

Green Synthesis of Mn-Doped ZnO Nanoparticles: Characterization, Hemocompatibility, and Cytotoxicity Effect Against C540 Melanoma Cells

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Abstract

In this study, we present a rapid and eco-friendly method for the preparation of ZnO nanoparticles (Zn-NP) and 5% Mn-doped ZnO nanoparticles (Mn-Zn-NP) using aqueous extract of *Prosopis fracta*. The synthesized nanoparticles were characterized using PXRD, FESEM, EDX, UV-Vis, DLS, FT-IR, and Raman spectroscopy. PXRD analysis confirmed the hexagonal wurtzite structure of both samples, with crystallite sizes of 35.16 nm and 39.52 nm for Zn-NP and Mn-Zn-NP, respectively. The successful doping of Mn²⁺ ions into the ZnO lattice was demonstrated by shifts in the PXRD peaks, EDX elemental analysis (4.52% Mn) and a blue shift in the UV-Vis absorption from 368 nm to 339 nm. FESEM imaging revealed a spherical morphology with particle sizes of ~30 nm (Zn-NP) and ~50 nm (Mn-Zn-NP). Excellent hemocompatibility was demonstrated for both samples using hemolysis assay. MTT cytotoxicity assay against C540 melanoma cells revealed concentration-dependent cell death, with Mn-Zn-NP presenting significantly higher toxicity (IC₅₀ ~25 µg/mL) compared to undoped Zn-NP (IC₅₀ ~50 µg/mL). Our findings indicate that manganese doping significantly improves the anticancer potential of ZnO-NP and suggest Mn-Zn-NP as a promising candidate for melanoma treatment.

1. Introduction

Nanotechnology is recognized as a highly dynamic field of scientific research [1,2], which in the past decade has witnessed significant progress in this field, presenting a variety of fabrication protocols designed to produce nanostructures with defined architectures and dimensions according to specific application demands [3,4]. For targeted biomedical applications, the development of biologically active nanostructures has become essential, driving extensive multidisciplinary research efforts [5-8].

Zinc oxide, as a semiconductor of considerable scientific and industrial importance, has a significant exciton binding energy (60 meV) [9] and a wide band gap (3.37 eV), distinguishing it as a highly versatile inorganic compound with multifunctional properties. Consequently, ZnO nanostructures have found wide application in various industrial fields, including UV-emitting devices [10], ethanol detection sensors [11], photocatalytic systems [12], and pharmaceutical/cosmetic formulations [13]. The dominant strategies for preparing ZnO nanostructures include diverse approaches such as precipitation chemistry [14,15], sol-gel transformations [16], solvothermal processing [17], hydrothermal synthesis [18,19], and others. Chemical synthesis routes usually produce harmful effects that are adsorbed onto

the nanostructured surfaces. These residual contaminants severely limit biomedical applicability. Increasing awareness of sustainable chemistry principles and biological synthesis paradigms has accelerated the development of environmentally friendly nanoparticle production methods [20-24].

Environmentally friendly methods for the fabrication of ZnO nanostructures have attracted increasing scientific attention, as conventional chemical protocols rely on expensive reagents and require organic or inorganic solvents and reducing agents [25]. The incorporation of plant extracts into synthetic protocols has emerged as straightforward yet highly effective alternative to conventional physicochemical techniques. Sustainable nanoparticle fabrication paradigms have been highlighted due to their ecological benefits, economic feasibility, and wide application in nanomedicine and catalytic drug synthesis [26-28].

Therefore, leveraging the advantages of green synthesis, this route has been used to prepare the desired nanoparticles. The medicinal plant *Prosopis farcta* (*P. farcta*) belongs to the Mimosaceae family and the genus *Prosopis*. This genus comprises 44 species of shrubs and trees, 11 of which have been reported in different countries [29]. Species within the genus *Prosopis* have

numerous uses in medicine. These plants are used in the prevention and treatment of many circulatory system abnormalities, endocrine system abnormalities, genitourinary system abnormalities, and subcutaneous tissue abnormalities [30]. Phytochemical analyses have shown that the properties of medicinal plants are related to the various compounds they contain, including alkaloids, carbohydrates, glycosides, tannins, reducing sugars, steroids, terpenoids, flavonoids, and saponins [31]. Specifically, 16 phenolic compounds, including tannins, quercetin, apigenin, rosin, myristin, viscin, vitexin, luteolin, and isovitexin, have been identified in the plant. This plant has various uses in human and animal nutrition [32]. The leaves and fruits of the plant are traditionally used in the treatment of many diseases and disorders, and a number of these effects have been proven by researchers.

In the field of cancer, nanotechnology encompasses a variety of approaches, including cellular detection and precise therapeutic delivery. This versatility is due to the unique physicochemical properties of nanostructures such as semiconductor quantum dots and nanocrystals. Scientific research has demonstrated the selective cytotoxicity of ZnO nanostructures towards malignant cell populations. For instance, Permanathan et al. evaluated the differential toxicity responses between human peripheral blood mononuclear cells and leukemia HL60 cell lines when exposed to ZnO nanostructures and studied the context-dependent cellular reactivity patterns. The biological results reflect the integration of multiple simultaneous signaling cascades in the cellular microenvironment. Importantly, malignant HL60 populations showed greater vulnerability to ZnO nanostructure-induced cytotoxicity than healthy mononuclear cells [33].

