

Biosynthesis of ZnO Nanoparticles and Multifarious Chemical Analysis for Innocuous Biological Prerogative and Anticancer Activity Against Human Cervical Cancer Cells (HeLa Cell Line)

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ABSTRACT

A nanoparticle is any particle between about 1 and 100 nanometers across, a size range where materials start behaving differently from their bulk counterparts. Zinc oxide nanoparticles (ZnO-NPs) is an inorganic metal oxide that meets as medicine, a preservative in packaging, as well as an antibacterial and anticancer agent without risk. Their effectiveness stems from a combination of their tiny size. The qualities of ZnO-NPs are influenced by their size, shape, concentration, and length of contact with the bacterial cell. There are many uses for ZnO including food technology, agriculture, cosmetology, optoelectronics, drug transporters, and disease control agents. In the present work, bioaugmented zinc oxide nanoparticles (ZnO-NPs) were prepared from aqueous leaf extracts of *Ionidium suffruticosum* plant. The ZnO-NPs were characterized by different techniques such as Fourier transform infrared (FTIR) spectroscopy, scanning electron microscopy (SEM), and dynamic light scattering (DLS). The crystallites exhibited a mean size of 52.47 nm measured via DLS and were highly pure, while SEM analyses of synthesized NPs confirmed their spherical shape. The functional groups responsible for stabilizing and capping of ZnO-NPs were confirmed using FTIR analysis. DLS technique can be considered as a moderately stable colloidal solution. Synthesized NPs were used to evaluate for their possible anticancer properties. The NPs were found to be highly active against cancerous HeLa cells. Due to their eco-friendly synthesis, nontoxicity, and biocompatible nature, ZnO-NPs synthesized from *Ionidium suffruticosum* can be exploited as potential candidates and making them one of the most versatile nanoparticle types in biomedical research.

Keywords: *Ionidium suffruticosum*, Cancer, Cytotoxicity, HeLa cell, MTT assay

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1. INTRODUCTION

The rapidly expanding subject of nanotechnology is focused on creating innovative materials at the nanoscale. In other terms, nanotechnology's objective is to create, describe, & control matter that is between 1 and 100 nm in size. The manufacture and modification of materials at nanometre scales is the focus on the branch of science known as nanotechnology[1]. The human body depends on zinc for a variety of processes, including protein synthesis, enzymatic reactions, learning, memory, and immunological function. Zinc is an essential trace element. Due to its numerous uses ZnO-NPs are used in water purification, photocatalysis and anti-microbial resistance they are extremely significant. ZnO-NPs exhibit characteristics that set them apart from conventional NPs. ZnO is used as an additive in cosmetics, sunscreen, LEDs, transparent transistors, solar cells, memory technology, and catalysis[2-4]. The molecular weight of ZnO-NPs, which is white and odourless, is 81.38 g/mol. Its crystalline structure is wurtzite, and its substantial band gap transistor (3.37 eV at room temperature). Because of their distinctive electrical, optical, catalytic, or photochemical capabilities ZnO-NPs are employed in both biological and commercial applications[5,6]. Wood and fabrics are protected against UV deterioration using ZnO-NPs. ZnO-NPs can control dangerous bacteria and are synthesized in a way that doesn't harm the environment[7]. As a cross-linking agent, ZnO-NPs can also be used &

treatment activator in the rubber industry, and they can speed up the vulcanization process in rubbers used to make balloons, tyres, rubber items, and industrial and medical gloves. The scientific world places a high value on the formation of ZnO-NPs due to their many uses[8]. In the past, chemical and physical techniques were mostly used to create ZnO-NPs. The top-down technique and the bottom-up techniques are the two methods that can be utilised to create NPs[9]. Top-down strategies include mechanical milling, electro explosion, ablation, sputtering, whereas bottom-up strategies include the three fundamental techniques for creating NPs, physical, chemical, and biological processes[10]. Conventional techniques can produce pure, superior nanoparticles. Nevertheless, the procedure is expensive and occasionally produces risky by-products that could have negative effects when used for medical purposes[11]. These methods also call for extra chemicals for capping and stabilising. This issue arises when NPs are created through the bottom-up green pathway, which causes an oxidation/reduction reaction. Green synthesis can utilise algae, fungi, bacteria, plants, and microorganisms[12]. They make it possible to manufacture considerable amounts of pure ZnO-NPs. To create NPs, a variety of components from plants are mixed throughout the green synthesis process.

2. MATERIALS AND METHODS

2.1. Biosynthesis of ZnO NPs

For the synthesis of ZnO nanoparticles, the crude *Ionidium Suffruticosum* leaves were collected from botanic garden. The extract was prepared by drying leaves at room temperature and washed in distilled water, 5 g of this powder was homogenized completely in 100 ml Milli-Q water and extracted at $\leq 80^\circ\text{C}$ for 20 min. The resultant was filtered using Whatman filter papers No. 1. Then, extract was stored at 4°C and used for generating biosynthesized zinc oxide nanoparticles. Zinc nitrate (99.999%) was purchased from Sigma. The synthesis of ZnO NPs was carried out by taking a 0.05 M of zinc nitrate in 100 ml Milli-Q water. Then, 2:2 (v/v) of leaf extract and zinc nitrate to obtain a mixture solution in a round-bottom flask, and incubated with constant stirring (100 rpm) at 40°C for 24 h. The solution was cooled to room temperature and filtered using Whatman filter papers No. 1.

