



Investigation of Antibacterial Activity and Cytotoxicity of ZnO nanoparticles Synthesized by a Novel Biological Method

Nada K. Abbas¹, Israa Al-Ogaidi^{2, 3}, Shurooq S. Mahmood^{1*}, Hamid N. Obied⁴

¹. Department of Physics, College of Science for Women, University of Baghdad, Baghdad, Iraq.

². Department of Biotechnology, College of Science, University of Baghdad, Baghdad, Iraq.

³. Department of Chemistry and biochemistry, Fulbright College of Arts and Sciences, University of Arkansas, Arkansas, USA.

⁴. Department of Pharmacology, College of Medical, University of Babylon, Babylon, Iraq.

*Corresponding Author: Shurooq S. Mahmood

Abstract

This research explains the biosynthesis, characterization, evaluation of the antibacterial activity and cytotoxicity of zinc oxide nanoparticles (ZnO NPs) prepared by a low-cost and simple procedure. Bio-inspired ZnO NPs were synthesized with the aid of a novel, non-toxic, ecofriendly biological material namely; *Banana* peels extract (BPE). Qualitative phytochemical screening of the aqueous fruit peels extract of *Banana* revealed the presence of many phytochemicals in it. The structural, morphological and optical properties of the synthesized nanoparticles have been characterized by using UV-Vis spectrophotometer, XRD, FE – SEM with EDX analysis, AFM, and FTIR. The synthesized ZnO NPs were characterized by a peak at 373 nm in the UV-Vis spectrum. The XRD of the sample revealed the hexagonal wurtzite structure with an average grain size 11.98 nm. Particle shapes and sizes were determined by FE-SEM. Surface morphology of the sample was studied by AFM. FT-IR confirmed the presence of functional groups of both leaf extract and ZnO NPs. The results showed the antibacterial activity of the ZnO NPs against Gram-positive bacteria (*Staphylococcus aureus*) and Gram-negative bacteria (*Pseudomonas aeruginosa* and *Escherichia coli*). The cytotoxicity of ZnO NPs was tested against vero101 normal cell line and skin A431 cancer cell line by Crystal violet (CV) assay. *Banana* peels mediated ZnO NPs showed no evidence of toxicity against normal cell line, the nanoparticles are biocompatible and are non-toxic and effective cytotoxic effect against cancer cell line. These results clearly support the benefits of using a biological method for synthesizing ZnO NPs with anticancer activities.

Keywords: Zinc Oxide NPs; Biosynthesis; *Banana* peels extract (BPE); Phytochemical; Antibacterial; Cytotoxicity

Introduction

In recent years, metal and metal oxide nanoparticles have been intensively studied. The nano-sized materials have been appealed considerable attention because of their peculiar physicochemical properties compared to the bulk materials. Nowadays, there are plenty of opportunities to fully employ in modern clinical technology the novel concepts and phenomena that have appeared in Nanoparticles research field [1].

Transition metal oxides have a wide range of applications as catalysts, sensors, superconductors, antimicrobial agents, etc. Researchers have extensively studied the role

of noble metals such as gold and silver nanoparticles as antimicrobial agents [2, 3]. Recently, the antimicrobial activity of nano-sized ZnO particles has attracted attention, [4] since the small size (less than 100 nm) and high surface to volume ratio of the nanoparticles allow for better interaction with bacteria.⁵ Recent studies have shown that these nanoparticles have selective toxicity to bacteria and exhibit minimal effects on human cells [6,7].

Since, most of the ZnO nanoparticles are produced synthetically; it has certain advantages, compared to silver nanoparticles,

such as lower cost and white appearance [6]. ZnO has received more attention due to its unique morphology and dimension dependent properties.

ZnO being a wide band gap semiconductor (3.3 eV) has received more attention as it possesses a wide range of useful properties including electrical, chemical, optical and magnetic properties [8, 9]. ZnO has gained much importance as it can be applied in many applications such as for gas sensing, catalyst, for semiconductors, UV-shielding materials, nano generators, an antibacterial agent, cosmetics as well as medicinal applications [10, 11].

In recent days controllable synthesis of ZnO nano-materials of desired size and shapes has been the subject of the investigation by researchers because it has been found that the properties of ZnO nanoparticles are size and morphology dependent. Several methods have been developed to synthesize ZnO nanoparticles such as precipitation, hydrothermal, combustion, sol-gel, chemical vapor deposition, spray pyrolysis, sonochemical [12, 14].

The biosynthesized ZnO nanoparticles are trusted to be friendly with environment, non-toxic, bio-safe and bio-compatible. It is desirable for biomedical application such as drug carriers, cosmetics and filling the medical materials [15]. Plants and/or their extracts provide a biological synthesis route of several metallic nanoparticles which are more eco-friendly and allow controlling the synthesis with well-defined size and shape [16]. The enzymes, [17] plant leaf extract [18] and bacteria [19] plays a vital role in the green synthesis of zinc oxide nanoparticles.

Based on the review of literature, the bio synthesis of ZnO NPs using various plants has been carried out, *Cassia fistula* plant extract, [20] *Trifolium pratense* flower extract, [21] *Aloe barbadensis miller* leaf extract, [22] *Candida albicans*, [23] *Vitex negundo* extract, [24] leaf extract of *Tamarindus indica* (L.), [25] *Nephelium lappaceum* L. peel extract, [26] *Solanum nigrum* leaf extract, [27] *Azadirachta indica*, [28] curry leaf *Murraya koenigii*, [29] *Borassus flabellifer* fruit extract, [30] *Artocarpus gomezianus* fruits extract, [31] *Ocimum basilicum* L. var. *purpurascens* Benth.-*Lamiaceae* leaf extract, [32] *Moringa oleifera* leaf extract [33].

