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Synthesis of zinc oxide nanoparticles using *Ficus thonningii* aqueous extract and evaluation of its anti-oxidant and anti-microbial activities

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Zinc oxide nanoparticles (ZnO-NPs-AE) were synthesized using *Ficus thonningii* aqueous extract as an efficient stabilizing agent. Anti-microbial and anti-oxidant activity of the synthesized ZnO-NPs-AE were carried out using disc diffusion and DPPH (2,2-diphenyl-1-picryl-hydrazyl-hydrate) free radical method, respectively. Characterization of the synthesized ZnO-NPs-AE was achieved using UV-vis spectroscopy (UV-vis), Fourier-transform infrared spectroscopy (FTIR), X-ray diffraction (XRD), Scanning electron microscope (SEM), and energy-dispersive X-ray spectroscopy (EDX). Absorption peak in the range of 370 nm was observed using UV–vis following a color change from yellow to brown; both UV–Vis and FTIR spectra confirmed the formation of ZnO-NPs-AE, and SEM analysis result revealed spherical morphology with an average size of 18 nm. Anti-microbial analysis result of the biosynthesized ZnO-NPs-AE showed significant activity against *Escherichia coli* and *B. cereus* with lower concentrations. Moreover, the anti-oxidant activity result obtained using DPPH free radical scavenging method of the ZnO-NPs-AE revealed an IC_{50} value of 35 μg/mL. Biosynthesized ZnO-NPs-AE displayed anti-oxidant activity and anti-microbial potential against both gram-negative and gram-positive bacteria.

Keywords: Zinc oxide nanoparticles; Characterization; Antimicrobial; Antioxidant; *Ficus thonningii.*

INTRODUCTION

Nanotechnology is a rapidly expanding field of research that employs biosynthetic and environmentally friendly technology to create metallic oxide nanoparticles that are nontoxic, chemically stable, and biocompatible and could be used as drug carriers, anti-microbial, anti-oxidant, anticancer agents, and antidiabetics due to their novel physicochemical properties [1]. Among the recently studied nanoparticles, zinc oxide revealed specific properties that might be useful in biomedical engineering. Because of their hexagonal phase, n-type semiconductor, and wurtzite structure, ZnO NPs have recently attracted much attention [2]. Metal oxide NPs are synthesized using expensive and harmful physical and chemical techniques, with antagonistic compounds utilized as stabilizing agents [3]. Nanomaterials can be modified by replacing certain atoms, which improves the optical, mechanical, and electrical properties of materials by altering their chemical surface properties [4]. Zinc oxide is rapidly being used in personal care items including cosmetics and sunscreens due to its excellent UV absorption characteristics [5]. Furthermore, zinc is widely recognized as an essential trace element that can be found in all bodily tissues, including brain, muscles, bones, and skin [2]. Apart from the biomedical application, zinc oxide can be utilized in a variety of different industries, such as concrete manufacture, photocatalysis, electronics, and electrotechnology [6]. Diarrhoea, urinary tract infections, diabetes mellitus, gonorrhea, respiratory infections, and mental problems are just a few of the diseases that *Ficus thonningii* is used to cure in African ethnomedicine [7]. *Ficus thonningii*, also known as the wild fig, is a multi-stemmed, evergreen or briefly deciduous tree with a dense, rounded to spreading crown that is primarily found in tropical and subtropical Africa's upland forests, at altitudes of 1,000–2,500 m, and thrives in light, deep, and well-drained soil [8]. The leaves are alternating or whorled, mid-dark green and sub-glossy above [9] and paler below, rounded or tapering, 4.5-12 cm long, hairless or finely hairy with a prominent midrib [10]. *Ficus thonningii* is a blooming tree that is pollinated by wasps who dwell in the syconium of its fruit in a symbiotic relationship. It is easily cultivated *via* seeds and cuttings [11].

The cost-effective green synthesis of ZnO-NPs-AE utilizing *Ficus thonningii* extract as a stabilizing agent is reported in this paper, as well as their characterization using several spectroscopic and microscopic techniques. Anti-oxidant potential of

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the synthesized ZnO-NPs-AE was studied using DPPH and anti-microbial activity using disc diffusion.

MATERIALS AND METHOD

Plant Materials

The stem bark of *Ficus thonningii* were obtained from Ajingi Local Government Area, Kano State, Nigeria and was authenticated at the Herbarium of the Department of Plant Biology, Bayero University Kano, Nigeria, and the specimen was given a voucher number BUKHAN 0110 and was deposited at the Herbarium of the institute.

