



Harnessing plant extracts for zinc oxide nanoparticles: a greener route to enhanced biocompatibility and therapeutic potential

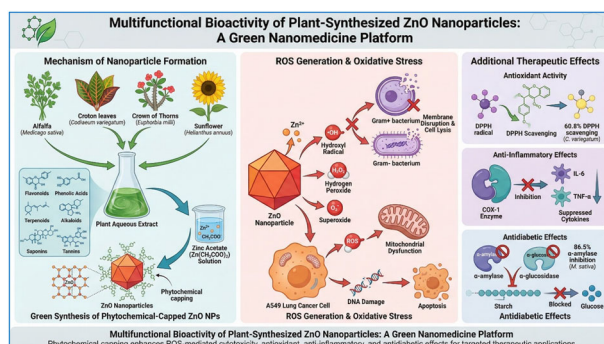
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Abstract

The green synthesis of metal oxide nanoparticles has emerged as a sustainable and eco-friendly alternative to conventional chemical methods. Utilizing plant-derived phytochemicals as natural reducing and stabilizing agents, this approach eliminates hazardous chemicals and energy-intensive processes. Beyond environmental benefits, green synthesis influences the physicochemical and biological properties of nanoparticles by enhancing surface functionality, stability, and bioactivity. Zinc oxide nanoparticles are widely favored in biomedical applications due to their biocompatibility, affordability, and low toxicity. Their therapeutic effects, especially in anticancer and antibacterial applications, arise from their ability to generate reactive oxygen species, release Zn^{2+} ions, and induce apoptosis. This study synthesized ZnO NPs via chemical and green routes using aqueous extracts from *Medicago sativa*, *Euphorbia milii*, *Codiaeum variegatum*, and *Helianthus annuus*. Structural analysis confirmed that green synthesis did not alter the ZnO crystalline structure. However, biological activities varied significantly by plant extract. *Codiaeum variegatum*-mediated ZnO NPs showed superior antioxidant activity (60.8% DPPH scavenging) and strong cytotoxicity against A549 lung cancer cells (94.7% reduction). Chemically synthesized ZnO NPs exhibited notable anti-inflammatory activity (90.4%). ZnO NPs from *Medicago sativa* demonstrated potent antidiabetic effects via α -amylase (86.5%) and α -glucosidase (85.7%) inhibition. All ZnO NPs inhibited the growth of *Escherichia coli*, *Klebsiella pneumonia*, *Streptococcus pyogenes*, and *Staphylococcus aureus* with maximum MBC value 47.4 mg/ml of chemogenic ZnO NPs against *S. aureus* and 44.4 mg/ml for biogenic ZnO NPs against *S. pyogenes*. Additionally, *Medicago sativa* ZnO NPs recorded the highest suppression of *S. pyogenes* biofilm formation by 79%. The obtained results highlight the role of green synthesis as an effective strategy to tailor ZnO NP bioactivity for biomedical applications.

Graphical Abstract



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Keywords Green synthesis · Zinc oxide nanoparticles · Anti-inflammatory · α -amylase · α -glucosidase · Antioxidant activity · Antibacterial

Highlights

- Green synthesis of ZnO NPs using four plant extracts provides a sustainable, eco-friendly alternative to chemical methods.
- Plant-mediated ZnO NPs show superior and tunable bioactivity: antioxidant, antibacterial, anticancer, anti-diabetic, and anti-biofilm effects.
- *Codiaeum variegatum* ZnO NPs exhibit strong anticancer (94.7% cell reduction) and antioxidant (60.8% DPPH scavenging) activity.
- *Medicago sativa* ZnO NPs display potent anti-diabetic action via α -amylase and α -glucosidase inhibition, outperforming acarbose.
- All green ZnO NPs inhibit bacterial growth and biofilm formation, with *Medicago sativa* NPs reducing *S. pyogenes* biofilm by 79%.
- Structural analysis confirms pure, crystalline ZnO NPs with phytochemical capping, enhancing biocompatibility.

1 Introduction

Recently, green nanotechnology has arisen as a transformative field that bridges sustainability and advanced material science. As concerns about ecological degradation and biomedical toxicity escalate, researchers are turning toward eco-friendly nanoparticles synthesis techniques that align with principles of green chemistry [1, 2]. Among metal oxide nanoparticles, zinc oxide nanoparticles (ZnO NPs) have attracted special interest due to their wide spectrum of applications in antimicrobial therapies, anticancer treatments, wound healing, and drug delivery systems [3, 4]. However, conventional routes of ZnO NPs synthesis often rely on hazardous chemicals, high temperatures, and energy-intensive procedures, which may compromise their biocompatibility and limit their clinical applications. As a result, there is an urgent need to develop alternative synthesis strategies that are not only sustainable and environmentally benign but also capable of enhancing the therapeutic efficacy of the nanoparticles. Plant-mediated green synthesis has emerged as a promising solution to these challenges. This approach utilizes phytochemicals such as flavonoids, alkaloids, terpenoids, and phenolics from plant extracts as reducing, capping, and stabilizing agents. This method eliminates the need for toxic chemicals and often functionalizes the nanoparticle surface, potentially increasing bioactivity and biocompatibility. Furthermore, it offers advantages in scalability, cost-effectiveness, and reduced environmental impact [5, 6].

A growing body of literature demonstrates that green-synthesized ZnO NPs exhibit enhanced biological activities. For instance, they have shown promising and selective anticancer efficacy in vitro, demonstrating potent cytotoxicity against a range of cancer types including lung, colon, and sarcoma models while showing significantly less

toxicity to normal cells, highlighting their potential as safer chemotherapeutic agents [7–9]. The antioxidant capacity of green ZnO NPs, frequently attributed to the phytochemicals from the mediating plant extract, has been consistently confirmed through assays like DPPH, showing free radical scavenging abilities comparable to standard antioxidants [10–14].

The antibacterial efficacy of biosynthesized ZnO NPs is well-documented, showing broad-spectrum activity against both Gram-positive and Gram-negative pathogens. Antibacterial action is often influenced by bacterial cell wall structure, with several studies reporting a stronger effect on Gram-negative bacteria. Additionally, these NPs have shown significant potential in inhibiting and disrupting bacterial biofilms [15–20]. Anti-inflammatory studies further reveal that green-synthesized ZnO NPs can achieve inhibition rates comparable to, or even surpassing, standard pharmaceutical drugs like diclofenac and indomethacin [11, 18, 21]. In the context of diabetes management, these NPs have demonstrated significant inhibitory effects on key enzymes like α -amylase and α -glucosidase, both in vitro and in vivo, suggesting a promising role as natural anti-diabetic agents [22, 23].

In the present study, we explore the green synthesis of ZnO NPs using extracts from four distinct medicinal and ornamental plants: *Medicago sativa* (alfalfa), *Euphorbia milii* (crown of thorns), *Codiaeum variegatum* (garden croton), and *Helianthus annuus* (sunflower). These plants were selected for their rich profiles of bioactive phytochemicals, which are expected to influence the physical properties and biological performance of the synthesized NPs. *Medicago sativa* L. (alfalfa) is known for its high content of flavonoids, saponins, and phytoestrogens, contributing to its notable antioxidant and antimicrobial properties [24, 25]. *Euphorbia milii* Des Moul. (crown of thorns)

contains diverse bioactive compounds, including terpenoids and phenolics, which underline its reported anti-inflammatory and anticancer potential [26, 27]. *Codiaeum variegatum* (L.) A. Juss. (garden croton) is rich in flavonoids and phenolic compounds, which are associated with strong antioxidants and antimicrobial activities [28, 29]. Finally, *Helianthus annuus* L. (sunflower) possesses a variety of phytoconstituents such as flavonoids, alkaloids, and saponins, making it a suitable candidate for mediating nanoparticle synthesis [30, 31].

This study aims to synthesize ZnO NPs using a green approach with the aforementioned plant extracts. A comparative analysis will be conducted between these green-synthesized NPs and those produced by conventional chemical methods to evaluate differences in their morphology, crystallinity, and surface characteristics. Furthermore, the biological activities of the synthesized NPs, including cytotoxicity, antioxidant capacity, antimicrobial efficacy, anti-inflammatory effects, and antidiabetic potential, will be comprehensively assessed.

2 Materials and methods

This work was approved by the Ethics Committee of South Valley University, Faculty of Science (Permit Number: 003/01/25). All procedures were conducted in accordance with the relevant institutional guidelines and regulations. No human or animal subjects were involved in this study.

2.1 Preparation of plant aqueous extract

The fresh leaves of *Helianthus annuus* (sunflower), *Euphorbia milii*, *Codiaeum variegatum* (croton), and *Medicago sativa* L. (alfalfa) were gathered and properly cleaned with tap water and then distilled water to get rid of any pollutants or dust that might have adhered. After being washed, the leaves were cut into little pieces and allowed to air dry. For every plant species, 50 grams of the prepared leaf were added to 500 mL of deionized water and heated on a hot plate with magnetic stirring for 30 min at 80 °C. The mixtures were allowed to reach room temperature before being filtered three times using Whatman No. 1 filter paper. Plant extracts were collected and stored at 4 °C for use in ZnO NPs biosynthesis [32].

2.2 Preparation of ZnO NPs

A total of five ZnO NPs samples were synthesized using the coprecipitation method, one via a conventional (chemogenic) route and four through green (biogenic) synthesis utilizing extracts from the selected plants [29]. All synthesis procedures were carried out under identical experimental

conditions, with the only variation being the reducing agent. In the chemogenic synthesis, sodium hydroxide (NaOH) was employed solely as both the precipitating and reducing agent. In contrast, the biogenic ZnO NPs were synthesized using 40 mL of aqueous plant extracts (from *Medicago sativa*, *Euphorbia milii*, *Codiaeum variegatum*, and *Helianthus annuus*) as the green reducing agents. In a typical procedure, 4.39 g of zinc acetate dihydrate was dissolved in 100 mL of distilled water and stirred at 500 rpm for 30 min at 80 °C. The plant extract or NaOH was then introduced, and the pH of the reaction mixture was adjusted to 12 by gradually adding sodium hydroxide. The reaction was allowed to proceed with continuous stirring for an additional 2 h to facilitate complete precipitation. The resulting precipitate was washed several times with a 5:1 mixture of distilled water and ethanol to remove residual impurities and then dried at 100 °C to obtain the final ZnO nanopowders (Fig. 1).