The white coloured precipitate was washed with deionized water and absolute ethanol for several times using centrifugation (5000 rpm for 5 min), and dried in an oven at 60°C for 24 h. Finally, the ZnO nanoparticle product was calcined at 600°C for 3 h[13].

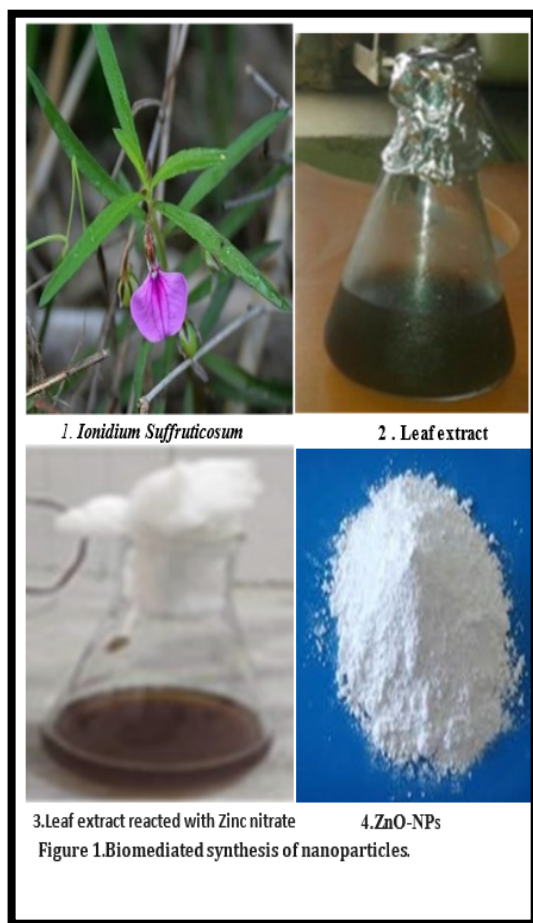


Figure 1. Biomediated synthesis of nanoparticles.

(b).FTIR analysis

FTIR Analysis of leaf extracts was used to identify the functional groups of compounds in crude powder of the leaves. The wavelength of light absorbed corresponds to features in the chemical bond, which can be observed in the annotated FTIR spectrum. The infrared absorption spectrum in FTIR is used to interpret the chemical bonds in the compound. For FTIR analysis, 10 mg dried powder of leaf sample was used to identify the functional groups of compounds in crude powder of the plants. The wavelength of light absorbed corresponds to features in the chemical bond, which can be observed in the annotated FTIR

(d).DLS and Zeta potential analysis

For ZnO NPs size distribution measurement in liquid and colloidal suspension, dynamic light scattering measurements technique was followed. In DLS Instrument 12 mm cell (DTS 0012) was used. One milliliter of distilled water (or ethylene glycol) was added to the cell and then 50 μ l from ZnO NPs stock dispersions were added. Samples were again sonicated for 5 minutes. Size distributions of the nanoparticles were determined

Scanning Electron Microscope (SEM), offers information about the crystal structure with high spatial precision. It generates a magnified image by focusing an electron beam on a sample. Objects can be magnified up to a million times with a SEM. Therefore, SEM is a highly effective method for characterizing materials. We can see objects to the order of a few angstrom (10-10 m). SEM instrument used for our characterization is Scanning Electron Microscope (Make: Philips, Model CM 200, Operating voltages : 20-200kv Resolution : 2.4 $\text{\AA}$). The resulted colloidal particles were characterized to determine their shape and size by high-resolution Scanning electron microscopy (SEM). For the preparation of the SEM grid, a carbon-coated copper grid was used. A drop of the ZnO NPs solution was placed over the grid and dried at room temperature. The solution was first dried for 1 hour in oven and then put 15 minute in front of IR lamp and after that SEM characterization is taken. The SEM image of different ZnO NPs nanoparticle morphology that was captured with 200Kv.

spectrum. The infrared absorption spectrum in FTIR is used to interpret the chemical bonds in the compound. For FTIR analysis, 10 mg dried powder of each sample was encapsulated in 100 mg of potassium bromide (KBr) pellet and translucent sample discs were prepared. The samples were run at infrared region between 400-4000 cm^{-1} and standard DLATGS detector was used at 2.8 mm/sec mirror speed. DLATGS stands for Deuterated L-Alanine-doped Triglycine Sulfate. It is an advanced organic crystal material widely used as a high-performance pyroelectric sensor in infrared (IR) detectors, especially for Fourier-transform infrared (FTIR) spectrometers.

with a Malvern Zetasizer Nano ZS (Malvern Instruments Ltd., GB) by the DLS technique. The suitable parameters (viscosity, absorption and refractive index) were chosen for each ZnO NPs nanoparticle dispersants. For ZnO NPs sample: absorption 0.1, refractive index 2.0, for water: viscosity 1.0031 cP, refractive index 1.33, for ethylene glycol viscosity 1.98 cP, refractive index 1.44. The pH value of each suspension was adjusted by adding either NaOH or HCl.

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Histograms with the distributions of sizes were recorded.

