

RESEARCH PAPER

Antioxidant and Cytotoxic Activity of Biosynthesized Zinc Oxide Nanoparticles and *Dombeya wallichii* Extract on Human Breast Cancer Cell Lines

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ARTICLE INFO

Article History:

Received 03 April 2026

Accepted 14 May 2026

Published 01 July 2026

Keywords:

Breast cancer

Dombeya wallichii

Green synthesis

Zinc oxide nanoparticles

ABSTRACT

This study investigated the green synthesis of zinc oxide nanoparticles using the aqueous leaf extract of *Dombeya wallichii* as a plant-based source of reducing and stabilizing agents. The synthesized ZnO-NPs were examined by Fourier Transform Infrared Spectroscopy, X-ray Diffraction, Field Emission Scanning Electron Microscopy, Energy Dispersive X-ray Spectroscopy, and Atomic Force Microscopy in order to confirm their formation and evaluate their main physicochemical characteristics. The biological activity of the aqueous leaf extract and the green-synthesized ZnO-NPs was assessed through antioxidant and cytotoxicity assays. The antioxidant potential was measured using the DPPH free radical scavenging assay, whereas cytotoxic activity was evaluated by the MTT assay against human breast cancer cells (MCF-7) and normal mammary epithelial cells (MCF-10). The obtained results showed that both treatments produced concentration-dependent effects. However, ZnO-NPs showed higher free radical scavenging activity and stronger inhibitory effects against MCF-7 cells than the crude plant extract. Their effect on MCF-10 cells was comparatively lower, which may indicate a selective action toward cancer cells. Based on these findings, the aqueous leaf extract of *D. wallichii* appears to be a suitable natural medium for the green synthesis of ZnO-NPs. The resulting nanoparticles showed promising antioxidant and anticancer properties, suggesting their potential value as bioactive nanomaterials for further breast cancer-related investigations.

How to cite this article

Aqeel Shihab A., Hameed Mohammed F., Muhi Hasson S. Antioxidant and Cytotoxic Activity of Biosynthesized Zinc Oxide Nanoparticles and *Dombeya wallichii* Extract on Human Breast Cancer Cell Lines. J Nanostruct, 2026; 16(3):3227-3239.

DOI: 10.22052/JNS.2026.03.019

INTRODUCTION

Nanotechnology is a new branch of biomedicine that employs materials at the nanoscale, which have special properties, including their small size and high surface area [1]. Such properties have also stimulated the application of nanoparticles to cancer-related applications, in particular, due to

their possible enhancement of cellular targeting and minimization of damage to normal tissues [2]. Physical, chemical, or biological methods can be used to create nanoparticles, but green synthesis has received more attention due to its simplicity, eco-friendliness, and reliance on natural biological resources [3]. Under this method, the phytochemical-enriched plant extracts can be

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involved in forming nanoparticles as reducing, stabilizing, and capping agents [4]. Zinc oxide nanoparticles are among the other metal oxide nanoparticles that have been of interest because of their biological properties, and potential biomedical uses such as anticancer activity. Plant extracts as ZnO-NPs synthesis can also enhance the stability and biological activity of nanoparticles by having active phytochemical compounds on the nanoparticle surface [5].

Medicinal plants are believed to be effective sources of natural bioactive compounds with antioxidant and anticancer properties [6]. Their biological activities are primarily linked to secondary metabolites like alkaloids, flavonoids, terpenes, and phenolic compounds [7]. *D. wallichii* is a Malvaceae plant that has been reported to have bioactive constituents that have the potential to be antioxidants and anti-inflammatory [8]. Nevertheless, its therapeutic potential has not been adequately explored, especially in terms of its anticancer properties and its potential

application in green synthesis of ZnO-NPs [9].

Cancer is one of the most critical health issues in the world and is identified by uncontrolled cell growth, infiltration of tissues and the capacity to invade other body organs [10]. Breast cancer is one of the most frequently occurring cancer types in women and is a significant cause of cancer-related mortality particularly in places with scarce healthcare facilities [11]. The disease occurs when there is malignant change in breast epithelial cell resulting in abnormal growth, invasion and spreading via lymphatic or blood circulation [12]. Despite the fact that surgery, radiotherapy and chemotherapy are common methods of cancer treatment and have enhanced patient survival, the treatment approaches have a number of limitations. These are systemic toxicity, lack of selectivity in targeting cancer cells, tissue damage and therapeutic resistance development [13,14].

Therefore, the present study aimed to characterize the phytochemical constituents of *D. wallichii* leaf extract, evaluate its anticancer



Fig. 1. *D. wallichii* plant.

activity, and investigate its application in the green synthesis of zinc oxide nanoparticles against breast cancer cell lines.

MATERIALS AND METHODS

Materials

Zinc nitrate, DPPH (2,2-diphenyl-1-picrylhydrazyl), MTT reagent, and dimethyl sulfoxide (DMSO) were purchased from HiMedia (India). Absolute ethanol was purchased from SRL (India). *Dombeya wallichii* leaves were collected from a local nursery in Al-Hillah city, Iraq, and used for the preparation of the aqueous extract and the green synthesis of zinc oxide nanoparticles. MCF-7 human breast cancer cells and MCF-10A normal human breast epithelial cells were used to evaluate the cytotoxic activity of the prepared treatments. All chemicals and reagents used in this study were of analytical grade.

Plant collection and identification

The leaves of *D. wallichii* were collected from a local nursery in Al-Hillah city during September 2025. The plant was identified and authenticated by Dr. Shaema Muhi Hasson, Professor of Plant

Taxonomy, Department of Biology, College of Science, University of Babylon. After collection, the leaves were cleaned to remove dust and other impurities, then prepared for the next experimental steps.

The aqueous extract of *D. wallichii* leaves was prepared.

