Biogenic ZnO/CuO Nanoparticles Synthesized Using Leucas Aspera Crude Extract: Structural Insights and Impact on Biocompatibility

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**Abstract:** Green-synthesized zinc and copper nanoparticles from *Leucas aspera* (La-ZnO/CuO NPs) exhibited a wide range of antibacterial properties against pathogenic and mutagenic gram-negative and gram-positive bacteria. Inorganic nano-metal oxides can serve as useful substitutes for organic antibiotics that are drug-resistant. The nanoparticles were synthesized using a wet chemical approach to produce zinc oxide (ZnO) and copper oxide (CuO) nanoparticles. FTIR, XRD, SEM, and TEM were used to characterize the nanoparticles. The disk diffusion method was employed to test the antibacterial properties of the nanoparticles against *S. aureus* and *E. coli*. TEM analysis demonstrated that the La-ZnO/CuO NPs were in the form of nanorods, with an average length of 100 nm, while the spherical nanoparticles had an average diameter of 55 nm. XRD confirmed the crystalline structure of the synthesized La-ZnO/CuO NPs, and functional compounds were identified using FTIR, which revealed the possible compounds present in La-ZnO/CuO NPs. In the antibacterial assays, the minimum zones of inhibition for the La-ZnO/CuO NPs were 13 mm and 14 mm against *E. coli* and *S. aureus*, respectively. La-ZnO/CuO NPs were also analyzed for free radical scavenging activity and in-vitro toxicity using zebrafish embryos, and they were found to be less toxic while producing a high level of free radicals. Additionally, La-ZnO/CuO NPs were tested for their anticancer effects against the MCF-7 cell line. According to the findings, the prepared La-ZnO/CuO NPs exhibited optimal biocompatibility, enhanced antibacterial activity, and acceptable anticancer properties. This type of composite nanoparticle, containing natural components, may be a promising material for biomedical applications.

**Keywords:** La-ZnO/CuO NPs; Green synthesis; Material characterization; Anti-microbial activity; Bio-compatibility; Toxicity analysis.

# Introduction

Zinc and copper oxide (ZnO-CuO) nanoparticles are particles with a size of one billionth of a meter, or nanometers. Materials frequently have distinct characteristics at this nanoscale that set them apart from their bulk counterparts [(Aparna et al., 2021; Poornima et al., 2021; Verma & Muthuswamy Pandian, 2021)](https://paperpile.com/c/yclMY5/wN0vk+S3KHj+3bGu0). These nanoparticles are of interest because of their potential as powerful antioxidants substances that can stop or lessen the toxicity that free radicals do to cells. Unstable chemicals known as free radicals can induce oxidative stress, which damages cells and has a role in several diseases [(Karthik et al., 2019)](https://paperpile.com/c/yclMY5/gWp9). The prevalence of bacteria resistant to antibiotics has prompted an ongoing research for novel substitutes. Water borne bacteria species are among the drug resistant pathogens that really threaten public health because they cause disorders like diarrhea, which kill 2000 approximately infants worldwide per day. Groups of pathogenic bacterial species have been shown to be toxic to inorganic nanoparticles. While inorganic nanomaterial are widely known for their broad spectrum biocidal activity, nothing is known about their bactericidal effect [(Merchant et al., 2022; Pandiyan et al., 2022)](https://paperpile.com/c/yclMY5/whXrn+lmpYF)[; Merchant et al., 2022; Pandiyan et al., 2022)](https://paperpile.com/c/yclMY5/whXrn+lmpYF+LgVhp),[(Chokkattu et al., 2022; Ramamurthy et al., 2022)](https://paperpile.com/c/yclMY5/scfrq+RiSFw). It has been suggested that reactive oxygen species, which are toxic to bacteria, are produced when ions are released into a solution. According to other research, due to their small size, nanoparticles can breach bacterial cell walls and target organelles, causing the cells to perish [(Chokkattu et al., 2022; Ramamurthy et al., 2022](https://paperpile.com/c/yclMY5/scfrq+RiSFw); [Jain & Verma, 2022; Marya et al., 2022)](https://paperpile.com/c/yclMY5/Es89a+c3jAB). Antibiotics that are inorganic, as opposed to organic, have many targeting routes that work by causing mutations in drug resistant organisms [(Cao, 2017)](https://paperpile.com/c/yclMY5/iDpx).

Metal oxide nanoparticles are an effective material among inorganic nanoparticles because of their antibacterial and bio-medical properties. This is largely explained by the fact that metal oxides are less expensive than metal nanoparticles like zinc and copper and have simple synthesis processes that allow for fine tuning the size and shape of the nanoparticles. Oxides of zinc and copper are thought to be acceptable substitutes for antimicrobials with an organic basis and numerous parameters, mostly influenced by the synthesis process, dictate their antibacterial properties. These variables include their shape, size, and surfactant content, among other things. Because of their micro size and strong reactivity, metal nanoparticles can readily penetrate through bacterial well walls and cling to interior proteins and organelles, which kills the bacteria. Surfactants on the surface of nanoparticles have an impact on their activities for biomedical application. Certain surfactants promote the proliferation of cells, while others suppress it and even cause the shrinking of cells [(Fedlheim & Foss, 2001)](https://paperpile.com/c/yclMY5/N8qd). A particular surfactant used in the synthesis of nanoparticles will have the impact of either accelerating or reducing cell proliferation. As an example, it has been reported that the cationic surfactant cetyltrimethylammonium bromide (CTAB) has antibacterial properties. The toxicity of nanoparticles can be influenced by their form and it was discovered that the spherical form of zinc and copper nanoparticles was less hazardous than the cubic form. This could be for a various reasons, the main one being that they each release ionic species into solution, which is the main antibacterial agent. Moreover, the diffusive rate of the nanoparticle via the cell wall pores. The size and form of the particles affect how easily they can pass through cell walls. As a result, the qualities of nanoparticles including their ability to combat bacteria are determined by their physical properties. The capacity of the nanoparticle to release ions into solution is especially important for the antibacterial application. A wide range of synthesis techniques and factors can be used to achieve particular nanoparticle characteristics [(Ozturk & Hakeem, 2018)](https://paperpile.com/c/yclMY5/e5tk).

The study uses two widely techniques, the H2O2 (hydrogen peroxide) assay and the DPPH (2,2-diphenyl-1-picrylhydrazyl) assay, to evaluate the antioxidant activity of Le-ZnO/CuO NPs. Substances has a capacity to neutralize free radicals by measuring its color change when an antioxidant reduces a stable free radical, as in the DPPH assay. On the other side, the H2O2 assay gauges a materials capacity to break down the reactive oxygen species hydrogen peroxide into water and oxygen. Free radical scavenging activity describes a substances ability to scavenge free radicals. This is an important function because oxidative stress, which is caused by free radicals, can harm cells and contribute to the onset of many diseases [(Wadhwani et al., 2022)](https://paperpile.com/c/yclMY5/MXNwP),[(Sreevarun et al., 2023)](https://paperpile.com/c/yclMY5/DrZNH),[(Adel et al., 2023)](https://paperpile.com/c/yclMY5/UkZo6). Thus, materials with potent free radical scavenging properties, like the Le-ZnO/CuO NPs under investigation, may be useful in reducing or eliminating oxidative damage [(Mahawar et al., 2024)](https://paperpile.com/c/yclMY5/Dlzy).

The main goal of this study is to synthesize Le-ZnO/CuO NPs for antioxidant activity and compare them with other well-known antioxidants like vitamin E and ascorbic acid. The study addresses several gaps, such as the need for more stable and effective antioxidants. The results of this study may pave the way for the synthesis of novel nanoparticles, potent antioxidant activity, anti-bacterial and anti-cancer (MCF-7) cell line for biomedical application, all of which may have widespread applications in the treatment of various health issues and diseases. The objective of this work is to advance the field of antioxidant, anti-bacterial and anti-cancer by understanding and utilizing the potential biomedical applications of Le-ZnO/CuO NPs [(Nekoukhou et al., 2024)](https://paperpile.com/c/yclMY5/Ru16).

# Materials and methods

## Collection of plant sample and crude extract preparation

The roots of *Leucas aspera* were collected from Attur (Salem district), a town located in Tamil Nadu, India. Its coordinates are latitude 11.598116 N and longitude 78.596802 E. *Leucas aspera* roots were collected, and they were shade dried for a week at room temperature. The roots were properly cleaned many times with D.D. water after drying to get rid of any contaminants [(Ghani, 1998)](https://paperpile.com/c/yclMY5/fsap). After boiling 20 g of the dried roots in 250 ml of D.D. water to an 80°C temperature, the root extract was filtered (with Whatman No. 1). The crude extract of *Leucas aspera* was used as reducing agent for the synthesis of Le-ZnO/CuO NPs [(Khare, 2004)](https://paperpile.com/c/yclMY5/0UH2).