2.3 Characterization of green synthesized ZnO NPs

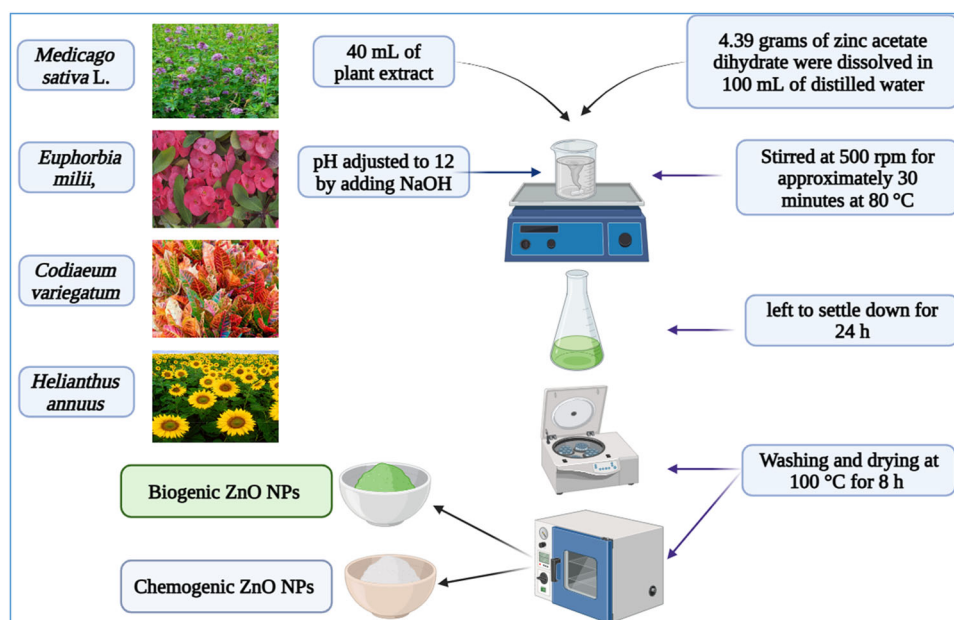
X-ray diffraction (XRD) analysis was performed using an X'Pert PRO PANalytical diffractometer, operating with Cu-K α radiation (wavelength $\lambda = 1.54056$ Å). The system was set to a voltage of 40 kV and a current of 30 mA. The synthesized samples were characterized by Fourier Transform Infrared (FTIR) spectroscopy using the KBr pellet technique to identify the functional groups present. Analysis was performed with an FTIR Model 6100 (Jasco, Japan), operating at a resolution of 4.00 cm⁻¹ over a wavenumber range of 4000 to 400 cm⁻¹. The surface morphology and structural features of the samples were examined using a high-resolution transmission electron microscope (JEOL JEM 2100, Japan). Additionally, optical absorption spectra of the ZnO nanoparticles were recorded using a UV-Visible spectrophotometer (SPECORD 200 PLUS, Analytik Jena, Germany).

2.4 Bioactivity of ZnO NPs

2.4.1 Anticancer activity

The anticancer activity of both chemogenic and biogenic ZnO NPs was assessed using a standard MTT assay on A549 human lung carcinoma cell lines (obtained from the Egyptian Holding Company for Biological Products and Vaccines (Vacsera), Giza, Egypt). Briefly, cells were seeded into 96-well tissue culture plates at a density of 10⁵ cells/mL (100 μ L per well) in complete RPMI medium supplemented with 10% fetal bovine serum (FBS) and 1% penicillin/streptomycin (Life Science Production, UK). The plates were incubated at 37 °C for 24 h to allow the formation of a confluent monolayer. Once confluency was achieved, the growth medium was removed, and the cells were washed twice with phosphate-buffered

Fig. 1 Schematic illustration of synthesis of Chemogenic and Biogenic ZnO NPs using different plant extracts (*Medicago sativa* L., *Euphorbia milii*, *Codiaeum variegatum*, and *Helianthus annuus*)



saline (PBS; Lonza, Germany). Serial dilutions of the ZnO NPs samples, ranging from 31.25 to 1000 $\mu\text{g/mL}$, were prepared in RPMI medium. Then, 100 μL of each concentration was added to the corresponding wells. Three wells containing only maintenance medium were used as negative controls, while doxorubicin served as a positive control. MTT solution (3-[4,5-dimethylthiazol-2-yl]-2,5-diphenyltetrazolium bromide; SERVA Electrophoresis GmbH, Heidelberg, Germany) was prepared at 5 mg/mL in PBS, and 20 μL was added to each well. To ensure uniform mixing, the plate was placed on a shaking platform and agitated at 150 rpm for 5 min, then incubated at 37 $^{\circ}\text{C}$ in a 5% CO_2 atmosphere to allow viable cells to metabolize MTT into formazan crystals. After incubation, the medium was carefully removed, and wells were gently blotted to remove any remaining liquid if necessary. Formazan crystals were solubilized by adding 200 μL of dimethyl sulfoxide (DMSO; Alfa Aesar, USA) to each well, followed by agitation at 150 rpm for 5 min to achieve complete dissolution. Absorbance was measured at 560 nm with background correction at 620 nm. The optical density values correlate directly with the number of viable cells [32]. The percentage of cell viability was determined using Eq. 1:

$$\text{Cell viability}(\%) = \frac{\text{OD of untreated cells} - \text{OD of treated cells}}{\text{OD of untreated cells}} \quad (1)$$

2.4.2 Antioxidant activity

The antioxidant activity of ZnO NPs was evaluated using the 2,2-diphenyl-1-picrylhydrazyl (DPPH) assay. DPPH

(SERVA Electrophoresis GmbH, Heidelberg, Germany) is a stable free radical compound characterized by a deep purple color, which makes it an excellent scavenger of other free radicals. In the presence of an antioxidant, DPPH radicals are reduced, leading to a color change from purple to colorless. For the assay, a stock solution from ZnO NPs samples, plants extract and ascorbic acid as positive control were prepared by dissolving 4 mg of each sample in 4 mL of methanol. From this stock, five serial dilutions were made at concentrations of 500, 250, 125, 62.5, and 31.25 $\mu\text{g/mL}$. Then, 3 mL of DPPH solution was added to each dilution, and the mixtures were incubated in the dark at room temperature for 30 min. After incubation, the absorbance (Abs) of both the samples and the control was measured at 517 nm using a spectrophotometer. The free radical scavenging activity (%) was calculated using Eq. 2 [33]:

$$\text{Free radical scavenging activity}(\%) = \left(\frac{\text{Abs}_{\text{control}} - \text{Abs}_{\text{sample}}}{\text{Abs}_{\text{control}}} \right) \times 100 \quad (2)$$

The IC_{50} value is obtained by graphically analyzing the plot of inhibition percentage against the extract. The IC_{50} is the minimum concentration of an antioxidant-containing material needed to neutralize 50% of the original DPPH radical.

2.4.3 Antibacterial activity

The antibacterial activity of ZnO NPs was assessed against four bacterial strains: *Staphylococcus aureus* (BLSVU9), *Streptococcus pyogenes* (BLSVU11), *Escherichia coli*

(BLSUV1), and *Klebsiella pneumoniae* (BLSVU2), all obtained from the Bacteriology Laboratory, Department of Botany and Microbiology, Faculty of Science, South Valley University, Qena, Egypt. The minimum inhibitory concentration (MIC) was determined using a microbroth dilution method with p-iodonitrotetrazolium violet chloride (INT) as a growth indicator. Overnight bacterial cultures grown on tryptic soy agar (TSA) were diluted to an optical density (OD) of 0.001 in tryptic soy broth (TSB). Subsequently, 100 μ L of each bacterial suspension was added to wells of a 96-well microtiter plate containing varying volumes (10–100 μ L) of ZnO NPs samples. TSB alone and TSB with bacteria but without ZnO NPs served as negative and positive controls, respectively. Plates were incubated at 37 °C for 24 h. After incubation, 40 μ L of INT solution (0.2 mg/mL) was added to each well, followed by an additional incubation of 2 h at 37 °C [34]. The MIC was recorded as the lowest nanoparticle concentration that prevented any visible color change, indicating bacterial growth inhibition. To determine the minimum bactericidal concentration (MBC), 50 μ L aliquots from wells showing no visible growth in the MIC assay were plated onto fresh TSA plates. After 24 h of incubation at 37 °C, viable colonies were counted. The MBC was defined as the lowest concentration at which a 99.9% reduction in colony-forming units (CFU), corresponding to a 3-log reduction from the initial inoculum, was observed. The detection limit of this assay was 10¹ CFU/mL [35].

2.4.4 Antibiofilm activity

The static biofilm-forming ability of the selected bacterial isolates was initially assessed using the crystal violet staining method in 96-well microtiter plates, as previously described [36]. Bacterial cultures grown in tryptic soy broth (TSB) were adjusted to an optical density (OD) of 0.02 at 595 nm. A volume of 130 μ L from each bacterial suspension was transferred into U-bottom 96-well microtiter plates (Sterilin, UK) and incubated at 37 °C for 24 h to allow biofilm formation. After incubation, wells were gently washed with distilled water to remove planktonic cells. The adherent biofilms were stained with 0.1% crystal violet solution for 10 min. Excess stains were rinsed off with distilled water, and the bound dye was solubilized with 96% ethanol. The optical density (OD) was then measured at 595 nm using an Infinite® F50 Robotic microplate reader (Austria) to quantify the biofilm biomass [37]. For biofilm inhibition assays, 130 μ L of the bacterial suspension was added to 96-well plates and incubated at 37 °C for 24 h to initiate biofilm formation. After this period, 30 μ L of the test sample extract was added to each well, followed by a second 24 h incubation. The wells were then washed six times with distilled water, stained with 160 μ L of 0.1% crystal

violet, and biofilm quantification was performed as previously described [38].

2.4.5 Anti-inflammatory activity

The anti-inflammatory activity of ZnO NPs was assessed using a cyclooxygenase-1 (COX-1) inhibition assay [39]. The COX-1 enzyme was pre-incubated with various concentrations of ZnO NPs (ranging from 0.5 to 1000 μ g/mL), dissolved in dimethyl sulfoxide (DMSO), for 5 min at room temperature (25 °C) in the presence of hematin (1 μ M). The reaction was initiated by the addition of a substrate mixture containing arachidonic acid (50 μ M), phenol (500 μ M), 1-leuco-dichlorofluorescein (20 μ M), and hematin, prepared in 1 mL of 0.1 M Tris-HCl buffer (pH 8.0). Following substrate addition, the absorbance was measured at 502 nm using a Milton Roy Spectronic 1201 UV-Vis spectrophotometer. Blanks were prepared under identical conditions without enzyme addition. Celecoxib was used as a positive control. The percentage of inhibitory was evaluated using Eq. 3 [39]:

$$(COX - 1)Inhibition\% = \left(\frac{Abs_{control} - Abs_{sample}}{Abs_{control}} \right) \times 100 \quad (3)$$

The IC₅₀ value (μ g/mL), representing the concentration required to inhibit 50% of COX-1 activity, was calculated from the inhibition curve.