Aqueous extract of the leaves of *D. wallichii* was prepared using a method reported elsewhere with some slight alterations [15]. The leaves collected were rinsed in distilled water to get dust and other surface impurities out and then dried in shade in room temperature till fully dry. The dried leaves were electrically grained and ground to a fine powder and kept in a sterile closed container till used. Dried leaf powder was added to distilled water in a 1:10 (w/v) ratio. The mixture was shaken in a tightly closed glass tube with the help of an electric shaker during 1 h to enhance the contact between the solvent and the powder of the plant. The mixture was then left to cool at room temperature after incubating it in a water bath at 40°C during 2 h. Using Whatman filter paper, the extract was filtered to eliminate the solid residues. A part of the filtrate was then utilized in the green

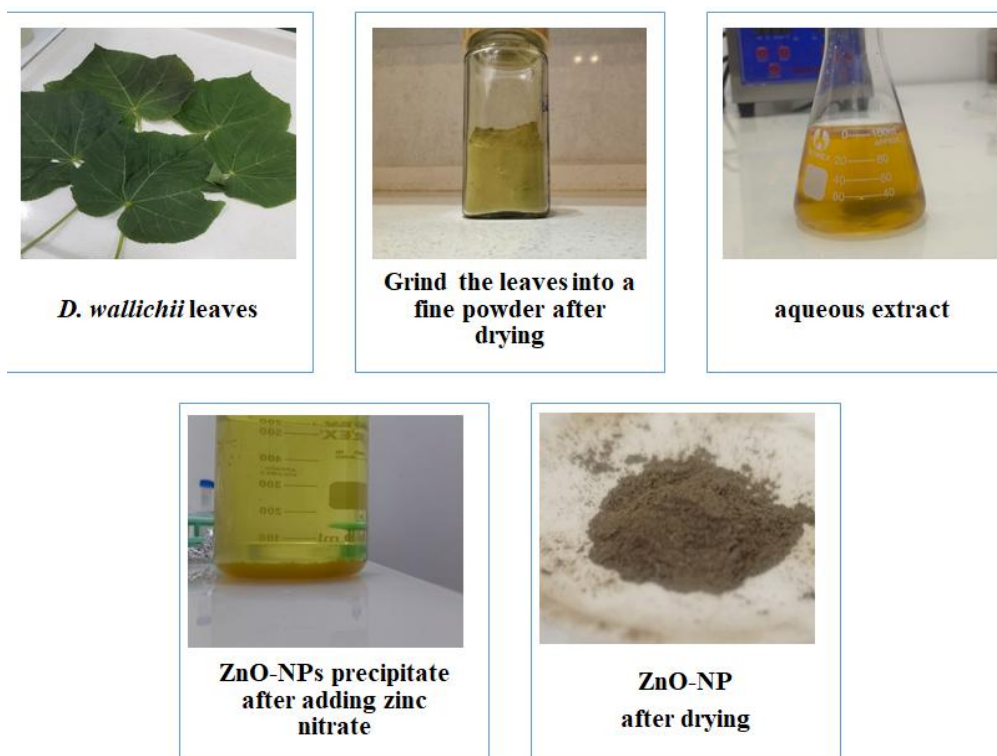


Fig. 2. Synthesis of zinc oxide nanoparticles.

synthesis of zinc oxide nanoparticles and the rest of the filtrate dried in an oven at 40 °C until a constant weight had been achieved. The dried extract was milled into a fine powder, sterilized under ultraviolet light (20 min) and then stored in a sterile airtight dark glass container in a cool and dry place until further usage.

Zinc oxide nanoparticles synthesis [16]

In short, 0.05 M solution of zinc nitrate was prepared by dissolving 4.73 g of Zn(NO₃)₂ in distilled water. To get a homogenous reaction mixture, aqueous extract of the *D. wallichii* leaves was added to the zinc nitrate solution in a ratio of 1:1 (v/v). This ratio was applied to mediate the reduction and stabilization processes with the help of plant phytochemicals. This mixture was then allowed to incubate in a shaking incubator at 100 rpm and 40 °C during 24 h to facilitate the formation of nanoparticles. The precipitate was then formed after incubation was over and centrifugation carried out at 5000 rpm/30 min. A series of washes (distilled water and absolute ethanol) were performed to clean the precipitate of the unreacted substances and leftover organic compounds. Lastly, the purified precipitate was heated in a hot air oven at 40 °C 24 h and the resulting powder was stored to be further characterized and to be analyzed biologically.

Characterization of zinc oxide nanoparticles (ZnO-NPs)

ZnO nanoparticles were characterized by FTIR, XRD, FE-SEM, EDX, and AFM analyses to evaluate their functional groups, crystalline structure, morphology, elemental composition, and surface topography.

Free radical scavenging activity test (DPPH)

The antioxidant activity of *D. wallichii* leaf extract and ZnO-NPs was determined using the DPPH radical scavenging assay. A 0.1 mM DPPH solution was prepared, and stock solutions of the plant extract and ZnO-NPs were prepared at 400 µg/mL. These solutions were then diluted to obtain concentrations of 3.125, 6.25, 12.5, 25, 50, 100, 200, and 400 µg/mL. For each concentration, 4 mL of the tested sample was mixed with 2 mL of DPPH solution. The mixtures were shaken thoroughly and kept in the dark at room temperature for 30 min. After incubation, the absorbance was recorded at 517 nm using a spectrophotometer.

The percentage of DPPH radical scavenging activity was calculated according to the Eq. 1:

$$Q = \frac{A_0 - AC}{A_0} \times 100 \quad (1)$$

Where, A₀ = control absorbance, AC = sample absorbance

The IC₅₀ value represent the concentration required to scavenging 50% of DPPH radicals [17].

Estimation the cytotoxicity of *D. wallichii* leaf extract and ZnO-NPs using MTT assay

A 96-well flat-bottom microplate was used for cell seeding at density of (1 × 10⁴ to 10⁶) cells/ml. The cells were incubated at 37°C in humidified atmosphere containing 5% CO₂ for 24 h to allow for attachment and stabilization. Following incubation, the culture medium with was carefully removed. Serial dilution of *D. wallichii* leaf extract and ZnO-NPs were prepared to obtain final concentrations of (25, 50, 100, 200, and 400 µg/ml) each concentration was tested in triplicate. The treated plates were incubated for an additional 24 h under the same conditions. After treatment, the cells were gently washed with phosphate-buffered saline (PBS) to remove residual compounds. Subsequently 20 µL of MTT solution was added to each well, followed by incubation for 4 h at 37°C to allow the formation of formazan crystals. After incubation, 100 µL of DMSO was added to each well to dissolve the formazan crystals. The absorbance of each wells was measured at 575 nm using microplate reader. The mean absorbance values were calculated for each treatment group.

Cell viability was determined using the Eq. 2:

$$\text{Cell viability (\%)} = \frac{\text{absorbance of treated cells}}{\text{absorbance of control}} \times 100 \quad (2)$$

Cytotoxicity (%) was subsequently calculated as Eq. 3:

$$\text{Cytotoxicity (\%)} = 100 - \text{Cell viability} \quad (3)$$

Where the control represents untreated cells, and treated cells correspond to those exposed to different concentrations of the tested compounds [18].

Statistical Analysis

All experimental data were expressed as

mean \pm standard deviation (SD) based on three replicates. Statistical analysis was performed using IBM SPSS Statistics software. One-way analysis of variance (ANOVA) was used to compare the differences among different concentrations within each treatment or cell line. When significant differences were detected, post hoc multiple comparison tests were applied to determine the differences between groups. In addition, the independent samples t-test was used to compare the responses between the two cell lines at the same concentration. Differences were considered statistically significant at $p \leq 0.05$.