Banana is one of the most common crops grown in almost all tropical countries; therefore it is an abundant and cheap agricultural product. Peels represent about 30-40 g / 100 g of fruit weight [34]. The *Banana* peels waste is normally of disposed in municipal landfills, which contribute to the existing environmental problems. However, the problem can be recovered by utilizing its high-added value compounds, including the dietary fibre fraction that has great potential in the preparation of functional foods. Dietary fibre has shown beneficial effects in the prevention of several diseases, such as cardiovascular diseases, constipation, irritable colon, diverticulosis, colon cancer, and diabetes [35].

The crude extract of this peel has anti-infection, anti-parasitic, antibacterial, antifungal and anti-inflammation activity [36, 37]. Aqueous extract of *Banana* peel has rich phytochemicals such as alkaloids, tannins, Flavonoids, phytosterols, phenols, terpenes and carbohydrates. Flavonoids are known to be synthesized by plants in response to microbial infections [38]. In this study, distilled water was used to obtain the peels extract of *Banana*.

The peels of these plants were qualitatively screened for phytochemicals using standard methods. The aim of the current study is to synthesize ZnO NPs through a green approach with the aid of a novel, non-toxic, eco-friendly biological material namely, abundantly available and as a waste; *Banana* peels extract (BPE) and the nanoparticles have been characterized utilizing UV-Visible spectroscopy, X-Ray diffraction (XRD), Field Emission Scanning Electron Microscopy (FE-SEM) with EDX analysis, Atomic Force Microscopy (AFM) and Fourier Transform Infrared Spectroscopy (FT-IR) analysis.

Besides, an application of these nanoparticles that are biologically synthesized as antibacterial against Gram-positive bacteria (*Staphylococcus aureus*) and Gram-negative bacteria (*Pseudomonas aeruginosa* and *Escherichia coli*) has also been investigated. Minimum inhibitory concentration (MIC) was determined by micro broth dilution technique. The potential toxicity of ZnO NPs against vero101 normal cell line and skin A431 cancer cell line was evaluated in this present work by Crystal violet (CV) assay.

Materials and Methods

Preparation of Fresh Banana Peels Extract (BPE)

Banana Peels Extract (BPE) was prepared according to [39] with slight modification; which includes the use of microwave oven to cut short the time also to preserve the vital resources in the Banana peels.

Qualitative Phytochemical Analysis of the Peels Extracts of Banana

The qualitative phytochemical analysis of the banana peels extract as an aqueous extraction were done by using different phytochemical screening to distinguish the chemical components existent in the extract in comparison with standard chemical reagents.

Test for Flavonoids

A mixture of One mL of the peels extract and 1 mL of 5% lead acetate in a test tube was allowed to stand at room temperature (25°C) for 2 min. Formation of white precipitate in the sample indicates that the extract contained flavonoids [40, 41].

Test for Tannins

A solution of two mL of peels extract was treated with 10% alcoholic ferric chloride. That observe of the formation of blue or green colour indicate to presence of tannins [42].

Test for Alkaloids

Mayer's reagent and Dragendorff's reagent were used in crude extract to test the presence of alkaloids. One mL of the peels extract was added with 5 mL of 1% hydrochloric acid, filtered and tested with the reagents. The formation of white or creamy precipitates was taken as a positive result for the presence of alkaloids [40, 41].

Test for Saponins

A mixing of 2 mL of the extract and 6 mL of distilled water in a test tube was shaken vigorously and observed formation of persistent foam that indicated the presence of saponins [41].

Test for Glycosides

A few drops of Keed reagent [3-5 dinitrobenzoic acid (0.5 gm) dissolved in 25 mL of 95% methanol and a few drops of 1N NaOH solution was added to 5 ml of peels

extract. A change in color of solution to blue indicated the presence of glycosides.

Test for Phenols

Five mL of the extract was dissolved in distilled water which was added to 3 mL of solution of lead acetate at a concentration of 10%. The formation of a dark green colour indicates the presence of phenolic compounds [40, 41].

Test for Terpenoids

One mL of the peels extract was treated with 1 mL of chloroform and 1 mL of concentrated sulphuric acid was added to form a layer. A reddish brown colour indicates the presence of terpenoids.

Test for Steroids

One mL of chloroform, few drops of concentrated H₂SO₄ and 1mL acetic anhydride were added and mixed with 5 ml of the peels extract. The observe of formation of dark red colour which indicates the presence of steroids [42].

Synthesis of the ZnO nanoparticles from BPE

Zinc nitrate (99%purity), sodium hydroxide (pellet.99%) and ethanol were used as the introductory material was supplied by Sigma-Aldrich chemicals. Zinc oxide nanoparticles were prepared by green synthesis method using zinc nitrate and sodium hydroxide precursors mediated *Banana* Peels Extract (BPE) as a reducing and capping agent. In this experiment, 0.02M Zinc nitrate (Zn (NO₃)₂·4H₂O) was added to 300 ml of distilled water under constant stirring using a magnetic stirrer to completely dissolve the zinc nitrate. Aqueous peel extract of *Banana* was introduced into the above solution after 10min stirring at 6ml.

After 1h to the same 2.0 M aqueous solution of sodium hydroxide (NaOH) was added to make pH 12 under high speed constant stirring, drop by drop (slowly for 15 min) touching the walls of the vessel resulted in a pale white aqueous solution. The reaction mixture was kept for incubation in a microwave oven (700 W, 2.45 GHz) for 1min under static conditions. This reaction was then placed in a magnetic stirrer for 2hrs. The beaker was sealed at this condition. After the completion of the reaction, the

supernatant solution was separated carefully. The remaining solution was centrifuged at 10,000 rpm for 10 min. The pale white precipitate was then taken out and washed three times with distilled water followed by ethanol to get free of the

impurities and remove the byproducts which were bound with the nanoparticles. Then a pale white powder of ZnO nanoparticles was obtained after drying at 400°C in oven for 2h (Figure 1). During drying, $Zn(OH)_2$ is completely converted in to ZnO.



