Study the effect of biosynthetic zinc oxide nanoparticles as anti-A549 lung cancer cell agent

S. K. Malaghi, B. A. Ghyadh *
Department of Biology, College of Science, University of Thi-Qar, 64001, Iraq

Nanoparticle targeting of cancer cells provides an alternative to conventional cancer treatments like chemotherapy and radiation. Some nanoparticles, such as zinc oxide nanoparticles (ZnO NPs), may be harmful to cancer cells in particular. Their nanomedicine biomanufacturing method is cost-effective and employs probiotic microbes like Lactobacillus plantarum, which are good for the environment. Zinc oxide nanoparticles (ZnO NPs) will be synthesised from cell filtrate of Lactobacillus plantarum. Then, their toxicity to lung cancer cells (A549) and selectivity to normal cells (WRL68) will be compared. Several analytical methods, including as scanning electron microscopy (SEM), ultraviolet-visible (UV-Vis) spectroscopy, and Fourier-transform infrared (FTIR) spectroscopy, were used to analyse the ZnO nanoparticles that were synthesised from Lactobacillus plantarum cell filtrate. The cytotoxicity of the particles was assessed by using an MTT test on both normal WRL68 cells and cancerous A549 cells. The effective biosynthesis of zinc oxide nanoparticles (ZnO-NPs) was confirmed by UV-Vis absorption measurement, which revealed a unique absorption spectrum at 370 nm. Several functional groups were shown to be actively involved in biosynthesis via Fourier transform infrared spectroscopy (FTIR) research. The signal at 3423.05 cm^{-1} indicates the existence of hydroxyl groups, which are caused by the stretching vibrations of the O-H bonds. Signals at 1734.11 cm^{-1} and 1621.69 cm^{-1} were corresponding to carbonyl (C=O) bond stretching vibrations and N-H bond bending and C=C bond stretching, respectively. A signal at 1074.17 cm^{-1} indicates vibrations caused by stretching of the C-O bond. The unique signal at 555.98 cm^{-1} confirms the formation of zinc oxide nanoparticles, showing that the biosynthetic method was successful. The scanning electron microscopy (FESEM) examination showed that the nanoparticles were round, evenly distributed, and about 45 nm in size. Human lung cancer cells (A549) were shown to have a concentration-dependent deadly impact from the particles, but normal liver cells (WRL68) showed only a modest effect, according to the MTT experiment used to evaluate their cytotoxicity. These results suggest that the generated nanoparticles may have cancer cell selectivity. Zinc oxide nanoparticles produced by Lactobacillus plantarum are an environmentally benign and potentially effective new method for treating lung cancer, according to the results. Additional investigation into their molecular mechanism of action is crucial, they stress.

(Received July 3, 2025; Accepted October 13, 2025)

Keywords: Biosynthesized ZnO NPs, *Lactobacillus plantarum*, A549 lung cells, Anticancer activity

1. Introduction

Lung cancer represents the primary cause of cancer mortality and ranks as the second most frequently diagnosed cancer in the United States. While tobacco smoking remains the primary risk factor for lung cancer, responsible for 80% to 90% of diagnoses, numerous other risk factors are inadvertently associated with its development. Among nonsmokers, there are limited causally associated risk factors for lung cancer [1]. Lung cancer ranks among the most common cancers in Iraq, notably in the Middle Euphrates region, with a higher incidence observed in men. Data from the Iraqi Cancer Registry and regional studies indicate a gradual increase in the prevalence of lung cancer. The increases are most likely attributed to heightened tobacco use, air pollution, and

* Corresponding author: bus.al.p@utq.edu.iq
https://doi.org/10.15251/DJNB.2025.204.1265