RESULTS AND DISCUSSION

Fourier transform infrared spectroscopy (FTIR)

FTIR analysis was performed to detect the functional groups associated with the biosynthesis and stabilization of ZnO-NPs, as shown in (Fig. 3). The spectrum displayed a broad absorption band at 3304.06 cm^{-1} , which is related to O–H stretching vibrations of phenolic and alcoholic compounds. The bands recorded at 2958.80 and 2927.94 cm^{-1} were assigned to C–H stretching vibrations, indicating the presence of organic compounds derived from the plant extract [19,20]. The absorption band at 1653 cm^{-1} corresponded to

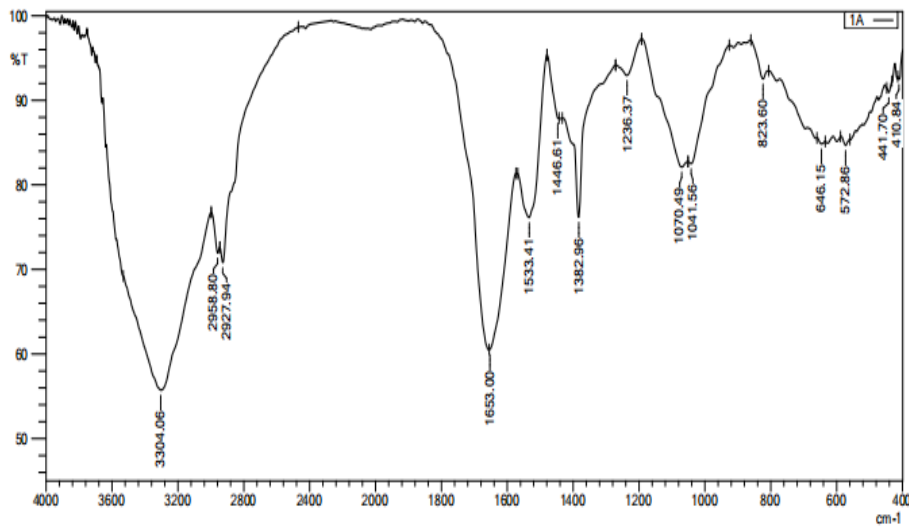


Fig. 3. FTIR spectrum of biosynthesized zinc oxide nanoparticles.

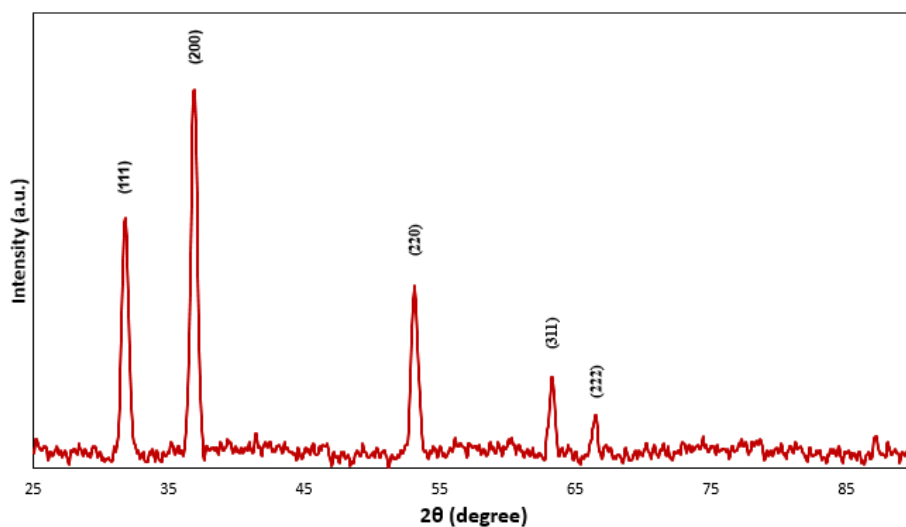


Fig. 4. XRD images of ZnO-NPs.

C=O stretching vibration, whereas the peak at 1533.41 cm^{-1} may be associated with N–H bending or C–N stretching vibrations. In addition, the bands observed at 1382.96 , 1070.49 , and 1041.56 cm^{-1} were related to C–H and C–O vibrations of alcohols, ethers, and phenolic compounds, suggesting their involvement in the reduction and stabilization of ZnO-NPs [21,22]. The band at 823.60 cm^{-1} may refer to aromatic structures. Furthermore, the low-frequency bands observed between 646.15 and 410.84 cm^{-1} confirmed ZnO stretching vibration, which supports the successful formation of ZnO nanoparticles [23]. These results indicate that the phytochemical constituents of *D. wallichii* leaf extract contributed to the formation, capping, and stabilization of ZnO-NPs through the green synthesis process.

X-ray diffraction (XRD) analysis

X-ray diffraction (XRD) analysis was used to evaluate the crystalline nature and phase structure of the synthesized ZnO-NPs, as shown in (Fig. 4). The XRD pattern showed several distinct diffraction peaks, indicating the crystalline nature

of the prepared nanoparticles. The sharp and clear peaks also confirmed the polycrystalline structure of the sample. The most intense peak was observed at 36.876° , suggesting a preferential growth orientation of the ZnO-NPs [24]. The diffraction peaks at 31.794° , 56.643° , 66.434° , and 72.556° were close to the standard reflections of hexagonal wurtzite ZnO according to JCPDS/ICDD card No. 36-1451, confirming the formation of crystalline ZnO-related structures. However, the peaks observed at 41.417° and 45.581° did not match the standard ZnO pattern, which may be related to additional reflections or minor secondary phases [25]. These results support the successful biosynthesis of crystalline ZnO-NPs using *D. wallichii* leaf extract.

Field emission scanning electron microscopy (FE-SEM)

The outer structure and nanoscale size of the ZnO-NPs biosynthesized were studied using field emission scanning electron microscopy (FE-SEM) as shown in (Fig. 5). In the micrograph, the prepared particles were observed to be

