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Waste-Derived Green Synthesis of Mn-Doped ZnO Nanoparticles using *Bos indicus* Cow Dung Extract for Antimicrobial and Anticancer Applications

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Abstract

In the present study, pure ZnO and manganese (Mn)-doped ZnO nanoparticles were synthesized via a green route using aqueous cow dung extract (*Bos indicus*). The synthesized nanomaterials were systematically characterized using powder X-ray diffraction (XRD), scanning electron microscopy (SEM), transmission electron microscopy (TEM), energy-dispersive X-ray spectroscopy (EDS), X-ray photoelectron spectroscopy (XPS), and UV–Visible diffuse reflectance spectroscopy (UV–Vis DRS). Zinc oxide (ZnO) is widely recognized as a promising antimicrobial agent due to its unique physicochemical properties. The antibacterial activity of the synthesized nanoparticles was evaluated against Gram-negative bacteria (*Escherichia coli*) and Gram-positive bacteria (*Staphylococcus aureus* and *Bacillus subtilis*). The results demonstrated significant antibacterial activity, particularly against *B. subtilis* and *S. aureus*. Furthermore, Mn-doped ZnO nanoparticles exhibited enhanced anticancer activity against the MCF-7 breast cancer cell line, with an IC₅₀ value of 219.56 µg/mL. The incorporation of Mn into the ZnO lattice improved the selective cytotoxicity compared to pure ZnO nanoparticles.

Keywords: ZnO nanoparticles, Green synthesis, Cow dung extract (*Bos indicus*), Antibacterial activity, Anticancer activity.

1. Introduction

Nanomaterial research has recently gained importance due to prospective applications in human life and the environment. Nowadays, nanoparticle research is unavoidable not only because of its importance but also due to the way of synthesis [1]. Consequently, extensive research efforts have been directed toward the synthesis of nanomaterials using various approaches, including physical, chemical, and biological methods [2]. Among these, biological synthesis has recently emerged as a preferred route because of its eco-friendly nature, safety, non-toxicity, and biocompatibility [3]. Cow dung can be defined as the undigested residue of consumed food material being excreted by herbivorous bovine animal species [4]. Cow dung, a naturally abundant and renewable resource, is rich in organic matter, microbial populations, and bioactive compounds such as proteins, flavonoids, and phenolic substances. These constituents act as effective reducing and stabilizing agents, making cow dung extract a promising yet relatively unexplored medium for nanoparticle synthesis [5]. The utilization of cow dung not only enables sustainable nanoparticle production but also adds value to agricultural waste, aligning with the principles of the circular bioeconomy [6][7].

Zinc oxide (ZnO) has attracted considerable interest due to its distinctive electronic, optical, chemical, and catalytic properties, leading to its application in advanced fields such as cosmetics, electronics, biology, and medicine [8][9]. However, pure ZnO nanoparticles exhibit certain limitations in photocatalytic performance, mainly due to their wide band gap and restricted absorption in the visible region [10]. To overcome these drawbacks, doping with transition metals such as iron (Fe) [11], manganese (Mn) [12], and nickel (Ni) [13] has been widely explored to tailor the band gap and enhance visible-light-driven photocatalytic activity [9]. The incorporation of dopant ions into the ZnO lattice generates point defects, including oxygen and zinc vacancies, which introduce intermediate energy levels within the band gap. These modifications significantly alter the physicochemical properties of ZnO, making Mn-doped ZnO particularly attractive for photocatalytic applications [14]. Zinc oxide nanoparticles (ZnO NPs) have been extensively investigated for a wide range of biological applications, including antibacterial [15][16], anticancer [17], antidiabetic [18], drug delivery [19], antifungal [20], and agricultural uses [21][22]. Among these, ZnO NPs have gained particular attention as effective antimicrobial agents due to their high thermal stability, low degradation rate, and relatively longer functional lifespan compared to conventional organic antimicrobial compounds [23]. ZnO exhibits strong antibacterial activity against both Gram-positive and Gram-negative bacteria, which is largely attributed to its ability to generate reactive oxygen species (ROS) [24]. These ROS are known to induce cytotoxic effects in microbial cells

[25][26], leading to their inhibition or destruction [27]. One of the key factors contributing to the antimicrobial efficiency of ZnO nanoparticles is their band gap energy, which facilitates enhanced ROS generation upon interaction with biological systems. The properties of ZnO can be further tuned to improve its biological performance, particularly through doping with suitable elements. Incorporation of manganese (Mn) into the ZnO lattice is one such approach that modifies its physicochemical characteristics. Mn doping can enhance the stability of ZnO nanoparticles and potentially reduce their rapid dissociation, thereby controlling excessive ROS generation. This controlled ROS release is advantageous for biomedical applications, such as topical antiseptics, where a gradual and sustained antimicrobial effect is desirable. Manganese (Mn) is the metal of choice for doping of ZnO nanoparticles because of the ready availability of its d electrons at the t_{2g} level, and also for the fact that these electrons can easily overlap with the valance bond of ZnO nanoparticles [28]. Furthermore, previous studies have demonstrated that Mn exhibits good solubility within the ZnO matrix, and their combination shows enhanced performance in various electronic and functional applications [29]. In addition, Mn-doped ZnO nanoparticles often exhibit reduced particle size compared to pure ZnO, which enhances their interaction with cellular systems and improves biological activity [30]. Magnetic nanomaterials have also attracted considerable attention due to their potential applications in antimicrobial and biomedical fields [31]. It has been reported that Mn doping not only improves the antibacterial efficiency of ZnO but also introduces magnetic properties, making these nanomaterials suitable for advanced applications such as spintronics and targeted biomedical systems.