2.4.6 Antidiabetic activity

The antidiabetic potential of the synthesized ZnO NPs was assessed by evaluating their inhibitory effects on two key carbohydrate-hydrolyzing enzymes: α -amylase and α -glucosidase. The α -amylase inhibition assay was performed using the 3,5-dinitrosalicylic acid (DNSA) method. ZnO NPs samples were initially dissolved in 10% DMSO and further diluted in phosphate buffer (0.02 M Na₂HPO₄/NaH₂PO₄ containing 0.006 M NaCl, pH 6.9) to obtain concentrations ranging from 1.9 to 1000 μ g/mL. A reaction mixture containing 200 μ L of α -amylase (2 U/mL) and 200 μ L of each test sample was incubated at 30 °C for 10 min. Subsequently, 200 μ L of 1% soluble starch was added, and the mixture was incubated for an additional 3 min. The reaction was terminated by adding 200 μ L of DNSA reagent, followed by heating at 85–90 °C for 10 minutes in a water bath. After cooling, the reaction mixtures were diluted with 5 mL of distilled water. Absorbance was measured at 540 nm using a Biosystem 310 UV-Vis spectrophotometer. Controls (without extract) and blanks (without enzymes) were included. The percentage of α -amylase inhibition was calculated using

Eq. 4 [40]:

$$\alpha - \text{amylase inhibition}(\%) = \frac{A_{\text{Control}} - A_{\text{Sample}}}{A_{\text{Control}}} \times 100 \quad (4)$$

Wherein A_{Control} is the absorbance of the control without protein extract, and A_{Sample} is the absorbance of sample containing the protein extract

For the α -glucosidase inhibition assay, yeast α -glucosidase and *p*-nitrophenyl- α -D-glucopyranoside (pNPG) were used as the enzyme and substrate, respectively. Test samples and acarbose (used as the positive control) were prepared in concentrations ranging from 0.5 to 5.0 mg/mL. In each reaction, 100 μ L of test sample was mixed with 50 μ L of α -glucosidase (1 U/mL) and 250 μ L of 0.1 M phosphate buffer (pH 6.9), followed by pre-incubation at 37 °C for 20 min. Next, 10 μ L of 10 mM pNPG was added, and the mixture was incubated for 30 min. The reaction was terminated by adding 650 μ L of 1 M sodium carbonate. Absorbance was recorded at 405 nm using an Amersham Biosciences spectrophotometer. The α -glucosidase inhibition percentage was calculated using Eq. 5 [40]:

$$\alpha - \text{glucosidase inhibition}(\%) = \frac{A_{\text{Control}} - A_{\text{Sample}}}{A_{\text{Control}}} \times 100 \quad (5)$$

Wherein A_{Control} is the absorbance of the control without protein extract, and A_{Sample} is the absorbance of sample containing the protein extract. In both assays, inhibition percentages were plotted against concentration to determine the IC_{50} values (the concentration at which 50% inhibition of enzymatic activity occurs).

2.5 Statistical analysis

Statistical variation among the obtained results was evaluated using one-way analysis of variance (ANOVA) performed with the Statistical Package for the Social Sciences (SPSS) software. All data are expressed as mean $\pm$ standard deviation (SD) from at least three independent experiments. Differences between groups were considered statistically significant at $P < 0.05$.

3 Results and discussion

3.1 Characterization of ZnO NPs

3.1.1 X-ray Diffraction (XRD)

The present study successfully demonstrated the biogenic synthesis of ZnO NPs using aqueous extracts from four different medicinal plants, *Medicago sativa* L., *Euphorbia*

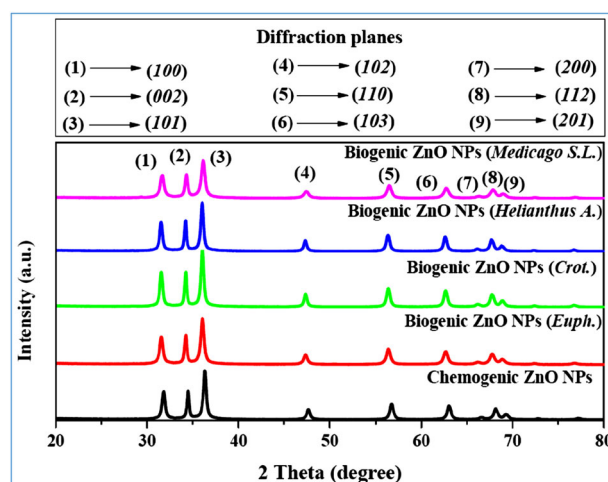


Fig. 2 XRD patterns of Chemogenic and Biogenic ZnO NPs

mili, *Croton*, and *Helianthus annuus*, in comparison to a chemogenic synthesized ZnO NPs. The crystallographic structure and phase purity of the synthesized ZnO NPs were examined by X-ray diffraction (XRD), and the resulting diffraction patterns are illustrated in Fig. 2. All samples exhibited distinct and sharp diffraction peaks, indicative of high crystallinity. The observed 2θ diffraction angles at approximately 31.6°, 34.2°, 36.0°, 47.3°, 56.3°, 62.5°, 66.1°, 67.8°, and 68.8° correspond to the (100), (002), (101), (102), (110), (103), (200), (112), and (201) planes, respectively. These reflections match the standard hexagonal wurtzite structure of ZnO NPs (COD card no. 9011662), confirming the formation of pure crystalline ZnO NPs in all synthesized samples [41]. Notably, the absence of extraneous peaks in all XRD patterns confirms the phase purity of the ZnO NPs, with no detectable secondary phases or impurities. This is a critical finding, as it indicates that the plant-derived phytochemicals used in the green synthesis processes did not induce any significant lattice distortion or phase transformation in the ZnO structure [42].

The obtained diffraction patterns were analyzed to determine the crystallographic planes of the ZnO NPs using standard reference data (COD card no. 9011662). Miller indices (*hkl*) were assigned accordingly, and the interplanar spacing (*d*) values were calculated using Bragg's law, as given in Eq. 6.

$$n\lambda = 2d \sin \theta \quad (6)$$

where *n* is the order of diffraction, λ is the wavelength of the incident X-ray, 1.5406 (Å), *d* is the interplanar spacing (Å), and θ is the peak position (radians).

The synthesized ZnO NPs exhibited a hexagonal wurtzite crystal structure, with lattice constants $a = b = 3.249$ Å and $c = 5.207$ Å. The lattice parameters of the biogenically

Table 1 Crystal structure parameters of Chemogenic and Biogenic ZnO NPs as extracted from XRD data

Sample	Crystal size (nm)	Lattice constant ($a = b$) (°Å)	Lattice constant (c) (°Å)	Unit cell volume (°Å) ³
Chemogenic ZnO NPs	32.78083 ± 8.06	3.25142 ± 0.001	5.2109 ± 0.0018	47.71 ± 0.05
Biogenic ZnO NPs				
<i>Crot.</i>	33.6600 ± 9.11	3.26419 ± 0.005	5.23135 ± 0.008	48.27 ± 0.22
<i>Euph.</i>	36.91839 ± 12.79	3.26416 ± 0.0039	5.2313 ± 0.0063	48.27 ± 0.17
<i>Helianthus A.</i>	37.44992 ± 11.05	3.27018 ± 0.01	5.24095 ± 0.016	48.54 ± 0.44
<i>Medicago S.L.</i>	37.207 ± 26.18	3.25659 ± 0.0043	5.21911 ± 0.007	47.93 ± 0.19

synthesized ZnO NPs were calculated using Eq. 7 [43]:

$$\frac{1}{d^2} = \frac{4}{3} \left(\frac{h^2 + hk + k^2}{a^2} \right) + \frac{l^2}{c^2} \quad (7)$$

where d is the Interplanar spacing (Å), $h k l$ is the miller indices and a, c are the Lattice parameters.

The crystal size of ZnONPs was determined using the well-known Scherer relationship Eq. 8 [44],

$$D = \frac{K\lambda}{\beta \cos \theta} \quad (8)$$

where D is the crystal size, K is the shape constant (~ 0.9), λ is the wavelength, θ is the Bragg angle and β is the observed peak width at half-maximum height.

The crystallite sizes of the synthesized ZnO NPs ranged from 32.78 nm to 37.45 nm, confirming the successful formation of nanoscale materials across all samples, as summarized in Table 1. These values are in close agreement with those reported by Anitha et al., who observed an average crystallite size of 35.6 nm for ZnO NPs synthesized using *Artocarpus gomezianus* fruit extract [45]. Among the samples, the ZnO NPs derived from *Helianthus annuus* exhibited the largest average crystallite size (37.45 ± 11.05 nm), whereas those chemogenic synthesized ZnO NPs had the smallest (32.78 ± 8.06 nm). These differences in crystallite size can be attributed to the nature and concentration of phytochemicals present in the respective plant extracts, which influence the nucleation and growth kinetics during nanoparticle formation [46]. The relatively uniform and nanoscale dimensions of the particles suggest the effective capping and stabilizing role of biomolecules, which help prevent agglomeration and maintain the crystalline integrity of the ZnO NPs [14]. The lattice constants ($a = b$ and c) of all ZnO NPs samples closely resembled the standard values for hexagonal wurtzite ZnO, with only minor variations. ZnO NPs derived from *Helianthus annuus* recorded the highest lattice constants ($a = 3.27018 \pm 0.01$ Å, $c = 5.24095 \pm 0.016$ Å), while chemogenic ZnO NPs exhibited the lowest ($a = 3.25142 \pm 0.001$ Å, $c = 5.2109 \pm 0.0018$ Å). These slight deviations may arise from microstrain or lattice distortions, potentially induced by the adsorption of

phytochemical residues or subtle changes in the reaction environment during green synthesis [47]. The unit cell volumes ranged from 47.71 Å³ for chemogenic ZnO NPs to 48.54 Å³ for biogenic ZnO NPs derived from *Helianthus annuus*, mirroring the trend observed in lattice constants. The slight increase in unit cell volume, particularly in biogenic samples, suggests marginal lattice expansion likely due to the incorporation of surface-bound organic functional groups or intrinsic structural defects introduced during the phytochemical-mediated growth process.

Collectively, the XRD analysis and the derived crystallographic parameters confirm the formation of highly crystalline ZnO NPs with a hexagonal wurtzite structure. The subtle variations in crystallite size and lattice parameters reflect the influence of synthesis conditions, especially the type of plant extract used, on the structural attributes of ZnO NPs. These structural differences are anticipated to impact the NPs biological performance and catalytic behavior, as further demonstrated in subsequent cytotoxicity and antioxidant assays.