The zeta potential was measured by analyzing 0.1 g of ZnO NPs nanoparticle in 10 ml of water (or additives solutions) using the Zetasizer Nano ZS (Malvern Instruments Ltd., GB). Before zeta potential measurements all samples were sonicated for 5 minutes. Zetasizer Nano ZS uses Laser Doppler Velocimetry to determine electrophoretic mobility. The zeta potential was obtained from the electrophoretic mobility by the Smoluchowski equation. The dynamic method consisted of using ZS Malvern Zetasizer device coupled with an automatic titrator (Malvern MPT-2). pH of the suspensions was automatically adjusted by this automatic titrator using hydrochloric acid (0.25 and 0.025 mol/l) and sodium hydroxide (0.25 mol/l). Concentrations of additives (SDS, CTAB, NaCMC) were 5mmol/l.

(e) Anticancer activity

(i) MTT assay

The MTT test (EZ4U, Biomedica, Austria), on different cell lines. For the experiment, the cells were plated in 96-well plates of 100 μ l at the concentrations of 5,000 cells/well (substrate-dependent cells) or 10,000–15,000 cells/well (suspension cells) overnight. Then, aliquots of 100 μ l of experimental ZnO NPs (0, 1, 10, and 100 μ g/ml) as well as the reference compound doxorubicin (0, 1, and 10 μ g/ml) in culture medium were added to the cells and incubated for the next 72 hours. Furthermore, the 3-(4,5-dimethylthiazol2-yl)-2,5-diphenyl tetrazolium bromide (MTT reagent) was appended to the cells following the manufacture recommendations (Biomedica, Austria). The results of the reaction were determined by an Absorbance Reader Bio Tek ELx800 (BioTek Instruments, Inc., Winooski, VT) at 490 and 630 nm. The relative number of living cells (parts per unit) was determined by the ratio of optical densities measured for the wells with tested compounds to the wells with control (non-treated) cells. The half-maximal inhibitory concentration (IC₅₀) of tested extracts was calculated as the extract concentration that reduced the cell viability to 50%. Doxorubicin (Actavis, Romania) was chosen as a reference drug as a positive control. The extracts were added to the cultured cells at the indicated concentrations (0–100 μ g/ml) and then were treated for 72 hours. The results were analyzed and illustrated using GraphPad Prism 6 software (GraphPad Software., San Jose, CA). The obtained data are presented as the average $\pm$ standard deviation (SD) of three replications in two parallels (n = 6).

(ii). Analysis of apoptosis

Cell line Human Cervical Cancer cells (HeLa)

were kindly provided by Cancer Institute, adayar, Chennai, The stored cells were first suspended in DMEM, to wash away DMSO, and collected by centrifugation at 1200rpm for 5min and 25°C. The washed cells were re-suspended and cultured in DMEM medium (Dulbecco's Modified Eagle Medium) supplemented by 10% heat-inactive Fetal Bovine Serum (FBS) and 1% Pen-Strep Antibiotic (100U/mL Penicillin and 100 μ g/mL streptomycin); at 37°C in 5% CO₂ incubator. Cells were sub-cultured after they formed a monolayer on the culturing flask and detached by treating with trypsin for 15min. cell suspension was mixed by pipetting to remove any clumps of cells just before a sample was taken. Then ZnO NPs nanoparticles determined earlier concentration were added and after 24 hours, complete medium was added to inhibit the further chemical reaction.

(iii).Cancer cell death image analysis

Morphological changes and death of HeLa cells treated with the different concentrations of ZnO NPs were observed by Operetta high-content imaging system (Perkin Elmer, Buckinghamshire, UK) for fluorescent microscopy. HeLa cells (2.5×10^4 cell/mL, 0.1 mL/well) were incubated in a 96-well plate with 0.2 mL/well of three different doses (62.5, 125.0, and 250.0 μ g/mL) of the selected ZnO NPs concentrations for 48 h at 37 °C with 5% CO₂. The cells were directly imaged for phase contrast microscopy, whereas Hoechst 33,342 dye (Invitrogen, Paisley, UK; Cat. No.: H1399) was applied for fluorescent microscopy following the manufacturer's instructions. The cells were stained with 0.5 μ L dye (0.4 mg/mL) and incubated for 15 min prior to viewing.

(iv)Evaluation cancer cell death by flow cytometric method

Flow cytometer with Cell cycle analysis software, ModFit LT™ (Verity Software House)

Cell Culture

1. Suspend 0.5×10^6 MCF-7 breast cancer or PE01 ovarian cancer cells in 25 mL RPMI/PS/10% FCS and transfer to a 75-cm² tissue culture flask. Place in a 5% CO₂ humidified incubator at 37 °C.
2. Allow cells to grow to confluence, feeding twice per week by aspirating the spent media, washing with 20 mL PBS, and then adding 25 mL fresh tissue culture media.