The enhancement in antimicrobial and antioxidant properties is mainly attributed to increased surface area and reduced particle size resulting from Mn incorporation [32]. Furthermore, ZnO doped with transition metal ions such as Co, Ni, Mn, V, and Fe has been widely explored for spintronic applications due to the modification of its electrical, optical, and magnetic properties [33]. Consequently, Mn-doped ZnO nanoparticles have emerged as promising materials for multifunctional applications. Therefore, the present study focuses on the synthesis and characterization of pure ZnO and Mn-doped ZnO nanoparticles, along with the evaluation of their antibacterial, anticancer and photocatalytic activities [28][34].

2. Experimental

2.1 Materials

$\text{Zn}(\text{NO}_3)_2 \cdot 6\text{H}_2\text{O}$ (Zinc nitrate hexahydrate), MnCl_4 (Manganese tetrachloride), (NaOH) sodium hydroxide, and ethanol were purchased from Sigma-Aldrich Company and applied

without further purification. Cow dung is collected from the local area and is derived from *Bos indicus*.

2.2 Preparation of Cow Dung Extract

To prepare the cow dung extract, 10 g of cow dung was added to 100 ml of distilled water. The mixture was then boiled for about 30 minutes, cooled, and filtered using a muslin cloth. The filter was centrifuged at 10000 rpm for 15 minutes. The pellet was discarded, and the supernatant was collected and filtered through Whatman filter paper No.1 [35].

2.3 Synthesis of Zinc oxide nanoparticles

An aqueous solution of zinc nitrate hexahydrate (1M, 50 ml) was prepared in a beaker. To this solution, 100ml of cow dung extract was added dropwise using a burette over a period of 1 hour under continuous stirring. The pH of the mixture was maintained at 12 by the dropwise addition of NaOH solution. The reaction mixture was stirred at 600-700 rpm and maintained at 80-90 °C throughout the process. The reaction was allowed to proceed for 4 hours under these conditions. After completion of the reaction, the resulting precipitate was collected by filtration and washed several times with ethanol to remove impurities. The obtained product was then dried at 70°C for several hours. Finally, the dried powder was calcined at 500 °C for 3 hours to obtain zinc oxide nanoparticles.

2.4 Synthesis of Mn-doped ZnO nanoparticles

20 ml of 1M zinc nitrate hexahydrate $\text{Zn}(\text{NO}_3)_2 \cdot 6\text{H}_2\text{O}$ and 20 ml of 1M manganese chloride tetrahydrate mix in beaker. The pH of the solution was adjusted to 8 to 9 by adding NaOH solution dropwise. The reaction mixture was heated to 60 to 70 °C on a magnetic stirrer at 600 to 700 rpm. Subsequently, 100 ml of the prepared cow dung (pH 8-9) was added dropwise over a period of 1 hour under continuous stirring. The reaction was allowed to proceed for 4 hours while maintaining the temperature. Furthermore, the resulting product was filtered through Whatman No. 41 filter paper and washed with distilled water and ethanol to remove impurities. The obtained precipitate was dried at 70°C for 4 hours. Finally, the dried powder was calcined at 500 °C for 3 hours to obtain zinc oxide nanoparticles.

2.5 Experimental procedure

2.5.1 Disk diffusion method

The antibacterial activity of pure and Mn-doped ZnO nanoparticles was evaluated against *Escherichia coli* and *Staphylococcus aureus* using the standard disk diffusion method as described by Rekha et al. Sterile nutrient agar plates were prepared and allowed to solidify under aseptic conditions. Fresh bacterial cultures were grown in nutrient broth, and the inoculum density was adjusted to the required turbidity standard. The bacterial suspension was

evenly spread over the agar surface using a sterile cotton swab to obtain a uniform lawn culture. Sterile discs (6 mm in diameter) impregnated with nanoparticle solutions at different concentrations (50, 100, and 150 $\mu\text{L/mL}$), prepared using DMSO as the solvent, were allowed to dry under aseptic conditions. These discs were then carefully placed onto the inoculated agar plates using sterile forceps. The plates were initially kept at 4 °C for 30–60 minutes to allow proper diffusion of the test compounds into the medium, followed by incubation at 37 °C for 24 hours. After incubation, the zones of inhibition were measured in millimeters. Streptomycin was used as the positive control (standard), and the observed antibacterial activity was recorded and presented in Table 1 [36].

