



Al-Qadisiyah Journal of Pure Science

Al-Qadisiyah Journal of Pure Science

ISSN(Printed): 1997-2490 ISSN(Online): 2411-3514

DOI: 10.29350/jops



## Biosynthesis of Zinc Oxid nanoparticles by truffles and their antimicrobial and anticancer effects

Farqad hasan falih AL-daemi 1,2 , Majid Kadhim Al-Shibly 2 , and Rana A. Ghaleb 3 1Al-Toosi University College. 2 Al-Qadisiya University - Faculty of Education 3 Department of Human Anatomy/College of medicine/University of Babylon-Iraq

### Abstract

Biosynthesis method of NPs is considered one of the most promising methods, aimed to biosynthesize ZnO NPs using edible truffles *Terfezia claveryi* and evaluate their antimicrobial activity by using the optimum concentration from among three different concentrations (8,10,13)  $\mu\text{g/ml}$  of ZnO NPs, as well as evaluation of their effectiveness as an antioxidant and lanti-degradation of red blood cells, The study was conducted during from November 11, 2020 to October 22, 2021 in the Research Laboratory of the College of Education, University of Al-Qadisiyah and the Al-Ameen Scientific Laboratory for Scientific Research , ZnO NPs were initially characterized by UV spectroscopy, ( AFM), (SEM),to detect the size, shape and distribution of nanoparticles, as the shape of the ZnO NPs manufactured by *Terfezia claveriy* , ZnO NPs was tested towards the isolates of Gram-positive bacteria (*Staphylococcus aureus*, *Enterococcus faecalis*) and Gram-negative bacteria (*Escherich coli*, *Klebsiella pneumonia* and isolate of *Candida albicans*). ZnONPs showed the anti-bacterial activity, as the largest inhibition diameter of ZnO NPs 23 mm. 21mm in *Escherichia coli* and *Klebsiella pneumonia* bacteria at a concentration of 13  $\mu\text{g} / \text{ml}$  respectively,. While there was no effect on the *Candida* yeast, and some antibiotics were tested to show their ability to inhibit pathogenic isolates, nanoparticles were used in synergy with three antibiotics that are considered the most inhibiting (Levofloxacin, Ciprofloxacin Amikacin), larger diameters were recorded when ZnO NPs was mixed with antibiotics. and its effectiveness as anti-cancer agents was evaluated using a series of nanoscale concentrations of ZnO NPs using the breast cancer cell line (MCF7) at 100  $\mu\text{g/ml}$  gave a more toxic effect towards the (MCF7) . While there was no significant effect on the orientation of normal breast cells.

### Introduction

*Terfeziy sp.* and *Tirmania sp.* are the most common desert truffle genera in the Middle East. Because of their proteins, carbs, lipids, fibres, and low energy content, desert truffles have nutritional value (Owaid, 2022), zinc nanoparticles are all distinct physicochemical features. Antibacterial activity of ZnO NPs has been observed against a variety of pathogens, including *Escherichia coli*, *Staph aureus*, *Pseudomonas aeruginosa*, (da Silva *et al.*, 2019). And antibacterial activity against pathogenic microbes without causing antibiotic resistance (Sánchez-López *et al.*, 2020). ZnO NPs possesses physical, chemical, and morphological features, as well as an effective and logical role as an antibacterial, anticancer, and cytotoxic agent. The aim of this study was to look into the biological impacts of ZnO NPs made from truffle extract, Study of some applications of Ag and ZnO NPs such as : antimicrobial (Synergistically with antibiotics) , Evaluation of the cytotoxic activity of ZnOnPs on mcf 7 breast cancer, and the trend of normal breast cells using MTT assay.

## Materials and methods.

### Fungal Supernatant Preparation

As a first step (15g) of truffle powder was taken and mixed with 150 ml of distilled water and heated stirrer for 30 to 50 minutes at a temperature of 45 C° and filtered through pieces of medical gauze, then filtered through Whitman No.1 filter paper (pore size 25 µm). The filtrate Centrifuge at 4000 rpm for 10 minutes, , The supernatant (115 ml) was taken into a clean 250 ml volume conical flask and stored at 7 C°; (Al-Askar *et al.*, 2013).

Purification of biosynthesized of ZnO nanoparticles After preparing the fungal supernatant as mentioned in the paragraph 3.2.4, 1mM Zinc acetate [Zn(O<sub>2</sub>CCH<sub>3</sub>)<sub>2</sub>(H<sub>2</sub>O)<sub>2</sub>] was dissolved in 45 mL deionized water and kept in a stirrer for 1 hour. After 20 min, 25 mL of NaOH solution (2 g NaoH in 50mL deionized water) was slowly added to zinc acetate, and then the two solutions were slowly added to 130 mL of truffle aqueous extract (Figure 1). The mixture was then incubated in the vibratory incubator at 180 rpm, 28 C° for 48 h for ZnO nanoparticle synthesis, the color change was observed visually and images were taken (Jamdagni *et al.*, 2018).