Despite extensive research on ZnO nanoparticles for anticancer applications, significant challenges remain regarding their therapeutic efficacy and biosafety. While doping with transition metals has shown promise in modulating the physicochemical and biological properties of ZnO, most reports employ conventional chemical synthesis methods that may introduce toxic residues. To the best of our knowledge, this is the first study reporting the green synthesis of Mn-doped ZnO nanoparticles using *P. farcta* extract and their systematic evaluation for melanoma cytotoxicity. The novelty of our approach lies in: (i) utilizing a plant extract with intrinsic bioactive compounds that may synergistically enhance anticancer activity; (ii) demonstrating that Mn doping significantly improves the selective cytotoxicity of ZnO nanoparticles against C540 melanoma cells; and (iii) establishing the hemocompatibility of these nanoparticles, a critical parameter for systemic anticancer applications. Accordingly, the present study aims to address these gaps by synthesizing and characterizing Mn-doped ZnO nanoparticles via a sustainable green route and evaluating their anticancer potential against melanoma cells.

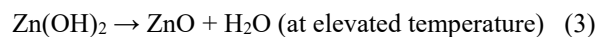
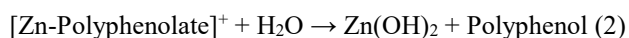
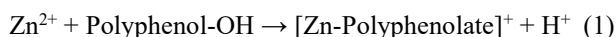
Therefore, in this study, an attempt was made to synthesize zinc oxide nanoparticles (Zn-NP) and zinc oxide nanoparticles doped with 5% manganese (Mn-Zn-

NP) via a green method using *P. farcta* extract. Considering the potential of zinc oxide nanoparticles in cancer treatment, the effects of the synthesized nanoparticles on the cytotoxic status of melanoma cancer cells (C540) were investigated.

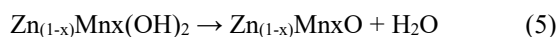
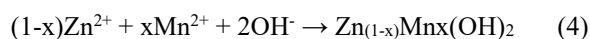
2. Experimental

2.1 Synthesis of Zn-NP and Mn-Zn-NP

In order to extract of *P. farcta* for beginning the synthesizing process, we weighted 1.0 gr of leaf powder of *P. farcta* and shaken its mixture with 100 mL of distilled water for 20 hours at 150 rpm. Subsequent to filtering the solution through Whatman No. 1, the resultant was set aside for the upcoming trials. To synthesis of nanoparticles, 10 mL of aqueous extract of *P. farcta* was dissolved with 50 mL of distilled water in two Erlenmeyer's, separately, and go through a water bath at 80 °C. The $Mn_{1-x}Zn_xO$ formula was followed to arrange 50 mL of solutions consisted of $Zn(NO_3)_2 \cdot 9H_2O$ (0 and 0.05 M) and $Mn(NO_3)_2$ as the applied percentages of manganese nitrate were 0 and 5, respectively. The obtained mixtures were stirred for 4 hours, and then they dried at 90 °C for 10 hours. The resulted raw powder calcinated by a furnace at 550 °C for 2 hours, separately. Two obtained white and light-yellow powders were Zn-NP and Mn-Zn-NP, respectively. The Synthesis Mechanism proposed in the following questions:



Mn Doping:



2.2 Characterization

The framework of our samples was characterized by the data of Powder X-ray diffraction (PXRD) (Netherlands, PANalyticalX'Pert PRO MPD system, Cu K α). Moreover, the morphological analysis required the utilization of Field Emission Scanning Electron Microscopy (FESEM) (MIRA3 TESCAN, Czech), while the scrutinization of elemental data was completed through the EDX spectra. Lastly, we configured the spectral responses and defect states by the results of UV-vis spectrometer that implicated diffuse reflectance attachments (Halo DB-20 UV-Vis double beam).

2.3 In vitro Hemolysis Assay

To conduct the hemolysis test, a mixture of fresh human blood and phosphate-buffered saline (PBS) was prepared in 3:5 ratio. Various concentrations of Zn-NP and 5% Mn-Zn-NP (1000, 500, 250, 50, and 10 $\mu\text{g/mL}$) were then ready using PBS. Subsequently, 250 μL of each concentration was placed into separate vials and allowed to incubate at 37 °C for 30 minutes. Positive and

negative controls were represented by H₂O and PBS, respectively. Following this, 100 μ L of diluted blood was introduced into all sample vials. The vials were subsequently placed in an incubator set at a temperature of 37 °C for 2 hours. The intact erythrocytes were separated through the vials to centrifugation at 5000 rpm for 10 minutes. Subsequently, the microplate reader was utilized to measure the absorbance of the liquid portion above the sediment. The recorded values were then used to calculate the hemolysis rate percentage (%HR) of the samples, which is determined by the formula of Equation 1:

$$\%HR = \frac{\text{sample absorbance} - \text{negative control absorbance}}{\text{positive control absorbance} - \text{negative control absorbance}} \quad (6)$$

2.4 Cell Culture

The melanoma cancer cells (C540) were utilized to evaluate the cytotoxic effects of our synthesized nanoparticles. These cells were obtained from the Pasteur Institute of Iran and thawed before being cultured in accordance with the research conducted by Sarani et al [34].

