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Green synthesis of zinc oxide nanoparticles using *Lagenaira breviflora* aqueous fruit extract and its antimicrobial activity

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Abstract

Green synthesis involves eco-friendly approaches to producing materials, including nanoparticles. It is gaining more interest due to its affordability and renewability. In this study ZnONPs was synthesized using aqueous extract of *Lagenaria breviflora* fruit and assessed for it antimicrobial activities. The Aqueous extract of *Lagenaria breviflora* fruit was obtained by simple solvent extraction with double distilled water and the extract obtained was used as reductant in the synthesis of ZnONPs via a one pot facial synthetic pathway. The synthesized ZnONPs were characterized using UV-VIS, FTIR, XRD and SEM. The ZnONPs were further screened for their Antimicrobial activities against *Escherichia coli, Salmonella typhi, Staphylococcus aureus*, *Candida albicans* and *Aspergillus niger* using the well-diffusion method. Phytochemical screen carried out on the aqueous extract showed the presence of alkaloids, tannnis, flavonoids, terpenoids, saponins and carotenoids. A UV-vis peak of 357nm was observed for the ZnONPs, FTIR results showed the presence of -OH, -NH, -CH, -C=O, -C=C and -CO functional groups. XRD data confirmed the particles to be crystalline, with average crystallite size of 17.33 nm and the SEM result showed that the crystalline particles are spherical with an average particle size of 82.10nm. The antimicrobial screening of the synthesized ZnONPs showed average inhibition zones of 11mm, 11mm, 12mm, 10mm and 9mm for *Escherichia coli, Salmonella typhi, Staphylococcus aureus*, *Candida albicans* and *Aspergillus niger* respectively. The synthesized ZnONPs showed better activity toward tested micro-organisms compared to the crude aqueous extract. ZnONPs as observed in comparison with the controls Ciprofloxacin and fluconazole can served as potential substitute.

Introduction

Over the years, there have been growing interests in the study and applications of nanomaterials due to the

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unique properties of nano-sized objects compared to their bulk material counterpart owing to their high surface to volume ratio. This fundamental shift in properties has opened up a wide array of applications of nanoparticles across various fields, particularly in electronics, environmental science, and biomedicine [1]. The use of plants extract to synthesize nanoparticles is increasingly promoted due to their remarkable ability to reduce and stabilize these nanomaterials. Plant phytochemicals, play a crucial role in this process [2]. Biologically, nanoparticles are utilized in areas such as bio-imaging, where they help visualize biological processes; diagnostics, for identifying diseases; and bio-sensing, which involves detecting specific biomolecules. Additionally, these nanomaterials are making strides in gene therapy and are being explored for their potential in developing antimicrobial and anticancer treatments [3]. Zinc oxide nanoparticles (ZnONPs) are fascinating nanomaterials that hold great potential in various applications, including food additive, cosmetics, and inducers of photo-catalytic degradation of environmental pollutants, electrochemical sensors, and medical devices due to their biocompatibility, ultraviolet light absorption and scattering, and antimicrobial properties [4].

There are several methods of synthesizing zinc oxide nanoparticles including, chemical, physical, and biological/ green process [5-6]. However, physical and chemical methods often involve toxic chemicals, high energy, high cost and waste production which can lead to harmful reactions in the environment and pose risks to both human and animal health. Additionally, the presence of these hazardous substances can limit the potential applications of the nanoparticles and compromise their biocompatibility [7]. To meet the growing demand for zinc oxide nanoparticles, there is need for the development of eco-friendly, affordable, and innovative synthesis methods. These approaches aim to minimize or completely eliminate the use of harmful chemicals, thereby reducing environmental pollution and waste [8]. One promising solution is the use of “green” synthesis methods, which involve gentle solvents and natural materials like plant extracts or biomolecules from plants, bacteria, and fungi for reducing or stabilizing agents. By leveraging on this natural capability of plants and other biological materials, zinc oxide nanoparticles can be created at low cost, high yield, effective and ecological toxic free [9].