### UV-Visible spectroscopy

the color change from yellow to dark brown color of the mixture was observed with the silver nitrate solution (Rai *et al.*, 2013) .

### Scanning electron microscope (SEM)

(SEM) was used to characterize the morphology and size of npNPs . Where powdered nanoparticles were sent to make dilutions on them with different concentrations (Biao *et al.*, 2017)

### Atomic force microscope(AFM)

technique that facilitates simultaneous mapping of surface morphology and mechanical parameters, such as adhesion and deformation (Moosburger-Will *et al.*, 2012)

### Collection of clinical isolates.

Five isolates were obtained divided into four bacterial isolates: two of them were Gram-negative bacteria (*K. pneumonia*, *E. coli.*), and two Gram-positive bacteria (*S.aureus*, *E. faecalis*) and one isolate of *C. albicans*,

### Combination of antibiotics with ZnO-NPs.

The Combination between ZnO-NPs 13mg/ml (30µl) and antibiotics against bacterial isolates were done by disc diffusion method (Birla *et al.*, 2009).

### Maintenance of cell cultures .

MCF-7 and HBL cells supplemented with 10% Fetal bovine serum, 100 unit's/mL penicillin, and 100 µg/mL streptomycin. (Khashan *et al.*, 2020).

MTT Assay: the MTT assay use is to determine the cytotoxicity. (MTT dye) into dark purple formazan crystals by NADH which can be solubilized for homogenous measurement. Thus, detected by measuring formazan concentration reflected in optical density(absorbance) using

a plate reader at 570 nm. The darker the solution, the greater the number of viable and metabolically active cells (Meerlo *et al.*, 2011).

## Result and Discussion

In general, the biosynthetic pathway of ZnONPs is a simple procedure in which a zinc salt, such as zinc nitrate or zinc acetate, is added to a previously produced biological extract. following the reaction, the solution is subjected to a heat treatments, yielding ZnO particles.(Król *et al.*, 2017; Mirzaei & Darroudi, 2017). ZnO NPs has attracted a lot of attention given to the visual characteristics of excellent. The first test for nanoparticle synthesis is visible color change. The production of ZnONP using truffle extract is shown in Figure 2. Color change The NP structure of ZnO goes from medium white to pale walnut to pale yellow.