Figure 1: ZnO nanoparticles and aqueous solution of ZnO nanoparticles

Characterization of the ZnO nanoparticles

In order to study the structural properties of ZnO NPs, the crystalline structure was recorded by X-ray diffractometer (XRD-6000, Shimadzu, Japan), the source of radiation is Cu (k_{α}) with wavelength of ($\lambda=1.5405\text{\AA}$), voltage 60 kV and current 80 mA, at scanning speed of 5 degrees/min in 2θ range from 20° to 80° , these measurements were carried out on dried and finely grounded samples on Nano-Filter paper. The size and shape of the ZnO nanoparticles were determined by FE-SEM with EDX analysis and AFM. The morphology of synthesized ZnO NPs was observed by Field emission scanning electron microscopy (FE-SEM, SIGMA VP-500, and ZEISS) and the elemental composition by recorded using (EDX, ZEISS).

The surface morphology, particle size distribution and root mean square of roughness of the ZnO NPs were performed by using atomic force microscope AFM (AA3000 Scanning Probe Microscope SPM, tip NSC35/AIBS from Angstrom Advanced Inc., USA). The Ultraviolet-Visible spectra of the bioreduction ZnO NPs were monitored periodically as a function of wavelength within a range from 190 to 1100 nm by using UV-VIS Spectrophotometer (UV-1800 Shimadzu, Japan). The UV-Vis spectra were monitored for the sample at a resolution of 1 nm at room temperature. In order to determine the functional groups which present in biomolecules in the plant extract surface and their possible involvement in the

synthesis of ZnO nanoparticles, Fourier Transform Infrared (FTIR) Spectroscopy measurements was carried out.

The test samples were independently dried and blended with KBr to obtain a pellet. The FTIR Spectrometer were detected by used (SENSOR 27, Bruker Optik GmbH, Germany) ranging from 400 to 4000 cm^{-1} .

Activation and Preparation of the Bacterial Isolates

The bacterial isolates used in this study were obtained from Department of Biotechnology / College of Science / University of Baghdad; they were Gram-positive bacteria (*Staphylococcus aureus*) and Gram-negative bacteria (*Pseudomonas aeruginosa* and *Escherichia coli*) were used to evaluate the antibacterial activity of prepared ZnO nanoparticles. Bacterial isolates were streaked on brain heart infusion agar and incubated for 18 hours at 37°C . Then single colony was picked up from media plate and inoculated into 5 mL of brain heart infusion broth then incubated for overnight at 37°C .

Determination of Minimum Inhibitory Concentration and Minimum Bactericidal Concentration (MIC/MBC) as an Antibacterial Activity of ZnO nanoparticle

The antibacterial activities of nano-sized zinc oxide were evaluated against Gram-positive bacteria (*Staphylococcus aureus*) and Gram-negative bacteria (*Pseudomonas aeruginosa* and *Escherichia coli*) by serial dilution method through the determination of the

minimum inhibitory concentration (MIC and MBC) in the culture broth. The method of

twofold serial dilutions (28) was used in this study for determination of the minimum inhibitory concentration (MIC) values, 1 ml of media was taken in a test tube, to which, 1ml of test solution (200 µg/ml) was added, thereafter, 0.1 ml of the bacterial strains prepared in 0.9% NaCl was added to the test tube containing media and test solution. Serial dilution was done nine times giving concentrations of (200, 100, 50, 25, 12.5, 6.2, 3.1, 1.5 and 0.7) µg/ml (Figure 2). Then 1 mL of each serial nanoparticles were added to the set of tubes except for that tubes of control. All tubes which contained tested samples and

controls were incubated for 24 h at 37 °C with shaking 200 rpm. The MIC values were taken as the lowest concentration required to arrest the growth of the bacteria in the test tube after incubation (showed no turbidity) while the minimum bactericidal concentration (MBC) was determined by sub culturing 50 µl from each test tube showing no apparent growth (clear), if there was no growth this concentration was taken as MBC. A sterile loop was used to inoculate and spread the solutions on Mueller Hinton Agar medium in Petri dishes. The Petri dishes were incubated at 37 °C for 24 h at the same conditions. Petri dishes of MBC were compared with control.



Figure 2: Determination of Minimum inhibitory concentration and Minimum Bactericidal concentration (MIC/MBC) as antibacterial activity of ZnO nanoparticle by serial dilution method against (A) *Staphylococcus aureus*, (B) *Pseudomonas aeruginosa* and (C) *Escherichia coli*

Cell Culture

Vero101 normal cell lines and skin A431 cancer cell lines were obtained from Cancer Research Lab., Department of Pharmacology, College of Medical, University of Babylon, Iraq. It was grown in 25 mL culture flask

with growth medium containing 10% FBS and antibiotics, incubated at 37°C.

Cytotoxicity Assays

The cytotoxicity assays were applied for determining the effect of ZnO NPs on Vero101 normal cell line and skin A431

cancer cell line culture, according to Freshney [44]. When the growth in the flask became as monolayer before it reached the exponential phase, the cell monolayer was harvested and re-suspended with a growth medium in a concentration of 5×10^5 cell / ml and seeded in each well of a 96-well microtiter plate and incubated for 24 hr at 37 °C in the incubator. Since the cell growth reaches 80%, the wells were exposed to serial dilutions of the ZnO NPs. The cells were then exposed to 200 µl of each of the serial dilutions of ZnO nanoparticles (200, 100, 50, 25, 12.5 and 6.2) µg/ml and incubated for 48 hr under similar conditions. Following incubation, the culture medium was changed and cells were incubated for another 24 hr. After the end of the exposure the wells washed with 200 µl of a sterile Phosphate buffered saline (PBS). The effect of the ZnO NPs on the cell lines growth was assessed by Crystal violet (CV) assay.

Crystal Violet (CV) Assay

Crystal violet (CV) assay was used to determine the optical density of the cell growth in each well of the microtiter plate, by using plate reader at 570 nm. After the end point of the cytotoxicity assay, the maintenance medium with the test substance was discarded out and the wells washed with 100 µl of cold PBS by automatic pipette. Then the cell cultures were fixed with 10 % buffered formalin for 20 min at room temperature. A fixative solution was discarded and 100 µl of 0.1 % aqueous CV solution was added to each well. The samples were incubated at room temperature for 20 min with gentle shaking.