Synthesis of Zinc Oxide Nanoparticles (ZnO NPs)

ZnO NPs using *Ficus thonningii* aqueous leaf extract were prepared using the method described by Huzaifa *et al.* with some minor modifications. Synthesis of ZnO NPs was carried out using $Zn(NO_3)_2 \cdot 6H_2O$ solutions in 90 mL of distilled water. *F. thonningii* extract (10 mL) was added dropwise to the mixture under constant stirring at 90°C for 8 hours and calcined for 3 hours at 300 ±10°C in a muffle furnace.

Characterization

The UV-vis spectrum of synthesized ZnO-NPs-AE was evaluated using a UV-vis spectrophotometer (Shimadzu UV-2450) to confirm the synthesis of the nanoparticles in a wavelength range between 300 and 800 nm at room temperature. Functional groups attached to the synthesized ZnO-NPs-AE were evaluated using Fourier transform infrared (FTIR) spectrometer at 4 cm^{-1} resolution and a frequency range of 4000–400 cm^{-1}. Crystalline structure of a powdered ZnO-NPs-AE sample was determined using a X-ray diffractometer (Rigaku ZSX Primus II) with CuKα radiation. Morphology of the synthesized ZnO NPs was determined using a scanning electron microscope (JOEL JSM 6335-F) with 150 kV acceleration voltage, and energy-dispersive X-ray spectroscopy (EDS Oxford Instruments AZTEC EDS).

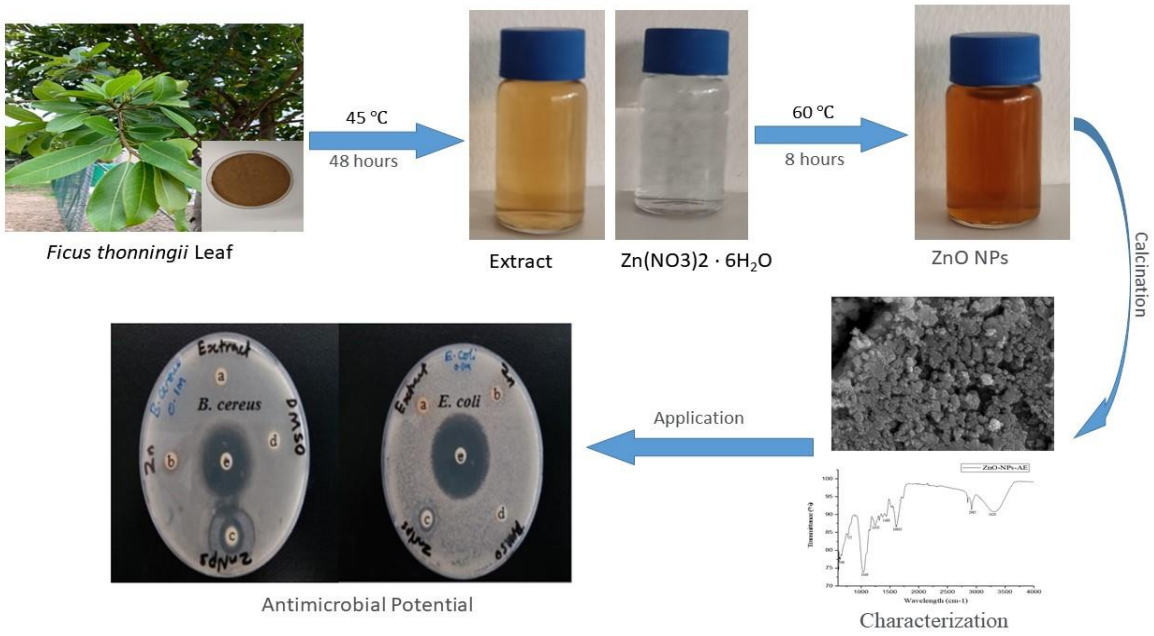

Figure 1. Overview of the synthesis of ZnO-NPs-AE, characterization and anti-microbial activity.

DPPH free radical scavenging activity

Blios (1958) method with minor modifications was utilized to determine the scavenging activity of synthesized ZnO-NPs and *Ficus thonningii* extracts using the 2,2-diphenyl-1-picryl hydrazyl (DPPH) free radical scavenging assay [12]. Different concentrations of *Ficus thonningii* extracts prepared in methanol (200, 150, 100, 75, 50, 37.5, 25, 17.5 g/mL) were combined with 3.0 mL of DPPH and incubated for 20 minutes at room temperature. The extracts' absorbance was measured at 517 nm using a UV-vis spectrophotometer and compared to that of a control sample (ascorbic acid).