3.1.2 High resolution transmission microscope (HRTEM)

HRTEM technique was used to investigate the morphological future and particle size distribution analyses of the prepared chemogenic and biogenic ZnO NPs. The results demonstrate distinct morphological and dimensional properties driven by the synthesis technique and nature of the reducing and capping agents present in the plant extracts as shown in Fig. 3. With an average particle size of 29.54 ± 4.78 nm, chemogenic ZnO NPs demonstrated a relatively uniform shape, suggesting controllable nucleation along with growth under controlled laboratory circumstances [48]. Conversely, the varied nature of phytochemicals in plant extracts was reflected in the greater diversity of morphology and particle size exhibited by biogenic derived ZnO NPs. *Croton*-derived ZnO NPs had the highest mean particle size (34.8 ± 3.69 nm) among the biogenic samples, indicating slower nucleation kinetics or less efficient capping agents that allow for further development [49]. The broadest size dispersion and a moderately high mean size (27.26 ± 5.56 nm) were displayed by ZnO NPs derived from *Helianthus A.*, which due to different

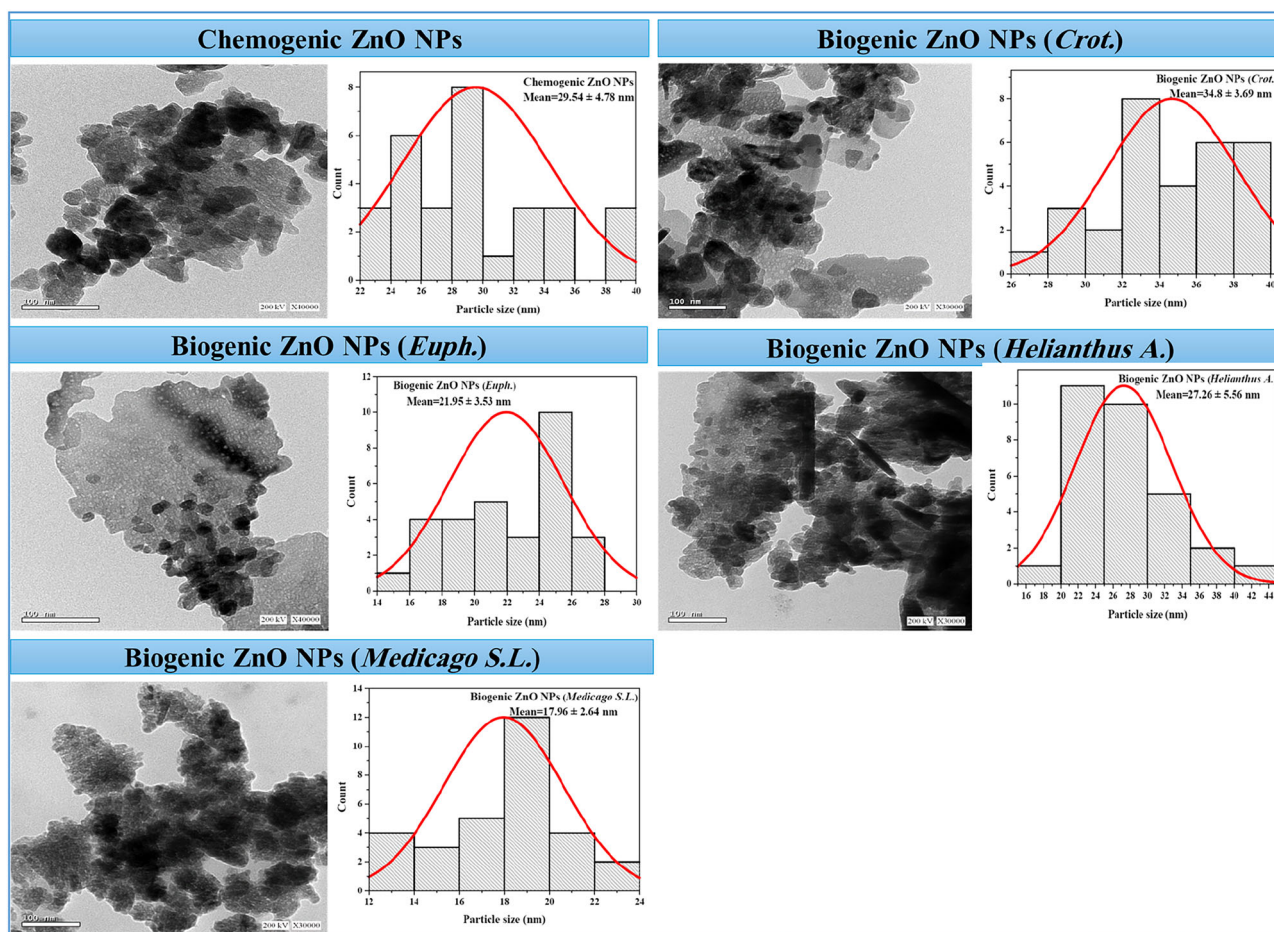


Fig. 3 HRTEM image and size distribution histogram of Chemogenic and Biogenic ZnO NPs using different plant extracts (*Medicago sativa L.*, *Euphorbia milii*, *Croton*, and *Helianthus annuus*). Images with

scalar 100 nm were recorded at 200 KV with magnification X40000. Particle size distribution was calculated with image j software ($n = 100$)

phytochemical concentrations or molecular weights that caused uneven stabilization during NPs formation. While *Medicago sativa L.* extract produced the smallest and most monodisperse ZnO NPs (17.96 ± 2.64 nm), *Euphorbia milii* extract produced smaller NPs (21.95 ± 3.53 nm), demonstrating its strong potential as a green reducing and stabilizing agent. Similar results were reported in the biosynthesis of green ZnO nanoparticles using various plant extracts [50–52]. Polyphenols, flavonoids, and saponins, known for their potent metal ion reduction and robust interaction with NPs surfaces, are responsible for this action [3].

Particle size has a significant impact on functional features of ZnO NPs. The higher surface area-to-volume ratios of smaller ZnO NPs, such as those from *Medicago sativa L.*, improve interactions with biological targets and boost antimicrobial efficacy. These findings are consistent with other studies that have shown that ZnO NPs inhibit quorum sensing and disrupt biofilms in foodborne pathogens (e.g., *Nigella sativa*-derived ZnO NPs, ~ 24 nm) [53]. In contrast, particles from *Croton* or *Helianthus A* that are a little bigger

and more polydisperse would be more appropriate for photocatalysis or UV filtering, where their controlled dissolution and light-scattering qualities are useful. Additionally, biogenic synthesis is an environmentally benign substitute for chemogenic pathways due to its lower energy requirements and lack of hazardous chemicals. Considering the inherent diversity in phytochemical composition across various plant sources and growing settings, the primary difficulty still stands in maximizing extract standardization and guaranteeing batch repeatability.

3.1.3 Fourier transformation Infrared spectroscopy (FTIR)

The functional groups found in the plant extracts (Fig. 4A) and the phytochemical interactions involved in the formation of ZnO nanoparticles (Fig. 4B) were both examined using FTIR spectroscopy. All of the plant extracts (*Crot.*, *Euphorbia milii*, *Helianthus annuus*, and *Medicago sativa L.*) showed multiple significant bands in their FTIR spectra, which correspond to important biomolecular vibrations involved in the creation and stabilization of nanoparticles.

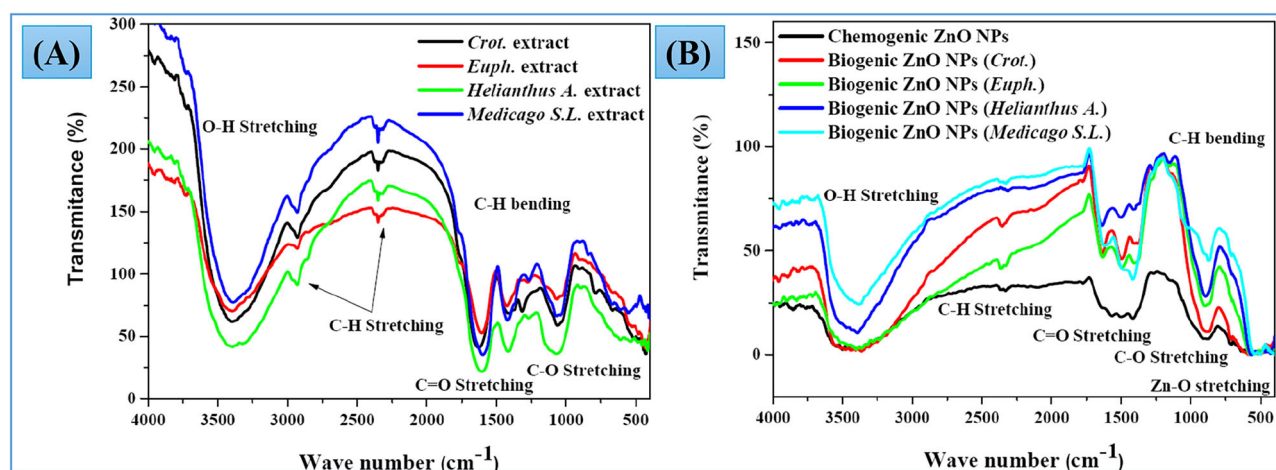


Fig. 4 FTIR spectra of plant extracts (A) and Chemogenic and Biogenic ZnO NPs (B)

In all plant extracts, a broad absorption band between 3200 and 3600 cm^{-1} is seen. This band corresponds to O–H stretching vibrations, which are a sign of the O–H stretching vibrations of hydroxyl groups, which are common in polyphenols and other antioxidant chemicals usually present in plant extracts as shown in Fig. 4A. These biomolecules are well-known for their potent antioxidants and reducing properties, which are essential for the reducing of zinc ions into ZnO NPs [54]. The existence of alkene and hydrocarbon structures, possibly derived from terpenoids or fatty acids, was indicated by peaks found in the 2320–2920 cm^{-1} range that were determined to be caused by C–H stretching and C=C vibrations. The presence of proteins or biomolecules containing amino acids, which may function as stabilizing or reducing agents during the production of nanoparticles, is suggested by the appearance of the amide I band, which is normally indicative of C=O stretching, in the 1620–1650 cm^{-1} range. Additionally, the existence of carbohydrates, flavonoids, and polyols involved in capping and stability was further confirmed by the observation of C–O stretching vibrations at 1050–1065 cm^{-1} and C–O–H bending vibrations in the 1390–1410 cm^{-1} area [31, 55].

Most remarkably, the successful production of ZnO NPs was confirmed by the appearance of the distinctive Zn–O stretching vibrations in the 475–570 cm^{-1} area across all ZnO NPs as shown in Fig. 4B. In contrast to chemogenic ZnO, biogenic ZnO NPs had stronger and somewhat sharper Zn–O bands, indicating enhanced crystallinity and biomatrix interaction [32]. Bioactive phytochemicals were incorporated onto the surfaces of the produced ZnO NPs, as demonstrated by their FTIR spectra. In all ZnO NPs, broad O–H stretching bands were noticeable (3200–3400 cm^{-1}), indicating that alcohol and polyphenolic chemicals were still adsorbed onto the NPs surfaces. In comparison to the

raw extracts, a modest shift and reduction in intensity suggest strong interactions or chemical participation during synthesis [46]. The presence of C–H stretching ($\sim 2900 \text{ cm}^{-1}$), C=O stretching ($\sim 1620\text{--}1650 \text{ cm}^{-1}$), and C–O stretching ($\sim 1050\text{--}1100 \text{ cm}^{-1}$) bands indicates the retention of organic molecules on the NPs surface, acting as natural capping agents that hinder agglomeration and improve colloidal stability through steric or electrostatic repulsion, and reducing agents, which facilitated the conversion of Zn^{2+} ions into ZnO NPs. [41, 56]. These findings align with earlier research that documented the critical role of functional groups derived from plants in the environmentally friendly synthesis of metal oxide NPs, where biomolecules offer enhanced biocompatibility and structural stability in addition to reducing metal ions. The variety of functional groups found demonstrates the extracts' phytochemical complexity and their crucial influence on the final ZnO NPs' physicochemical characteristics [57].