exposure to environmental pollutants resulting from industrial activities and regional conflicts [2,3]. Lung cancer ranks as the third most prevalent cancer in Iraq, with the highest incidence observed in men. In 2023, a total of 3,020 new cases were documented. Lung cancer affected 2,129 cases. Males represented 70.5% of cases, whereas females constituted 29.5%, totalling 891 individuals [4]. Lung cancer can be categorised into two primary types: small cell lung cancer (SCLC), representing approximately 13% of cases, and non-small cell lung cancer (NSCLC), comprising about 84% of occurrences. Although non-small cell lung cancer (NSCLC) generally has a more favourable prognosis compared to small cell lung cancer (SCLC), it still presents significant challenges in advanced stages [5]. The incidence of cancer has significantly increased over the past three decades due to advancements in diagnostic techniques, an increase in the number of individuals registering for cancer, and changes in risk factor profiles [6] The majority of cancer therapies, including traditional surgical procedures, radiation therapy, and chemotherapy, are very harmful to patients and do not alleviate their disease [7,8]. Research and development of novel drugs is crucial for enhancing treatment results. The unique chemical and physical characteristics of nanoparticles have sparked renewed interest in cancer therapy in recent years, giving birth to a whole new subfield of oncology known as cancer nanomedicine [9,10].

Nanoparticles of zinc oxide, or ZnO NPs, are relatively new and have promising uses in a variety of fields. Small particle size, particular surface area, molecular arrangement, and electrical structure provide ZnO NPs their unique properties, which include excellent biocompatibility, high stability, and adjustable surface and particle size. In addition, ZnO NPs are extensively employed due to their inexpensive cost and low toxicity [11,12]. One innovative approach in nanomedicine, especially for cancer therapy, is the generation of zinc oxide nanoparticles (ZnO NPs) by bacterial mediation. This method employs bioactive chemicals and bacterial enzymes to transform zinc ions into ZnO NPs via intracellular or extracellular pathways; it is both economical and ecologically benign [13]. Research on the potential of bacterially produced ZnO NPs to selectively kill lung cancer cells is ongoing. 14[. Not only is the genus Lactobacillus famous for its fermentation-related roles in food, but it has also become an integral element of many biotechnological and therapeutic processes. 15[. Gut health, improved mucosal immunity, and bioactive chemical generation are all supported by these non-pathogenic probiotic microorganisms [16]. Particularly, the potential use of Lactobacillus sp. in cancer therapy is the subject of active research [17]. They have the potential to cure cancer because of their impact on the host immune system and their capacity to produce metabolites that kill tumour cells [18]. This opens up new possibilities for using Lactobacillus strains. Their byproducts serve as reducing agents and stabilising agents for the creation of nanoparticles in innovative therapeutic methods such nanoparticle-mediated treatments [19]. Enzymes, proteins, and bioactive metabolites serve as reducing and capping agents; the green synthesis process employs non-pathogenic bacterial species such as Lactobacillus plantarum. Improved biocompatibility, decreased toxicity, and environmental friendliness are outcomes of these procedures, which make nanoparticle manufacturing simpler. [20].

2. Material and methods

2.1. Preparation of cultural media

2.1.1. De Man Rogosa and sharpe (MRS) Agar

6.7 grammes of medium were suspended in one hundred millilitres of distilled water. After a thorough mixing, the mixture should be heated while being stirred continuously. Until the substance is entirely dissolved, boil for one minute. 15 minutes of autoclaving at 121 degrees Celsius. Mix completely, then distribute onto plates after cooling to 45–50 degrees Celsius [21].

2.1.2. De Man Rogosa and sharpe (MRS) broth

All types of Lactobacillus are cultured and preserved in a liquid medium. Dissolve 5.51 g in 100 ml of water that has been distilled. Warm the medium until it dissolves completely, if necessary. Transfer to containers of choice, such as bottles, flasks, or tubes [22].

2.2. Bacterial isolation and identification

Cultured lactic acid bacteria were isolated from a local yoghurt sample, incubated under the right conditions, and then cultivated on MRS agar plates to create pure colonies. At first, morphological analysis was used to identify the bacterium. Gramme stain and polymerase chain reaction (PCR) were used to confirm their molecular identification. For gene sequencing, the PCR output was sent to a specialised facility in Korea. In order to confirm that the isolate was really a member of the Lactobacillus plantarum species, the sequencing results were run through the NCBI database and analysed using BLAST (Basic Local Alignment Search Tool) [23].

2.3. Preparation of cell-free supernatant of *Lactobacillus plantarum*

The cultures were cultured in nutrient broth at 37°C for 24 hours after acquiring a sample of Lactobacillus plantarum from yoghurt from neighboring markets. Biosynthesis of zinc oxide nanoparticles made use of the top liquid produced by centrifuging the cultures at 10,000 rpm for 10 minutes [24].