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- When cells become confluent, remove spent tissue culture media and wash cells in 10 mL PBS. Remove excess PBS.
- Add 5 mL Trypsin/EDTA to each flask and place back in the incubator until the cells have become detached (Note 5).
- Transfer cell suspension to a sterile Universal container using a sterile pipet; pour 20 mL RPMI/PS/10% FCS (to deactivate the Trypsin) into the flask in order to wash it out and then pool with the cell suspension in the Universal container (total volume of 25 mL).
- Centrifuge at 600 g for 5 minutes.
- Pour off the media and resuspend the pellet in 5 mL RPMI/PS/10% FCS. Syringe with a 21G needle three times to break up the pellet, and then make up to 25 mL with RPMI/PS/10% FCS
- Count total number of cells using a hemocytometer.
- Transfer aliquots of 1×10^6 cells to FACS tubes (regardless of volume) and then centrifuge again at 600 g for 5 minutes.
- Resuspend pellet in 100 μ L citrate buffer, cover tubes, and store at -40°C prior to analysis.

multiple comparisons. Statistical evaluations were performed using Origin software (Version 2025, OriginLab Corporation, Northampton, MA, USA), with significance at $p \leq 0.05$.

3. RESULTS AND DISCUSSION

It was found that the ZnO NPs with spherical shape and the size in the range from 46.32 to 54.47 nm was obtained. SEM analysis was performed to know the morphological shape and size of the synthesized ZnO NPs nanoparticle, the particles are spherical in shape and size was averagely 52.47 nm. This result indicates that the synthesized ZnO NPs were crystalline and monodispersed. Selected area electron diffraction (SAED) pattern of ZnO NPs were shown in Figure 2.

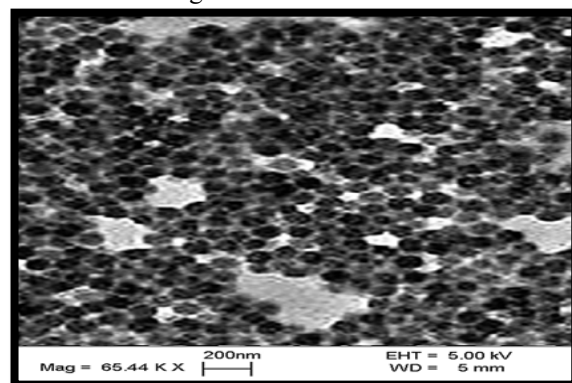


Figure 2. Scanning electron microscopic image of ZnO NPs shows spherical morphology.

The surface potential of nanoparticles is the potential difference between the medium where nanoparticles are dispersed and the accessible surface of dispersed nanoparticles, which can be analyzed using a zeta sizer. The figure 3, demonstrates the zeta potential of the biosynthesized ZnO NPs which was found to be -30.8 mV . This shows that the ZnO NPs synthesized from the leaf extract of *Ionidium Suffruticosum* are highly stable.

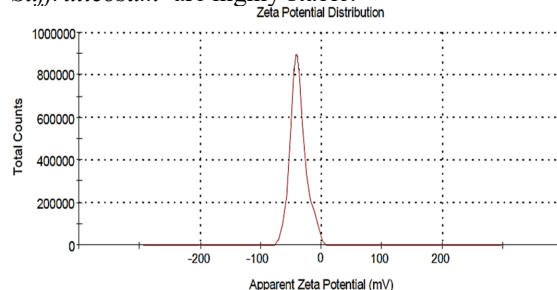


Figure 3. Zeta potential of ZnO NPs

The bioactive compound of methanol extract of leaves of *Ionidium Suffruticosum* plant was traced out by FTIR spectrophotometer (Thermo electron Scientific). Totally 21 peaks were

Preparation of Samples for Analysis

- Thaw Solutions A and B, along with the frozen whole cell suspensions (in 100 μ L) (but not heated) in a water bath at 37° before preparing and staining nuclei for DNA analysis. Allow Solution C to thaw at room temperature before placing on ice.
- Digest cell suspensions down to nuclei by adding 450 μ L of 0.003% Trypsin solution (Solution A), mix and leave at room temperature for 10 minutes (Notes 6 and 7).
- Prevent further degradation by adding 0.05% (w/v) Trypsin inhibitor solution and 0.01% (w/v) RNase A (Solution B) in a final volume of 375 μ L, mix, and leave for 10 minutes.

Finally, stain cells by adding 416 μ L/m Lice-cold propidium iodide/1.16 mg/mL spermine tetrahydrochloride solution (Solution C) in a final volume of 250 μ L and leave the samples on ice in the dark for an additional 10 minutes prior to analysis.

2.5. Statistical analysis

All experiments were conducted in triplicate. The data were assessed through one-way and two-way ANOVA, followed by Tukey's post-hoc test for

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obtained for the FTIR analysis of *Ionidium Suffruticosum* methanol extract. The IR spectrum of sample showed an absorption band at 564.08 cm⁻¹ which corresponds to C-Cl stretching showed the presence of alkyl halides, band at 807.06 cm⁻¹ is C-H stretching exhibit the presence of, aromatic, bands at 1167.69 cm⁻¹, 1199.51 cm⁻¹, 1307.50 cm⁻¹, 1354.75 cm⁻¹, 1452.14 cm⁻¹, 1506.13 cm⁻¹, 1600.63 cm⁻¹, 1655.59 cm⁻¹, 1751.05 cm⁻¹, 1999.82 cm⁻¹, 2165.67 cm⁻¹, 2294.88 cm⁻¹, 2338.27 cm⁻¹, 2363.34 cm⁻¹, 2927.41 cm⁻¹, 2974.66 cm⁻¹, 3332.39 cm⁻¹, 3726.76 cm⁻¹ and 3823.19 cm⁻¹ indicate the presence of bioactive compounds such as sulfones, sulfonyl, chlorides, sulphates, sulfones, sulfates, sulfonamides, alkanes, aromatic, alkenes, ester, ketenes, isocyanates, isothiocyanates, acetylene, nitrile, phosphine, aldehyde, alkane, amide, and alcohol.