The inhibition % of DPPH radical scavenging activity was estimated using the following equation:

$$\text{DPPH free radicals (\%) Ac} = (As/Ac) \quad (1)$$

where the absorbance of the control is Ac, and the absorbance of the standard or sample is As.

The IC_{50} value was calculated as the sample concentration required to scavenge 50% of DPPH free radicals.

Experiments were done in triplicate, and the results were provided as mean ± standard deviation.

Anti-microbial activity

Anti-microbial activity of the sythesized ZnO-NPs-AE and *Ficus thonningii* extracts was determined on Muller Hinton agar using the method of Jorgensen and Turnidge (2015) with some modifications [13]. Inhibition zones were measured after incubation, and results were interpreted in accordance with the Clinical and Laboratory Standards Institute (CLSI) protocol, (2007), for anti-microbial testing. Experiments were done in triplicate and the results were provided as mean ± standard deviation.

Statistical Analysis

Data are given as means ± standard errors of the mean (SEMs). Statistical comparisons were performed using unpaired Student's t-test, ANOVA followed by Newman-Keuls *post hoc* analysis, where necessary using IBM SPSS Statistics version 21. All experiments were carried out in triplicate ($n = 3$). $P < 0.05$ was considered significant or $P > 0.05$ insignificant and $P < 0.0001$ was considered highly significant.

RESULTS

ZnO-NPs-AE were synthesized from zinc nitrate solution using *Ficus thonningii* leaves aqueous extract as a stabilizing agent. The synthesis was confirmed and characterized using various spectroscopic and microscopic techniques.

ZnO-NPs-AE were confirmed using UV-Vis spectroscopic technique due to the rapid color formation from yellow to dark brown as a result of surface plasmon vibration (Figure 1). The UV-vis spectrum of the synthesized ZnO-NPs-AE revealed an absorption peak at 370 nm in agreement with zinc oxide nanoparticle's absorption peak range (360-380) as reported by [14].

FTIR analysis was conducted at room temperature in a frequency range between 4,500 and 400 cm^{-1} and the result revealed the composition of bioactive molecules of *Ficus thonningii* and their distribution on the surface of the synthesized ZnO-NPs-AE. Broad absorption bands at 3420 cm^{-1} are associated with O-H bond from the water that was absorbed during the synthesis. In addition, bands were seen at 1450 and 1049 cm^{-1}, corresponding to C=CH stretching of methyl group and C=O of saturated alcohol, respectively. Presence of alcohol and methyl group is among the characteristics of the NPs ability to bind to metals which could prevent agglomeration [15].