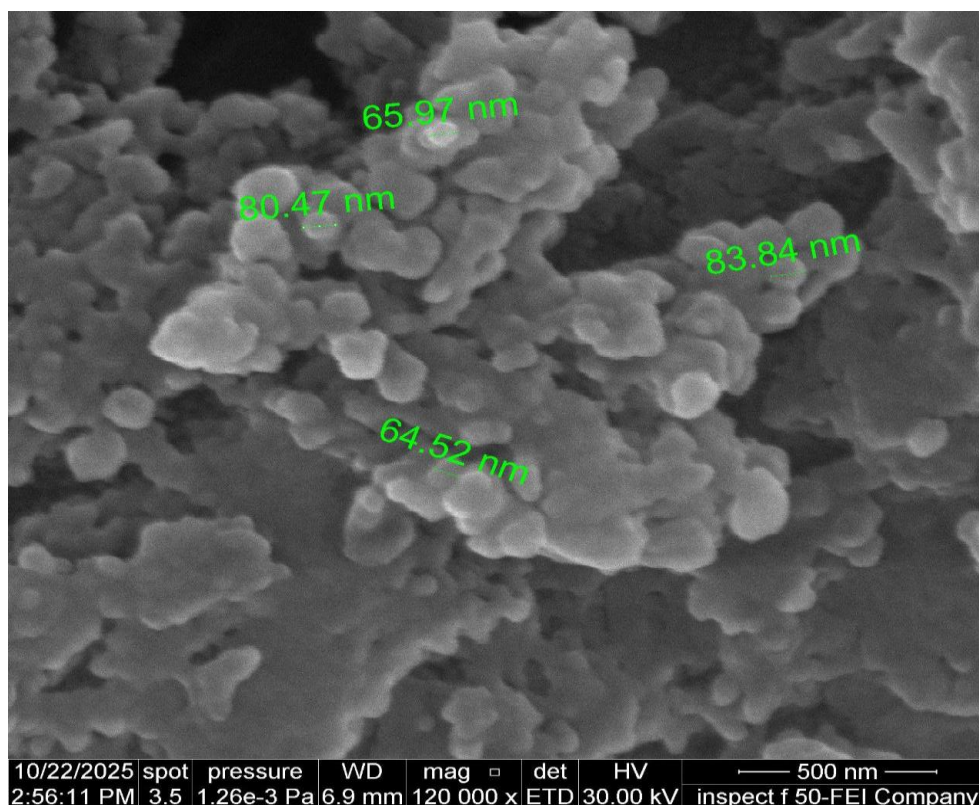


Fig. 5. Field emission electron microscopy analysis.

predominantly rounded with some visible inter-particle aggregation. Such aggregation can be due to the close proximity of nanoparticles during the biosynthesis and drying process, or because of biomolecules of plant origin on their surfaces. The size of the particles was estimated to be between 64.52-83.84 nm, which means that the particle ZnO synthesized was in the nanoscale range [26]. These variations in particle size and shape could be connected with the impact of phytochemical components in *D. wallichii* leaf extract that is capable of modulating the nucleation process, particle development, and surface stabilization (in the green synthesis process) [27,28]. These FE-SEM results show that the nanosized ZnO particles were successfully prepared in the *D. wallichii* leaf

extract as a biomaterial.

Energy-dispersive X-ray Spectroscopy (EDX)

To identify the elemental profile of the biosynthesized ZnO-NPs, energy-dispersive X-ray spectroscopy (EDX) was conducted, which is depicted by (Fig. 6). The EDX pattern showed clear signs of z and oxygen which indicates the presence of ZnO nanoparticles [29]. The high carbon percentage could be related to organic residues of *D. wallichii* leaf extract on the surface the nanoparticles. Such plant-based compounds are capable of increasing the surface coating, particle stabilization and diminishing uncontrolled aggregation during biosynthesis [30]. Some phosphorus signal was also detected and this

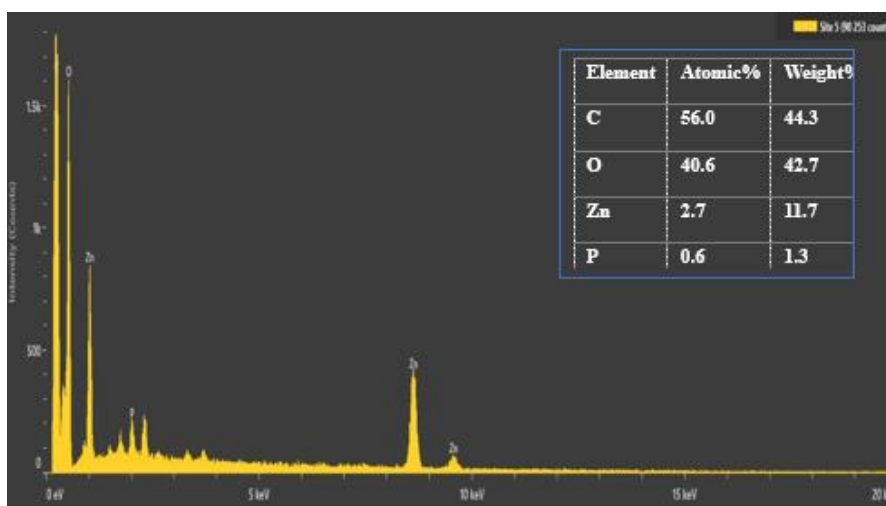


Fig. 6. X-ray diffraction of biosynthetic zinc oxide nanoparticles.

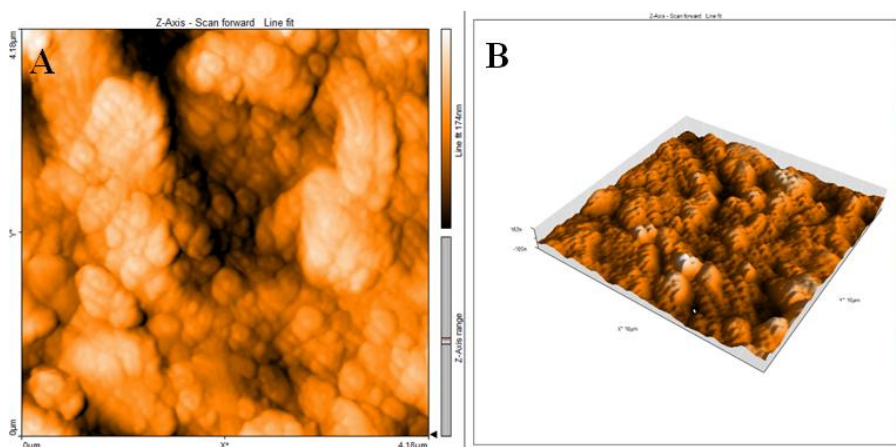


Fig. 7. AFM images for nanoparticles; (A) 2D and (B) 3D.

could be attributed to the presence of phosphorus-containing compounds left behind by the plant extract after the formation of the nanoparticle [31]. Thus, the EDX analysis confirms the effective synthesis of ZnO-NPs and suggests the role of the phytochemicals in the capping and stabilization of the phytochemicals.