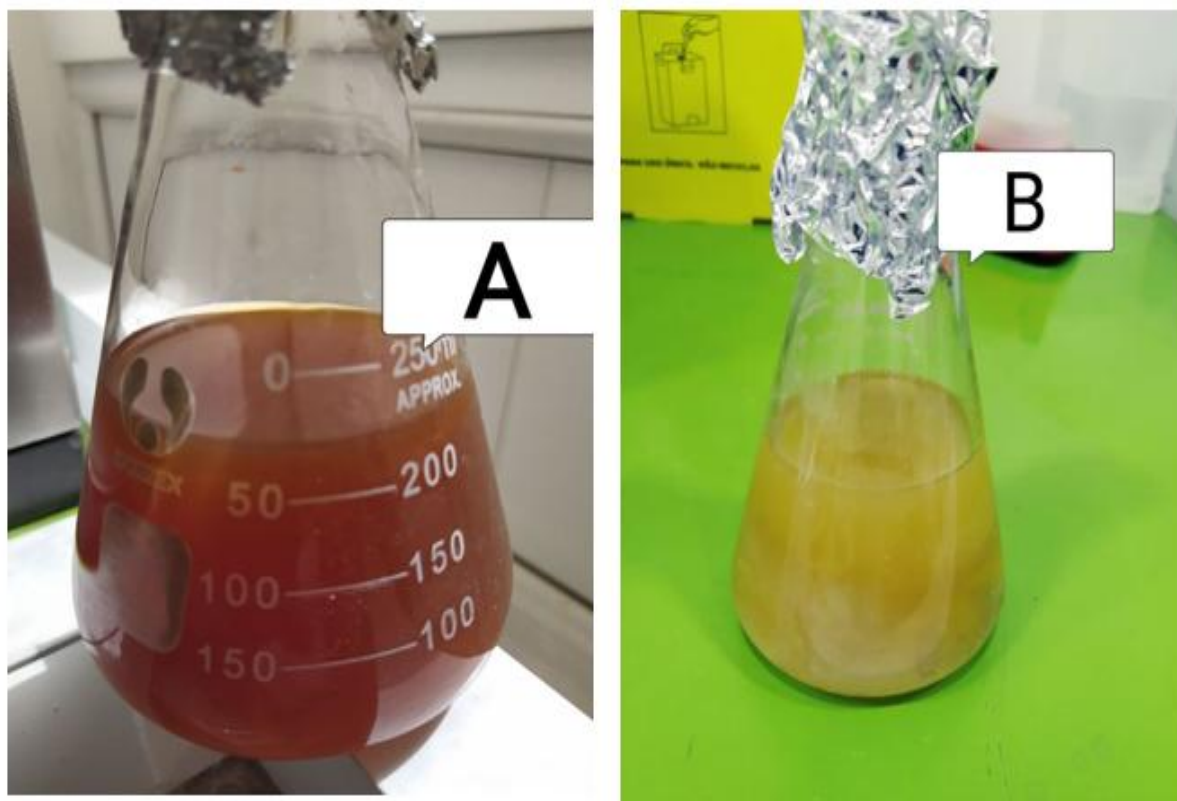


Figure (2): The change color caused by adding zinc acetate to truffles *T. claveryi* ) truffle extract at 28C°, centrifuged at 10000 rpm, and incubator rocking at 160 rpm; (a) - before (b) after reaction time 72 hours.

According to the research, Yan *et al.* (2017) *T. claveryi* contain a high concentration of bioactive substances such as , protein, ascorbic acid, ergosterol, phenolic, flavonoids, terpenoids,

phytosterol, and polysaccharides. Truffle, in particular, have an abundance of flavonoids, which are valued as a significant secondary metabolite of natural goods due to their various biological characteristics comprising (Gil-Ramírez *et al.*, 2016).

UV-Vis spectroscopy The production of ZnO NPs was confirmed using UV-visible spectroscopy. Due to the effect of surface plasmon resonance (SPR) and the interaction of chemical compounds in the *T. claveryi*, the conducting electrons begin to vibrate at a certain wavelength range. (Fig.3) after 24 hours, represents the UV-visible spectra of *T. claveryi*-prepared ZnO NPs The signal at 360 nm confirms the presence of ZnO NPs in the reaction mixture.

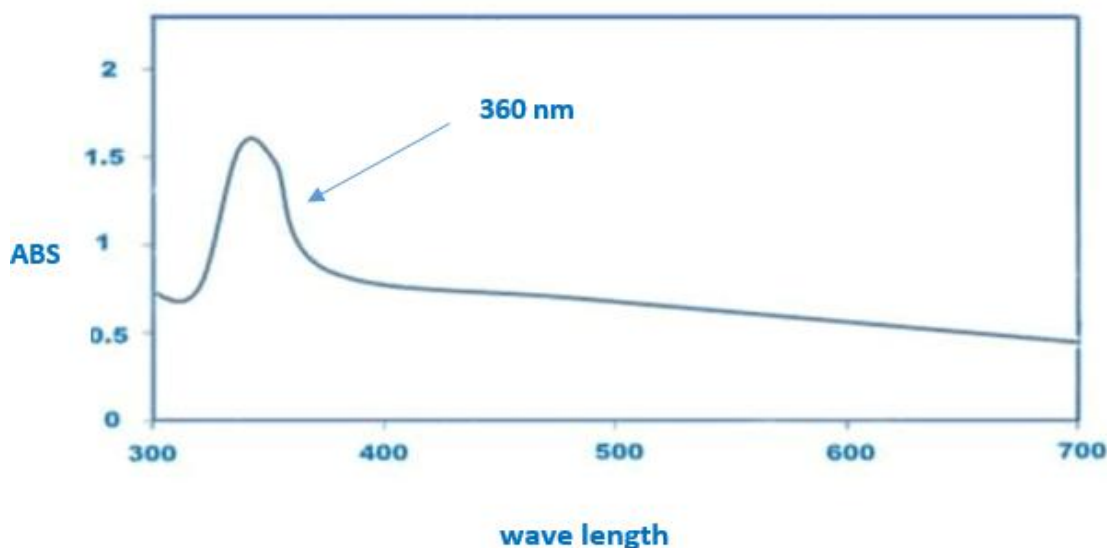


Figure (3): UV-visible spectrophotometry was used to estimate the maximum absorption range of the generated ZnONPs. A strong resonance for *T. claveryi*-derived ZnO NPs nanoparticles could be seen at 360 nm.

nanoparticles Rabeea *et al.* (2021). Concluded that the color change in ZnO NPS that synthesized by extracted natural organic compounds is due to those organic compounds where the UV absorption level was recorded at 3.650 nm. Tomaev *et al.* (2019). Who mentioned the production of ZnNPs in the range of 200-400.

