. The reduction of bulk zinc acetate dihydrate to ZnO NPs resulted in a colour change from brown to milky, with UV absorption peaks observed at 371 nm. The SEM analysis revealed that NPs exhibited a spherical morphology with an average size of 23.43 nm. XRD analysis indicated that the ZnO nanoparticles are crystalline, adopting a hexagonal wurtzite structure with an average crystal size of 29.876 nm. Furthermore, the study demonstrated that ZnO nanoparticles synthesized from avocado seed extract possess antibacterial activity, effectively inhibiting the growth of *Staphylococcus aureus*. Therefore, the application of this plant-based NP in the biomedical sector represents a promising developmental approach.

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