Atomic Force Microscopy (AFM)

The surface morphology and size distribution of the biosynthesized ZnO-NPs were observed with the help of atomic force microscopy (AFM) as illustrated in (Fig. 7). The AFM images (two-dimensional and three-dimensional) revealed that the particles had different sizes and uneven surface

characteristics. The aggregation observed could be connected with the fact that phytochemical compounds of *D. wallichii* leaf extract may serve as the stabilizing and capping agents during the formation of nanoparticles. Mean particle size was 65.87 nm with majority of the particles less than 100 nm in diameter as displayed in (Fig. 8). AFM images also demonstrated rough topography of the surface featuring hill-like and valley-like features. Such ruggedness could be attributed to the input of plant-based compounds in reducing, stabilizing, and capping, which results in the aggregation of ZnO nanostructures. These results validate the fact that ZnO-NPs have been formed in the presence of phytochemicals in the aqueous

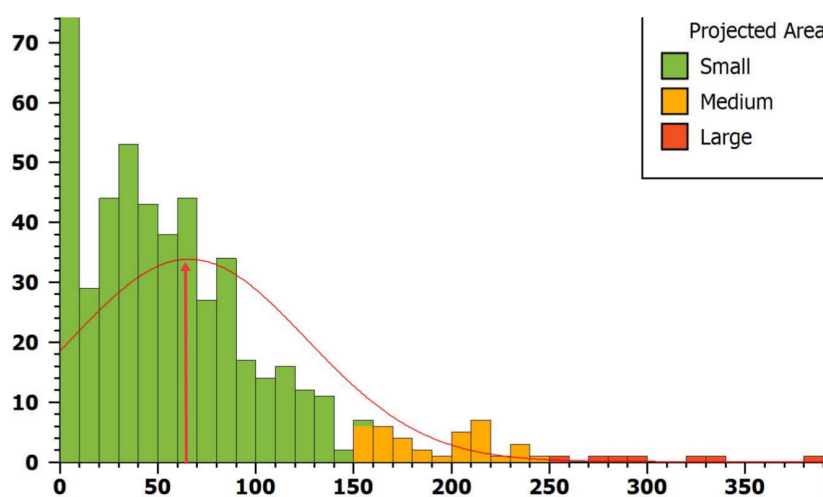


Fig. 8. Particle analysis using an AFM microscope.

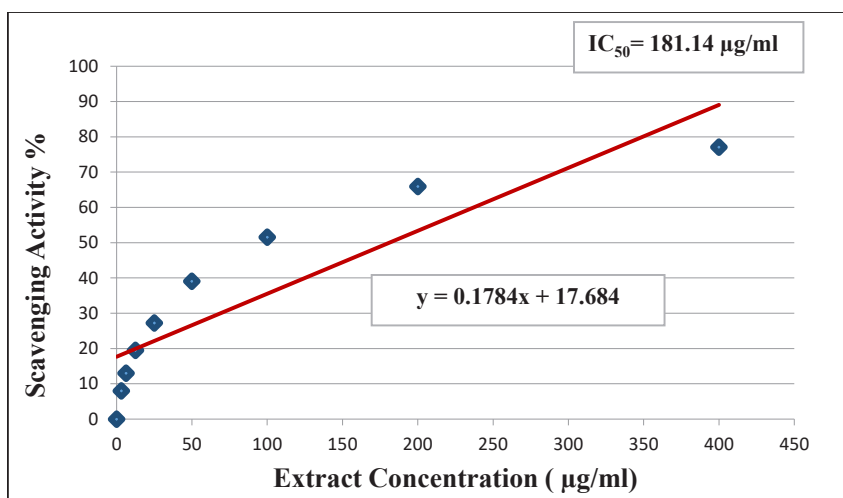


Fig. 9. Activity of the aqueous extract of *D. wallichii* leaves in DPPH free radical scavenging.

extract of *D. wallichii* [32].

DPPH free radical scavenging activity

The antioxidant activity of the aqueous extract of *D. wallichii* leaves and ZnO-NPs was evaluated using the DPPH free radical scavenging assay. As shown in (Table 1 and Fig. 9), the plant extract exhibited a dose-dependent scavenging activity, with inhibition percentages of (8.02, 13.01, 19.45, 27.24, 39.09, 51.53, 65.9, and 77.08) at concentrations of 3.125, 6.25, 12.5, 25, 50, 100, 200, and 400 µg/mL, respectively. The highest scavenging activity of the extract was recorded at 400 µg/ml, with an IC₅₀ value of 181.14 µg/mL.

The ZnO-NPs also showed a dose-dependent antioxidant activity, as presented in (Table 1 and

Fig. 10). The scavenging percentages were (12.05, 18.61, 25.17, 34.72, 46.01, 59.34, 70.5, and 81.48)% at concentrations of 3.125, 6.25, 12.5, 25, 50, 100, 200, and 400 µg/ml, respectively. The highest activity was observed at 400 µg/mL, with an IC₅₀ value of 152.20 µg/mL. These results indicate that ZnO-NPs had stronger antioxidant activity than the plant extract, as reflected by their higher scavenging percentage and lower IC₅₀ value.

The antioxidant activity of the plant extract may be related to its phytochemical constituents, especially phenolic and flavonoid compounds, which can neutralize free radicals by donating hydrogen atoms or electrons [33,34]. The higher activity of ZnO-NPs may be attributed to their nanoscale size and large surface area, which

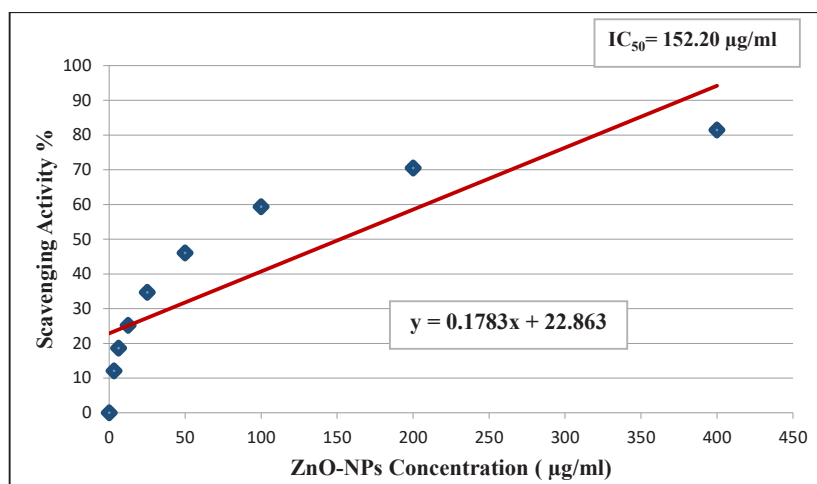


Fig. 10. Activity of ZnO-NPs in DPPH free radical scavenging.

Table 1. The percentage of DPPH scavenging activity for the extract and ZnO-NP.