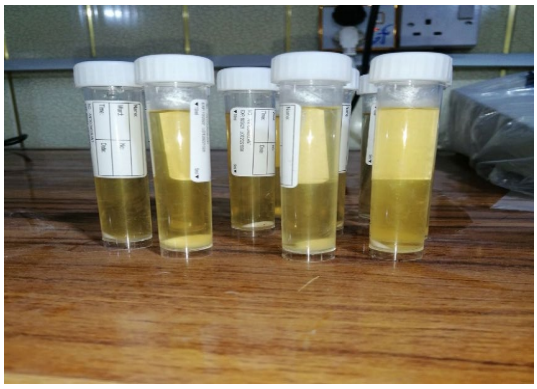
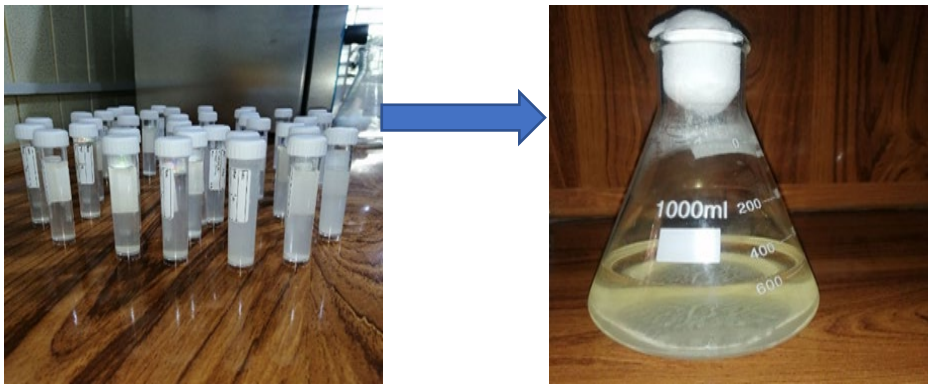


Fig. 1. Lactobacillus plantarum cultures after centrifugation at 10,000 rpm for 10 minutes.

2.4. Biosynthesis of zinc oxide nanoparticles (green manufacturing)

The top supernatant of cultures of Lactobacillus plantarum was used to make zinc oxide nanoparticles, as stated by Mohd Yusof [25]. In order to do this, 45 milliliters of Lactobacillus plantarum upper supernatant and 495 milliliters of a zinc sulphate solution with a concentration of 10 mM were mixed and then agitated in an incubator set at 37°C for three days. White precipitate at the base of the flask is an early sign of creating nanoparticles of zinc oxide.



(a) (b)

Fig. 2. A. After 3 days of adding the upper liquid of the Lactobacillus plantarum cultures to the zinc sulfate saline solution.B. After three days, the precipitate was isolated from the filtrate using centrifugation.

2.5. Characterization of zinc oxide nanoparticles

By analysing the reaction mixture's wavelength composition using UV-Vis spectroscopy, the existence of zinc oxide nanoparticles was established. Nanoparticles of zinc oxide showed a spectrum range of 200–1100 nanometres. The presence of zinc oxide nanoparticles may be verified by this procedure [26]. With the use of Fourier transform infrared spectroscopy, the functional groups were found. Recorded in the region of 450-4000 cm^3 was the infrared spectra of the produced zinc oxide particles [27]. The scanning electron microscopy was used to record and confirm the nanostructural morphology of the produced ZnO particles [28].

2.6. *Lactobacillus* mediated ZnO NPs anticancer properties against a human cancer cell line

2.6.1. Cell cultures

A549 human lung cancer cells were obtained from India's National Centre for Cell Sciences (NCCS) in Pune. As part of the bioactives utilised to develop these cells, Roswell Park Memorial Institute-1640 Medium (RPMI) included penicillin G, streptomycin, sodium bicarbonate, and foetal bovine serum. Careful preparation of the medium provided optimal conditions for cell growth and maintenance. The cells were cultured in an environment with elevated levels of carbon dioxide and humidity at 37°C to promote optimal development [29].