SEM (Scanning Electron Microscope) ZNO NPs give different shapes of nanoparticles by ESM, For the purpose of examining the shape and size of the synthesized nanoparticles, images were taken at different magnifications, *T. claveryi* were used to make nanoparticles. and their diameters range from 20.35nm to 100.44 nm, as shown in Fig 4.

The production of nanoparticles in their crystalline to prism form is confirmed by their surface morphology.

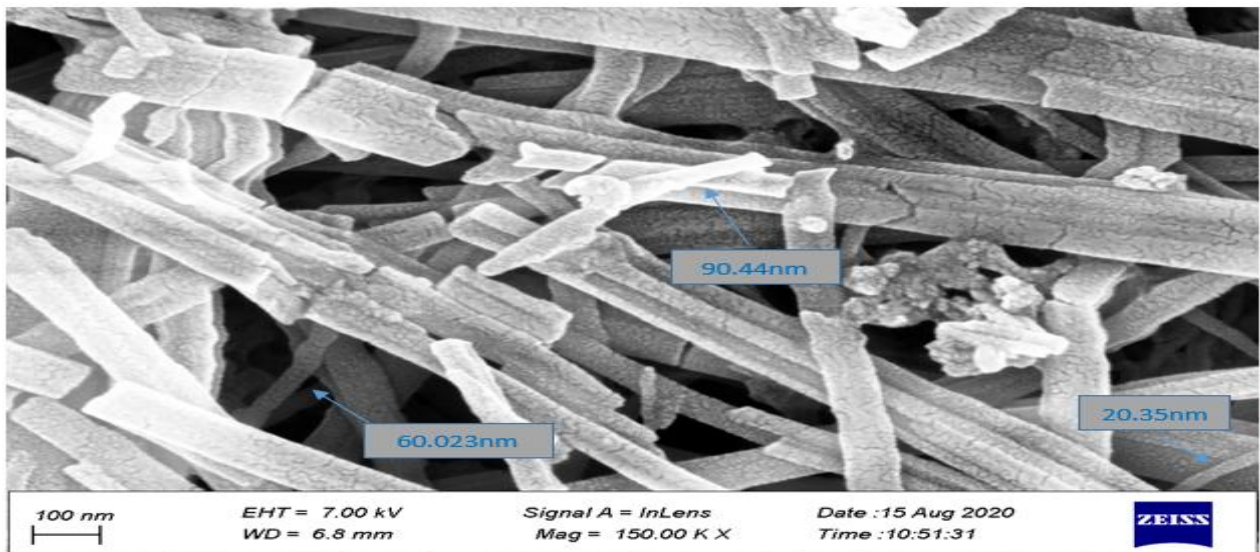
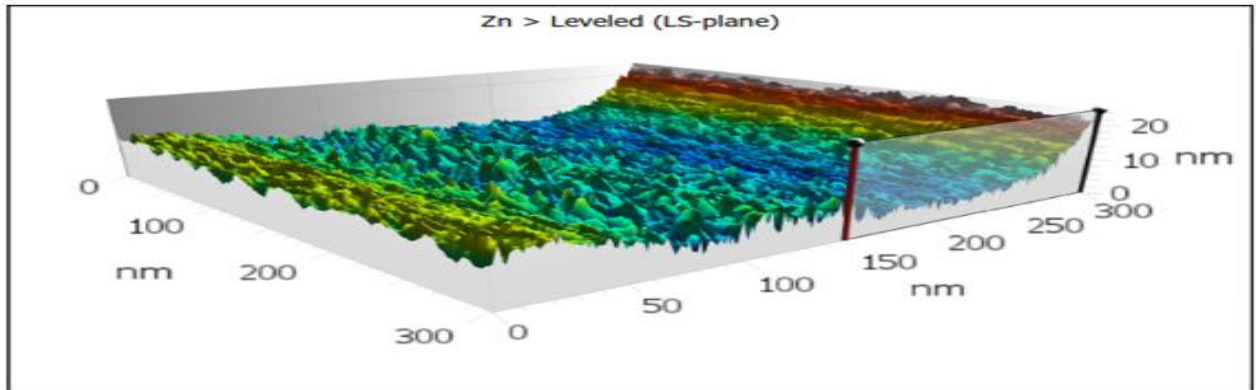


Figure (4): Electron microscopy image of ZnO NPs produced from *T. claveryi*. Magnification force is 150.00 k x.