Utilization of micro-organism for synthesizing nanoparticles including maintaining cell culture of the respective virus, bacteria and fungi; a slower process compared to plant mediated nanoparticles [10]. Plants, aside from being considered essential resources of food, fuel, and shelter, are also a great source of medicine [11]. Active ingredients derived from plants play crucial role in the synthesis of metal nanoparticles. Considering the potential of plant sources, this work aim at using green technique for the synthesis of zinc oxide nanoparticles using fruit extract of *Lagenaria breviflora*; a gourd plant that thrive predominantly across West Africa. It belongs to the family of Cucurbitaceae. The fruits are dark green with creamy blotches and are ovoid and about 9cm long; used as herbal remedy for the treatment of conditions such as measles, chickenpox, and intestinal worms, wound and also very valuable to livestock farmers for the treatment of Coccidiosis and Newcastle disease especially in poultry [12].

Experimental

The process in green synthesis involves mixing a metal salt solution with an aqueous plant extract under mild conditions, which not only safe time, reduces energy consumption but also minimizes waste generation.

Preparation of plant extract

Zinc acetate dihydrate ($Zn(CH_3CO_2)_2.2H_2O$) and sodium hydroxide (NaOH) were obtained from Sigma-Aldrich. Fresh fruits of *Lagenaria breviflora* were collected from the botanical garden, Joseph Sarwua Tarka

University, Nigeria. The fruits were thoroughly washed with tap and double-distilled water to remove contaminants, and then shade dried to constant weight and pulverized. 20g of the sample was measured into a 500cm^3 Erlenmyer flask containing 200cm^3 double distilled water and covered with a cork. The mixture was then left for 48hrs, with occasional shaking. The extract was filtered using Whatman filter paper No. 1 and then stored in the refrigerator for future use.

Qualitative phytochemical screening

Phytochemical screening was conducted on the aqueous extract of *Lagenaria breviflora* fruits. This involved testing for various bioactive compounds, including alkaloids, saponins, flavonoids, tannins, terpenoids, and carotenoids, following the methods outlined by [13].

Green synthesis of zinc oxide nanoparticles (ZnONPs)

Zinc oxide nanoparticles (ZnONPs) were prepared using aqueous extract of *Lagenaira breviflora* fruit as a bioreductant according to the procedure reported in literature with slight modification [14]. A solution of zinc acetate dehydrate (120cm^3, 0.02M) was prepared and mixed with 80 cm^3 aqueous extract of *Lagenaira breviflora* fruit. The mixture was stirred for 1h; then, few drops of 2.0M NaOH solution were added into the mixture to make pH 12 under constant stirring for another 1h till the colour changes from yellow to pale white. The change in colour of the reaction mixture was duly observed. The reaction progress was also monitored with UV-Visible spectrophotometer ranging from 300nm–700nm. The pale white precipitate solution obtained was centrifuged at 3500rpm for 30 minutes; washed with double distilled water and ethanol to make it free from impurities. The crystals were dried in the oven at 40°C overnight and then set aside for characterization and antimicrobial screening.

Characterization of synthesized zinc oxide nanoparticles

The synthesized zinc oxide nanoparticles were characterized by *scan Jen-way* (*Model 7415*) *Single beam UV-visible Spectrophotometer*. FTIR spectra were recorded using an *Agilent Cary 630* FTIR spectrophotometer to identify possible functional group in the plant extract. The average crystallite size was identified by *X-ray diffraction* (*XRD*) *Thermo scientific model: ARL'XTRA X-ray and serial number 197492086.* The particle size and shape were examined using *Scanning Electron Microscopy (SEM) model PhenomProX, by PhenomWorldEinhoven; The Netherlands*.