Concentration (µg/ml)	Scavenging activity of Extract % Mean +SD	Scavenging activity of ZnO-NPs Mean +SD
3.125	8.02 ± 0.42	12.05 ± 0.56
6.25	13.01 ± 0.61	18.61 ± 0.84
12.5	19.45 ± 0.88	25.17 ± 1.13
25	27.24 ± 1.21	34.72 ± 1.53
50	39.09 ± 1.76	46.01 ± 2.07
100	51.53 ± 2.29	59.34 ± 2.65
200	65.9 ± 2.91	70.5 ± 3.14
400	77.08 ± 3.44	81.48 ± 3.63

increase the number of active sites available for interaction with free radicals [35,36]. Therefore, the DPPH results suggest that green-synthesized ZnO-NPs possess better free radical scavenging efficacy than the aqueous extract of *D. wallichii* leaves.

Toxic Activity Testing Using MTT Assay

The cytotoxic activity of the aqueous extract of *D. wallichii* leaves and ZnO-NPs was evaluated against MCF-7 breast cancer cells and MCF-10 normal breast epithelial cells using the MTT assay.

Cells were treated with different concentrations of each treatment for 24 h, as shown in (Table 2). The results showed a concentration-dependent increase in cell inhibition for both treatments.

The aqueous extract showed inhibition percentages of 5.86, 11.61, 21.52, 34.02, and 46.06% against MCF-7 cells at concentrations of 25, 50, 100, 200, and 400 µg/mL, respectively, with an IC₅₀ value of 437 µg/ml, as shown in (Fig. 11). In MCF-10 normal cells, the extract showed lower inhibition percentages of 2.08, 4.60, 5.40, 9.33, and 14.62% at the same concentrations,

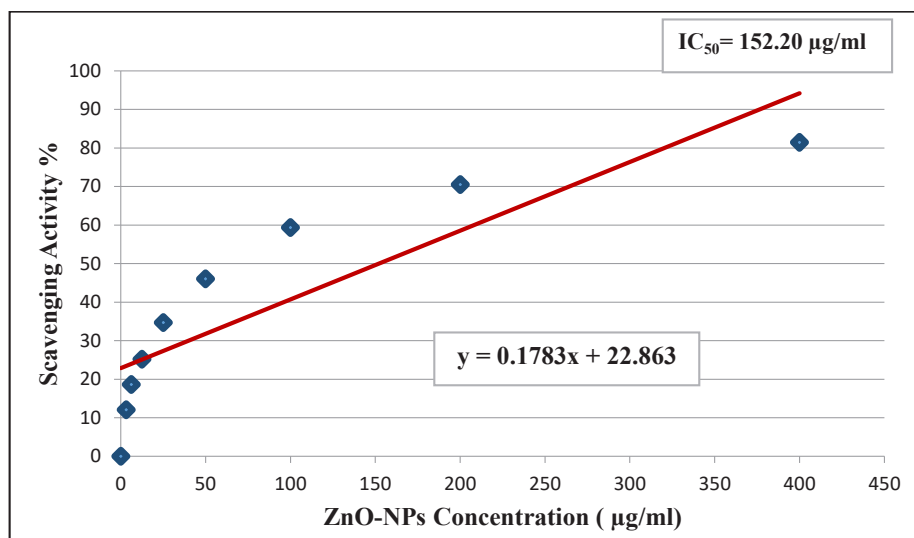


Fig. 11. Cytotoxicity effect of *D. wallichii* extract on MCF-7 and MCF-10 cell lines.

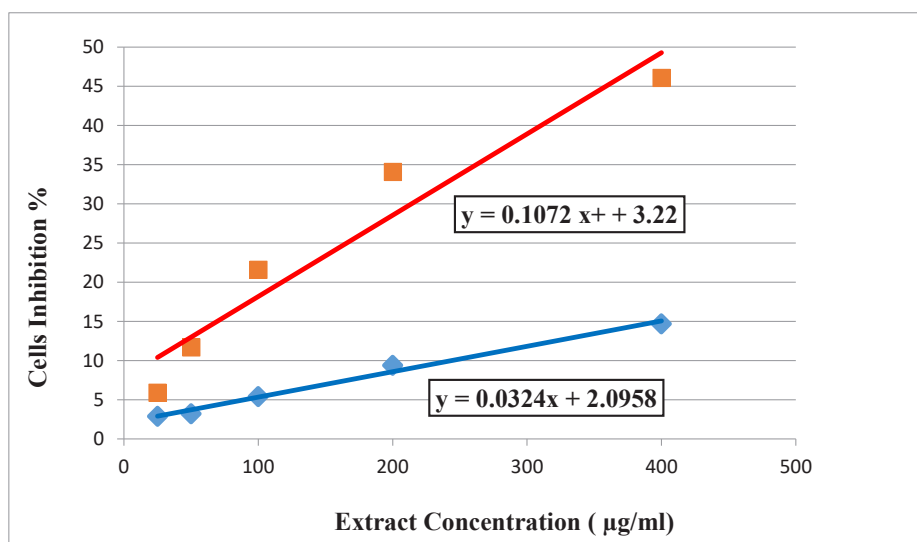


Fig. 12. Cytotoxicity effect of ZnO-NPs on MCF-7 and MCF-10 cell lines.

Table 2. Cytotoxic effect of *D. wallichii* extract and ZnO-NPs on (MCF-7) and (MCF-10) cell lines in vitro by using MTT assay.

Type of Treatments	Type of Cell Line	Cells Inhibition %				
		The Concentrations ($\mu\text{g/ml}$)				
		25	50	100	200	400
The Extract	MCF-7	5.86 \pm 0.5	11.61 \pm 2.2	21.52 \pm 2.4	34.02 \pm 1.9	46.06 \pm 4.1
		a(A)	b(A)	c(A)	d(A)	e(A)
	MCF-10	2.08 \pm 0.4	3.20 \pm 0.5	5.40 \pm 1.0	9.33 \pm 0.8	14.62 \pm 1.4
		a(B)	a(B)	b(B)	c(B)	d(B)
ZnO-NPs	MCF-7	11.22 \pm 3.4	24.34 \pm 1.0	38.65 \pm 1.1	50.42 \pm 1.1	59.49 \pm 2.9
		a(A)	b(A)	c(A)	d(A)	e(A)
	MCF-10	2.20 \pm 0.3	4.60 \pm 0.3	5.90 \pm 0.5	11.03 \pm 1.6	20.71 \pm 1.0
		a(B)	b(B)	b(B)	c(B)	d(B)

Similar letters indicate no significant differences between groups ($p > 0.05$), whereas different letters indicate significant differences between groups ($p \leq 0.05$). Capital letters were used for comparisons between the two cell lines at the same concentration within each treatment, while small letters were used for comparisons among different concentrations within each cell line for each treatment.

respectively.