2.5 MTT Test

To evaluate the cytotoxic performance of biosynthesized nanoparticles using the MTT ((3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide) assay, three samples were analyzed at concentrations of 1, 5, 10, 50, 100, 250, 500, and 1000 μ g/mL. Initially, C540 cells per well were added to a 96-well plate and incubated for 24 hours. Prior to removing the culture medium from each well, it was important to confirm cell adhesion to the

plate surface. Afterwards, we combined 100 μ L of nanoparticles with the corresponding wells and proceeded to incubate the plate for 24 hours at 37 °C under 5% CO₂. Following the dispatch of the plate, we added 10 microliters of MTT solution to each well of the 96-well plate, requiring a re-incubation period of 4 hours. To complete the process, we assessed the adsorption of the samples using an ELISA reader at a wavelength of 490 nm. This was followed by the utilization of the Equation 2:

$$\text{Cell survival} = \frac{\text{Optical density of treated sample}}{\text{Optical density of control sample}} \times 100 \quad (7)$$

3. Results and Discussion

3.1 PXRD

Figure 1 presents the PXRD patterns of Zn-NP and Mn-Zn-NP, which the diffraction peaks of every pattern seemed to be suitably in correlation to the standard reference data (JCPDS data card No. 36-1451) of the hexagonal wurtzite construction of ZnO. This observation affirms the crystalline nature of the sample as the most stable mode of ZnO. Furthermore, doping of manganese ions into the lattice sites of Zn²⁺ resulted in reduced the peak intensity than Zn-NP of PXRD pattern [35]. We determined the crystallite sizes of our samples by Scherrer's equation [36] to be 35.16 and 39.52 nm for Zn-NP and Mn-Zn-NP, respectively, and realized that the enlargement in doped particles sizes was caused by the difference in ionic radius of Manganese (0.81 Å) and zinc (0.74 Å). Miri et al. presented similar PXRD result for ZnO nanoparticles with wurtzite structure [35]. The similar findings observed in Grace et al. study [37].

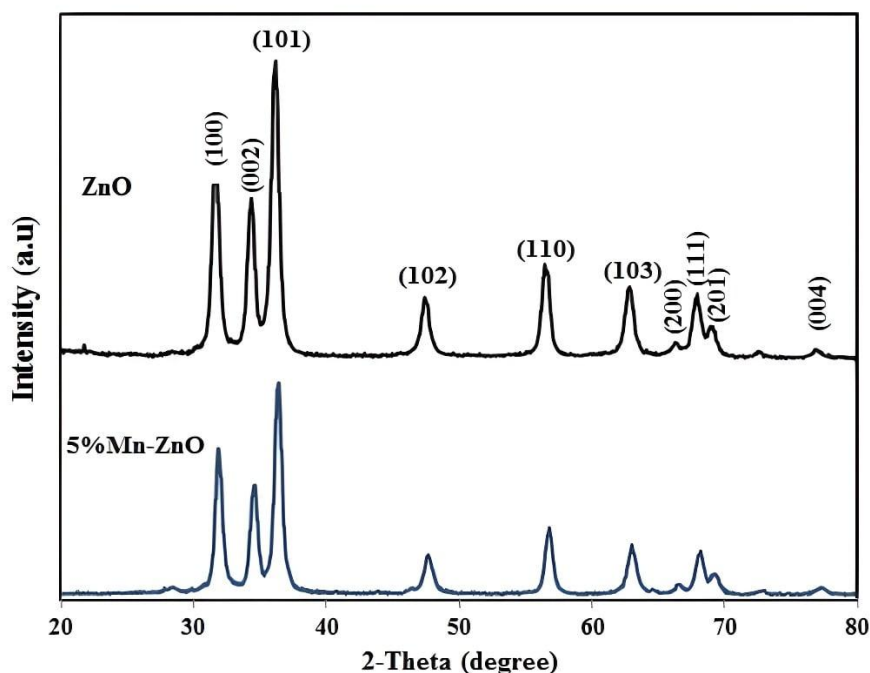


Figure 1. PXRD patterns of Zn-NP and Mn-Zn-NP.

3.2 FESEM and EDX

Figure 2 exhibits that the average particle size of Zn-NP to be around 30 nm, while the appearance of Mn throughout the crystalline lattice of Zn-NP increased the particle size of doped particles up to 50 nm. As shown in Figure 2, the size distribution presented 30.78 nm and 60.34 nm for the Zn-NP and Mn-Zn-NP, respectively. The data of PXRD section pointed out the relation between this observation and the ionic radii of the

substituting Mn^{2+} and Zn^{2+} ions. Furthermore, the spherical morphology of both samples was recorded as well. The satisfactory ingress of Mn into the construction of Zn-NP was approved by Energy Dispersive X-ray (EDX) of synthesized products. The rate of existing impurity in the samples was close to zero since the percentages of Mn atom in Zn-NP and Mn-Zn-NP were 0 and 4.52%, respectively, which is perceivable in Figure 3.