2.6.2. Cytotoxicity evaluation

The MTT test was used to determine the inhibitory concentration (IC50) value. Seeded onto a 96-well plate at a density of 1x104-1x106 cells/ml per well, A549 lung cancer cells were cultured for 24 hours until they achieved around 80% confluence. A solution containing ZnO-NPs at varying concentrations (400, 200, 100, 50, and 25 mg/ml) was added to each well in a new medium. The cells were incubated for an extra twenty-four hours. Every well was supplemented with 10 µL of MTT solution after the medium was removed. After that, let it incubate at 37°C for 4 hours. Once the top liquid was removed, 100 µL of DMSO was added to every well in order to dissolve the formazan crystals. The wells were then incubated for a duration of 10 minutes. Using a Thermo-Multiskan EX ELISA plate reader, the optical density (OD) was measured at 575 nm [30]. The optical density data were then used to calculate cell viability percentages using the procedure below:

$$\%\, cell\, viability = \frac{OD\, of\, treated\, samples}{OD\, of\, untreated\, sample} \times 100.$$

The ZnO NPs half-inhibitory concentrations © were calculated with the help of Graph Pad Prism version 9.4 (La Jolla, CA: Graph Pad Software Inc.).

3. Result and discussion

3.1. Isolation and identification of bacterial

Lactobacillus bacteria were isolated from a local yogurt sample and identified as Gram-positive bacteria through microscopic analysis. Using an immersion oil light microscope at 100× magnification, the cells were found to be long or short purple rods, grouped singly or in pairs. These findings are compatible with the morphological description of the genus [31]. The National Center for Biotechnology Information (NCBI) BLAST tool was used to evaluate the 16S rRNA gene sequence that was obtained by the Sanger procedure in order to verify the isolate's identification. With no mutations or gaps in the sequence, the sequence demonstrated 100% identity (162/162 bp) with *Lactobacillus plantarum LB11* (GenBank accession number: KJ775808.1), an E value of 5e-77, and a matching score of 300, improving the molecular diagnosis's precision. The matched region in the reference sequence was 358 to 519 nucleotides, with a positive/positive read orientation. The isolate was confirmed to be *Lactobacillus plantarum*

based on microscopic and molecular identification results. The DNA Data Bank of Japan has assigned this strain the accession number LC874613 [32].

Fig. 3. Lactobacillus cultured on MRS agar medium.

3.2. Biosynthesis of zinc oxide nanoparticles

When the *Lactobacillus plantarum* bacteria's supernatant solution was combined with zinc sulfate solution, the zinc sulfate salts were reduced to zinc oxide nanoparticles, and the reaction mixture's percentage of ZnONPs was approximately 0.4 grams of ZnO-NPs [33]. This demonstrated the bacteria's capacity to biosynthesize zinc oxide nanoparticles. According to the study's findings, a precipitate formed at the reaction flask's bottom, indicating the creation of secondary zinc oxide particles. The following methods were used to verify the produced zinc oxide's nanoscale characteristics.

Fig. 4. The resulting ZnoNPs from the reaction mixture were approximately 0.4 g.

3.3. ZnO nanoparticles characterization

3.3.1. UV–visible spectroscopic analysis of ZnO nanoparticles

Figure 4 shows that zinc oxide nanoparticles produced via green synthesis had a strong absorption peak at 370 nm when subjected to UV-visible spectroscopy. According to earlier scientific studies, this clear peak is a typical indicator of the effective creation of zinc oxide nanoparticles and a marker of successful nanoparticle synthesis [34,35].

