Biosynthesis of Zinc Oxide Nanoparticles Using *Solenostemon Monostachyus* Leaf Extract and its Antimicrobial Activity

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Abstract

A simple and rapid biosynthesis of zinc oxide nanoparticles (ZnO NPs) obtained using Solenostemon monostachyus leaf extract. The ZnO NPs was characterised by UV-visible spectroscopy, scanning electron microscopy/Energy dispersed X-ray, Fourier Transform Infra-red and X-ray Diffraction techniques. The UV-visible spectrum showed a maximum absorption peak at 350 nm which is typical for ZnO NPs and was ascribed to the excitation of Surface Plasmon Resonance phenomenon. FTIR Spectroscopy revealed a broad peak around 3455 cm^{-1} which was attributed to OH stretching vibration (υOH) that is probably from alcohols, flavonoids and phenols. X-ray Diffraction studies were observed at 2θ = 11.1°, 13.8°, 16.7°, 25.1°, 28.9° and 44.0°. The X-ray spectrum indicated typical for crystalline nanoparticles. Calculated nano particle size was 23.06 nm and was within the literature range for nano zinc oxide. The synthesized NPs exhibited significant antibacterial activity against Escherichia coli and Staphylococcus aureus but exhibited moderate activity against Klebsiella pneumonia and Salmonella typhimurium. Significant antifungal activity was also observed against Aspergellus niger and Candida albican.

Key Words: *Antimicrobial activity, Characterization, Nano zinc oxide, Solenostemon monostachyus*

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1.0 Introduction

Nanotechnology is an emerging field of technology that has found tremendous applications in science, engineering, pharmacy, water purification etc Govindappa *et al*., 2016. Several chemical methods are available for synthesis of nanoparticles but most of the methods are not environmentally friendly because they involve the use or generation of toxic chemicals that may ultimately contribute adversely to environmental health (Odiongenyi and Afangide 2019). In search for eco-friendly synthetic route, current research direction is gear toward green synthesis (Odiongenyi and Afangide, 2019). Green syntheses of metal oxide nanoparticles (NPs) have emerged over the years. Particles within the size of 1-100 nm are regarded as nanoparticles (Laurent *et al.*, 2008).

It has been established that zinc oxide (ZnO) is a good candidate for green synthesis because of its non-toxic, inexpensive and antimicrobial activity amongst other properties (Vijayakumar *et al*., 2018; Yedurkar *et al.,* 2016). And the green synthesis widely studied involves the use of different plants part (Bark, Leave and root) using different solvents (Ahmed *et al.,* 2017; Ogunyemi *et al.,* 2019; Alrubaie *et al.,* 2019; Ezealisiji *et al.*, 2019; Mydeen *et al.*, 2019; Umar *et al*., 2019). Sundrarajan and co-workers (2015) reported the synthesis of ZnO NPs using *Pongamia pinnata* leaf extract and observed spherical morphology through X-ray diffraction (XRD) instrument.

The bacterial activity of the synthesised nano materials was tested against *Staphylococcus aureus* (gram positive) and *Escherichia coli* (gram negative) and the results obtained showed strong and comparative bioactivity. Shah *et al.,* (2015) also synthesised ZnO NPs using leaf extract of *Camellia sinesis* for the reduction process. They noticed significant antimicrobial activity against gram-positive and gram-negative bacteria and against fungal strain. A biosynthesis of ZnO NPs from *Albizia lebebbeck* stem bark and the evaluation of its antimicrobial and antioxidant and cytotoxic activities on human breast cancer cell lines were studied by Umar *et al.,* (2019). The results obtained indicated that the biosynthesised ZnO NPs, which had a nano dimension of 66.25 nm displayed cytotoxic activity against strongly and weakly metastatic breast cancer cell lines, in addition to antimicrobial and antioxidant activities.

In biosynthesis, the plant extract act as reducing agent and also as a stabilizing agent. The presence of compounds that have hydroxyl and carbonyl functional groups in plant extracts has been reported to be the major factors that contribute to their reducing ability (Ohamad *et al*., 2013). *Solenostemon monostachyus* (*S. monostachyus*) has been reported to have strong antioxidant properties and can therefore reduce metal oxide to nano oxide (Osikoya *et al*., 2017; Kumar and Pandey, 2013). *Solenostemon monostachyus* leaf extract contains saponins, alkaloids, flavonoids and other phytochemicals which are rich in carbonyl, hydroxyl and other functional groups that can enhance its reducing properties (Odika *et al*., 2007). In our research group, we successfully used leaf extract of *Solenostemon monostachyus* to synthesized silver nanoparticles and found that the synthesized nano materials exhibited strong antifungal and antibacterial activities (Karu *et al*., 2020). Similar research on the synthesis of silver nanoparticles and their antimicrobial activity had earlier been reported by Jain *et al*., (2009). In spite of the documented research on phytochemical constituents of *Solenostemon monostachyus* leaf extract, literature is scanty on the use of this plant leaf for synthesis of nano zinc oxide. Therefore, the aim of the present study is to use the extract of this plant to reduce zinc oxide to nano zinc oxide, characterise the nanomaterials and investigate its antimicrobial properties.