3.1.4 UV–Vis spectroscopy

UV–Vis spectroscopy was used to examine the optical behavior of plant extracts and the ZnO NPs that corresponded to them, as illustrated in Fig. 5A–C. The absorption spectra of aqueous plant extracts (*Medicago sativa L.*, *Helianthus annuus*, *Euphorbia milii*, and *Croton*) are shown in Fig. 5A. These extracts were shown to have an absorption edge in the 280–350 nm region, which is mostly representative of the $\pi \rightarrow \pi^*$ and $n \rightarrow \pi^*$ transitions in flavonoids, phenolic compounds, and other polyaromatic molecules [58]. The UV–Vis absorption spectra of chemogenic and biogenic ZnO NPs are displayed in Fig. 5B. The inherent bandgap absorption of ZnO resulting from electron transitions from the valence band to the conduction band was represented by a clear absorption peak in the

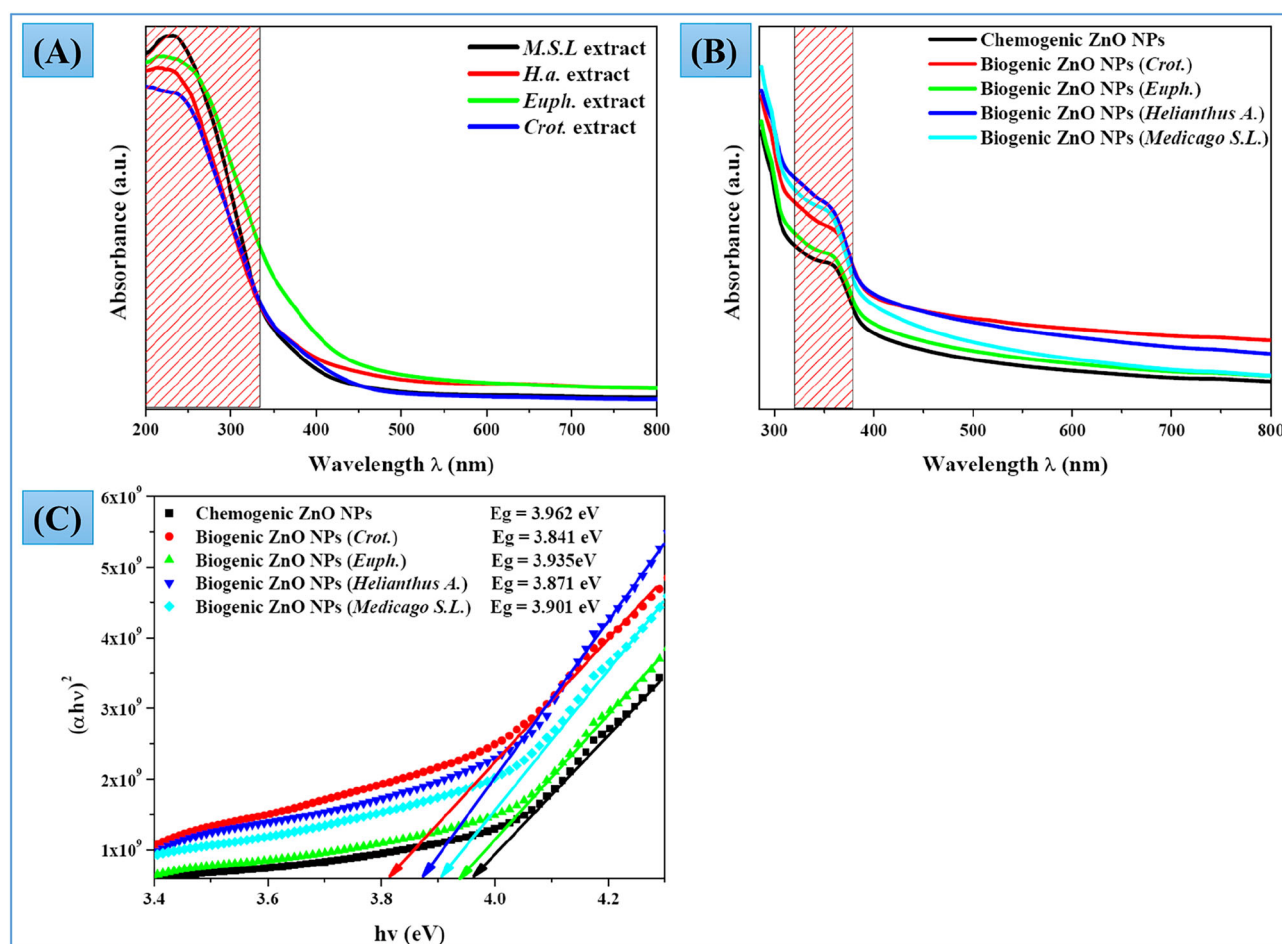


Fig. 5 UV-vis Absorption Spectra of plant extracts (A), Chemogenic and Biogenic ZnO NPs (B) and optical bandgap energy (E_g) as determined according to Tauc's equation (C)

360–380 nm area for all ZnO NPs [59]. Changes in particle size, shape, and surface chemistry due to phytochemical interactions were shown by the minor red- or blue-shift in the absorption edge that biogenic ZnO NPs displayed, depending on the plant utilized [60]. Significantly, compared to chemogenic ZnO, the biogenic ZnO produced with *Helianthus annuus* and *Euphorbia milii* showed somewhat higher absorption in the UV spectrum. This could be explained by better crystallinity and size reduction, which is in line with quantum confinement effects [61].

The optical bandgap energy (E_g) of the synthesized NPs was determined according to Tauc's Eq. 9 [62]:

$$\alpha(h\nu) = A(h\nu - E_g)^{m/2} \quad (9)$$

where α is the absorption coefficient, $h\nu$ is the photon energy, A is a proportionality constant, and m is an exponent that depends on the nature of the electronic transition. For direct allowed transitions, $m = 1$, whereas for indirect allowed transitions, $m = 4$. Subsequently, plots of $(\alpha h\nu)^2$ versus $h\nu$ (photon energy) were constructed, and the

optical bandgap was then determined by projecting the linear section of the curves to intercept the energy axis ($h\nu$) at $(\alpha h\nu)^2 = 0$.

Tauc plot extrapolation from UV-Vis absorption spectra were used to determine the optical bandgap E_g of chemogenic and biogenic ZnO NPs, as illustrated in Fig. 5C. The bandgap values varied between 3.841 eV for biogenic ZnO NPs from *Croton* extract and 3.962 eV for chemogenic ZnO NPs. Comparing biogenically created samples to their chemically synthesized counterparts, these findings show a modest bandgap narrowing. The bandgap variance among the various ZnO NPs samples can be caused by several factors. Despite ZnO NPs straight wide-bandgap semiconductor nature, which has a bulk bandgap of around 3.37 eV, the quantum confinement effect usually causes NPs to show a blue shift in the bandgap [63]. In contrast to the chemogenic sample, the biogenic ZnO NPs showed a lower bandgap, indicating that strain, defect states, and surface functionalization might outweigh size-related quantum effects [64]. A key factor in adjusting the optical characteristics of plant extracts is the presence of different

phytochemicals, including alkaloids, terpenoids, phenolic acids, and flavonoids. In addition to reducing Zn^{2+} ions during synthesis, these biomolecules also cap the surface of the NPs, introducing surface states and defect levels that aid in bandgap narrowing [65]. According to the observed pattern, ZnO NPs mediated by *Croton* and *Euphorbia milii* might have a greater density of surface defects or intermediate energy states close to the valence or conduction bands, which would enable lower energy electronic transitions [27, 29]. Furthermore, the electronic structure is probably changed by the interaction of ZnO with organic ligands from plant extracts, which induces zinc interstitials and oxygen vacancies, which further modify the band structure [49]. These modifications improve the absorption in the UV-visible region, making biogenic ZnO NPs more appropriate for photocatalytic and biomedical applications, where effective photon absorption and ROS generation are crucial [31].

3.2 Bioactivity of green ZnO NPs

3.2.1 Anticancer activity

The MTT assay was used to evaluate the cytotoxic capacity of both biogenic and chemogenic ZnO NPs against Lung cancer cell lines (A549) at different concentrations (0–1000 $\mu\text{g/mL}$) as shown in Fig. 6. A significant reduction in cell viability was noted for all ZnO NPs samples demonstrated a dose-dependent cytotoxic impact. In comparison to their chemogenic equivalent, biogenic ZnO NPs samples showed increased cytotoxicity. Interestingly, at 100 $\mu\text{g/mL}$, the chemogenic ZnO NPs demonstrated marginally greater survival rates, whereas the biogenic ZnO NPs caused cell viability reductions to less than 20%. The

most powerful cytotoxic effect was shown by *Croton*-derived ZnO NPs, which reduced viability to over 10% at 100 $\mu\text{g/mL}$ among the biogenic samples. As positive control, the well-known anticancer drug doxorubicin was added for comparison. ZnO NPs, particularly the biogenic samples, showed excessive viability decreases at lower concentrations, indicating superior early cytotoxic efficacy, whereas doxorubicin maintained a progressive cytotoxic profile. These findings are in agreement with earlier studies demonstrating the anticancer activity of biogenic ZnO nanoparticles derived from diverse biosources [66]. There are several possible synergistic variables that contribute to the increased cytotoxicity of biogenic ZnO NPs. First, oxidative stress-mediated apoptosis may result from the appearance of phytochemicals originating from plants on the surface of nanoparticles, such as flavonoids, phenolics, and terpenoids, which may improve cellular absorption and encourage the production of reactive oxygen species (ROS) [67]. Second, higher surface area, defect density, and functionalization are frequently found in biogenic ZnO NPs, which may enhance their interaction with intracellular organelles and cellular membranes [68].

Using the dose-response curves from the MTT data, IC_{50} values, the concentration needed to decrease cell viability by 50%, were calculated to measure the antiproliferative activity of the produced NPs and presented in Table 2. The IC_{50} values, which range from ~53 to 62 $\mu\text{g/mL}$, show that the lethal effects of both chemogenic and biogenic ZnO NPs are similar. The chemogenic ZnO NPs had the lowest IC_{50} (53.47 $\mu\text{g/mL}$) of all the examined samples, marginally surpassing the doxorubicin (55.76 $\mu\text{g/mL}$) standard chemotherapeutic drug. Nevertheless, the cytotoxicity of all biogenic ZnO NPs was equally strong, indicating that green production did not lessen therapeutic effectiveness. The IC_{50} values of the biogenic ZnO NPs were somewhat greater than those of their chemogenic counterpart, although the differences were not statistically significant ($p > 0.05$). This suggests that NPs with similar anticancer potential and potential extra advantages of biocompatibility and environmental friendliness are produced by phytochemical-mediated synthesis.