ZnO-NPs exhibited stronger cytotoxic activity against MCF-7 cells, with inhibition percentages of 11.22, 24.34, 38.65, 50.42, and 59.49% at concentrations of 25, 50, 100, 200, and 400 $\mu\text{g/ml}$, respectively, and an IC_{50} value of 269.47 $\mu\text{g/ml}$. In contrast, their effect on MCF-10 cells was lower, with inhibition percentages of 2.20, 4.60, 5.90, 11.03, and 20.71% at the same concentrations, as shown in (Fig. 12). These results indicate that ZnO-NPs were more effective than the plant extract against MCF-7 cells, while both treatments showed lower toxicity toward normal MCF-10 cells.

The cytotoxic activity of the plant extract may be related to its phytochemical constituents, especially phenolic compounds and their derivatives, which can interfere with cancer cell survival mechanisms [37]. These compounds may affect reactive oxygen species pathways, modulate inflammatory signaling such as NF- κ B, and promote apoptosis in cancer cells [38]. The stronger activity of ZnO-NPs may be attributed to their ability to interact with cancer cells, disturb mitochondrial function, and increase ROS production, leading to oxidative stress and cell death [39]. In addition, ZnO-NPs may dissolve in acidic intracellular compartments such as lysosomes, releasing Zn^{2+} ions and disturbing ionic balance, which can further activate cell death pathways [40].

Similar letters indicate no significant differences between groups ($p > 0.05$), whereas different letters indicate significant differences between groups ($p \leq 0.05$).

Capital letters were used for comparisons between the two cell lines at the same concentration within each treatment, while small letters were used for comparisons among different concentrations within each cell line for each treatment.

CONCLUSION

The aqueous extract of *D. wallichii* was a potent antioxidant and was rich in various phytochemical compounds with potential biological activity. The extract exhibited anticancer and cytotoxic effects against the human breast cancer cell line MCF-7, while the normal human breast epithelial cell line MCF-10 was not significantly affected, indicating a possible degree of safety toward normal cells. The green synthesized zinc oxide nanoparticles (ZnO-NPs) prepared using *D. wallichii* extract showed enhanced cytotoxic and anticancer activity against MCF-7 cells compared with the plant extract alone. The combination of *D. wallichii* extract with ZnO nanoparticles demonstrated a greater inhibitory effect on MCF-7 cancer cells than either treatment alone, suggesting a synergistic therapeutic potential.

CONFLICT OF INTEREST

The authors declare that there is no conflict of interests regarding the publication of this manuscript.

REFERENCES

1. Hadi DJ, Younus MA. Bio Synthesis, Characterization, and

- Evaluation of the Anticancer Activity of Gold and Silver Nanoparticles and Their Polyacetal Nanocomposite. *Iraqi Journal of Science*. 2024;6824-6841.
2. Zeinali R, Zaeifi D, Zolfaghari-Moghaddam SY, Paul M, Biazar E. Current Advances in Nanocarriers for Cancer Therapy. *International Journal of Nanomedicine*. 2025;Volume 20:12217-12262.
 3. Perveen F, Farmn U, Assad R, Haidar Z, Waseem A, Muhammad K, et al. A Review on Nanotechnology-Driven Green Synthesis of Silver Nanoparticles Using *Nigella Sativa*. *Insights-Journal of Health and Rehabilitation*. 2025;3(3 (Health and Rehab)):121-128.
 4. Jafarzadeh S, Nooshkam M, Zargar M, Garavand F, Ghosh S, Hadidi M, et al. Green synthesis of nanomaterials for smart biopolymer packaging: challenges and outlooks. *Journal of Nanostructure in Chemistry*. 2023;14(2):113-136.
 5. Al-darwesh MY, Ibrahim SS, Mohammed MA. A review on plant extract mediated green synthesis of zinc oxide nanoparticles and their biomedical applications. *Results in Chemistry*. 2024;7:101368.
 6. Davis CC, Choisy P. Medicinal plants meet modern biodiversity science. *Curr Biol*. 2024;34(4):R158-R173.
 7. Al-Amery SMH, Naji NM, Kadhim RE, Merhij EI, Jassim YA. Taxonomical, Phytochemical, Antioxidant and Antibacterial Study of Some Medicinal Plants of the Myrtaceae Family. *Nativa*. 2024;12(4):795-805.
 8. Tariq H, Ishfaq K, Chauhdari T, Sarwar F, Ismail F. Neuroprotective potential of dombeya wallichii ameliorates Parkinson's activity in paraquat-induced Rat's model via modulation of neuro-inflammatory cytokines. *J Ayurveda Integr Med*. 2025;16(4):101162.
 9. Herur S, Kashyap S, Vashisht S, Shankara D, Shree P, Bayineni V. Antibacterial activity by Dombeya wallichii plant extracts obtained by ultrasound-assisted extraction. *Journal of Emerging Investigators*. 2023.
 10. Molla G, Bitew M. The Future of Cancer Diagnosis and Treatment: Unlocking the Power of Biomarkers and Personalized Molecular-Targeted Therapies. *Journal of Molecular Pathology*. 2025;6(3):20.
 11. Durrani S, Alamri S, Zaman S, Alobaisi Y, Hamdan A, Alharbi M, et al. Differences in Clinical, Epidemiological, and Pathological Features of Breast Cancer in the Saudi Population: An Analytical Cross-Sectional Single Institution Study. *Healthcare*. 2025;13(7):737.
 12. Łukasiewicz S, Czezelewski M, Forma A, Baj J, Sitarz R, Stanisławek A. Breast Cancer—Epidemiology, Risk Factors, Classification, Prognostic Markers, and Current Treatment Strategies—An Updated Review. *Cancers (Basel)*. 2021;13(17):4287.
 13. Zafar A, Khatoun S, Khan MJ, Abu J, Naeem A. Advancements and limitations in traditional anti-cancer therapies: a comprehensive review of surgery, chemotherapy, radiation therapy, and hormonal therapy. *Discover Oncology*. 2025;16(1).
 14. Xu W, Lee M-K. Development and evaluation of lipid nanoparticles for paclitaxel delivery: a comparison between solid lipid nanoparticles and nanostructured lipid carriers. *Journal of Pharmaceutical Investigation*. 2015;45(7):675-680.
 15. Al-Sultany FH, Al-Hussaini IM, Al-Saadi AH. Studying Hypoglycemic Activity of *Cuscuta chinesis* Lam. on Type 1 Diabetes Mellitus in White Male Rats. *Journal of Physics: Conference Series*. 2019;1294(6):062020.
 16. Salih AM, Al-Qurainy F, Khan S, Tarroum M, Nadeem M, Shaikhaldein HO, et al. Biosynthesis of zinc oxide nanoparticles using *Phoenix dactylifera* and their effect on biomass and phytochemical compounds in *Juniperus procera*. *Sci Rep*. 2021;11(1).
 17. Hussien EM, Endalew SA. In vitro antioxidant and free-radical scavenging activities of polar leaf extracts of *Vernonia amygdalina*. *BMC Complementary Medicine and Therapies*. 2023;23(1).
 18. Mohammed FH, Al-saily HMN, Jassim YA, Hassan WS. Evaluation of the anticancer potential of Iraqi Date *Phoenix dactylifera* L. seed extract on breast cancer MCF7 and prostate cancer PC3 cell lines. *Journal of Applied and Natural Science*. 2024;16(3):1308-1316.
 19. Sasidharan S, Tey L-H, Djearamane S, Mahmud Ab Rashid NK, Pa R, Shing Wong L, et al. Development of novel biofilm using *Musa acuminata* (waste banana leaves) mediated biogenic zinc oxide nanoparticles reinforced with chitosan blend. *Journal of King Saud University - Science*. 2024;36(3):103080.
 20. Al-Mur BA. Green Zinc Oxide (ZnO) Nanoparticle Synthesis Using Mangrove Leaf Extract from *Avicenna marina*: Properties and Application for the Removal of Toxic Metal Ions (Cd^{2+} and Pb^{2+}). *Water*. 2023;15(3):455.
 21. Vithalani P, Bhatt N. Green Synthesis of Zinc Oxide Nanoparticles using *Allium sativum* Plant Extract and Photocatalytic Activity of Rhodamine B. *Environ Ecol*. 2024;42(2):374-382.
 22. Wan Mat Khalir WKA, Shameli K, Jazayeri SD, Othman NA, Che Jusoh NW, Hassan NM. Biosynthesized Silver Nanoparticles by Aqueous Stem Extract of *Entada spiralis* and Screening of Their Biomedical Activity. *Frontiers in Chemistry*. 2020;8.
 23. Jayachandran A, T.R A, Nair AS. Green synthesis and characterization of zinc oxide nanoparticles using *Cayratia pedata* leaf extract. *Biochemistry and Biophysics Reports*. 2021;26:100995.
 24. Fouda A, Saied E, Eid AM, Kouadri F, Alemam AM, Hamza MF, et al. Green Synthesis of Zinc Oxide Nanoparticles Using an Aqueous Extract of *Punica granatum* for Antimicrobial and Catalytic Activity. *Journal of Functional Biomaterials*. 2023;14(4):205.
 25. Hsieh K-P, Naruphontjirakul P, Chen J-H, Ko C-S, Lin C-W, Su W-T. Incorporation of Zinc Oxide Nanoparticles into PCL Nanofibers to Enhance Osteogenic Differentiation of Periodontal Ligament Stem Cells. *Materials*. 2025;18(10):2295.
 26. Mohammed LY. Antioxidant Activity and Physico-Chemical Properties of Green Synthesized Zinc Oxide Nanoparticles Using *Eruca Sativa* Leaf Extract. *Science Journal of University of Zakho*. 2023;11(4).
 27. Aldeen TS, Ahmed Mohamed HE, Maaza M. ZnO nanoparticles prepared via a green synthesis approach: Physical properties, photocatalytic and antibacterial activity. *Journal of Physics and Chemistry of Solids*. 2022;160:110313.
 28. Kamal A, Akhtar MS, Nazish M, Tahira KT, Rahman KU, Iqbal A, et al. Plant phytochemicals-mediated synthesis of zinc oxide nanoparticles with antimicrobial, pharmacological, and environmental applications. *An Acad Bras Cienc*. 2024;96(4).
 29. Sohrabi S, Kargar M, Ramezani A, Moazamian E,