2.5.2 Anticancer activity

The cytotoxic potential of the synthesized undoped and Mn-doped ZnO nanoparticles was evaluated using the MTT (3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyl-2H-tetrazolium bromide) assay (Bio Basic Inc., Canada) following a standard protocol [37]. The human breast cancer cell line MCF-7 [at INFINITE BIOTECH Laboratory, Institute of Research and Analytics, Sangli, Maharashtra, India] was cultured and seeded into 96-well tissue culture plates at a density of 1×10^4 cells/mL. The cells were treated with different concentrations (25, 50, 75, 100, and 125 $\mu\text{g/mL}$) of the test samples. Control wells contained cells treated with 0.2% DMSO in PBS. All experiments were performed in triplicate to ensure reproducibility. Control was maintained to determine the control cell survival and the percentage of live cells after culture. The plates were incubated at 37°C in a humidified atmosphere containing 5% CO₂ for 24 h. After incubation, the culture medium was carefully removed, and 20 μL of MTT solution (5 mg/mL in PBS) was added to each well. The plates were further incubated for 4 h under the same conditions to allow the formation of formazan [38] metabolically active (viable) cells, which was confirmed by microscopic observation. Following incubation, the medium was discarded, and 200 μL of DMSO was added to each well to dissolve the formazan crystals. The plates were kept for 10 min (protected from light using aluminum foil) to ensure complete solubilization. The absorbance of each well was then measured at 550 nm using a microplate reader. Cell viability and inhibition percentages were calculated using the following equations [39]:

$$\begin{aligned} \text{Viability (\%)} &= (\text{Optical density of sample} / \text{Optical density of control}) \times 100 \\ \text{Inhibition (\%)} &= 100 - \text{Viability (\%)} \end{aligned}$$

3 Results and Discussion

3.1 X-Ray Diffraction Analysis and UV-Vis diffuse reflectance spectroscopy (DRS) of as-synthesized Mn-doped ZnO nanoparticles:

Figure 1 illustrates the typical XRD patterns of the synthesized uncapped ZnO and Mn-doped ZnO nanopowders. The diffraction peaks observed at Bragg angles of 31.85°, 34.55°, 36.35°, 47.69°, 56.75°, 62.86°, 66.37°, 67.96°, 69.10°, 72.87°, and 77.21° correspond to the crystallographic planes (100), (002), (101), (012), (110), (013), (200), (112), (004), and (202), respectively. These peaks are sharp and intense, indicating the high crystallinity of the synthesized materials. All observed diffraction peaks are in good agreement with the standard JCPDS card no. 36-1451, confirming the hexagonal wurtzite structure of ZnO with lattice parameters $a = 3.2417 \text{ \AA}$ and $c = 5.1876 \text{ \AA}$. The prominent diffraction peaks located at 32.09°, 34.79°, 36.63°, 48.05°, 57.20°, 63.58°, and 67.12° are indexed to the (100), (002), (101), (102), (110), (103), and (200) planes, respectively, which further supports consistency with standard ZnO data (JCPDS no. 36-1451).

Figure 1(b) represents the XRD pattern of Mn-doped ZnO nanoparticles. Compared to pure ZnO, the doped samples exhibit a noticeable decrease in peak intensity along with slight peak shifting and broadening. This broadening effect suggests a reduction in crystallite size and the formation of smaller nanoparticles. Similar observations have been previously reported for ZnO-based nanostructures[40][34]. The overall diffraction pattern of Mn-doped ZnO remains comparable to that of pure ZnO, indicating that the crystal structure is preserved after doping [41]. However, the reduction in peak intensity with increasing Mn^{2+} incorporation suggests partial substitution of Zn^{2+} ions within the ZnO lattice [42]. This substitution introduces lattice imperfections and increases defect concentration, leading to slight deterioration in crystallinity. Such structural modifications are typical in doped semiconductor systems and confirm successful incorporation of Mn ions into the ZnO matrix.

The crystallite size (D) of the nanoparticles was estimated using the Debye–Scherrer equation [43]:

$$D = \frac{0.94\lambda}{\beta \cos \theta} \quad (1)$$

where λ is the wavelength of Cu K α radiation, β is the full width at half maximum (FWHM) in radians, and θ is the Bragg diffraction angle. The average crystallite sizes of pure ZnO and Mn-doped ZnO were calculated to be 66.84 nm and 37.67 nm, respectively, confirming a decrease in particle size upon Mn doping.

The lattice constants (a and c) were determined using the following relation [34] :

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

where d is the interplanar spacing and (h, k, l) are the Miller indices. The calculated lattice parameters for both pure and Mn-doped ZnO are found to be very close to the standard values (JCPDS: 36-1451), indicating that Mn incorporation does not significantly alter the lattice structure. This suggests that Mn ions are successfully incorporated into the ZnO lattice without causing major structural distortion.