## Zinc oxide/Copper oxide nanoparticle synthesis

All chemicals used were of analytical grade. Precursors included zinc acetate (zinc acetate dihydrate) and copper acetate (copper (II) acetate dihydrate). Sodium hydroxide of analytical grade was used as the precipitating reagent. All chemicals used in the synthesis were obtained from Sigma Aldrich, and deionized water for all aqueous solutions was provided by MilliQ (Milli-Q Water Systems, USA). The simple wet chemical precipitation approach was used to synthesize oxide nanoparticles, also known as nanometal oxide particles. In this process, the appropriate ionic solution is transformed into its insoluble nanometal oxide precipitate with the help of a precipitating reagent.

150 milliliters of deionized water contained 0.1 milligrams of copper acetate and zinc acetate. The solution was continuously stirred at 700 rpm on a magnetic stirrer hot plate (REMI – 1MLH) at 80 ºC. The pH of the homogeneous solution was adjusted to 11 using 2 M NaOH. The mixture was continuously stirred at the same speed and temperature for an additional twelve minutes. The formation of precipitates and a black solution indicated the formation of zinc and copper oxide [(Markert et al., 2015)](https://paperpile.com/c/yclMY5/y867). While stirring continuously, the temperature was gradually reduced to room temperature. Centrifugation was used to separate the precipitates, and the extra ions were removed by repeatedly washing the precipitates with pure water. The resulting particles were dried overnight at 80 ºC in an oven. Afterward, they were calcinated at 500 ºC to remove any contaminants that might have been present on the particle surfaces. The synthesized particles then underwent a series of characterization analyses in the appropriate sequence [(Niederberger & Pinna, 2009)](https://paperpile.com/c/yclMY5/lT7x).

## Characterization of Le-ZnO/CuO NPs

The phase purity of the obtained Le-ZnO/CuO NPs was assessed by powder X-ray diffraction (XRD) using an X-ray diffractometer (Difray, Saint-Petersburg, Russia) equipped with a chromium (CrKα, λ = 2.2909 Å) radiation source, operated at 40 kV and 30 mA in Bragg–Brentano geometry [(Egamberdieva et al., 2015)](https://paperpile.com/c/yclMY5/Ymnw). XRD spectra of the Le-ZnO/CuO NPs were obtained at 293 K, with a 2θ range of 10 to 140 and a step-scan mode of 0.02. The functional groups of the Le-ZnO/CuO NPs were determined using Fourier transform infrared (FT-IR) spectroscopy (Spectrum 100, PerkinElmer, Waltham, MA, USA) at room temperature, within a wavelength range of 4000–800 cm−1 and a resolution of 4 cm−1. The size, structural morphology, and elemental purity of the Le-ZnO/CuO NPs were examined using scanning electron microscopy (SEM) with silicon drift detectors and X-ray microscope integration and automation software (SDD-XMAS) (Japan). Approximately 0.001 g of Le-ZnO/CuO NPs was added to 10 milliliters of ethanol and sonicated for 30 minutes. After sonication, a drop of the sonicated suspension was placed on carbon tape affixed to the sample holder for SEM analysis. Once the nano-suspension dried, SEM analysis was performed at a voltage of 10 kV. The brightness and contrast of the image were adjusted to clearly detect the Le-ZnO/CuO NPs. The synthesized Le-ZnO/CuO NPs were also examined using a transmission electron microscope (JEOL, JEM-2010, JEOL, Japan) to determine their size and shape [(Khare, 2004)](https://paperpile.com/c/yclMY5/0UH2).

## Free radical scavenging activity (Anti-oxidant assay)

## DPPH assay

The DPPH assay technique was used to assess the radical scavenging capacity of Le-ZnO/CuO NPs. A stock solution was prepared by combining 22 mg of DPPH with 100 mL of methanol, which was then filtered to obtain a solution with an absorbance of approximately 0.892 at 517 nm. Different concentrations of Le-ZnO/CuO NPs (Control, 5, 10, and 15 µg/mL) were mixed with DPPH. A reference solution was made using 3 mL of DPPH and 100 μL of methanol. After 30 minutes, the absorbance of the solution was measured at 517 nm. The percentage of antioxidant activity was calculated and plotted [(Dehghani et al., 2023)](https://paperpile.com/c/yclMY5/fwgA).

## H2O2 assay

The H₂O₂ scavenging activity of Le-ZnO/CuO NPs was measured at 230 nm using the traditional UV technique. The H₂O₂ scavenging activity was assessed by calorimetry. A pink quinoneimine color is produced when H₂O₂ reacts with phenol and 4-aminoantipyrine in the presence of horseradish peroxidase [(Venezia et al., 2024)](https://paperpile.com/c/yclMY5/9TKw). The scavenging activity was determined by evaluating this color shift. This calorimetric experiment was performed with selected concentrations of Le-ZnO/CuO NPs (Control, 5, 10, and 15 µg/mL) as well as the standard antioxidant ascorbic acid. After the solutions were incubated for 30 minutes at room temperature and their absorbance was measured at 504 nm [(Ullah et al., 2023)](https://paperpile.com/c/yclMY5/YCUt).

## Anti-bacterial activity of synthesized Le-ZnO/CuO NPs

The antibacterial activities of the green-synthesized Le-ZnO/CuO NPs were evaluated against *Escherichia coli* MTCC 1692 and *Staphylococcus aureus* MTCC 2639. Mueller-Hinton agar plates were used for cultivating the bacteria. Le-ZnO/CuO NPs and antibiotic control discs were placed on the agar plates [(Kalpana & Devi Rajeswari, 2018)](https://paperpile.com/c/yclMY5/rLA7). The control consisted of 25 mg of erythromycin and 25 mg of amoxicillin mixed in a 1:1 ratio. Le-ZnO/CuO NPs were tested with both positive and negative controls at two distinct concentrations. To assess the antibacterial activity, the zone of inhibition around the discs, where bacterial growth was inhibited, was measured after incubation [(Babapour et al., 2021)](https://paperpile.com/c/yclMY5/eWip).

## *In-vitro* analysis of Le-ZnO/CuO NPs against MCF-7

## MTT assay

The MTT assay was used to assess the viability of MCF-7 cells. The cells were seeded in 96-well plates at a density of approximately 2 × 10⁵ cells/mL. After adding 100 μL of radioimmuno precipitation buffer to each well, the cells were incubated for one day at 5% CO₂. Following the incubation period, the samples were added to the wells, and Le-ZnO/CuO NPs (Control, 5, 10, and 15 µg/mL) were dissolved in dimethyl sulfoxide (DMSO). After another day of incubation, 100 μL of MTT solution (0.5 mg/mL in Dulbecco's Modified Eagle Medium (DMEM)) was added to each well, and the cells were incubated for an additional three hours. Cell growth was measured based on the ability of live cells to convert the MTT yellow dye into formazan [(Nahhas, 2019)](https://paperpile.com/c/yclMY5/KZAw). After 100 μL of DMSO was added to each well to dissolve the metabolic products, the medium was observed. The 96-well plates were then shaken for 10 minutes at 150 rpm, and the optical densities were measured at approximately 570 nm. The inhibition percentage was calculated by plotting the concentration graph using the formula: 100 - (absorbance of test wells/absorbance of control wells) × 100 [(Karthik et al., 2019)](https://paperpile.com/c/yclMY5/gWp9).

## XTT assay

Le-ZnO/CuO NPs were added to breast cancer cells at varying concentrations (Control, 5, 10, and 15 µg/mL) for different durations. The analysis was based on the uptake of XTT salt. The breakdown of XTT by the mitochondria of live cells resulted in the production of soluble formazan, and two duplicates of each concentration were assessed. The amount of formazan in the media was measured using spectrophotometry at an absorption wavelength of 450 nm [(Karthik et al., 2019; Shukla & Iravani, 2018)](https://paperpile.com/c/yclMY5/gWp9+jRj2).

## *In-vitro* toxicity analysis using zebrafish embryos

To assess mortality levels and determine the cytotoxic effects of the synthesized Le-ZnO/CuO NPs, zebrafish eggs were exposed to varying doses of Le-ZnO/CuO NPs. Different concentrations of Le-ZnO/CuO NPs dilutions were used, with saline serving as the control. Twenty zebrafish eggs were placed in a tank filled with Hank's solution. Le-ZnO/CuO NPs were added in varying amounts to each well containing a zebrafish egg, and the embryos were allowed to develop [(Machado et al., 2021)](https://paperpile.com/c/yclMY5/A8rs). The wells were maintained at a constant room temperature of 24°C. The formation and development of organs were observed. Zebrafish embryos were monitored using an optical microscope (CX41; Olympus Corporation, Tokyo, Japan). Every eight hours, the viability of the embryos was assessed, and any dead ones were removed to prevent contamination [(Machado et al., 2021; Sivalingam et al., 2024)](https://paperpile.com/c/yclMY5/A8rs+GTe8).