It is obvious that the nanoparticles are more dependent on the zinc acetate concentration in general. The particles are mostly crystalline, according to the results. Bing *et al.* the effect of surface shape and interaction on the synergistic activity of ZnO in several. (Ovais *et al.*, 2018) found that field-emission scanning electron microscopy (SEM) detected rod-shaped and cube-shaped ZnONPs with diameters ranging from 45 to 95 nm. Atomic Force Microscopy (AFM) assay The average diameter ZnO NPs synthesis by *T. claveryi* is 9.07 nm, as shown in Figures (5).



Avg. Diameter: 9.07 nm

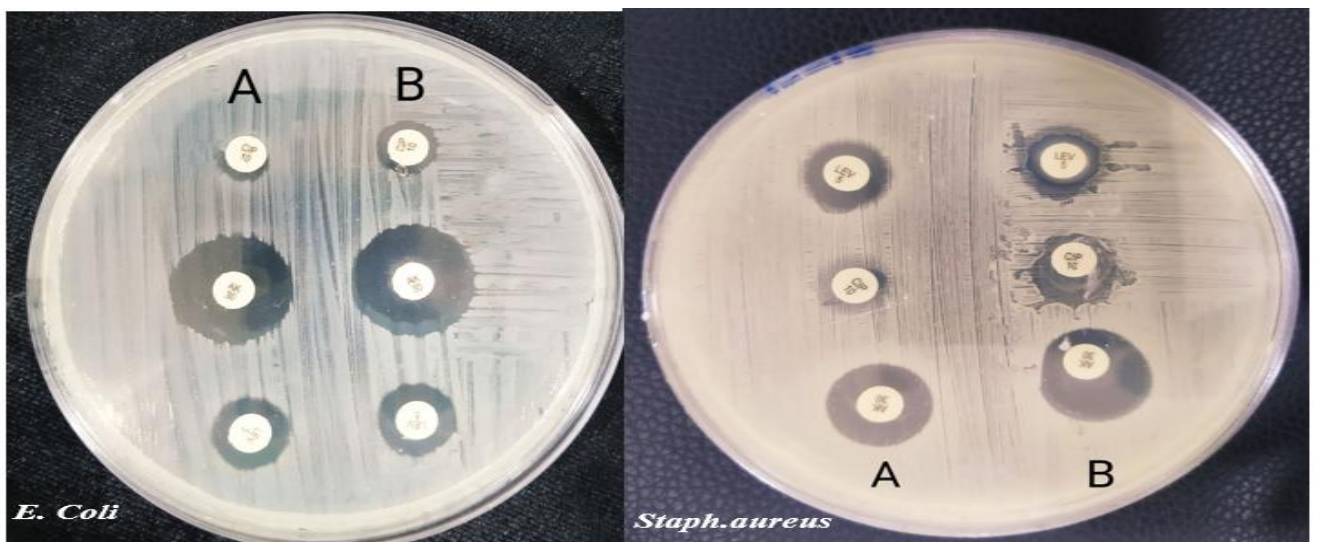
≤5% Diameter: 24.00 nm

≤50% Diameter: 12.03 nm

≤90% Diameter: 7.05 nm

**Figure (5) Atomic Force Microscopic analysis of biosynthesized ZnO NPs from *Terfezia claveryi*.**

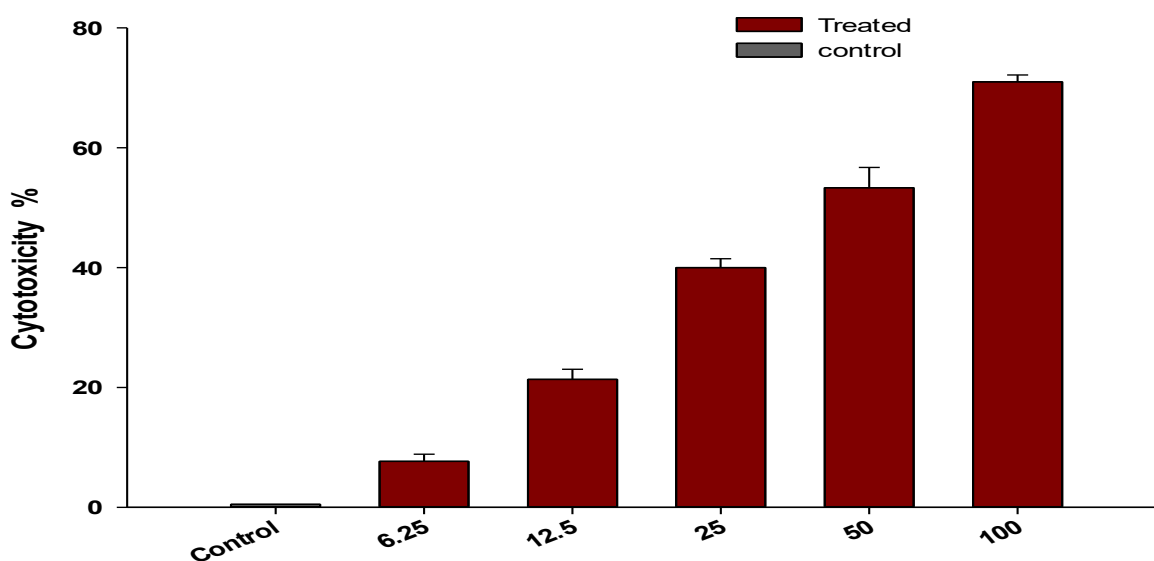