- Ebrahiminezhad A, Berenjian A. Phytochemical-Mediated Synthesis of Zinc Oxide Nanoparticles with Enhanced Antimicrobial Properties. *Mol Biotechnol.* 2025.
30. Alprol AE, Eleryan A, Abouelwafa A, Gad AM, Hamad TM. Green synthesis of zinc oxide nanoparticles using *Padina pavonica* extract for efficient photocatalytic removal of methylene blue. *Sci Rep.* 2024;14(1).
31. Abbas Z, Irshad M, Ali S, Summer M, Rasheed A, Jawad M. Radical scavenging potential of spectrophotometric, spectroscopic, microscopic, and EDX observed zinc oxide nanoparticles from leaves, buds, and flowers extract of *Bauhinia Variegata* Linn: A thorough comparative insight. *Microscopy Research and Technique.* 2024;87(9):2121-2133.
32. Ali Ibrahim T, A. Salman T, al-Rudha Abbas S. Biosynthesis of Zinc Oxide Nanoparticles Using Orange Peels Extract for Biological Applications. *Plant Archives.* 2021;21(Suppliment-1):329-332.
33. Gulcin I, Alwaseel SH. DPPH Radical Scavenging Assay. *Processes.* 2023;11(8):2248.
34. Ahmad Z, Rauf A, Orhan IE, Mubarak MS, Akram Z, Islam MR, et al. Antioxidant Potential of Polyphenolic Compounds, Sources, Extraction, Purification and Characterization Techniques: A Focused Review. *Food Science and Nutrition.* 2025;13(12).
35. Jaishi DR, Ojha I, Bhattarai G, Baraili R, Pathak I, Ojha DR, et al. Plant-mediated synthesis of zinc oxide (ZnO) nanoparticles using *Alnus nepalensis* D. Don for biological applications. *Heliyon.* 2024;10(20):e39255.
36. Haiouani K, Hegazy S, Alsaeedi H, Bechelany M, Barhoum A. Green Synthesis of Hexagonal-like ZnO Nanoparticles Modified with Phytochemicals of Clove (*Syzygium aromaticum*) and *Thymus capitatus* Extracts: Enhanced Antibacterial, Antifungal, and Antioxidant Activities. *Materials.* 2024;17(17):4340.
37. Bakrim S, El Omari N, El Hachlafi N, Bakri Y, Lee L-H, Bouyahya A. Dietary Phenolic Compounds as Anticancer Natural Drugs: Recent Update on Molecular Mechanisms and Clinical Trials. *Foods.* 2022;11(21):3323.
38. Tubtimsri S, Chuenbarn T, Manmuan S. Quercetin triggers cell apoptosis-associated ROS-mediated cell death and induces S and G2/M-phase cell cycle arrest in KON oral cancer cells. *BMC Complementary Medicine and Therapies.* 2025;25(1).
39. Yan Y, Huang W, Lu X, Chen X, Shan Y, Luo X, et al. Zinc oxide nanoparticles induces cell death and consequently leading to incomplete neural tube closure through oxidative stress during embryogenesis. *Cell Biology and Toxicology.* 2024;40(1).
40. Cho W-S, Duffin R, Howie SEM, Scotton CJ, Wallace WAH, MacNee W, et al. Progressive severe lung injury by zinc oxide nanoparticles; the role of Zn²⁺ dissolution inside lysosomes. *Part Fibre Toxicol.* 2011;8(1).