3.2.2 Antioxidant activity

Figure 7 shows the evaluation and comparison of the radical scavenging capacity (SCV%) of chemogenic and biogenic ZnO NPs with ascorbic acid, a recognized antioxidant standard, at various doses (25–500 $\mu\text{g/mL}$). Scavenging activity increased in all evaluated ZnO NPs in a concentration-dependent manner. However, ascorbic acid's antioxidant efficacy was noticeably better, demonstrating a quick and notable increase in SCV% that rose above 100% at the highest concentration examined. In contrast, ZnO NPs, both biogenic and chemogenic, showed moderate

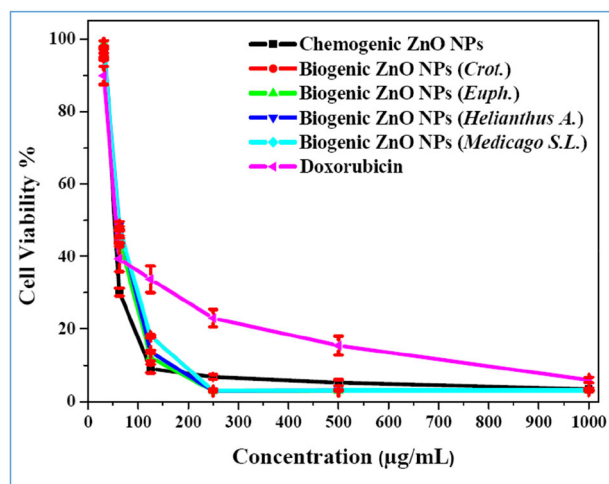


Fig. 6 Cell viability (%) of A549 cell lines after treatment with Chemogenic and Biogenic ZnO NPs at different concentrations (31.5–1000 $\mu\text{g/mL}$). doxorubicin was used as positive control

Table 2 Summary of the calculated IC₅₀ of different bioactivities (Anticancer, Antioxidant, Anti-inflammatory and Antidiabetic) for Chemogenic and Biogenic ZnO NPs

Sample	Anticancer activity (cell viability)	Antioxidant activity (DPPH radical scavenging)	Anti-inflammatory activity (COX-1 inhibition)	Antidiabetic activity	
				(α -amylase inhibition)	(α -glucosidase inhibition)
Chemogenic ZnO NPs	53.47 $\pm$ 2.49	164.9 $\pm$ 8.56	37.18 $\pm$ 3.27	20.87 $\pm$ 2.75	11.11 $\pm$ 1.33
Biogenic ZnO NPs <i>Crot.</i>	59.13 $\pm$ 4.75	207.43 $\pm$ 9.56	392.74 $\pm$ 11.29	83.06 $\pm$ 3.65	23.59 $\pm$ 3.87
<i>Euph.</i>	59.15 $\pm$ 3.93	338.55 $\pm$ 12.45	119.79 $\pm$ 6.39	196.13 $\pm$ 7.38	19.23 $\pm$ 2.48
<i>Helianthus A.</i>	61.63 $\pm$ 5.22	197.64 $\pm$ 8.93	101.68 $\pm$ 8.99	116.98 $\pm$ 7.92	53.77 $\pm$ 5.17
<i>Medicago S.L.</i>	60.89 $\pm$ 4.68	217.48 $\pm$ 7.25	174.71 $\pm$ 6.46	15.22 $\pm$ 2.69	38.97 $\pm$ 3.65
Doxorubicin	55.76 $\pm$ 4.22	-	-	-	-
Ascorbic acid	-	20.49 $\pm$ 2.78	-	-	-
Celecoxib	-	-	5.88 $\pm$ 1.39	-	-
Acarbose	-	-	-	49.14 $\pm$ 4.39	50.28 $\pm$ 6.52

The presented data were the mean $\pm$ standard deviation

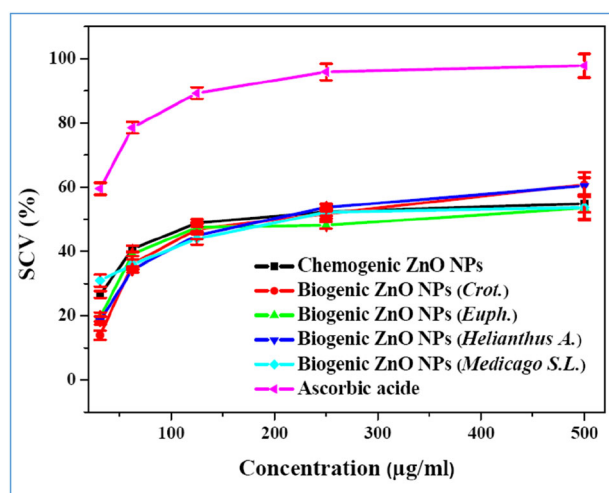


Fig. 7 DPPH radical scavenging activity of ZnO NPs synthesized via chemical and green routes

antioxidant activity. At 500 $\mu\text{g/mL}$, SCV values increased steadily to a high of roughly 50–60%. *Helianthus annuus* extract-synthesized ZnO NPs showed a somewhat greater scavenging effectiveness among the biogenic samples, followed by extracts from *Medicago sativa L.*, *Croton*, and *Euphorbia*. The performance of all biogenic ZnO NPs was notably comparable to or slightly superior to that of their chemogenic equivalent, indicating that phytochemical capping from plant extracts may improve the capacity to scavenge radicals [49]. The observed antioxidant activity most likely results from the NPs surface chemistry, where functional groups or leftover phytochemicals from green synthesis provide electron-donating or hydrogen-transfer properties [69]. The limited but noteworthy antioxidant capability of ZnO NPs was highlighted by the fact that the activity was still much lower than that of ascorbic acid.

For further confirmation the IC₅₀ was calculated and listed in Table 2. As predicted, ascorbic acid demonstrated

the strongest antioxidant activity, confirming its function as a strong positive control by attaining IC₅₀ at an exceptionally low concentration of $20.49 \pm 2.78 \mu\text{g/mL}$. However, the antioxidant effectiveness of all ZnO NPs samples was comparatively lower. Comparing the chemogenic ZnO NPs to their biogenic counterparts, the chemogenic ones showed the lowest IC₅₀ ($164.9 \pm 8.56 \mu\text{g/mL}$), indicating greater scavenging activity. Some green-synthesized ZnO NPs, however, showed encouraging antioxidant behavior. In particular, the ZnO NPs mediated by *Helianthus annuus* (IC₅₀ = $197.64 \pm 8.93 \mu\text{g/mL}$) and *Medicago sativa L.* (IC₅₀ = $217.48 \pm 7.25 \mu\text{g/mL}$). Notably, among the studied samples, *Euphorbia*-derived ZnO NPs exhibited the poorest radical scavenging performance, with the greatest IC₅₀ ($338.55 \pm 12.45 \mu\text{g/mL}$). The kind and quantity of surface-bound functional groups and leftover phytochemicals from various plant extracts used in the biogenic synthesis are probably the causes of the variance in antioxidant activity [70]. These biomolecules might alter the NPs ability to donate electrons, which would affect how well they can suppress free radicals [71]. The results indicate that green-synthesized ZnO NPs have moderate and adjustable antioxidant potential, which may be advantageous in biomedical or cosmeceutical applications where oxidative stress modulation is desired, even though all ZnO NPs samples demonstrated lower antioxidant activity when compared to ascorbic acid. various studies have reported notable antioxidant activities of ZnO NPs synthesized using different plant extracts. Among them, *Knoxia sumatrensis* exhibited the highest activity at 95.8% [72], *Azadirachta indica* (92%) [73], *Rosa canina* (>90%) [74], and *L. pruinosa* (86.5%) [12]. ZnO NPs synthesized from *Eucalyptus globulus* also showed strong antioxidant potential (82%) [75]. Moderate activities were observed for *Trianthema portulacastrum* and *Costus igneus* (75%) [42, 76], and *Ceropegia candelabrum* (55.43%) [77].

Table 3 Antibacterial efficacy of chemogenic and biogenic ZnO NPs against some Gram-positive and negative pathogenic strains*

Sample		<i>S. aureus</i>		<i>S. pyrogenes</i>		<i>E. coli</i>		<i>K. pneumoniae</i>	
		MIC	MBC	MIC	MBC	MIC	MBC	MIC	MBC
Chemogenic ZnO NPs		16.7	47.4	9.1	37.5	9.1	37.5	9.1	41.2
Biogenic ZnO NPs	<i>Crot.</i>	9.1	41.2	33.3	44.4	9.1	9.1	9.1	23.1
	<i>Euph.</i>	16.7	28.6	23.1	44.4	9.1	37.5	9.1	-
	<i>Helianthus A.</i>	9.1	-	33.3	50	9.1	28.6	9.1	28.6
	<i>Medicago S.L.</i>	16.7	37.5	9.1	37.5	9.1	41.2	9.1	33.3

*Values expressed in mg/ml

3.2.3 Antibacterial activity

Chemogenic and biogenic ZnO NPs were investigated in terms of their antibacterial activity on Gram-positive (*S. aureus* and *S. pyrogenes*) and Gram-negative (*E. coli* and *K. pneumoniae*) bacteria. The MIC and MBC were performed by broth microdilution and the results are presented in Table 3. All ZnO NPs showed broad-spectrum antibacterial activity, however the potencies varied noticeably across the strains and synthesis methods. The biogenic ZnO NPs from *Croton* and *Helianthus annuus* showed strong growth inhibition at comparatively low doses against all tested strains, with MIC of 9.1–33.3 mg/mL and bactericidal activity with MBC of 23.1–50 mg/mL against all tested bacterial strains except *Helianthus* against *S. pyrogenes* needed higher doses than tested one. The antibacterial effectiveness of biogenic ZnO NPs made from *Euphorbia milii* and *Medicago sativa L.* was strong also and has bacteriostatic impact with MIC of 9.1–23.1 mg/mL against all tested bacterial strains and bactericidal effect with MBC of 28.6–44.4 mg/mL against all tested bacterial strains except *Euphorbia milii* against *K. pneumoniae* needed higher doses than tested one. Additionally, the chemogenic ZnO NPs consistently showed antibacterial activity against all pathogens, with MIC values ranging from 9.1 to 16.7 mg/mL. Their MBC values, however, were typically greater dose up to 47.4 mg/mL, suggesting that higher dosages were required to completely eradicate the bacterium.

Overall, the evidence points to the superior antibacterial properties of plant-mediated ZnO NPs over their chemically produced counterparts, especially those synthesized from extracts of *Croton* and *Helianthus*. This increased activity could be explained by bioactive phytochemicals on the surface of the NPs, which may work in concert with ZnO NPs natural antibacterial properties [49]. Recent studies have demonstrated the potent antibacterial efficacy of green-synthesized ZnO NPs. *Aristolochia indica* mediated ZnO NPs exhibited MIC and MBC values ranging from 25 to 400 µg/mL against multidrug-resistant wound pathogens, including *S. aureus* and *E. coli* [78]. Similarly, Spirulina-embedded ZnO NPs showed MICs of 31.25 µg/mL against

S. aureus and 62.5 µg/mL against *K. pneumoniae*, with corresponding MBCs of 125 and 250 µg/mL [79]. Additionally, green ZnO NPs isolated from multidrug-resistant *E. coli* strains demonstrated MIC values between 12.5 and 32 µg/mL and MBCs of 25 to 64 µg/mL [80].

The chemogenic and biogenic antibacterial activity of ZnO NPs is mainly ascribed to a number of synergistic processes that compromise the integrity and physiology of bacteria. ROS generation, membrane rupture, Zn^{2+} ion release, and interaction with intracellular components are the main mechanisms [3]. Particularly when exposed to UV or visible light, ZnO NPs are known to generate reactive oxygen species, including hydrogen peroxide (H_2O_2), superoxide anions ($\text{O}_2^{\cdot-}$), and hydroxyl radicals ($\cdot\text{OH}$). These extremely reactive ROS put bacterial cells under oxidative stress by: Cellular membrane damage caused by lipid peroxidation, Enzyme deactivation due to protein oxidation and fragmenting DNA, preventing transcription and replication. This oxidative damage is regarded as one of the main mechanisms of ZnO NPs antibacterial activity and jeopardizes bacterial viability [32].