combination effect of antibiotic with ZnO NPs, In comparison to antibiotics alone, the combination impact of antibiotics with ZnO NPs via disc diffusion method resulted in increased fold widths of inhibitory zones of bacterial isolates, combinations of ZnO NPs and antibiotics Antibacterial activity was increased on an average fold-area basis. were added 30  $\mu$ L at a concentration of 13  $\mu$ g/ml ZnO NPs was added to each antibiotic loan as show in ( figure 6).



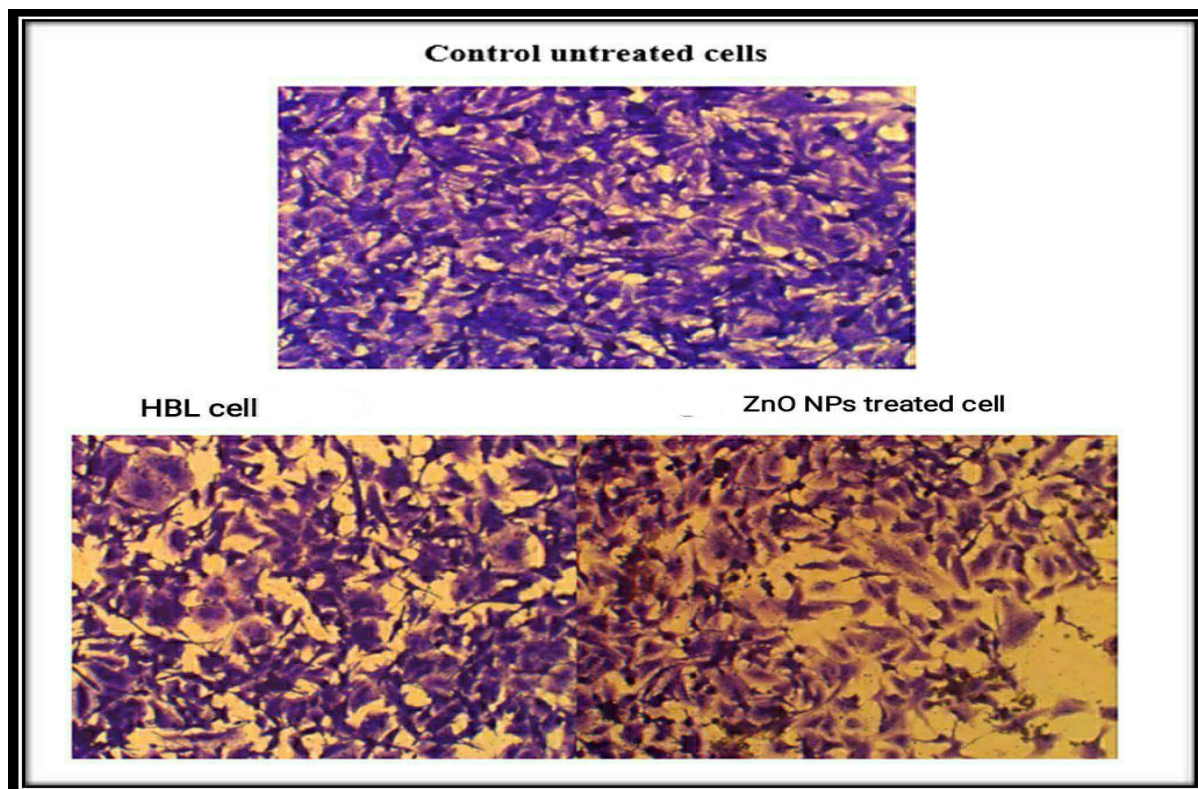
**Figure (6) Zone of growth inhibition (mm) of; *E. coli* and *Staph. aureus* with antibiotic alone and in combination with ZnO-NPs content of 30  $\mu$ l per disc, concentration of ZnO-NPs 13  $\mu$ g/ml). (A= Antibiotic) (B=antibiotic with AgNPs), Levofloxacin =LEV. Ciprofloxacin= Cip. Amikacin=AK.**