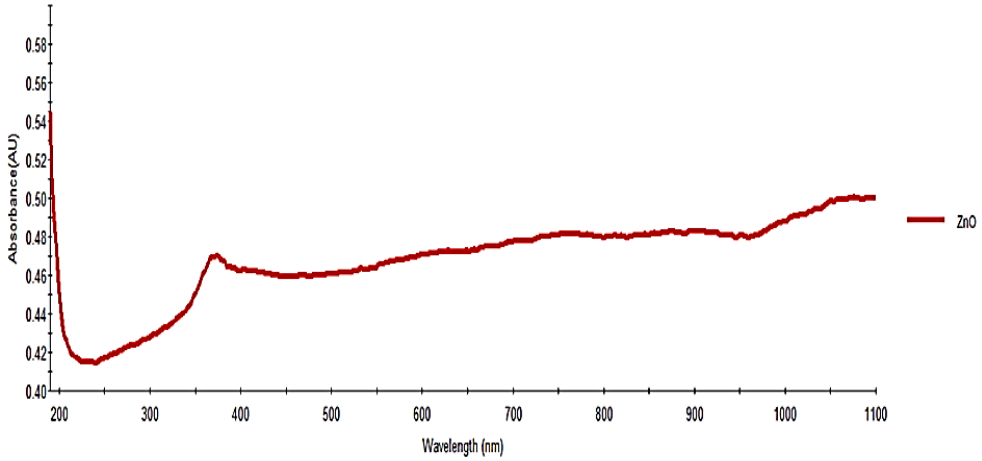


Fig. 4. Absorption spectrum of zinc oxide nanoparticles synthesized using Lactobacillus plantarum culture filtrate.

3.3.2. Functional groups and Fourier transform infrared spectroscopy

We looked at the FTIR spectra of ZnONPs made using Lactobacillus plantarum filtrate to identify the functional groups that were active throughout biosynthesis. Here is how the data might be understood: The spectrum showed notable peaks at 3423.05, 1734.11, 1621.69, 1074.17, and 555.98 cm^{-1}.

The stretching vibrations of the O-H bond, detected at 3423.05 cm^{-1}, indicate the presence of hydroxyl groups originating from alcohols, phenols, or absorbed water. This points to the potential involvement of biomaterials like proteins or polysaccharides in the particle encasing and stabilisation process.

Organic acids or esters exhibit carbonyl bond (C=O) stretching vibrations around 1734.11 cm^{-1}, which may indicate that acids generated by bacterial metabolism contribute to the reduction of zinc ions.

Proteins or aromatic compounds may be detected in biocontaining environments by bending vibrations of N-H or C=C bonds, as shown by the signal at 1621.69 cm^{-1}. C-O bond stretching vibrations, which are shown by the signal at 1074.17 cm^{-1}, suggest the existence of carbs or ether molecules. This proves that proteins or carbohydrates are involved in the stabilising mechanism. The effective biogenesis of ZnO nanoparticles is shown by the signal at 555.98 cm^{-1}.

These results are in agreement with other studies that have discovered comparable peaks, demonstrating that bioactive compounds play a significant role in the formation of ZnONP [36,37].

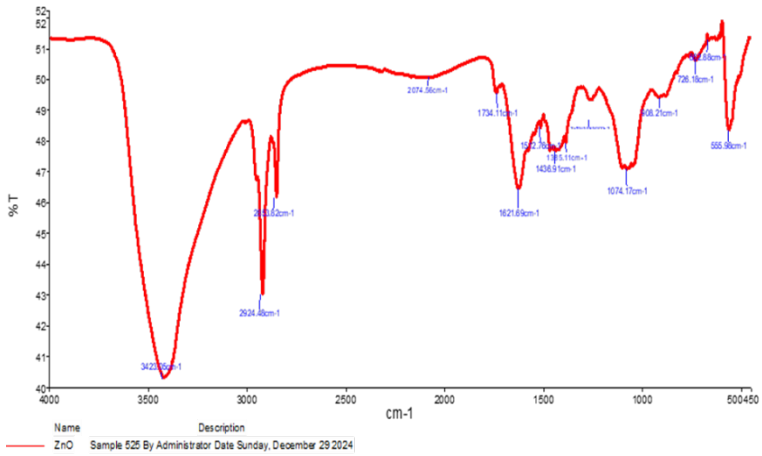


Fig. 5. Fourier transform infrared (FTIR) spectrum of zinc oxide nanoparticles synthesized using Lactobacillus plantarum filtrate.

3.3.3. FESEM analysis for morphology and particle size

FESEM analysis at 135,000x magnification revealed that the zinc oxide nanoparticles were spherical in shape, with a rate diameter of around 45 nanometers and a homogeneous size distribution. These morphological traits are congruent with prior research [38].