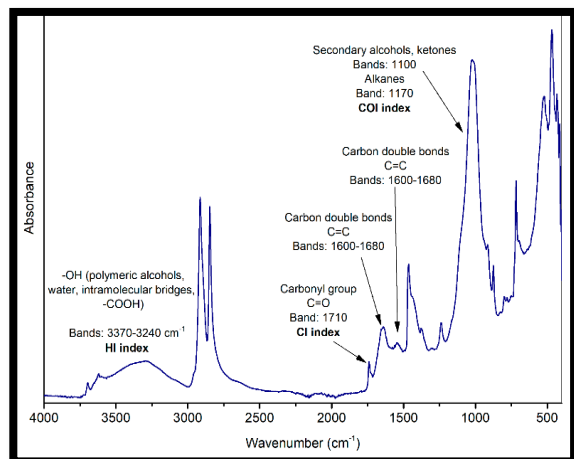


Figure 4. FTIR shows the functional groups
Table-1 : Identified functional groups

Wavelength [nm]	Wavenumber [cm ⁻¹]	Assignment
2857	3500	O-H stretch etch of hydroxyl groups
3125	3200	N-H stretch (amide A) of proteins
3379	2959	C-H stretch (asymmetric) of -CH ₃
3408	2934	C-H stretch (asymmetric) of >CH ₂
3423	2921	C-H stretch (asymmetric) of >CH ₂ in fatty acids
3450	2898	C-H stretch of C-H methine
3481	2872	C-H stretch (symmetric) of CH ₃
3506	2852	C-H stretch (symmetric) of >CH ₂ in fatty acids
5743	1741	>C=O stretch of esters
5830	1715	>C=O stretch of esters, RNA/DNA, OH-C=O
5899	1695	Amide I band components
5934	1685	resulting from antiparallel
5970	1675	pleated sheets and b-turns of proteins
6042	1655	Amide I of a-helical stretch uctures

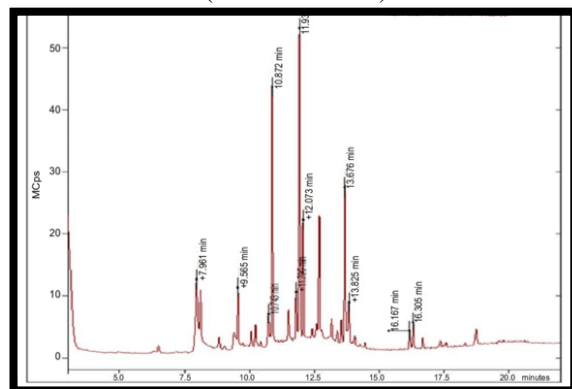


Figure 5. GC-MS analysis of *Ionidium Suffruticosum* leaf extract indicated the presence of biochemical compounds

The GCMS outline of *Ionidium Suffruticosum* indicated the presence of compounds in the plant extract shows, the retention time and compounds of 21.066- 4,8,12,16- Tetramethylheptadecan-4- olide (C₂₁H₄₀O₂), 22.591 - Bis(2-ethylhexyl) phthalate (C₁₂H₁₄O₄), 24.232 - adamantane-1-carboxylic acid methyl ester (C₁₂H₁₈O₂), 25.815 - Benzopyran-6-ol,3,4-dihydro-(C₂₂H₃₆O₂), 26.193 - gamma-Tocopherol (C₂₈H₄₈O₂), 26.411 - dimethyl-5,5'-diphenyl-1H, (C₁₄H₇NO₃), 28.56 - 1Hbenzo[b]furo[2,3-f]indole (C₁₈H₁₆N₆S₂) and minimum presented retention time and compounds 16.303 Bicyclo [3.1.1]heptane, 2,6,6- trimethyl- (C₁₀H₁₈), 16.361 - 2- Pentadecanone, 6,10,14-trimethyl- (C₁₈H₃₆O), 16.564 - Bicyclo [3.1.1]heptane, 2,6,6- trimethyl- (C₁₀H₁₈), 16.637 phthalic acid (C₈H₆O₄), 16.739-3,7,11,15-Tetramethyl-2-hexadecen-1-ol (C₂₀H₄₀O), 17.145-5,9,13-Pentadecatrien-2-one, 6,10,14-trimethyl (C₁₈H₃₀O), 17.595-2(3H)-Furanone,dihydro-5-methyl 5- pentyl (C₁₀H₁₈O₂), 17.828 - Hexamethyl-2,6,10,14,18,22-tetracosahexaen-1-yl]-6-methox (C₃₀H₅₀O), 18.525 - Oxalic acid, cyclohexyl isohexyl ester (C₁₄H₂₄O₄), 18.844 - 6-Octadecenoic acid (C₁₈H₃₄O₂), 19.077 - Phytol (C₂₀H₄₀O) were detected in the samples IUPAC name and 2D structure of bioactive compounds.