After that the plates were washed by submersion in flowing tap water until there is no diffusion of the crystal violet stain from the wells. The plates were allowed to dry in the air and 0.2 % Triton X-100 in water was added to each well and incubated for 30 min at room temperature with gentle shaking to dissolve the dye. Then, 100 ml from each well

was transferred into a new 96-well microplate and the absorbance was read at 570 nm by a micro plate reader [43].

The percentage of inhibition was calculated according to the following equation:

$$\text{Inhibition \%} = \frac{[(\text{optical density of control wells} - \text{optical density of test wells}) / \text{optical density of control wells}] \times 100}{1}$$

The percentage of viability was calculated according to the following equation:

$$\text{Viability \%} = 100 - \frac{[(\text{optical density of control wells} - \text{optical density of test well}) / \text{optical density of control wells}] \times 100}{1}$$

Results and Discussion

To the researchers' knowledge, this report has been the first one on the preparation of ZnO NPs by using *Banana* peels to avoid toxicity problems without a probability of cytotoxicity for living tissues after penetrating the target tissues. Conversely, other kinds of chemical reducing agent materials such as ammonia (NH₃) can cause significant toxicity for normal tissues. Interestingly the prepared ZnO NPs has selectivity toxic effect to the cancer cell.

Phytochemical Properties of Banana Peels Extract (BPE)

The phytochemicals present in the *Banana* peels were revealed by standard methods before using in ZnO nanoparticles synthesis. The results proved the qualitative presence of alkaloids, flavonoids, Tannins, saponins, glycosides, phenols and terpenoids whereas Steroids was absence in the extract. These phytochemicals in *Banana* peels served as reducing, stabilizing and capping agents for the microwave assisted synthesis of ZnO nanoparticles from ZnNO₃. As illustrated in (Table 1), the phytochemical screening of *Banana* peels extract exhibited the presence a complex mixture of phytochemical components.

Table 1: Qualitative Phytochemical analysis of peels extracts of *Banana*

Phytochemical	Aqueous extract of <i>Banana</i> peels
Flavonoids	+
Tannins	+
Alkaloids	+
Saponins	+
Glycosides	+
phenols	+
Steroids	-
Terpenoids	+

Ultraviolet-Visible Spectra Analysis

Figure 3 exhibits the absorption spectrum of the bio-synthesized ZnO NPs with a strong absorption peak around 373 nm. This result indicating that ZnO NPs displays exciton absorption at 373 nm due to their large exciton binding energy at room temperature.

$$E_g = hc / \lambda_m \quad \text{eV} \quad (1)$$

Where, h is the planks constant, c is the speed of light and λ_m is the wavelength corresponding to the maximum wavelength.

The wavelength of 373 nm absorption peak confirms the occurrence of blue-shifted absorption spectrum with respect to the bulk value (377 nm) of the ZnO NPs, due to the quantum confinement effect, which in a good agreement of the previous report [45]. The energy band gap (E_g) value of the sample was calculated using the equation: [46]

The energy band gap (E_g) of ZnO NPs was calculated to be 3.33 eV. The appearance of the sharp excitonic peak indicates the high crystalline quality of the grown nanostructures [47].

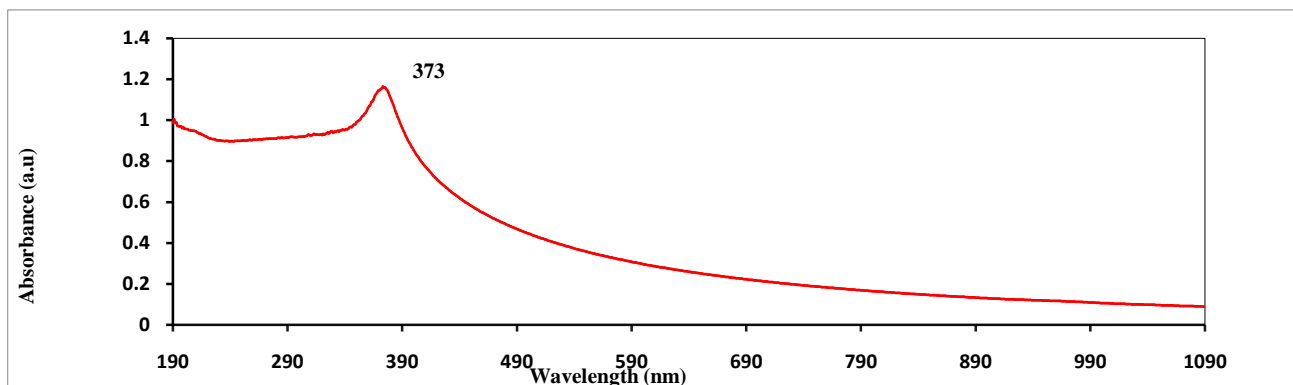


Figure 3: UV - Vis spectrum of ZnO nanoparticles

X-ray Diffraction (XRD) Analysis

XRD pattern of bio-synthesized ZnO nanoparticles from peels extract *Banana* is shown in (Figure 4). The ZnO NPs diffraction peaks clearly indicate highly crystalline structure. The sharp and narrow diffraction peaks appearing at about 2θ of 31.95, 34.60, 36.42, 47.70, 56.75, 63.02, 66.62, 68.13 and 69.22 (deg) were assigned to (100), (002),

(101), (102), (110), (103), (200), (112) and (201) hkl plane values of ZnO nanoparticles. The peak intensity profiles were confirmed the hexagonal wurtzite structure by comparison with the data from JCPDS card No. 89-7102. The XRD pattern obtained is consistent with earlier reports (Parthiban and Sundaramurthy [48], Chitra and Annadurai [49]).