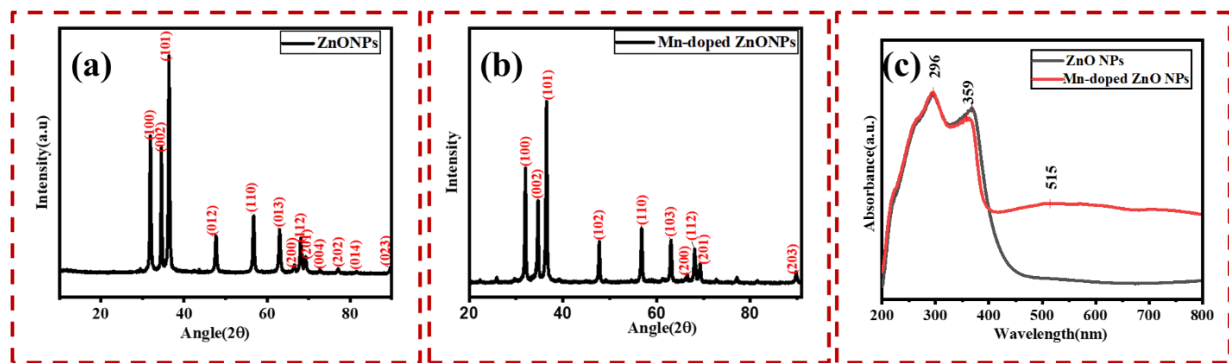


Fig. 1. XRD patterns of (a) pure ZnO NPs, (b) Mn-doped ZnO nanoparticles and (c) UV - Vis DRS spectrum of the ZnO and Mn-doped ZnO nanoparticles.

UV–visible absorption spectroscopy is a rapid and effective technique for the preliminary investigation of biologically synthesized nanoparticles [44]. **Figure 1c** presents the UV–Vis absorption spectra of ZnO nanoparticles recorded over the wavelength range of 300–1100 nm. The optical properties of pure and Mn-doped ZnO nanostructures were analysed to determine their band gap energies. The absorption spectra of pure and Mn-doped ZnO are illustrated in Figure 2. Pure ZnO nanoparticles exhibit characteristic absorption peaks at 293 nm and 368 nm [45]. Upon Mn doping, these peaks shift to 296 nm and 359 nm, respectively, along with the emergence of a broad absorption band centered around 515 nm. The shift from 293 nm to 296 nm indicates a slight red shift in the higher-energy region, whereas the shift from 368 nm to 359 nm corresponds to a blue shift near the band-edge region. The observed blue shift suggests a marginal increase in the band gap energy after Mn incorporation. This behavior can be attributed to $sp-d$ exchange interactions between the conduction band electrons of ZnO and the localized d -electrons of Mn^{2+} ions, resulting in modification of the electronic

band structure. Furthermore, the appearance of the broad absorption band at 515 nm is associated with d–d electronic transitions of Mn²⁺ ions within the ZnO lattice, confirming the successful incorporation of Mn into the crystal structure [34].

The band gap energy (E_{g}) of the nanostructures was estimated from the absorption edge using the following relation [46]:

$$E_{\text{g}} = \frac{1240}{\lambda_{\text{g}}} \quad (3)$$

where E_{g} is the band gap energy (eV) and λ_{g} is the absorption edge wavelength (nm). In addition, the optical band gap was further evaluated using Tauc's relation:

$$(\alpha h\nu)^{1/n} = C(h\nu - E_{\text{g}}) \quad (4)$$

where α is the absorption coefficient, $h\nu$ is the photon energy, h is Planck's constant, ν is the frequency ($\nu = c/\lambda$), and c is the speed of light. The constant C represents the proportionality factor, while E_{g} denotes the optical band gap. The value of n is 1/2 for indirect transitions and 2 for direct allowed transitions. The optical band gap of pure ZnO nanoparticles was determined to be 3.10 eV. After Mn doping, the band gap increased to 3.20 eV, indicating a clear blue shift in the absorption edge. This enhancement in band gap energy can be attributed to sp–d exchange interactions between the conduction band electrons of ZnO and the localized d -electrons of Mn²⁺ ions, which lead to modifications in the electronic band structure [42][47]. The observed band gap widening may also be influenced by several contributing factors, including variations in structural parameters, changes in carrier concentration, and the presence of intrinsic defects such as oxygen vacancies. Additionally, the shift can be explained by the Burstein–Moss effect, where an increase in charge carrier density results in the filling of lower energy states in the conduction band, thereby requiring higher energy for electronic transitions [48][49].