2.0 Materials and Methods

2.1 Preparation of aqueous extract of S. monostachyus leaf

Leaves of *S. monostachyus* plant were collected from Gombe State University Campus and were air dried to constant weight. 10 grams of the dried leaves was pulverized using pestle and mortal and soaked in 50 ml of deionized water. The system was heated at 60 ºC for one hour and then filtered with a Whatman No.1 filter paper. The filtrate was stored in a refrigerator prior to analysis.

2.2 Preparation of solutions

Solution of 0.1 M $Zn(NO_3)_2$ was prepared by dissolving 7.4 g of the salt in a 100 ml distilled water, contained in a flask and made upto the 1 L mark with deionized water.

2.3 Synthesis of zinc oxide nanoparticles (ZnO NPs)

ZnO NPs was synthesized according to the method reported by Karu *et al*., 2020. 5 ml aqueous extract of *S. monostachyus* was mixed with 50 ml of 0.1 M $Zn(NO_3)_2$. The mixture was heated (with constant stirring) at 70 °C using a hot plate. A colour change was observed from pale yellow to dark yellow which signalled the reduction of zinc. The mixture was allowed to stand for five hours in order to stabilized the product. The supernatant was decanted to a clean dried beaker and the paste was transferred into crucible**.** The paste obtained were dried in an oven at 70 ºC for two hours to obtain ZnO NPs, which was preserved in a sample bottle.

2.4 Characterization of ZnO NPs

The synthesized ZnO NPs was characterised by UV-visible, IR and X-ray diffraction (XRD). Microscopic analysis, particle size and morphology were determined by scanning electron microscopy (SEM) and energy dispersive X-ray analysis (EDX) analysis. UV-visible spectrum was scanned with a Perkin Elmer UV-vis spectrophotometer 725 in the wavelength range 350-700 nm. The UV-visible spectrum provides information on the actual formation of the metal nanoparticles by surface plasmon resonance effect. To confirm the phytochemical agent responsible for the reduction, capping and stabilization of the ZnO NPs, Fourier transform infra-red spectroscopy was employed. The infrared spectra measurements were carried using a Perkin Elmer spectrophotometer model 10.03.09.

2.5 Antibacterial analysis

The antibacterial activity of ZnO NPs was investigated against one gram-positive bacteria (*Staphylococcus aureus*) and three gram-negative bacteria (*Salmonella typhimurium*, *Escherichia coli* and *Klebsiella pneumonia*). Agar well diffusion method was used to determine the antibacterial activity. Mueller Hinton Agar was prepared according to the manufacturer's instructions and sterilized by autoclaving at 121°C for 15 minutes. Then, it was poured on petri dish and allowed to solidify for 30 minutes. The isolates were inoculated on the medium (Mueller Hinton agar). Appearance of turbidity in broth culture was adjusted equivalent to 0.5 McFarland standards. A sterile cork borer was used to make a 6 mm diameter wells on the agar plates. Various concentrations; 100 µg/L, 200 µg/L, 300 µg/L and 400 µg/L of ZnO NPs were prepared in 50% DMSO. These concentrations were applied on each of the well in the culture plates previously inoculated with the test organisms. Thereafter, the plates were incubated at 37°C for 24 hours. Antibacterial activity was determined by measuring the zone of inhibition around each well (taking the average of the length and breadth including that of the well). For *Staphylococcus aureus*, *Salmonella typhimurium* and *Escherichia coli*, ofloxucin 5 mg was used as a control whereas, augmentin 25 mg was used as a control for *Klebsiella pneumonia*.