Assessment of antimicrobial assay

The antibacterial assays were done on a (Gram-negative pathogens, *Escherichia coli* and *Salmonella typhi*, and Gram positive *Staphylococcus aureus,*), *Candida albicans and Aspergillus niger* by well diffusion method. Nutrient Agar and potato dextrose plates were swabbed with 24h old broth culture of respective micro-organisms. After which, wells of about 2cm apart were bored on the surface of inoculated agar plates using 10mm cork borer. 0.2cm^3 of different concentrations; 500mg/mL, 250mg/mL and 125mg/mL of plant extracts, zinc oxide nanoparticles, Ciprofloxacin and fluconazole were introduced respectively to the wells. The plates were incubated at 37^{o}C for 24 h for bacterial isolates and 48 h for fungi isolates. The experiment was conducted in triplicate and the antibacterial activity was assessed based on the inhibition zone diameters recorded around the well treated with plant extract and synthesized zinc oxide nanoparticle.

Results and Discussion

Phytochemical analysis

The result of the phytochemical screening of the *Lagenaira breviflora* fruit extracts revealed the presence of terpenoids, alkaloids, flavonoids, carotenoids, saponins and tannis as presented in Table 1. The presence of these bio-molecules especially flavonoids and terpenoids in *Lagenaira breviflora* are responsible for the bio-reduction zinc ions (Zn^{2+}) to zinc oxide nanoparticles (ZnONPs).

Table 1. Phytochemical screening of aqueous extract of *Lagenaira breviflora* fruit.

Phytochemical constituents	Test performed	Aqueous extract
Alkaloids	Mayer's and Dragendorffs tests	+
Tannis	Ferric chloride test	+
Flavonoids	Ammonia Test	+
Saponins	Frothing test	++
Terpenoids	Salkowski Test	+
Carotenoids	Carr-price reaction test	+

Key: (+, present) (++, very present)

UV-Vis spectroscopy analysis

The successful synthesis of zinc oxide nanoparticles (ZnONPs) using fruit extracts of *Lagenaria breviflora* was confirmed through noticeable color changes and UV-vis spectroscopy analysis of the reaction mixture. After mixing zinc acetate dehydrate solution with the plant extracts and stirring, the color of the reaction mixture changed progressively from yellow to pale white after no further change in colour was observed. (Figure 1). This change indicates that ionic zinc (Zn^{2+}) has been converted into zinc oxide nanoparticles (ZnONPs).This finding is consistent with the report in literature [15]. The pale white precipitate colour of ZnONPs arises from vibration of free electrons of the metallic zinc, which resonated with the light wave. This explains surface plasmon resonance (SPR) absorption attributed with metallic nanoparticles, often confirmed using UV-vis spectroscopy to provide clearer understanding of the visual observation in ZnONPs formation. As illustrated in Figure 2, the synthesized ZnONPs showed maximum absorbance peak at 357 nm within the range of 300 to 700.

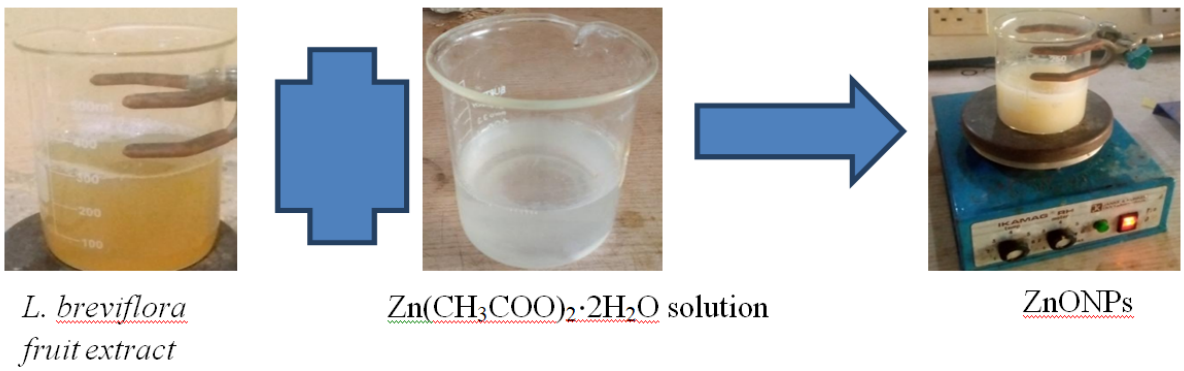

Figure 1. Image of synthesized ZnONPs.