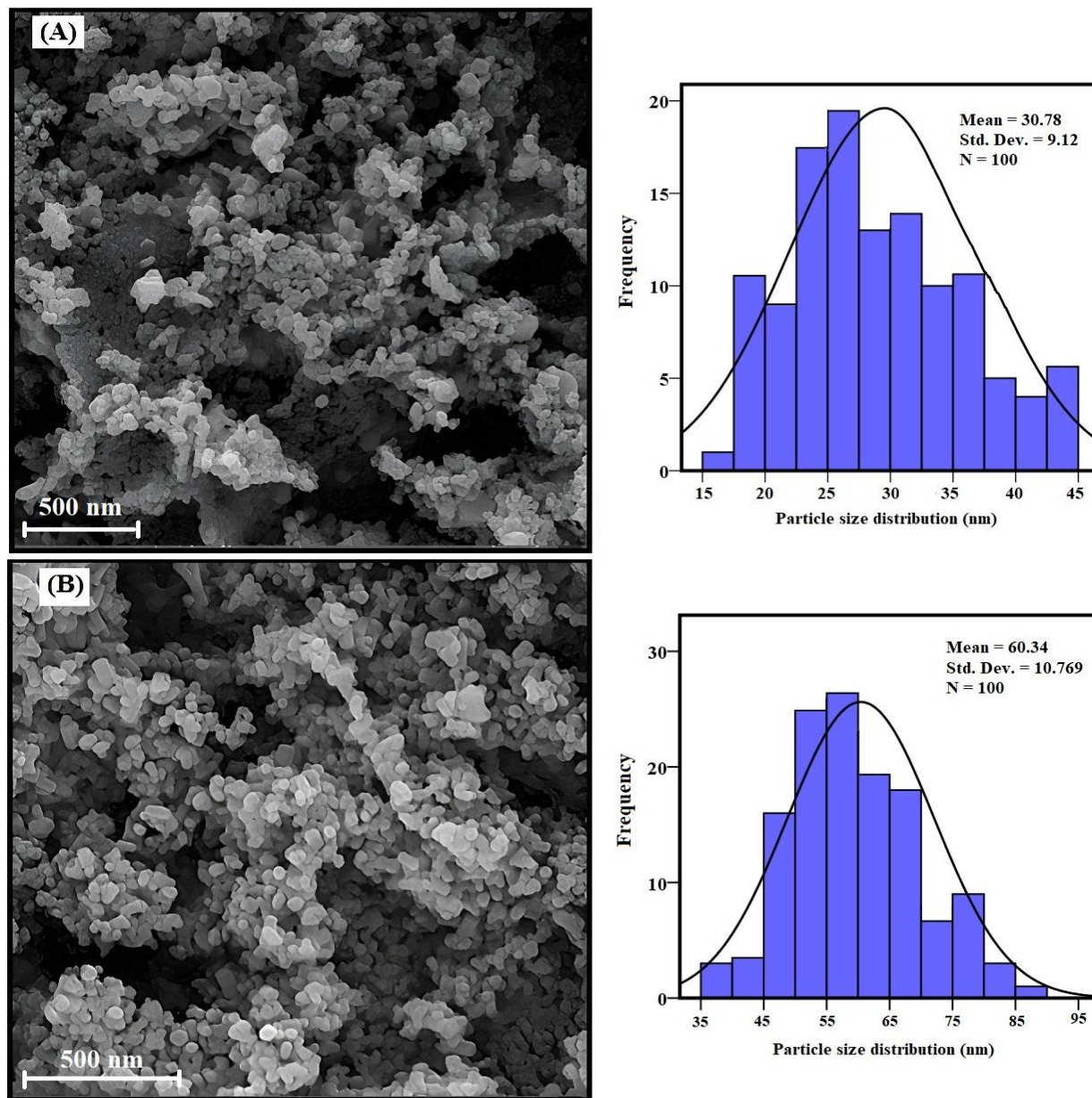


Figure 2. FESEM images and size distribution of (A) Zn-NP and (B) Mn-Zn-NP.

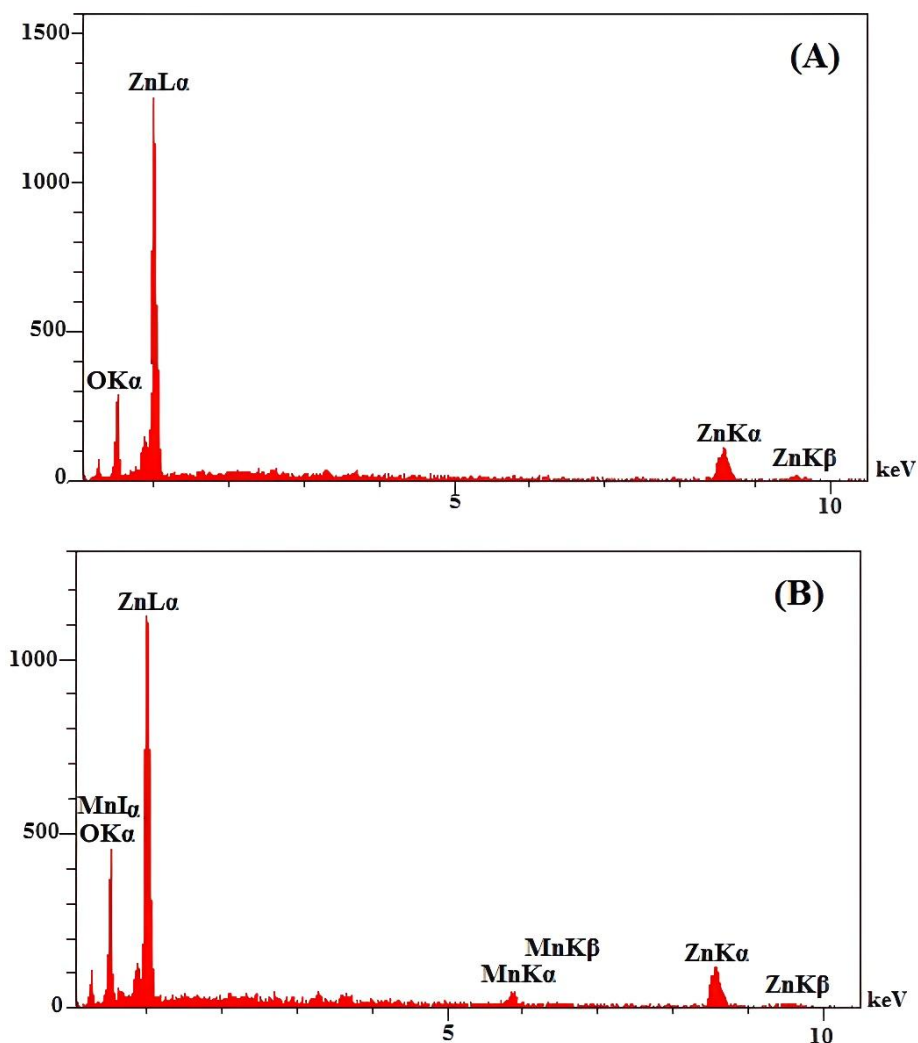


Figure 3. EDX patterns of (A) Zn-NP and (B) Mn-Zn-NP.