Dynamic light scattering (DLS) was used to evaluate the average size distribution, and possible aggregation of the synthesized ZnO NPs in liquid medium. So the particle size in liquid medium is just few nano meters increased. As observed from the particle size distribution graph, an average particle size of 78.3 ± 0.9 nm and polydispersity index (PDI) of 0.574 were obtained. Regarding the zeta potential, the value found was - 37.8 mV .

Poly dispersity describes the degree of “non-uniformity” of a distribution in liquid medium.

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This value was calculated based on the width of the size distribution. The range is from 0.0 (a perfect uniform sample) to 1.0 (highly polydisperse sample with multiple particle size). PDI values above 0.7 indicate a broad particle size distribution profile. The obtained PDI of 0.174 had a narrow particle size distribution and can be considered good polydispersity, with an acceptable value indicating moderate homogeneity. When particles are present in a liquid, an electrical double layer (EDL) is usually formed, consisting of ions in the liquid, as shown in figure 6. This model explains the operation of the electrical repulsive force. The EDL surface consists of two layers. Usually, particles with ZP in the range of -30 mV to $+30$ mV are considered to be normally stable.

After treatment of HeLa cells with different concentration of ZnO NPs nanoparticles for 48 h, 46% of the cells were viable, 54% were in early apoptosis, 5% were in late apoptosis, and 1% exhibited necrosis (cell death). Treatment with $125 \mu\text{g/mL}$ of the ZnO NPs fraction increased the percentage of cell death in late apoptosis, 70% of cells were died in late apoptosis when treated with $250 \mu\text{g/mL}$ of the ZnO NPs. HeLa cells treated with 62.5 , 125.0 , and $250.0 \mu\text{g/mL}$ of the ZnO NPs displays reduced cell viability with increasing dose when compared with untreated cells (figure 6), microscopic image showed that untreated HeLa cells were characterized by an organized monolayer with a regular polygonal appearance. After treatment, the cells became rounded and disconnected from each other and exhibited cellular shrinkage and nuclear condensation. Hoechst 33,342 staining then was used to determine if the ZnO NPs nanoparticles induced apoptosis in HeLa cells. Cells treated with $62.5 \mu\text{g/mL}$ of the extract showed some chromatin condensation and DNA fragmentation, which are the hallmarks of apoptotic cells. A number of cells with similar changes were observed in the $125 \mu\text{g/mL}$ treatment. At $250 \mu\text{g/mL}$ of the extract, most cells showed apoptotic body formation with decreased cell viability, indicating that induction of apoptosis and cell death was ZnO NPs dose-dependent. Flow cytometry result based, Dot Plots of untreated HeLa cells (i) and treated HeLa cells (ii, iii, and iv) treated with 62.5 , 125 , and $250 \mu\text{g/mL}$ of the ZnO NPs nanoparticles signify statistical significance at $p < 0.05$ compared to untreated HeLa cells.

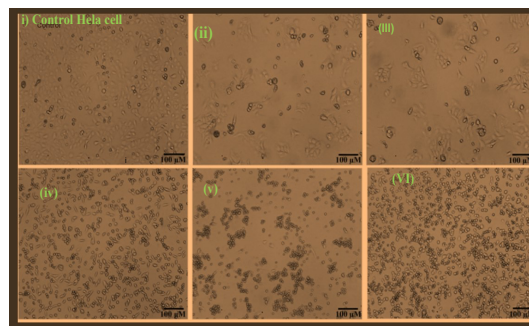


Figure 6. Anticancer activity of ZnO NPs against HeLa cells (i) Control HeLa cell (ii-vi) Various concentration of ZnO NPs nanoparticles shows apoptosis, and cell death in HeLa cells.

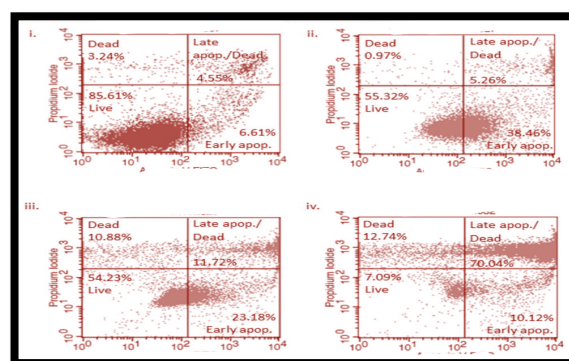


Figure 7. Flow cytometry analysis of ZnO NPs induced anticancer activity.

ZnO NPs nanoparticles made of inorganic molecules that act as linkers and one or more types of metal ions. Due to the versatility of its chemical composition, there are many zeolite-like structures (ZIF) using imidazoles as ligands, with nanoparticles in drug delivery systems due to their adjustable pore shape and size, ultrahigh surface area, and versatile functionalities. An endogenous copper metal-organic framework nano enzyme has been demonstrated to mediate the synergistic interaction between H₂S-activated NIR PTT and chemodynamic therapy in the successful treatment of colon cancer, according to a recent study[14-15].