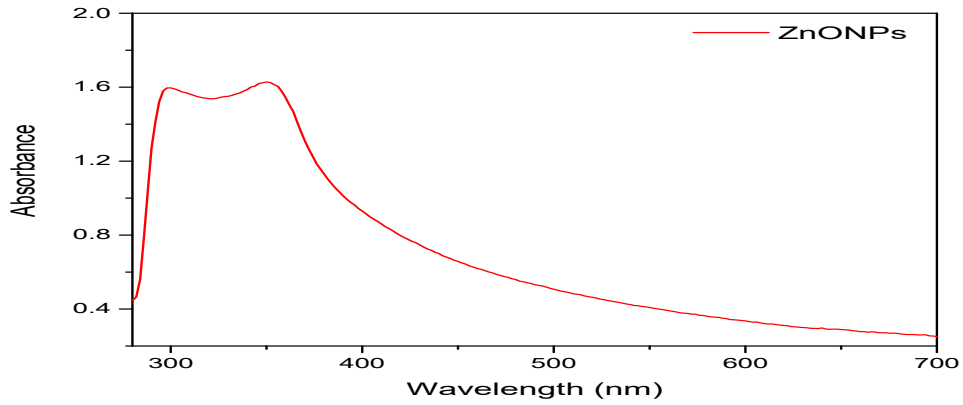

Figure 2. UV-vis absorption spectrum of the synthesized ZnONPs.

FTIR analysis

The FTIR spectrum (Figure 3) shows various functional groups, including hydroxyl (O-H stretching) at 3373 cm^{-1}. The peak at 2920 cm^{-1}, corresponding to C-H stretching of methyl groups and the peak at 2847cm^{-1} has been ascribed C-H of the aldehydes. The band at 1544cm^{-1} is attributed to C=O stretching vibration of carbonyl compounds, alkene (C=C stretching) at 1500cm^{-1} and 1028cm^{-1} assigned to stretching of C-O bonds. The peaks at 3373 and 1028 cm^{-1} showed the activity of flavonoids and terpenoids with a functional group of alcohols, and they are responsible for the bio-conversion of zinc ions (Zn^{2+}) to ZnONPs.

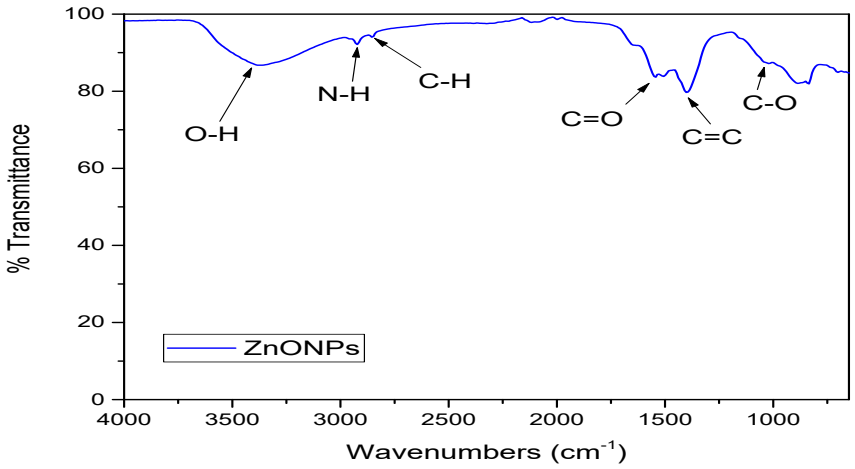

Figure 3. FTIR spectrum of synthesized ZnONPs.