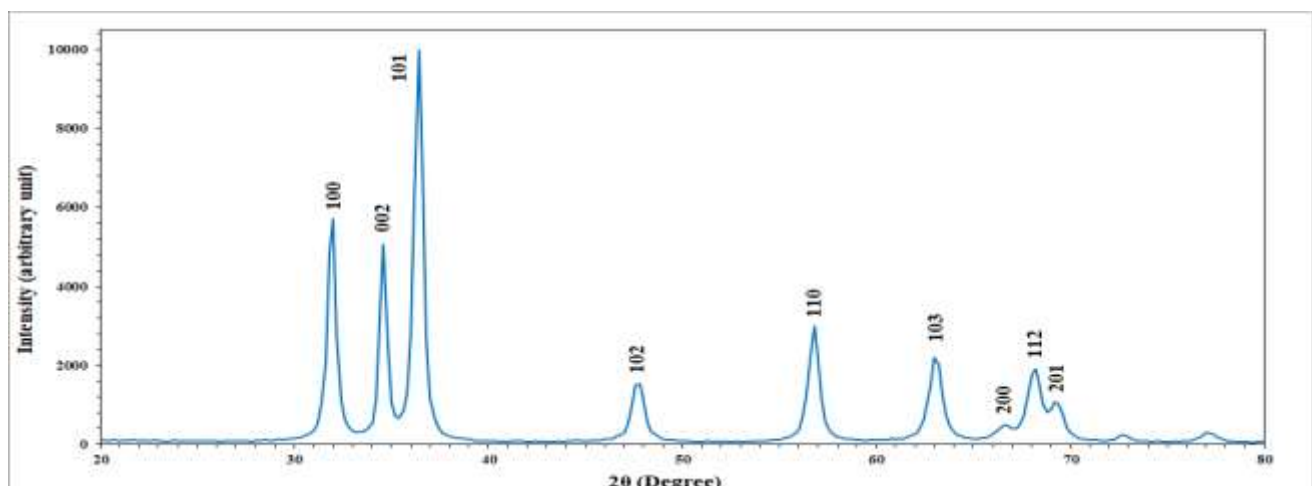


Figure 4: XRD pattern of ZnO nanoparticles

The calculation of average grain size was

$$D = \frac{K\lambda}{\beta \cos\theta} \text{ \AA} \quad (2)$$

Where D is the average crystallite size in \AA , K is the shape factor, λ is the wavelength of X-ray (1.5406 \AA) Cu -K α radiation, θ is the Bragg angle, and β is the corrected line

attained by using Scherer's equation: [50]

broadening of the nanoparticles. The average powder particle size calculated by Scherer's equation was found to be 11.98 nm. The calculated values are listed in (Table 2).

Table 2: The structure and geometric parameters of ZnO nanoparticles

2 θ ($^{\circ}$)	d-spacing (\AA)	FWHM (β)	Grain Size (nm)
31.9537	2.798	0.6320	13.076
34.6051	2.589	0.5900	14.104
36.4245	2.464	0.6288	13.301
47.7053	1.904	0.7890	11.009
56.7595	1.620	0.7132	12.660
63.0208	1.473	0.7724	12.063
66.6222	1.402	1.0000	9.505
68.1318	1.375	0.8911	10.761
69.2215	1.356	0.8466	11.400
		average	11.986

FE-SEM with EDX Analysis

After the substantiation of XRD result the sample was proceeded for the further FE-SEM with EDX studies. The size, shape and surface morphology of the ZnO nanoparticles are clearly indicated FE-SEM image as shown in (Figure 5, A), the micrograph images of ZnO nanoparticles prove that they have granular nano-sized range and have a uniform distribution with agglomerated particles of spherical shape [45].

The particle size ranges from 10 to 24 nm as shown in (Figure 5, A). Energy Dispersive X-ray (EDX) spectrometer analysis is confirmatory presence of elemental Zinc and Oxygen signals of the ZnO NPs. The vertical axis displays the number of x-ray counts although the horizontal axis displays energy in KeV (Figure 5, B). The weight and the atomic percentage of Zinc, Oxygen was found to be 77.9 and 22.1 these corresponds, the spectrum without impurities peaks.