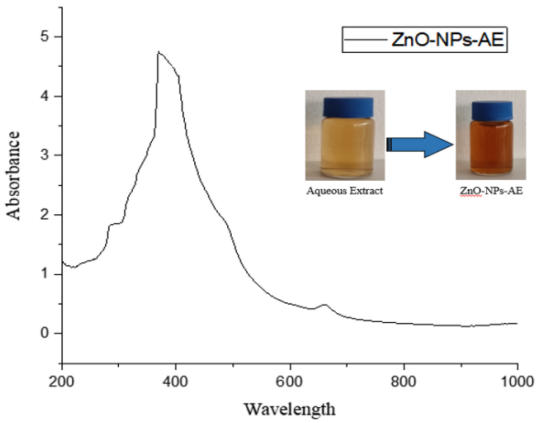

Figure 2. UV–visible spectrum of the synthesized ZnO-NPs-AE

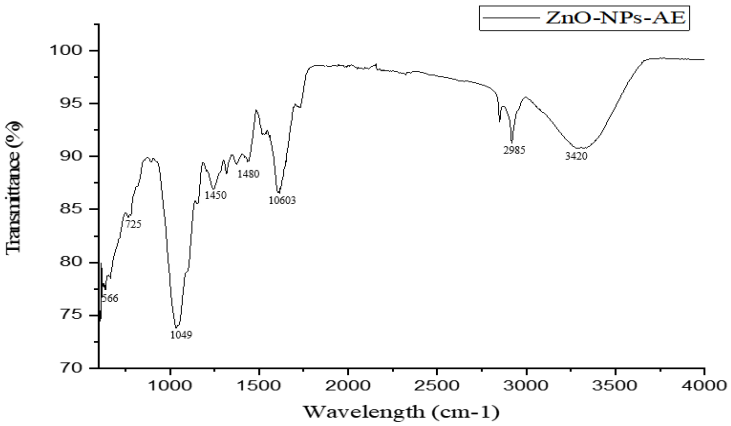

Figure 3. FTIR spectrum of the synthesized ZnO-NPs-AE

XRD pattern of the synthesized ZnO-NPs-AE revealed the various phase purity of the nanoparticles as shown in Fig. 4. Broad peaks obtained are 100, 002, 101, 102, 110, 103, 112, 004, and 104 corresponding to Bragg reflections with 2θ values of 31.70°, 34.34°,36.16°, 47.54°, 56.48°, 62.78°, 67.66°, 72.53°, and 76.58°, respectively. In addition, this confirms the presence of purified ZnO-NPs. Nanostructures can be observed and confirmed from XRD pattern of the particles, and the patterns observed in our particles confirmed the crystalline structure of the particles [16].

SEM analysis of the synthesized ZnO-NPs-AE revealed agglomerated particles with sharp spherical morphology and average size of 18 nm which could be responsible for the stability and biomedical potential of the NPs (Figure 5). Studies have reported that the size of NPs plays an important role in the the properties of these nanoparticles and it could lead to change in the potential of the synthesized nanoparticles [17].

EDX spectra showed the surface chemical composition of the synthesized ZnO-NPs-AE. Various signals oe Zn and of oxygen appeared on the surface of the synthesized ZnO-NPs-AE, and the appearance of C, P, and Na also confirmed the plant extract's interference in the particle synthesis.

In vitro anti-oxidant potential of *Ficus thonningii*, ZnO-NPs-AE, and ascorbic acid (control) was determined. The result revealed the increased anti-oxidant potential of ZnO-NPs-AE when compared with the plant extract (Figure 6). The synthesized zinc oxide revealed an IC_{50} value of 35 µg/mL which is close to that of ascorbic acid with IC_{50} of 8 µg/mL. It has been observed that enzymes that use the metal ion (Zn) as a co-factor scavenge H_2O_2 free radicals and that the presence of Zn ion in the particles may be responsible for more vital H_2O_2 free radical scavenging activity than the plant [18]. In addition, phenolic chemicals found in plant extracts have consistently high anti-oxidant activity and play a vital role in the green manufacturing of nanoparticles [19].