X-ray diffraction analysis

The XRD patterns of the synthesized ZnO nanoparticles using fruit extract of *Lagenaira breviflora* is shown in Figure 4. The analysis of the XRD spectrum showed seven peaks at 2θ value, ranging from 30-50°. These have been indexed as (111), (200), (220), (222), (300), (301), and (311) hexagonal plane of the ZnO. *These peaks indicate crystalline structure* and the most prominent peak occurred at 2θ value of 36.27° with a *d*-spacing of 0.25 nm and an average crystallite size of 17.33 nm. Consequently, high crystallinity is inferred

due to the presence of high-intensity peaks. The crystalline zinc oxide nanoparticle size was determined using Debye-Scherrer equation as stated by [16].

Debye-Scherrer equation: $D = k\,\lambda/\beta\cos\theta$ --------- (1)

where D = size of the nano-crystal

k = Scherrer constant (0.9),

λ = X-ray wavelength (1.5406 Å = 1.5406×10^{-10}),

β= full width at half maxima of (220) reflection at Bragg's angle 2θ,

θ = Bragg angle (Particle angle 36.27°)

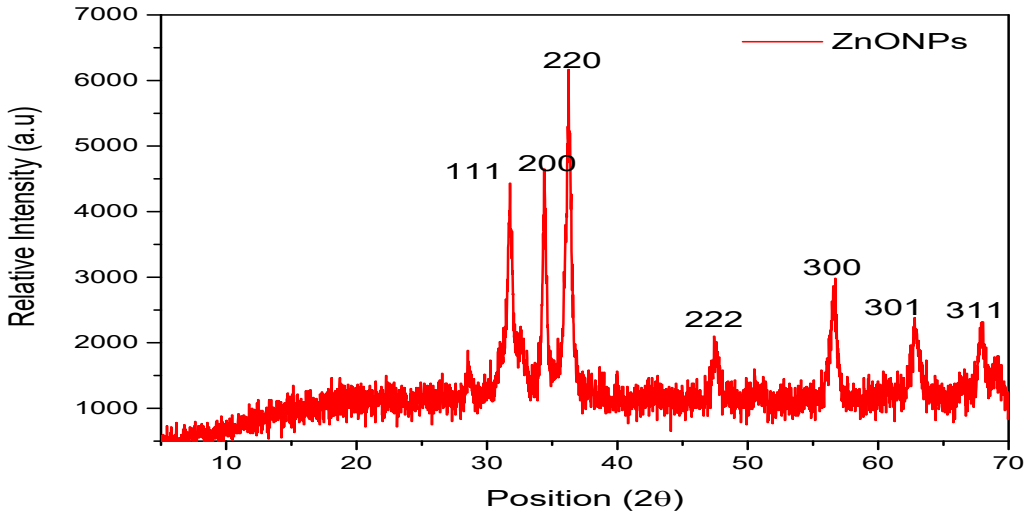

Figure 4. X-ray diffractogram of synthesized ZnONPs.

Scanning electron microscopy (SEM) analysis

The SEM analysis was used to determine the shape and particle size of the synthesized ZnO nanoparticles as presented in (Figure 5). The histogram displays the size distribution while the micrographs depict the shape. The SEM image revealed that the shapes of the synthesized ZnO nanoparticles were spherical. The average particle size of the ZnONPs was determined to be 82.10 nm at an average area 42.59 nm^2.