Among inorganic NPs, ZnO NPs have attracted significant attention in biomedical applications in recent years, particularly due to their antimicrobial, antioxidant, and anticancer properties. However, to safely exploit this potential, a detailed understanding of the cytotoxic effects of ZnO NPs nanoparticles is necessary. Such effects are especially pronounced in cancer

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cells, where they can trigger apoptosis and necrosis, thereby supporting the applicability of ZnNPs, promising anticancer agents [16-19]. Although the concepts of cytotoxicity and anticancer activity are related, the most fundamental difference between them is target specificity [20]. Previous studies have shown that ZnO NPs exhibit remarkable anticancer potential by inducing cytotoxic effects in various human cell lines. It is evidenced by us particularly, nano sized ZnO NPs demonstrate enhanced ability to trigger apoptosis, necrosis, or autophagy in cancer cells, leading to effective cancer or tumor cell elimination. Additionally, their size-dependent interactions can cause DNA strand breaks, ROS-mediated oxidative stress, and modulation of inflammatory cytokines, all contributing to their anticancer efficacy. Therefore, precise control over nanoparticle (NP) dose, size, and exposure conditions is crucial to maximize therapeutic benefits while minimizing toxicity.

4. Conclusion:

In conclusion, the development of eco-friendly Green synthesis of ZnO NPs is an important aspect in the field of nanotechnology and is also easily available starting materials, inexpensive method for nanoparticles biosynthesis and also easy to carry out in any laboratory, use of toxic reagent is avoided and pollution free. From our Study we report eco-friendly synthesis of ZnO NPs nanoparticles using a leaf extract of *Ionidium Suffruticosum* and in our first research paper UV-Visible Spectrophotometric Analysis ZnO NPs nanoparticle was done in the wavelength range of 200-600 nm. In our study, various analysis methods, including MTT (3-(4,5-dimethylthiazol-

2-yl)-2,5-diphenyltetrazolium bromide), DHE (dihydroethidium) staining, were used on ZnO NPs. The results showed that ZnO NPs exhibited the strongest effects on HeLa cancer cells. Furthermore, they exhibited higher toxicity in HeLa cells than in normal cells. Additionally, as ZnO NPs decreased in size, their ability to inhibit cell migration and invasion also decreased. Our study observed, that spherical ZnO NPs with a size ranging from 47 to 52 nm had higher anticancer activity. This study also emphasized the importance of ZnO NPs morphology, beyond size, in biological activity. However, their cytotoxicity has led to debates about the use of this potent anticancer agent in clinical trials. While many studies have demonstrated selective cytotoxicity of ZnO NPs, especially compared to some chemotherapeutic drugs, some studies have observed that ZnO NPs and to overcome this, researchers have shown that selective cytotoxicity can be increased through methods such as using green synthesis methods. Our green synthesized, smart ZnO NPs nanoparticles are being used or have the potential to be exploited as drug delivery systems for advanced cancer therapies. Due to their special qualities, clinicians are now able to provide new treatments or add them as supplements to already-effective therapies. As this research paper has demonstrated, ZnO NPs have shown promise for developing safer and more effective cancer therapies, including targeted drug delivery, stimuli-responsive drug release, and co-delivery of combinational drugs. These capabilities may support long-term perspectives and the creation of novel cancer control maneuvers.

REFERENCES:

1. Divya M., Chen J., Durán-Lara E.F., Kim K.-S., Vijayakumar S. Revolutionizing healthcare: Harnessing nano biotechnology with zinc oxide nanoparticles to combat biofilm and bacterial infections-A short review. *Microb. Pathog.* 2024;191:106679. doi: 10.1016/j.micpath.2024.106679. [DOI] [PubMed] [Google Scholar]
2. Shaikh T.N., Patel B.H. Nanotechnology Based Advanced Medical Textiles and Biotextiles for Healthcare. CRC Press; Boca Raton, FL, USA: 2024. Nanotechnology in hospital clothing and odor control of medical textiles; pp. 177–194. [Google Scholar]
3. El Shahawi A.M. Incorporation of zinc oxide nanoparticles and its antibacterial effect on toothpaste. *Bull. Natl. Res. Cent.* 2023;47:2. doi: 10.1186/s42269-022-00975-x. [DOI] [Google Scholar]
4. Keerthana P., Vijayakumar S., Vidhya E., Punitha V.N., Nilavukkarasi M., Praseetha P.K. Biogenesis of ZnO nanoparticles for revolutionizing agriculture: A step towards anti-infection and growth promotion in plants. *Ind. Crops Prod.* 2021;170:113762. [Google Scholar]
5. Gomathi R., Paradesi D. Antibacterial efficacy of a topical skin cream loaded with nano zinc oxide, cetylpyridinium chloride and chlorhexidine gluconate. *Mater. Today Proc.* 2023;93:54–60. doi: 10.1016/j.matpr.2023.10.001. [DOI] [Google Scholar]
6. Ma Y., Yi J., Ma J., Yu H., Luo L., Wu W., Jin L., Yang Q., Lou T., Sun D., et al. Hand sanitizer gels: Classification, challenges, and the future of multipurpose hand hygiene products. *Toxics.* 2023;11:687. doi: 10.3390/toxics11080687. [DOI] [PMC free article] [PubMed] [Google Scholar]