3.2 Scanning electron microscopy, energy-dispersive X-ray spectroscopy, and transmission electron microscopy studies of as-synthesized Mn-doped ZnO nanoparticles:

The surface morphology and microstructural features of the synthesized materials were examined using scanning electron microscopy (SEM), as presented in **Figure 2 (a–d)**. The

SEM images of pristine ZnO (**Figure 2a and 2b**) reveal the formation of densely packed nanoparticulate assemblies with an overall quasi-spherical appearance. At lower magnification (5 μm scale), the material appears as large, irregular clusters composed of finer particles, while higher magnification (1 μm scale) clearly shows that these clusters consist of numerous closely packed nanograins. The pronounced agglomeration observed can be attributed to interparticle attractive forces such as van der Waals interactions, which are typically significant for nanosized oxides. The individual primary particles appear relatively uniform, although their boundaries are not sharply distinguishable due to coalescence [50]. In contrast, the Mn-doped ZnO samples (**Figure 2c and 2d**) exhibit a distinctly modified morphology. Instead of purely spherical aggregates, the micrographs show the emergence of elongated, rod-like and needle-shaped structures interspersed within the particulate matrix. These anisotropic features are distributed throughout the sample and appear to originate from directional crystal growth induced by Mn incorporation. The coexistence of rod-like entities along with smaller granular particles suggests a change in growth kinetics and crystallization behavior in the doped system.

Furthermore, a clear increase in the overall particle dimension is observed upon Mn doping. The average particle size increases from approximately 125 nm for pure ZnO to about 194 nm for Mn-doped ZnO. This enlargement can be associated with enhanced grain growth and possible coalescence of adjacent particles during synthesis. The presence of Mn ions may alter the surface energy of specific crystallographic planes, thereby promote anisotropic growth and facilitate the formation of larger structures. Additionally, dopant-induced diffusion processes and localized thermal effects during synthesis could contribute to particle fusion and growth. The Mn-doped sample also appears comparatively less compact than pure ZnO, with a more open and loosely packed architecture. This structural variation may influence surface-related properties such as adsorption, catalytic activity, and interaction with biological systems. Overall, the SEM analysis demonstrates that Mn incorporation not only affects particle size but also significantly alters the morphological evolution from predominantly spherical aggregates to mixed granular–rod-like structures, indicating a strong influence of dopant ions on nucleation and growth mechanisms.

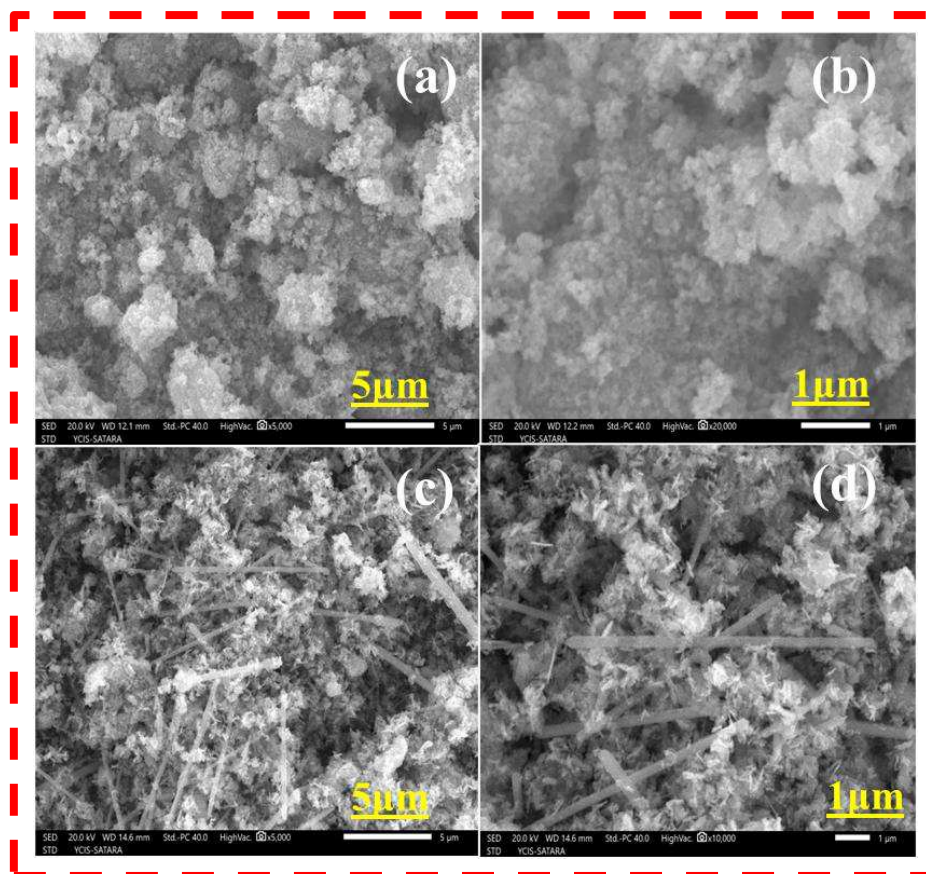


Fig. 2. The FESEM micrographs of (a and b) pure ZnO NPs and (c and d) Mn-doped ZnO nanoparticles.