Combinations of ZnO NPs with antibiotic agents and anti-inflammatory agents have been studied to enhance antimicrobial activity against pathogenic microorganisms (Abo-Shama *et al.*, 2020; Donnadio *et al.*, 2019), Zinc oxides, such as ZnO NPs, have gotten a lot of interest as antibacterial materials in recent years because of their durability under rigorous processing conditions; they've been utilized as a mild topical astringent in medicine for decades (Ghosh *et al.*, 2015).

Effect of ZnO NPs on the proliferation of Cancer Cells Line and HBL cells. The results of this study showed highly significant cytotoxic activity of ZnO NPs against the human breast cancer cell lines MCF-7 and no effect on human normal breast epithelial cells as showed in Figures 7 and 8 respectively, The MCF-7 cells was affected by the exposure to NPs. The results suggest the ability of ZnO NPs to suppress the growth of cell lines and this effect is concentration dependent manner. Therefore, the findings demonstrated that the NPs could induce cell death. Taken together, the results suggest a selective mode of inhibition by the NPs on MCF-7 cells.



**Figure (7): Cytotoxic activity of ZnO NPs against MCF-7 cells is concentration dependent manner.**



ZnONPs activities on human breast cancer cell lines) (Rai *et al.*, 2013); Umar *et al.* (2019) Conclude in a concentration-dependent manner, biosynthesized ZnO NPs displayed substantial lethal effects on MDA-MB 231 and MCF-7 breast cancer cell lines, ZnO NPs were discovered to have a lethal effect on colon cancer cell lines HT29, with the effect increasing with particle concentration (Bai Aswathanarayan *et al.*, 2018). the cytotoxic effect of ZnO nanopowders generated utilising *Punica granatum* and *Tamarindus indica* L. by a simple combustion-assisted technique on the MCF-7 Bca cell line; the cytotoxic effects were shown to be concentration dependant (Prashanth *et al.*, 2015)

## References

Abo-Shama, U. H., El-Gendy, H., Mousa, W. S., Hamouda, R. A., Yousuf, W. E., Hetta, H. F., & Abdeen, E. E. (2020). Synergistic and antagonistic effects of metal nanoparticles in combination with antibiotics against some reference strains of pathogenic microorganisms. *Infection and Drug Resistance*, 13, 351.

- Al-Askar, A., Hafez, E., Kabeil, S., & Meghad, A. (2013). Bioproduction of silver-nano particles by *Fusarium oxysporum* and their antimicrobial activity against some plant pathogenic bacteria and fungi. *Life Sci J*, *10*(3), 2470-2475.
- Bai Aswathanarayan, J., Rai Vittal, R., & Muddegowda, U. (2018). Anticancer activity of metal nanoparticles and their peptide conjugates against human colon adenorectal carcinoma cells. *Artificial cells, nanomedicine, and biotechnology*, *46*(7), 1444-1451.
- Biao, L., Tan, S., Wang, Y., Guo, X., Fu, Y., Xu, F., . . . Liu, Z. (2017). Synthesis, characterization and antibacterial study on the chitosan-functionalized Ag nanoparticles. *Materials Science and Engineering: C*, *76*, 73-80.
- Birla, S., Tiwari, V., Gade, A., Ingle, A., Yadav, A., & Rai, M. (2009). Fabrication of silver nanoparticles by *Phoma glomerata* and its combined effect against *Escherichia coli*, *Pseudomonas aeruginosa* and *Staphylococcus aureus*. *Letters in Applied Microbiology*, *48*(2), 173-179.
- da Silva, B. L., Abuçafy, M. P., Manaia, E. B., Junior, J. A. O., Chiari-Andréo, B. G., Pietro, R. C. R., & Chiavacci, L. A. (2019). Relationship between structure and antimicrobial activity of zinc oxide nanoparticles: An overview. *International journal of nanomedicine*, *14*, 9395.
- Donnadio, A., Cardinali, G., Latterini, L., Roscini, L., & Ambrogi, V. (2019). Nanostructured zinc oxide on silica surface: Preparation, physicochemical characterization and antimicrobial activity. *Materials Science and Engineering: C*, *104*, 109977.
- Ghosh, T., Das, A. B., Jena, B., & Pradhan, C. (2015). Antimicrobial effect of silver zinc oxide (Ag-ZnO) nanocomposite particles. *Frontiers in Life Science*, *8*(1), 47-54.
- Gil-Ramírez, A., Pavo-Caballero, C., Baeza, E., Baenas, N., Garcia-Viguera, C., Marín, F. R., & Soler-Rivas, C. (2016). Mushrooms do not contain flavonoids. *Journal of Functional Foods*, *25*, 1-13.
- Jamdagni, P., Khatri, P., & Rana, J. (2018). Green synthesis of zinc oxide nanoparticles using flower extract of *Nyctanthes arbor-tristis* and their antifungal activity. *Journal of King Saud University-Science*, *30*(2), 168-175.
- Khashan, K. S., Sulaiman, G. M., Hussain, S. A., Marzoog, T. R., & Jabir, M. S. (2020). Synthesis, characterization and evaluation of anti-bacterial, anti-parasitic and anti-cancer activities of aluminum-doped zinc oxide nanoparticles. *Journal of Inorganic and Organometallic Polymers and Materials*, *30*(9), 3677-3693.