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- 7.Djearmane S., Xiu L.-J., Wong L.-S., Rajamani R., Bharathi D., Kayarohanam S., De Cruz A.E., Tey L.-H., Janakiraman A.K., Aminuzzaman M., et al. Antifungal properties of zinc oxide nanoparticles on *Candida albicans*. *Coatings*. 2022;12:1864. doi: 10.3390/coatings12121864. [DOI] [Google Scholar]
- 8.Shams A.H.M., Helaly A.A., Algeblawi A.M., Awad-Allah E.F.A. Efficacy of seed-biopriming with *trichoderma* spp. and foliar spraying of ZnO-nanoparticles induce cherry tomato growth and resistance to *Fusarium* wilt disease. *Plants*. 2023;12:3117. doi: 10.3390/plants12173117. [DOI] [PMC free article] [PubMed] [Google Scholar]
- 9.Fouda A., Abdel-Rahman M.A., Eid A.M., Selim S., Ejaz H., Alruwaili M., Manni E., Almuhayawi M.S., Al Jaouni S.K., Hassan S.E.-D. Investigating the Potential of Green-Fabricated Zinc Oxide Nanoparticles to Inhibit the Foodborne Pathogenic Bacteria Isolated from Spoiled Fruits. *Catalysts*. 2024;14:427. doi: 10.3390/catal14070427. [DOI] [Google Scholar]
- 10.Chen J., Jing Q., Xu Y., Lin Y., Mai Y., Chen L., Wang G., Chen Z., Deng L., Chen J., et al. Functionalized zinc oxide microparticles for improving the antimicrobial effects of skin-care products and wound-care medicines. *Biomater. Adv.* 2022;135:212728. doi: 10.1016/j.bioadv.2022.212728. [DOI] [PubMed] [Google Scholar]
- 11.Aburto-Medina A., Le P.H., MacLaughlin S., Ivanova E. Diversity of experimental designs for the fabrication of antifungal surfaces for the built environment. *Appl. Microbiol. Biotechnol.* 2021;105:2663–2674. doi: 10.1007/s00253-021-11214-0. [DOI] [PubMed] [Google Scholar]
- 12.Giedraitienė A., Ružauskas M., Šiugždinienė R., Tučkutė S., Grigonis K., Milčius D. ZnO Nanoparticles Enhance the Antimicrobial Properties of Two-Sided-Coated Cotton Textile. *Nanomaterials*. 2024;14:1264. doi: 10.3390/nano14151264. [DOI] [PMC free article] [PubMed] [Google Scholar]
- 13.Almusallam A.H., Ismail A.M., El-Gawad M.A. Green Synthesized Zinc Oxide Nanoparticles: Antifungal Activity against Powdery Mildew Disease of Pepper (*Capsicum annuum* L.) and Genotoxicity Potentials. *Biopestic. Int.* 2024;20:193–206. doi: 10.59467/BI.2024.20.193. [DOI] [Google Scholar]
- 14.Gu L., Lin J., Wang Q., Meng F., Niu G., Lin H., Chi M., Feng Z., Zheng H., Li D., et al. Mesoporous zinc oxide-based drug delivery system offers an antifungal and immunoregulatory strategy for treating keratitis. *J. Control. Release*. 2024;368:483–497. doi: 10.1016/j.jconrel.2024.03.006. [DOI] [PubMed] [Google Scholar]
- 15.Khan S.A., Shahid S., Mahmood T., Lee C.-S. Contact lenses coated with hybrid multifunctional ternary nanocoatings (Phytomolecule-coated ZnO nanoparticles: Gallic Acid: Tobramycin) for the treatment of bacterial and fungal keratitis. *Acta Biomater.* 2021;128:262–276. doi: 10.1016/j.actbio.2021.04.014. [DOI] [PubMed] [Google Scholar]
- 16.Yassin M.T., Elgorban A.M., Al-Askar A.A., Sholkamy E.N., Ameen F., Maniah K. Synergistic anticandidal activities of green synthesized ZnO nanomaterials with commercial antifungal agents against candidal infections. *Micromachines*. 2023;14:209. doi: 10.3390/mi14010209. [DOI] [PMC free article] [PubMed] [Google Scholar]
- 17.Baglietto C. *Antiviral Nanomaterials*. Politecnico di Torino; Turin, Italy: 2021. [Google Scholar]
- 18.Goharshadi E.K., Goharshadi K., Moghayed M. The use of nanotechnology in the fight against viruses: A critical review. *Coord. Chem. Rev.* 2022;464:214559. doi: 10.1016/j.ccr.2022.214559. [DOI] [Google Scholar]
- 19.Melk M.M., El-Hawary S.S., Melek F.R., Saleh D.O., Ali O.M., A El Raey M., Selim N.M. Antiviral activity of zinc oxide nanoparticles mediated by *Plumbago indica* L. extract against herpes simplex virus type 1 (HSV-1) *Int. J. Nanomed.* 2021;16:8221–8233. doi: 10.2147/IJN.S339404. [DOI] [PMC free article] [PubMed] [Google Scholar]
- 20.Wolfgruber S., Rieger J., Cardozo O., Punz B., Himly M., Stingl A., Farias P.M.A., Abuja P.M., Zatloukal K. Antiviral activity of zinc oxide nanoparticles against SARS-CoV-2. *Int. J. Mol. Sci.* 2023;24:8425. doi: 10.3390/ijms24098425. [DOI] [PMC free article] [PubMed] [Google Scholar]