ZnO NPs can stick directly to the surfaces of bacterial cells through electrostatic interactions between the positively charged ZnO surface and the negatively charged bacterial membranes. The interaction's outcomes include increased membrane permeability, which encourages more NPs internalization, decreased membrane potential, which impedes essential functions like ATP synthesis and nutrition transfer, and cytoplasmic contents leaking as a result of physical membrane structure disintegration [81]. ZnO NPs can partially dissolve in aqueous solutions, producing Zn^{2+} ions that infiltrate bacterial cells and interfere with several metabolic processes. By attaching themselves to the sulfhydryl groups of proteins, these ions disrupt their function, disrupt DNA and ribosomes to stop protein synthesis and replication, and increase oxidative stress by producing reactive oxygen species (ROS) within cells. ZnO NPs of a smaller size, particularly those produced biogenically, have the ability to pass through bacterial membranes and gather in the cytoplasm. They directly interact with ribosomes, enzymes, and genetic material after internalization, which

results in apoptosis-like behavior in bacteria, inhibition of protein biosynthesis, and genotoxic stress [82].

The synergistic effects of surface-bound phytochemicals contribute to the improved antibacterial activity of biogenic ZnO NPs. Plant-derived biomolecules such as flavonoids, alkaloids, terpenoids, and polyphenols: Increase the generation of ROS; provide extra antibacterial activities (e.g., inhibition of enzymes, destabilization of membranes); and improve the dispersion of ZnO NPs and cellular contact. In addition to improving microbial selectivity, these bio-capping molecules may lessen cytotoxicity against mammalian cells [83].

3.2.4 Antibiofilm activity

A key component of ZnO NPs antibacterial efficiency is their antibiofilm capability, particularly when it comes to fighting biofilm-forming microorganisms that are generally resistant to traditional antibiotics [84]. With distinct variations between investigated strains and NPs type, the optical density (OD) values representing biofilm biomass in the presence of different ZnO NPs are shown in Fig. 8 Panels (A) for Gram-negative and (B) for Gram-positive. In comparison to both control and chemogenic ZnO NPs, biogenic ZnO NPs, especially those synthesized using extracts from *Medicago sativa* L. and *Helianthus annuus*, showed the strongest antibiofilm action, dramatically lowering OD values for all tested bacterial strains. Among the tested samples, biogenic ZnO NPs synthesized from *Helianthus annuus* exhibited the most pronounced inhibition of *S. aureus* biofilm formation, achieving a reduction of 71.8% as illustrated in Fig. 8A. Biogenic ZnO NPs derived from *Euphorbia* and *Croton* also demonstrated substantial

anti-biofilm activity, with inhibition percentages of 70.5% and 68.8%, respectively. In contrast, chemogenic ZnO NPs and biogenic ZnO NPs synthesized from *Medicago sativa* L. showed comparatively lower reductions of 62.9% and 60.7%, respectively. Interestingly, the highest anti-biofilm activity against *S. pyogenes* was observed with biogenic ZnO NPs synthesized from *Medicago sativa* L., which achieved a reduction of 79%. Other biogenic ZnO NPs produced from *Euphorbia* and *Croton* reduced biofilm formation by 73.67% and 52.3%, respectively. However, *Helianthus annuus*-derived NPs showed only 7.4% inhibition, similar to the chemogenic ZnO NPs, which recorded the lowest reduction at 1.7%.

In the case of *K. pneumoniae*, as described in Fig. 8B, biogenic ZnO NPs from *Helianthus annuus* again showed superior biofilm inhibition at 66.1%, followed by those synthesized from *Croton* (56.8%), chemogenic ZnO NPs (55.9%), *Euphorbia* (52.4%), and *Medicago sativa* L. (40.2%). For *E. coli*, the chemogenic ZnO NPs demonstrated the most effective reduction in biofilm formation at 72.5%. Among the biogenic counterparts, ZnO NPs from *Medicago sativa* L., *Helianthus annuus*, *Croton*, and *Euphorbia* exhibited comparable anti-biofilm effects, with inhibition percentages of 66.4%, 62.0%, 61.2%, and 60.2%, respectively.

Numerous factors contribute to biogenic ZnO NPs strong antibiofilm action. One important contributing factor is the increased production of ROS, which can pierce the biofilm matrix's extracellular polymeric substance (EPS) and cause oxidative breakdown and bacterial cell death [85]. Moreover, ZnO NPs have the ability to disrupt quorum sensing and bacterial adhesion pathways, both of which are essential for the early phases of biofilm development. Bacterial

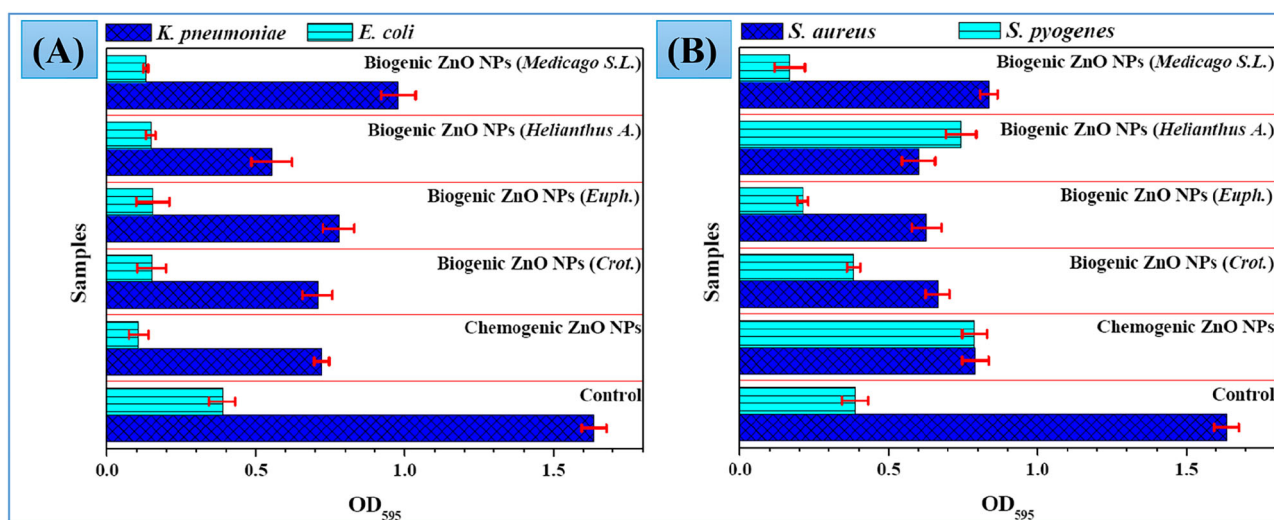


Fig. 8 Antibiofilm activity of Chemogenic and Biogenic ZnO NPs against Gram-negative (*E. coli* and *K. pneumoniae*) (A) and Gram-positive (*S. aureus* and *S. pyogenes*) (B) bacterial strains

integrity and viability are further jeopardized by ZnO NPs induced membrane rupture [86]. Remarkably, by strengthening their anti-adhesive and antibacterial properties, phytochemicals generated from plants on the surface of biogenic ZnO NPs may work in concert to suppress biofilms [49]. These results demonstrate the potential of biogenic ZnO NPs, especially those derived from *Helianthus annuus* and *Medicago sativa* L., as viable options for managing illnesses linked to biofilms in both clinical and environmental contexts. Similar activities were reported for *Plumbago zeylanica*-derived ZnO NPs, with average size ~25 nm, which showed biofilm inhibition for *E. coli*, *S. aureus*, and *P. aeruginosa* by 62.8%, 71.6%, and 77.7%, respectively, and eradicated pre-formed biofilms by up to 67.2% [87]. Furthermore, *Spirulina platensis*-derived ZnO NPs (~19 nm) demonstrated pronounced antibiofilm effects alongside MICs of 31.25 µg/mL (*S. aureus*) and 62.5 µg/mL (*K. pneumoniae*) [79]. Additionally, *Saraca asoca*-mediated ZnO NPs achieved approximately 83% inhibition of *Bacillus subtilis* biofilm formation and reduced mature biofilms by ~33% [88].

3.2.5 Anti-inflammatory activity

The anti-inflammatory potential of chemogenic and biogenic ZnO NPs was examined by inhibiting cyclooxygenase-1 (COX-1) enzyme activity, with celecoxib serving as positive control. Figure 9 shows the concentration-dependent suppression of COX-1 in all tested samples from 0–1000 µg/mL. The strongest COX-1 inhibitory action was demonstrated by the chemogenic ZnO NPs, which were close to 90% inhibition at the maximal concentration and trailed the positive control, celecoxib,

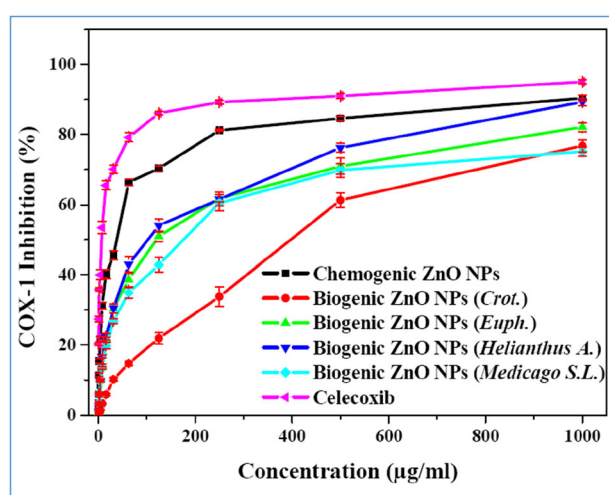


Fig. 9 Anti-inflammatory activity of Chemogenic and Biogenic ZnO NPs showing COX-1 inhibition rate at different concentrations (50–1000 µg/ml) of the test samples with Celecoxib serving as a positive control

which demonstrated >95% inhibition. With a moderate to high COX-1 inhibition of ~80% at 1000 µg/mL, biogenic ZnO NPs derived from *Helianthus annuus* and *Medicago sativa* L. showed encouraging anti-inflammatory potential. Notably, the inhibitory profiles of biogenic ZnO NPs produced from *Euphorbia milii* and *Croton* were lower, with *Croton*-based NPs demonstrating the least amount of activity among the biogenic samples.