3.3 Raman Analysis

Figure 4 illustrates the Raman spectra of ZnO-NP and Mn-ZnO-NP in the range of 200 to 800 cm^{-1} at ambient temperature. The high frequency branch of E_2 (E_2^{high}) of ZnO as the strongest mode of wurtzite crystal construction caused the appearance of the sharpest and strongest peak at about 435 cm^{-1} . Moreover, the peak at

around 580 cm^{-1} was attributed to A_1 longitudinal optical (LO) mode, while the detected peak at about 280 cm^{-1} and the broad shoulder centered at about 752 cm^{-1} were induced by the two-phonon process, which corroborates experimental findings for Raman scattering by Cheng [38] and Yoshikawa [39]. In the Raman spectrum of Mn-ZnO observed similar peaks, but these peaks shifted due to doping of Mn into ZnO structure.

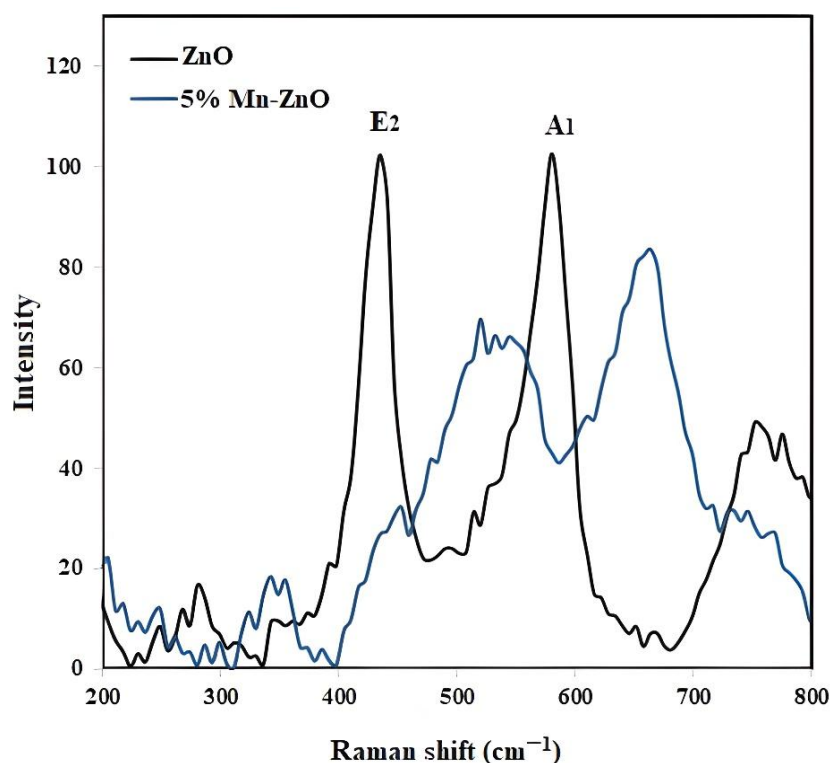


Figure 4. Raman patterns of Zn-NP and Mn-Zn-NP.

3.4 UV-vis Analysis

The Ultraviolet-Visible (UV-vis) Spectroscopy absorption spectra of Zn-NP and Mn-Zn-NP were displayed in Figure 5, which the optical absorption peaks observed at 368 and 339 nm, respectively. The shift in absorption towards a lower wavelength was observed due to the introduction of Mn doping into ZnO. According to the absorption data, the energy gap (E_g) of Zn-NP and Mn-Zn-NP was estimated using the Tauc

equation ($(\alpha h\nu)^2 = A(h\nu - E_g)$, Where ' α ', ' $h\nu$ ', ' A ' indicates to the absorption coefficient, the photon energy, a constant value independent of photon energy, respectively). The band gap of Zn-NP and Mn-Zn-NP was achieved 3.2 eV and 2.9 eV, respectively [39]. This observation further supports the successful substitution of Mn cations in the wurtzite structure of ZnO, replacing the Zn cation sites. These findings matched with Khudhair et al [40] and Mazhir et al [41] studies.