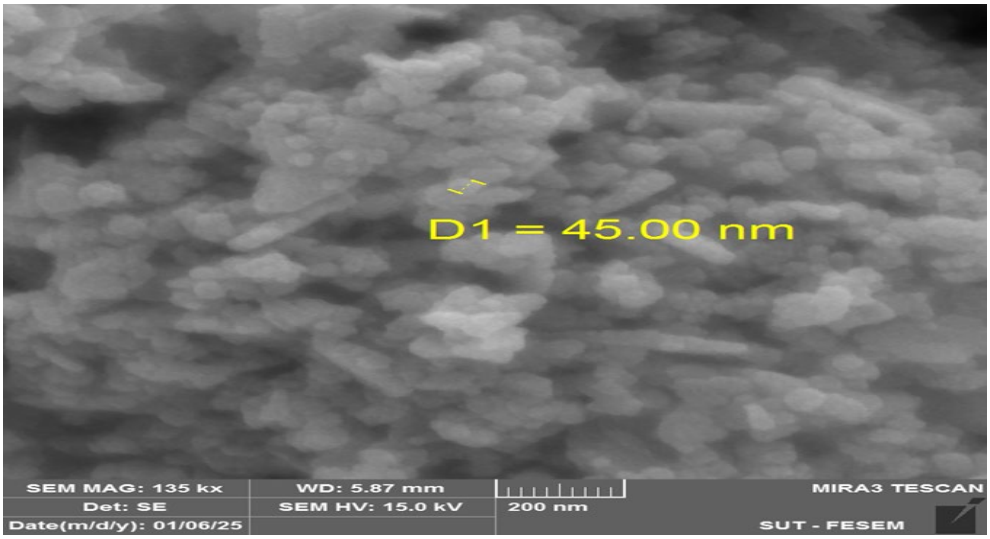


Fig. 6. Nanoparticles of zinc oxide made from Lactobacillus plantarum filtrate, as seen using a scanning electron microscope.

3.4. Anticancer activity

A higher quantity of zinc oxide reduces cell viability. Figure 7 shows that the IC50 values for A549 cells were 161.3 µg/ml, whereas for WRL cells they were 229.9 µg/ml. Zinc oxide nanoparticles were shown to decrease A549 cell viability, with the decrease being highly correlated with zinc oxide concentration.

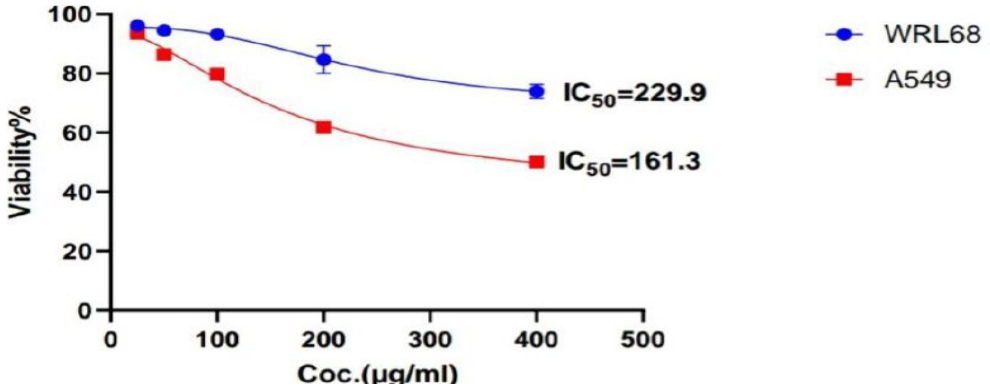


Fig. 7. A549 and normal WRL 68 cytotoxicity of ZnO NPs. Every point is the average of three replicates.

With concentrations of 400, 200, 100, 50, and 25, the viability rate decreased by 50.1 ± 0.9, 61.7 ± 1.3, 79.8 ± 0.2, 86.3 ± 1.6, and 93.6 ± 0.4, respectively. Table 1 shows that applying the same concentration to WRL 68 cells did not significantly change their viability rate, with percentages ranging from 73.9 ± 2.3 to 96.1 ± 0.2, with concentrations of 84.7 ± 4.6, 93.3 ± 1.4, 94.5 ± 0.5, and 96.1 ± 0.2 respectively.

By using in vitro cell cultures, Suhad et al. [39] examined the detrimental impacts of zinc oxide nanoparticles (ZnO) on human cell lines (A549, WRL68) and found that the cytotoxicity of ZnO nanoparticles was dose-dependent.

Table 1. The cytotoxic effect of ZnO-NPs on A549 and WRL68 cell line.