The elemental composition and chemical purity of the synthesized nanomaterials were further examined using energy-dispersive X-ray (EDX) spectroscopy, and the corresponding spectra are presented in **Figure 3 (a–b)**. The EDX spectrum of the undoped ZnO sample (**Figure 3a**) exhibits prominent and well-resolved peaks corresponding to zinc (Zn) and oxygen (O), confirming the successful formation of ZnO without the presence of any unintended metallic impurities. The characteristic Zn signals observed at higher energy regions, along with the O peak at lower energy, are consistent with the expected elemental fingerprint of ZnO [30]. In the case of Mn-doped ZnO (**Figure 3b**), along with Zn and O peaks, additional signals corresponding to manganese (Mn) are clearly detected, verifying the successful incorporation of Mn ions into the ZnO matrix. The Mn peaks appear with relatively lower intensity compared to Zn, which is expected due to the lower doping concentration. Importantly, no extra peaks related to secondary phases or impurity oxides are observed, suggesting that Mn is well integrated into the host lattice rather than forming separate phases [28]. The inset quantitative analysis (weight percentage) further supports these observations. For pure ZnO (**Figure 3a**

inset), the composition is dominated by Zn (~78.27 wt%) and O (~21.73 wt%), which is close to the theoretical stoichiometry of ZnO considering instrumental limitations of EDX for light elements. For the Mn-doped sample (**Figure 3b inset**), Zn remains the major component (~74.34 wt%), while O (~20.94 wt%) and Mn (~4.72 wt%) are also present. This confirms the successful doping of Mn into ZnO and indicates a slight reduction in Zn content due to substitution by Mn ions.

Additionally, minor peaks corresponding to elements such as carbon (C) and potassium (K) are observed with low intensity. These signals are likely associated with residual organic constituents and mineral content derived from the cow dung extract used during the green synthesis route. Such biomolecules can act as reducing, stabilizing, and capping agents, facilitating controlled nucleation and growth of nanoparticles while preventing excessive agglomeration. The relatively sharp and distinct peaks observed in both spectra also indicate good crystallinity and strong X-ray emission from the constituent elements. Moreover, the absence of significant extraneous peaks highlights the high purity of the synthesized materials. Overall, the EDX analysis, along with inset compositional data, confirms the successful formation of ZnO and Mn-doped ZnO nanostructures with effective incorporation of Mn and minimal contamination.

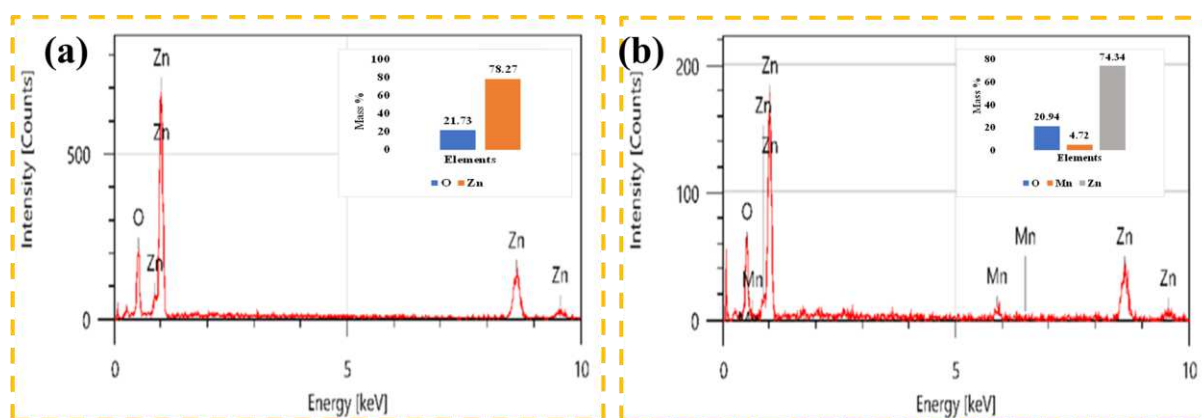


Fig. 3. EDX analysis of the ZnO and Mn-doped ZnO nanoparticles.

For an in-depth evaluation of morphology, particle size, and dispersion characteristics, transmission electron microscopy (TEM) analysis was carried out, as illustrated in **Figure 4 (a–d)**. The TEM micrographs of the undoped ZnO nanoparticles (**Figure 4a and 4b**) demonstrate a heterogeneous morphology consisting of irregularly shaped particles with the coexistence of short rod-like features. These nanostructures appear highly agglomerated, which can be attributed to the high surface energy and intrinsic tendency of ZnO nanoparticles to

cluster during synthesis and drying. The particle size distribution in pure ZnO is relatively broad, with measured dimensions ranging roughly from ~30 nm to over 100 nm, indicating non-uniform growth behaviour. In contrast, the Mn-incorporated ZnO samples (**Figure 4c and 4d**) exhibit a noticeable modification in morphology. The particles predominantly appear quasi-spherical to slightly elongated with smoother outlines and comparatively narrower size distribution. A significant reduction in agglomeration is also observed, suggesting improved dispersion possibly due to the presence of Mn ions influencing interparticle interactions. The particle size in Mn-doped ZnO is found to lie within a lower range, typically between ~25 nm and ~70 nm. The average particle sizes calculated from multiple measurements are approximately 46.81 nm for pristine ZnO and 34.27 nm for Mn-doped ZnO nanoparticles. This observable decrease in particle size upon Mn incorporation indicates that Mn^{2+} ions likely interfere with the crystal growth kinetics. Specifically, Mn doping may act as a growth inhibitor by introducing lattice strain or defect sites, which restrict grain coalescence and limit particle enlargement. Additionally, the dopant ions can enhance nucleation rates while suppressing subsequent growth, resulting in finer and more uniformly distributed nanoparticles. This reduction in particle size upon Mn doping suggests that the dopant plays a role in controlling nucleation and growth during the synthesis process [51].