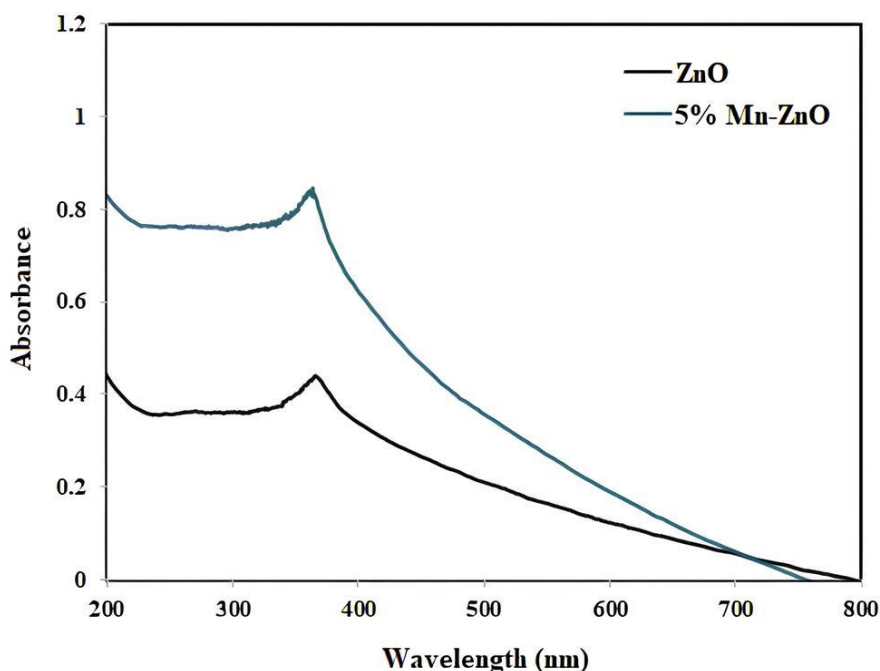


Figure 5. UV-vis spectra of Zn-NP and Mn-Zn-NP.

3.5 DLS Analysis

Dynamic light scattering (DLS) exams to study the hydrodynamic size of Zn-NP and Mn-Zn-NP in suspension. For Zn-NP, the average diameter is observed to be 25.9 nm as presented in Figure 6, which is consistent with the SEM results with a particle size of 30 nm. After doping with manganese, the hydrodynamic

size of the particles increased to 60.5 nm, which is in good agreement with the SEM findings. The increase in hydrodynamic size relative to the crystallite size indicates that Mn-Zn-NP in dispersion form stable and lose agglomerates, while their primary units remain small and defect-rich, which is a desirable property for adsorption applications.

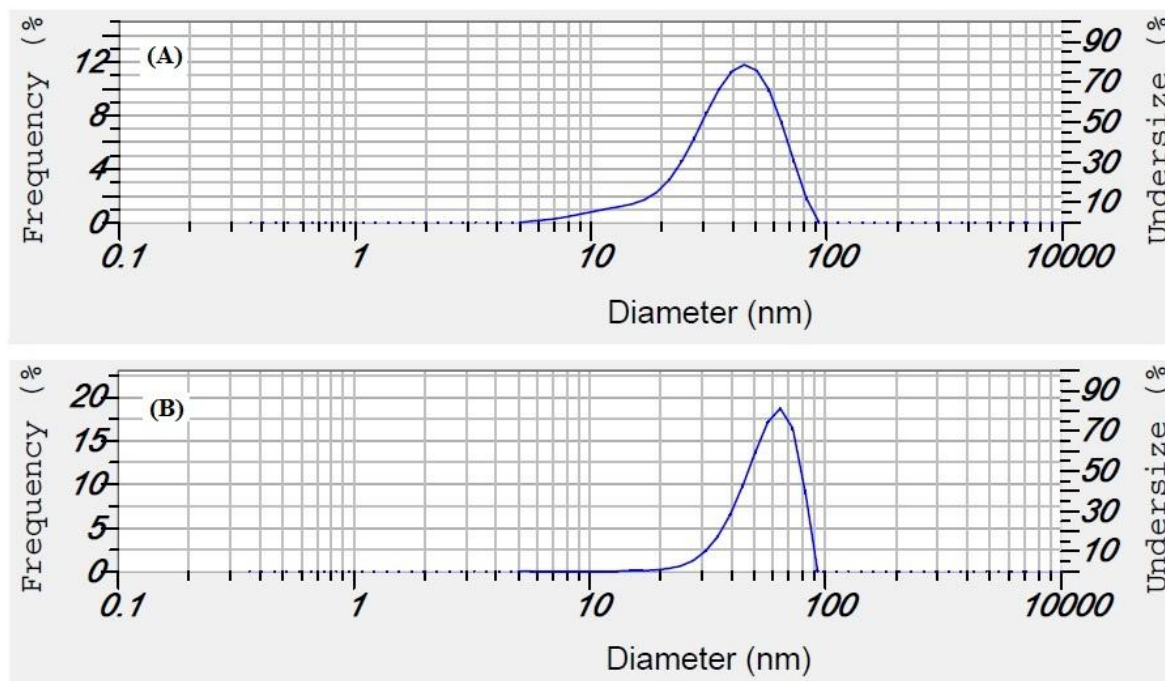


Figure 6. DLS graphs of (A) Zn-NP and (B) Mn-Zn-NP.

3.6 FT-IR Analysis

Figure 7 illustrated the Fourier transform infrared (FTIR) spectra obtained for Zn-NP and Mn-Zn-NP samples. These spectra show a broad peak associated with hydroxyl (OH) groups in the range of 3400 cm^{-1} , which is due to the moisture adsorbed on the nanoparticles. In addition, a peak at 471.12 cm^{-1} is associated with the Zn-

O stretching vibrations in the Zn-NP spectrum. This peak shifts to 458.96 cm^{-1} in Mn-Zn-NP spectrum due to the introduction of manganese into the ZnO structure. In addition, a peak at 670.24 cm^{-1} appears, which is due to the presence of manganese in the ZnO structure. Dulta et al. have attributed the vibration band in the range of $400\text{--}600\text{ cm}^{-1}$ to ZnO, which is consistent with our results [42].