## Analytical statistics

IBM SPSS Statistics for Windows, Version 23.0 (Released 2015; IBM Corp., Armonk, NY, USA), was used to gather, tabulate, and analyze the data. Le-ZnO/CuO NPs antibacterial activity was evaluated using a one-way ANOVA compared to the antibiotic standard, with a p value of < 0.05 being statistically significant. Le-ZnO/CuO NPs were tested for antioxidant activity and cytotoxicity against the standard using an unpaired t test; a p value of < 0.05 was considered statistically significant.

# Results and Discussion

## Characterization of synthesized CuO-ZnO NPs from *Leucas aspera*

The synthesized Le-ZnO/CuO NPs from Leucas aspera were fine and free from impurities, according to the XRD patterns shown in Figure 1 (A). There were no impurity-related spectral peaks in the pattern. Sharp peaks observed in the diffraction patterns indicate that the nanopowders have good crystallinity [(Raja et al., 2023)](https://paperpile.com/c/yclMY5/sWp9)[(Subramanian & Harikrishnan, 2023)](https://paperpile.com/c/yclMY5/dYjkW), [(Solanki et al., 2023)](https://paperpile.com/c/yclMY5/WXder), [(Raja et al., 2023)](https://paperpile.com/c/yclMY5/sWp9). The phase of the Le-ZnO/CuO NPs from Leucas aspera matched exactly with the produced XRD patterns. The spectral peaks corresponded precisely to the monoclinic phase. The XRD spectrum pattern of the Le-ZnO/CuO NPs from Leucas aspera is displayed in Figure 1 (A). The diffraction peaks of Le-ZnO/CuO NPs accurately matched the hexagonal wurtzite structure(Rafi et al., 2024). The crystal diameters of Le-ZnO/CuO NPs were calculated using Scherer’s equation to be 53.55 nm and 34 nm, respectively. Agglomeration is one reason for the discrepancy between the particle size measured by TEM analysis and the crystal size recorded by XRD [(Kitala et al., 2023)](https://paperpile.com/c/yclMY5/7Z0O)[(Chokkattu et al., 2023)](https://paperpile.com/c/yclMY5/C6E7Q),[(Laghari et al., 2023; Ramakrishnan et al., 2023)](https://paperpile.com/c/yclMY5/gIRgX+ckxtT),[(Muthuswamy Pandian et al., 2022)](https://paperpile.com/c/yclMY5/8ZPb)[(Kitala et al., 2023)](https://paperpile.com/c/yclMY5/7Z0O).

Transmission electron microscopy (TEM) of Le-ZnO/CuO NPs from Leucas aspera, synthesized via the wet chemical aqueous precipitation synthesis process, is demonstrated by the TEM micrographs. The synthesized nanoparticles aggregated into bundles or clusters, as seen in Figure 1 (C). The lengths of the nanoparticles ranged from 10 nm to 100 nm, with an average length of 40 to 75 nm. The widths of the nanoparticles varied from 4 nm to 22 nm, with an average width of 12 nm. Figure 1 (C) displays the TEM micrograph of the Le-ZnO/CuO NPs from Leucas aspera. The Le-ZnO/CuO NPs synthesized by wet chemical aqueous precipitation exhibited a more spherical shape compared to copper oxide; however, the size distribution of the particles was also more widely spread. ImageJ analysis of the size revealed that it varied between 12 and 25 nm [(Dorjee et al., 2023)](https://paperpile.com/c/yclMY5/L4tz)[(Muthuswamy Pandian et al., 2022; Ramakrishnan et al., 2023)](https://paperpile.com/c/yclMY5/8ZPb+gIRgX)[(Dorjee et al., 2023)](https://paperpile.com/c/yclMY5/L4tz).

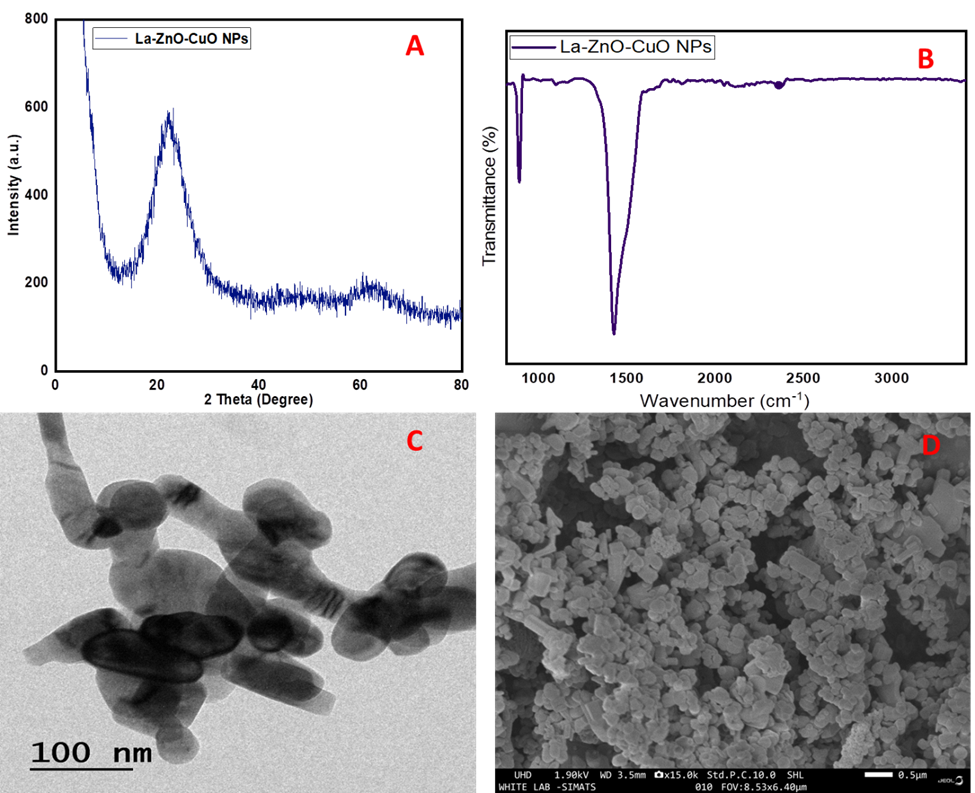
FTIR analysis (Figure 1 – B) displays the infrared spectra of Le-ZnO/CuO NPs from Leucas aspera. A peak at 596 cm⁻¹ is associated with the stretching vibration of Cu–O bonds (Tuluwengjiang et al., 2024). The absorption bands at 1350 cm⁻¹ and 1650 cm⁻¹ are markers of atmospheric CO₂. The O-H bond is represented by spectral bands that occur between 2800 and 3500 cm⁻¹ [(Yılmaz, 2023)](https://paperpile.com/c/yclMY5/x0MO). O-H bonds with very low band counts result from minimal moisture content [(Antonio-Pérez et al., 2023)](https://paperpile.com/c/yclMY5/dtV3). The presence of a Cu and Zn link in the FTIR spectrum in Figure 1 (B) verifies the synthesis of Le-ZnO/CuO NPs from Leucas aspera. The analysis of the FTIR spectra in Figure 1 (B) shows that the Zn–Cu–O vibration is indicated by the absorption band at 896 cm⁻¹ [(Berne et al., 1998)](https://paperpile.com/c/yclMY5/lnnU).

The SEM images of Le-ZnO/CuO NPs from Leucas aspera, synthesized using the green synthesis method, are displayed in Figure 1 (D). These images confirm that the Le-ZnO/CuO NPs from Leucas aspera, which range in size from 80 to 120 nm, have an irregular, square-like arrangement. The synthesized Le-ZnO/CuO NPs from Leucas aspera exhibit an absorption spectrum (Figure 1 - D) at 175 nm, and their absorption wavelength is in good agreement with that reported in the literature [(Di et al., 2023)](https://paperpile.com/c/yclMY5/5Ez4).