Concentration Of ZnO-NPs (µg/ml)	Mean viability (%) ± SD	
	A549	WRL-68
400	50.1±0.9	73.9±2.3
200	61.7±1.3	84.7±4.6
100	79.8±0.2	93. 3±1.4
50	86.3±1.6	94. 5±0.5
25	93.6±0.4	96.1±0.2

4. Conclusion

In the MTT test, zinc oxide nanoparticles produced by Lactobacillus plantarum showed anticancer activity against A549 lung cancer cells with just a little amount of damage inflicted on WRL68 normal cells. Based on these results, ZnO NPs might be a promising substance for use in cancer treatment and other medical fields.

References

[1] M. Schabath, M.B. Cote, Cancer Epidemiol Biomarkers Prev., 28 (2019) 1563–1579; https://doi.org/10.1158/1055-9965.EPI-19-0347
[2] J.M. Al-Musawi, F. Al-Shadeedi, N.A. Shakir, S.Q. Ibrahim, Al-Rafidain J Med Sci., 8 (2025) 63–70; https://doi.org/10.32341/ajms.v8i2.158
[3] M.M. Al-Hashimi, M.Q.Y. Alawjar, Int J Public Health., 13 (2024) 1063–1070; https://doi.org/10.1007/s00038-024-01786-0
[4] Ministry of Health – Iraq, Iraqi Cancer Board, (2022); https://storage.moh.gov.iq/2024/03/31/2024_03_31_11983087032_3940351786864953.pdf
[5] A.M. Wolf, K.C. Oeffinger, T.Y. Shih, L.C. Walter, T.R. Church, E.T. Fontham, et al., CA Cancer J Clin., 74 (2024) 50–81; https://doi.org/10.3322/caac.21897
[6] A.K. Ganti, A.B. Klein, I. Cotarla, B. Seal, E. Chou, JAMA Oncol., 7 (2021) 1824–1832; https://doi.org/10.1001/jamaoncol.2021.3826
[7] H. Barabadi, H. Vahidi, K. Damavandi Kamali, M. Rashedi, O. Hosseini, A.R. Golnaraghi Ghomi, et al., J Clust Sci., 31 (2020) 311–321; https://doi.org/10.1007/s10876-019-01616-2
[8] N.A. Askouri, Univ Thi-Qar J Sci., 4 (2014) 114–119; https://doi.org/10.22075/ujns.2023
[9] S. Raj, S. Khurana, R. Choudhari, K.K. Kesari, M.A. Kamal, N. Garg, et al., Semin Cancer Biol., 69 (2021) 166–177; https://doi.org/10.1016/j.semcancer.2020.11.001
[10] H. Hasan Tuhmaz, Univ Thi-Qar J Sci., 7 (2020) 84–86; https://doi.org/10.22075/ujns.2020
[11] A.I. Khudiar, Univ Thi-Qar J Sci., 4 (2014) 75–78; https://doi.org/10.22075/ujns.2023
[12] C. Hu, W. Du, Aging (Albany NY), 12 (2020) 25767; https://doi.org/10.18632/aging.103344
[13] R. Shanmugam, T. Munusamy, S. Jayakodi, K.A. Al-Ghanim, M. Nicoletti, N. Sachivkina, et al., Fermentation, 9 (2023) 413; https://doi.org/10.3390/fermentation9050413
[14] G. Bisht, S. Rayamajhi, B. Kc, S.N. Paudel, D. Karna, B.G. Shrestha, Nanoscale Res Lett., 11 (2016) 1–11; https://doi.org/10.1186/s11671-016-1777-6
[15] G. Hamad, R.A. Ombarak, M. Eskander, T. Mehany, F.R. Anees, R.A. Elfayoumy, et al., LWT, 163 (2022) 113603; https://doi.org/10.1016/j.lwt.2022.113603
[16] J. Sharifi-Rad, C.F. Rodrigues, Z. Stojanović-Radić, M. Dimitrijević, A. Aleksić, K. Neffe-Skocińska, et al., Medicina, 56 (2020) 433; https://doi.org/10.3390/medicina56090433
[17] S. Samanta, Anti-Cancer Agents Med Chem., 22 (2022) 605–628; https://doi.org/10.2174/1871520621666210226103505
[18] A.Q. Yu, L. Li, Nutr Cancer, 68 (2016) 535–544; https://doi.org/10.1080/01635581.2016.1153746