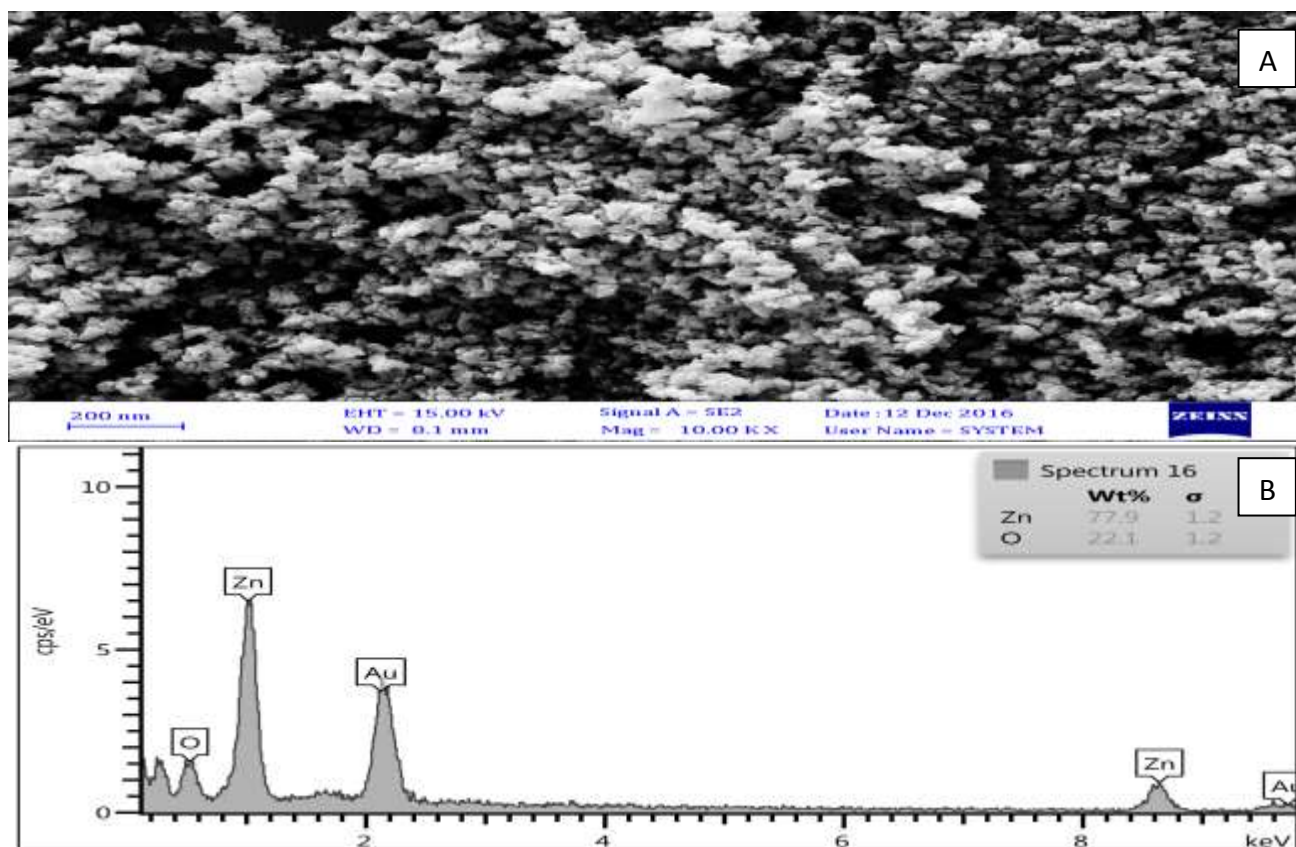


Figure 5 A: FE-SEM image of synthesized ZnO nanoparticles, (B) EDX spectrum

Atomic Force Microscopy (AFM) analysis

The AFM images were used to characterize the structure morphology and size of the ZnO NPs. Figure 6 showed lateral and 3D AFM image for the biosynthesis ZnO nanoparticle. From AFM pictures we can see that the size of nanoparticles is bigger. The sizes of nanoparticles obtained from the AFM images appear bigger than the values that we get from XRD and FE-SEM measurements. We interpret those results to several reasons;

first explanation relates the ZnO nanoparticles tend to form aggregates on the surface during deposition. Second explanation related to the shape of the tip AFM may cause misleading cross sectional views of the sample [51, 52]. So; the width of the nanoparticle depends on probe shape. The resultant images for ZnO NPs were showed have spherical shape. The particle size of the ZnO NPs was found to be 51 nm and is also used to verify that the ZnO NPs were more or less homogenous in size.

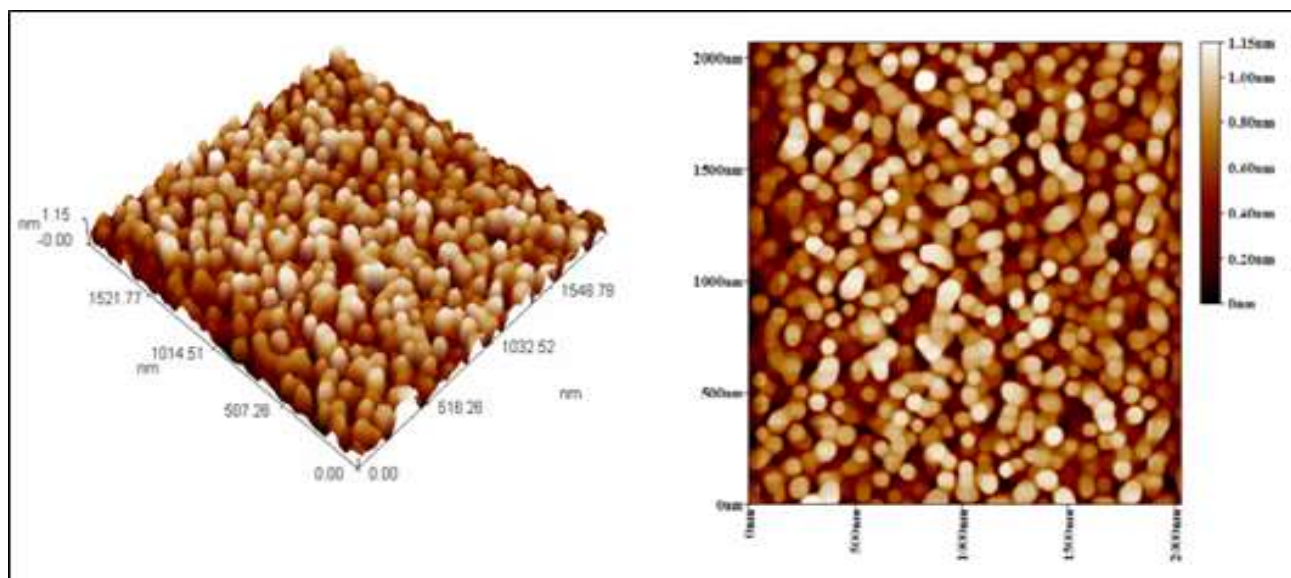


Figure 6: Atomic force microscopy images show the particle size distribution of ZnO nanoparticles

Fourier Transform Infrared Spectroscopy (FTIR)

(Figure 7) presents the FTIR spectra of biosynthesis ZnO NPs and *Banana* peel extract, which showed the composition and quality of the product. The absorption bands at 3427, 2928, 2354, 1605, 1408, 1056, and 524 cm^{-1} in *Banana* peel extract would be shifted into 3442, 2945, 2309, 1516, 1410, 1099, and 436 cm^{-1} of synthesized ZnO NPs sample. The strong absorption peaks at 436 cm^{-1} correspond to the Zn-O bonding and confirm

the presence of ZnO particles [53, 54]. The band observed at the region of 3442 cm^{-1} may be due to O-H stretching and deforming, probably due to atmospheric moisture. The other bands at 582 cm^{-1} and 765 cm^{-1} were probably due to the carbonate moieties that are generally observed when FTIR samples are measured in air [55]. The absorption band near 1410-1516 cm^{-1} were due to C=O stretching mode and the band near 2309 cm^{-1} was due to absorption of atmospheric CO_2 on the metallic cations.