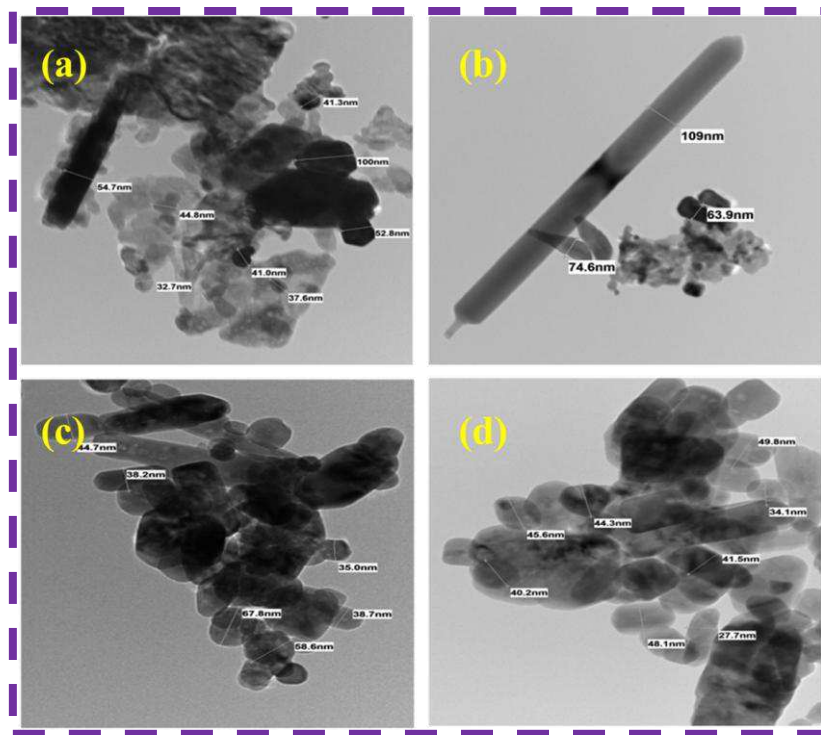


Fig. 4. Transmission electron microscopy (TEM) images of (a and b) pure ZnO NPs and (c and d) Mn-doped ZnO nanoparticles.

3.3 X-ray photoelectron spectroscopy analysis of as-synthesized Mn-doped ZnO nanoparticles:

X-ray photoelectron spectroscopy (XPS) was employed to investigate the surface chemical composition, elemental states, and bonding characteristics of the synthesized undoped and Mn-doped ZnO nanoparticles. Figure 6a presents the survey spectrum of undoped ZnO, which confirms the presence of Zn 2p, O 1s, and C 1s peaks, corresponding to the expected elemental composition. The absence of any additional peaks indicates the high purity of the synthesized ZnO nanoparticles. The high-resolution C 1s spectrum (**Figure 5a**) exhibits a peak centered at a binding energy of approximately 284 eV, which is attributed to surface carbon contaminants. This carbon signal is mainly associated with organic residues originating from the cow dung extract used during the green synthesis process. The O 1s spectrum of undoped ZnO nanoparticles (**Figure 5b**) was deconvoluted into three distinct components, representing different oxygen environments. These include lattice oxygen (O^{2-}) in the hexagonal wurtzite structure, oxygen ions associated with defect regions (oxygen vacancies), and loosely bound or adsorbed oxygen species present on the nanoparticle surface. **Figure 5c** illustrates the Zn 2p spectrum, which shows two prominent peaks at binding energies of approximately 1022 eV and 1045 eV, corresponding to Zn 2p_{3/2} and Zn 2p_{1/2}, respectively. These peaks confirm that zinc exists predominantly in the Zn²⁺ oxidation state within the ZnO lattice [30][52][53].

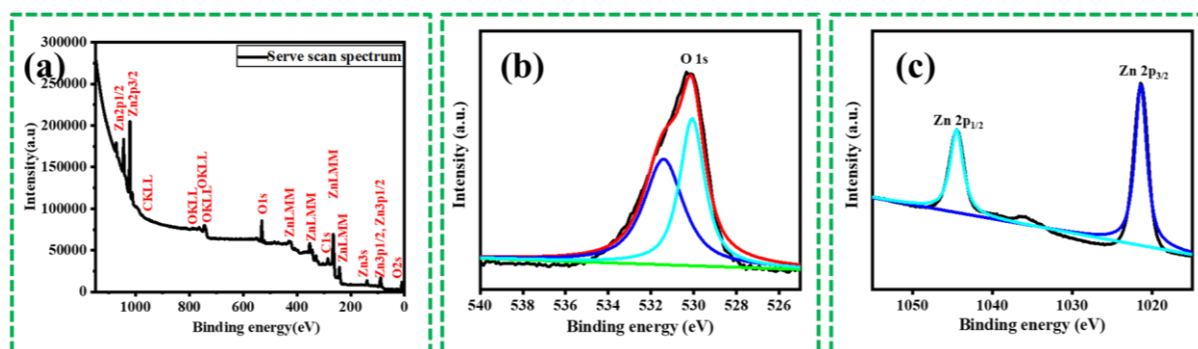


Fig. 5. Deconvoluted XP spectra of the ZnO NPs of (a) survey spectra, (b) O 1s, (c) Zn 2p