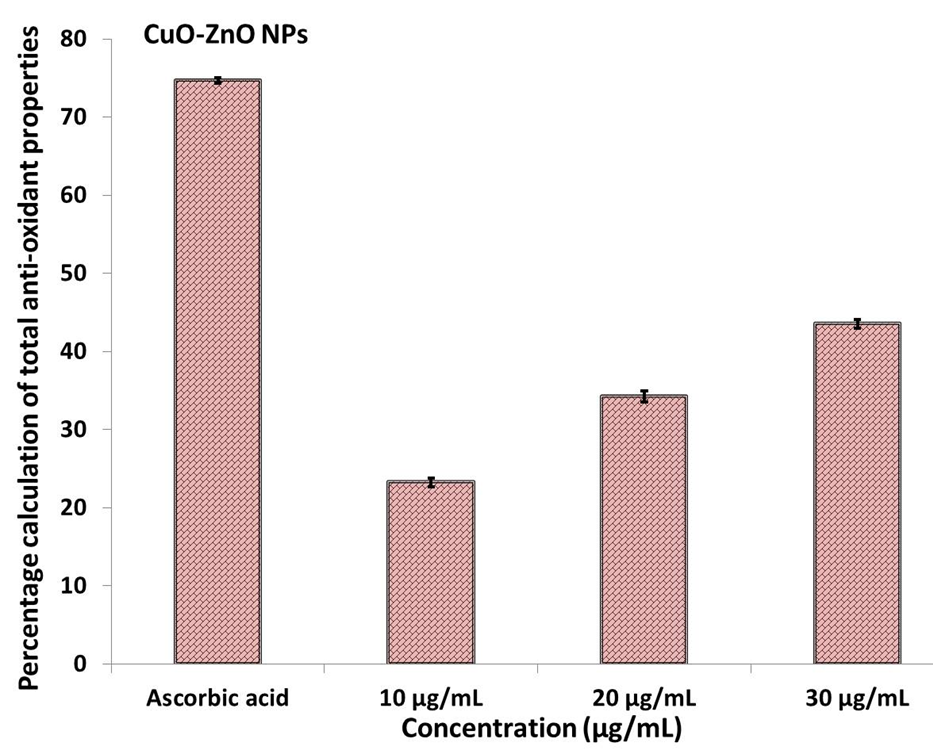
## Anti-oxidant properties of synthesized Le-ZnO/CuO NPs from *Leucas aspera*

## DPPH assay (2,2-Diphenyl-1-picrylhydrazyl)

The antioxidant activity of synthesized Le-ZnO/CuO NPs from Leucas aspera was analyzed through the DPPH assay, with vitamin C used as the control (Control, 5, 10, and 15 μg/mL) to evaluate the percentage of antioxidant properties present in the sample using wet chemical methods [(Pourchez & Laurence, 2017)](https://paperpile.com/c/yclMY5/9Ens). The results of the DPPH assay are displayed in Figure 2, showing that the concentration of synthesized Le-ZnO/CuO NPs from Leucas aspera increased in tandem with an increase in antioxidant activity [(Crini & Lichtfouse, 2019; Pourchez & Laurence, 2017)](https://paperpile.com/c/yclMY5/9Ens+lmQ0). The synthesized Le-ZnO/CuO NPs from Leucas aspera exhibit good antioxidant activity that is comparable to that of the standard free radical-producing compound (vitamin C) [(Crini & Lichtfouse, 2019; Pourchez & Laurence, 2017; Shukla & Iravani, 2018)](https://paperpile.com/c/yclMY5/9Ens+lmQ0+jRj2). No statistically significant difference was observed between the antioxidant activity of the synthesized Le-ZnO/CuO NPs from Leucas aspera and the control at any concentration [(Shukla & Iravani, 2018)](https://paperpile.com/c/yclMY5/jRj2).



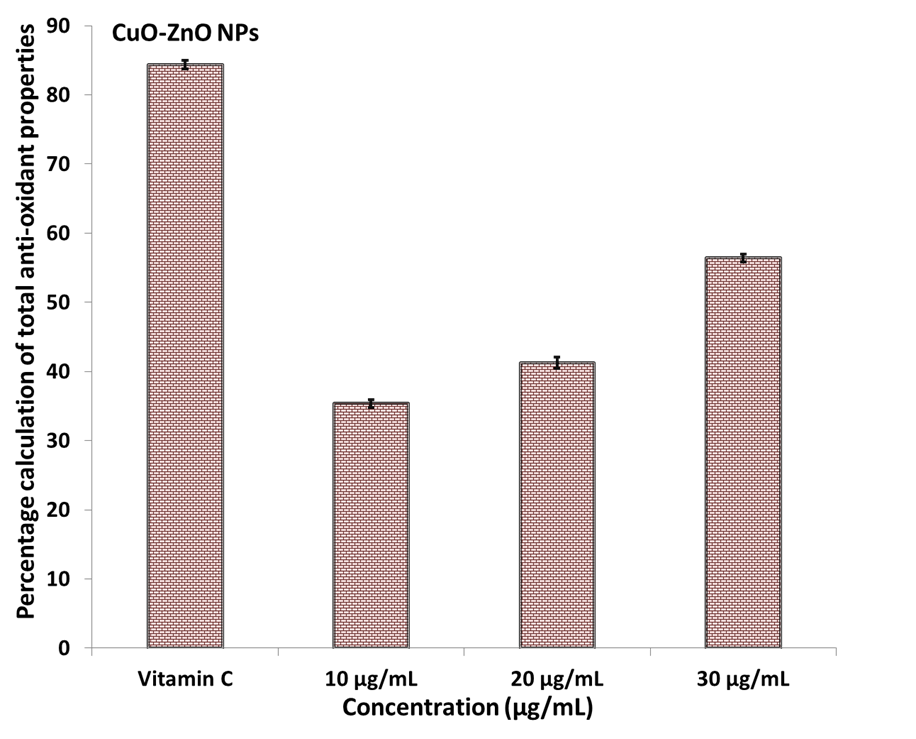
**Figure 1:** Characterization of synthesis Le-ZnO/CuO NPs from *Leucas aspera* (A): X-ray differaction (XRD) analysis, (B): Fourier transform infrared spectroscopy analysis (FT-IR), (C) High resolution transmission electron microscopy analysis (HR-TEM), and (D) Field emission scanning electron microscopy (FE-SEM).



**Figure 2:** DPPH assessment of synthesized Le-ZnO/CuO NPs with different concentration (μg/mL).

## H2O2 assay (Hydrogen Peroxide)

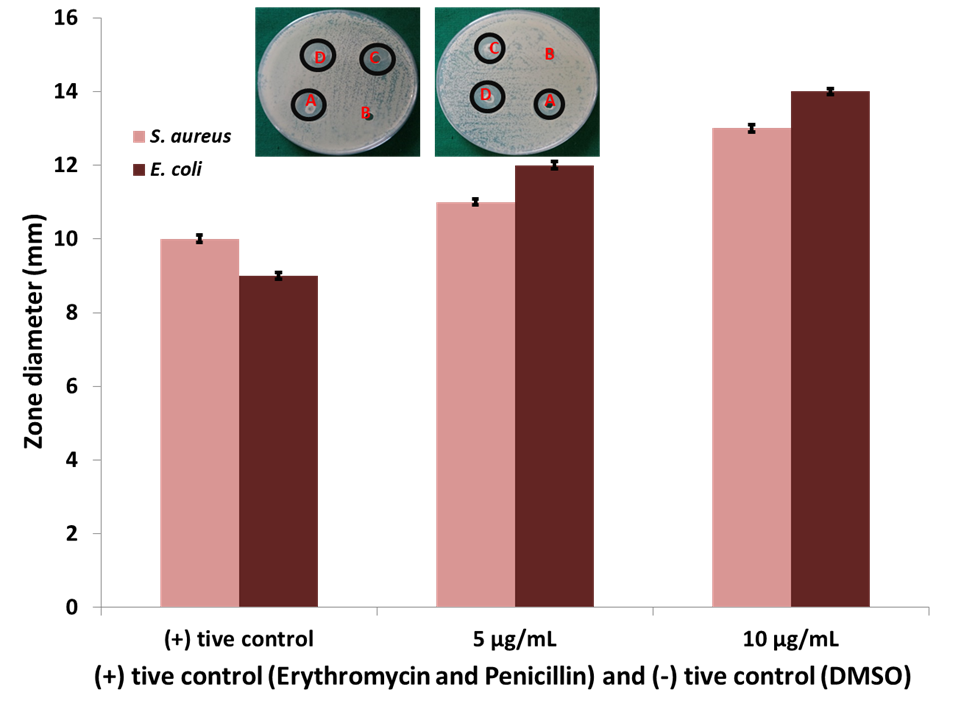
The H₂O₂ assay was used to measure the antioxidant activity of synthesized Le-ZnO/CuO NPs from Leucas aspera, as shown in Figure 3. Vitamin E served as the reference control to compare the total free radical scavenging activity. As the concentration of synthesized Le-ZnO/CuO NPs from Leucas aspera increased, the percentage of antioxidant activity also gradually increased [(Geremew et al., 2023)](https://paperpile.com/c/yclMY5/pxLO). No statistically significant difference was observed between the antioxidant activity of synthesized Le-ZnO/CuO NPs from Leucas aspera and vitamin E at different concentrations, despite the fact that the synthesized Le-ZnO/CuO NPs from Leucas aspera exhibited greater peroxidase activity than vitamin E [(Srivastava & Bhargava, 2021)](https://paperpile.com/c/yclMY5/EjHa).