[19] A. Latif, A. Shehzad, S. Niazi, A. Zahid, W. Ashraf, M.W. Iqbal, et al., Front Microbiol., 14 (2023) 1216674; https://doi.org/10.3389/fmicb.2023.1216674
[20] M.H. Kalaba, G.M. El-Sherbiny, E.A. Ewais, O.M. Darwesh, S.A. Moghannem, BMC Microbiol., 24 (2024) 254; https://doi.org/10.1186/s12866-024-02776-7
[21] J.D. De Man, D. Rogosa, M.E. Sharpe, J Appl Microbiol., 23 (1960) 130–135; https://doi.org/10.1111/j.1365-2672.1960.tb00188.x
[22] P. Laloi, D. Atlan, B. Blanc, C. Gilbert, R. Portalier, Appl Microbiol Biotechnol., 36 (1991) 196–204; https://doi.org/10.1007/BF00163743
[23] S.J. Mannan, R. Rezwan, M.S. Rahman, K. Begum, Bangladesh Pharm J., 20 (2017) 27–33; https://doi.org/10.3329/bpj.v20i1.31466
[24] A. Manzoor, J.I. Qazi, I.U. Haq, H. Mukhtar, A. Rasool, J Biol Eng., 11 (2017) 1–10; https://doi.org/10.1186/s13036-017-0070-9
[25] H. Mohd Yusof, N.A. Abdul Rahman, R. Mohamad, U.H. Zaidan, A.A. Samsudin, Sci Rep., 10 (2020) 19996; https://doi.org/10.1038/s41598-020-76989-7
[26] R. Chauhan, A. Reddy, J. Abraham, Appl Nanosci., 5 (2015) 63–71; https://doi.org/10.1007/s13204-014-0328-6
[27] A. Rajan, E. Cherian, G. Baskar, Int J Mod Sci Technol., 1 (2016) 52–57; https://doi.org/10.22271/ijmst.2016.v1.i2.52
[28] A. Mashrai, H. Khanam, R.N. Aljawfi, Arabian J Chem., 10 (2017) 1530–1536; https://doi.org/10.1016/j.arabjc.2014.12.002
[29] Z.V.M. Hussain, S.K. Sivanandhan, Indian J Pharm Educ Res., 55 (2021) 566–573; https://doi.org/10.5530/ijper.55.3.130
[30] S.Z.A. Khader, S.S.Z. Ahmed, M.R. Mahboob, S.B. Prabaharan, S.O. Lakshmanan, K.R. Kumar, et al., Braz J Pharm Sci., 58 (2022) e18594; https://doi.org/10.1590/s2175-97902022000118594
[31] D.A. Qasim, I.J. Lafta, O.A. Iyiola, Iraqi J Vet Med., 47 (2023) 44–51; https://doi.org/10.30539/ijvm.v47i1.1547
[32] P.K. Sidhu, K. Nehra, LWT, 139 (2021) 110546; https://doi.org/10.1016/j.lwt.2020.110546
[33] J. Jeevanandam, S.F. Kiew, S. Boakye-Ansah, S.Y. Lau, A. Barhoum, M.K. Danquah, et al., Nanoscale, 14 (2022) 2534–2571; https://doi.org/10.1039/D1NR06775H
[34] W. Ahmad, D. Kalra, J King Saud Univ Sci., 32 (2020) 2358–2364; https://doi.org/10.1016/j.jksus.2019.12.013
[35] F. Rahman, M.A. Patwary, M.B. Siddique, M.S. Bashar, M.A. Haque, B. Akter, et al., R Soc Open Sci., 9 (2022) 220858; https://doi.org/10.1098/rsos.220858
[36] Z.S. Hamza, Indian J Forensic Med Toxicol., 14 (2020) 2708–2715; https://doi.org/10.37506/ijfmt.v14i4.1148
[37] Z. Salman, W.R. Taj Al-Deen, Biochem Cell Arch., 19 (2019) 1–3; https://doi.org/10.1007/s13205-019-1813-2
[38] M. Khan, P. Ware, N. Shimpi, SN Appl Sci., 3 (2021) 1–17; https://doi.org/10.1007/s42452-021-00544-y
[39] H. Suhad, H.Z. Neihaya, A.L. Raghad, Caspian J Environ Sci., 19 (2021) 809–815; https://doi.org/10.22124/cjes.2021.5985