- Król, A., Pomastowski, P., Rafińska, K., Railean-Plugaru, V., & Buszewski, B. (2017). Zinc oxide nanoparticles: Synthesis, antiseptic activity and toxicity mechanism. *Advances in colloid and interface science*, 249, 37-52.
- Meerlo, J. V., Kaspers, G. J., & Cloos, J. (2011). Cell sensitivity assays: the MTT assay. In *Cancer cell culture* (pp. 237-245): Springer.
- Mirzaei, H., & Darroudi, M. (2017). Zinc oxide nanoparticles: Biological synthesis and biomedical applications. *Ceramics International*, 43(1), 907-914.
- Moosburger-Will, J., Jäger, J., Horn, S., & Wellhausen, C. (2012). Investigation of phase morphology of polyetherimide-toughened epoxy resin by scanning probe microscopy. *Polymer testing*, 31(8), 1008-1018.
- Ovais, M., Khalil, A. T., Islam, N. U., Ahmad, I., Ayaz, M., Saravanan, M., . . . Mukherjee, S. (2018). Role of plant phytochemicals and microbial enzymes in biosynthesis of metallic nanoparticles. *Applied microbiology and biotechnology*, 102(16), 6799-6814.
- Owaid, M. N. (2022). Biosynthesis of Silver Nanoparticles from Truffles and Mushrooms and Their Applications as Nanodrugs. *CURRENT APPLIED SCIENCE AND TECHNOLOGY*, 11 pages-11 pages.
- Prashanth, G., Prashanth, P., Bora, U., Gadewar, M., Nagabushana, B., Ananda, S., . . . Sathyananda, H. (2015). In vitro antibacterial and cytotoxicity studies of ZnO nanopowders prepared by combustion assisted facile green synthesis. *Karbala International Journal of Modern Science*, 1(2), 67-77.
- Rabeea, M. A., Owaid, M. N., & Muslim, R. F. (2021). Synthesis and characterization of silver nanoparticles by natural organic compounds extracted from Eucalyptus leaves and their role in the catalytic degradation of methylene blue dye. *Songklanakarin Journal of Science & Technology*, 43(1).
- Rai, M., Ingle, A. P., Gupta, I. R., Birla, S. S., Yadav, A. P., & Abd-Elsalam, K. A. (2013). Potential role of biological systems in formation of nanoparticles: mechanism of synthesis and biomedical applications. *Curr Nanosci*, 9(6), 576-587.
- Sánchez-López, E., Gomes, D., Esteruelas, G., Bonilla, L., Lopez-Machado, A. L., Galindo, R., . . . Camins, A. (2020). Metal-based nanoparticles as antimicrobial agents: an overview. *Nanomaterials*, 10(2), 292.
- Tomaev, V., Polishchuk, V., Vartanyan, T., & Vasil'ev, E. (2019). Surface Plasmon Resonance in Zinc Nanoparticles. *Glass Physics and Chemistry*, 45(3), 238-241.

- Umar, H., Kavaz, D., & Rizaner, N. (2019). Biosynthesis of zinc oxide nanoparticles using *Albizia lebbeck* stem bark, and evaluation of its antimicrobial, antioxidant, and cytotoxic activities on human breast cancer cell lines. *International journal of nanomedicine*, 14, 87.
- Yan, X., Wang, Y., Sang, X., & Fan, L. (2017). Nutritional value, chemical composition and antioxidant activity of three Tuber species from China. *AMB Express*, 7(1), 1-8.