**Figure 3:** Hydrogen Peroxide assessment of synthesized Le-ZnO/CuO NPs with different concentration (μg/mL).

## Anti-bacterial activity of synthesized Le-ZnO/CuO NPs NPs from *Leucas aspera* using different bacteria

Figure 4 show the antibacterial activity of synthesized Le-ZnO/CuO NPs from Leucas aspera. The standard antibiotic control consisted of 25 mg of erythromycin and 25 mg of amoxicillin mixed in a 1:1 ratio. As the concentration of synthesized Le-ZnO/CuO NPs from Leucas aspera increased, the zone of inhibition also gradually increased based on concentration [(Willander, 2014)](https://paperpile.com/c/yclMY5/v9ug).A statistically significant difference was observed for S. aureus when the zone of inhibition of the antibiotic standard was compared with a high concentration of synthesized Le-ZnO/CuO NPs from Leucas aspera. Similarly, a statistically significant difference was noted for E. coli when comparing the zones of inhibition of the antibiotic standards with low and high concentrations of synthesized Le-ZnO/CuO NPs from Leucas aspera. In comparison to the antibiotic standard, synthesized Le-ZnO/CuO NPs from Leucas aspera demonstrated notable antibacterial activity against S. aureus and E. coli [(Velsankar et al., 2022)](https://paperpile.com/c/yclMY5/9RSi) [(Pooja et al., 2019)](https://paperpile.com/c/yclMY5/T69h).

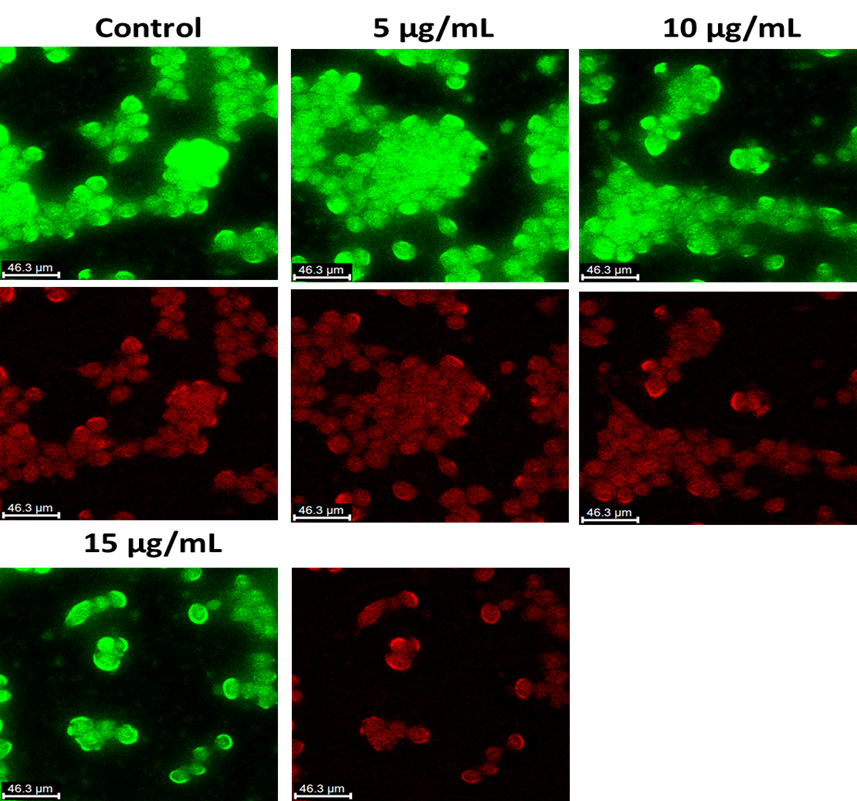


**Figure 4:** Anti-bacterial activity of synthesized Le-ZnO/CuO NPs using two different microbes and compare with standard antibiotics.

## Anticancer activity of synthesized Le-ZnO/CuO NPs from *Leucas aspera*

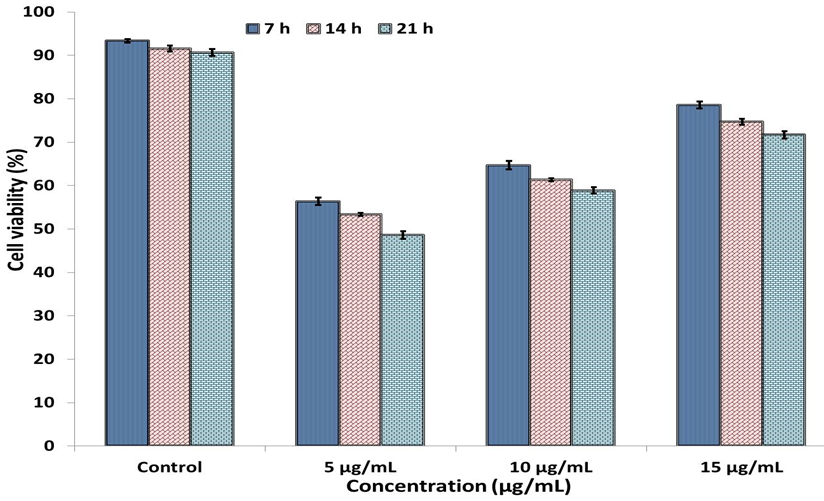
## MTT assay synthesized Le-ZnO/CuO NPs from *Leucas aspera*

The MTT assay was conducted on the MCF-7 human breast cancer cell line to assess cell survival by measuring its mitochondrial activity. Cell viability was evaluated using the colorimetric approach after the cells absorbed the MTT (3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyl tetrazolium bromide) salt, which was subsequently reduced by the mitochondrial enzyme succinate to form formazan. MCF-7 cancer cell lines exposed to synthesized Le-ZnO/CuO NPs from *Leucas aspera* exhibited mitochondrial damage or cellular death due to the presence of larger and smaller particles inside the cells, leading to increased oxidative stress and ultimately cellular apoptosis [(Boroumand Moghaddam et al., 2017)](https://paperpile.com/c/yclMY5/3Fvm).



**Figure 5:** Con-focal imaging of MCF-7 (Breast cancer cell line) after the treatment with Le-ZnO/CuO NPs.

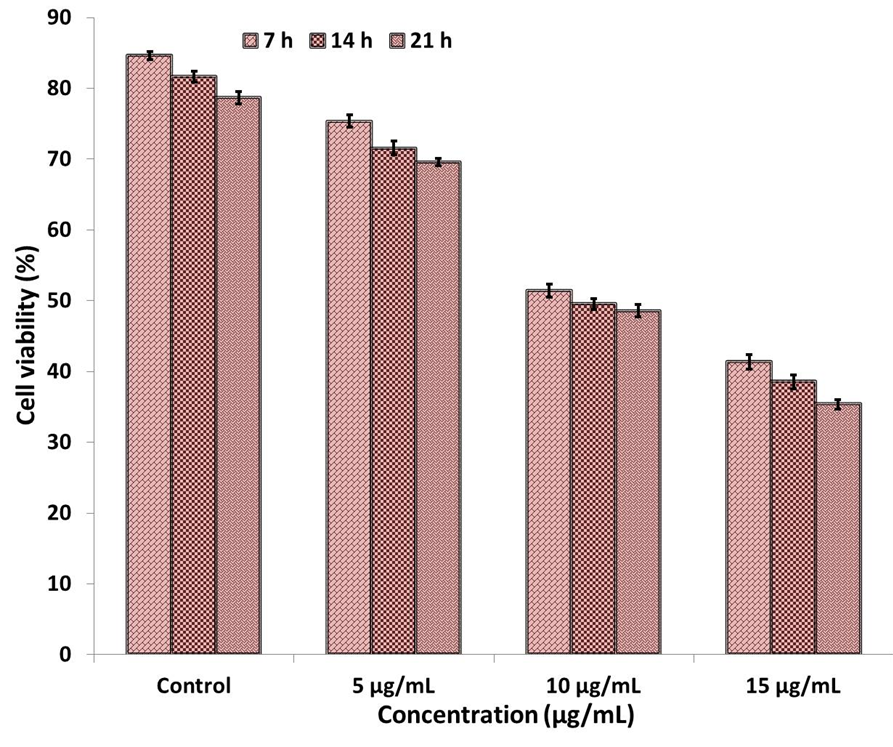
A drop in MTT of 23.35% was observed at low concentrations (5 μg/mL), whereas a reduction of up to 69.47% was noted at high concentrations (15 μg/mL) during a 24-hour period (Figure 6). According to these observations, the effect of Le-ZnO/CuO NPs appears to be dose- and time-dependent in decreasing the proportion of MTT. It was determined Le-ZnO/CuO NPs can cause cellular death in the MCF-7 breast cancer cell line [(Reed & Audisio, 2010)](https://paperpile.com/c/yclMY5/fB4i).



**Figure 6:** MTT analysis of synthesized Le-ZnO/CuO NPs with different concentration and time duration.

## XTT assay synthesized Le-ZnO/CuO NPs from *Leucas aspera*

The calorimetric approach was utilized to assess cell viability using the XTT assay. The XTT assay is an advanced test for MTT viability in which the mitochondrial enzyme transforms XTT into XTT formazan. The XTT assay was time- and dose-dependent, just like the MTT assay. A drop in XTT of 32.63% was observed at low concentrations (5 μg/mL), whereas a reduction of up to 84.74% was noted at high concentrations (15 μg/mL) during a 24-hour period (Figure 7). When Le-ZnO/CuO NPs enter the cell, they may accumulate and cause oxidative stress (the production of ROS), which could contribute to the cytotoxicity. Oxidative stress may result in loss of membrane integrity or programmed cell death, leading to apoptosis or necrosis (Figure 7) [(*Colloidal Metal Oxide Nanoparticles: Synthesis, Characterization and Applications*, 2019)](https://paperpile.com/c/yclMY5/asbI).