2.5 Antifungal analysis

ZnO NPs antifungal activity was tested against some fungi namely: *Aspergillus niger* (*A. niger*) and *Candida albican* (*C. albican*). The microbial cultures were procured from the Microbiology laboratory of the Federal Teaching Hospital, Gombe, Nigeria. About 38 g of potato dextrose agar (PDA) was dissolved in 1000 ml of deionized water, the mixture was heated on a hot plate and then autoclaved for 15 minutes at the temperature of 121 ˚C. The mixture was allowed to cool and then poured on petri dishes after the nutrient agar has solidified inside the petri dish, pure isolate of the fungi; *A. niger* and *C. albican* was respectively ~~each~~ grown on different agar plate at 27 ˚C for 48 hours in an incubator. Appearance of turbidity in broth culture was adjusted to be equivalent to 0.5 McFarland standards. A sterile cork borer was used to make a 6 mm diameter wells on the agar plates. Various concentrations; 100 µg/L, 200 µg/L, 300 µg/L and 400µg/L of ZnO NPs were prepared in 50% DMSO. These concentrations were applied on each of the well in the culture plates previously inoculated with the test organisms. Thereafter, the plates were incubated at 37°C for 24 hours. Antifungal activity was determined by measuring the zone of inhibition around each well (taking the average of the length and breadth including that of the well). Fluconazole was used as control.

3.0 Results and Discussion

3.1 UV-vis spectroscopy

The ZnO NPs obtained after extraction with the plant was colourless indicating that the produced nano zinc oxide is colourless. In zinc oxide and zinc nano oxide, the highest occupied molecular orbital (HOMO) is a fully filled d-orbital and from the theory of light absorption and colour, d-d transition is forbidden in zinc oxide, hence is it a colourless compound, which confirm the purity of the produced nano zinc oxide. Similar findings have been reported by Mydeen *et al*., (2020), who synthesised nano zinc oxide using leaf extract of *Prosopis juliflora* plant as a reducing agent. However, the UV-vis spectrum of the biosynthesized ZnO NPs (Fig. 1) showed a maximum absorption peak at 350 nm that can also be seen as typical for the range of values expected for nano zinc oxide and ascribed to the excitation of Surface Plasmon Resonance phenomenon. Ezealisiji *et al*. (2019) and Pal *et al.* (2018) reported ~~the~~ maximum wavelength of absorption values of 359 nm and 361 nm for nano zinc oxide synthesied through the use of leaves extracts of *Solanum torvum* (L) and *Moringa oleifera* leaf extracts respectively. Talam *et al*., (2012) also reported absorption maxima at 355 nm for nano zinc oxide synthesized through precipitation of zinc nitrate.

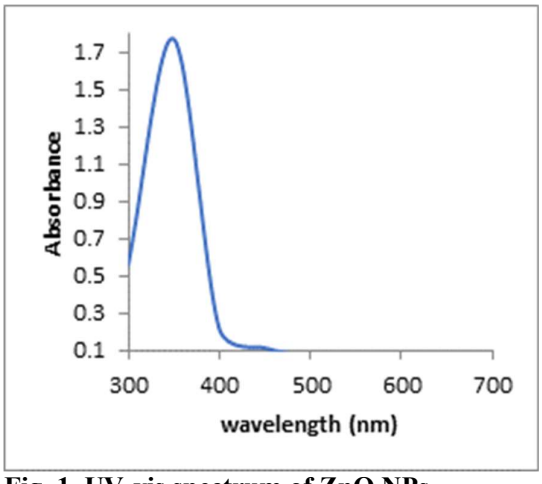

Fig. 1. UV-vis spectrum of ZnO NPs

3.2 FTIR analysis

Infra-red spectra were acquired by using KBr pellet technique of sample preparation on FT-IR Perkin Elmer spectrophotometer model 10.03.09, which operated through a scan range of 4000-400 cm^{-1} (Fig. 2). The IR spectroscopy was undertaken to ascertain the phytochemicals involved in the reduction, capping and stabilisation of the ZnO NPs. A broad peak around 3455 cm^{-1} corresponding to OH stretching vibration (υOH) in alcohols, flavonoids and phenols. A very sharp peak observed at 1364 cm^{-1} and another medium band at 1633 cm^{-1} are due to the C=O (υCO) and C=N (υCN) stretching vibrations respectively of a primary amide. The medium band at 1031 cm^{-1} is due to the stretching vibration of C-N (υCN) of an aromatic ring. These band assignments justify the presence of phenols, polyphenols and primary amide in the plant extract and can thus be concluded that they are involved in the reduction, capping and stabilisation of the bio-synthesized ZnO NPs. These findings are in agreement with previous studies reported by Ezealisiji *et al.* (2019) and Kaviya *et al.* (2011).