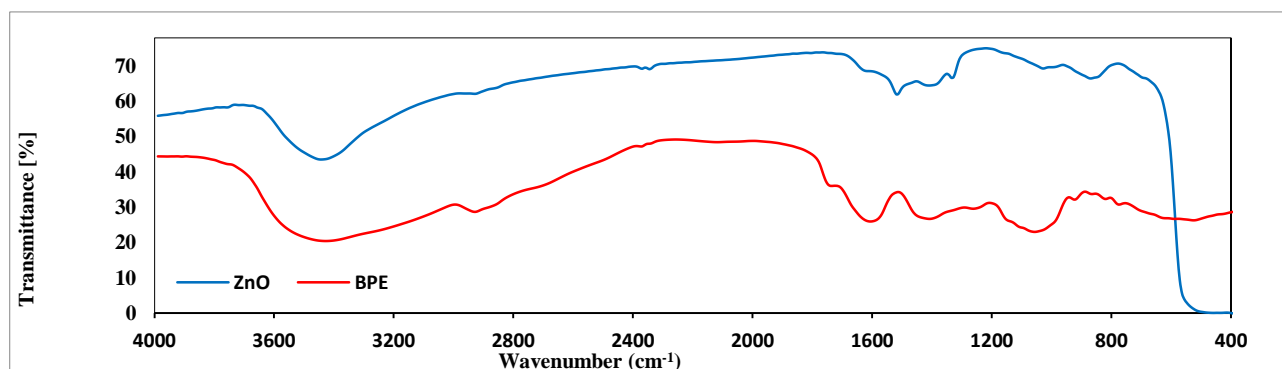


Figure 7: FT-IR spectra of peels extract of *Banana* and Green synthesized ZnO nanoparticles

Determination of Minimum Inhibitory Concentration and Minimum Bactericidal Concentration (MIC/MBC) as Antibacterial Activity of ZnO nanoparticle

In our study, the relative antibacterial activities of ZnO NPs suspensions against pathogenic isolates (*Staphylococcus aureus*, *Pseudomonas aeruginosa* and *Escherichia coli*) were studied in nutrient broth quantitatively by determination of the MIC and MBC. Here, nine ZnO NPs suspensions with different concentrations were tested of (200, 100, 50, 25, 12.5, 6.2, 3.1, 1.5 and 0.7 µg/ml) and the results are given in (Table 3).

The result showed that the Gram-negative bacteria isolates (*Pseudomonas aeruginosa* and *Escherichia coli*) were completely inhibited at the concentration about (12.5 µg/ml) of ZnO NPs (Minimum inhibitory concentration MIC) but no significant antibacterial activity was observed at concentrations less than (6.2 µg/ml) of ZnO NPs, while that the Gram-positive bacteria isolates (*Staphylococcus aureus*) were completely inhibited at the concentration about (6.2 µg/ml) of ZnO NPs but no significant antibacterial activity was

observed at concentrations less than (3.1 µg/ml) of ZnO NPs. The result also showed that the minimum bactericidal concentration (MBC) was same as MIC for all isolates. Previously it has been reported that the antibacterial mechanism of nanoparticles against Gram-positive and Gram-negative bacteria relies on the difference in the cell-membrane structural composition, most distinctively peptidoglycan layer thickness [56].

Gram-positive bacteria possess thick peptidoglycan layer composed of linear polysaccharide chain cross-linked with small peptides, forming a rigid cell membrane to penetrate ZnO NPs, thus no inhibition zone was formed, whereas the cell wall Gram-negative bacteria has thinner polysaccharide layer, showed significant bactericidal effect. Our results identical to the results of Nagarajan and Rajagopalan [57] that ZnO nanoparticles have bactericidal activity in addition they interpreted in their study that once hydrogen peroxide is generated by ZnO nanoparticles, the nanoparticles remains in contact with the deadly bacteria to prevent further bacterial action and continue to generate and discharge hydrogen peroxide to the medium.

Table 3: Antibacterial activity MIC/MBC (µg/mL) of ZnO against different type from bacteria

Antibacterial activity of ZnO NPs	Staphylococcus aureus	Escherichia coli	Pseudomonas aeruginosa
MIC	≥ 6.2 µg/ml	≥ 12.5 µg/ml	≥ 12.5 µg/ml
MBC	≥ 6.2 µg/ml	≥ 12.5 µg/ml	≥ 12.5 µg/ml

Assessment of Cytotoxicity

The cytotoxicity of the zinc oxide nanoparticles was evaluated against Vero101 normal cell lines and Skin A431 cancer cell lines at various concentrations (200, 100, 50, 25, 12.5 and 6.2) µg/ml. Toxicity of ZnO NPs depends on multiple factors such as surface oxidation and the bioactivity of the outer coating, resulting in release of zinc ions and

oxygen to the system leading to toxicity [58]. Figure 8 shows the cell viability evaluated after 72 h exposure to ZnO NPs of various concentrations (200, 100, 50, 25, 12.5 and 6.2 µg/ml). Vero101 normal cells exposed to 12.5-100 µg ZnO/ml showed no evidence to the toxicity more than 72 h (Figure 8, A) and our results indicate that even at 100 µg ZnO/ml the nanoparticles are non-toxic *in vitro*.

Table 4: LSD test to show the differences between control group and different treatment group

(I) group	(J) group	Mean Difference (I-J)	Std. Error	P value	95% Confidence Interval	
					Lower Bound	Upper Bound
control	6.25 µg	84.17325*	1.30837	.000	81.3671	86.9794
	12.5 µg	87.30726*	1.30837	.000	84.5011	90.1134
	25 µg	92.57240*	1.30837	.000	89.7662	95.3786
	50 µg	93.10518*	1.30837	.000	90.2990	95.9114
	100 µg	93.19920*	1.30837	.000	90.3930	96.0054
	200 µg	96.55259*	1.30837	.000	93.7464	99.3588

* The mean difference is significant at the 0.05 level.