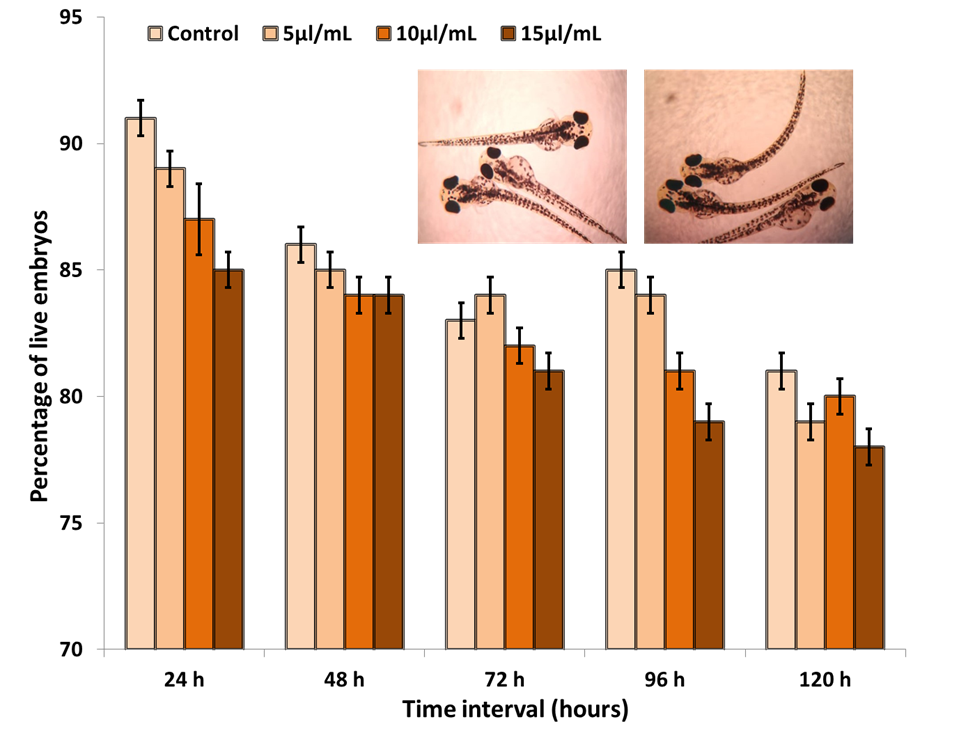


**Figure 7:** XTT analysis of synthesized Le-ZnO/CuO NPs with different concentration and time duration.

Additionally, the enhanced permeability and retention (EPR) effect allows nanoparticles to preferentially accumulate in tumor tissues, which often have leaky vasculature and poor lymphatic drainage. This selective accumulation reduces systemic toxicity and enhances drug delivery efficiency to cancerous cells [(Abdelhakim et al., 2020)](https://paperpile.com/c/yclMY5/vc9v). Nanoparticles can also be functionalized with ligands (such as antibodies, peptides, or small molecules) that bind specifically to receptors overexpressed on cancer cells, enabling targeted drug delivery and minimizing harm to healthy cells [(Shukla & Iravani, 2018)](https://paperpile.com/c/yclMY5/jRj2).

## *In-vitro* toxicity analysis of synthesized Le-ZnO/CuO NPs from *Leucas aspera* against zebra fish embryos

Studies on In-vitro toxicity were carried out using zebrafish embryos, with saline as the control. The toxicity was examined based on the viable proportion of embryos following Le-ZnO/CuO NPs treatment. The zebrafish embryos were examined under a light-field microscope at 40x magnification. The viability of the embryos treated with Le-ZnO/CuO NPs was similar to that of the control.



**Figure 8:** *In-vitro* toxicity analysis of synthesized Le-ZnO/CuO NPs against zebrafish embroys with different time interval (hpf).

As shown in Figure 8, the zebrafish embryos treated with Le-ZnO/CuO NPs were well-formed, exhibiting proper head, tail, eye, and internal organ development at the appropriate time intervals. Figure 8 shows that multiple embryos had hatched and were exhibiting different growth stages at 92 and 120 hours. No statistically significant difference was found when comparing the cytotoxicity effect of Le-ZnO/CuO NPs with the control on the viability of zebrafish embryos [(Guo et al., 2021)](https://paperpile.com/c/yclMY5/KFJV). The findings indicated that the Le-ZnO/CuO NPs were highly biocompatible and exhibited very little toxicity to the developing zebrafish embryos [(Guo et al., 2021; Han et al., 2021)](https://paperpile.com/c/yclMY5/KFJV+gmMF).

# Conclusion

In conclusion, *Leucas aspera* crude extract was used to synthesize ZnO/CuO nanoparticles (Le-ZnO/CuO NPs). The nanoparticles exhibited a dominant copper oxide crystalline phase with minute ZnO attributions by XRD analysis, showing a densely packed hexagonal arrangement was observed in HR-TEM. FTIR analysis validated the presence of Cu-O and Zn-O functional groups, while SEM images revealed that the nanoparticles are more amorphous in nature and resemble typical nanoparticle structures as observed from XRD. Le-ZnO/CuO NPs demonstrated excellent antioxidant and antimicrobial activity, which varied based on reaction time and dose. These nanoparticles can penetrate microbial cells and interact with intracellular components such as proteins and DNA. This interaction may lead to alterations in protein function, inhibition of DNA replication, or DNA damage (e.g., double-strand breaks), ultimately resulting in cell death. Notably, despite their small size, Le-ZnO/CuO NPs did not affect the mortality of zebrafish embryos, suggesting minimal toxicity in non-target organisms. The anticancer efficacy of Le-ZnO/CuO NPs was tested on the MCF-7 breast cancer cell line. Cell viability was assessed using MTT and XTT assays, both of which showed a dose and time dependent reduction in cell viability, confirming that the cytotoxicity was due to DNA damage. The increased generation of ROS within the cancer cells, demonstrated by oxidative stress parameters, further supports this. Cancer cells, with their altered metabolism, are more susceptible to oxidative stress, and ROS can induce DNA damage, disrupt cellular structures, and trigger apoptosis (programmed cell death). Overall, Le-ZnO/CuO NPs exhibit promising antimicrobial and anticancer properties, with their ability to generate ROS, induce DNA damage, and selectively accumulate in tumor tissues. These novel nanoparticles was not explicates significant crystalline pattern; however the biological activity was noticeable. This material can serve as an effective agent for biomedical applications targeting both cancer cells and microbes.