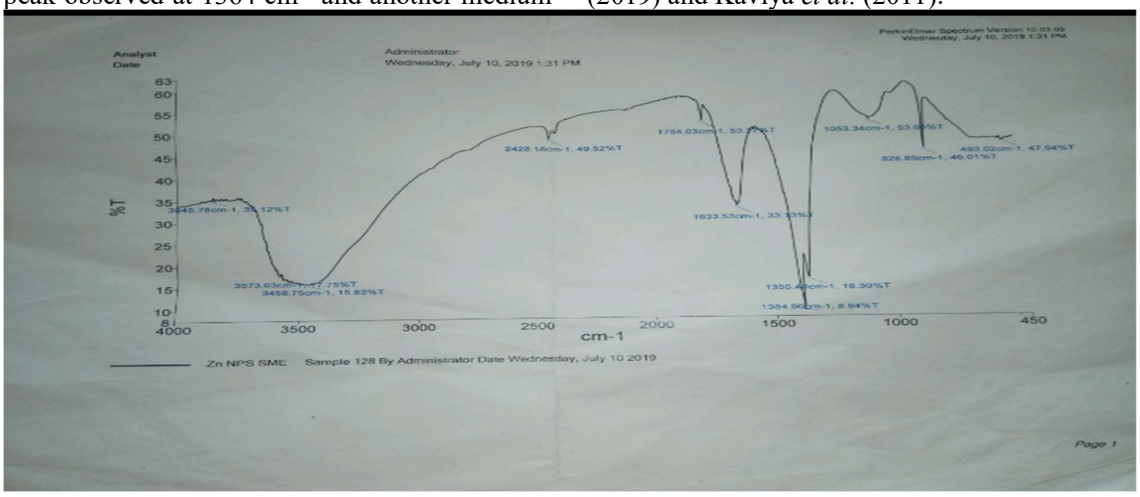

Fig. 2. FTIR spectrum of ZnO NPs sample

4.3 Scanning electron microscopy (SEM)

Scanning electron microscopy analysis of ZnO NPs is shown in Fig. 3, From the figure, it is evident that the size and surface morphology of the nanoparticles is spherical in shape, having smooth surface and well dispersed with close compact arrangement. The observed micrograph conformed with the information deduced by Talam *et al.,* (2012) who obtained spherical shape micrograph for nano zinc oxide, whose particles exhibited some faceting.

Dobrucka and Dlugaszewska, (2016) however observed agglomerated SEM image of ZnO NPs synthesized from *Trifolium prantense* flower extract.

3.4 X–ray diffraction analysis (XRD)

The XRD of the ZnO NPs is presented in Fig. 4. Diffraction lines were observed at 2θ with the following angles, 11.1, 13.8, 16.7, 25.1, 28.9 and 44.0°. The sharp lines indicate crystalline nanoparticles. The size of the nanoparticles was calculated using Debye-Scherrer equation

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

where D = particle size in nm, K is a constant (Scherrer constant = 0.9), λ is the wavelength of the X –ray diffraction, θ is Bragg's angle and β is Full Width Half Maximum (FWHM). The calculated mean crystalline particle size was found to be 23.06 nm. This falls within range of nanoparticles and reported by other workers (Fakhari *et al.,* 2019 and Alrubaie *et al*., (2019) who reported mean particle size of 21.49-25.26 nm and 19.22 nm for ZnO NPs obtained from *Laurus nobilis*. *L* and olive plant extracts respectively.

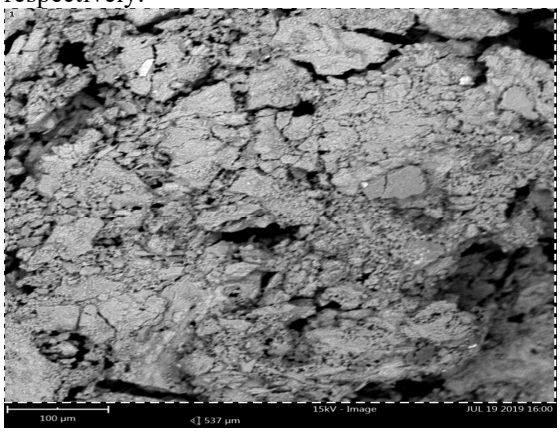

Fig. 3. SEM micrographs of ZnO NPs synthesized with *S. monostachyus* extract.