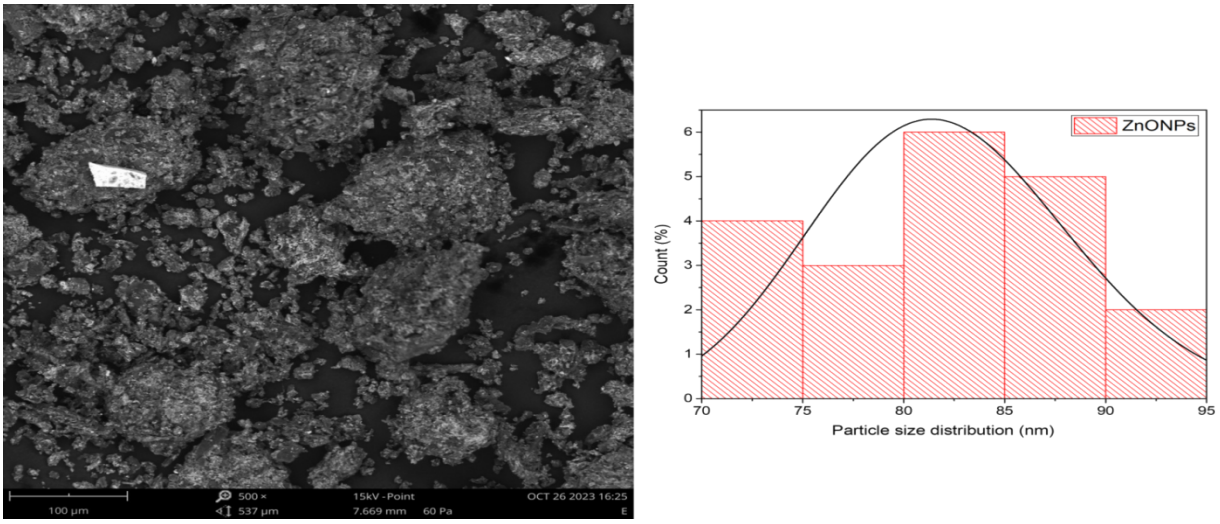

Figure 5. SEM image of synthesized zinc oxide nanoparticles (ZnONPs).

Antimicrobial activity

The antimicrobial activities of the plant extract and the mediated ZnONPs were tested against three bacterial pathogens; (Gram-negative *Escherichia coli* and *Salmonella typhi*) and (Gram-positive *Staphylococcus aureus*), showed inhibition zone diameter (IZD) of 14, 13 and 14 mm respectively while the crude extract showed IZD of 9, 9 and 10mm and this showed that the conversion to nanoparticles has increased the activity. The two fungi isolates; *Candida albicans* and *Aspergillus niger*; showed zone of inhibition diameter of 12 and 11mm while the crude extract showed IZD of 9 and 10mm. However the control, Ciprofloxacin and fluconazole showed higher activities than the ZnONPs at all the concentration measured. This effectiveness of ZnONPs of the extract in fighting the tested microbes could be attributed to its ability to induce oxidative stress by generating reactive oxygen species, and larger surface area of the nanoparticles that enhances their contact with the cell walls causing damage to the cellular component of the microorganisms.

Table 2. Antibacterial activity of plant extract, ZnONPs and the control.

Samples	**Concentration** (mg/mL)	**Zone of inhibition (mm)**		
		Gram negative		**Gram positive**
		E. coli	*S. typhi*	*S. aureus*
Plant extract	500	9	9	10
	250	7	7	8
	125	-	-	-
ZnONPs	500	14	13	14
	250	11	11	12
	125	8	9	9
Ciprofloxacin	500	15	14	16
	250	12	12	14
	125	10	9	12

Table 3. Anti-fungi activity of plant extract, ZnONPs and the control.

Samples	**Concentration** (mg/mL)	**Zone of inhibition** (mm) *Candida albicans*	*Aspergillus niger*
Plant extract	500	9	10
	250	7	8
	125	-	-
ZnONPs	500	12	11
	250	10	9
	125	8	7
Fluconazole	500	15	16
	250	13	14
	125	11	12

Conclusion

A simple one-pot green synthesis of zinc oxide nanoparticles using *Lagenaira breviflora* fruits extract was reported in this study. Synthesis was found to be efficient in terms of reaction time as well as stability of the synthesized nanoparticles. It proves to be an eco-friendly, providing a cost effective and an efficient way for the synthesis of zinc oxide nanoparticles. It also demonstrated that the ZnOPs of the extract could be used as a viable alternative in treating the diseases caused by the tested organisms which are susceptible to it. This method is cheap, eco-friendly and safe making it better alternative to conventional physical and chemical methods used for synthesis of zinc oxide nanoparticles.

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