# References

1. [Abdelhakim, H. K., El-Sayed, E. R., & Rashidi, F. B. (2020). Biosynthesis of zinc oxide nanoparticles with antimicrobial, anticancer, antioxidant and photocatalytic activities by the endophytic Alternaria tenuissima. *Journal of Applied Microbiology*, *128*(6), 1634–1646.](http://paperpile.com/b/yclMY5/vc9v)
2. [Adel, S. M., El-Harouni, N., & Vaid, N. R. (2023). White Spot lesions: State of the art biomaterials and workflows used in prevention, progression and treatment. *Seminars in Orthodontics*. https://doi.org/](http://paperpile.com/b/yclMY5/UkZo6)[10.1053/j.sodo.2023.01.002](http://dx.doi.org/10.1053/j.sodo.2023.01.002)
3. [Antonio-Pérez, A., Durán-Armenta, L. F., Pérez-Loredo, M. G., & Torres-Huerta, A. L. (2023). Biosynthesis of Copper Nanoparticles with Medicinal Plants Extracts: From Extraction Methods to Applications. *Micromachines*, *14*(10). https://doi.org/](http://paperpile.com/b/yclMY5/dtV3)[10.3390/mi14101882](http://dx.doi.org/10.3390/mi14101882)
4. [Aparna, J., Maiti, S., & Jessy, P. (2021). Polyether ether ketone - As an alternative biomaterial for Metal Richmond crown-3-dimensional finite element analysis. *Journal of Conservative Dentistry : JCD*, *24*(6), 553–557.](http://paperpile.com/b/yclMY5/wN0vk)
5. [Babapour, H., Jalali, H., & Mohammadi Nafchi, A. (2021). The synergistic effects of zinc oxide nanoparticles and fennel essential oil on physicochemical, mechanical, and antibacterial properties of potato starch films. *Food Science & Nutrition*, *9*(7), 3893–3905.](http://paperpile.com/b/yclMY5/eWip)
6. [Berne, B. J., Ciccotti, G., & Coker, D. F. (1998). *Classical And Quantum Dynamics In Condensed Phase Simulations: Proceedings Of The International School Of Physics*. World Scientific.](http://paperpile.com/b/yclMY5/lnnU)
7. [Boroumand Moghaddam, A., Moniri, M., Azizi, S., Abdul Rahim, R., Bin Ariff, A., Navaderi, M., & Mohamad, R. (2017). Eco-Friendly Formulated Zinc Oxide Nanoparticles: Induction of Cell Cycle Arrest and Apoptosis in the MCF-7 Cancer Cell Line. *Genes*, *8*(10). https://doi.org/](http://paperpile.com/b/yclMY5/3Fvm)[10.3390/genes8100281](http://dx.doi.org/10.3390/genes8100281)
8. [Cao, H. (2017). *Silver Nanoparticles for Antibacterial Devices: Biocompatibility and Toxicity*. CRC Press.](http://paperpile.com/b/yclMY5/iDpx)
9. [Chokkattu, J. J., Mary, D. J., Shanmugam, R., & Neeharika, S. (2022). Embryonic Toxicology Evaluation of Ginger- and Clove-mediated Titanium Oxide Nanoparticles-based Dental Varnish with Zebrafish. *The Journal of Contemporary Dental Practice*, *23*(11), 1157–1162.](http://paperpile.com/b/yclMY5/scfrq)
10. [Chokkattu, J. J., Neeharika, S., & Rameshkrishnan, M. (2023). Applications of Nanomaterials in Dentistry: A Review. *Journal of International Society of Preventive & Community Dentistry*, *13*(1), 32–41.](http://paperpile.com/b/yclMY5/C6E7Q)
11. [*Colloidal Metal Oxide Nanoparticles: Synthesis, Characterization and Applications*. (2019). Elsevier.](http://paperpile.com/b/yclMY5/asbI)
12. [Crini, G., & Lichtfouse, E. (2019). *Sustainable Agriculture Reviews 35: Chitin and Chitosan: History, Fundamentals and Innovations*. Springer.](http://paperpile.com/b/yclMY5/lmQ0)
13. [Dehghani, F., Mosleh-Shirazi, S., Shafiee, M., Kasaee, S. R., & Amani, A. M. (2023). Antiviral and antioxidant properties of green synthesized gold nanoparticles using leaf extract. *Applied Nanoscience*, *13*(6), 4395–4405.](http://paperpile.com/b/yclMY5/fwgA)
14. [Di, X., Fu, Y., Huang, Q., Xu, Y., Zheng, S., & Sun, Y. (2023). Comparative effects of copper nanoparticles and copper oxide nanoparticles on physiological characteristics and mineral element accumulation in Brassica chinensis L. *Plant Physiology and Biochemistry : PPB*, *196*, 974–981.](http://paperpile.com/b/yclMY5/5Ez4)
15. [Dorjee, L., Gogoi, R., Kamil, D., Kumar, R., Mondal, T. K., Pattanayak, S., & Gurung, B. (2023). Essential oil-grafted copper nanoparticles as a potential next-generation fungicide for holistic disease management in maize. *Frontiers in Microbiology*, *14*, 1204512.](http://paperpile.com/b/yclMY5/L4tz)
16. [Egamberdieva, D., Shrivastava, S., & Varma, A. (2015). *Plant-Growth-Promoting Rhizobacteria (PGPR) and Medicinal Plants*. Springer.](http://paperpile.com/b/yclMY5/Ymnw)
17. [Fedlheim, D. L., & Foss, C. A. (2001). *Metal Nanoparticles: Synthesis, Characterization, and Applications*. CRC Press.](http://paperpile.com/b/yclMY5/N8qd)
18. [Ganapathy, D., (2021). Awareness of hazards caused by long-term usage of polyethylene terephthalate (PET) bottles. *International Journal of Dentistry and Oral Science*, 2976–2980.](http://paperpile.com/b/yclMY5/LgVhp)
19. [Geremew, A., Carson, L., Woldesenbet, S., Wang, H., Reeves, S., Brooks, N., Jr, Saganti, P., Weerasooriya, A., & Peace, E. (2023). Effect of zinc oxide nanoparticles synthesized from leaf extract on growth and antioxidant properties of mustard (). *Frontiers in Plant Science*, *14*, 1108186.](http://paperpile.com/b/yclMY5/pxLO)
20. [Ghani, A. (1998). *Medicinal Plants of Bangladesh: Chemical Constituents and Uses*.](http://paperpile.com/b/yclMY5/fsap)
21. [Guo, X., Zhang, S., Liu, X., Lu, S., Wu, Q., & Xie, P. (2021). Evaluation of the acute toxicity and neurodevelopmental inhibition of perfluorohexanoic acid (PFHxA) in zebrafish embryos. *Ecotoxicology and Environmental Safety*, *225*, 112733.](http://paperpile.com/b/yclMY5/KFJV)
22. [Han, Y., Ma, Y., Yao, S., Zhang, J., & Hu, C. (2021). In vivo and in silico evaluations of survival and cardiac developmental toxicity of quinolone antibiotics in zebrafish embryos (Danio rerio). *Environmental Pollution (Barking, Essex : 1987)*, *277*, 116779.](http://paperpile.com/b/yclMY5/gmMF)
23. [Jain, R. K., & Verma, P. (2022). Visual assessment of extent of White Spot lesions in subjects treated with fixed orthodontic appliances: A retrospective study. *World Journal of Dentistry*, *13*(3), 245–249.](http://paperpile.com/b/yclMY5/c3jAB)
24. [Kalpana, V. N., & Devi Rajeswari, V. (2018). A Review on Green Synthesis, Biomedical Applications, and Toxicity Studies of ZnO NPs. *Bioinorganic Chemistry and Applications*, *2018*, 3569758.](http://paperpile.com/b/yclMY5/rLA7)
25. [Karthik, L., Vishnu Kirthi, A., Ranjan, S., & Mohana Srinivasan, V. (2019). *Biological Synthesis of Nanoparticles and Their Applications*. CRC Press.](http://paperpile.com/b/yclMY5/gWp9)
26. [Khare, C. P. (2004). *Encyclopedia of Indian Medicinal Plants: Rational Western Therapy, Ayurvedic and Other Traditional Usage, Botany*.](http://paperpile.com/b/yclMY5/0UH2)
27. [Kitala, K., Tanski, D., Godlewski, J., Krajewska-Włodarczyk, M., Gromadziński, L., & Majewski, M. (2023). Copper and Zinc Particles as Regulators of Cardiovascular System Function-A Review. *Nutrients*, *15*(13). https://doi.org/](http://paperpile.com/b/yclMY5/7Z0O)[10.3390/nu15133040](http://dx.doi.org/10.3390/nu15133040)
28. [Laghari, I. A., Pandey, A. K., Samykano, M., Aljafari, B., Kadirgama, K., Sharma, K., & Tyagi, V. V. (2023). Thermal energy harvesting of highly conductive graphene-enhanced paraffin phase change material. *Journal of Thermal Analysis and Calorimetry*, *148*(18), 9391–9402.](http://paperpile.com/b/yclMY5/ckxtT)
29. [Machado, S., González-Ballesteros, N., Gonçalves, A., Magalhães, L., Sárria Pereira de Passos, M., Rodríguez-Argüelles, M. C., & Castro Gomes, A. (2021). Toxicity in vitro and in Zebrafish Embryonic Development of Gold Nanoparticles Biosynthesized Using Macroalgae Extracts. *International Journal of Nanomedicine*, *16*, 5017–5036.](http://paperpile.com/b/yclMY5/A8rs)
30. [Mahawar, L., Živčák, M., Barboricova, M., Kovár, M., Filaček, A., Ferencova, J., Vysoká, D. M., & Brestič, M. (2024). Effect of copper oxide and zinc oxide nanoparticles on photosynthesis and physiology of Raphanus sativus L. under salinity stress. *Plant Physiology and Biochemistry : PPB*, *206*, 108281.](http://paperpile.com/b/yclMY5/Dlzy)
31. [Markert, B., Fränzle, S., & Wünschmann, S. (2015). *Chemical Evolution: The Biological System of the Elements*. Springer.](http://paperpile.com/b/yclMY5/y867)