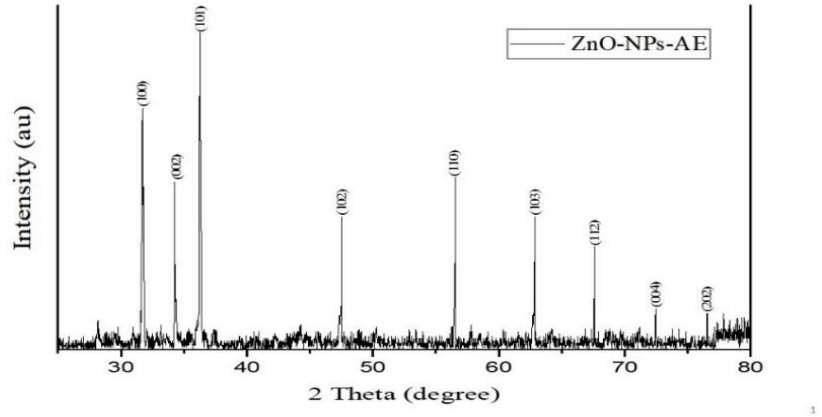

Figure 4. XRD pattern of the synthesized ZnO-NPs-AE

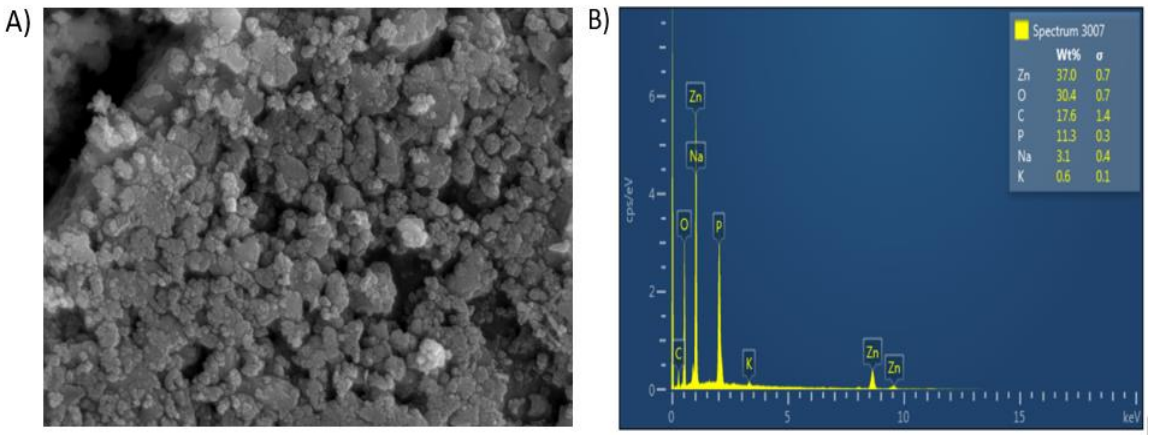

Figure 5. (A) SEM image of the synthesized ZnO-NPs-AE; (B) EDX spectrum of the synthesized ZnO-NPs-AE

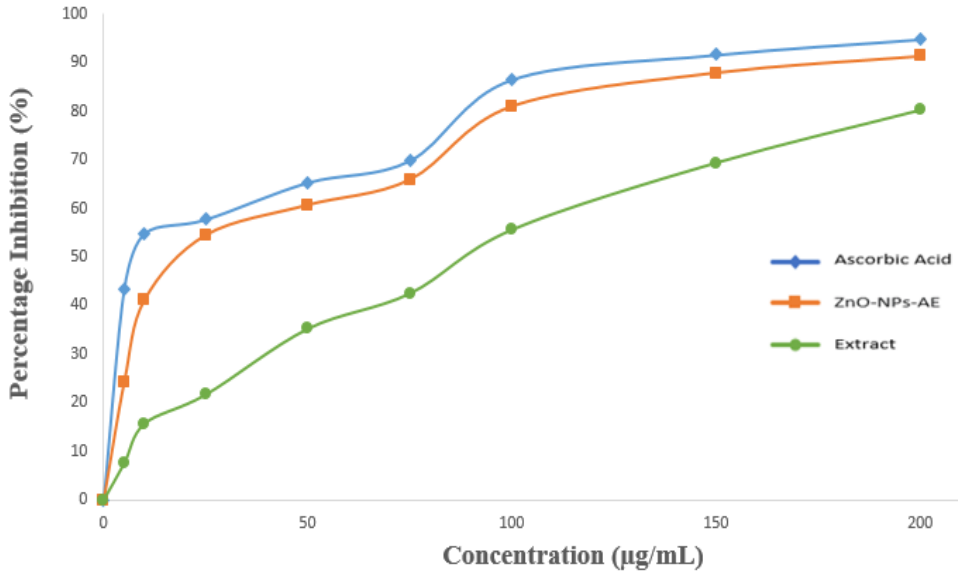

Figure 6. DPPH free radical scavenging potential of the various concentrations of the synthesized ZnO-NPs-AE, *Ficus thonningii* extract, and ascorbic acid. Data are presented as mean ± SD of at least three replicates ($n \geq 3$).

The results of the anti-microbial activities of the synthesized ZnO-NPs-AE evaluated using the disc diffusion method against *Escherichia coli*, and *Bacillus cereus* revealed highly significant differences when compared with the control group (ciprofloxacin) and the plant extract ($P < 0.05$; $n \geq 3$; Table 1). The change in the anti-microbial potential of the synthesized ZnO-NPs-AE might result from increased stability and reactivity of the particles. When the particle size of ZnO is lowered to the nanometer range, it can interact with the bacterial surface or the bacterial core, where it enters the cell and exhibits unique bactericidal properties [20]. The anti-microbial activity revealed by the synthesized ZnO-NPs-AE conforms with several studies.

Table 1. Anti-microbial activity of synthesized ZnO-NPs-AE

	Zone of Inhibition (mm)	
Organism	*Escherichia coli*	*Bacillus cereus*
Standard ciprofloxacin (10 ug/disc)	11.33 ± 0.29	13.00 ± 1.00
Ficus thonningii extract	1.33 ± 0.58***	2.67 ± 0.58***
ZnO-NPs-AE	2.00 ± 0.50***	3.67 ± 0.58***

CONCLUSION

Overall, stable ZnO-NPs-AE were synthesized *via* the green route using *Ficus thonningii* as a stabilizing agent. Following characterization of the synthesized nanoparticles, FTIR research indicated that the extract functions as a reducing and stabilizing agent. An absorption peak in the region of 370 nm and agglomerated spherical morphology with an average size of 18 nm was discovered using UV–vis spectroscopy. Anti-oxidant and anti-microbial analysis results of the synthesized ZnO-NPs-AE revealed IC_{50} values of 35 µg/mL and strong anti-microbial potential.

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