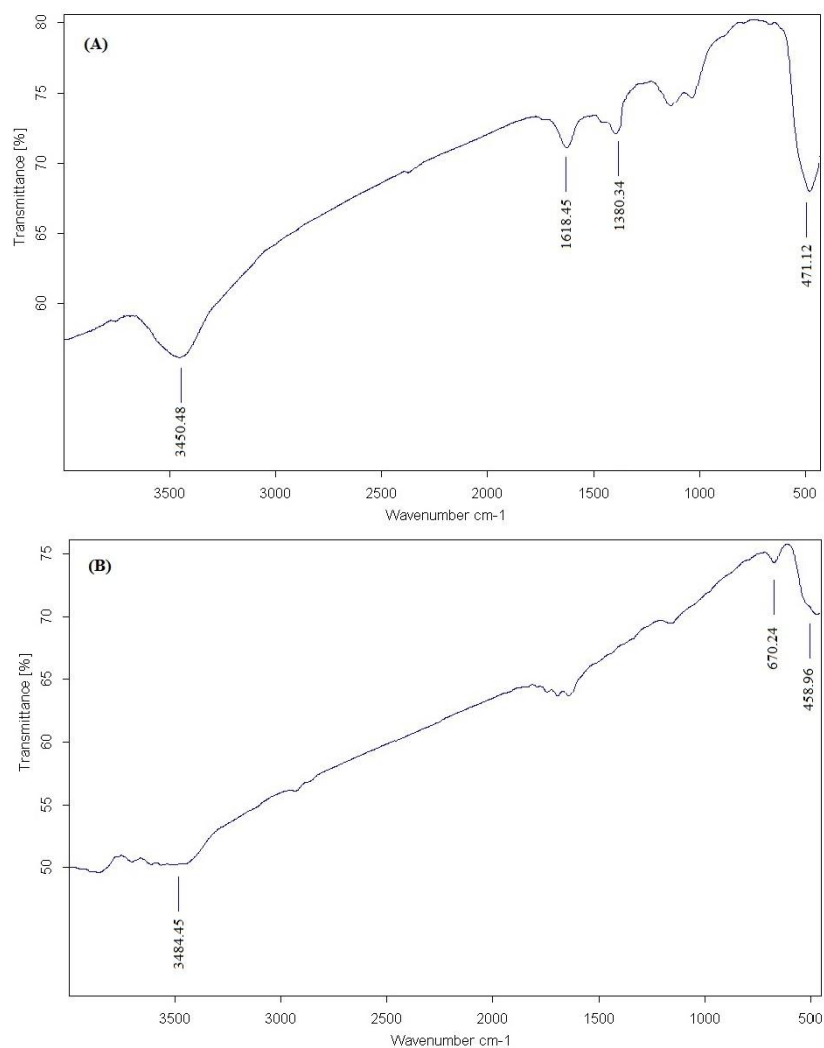


Figure 7. FT-IT graphs of (A) Zn-NP and (B) Mn-Zn-NP.

3.7 Hemolysis

The evaluation of the hemocompatibility of Zn-NP and Mn-Zn-NP with blood was conducted by utilizing the hemolytic assay. The assessment of hemocompatibility holds critical in the evaluation of biomaterials, as introduction to the blood milieu carries the potential to cause harm to erythrocytes or lead to the arrangement of thrombus. An *in vitro* hemolytic test was carried out to ensure the safety of Zn-NP and Mn-Zn-NP. The results demonstrated that the level of hemolysis percentage for the tested samples was less than 5% (Table 1), an esteem

that adjusts with the basic secure hemolytic limit for biomaterials as sketched out within the ISO rules (ISO/TR 7406). This indicates that the harm dispensed upon erythrocytes by the test was immaterial. Consequently, the outcomes about illustrate the biocompatibility and safe of Zn-NP and Mn-Zn-NP for systemic administration. Furthermore, the nonappearance of striking poisonous quality concerns can be concluded from these comes about. In any case, assist broad considers on poisonous quality are basic for the foundation of a total security profile.

Table 1. Percentage of hemolysis rate of Zn-NP and Mn-Zn-NP

Concentration ($\mu\text{g/mL}$)	Hemolysis Rate%		Control	
	Zn-NP	Mn-Zn-NP	Positive (H_2O)	Negative (PBS)
1000	0.415159 ± 0.10	1.10828 ± 0.11	1.199 ± 0.12	0.054 ± 0.05
500	0.323150 ± 0.12	0.90128 ± 0.15	1.199 ± 0.12	0.054 ± 0.05
250	-0.57165 ± 0.10	0.515416 ± 0.09	1.199 ± 0.12	0.054 ± 0.05
50	-0.89524 ± 0.13	-0.29146 ± 0.08	1.199 ± 0.12	0.054 ± 0.05
10	-0.88235 ± 0.10	-0.518 ± 0.08	1.199 ± 0.12	0.054 ± 0.05

3.8 Cytotoxic Performance

We assayed the cytotoxic performance of Zn-NP and Mn-Zn-NP on melanoma cancer cell by analyzing the results of MTT trial, which revealed a dose-dependent reduction in the cell viability of doped NP, as shown Figure 8. Additionally, Mn-Zn-NP exhibited a stronger cytotoxic effect on C540 cells compared to Zn-NP. Certain investigations pointed out the cytotoxic attitude of zinc oxide nanoparticles towards highly proliferating

cells as a result of intensifying the formation of ROS [43]. Specifically, these nanoparticles can perform as the redox system of cancer cells, generating substantial quantities of ROS by facilitating the induction of reactions with chemical species of cells for producing oxidative stress. Consequently, this phenomenon can influence biomolecules and injure cell membranes by peroxidizing the lipids and denaturing the proteins, ultimately leading to necrosis, DNA damaging, and lastly cell annihilation through apoptosis [44].