32. [Marya, A., Venugopal, A., Karobari, M. I., & Rokaya, D. (2022). White Spot lesions: A serious but often ignored complication of orthodontic treatment. *The Open Dentistry Journal*, *16*(1). https://doi.org/](http://paperpile.com/b/yclMY5/Es89a)[10.2174/18742106-v16-e2202230](http://dx.doi.org/10.2174/18742106-v16-e2202230)
33. [Merchant, A., Ganapathy, D. M., & Maiti, S. (2022). Effectiveness of local and topical anesthesia during gingival retraction. *Brazilian Dental Science*, *25*(1), e2591.](http://paperpile.com/b/yclMY5/lmpYF)
34. [Muthuswamy Pandian, S., Subramanian, A. K., Ravikumar, P. A., & Adel, S. M. (2022). Biomaterial testing in contemporary orthodontics: Scope, protocol and testing apparatus. *Seminars in Orthodontics*. https://doi.org/](http://paperpile.com/b/yclMY5/8ZPb)[10.1053/j.sodo.2022.12.011](http://dx.doi.org/10.1053/j.sodo.2022.12.011)
35. [Nahhas, D. A. (2019). *Zinc Oxide Based Nano Materials and Devices*. BoD – Books on Demand.](http://paperpile.com/b/yclMY5/KZAw)
36. [Nekoukhou, M., Fallah, S., Pokhrel, L. R., Abbasi-Surki, A., & Rostamnejadi, A. (2024). Foliar co-application of zinc oxide and copper oxide nanoparticles promotes phytochemicals and essential oil production in dragonhead (Dracocephalum moldavica). *The Science of the Total Environment*, *906*, 167519.](http://paperpile.com/b/yclMY5/Ru16)
37. [Niederberger, M., & Pinna, N. (2009). *Metal Oxide Nanoparticles in Organic Solvents: Synthesis, Formation, Assembly and Application*. Springer Science & Business Media.](http://paperpile.com/b/yclMY5/lT7x)
38. [Ozturk, M., & Hakeem, K. R. (2018). *Plant and Human Health, Volume 1: Ethnobotany and Physiology*. Springer.](http://paperpile.com/b/yclMY5/e5tk)
39. [Pandiyan, I., Sri, S. D., Indiran, M. A., Rathinavelu, P. K., Prabakar, J., & Rajeshkumar, S. (2022). Antioxidant, anti-inflammatory activity of -mediated selenium nanoparticles: An study. *Journal of Conservative Dentistry : JCD*, *25*(3), 241–245.](http://paperpile.com/b/yclMY5/whXrn)
40. [Pooja, D., Kumar, P., Singh, P., & Patil, S. (2019). *Sensors in Water Pollutants Monitoring: Role of Material*. Springer Nature.](http://paperpile.com/b/yclMY5/T69h)
41. [Poornima, P., Krithikadatta, J., Ponraj, R. R., Velmurugan, N., & Kishen, A. (2021). Biofilm formation following chitosan-based varnish or chlorhexidine-fluoride varnish application in patients undergoing fixed orthodontic treatment: a double blinded randomised controlled trial. *BMC Oral Health*, *21*(1), 465.](http://paperpile.com/b/yclMY5/3bGu0)
42. [Pourchez, & Laurence. (2017). *Women’s knowledge: traditional medicine and nature; Mauritius, Reunion and Rodrigues*. UNESCO Publishing.](http://paperpile.com/b/yclMY5/9Ens)
43. Rafi, D. M., Lakshmi, T. V., Shirley, C. P., Ravivarman, G., & Senthilkumar, G. (2024, April). Improving Prostate Cancer Diagnosis with Weakly Supervised Learning and Radiology-Confirmed Negative MRI Data. In 2024 International Conference on Inventive Computation Technologies (ICICT) (pp. 1183-1188). IEEE.
44. [Raja, F. N. S., Worthington, T., & Martin, R. A. (2023). The antimicrobial efficacy of copper, cobalt, zinc and silver nanoparticles: alone and in combination. *Biomedical Materials (Bristol, England)*, *18*(4). https://doi.org/](http://paperpile.com/b/yclMY5/sWp9)[10.1088/1748-605X/acd03f](http://dx.doi.org/10.1088/1748-605X/acd03f)
45. [Ramakrishnan, M., Shanmugam, R., Neeharika, S., Selvaraj, S., Chokkattu, J. J., & Thangavelu, L. (2023). Anti-inflammatory potential of a mouthwash formulated using clove and ginger mediated by zinc oxide nanoparticles: An in vitro study. *World Journal of Dentistry*, *14*(5), 394–401.](http://paperpile.com/b/yclMY5/gIRgX)
46. [Ramamurthy, S., Thiagarajan, K., Varghese, S., Kumar, R., Karthick, B. P., Varadarajan, S., & Balaji, T. M. (2022). Assessing the in vitro antioxidant and anti-inflammatory activity of Moringa oleifera crude extract. *The Journal of Contemporary Dental Practice*, *23*(4), 437–442.](http://paperpile.com/b/yclMY5/RiSFw)
47. [Reed, M. W., & Audisio, R. A. (2010). *Management of Breast Cancer in Older Women*. Springer Science & Business Media.](http://paperpile.com/b/yclMY5/fB4i)
48. [Shukla, A. K., & Iravani, S. (2018). *Green Synthesis, Characterization and Applications of Nanoparticles*. Elsevier.](http://paperpile.com/b/yclMY5/jRj2)
49. [Sivalingam, A. M., Pandian, A., Rengarajan, S., Boopathy, N., & Selvaraj, K. R. N. (2024). A comparative study of in vivo toxicity in zebrafish embryos synthesized CuO nanoparticles characterized from Salacia reticulata. *Environmental Geochemistry and Health*, *46*(9), 311.](http://paperpile.com/b/yclMY5/GTe8)
50. [Solanki, L. A., Dinesh, S. P. S., Jain, R. K., & Balasubramaniam, A. (2023). Effects of titanium oxide coating on the antimicrobial properties, surface characteristics, and cytotoxicity of orthodontic brackets - A systematic review and meta analysis of in-vitro studies. *Journal of Oral Biology and Craniofacial Research*, *13*(5), 553–562.](http://paperpile.com/b/yclMY5/WXder)
51. [Sreevarun, M., Ajay, R., Suganya, G., Rakshagan, V., Bhanuchander, V., & Suma, K. (2023). Formulation, Configuration, and Physical Properties of Dental Composite Resin Containing a Novel 2π + 2π Photodimerized Crosslinker - Cinnamyl Methacrylate: An Research. *The Journal of Contemporary Dental Practice*, *24*(6), 364–371.](http://paperpile.com/b/yclMY5/DrZNH)
52. [Srivastava, S., & Bhargava, A. (2021). *Green Nanoparticles: The Future of Nanobiotechnology*. Springer Nature.](http://paperpile.com/b/yclMY5/EjHa)
53. [Subramanian, A., & Harikrishnan, S. (2023). 3D printing in orthodontics: A narrative review. *Journal of International Oral Health: JIOH*, *15*(1), 15.](http://paperpile.com/b/yclMY5/dYjkW)
54. Tuluwengjiang, G., Rasulova, I., Ahmed, S., Kiasari, B. A., Sârbu, I., Ciongradi, C. I., & Samaniego, S. S. C. (2024). Dendritic cell-derived exosomes (Dex): Underlying the role of exosomes derived from diverse DC subtypes in cancer pathogenesis. Pathology-Research and Practice, 254, 155097.
55. [Ullah, S., Khalid, R., Rehman, M. F., Irfan, M. I., Abbas, A., Alhoshani, A., Anwar, F., & Amin, H. M. A. (2023). Biosynthesis of phyto-functionalized silver nanoparticles using olive fruit extract and evaluation of their antibacterial and antioxidant properties. *Frontiers in Chemistry*, *11*, 1202252.](http://paperpile.com/b/yclMY5/YCUt)
56. [Velsankar, K., Parvathy, G., Mohandoss, S., & Sudhahar, S. (2022). Effect of green synthesized ZnO nanoparticles using Paspalum scrobiculatum grains extract in biological applications. *Microscopy Research and Technique*, *85*(9), 3069–3094.](http://paperpile.com/b/yclMY5/9RSi)
57. [Venezia, V., Pota, G., Argenziano, R., Alfieri, M. L., Moccia, F., Ferrara, F., Pecorelli, A., Esposito, R., Di Girolamo, R., D’Errico, G., Valacchi, G., Luciani, G., Panzella, L., & Napolitano, A. (2024). Design of a hybrid nanoscaled skin photoprotector by boosting the antioxidant properties of food waste-derived lignin through molecular combination with TiO nanoparticles. *International Journal of Biological Macromolecules*, *280*(Pt 3), 135946.](http://paperpile.com/b/yclMY5/9TKw)
58. [Verma, P., & Muthuswamy Pandian, S. (2021). Bionic effects of nano hydroxyapatite dentifrice on demineralised surface of enamel post orthodontic debonding: in-vivo split mouth study. *Progress in Orthodontics*, *22*(1), 39.](http://paperpile.com/b/yclMY5/S3KHj)
59. [Wadhwani, V., Sivaswamy, V., & Rajaraman, V. (2022). Surface roughness and marginal adaptation of stereolithography versus digital light processing three-dimensional printed resins: An study. *Journal of Indian Prosthodontic Society*, *22*(4), 377–381.](http://paperpile.com/b/yclMY5/MXNwP)
60. [Willander, M. (2014). *Zinc Oxide Nanostructures: Advances and Applications*. CRC Press.](http://paperpile.com/b/yclMY5/v9ug)
61. [Yılmaz, H. (2023). *Recent Progress in Pharmaceutical Nanobiotechnology: A Medical Perspective*. Bentham Science Publishers.](http://paperpile.com/b/yclMY5/x0MO)