Due to their comparatively higher surface reactivity and decreased presence of phytochemical surface capping agents, chemogenic ZnO NPs exhibit superior inhibition, which may be explained by their increased ability to interact with the COX-1 enzyme [89]. However, biogenic ZnO NPs benefit from bioactive phytochemicals obtained from plants that are adsorbed onto their surface. Depending on their chemical makeup and affinity for the enzyme's active site, these phytochemicals may work in concert or in addition to reduce COX-1 [90]. Through a variety of mechanisms, ZnO NPs may inhibit COX-1, including the production of ROS, which can interfere with cyclooxygenase activity and impair prostaglandin synthesis. By preventing the enzymatic cascade or disrupting the availability of cofactors, Zn²⁺ ion release may also alter inflammatory signals [21]. These results suggest the possible use of ZnO NPs as anti-inflammatory medicines since they show considerable COX-1 inhibitory action in both chemogenic and biogenic forms. The differences in effectiveness between the biogenic samples demonstrate how components generated from plants might modify the behavior of nanoparticles and biological interactions. Several studies confirmed the strong anti-inflammatory activity of green ZnO NPs. Biogenic ZnO NPs derived from *Polygala tenuifolia* root extract significantly reduced the expression of key inflammatory markers such as iNOS, COX-2, IL-1β, IL-6, and TNF-α in LPS-stimulated RAW 264.7 macrophages [91]. Similarly, ZnO NPs synthesized using *Senecio chrysanthemoides* leaf extract showed 73% inhibition of inflammation at 100 µg/mL in a human red blood cell (HRBC) membrane stabilization assay, an effect comparable to that of diclofenac [92]. Furthermore, ZnO NPs synthesized from *Equisetum diffusum* displayed a dose-dependent inhibition of protein denaturation, ranging from 23.7% at lower concentrations to 91.2% at 500 µL of a 1 mg/mL solution [93].

3.2.6 Antidiabetic activity

The inhibitory potential of both chemogenic and biogenic ZnO NPs against two important enzymes that hydrolyze carbohydrates, α-amylase and α-glucosidase, which are therapeutic targets in the treatment of type 2 diabetes mellitus, was assessed in order to assess their antidiabetic efficacy [94]. The biogenic ZnO NPs synthesized from *Medicago sativa* extract showed the most remarkable

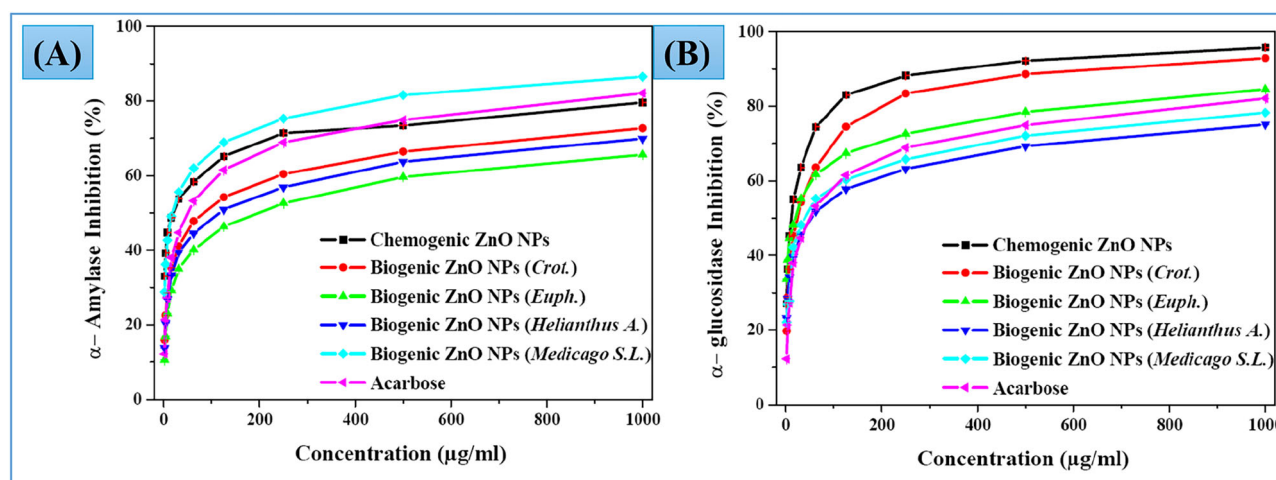


Fig. 10 Antidiabetic activity of Chemogenic and Biogenic ZnO NPs at different concentrations (50–1000 $\mu\text{g/ml}$) of the test samples with Celecoxib serving as a positive control showing α -amylase inhibition percentage (A) and α -glucosidase inhibition percentage (B)

performance for α -amylase inhibition, with the lowest IC_{50} value of $15.22 \pm 2.69 \mu\text{g/ml}$ as shown in Fig. 10A. In comparison to the positive control acarbose ($\text{IC}_{50} = 49.14 \pm 4.39 \mu\text{g/ml}$) and chemogenic ZnO NPs ($\text{IC}_{50} = 20.87 \pm 2.75 \mu\text{g/ml}$), this suggests a noticeably stronger inhibition, Table 2. The inclusion of bioactive phytochemicals such as flavonoids, phenolic acids, and saponins, which serve as capping and stabilizing agents during NPs formation, is probably what gives *Medicago*-derived ZnO NPs their improved performance. Higher inhibitory efficiency may result from these chemicals' ability to strengthen contacts with the α -amylase active site through electrostatic forces and hydrogen bonds [95]. Conversely, biogenic ZnO NPs from *Helianthus annuus* and *Euphorbia* demonstrated noticeably less α -amylase inhibition, with IC_{50} values of $116.98 \pm 7.92 \mu\text{g/ml}$ and $196.13 \pm 7.38 \mu\text{g/ml}$, respectively. This implies that the final bioactivity of the NPs is significantly influenced by the type and makeup of the plant extract utilized in synthesis [49]. The decreased performance of these samples could have been caused by larger particle sizes, decreased surface reactivity, or ineffective phytochemical capping [54]. Interestingly, the chemogenic ZnO NPs showed superior inhibitory effect compared to acarbose. This suggests that ZnO NPs can display significant α -amylase inhibition even without plant-based functionalization, probably because of their ionic contacts and potential for Zn^{2+} release [96].

In the case of α -glucosidase inhibition, chemogenic ZnO NPs were the most potent, beating all biogenic samples and the reference medication acarbose, which had an IC_{50} of $50.28 \pm 6.52 \mu\text{g/ml}$ as shown Fig. 10B. According to this, chemogenic ZnO and α -glucosidase interact in a unique way that may involve direct ionic binding or the production of ROS, which can alter the structure of the enzyme and

lower its catalytic activity. In contrast to *Medicago*-based NPs, which were less efficient ($\text{IC}_{50} = 38.97 \pm 3.65 \mu\text{g/ml}$), *Euphorbia*-derived ZnO NPs showed the greatest α -glucosidase inhibition among the biogenic samples ($\text{IC}_{50} = 19.23 \pm 2.48 \mu\text{g/ml}$), followed closely by *Croton* ($\text{IC}_{50} = 23.59 \pm 3.87 \mu\text{g/ml}$) [94].

Enzyme-specificity in NPs interactions is highlighted by the divergent behavior of α -amylase and α -glucosidase inhibition across various ZnO NPs [97]. Because of their high activity against α -amylase and limited activity against α -glucosidase, *Medicago*-based NPs may be more effective at blocking or interacting with the α -amylase active site due to their phytochemical surface groups [98]. In contrast, chemogenic ZnO NPs are better adapted to breaking down the structure of α -glucosidase due to their stronger oxidative capabilities and perhaps larger density of surface Zn^{2+} ions [99]. ZnO NPs' mechanism of inhibiting enzymes most likely combines surface binding, Zn^{2+} ion release, and ROS production. Through stabilizing the NPs, changing surface charge, and facilitating specific interactions with enzyme active sites, the phytochemicals included in biogenic NPs may modify these effects [100]. These variations highlight how crucial surface chemistry and particle size of NPs are in influencing biological activity. In line with these findings ZnO NPs synthesized from *Ficus palmata* showed strong in vitro inhibition—with IC_{50} values of $70.07 \mu\text{g/ml}$ for α -amylase and $68.49 \mu\text{g/ml}$ for α -glucosidase—and in vivo they significantly lowered blood glucose from day 21 to 42, also improving lipid profiles by reducing triglycerides, total and LDL cholesterol and increasing HDL [101]. Meanwhile, ZnO NPs from *Mussaenda frondosa* exhibited α -glucosidase inhibition comparable to acarbose ($\text{IC}_{50} \sim 14.85 \mu\text{g/ml}$ vs. $11.69 \mu\text{g/ml}$ for acarbose) and reasonable α -amylase inhibition [102]. In addition, in diabetic rat

models, ZnO NPs synthesized from *Tridax procumbens* leaf extract significantly reduced blood glucose, ameliorated weight loss, improved HbA_{1c} and lipid profiles, and protected pancreatic and hepatic tissue. At 200 mg/kg, these NPs surpassed both the extract alone and glibenclamide in hypoglycemic efficacy [103].

4 Conclusion

This study successfully demonstrates the viability of a green synthesis approach for producing biofunctional zinc oxide nanoparticles using extracts from *Medicago sativa*, *Helianthus annuus*, *Euphorbia milii*, and *Codiaeum variegatum*. The distinct phytochemical profiles of these plants, rich in flavonoids, tannins, alkaloids, and phenolic acids, were instrumental in mediating the formation of ZnO NPs with unique physicochemical characteristics, including reduced crystallite size and enhanced surface reactivity, which contributed to their superior biological performance.

The biosynthesized ZnO NPs exhibited multifaceted and enhanced bioactivities compared to their chemosynthetic counterparts. Notably, they demonstrated potent, dose-dependent cytotoxicity against A549 lung cancer cells, suggesting promising anticancer properties. In antidiabetic assays, the NPs showed significant enzyme inhibition, with *Medicago sativa*-derived NPs exhibiting strong α -amylase inhibition and *Euphorbia milii*-based NPs showing notable α -glucosidase inhibition, outperforming the standard drug acarbose in these specific tests. All biogenic NPs also displayed considerable free radical scavenging capacity, confirming potent antioxidant properties. While the chemosynthetic ZnO NPs showed excellent anti-inflammatory potential via COX-1 inhibition, the green-synthesized variants displayed broad-spectrum antibacterial activity against both Gram-positive and Gram-negative pathogens. This antimicrobial efficacy is likely mediated by a combination of mechanisms, including Zn²⁺ ion release, reactive oxygen species (ROS) generation, and membrane disruption, which also contributed to their significant ability to suppress microbial biofilm formation.

As a practical and environmentally responsible substitute for chemical synthesis routes, our study confirms the green synthesis of ZnO NPs utilizing extracts from medicinal plants. Depending on the plant source, the resultant NPs have tunable biological activity in addition to maintaining exceptional physicochemical stability. These multifunctional properties make them attractive options for potential future uses in antimicrobial coatings, wound healing, targeted drug delivery, and treatments for metabolic disorders. Future research should focus on molecular docking, in vivo pharmacokinetics, and clinical safety profiling to investigate the mechanistic basis of nanoparticle–biomolecule interactions in order to move these nanomaterials closer to translational medicine.

Data availability

No datasets were generated or analysed during the current study.

Author contributions Fatma Shaibah did the experimental part and Alaa Hassan Said analyzed the data and wrote the original manuscript. Moustafa M, Rokaia B. Elamary revised the original manuscript. All authors read and approved of the final manuscript.

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Compliance with ethical standards

Conflict of interest The authors declare no competing interests.

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