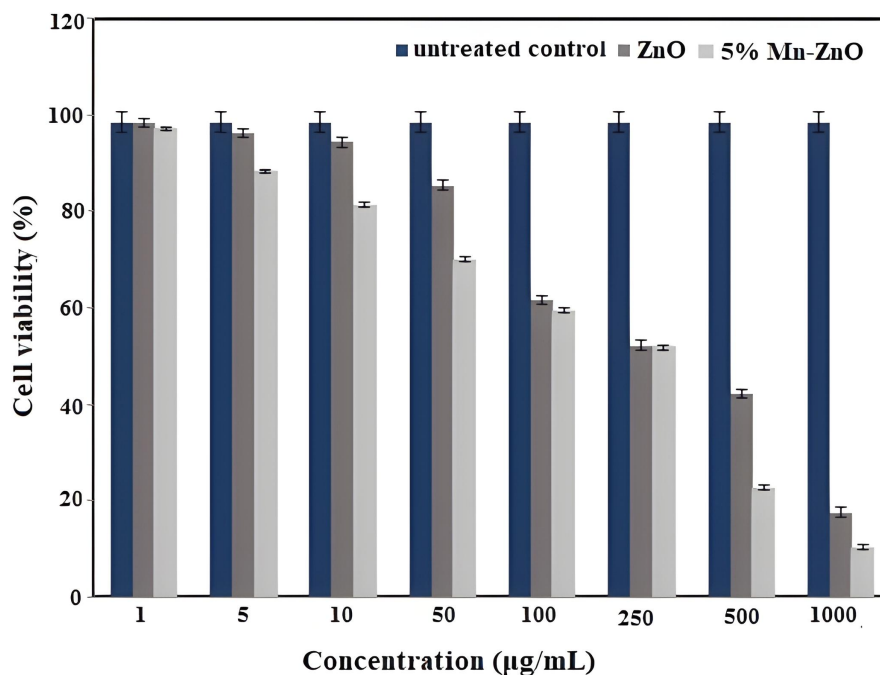


Figure 8. Cell viability of Zn-NP and Mn-Zn-NP on C540 cell line after 24 h incubation.

For instance, Rajendran et al. implicated the application of sol-gel technique to synthesize silver-doped zinc oxide nanoparticles, which were subsequently evaluated in terms of cytotoxicity towards the HaCaT cell line through the data of MTT trial. They reported their observations on the induced apoptotic and morphological changes in the cells that were treated with nanoparticles. According to their explanations, this occurrence was associated with the heightening of ROS levels and a decrease in the potential of mitochondrial membrane as a result of Ag and ZnO NPs cooperation in cancer cells. Furthermore, they confirmed that exposure to Ag-ZnO NPs on providing the induction of cell destruction in UVB-induced HaCaT cells, which is attributable to the reactive oxygen species-mediated apoptotic [45]. Similarly, Enoch et al. studied the synergistic effect of Mn₃O₄ nanoparticles and Carbopol on A431 skin cancer cells, and their results were demonstrated anticancer potential of synthesized nanoparticles with cell viability to $22.38 \pm 0.7\%$ [46]. In comparison to Zn-NP, the outcomes of their study denoted the superior toxic impacts of Mn-Zn-NP towards C540 cells compared to undoped Zn-NP. Nevertheless, the impact of Mn addition to ZnO lattice in enhancing cytotoxicity. Considering these descriptions, one can affirm the better anti-cancer property of Mn-Zn-NP in opposition to C540 cells.

4. Conclusion

In this study, we successfully synthesized ZnO nanoparticles (Zn-NP) and 5% manganese-doped ZnO nanoparticles (Mn-Zn-NP) via sustainable green approach using an aqueous extract of *Prosopis fratta* as both a reducing and stabilizing agent. Comprehensive characterization confirmed the formation of phase-pure hexagonal wurtzite ZnO structures with crystallite sizes of 35.16 nm (Zn-NP) and 39.52 nm (Mn-Zn-NP). The successful incorporation of Mn²⁺ ions (4.52% atomic composition by EDX) into the ZnO lattice was evidenced by lattice expansion (PXRD), a reduction in the optical band gap from 3.2 eV to 2.9 eV (UV-Vis), and characteristic Raman peak shifts. The evaluation of the hemocompatibility of Zn-NP and Mn-Zn-NP showed that biocompatibility and safe of our nanoparticles for systemic administration. The MTT assay revealed dose-dependent cytotoxicity against C540 melanoma cells, with Mn-Zn-NP demonstrating significantly superior anticancer activity ($IC_{50} \sim 25 \mu\text{g/mL}$) compared to undoped Zn-NP ($IC_{50} \sim 50 \mu\text{g/mL}$). This 50% enhancement in cytotoxic efficacy is attributed to increased ROS generation facilitated by Mn-induced defect states within the ZnO bandgap. The practical suitability of these NP in biological utilizations and cancer therapy is further supported by the comprehensive findings presented in this study.

List of Abbreviations

PXRD	Powder X-ray diffraction
FESEM	Field emission scanning electron microscopy
EDX	Energy Dispersive X-Ray
C540	Melanoma Cancer Cells
MTT	(3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide
Zn-NP	Zinc Oxide Nanoparticles
Mn-Zn-NP	Manganese doped zinc oxide nanoparticles
PBS	Phosphate-buffered saline

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Funding

Not applicable.

Ethics Statement

Not applicable.

Data Availability Statement

The data supporting the findings of this study are available within the article.

Author Contributions

Mostafa Azizi: Supervision, Methodology, Formal analysis, Investigation, Writing – original draft, Writing – review & editing.

Competing Interests

There is no conflict of interest.

Generative AI Statement

The authors disclose that generative AI was just used for correct spelling errors and format references in this manuscript.

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