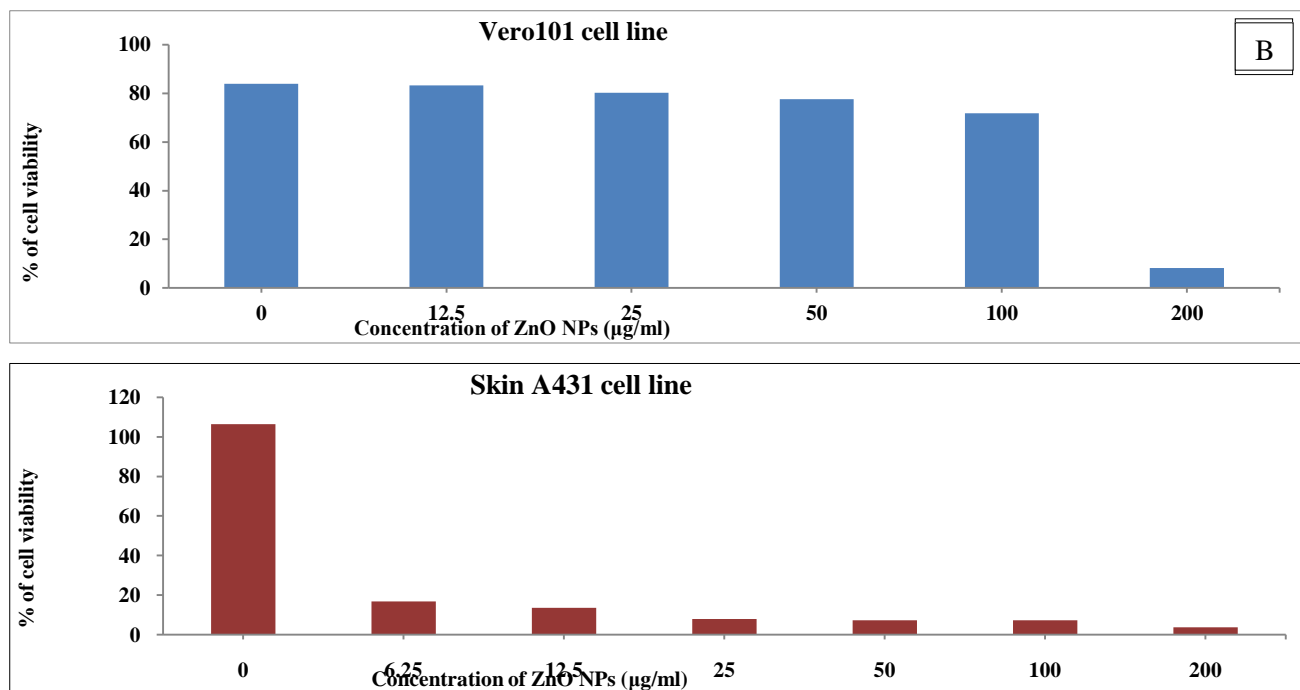


Figure 8: Cytotoxic effect of ZnO nanoparticles against (A) Vero101 normal cell lines, (B) Skin A431 cancer cell lines. Cells were treated with various concentrations (200, 100, 50, 25, 12.5 and 6.2 µg/ml) of ZnO NPs for 72 h grown in a serum free media. The percentage of cell death induced was determined using the CV assay

ZnO NPs were selectively induced cytotoxicity on Skin A431 cancer cell lines in dose depended manner of ZnO NPs with a significant p value 0.05 (Table 4). Maximum concentration of zinc oxide nanoparticles (200 µg/ml) effectively inhibited the growth of cell by more than 98% and our results indicate that even at 6.2 µg ZnO/ml the nanoparticles are toxic *in vitro*.

The toxicity mechanisms of ZnO NPs may be depend on the interaction between nanoparticles and biomolecules, and toxicity mainly involves protein unfolding [59], fibrillation, thiol cross-linking, and loss enzymatic activity. There are three mechanisms to explain why ZnO NPs exert toxic effects: oxidative stress, coordination effects and non-homeostasis effects [60].

According to the previous report Da Dong Guo *et al* [61]. ZnO NPs induced increasing the level of hydrogen peroxide and hydroxyl radicals, decreased the level of molecular oxygen and glutathione, and reduced interleukin-8 (IL-8) release (a signal for proinflammatory mediator release). This report can be explain our results which indicated that the bio-synthesized ZnO nanoparticles are non-toxic on human healthy cells at the highest concentration with $IC_{50} = 63.615 \mu\text{g/ml}$ while it has toxicity on human cancer cells with least concentration with $IC_{50} = 31.122 \mu\text{g/ml}$ since

the cancer cells already have high level of hydrogen peroxide and hydroxyl radicals [61]. There for bio-synthesized ZnO nanoparticles allowing them to be used as an alternative agent to chemical treatment that cause human cells damage.

Conclusion

This study has reported the bio-synthesized of zinc oxide nanoparticles by using *Banana* peels for the first time. It was cheap, simpler and better choice than physical and chemical methods as it is fast, clean and eco-friendly method. The XRD results indicated that the synthesized ZnO NPs have hexagonal wurtzite structure with the crystallite size of 11.98 nm. The diffuse reflectance spectrum showed a sharp increase at 373 nm with the band gap energy value of 3.33 eV.

FTIR spectrum indicated the involvement of functional activities of peels extract in the reduction of ZnO NPs. The final preparation showed that zinc oxide nanoparticles had strong antibacterial activity with no cytotoxic effect on normal cell, whereas it showed anticancer activity in dose dependent manner. Therefore a major conclusion of this study which suggests that biologically synthesized ZnO NPs might be used as novel anticancer agents for the treatment of skin cancer.

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