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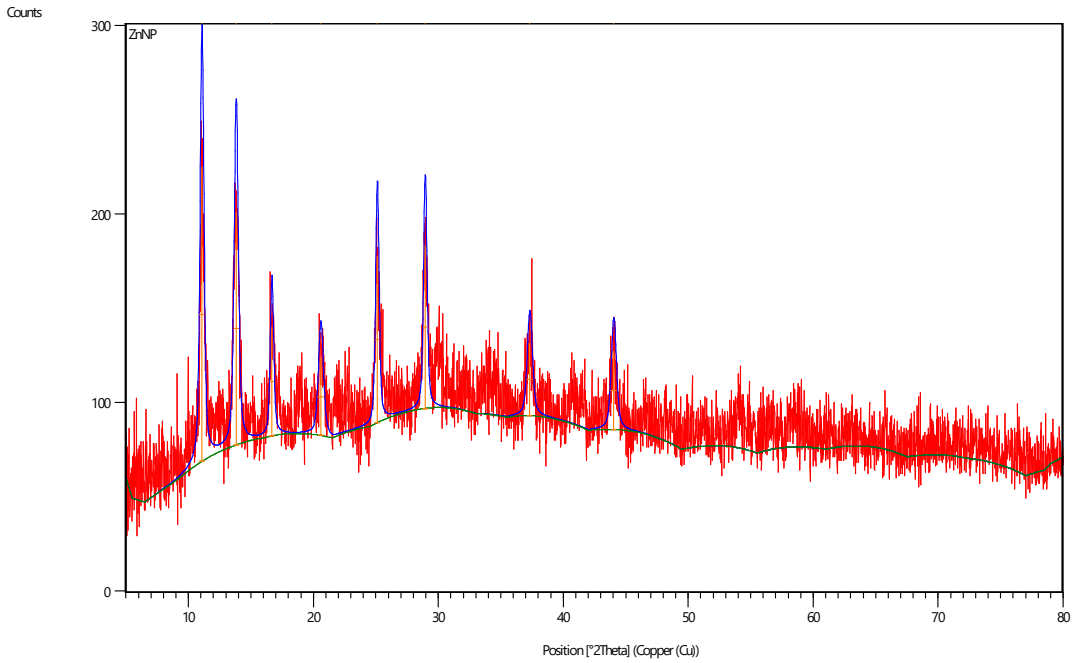

Fig. 4. X-ray diffraction of ZnO NPs synthesized with *S. monostachyus* extract

3.5 Antibacterial evaluation

Table 1 shows inhibition zones (mm) of ZnO NPs against bacterial isolate. The synthesized NPs exhibited significant antibacterial activity against *Escherichia coli* (*E. coli*) and *Staphylococcus aureus* (*S. aureus*), but exhibited a moderate activity against *Klebsiella pneumonia* (*K. pneumoniae*) and *Salmonella typhimurium* (*S. typhimurium*). The data obtained indicated that the antibacterial activity of the synthesized ZnO NPs increase with increase in concentration.

Table 1: Zone of inhibition (mm) of ZnO NPs against some bacterial strains

Tested Organism	Concentration µg/L mm					Name of control
	100	200	300	400	Control	
S. typhimurium	6.00	6.00	6.00	6.00	26.50	Ofloxucin (5 mg)
E. coli	8.50	10.00	11.00	17.50	29.00	Ofloxucin (5 mg)
S. aureus	9.50	15.50	19.00	22.00	10.50	Ciprofloxacin (10 mg)
K. pneumonia	6.00	6.00	6.00	6.00	20.00	Ciprofloxucin (10 mg)

3.6 Antifungal activity of the synthesized NPs

ZnO NPs exhibited strong antifungal activity against *Candida albican* (*C. albican*) and *Aspergellus niger* (*A. niger*) as compared with fluconazole standard drug as reveal by data presented in Table 2. The activity of ZnO NPs shows concentration dependent for both *C. albican* and *A. niger*.

Table 2. Zone of inhibition (mm) of ZnO NPs against some ~~selected~~ fungi

Tested Organism	Concentration µg/L					Name of control (50 mg)
	100	200	300	400	Control	
A. niger	12.50	14.50	17.50	24.00	16.50	Fluconazole
C. albicans	6.00	7.00	7.50	8.00	7.50	Fluconazole

4.0 Conclusion

In this work, ZnO NPs was synthesized by cost effective green synthesis method using *Solenostemon monostachyus*. The nanoparticles were characterized with UV-visible spectroscopy, FTIR, SEM and XRD techniques. The results obtained indicated that leaf extract of *Solenostemon monostachyus* effectively reduced zinc nitrate to nano zinc oxide and that ZnO NPs have good antibacterial and antifungal activity against *E. coli*, *S. aureus, C. albican* and *A. niger.*

5.0 Acknowledgment

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