The XPS spectra of Mn-doped ZnO are presented in **Figure 6**. The survey spectrum (**Figure 6a**) reveals the presence of Zn 2p, Mn 2p, O 1s, and C 1s peaks, confirming the successful incorporation of Mn into the ZnO matrix. The C 1s peak observed at ~284 eV is again attributed to surface carbon species, consistent with the presence of residual organic compounds from the biological extract used during synthesis [52]. The presence of carbon in

the spectra is attributed to residual biochemical compounds from the cow dung extract, which remain adsorbed on the surface of the synthesized nanoparticles. **Figure 6c** illustrates the high-resolution Zn 2p spectrum of Mn-doped ZnO, where two distinct peaks are observed at binding energies of approximately 1022 eV and 1045 eV. These peaks correspond to $\text{Zn}^{2+} 2p_{3/2}$ and $\text{Zn}^{2+} 2p_{1/2}$, respectively, confirming that zinc exists in the +2 oxidation state within the lattice [54]. **Figure 6b** shows the Mn 2p spectrum, which exhibits two characteristic peaks at binding energies of 641 eV and 651 eV. These peaks are assigned to $\text{Mn}^{2+} 2p_{3/2}$ and $\text{Mn}^{2+} 2p_{1/2}$, respectively, indicating that manganese is present in the +2 oxidation state and has been successfully incorporated into the ZnO crystal structure. To further investigate the oxygen bonding environment in Mn-doped ZnO, the O 1s spectrum was deconvoluted into three peaks centered at binding energies of 530.52 eV, 532.22 eV, and 528.06 eV, as shown in **Figure 6d**. These peaks correspond to different oxygen states within the material, including lattice oxygen (O^{2-}) in the hexagonal wurtzite structure, oxygen ions associated with defect regions (such as oxygen vacancies), and loosely bound or adsorbed oxygen species on the nanoparticle surface. Overall, the O 1s spectral profile suggests the coexistence of multiple oxygen environments, which is typical for ZnO-based nanomaterials and confirms the presence of structural defects and surface-active oxygen species [30].

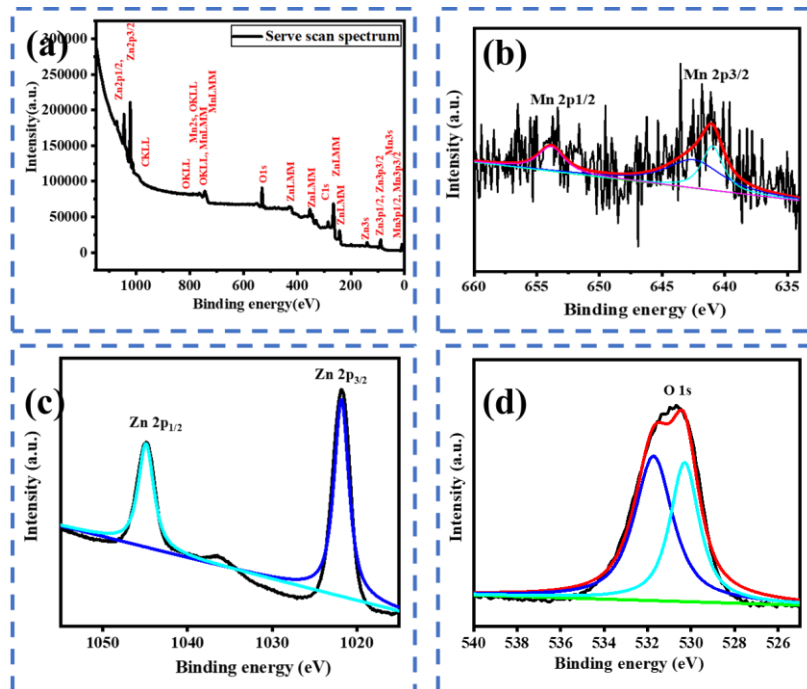


Fig. 6. Deconvoluted XP spectra of the Mn-doped ZnO nanoparticles (a) survey spectra, (b) Mn 2p, (c) Zn 2p, (d) O 1s

3.4 Antibacterial activity of Mn-doped ZnO nanoparticles

The antibacterial activity of cow dung extract-mediated Mn-doped ZnO nanoparticles was evaluated against *Escherichia coli* and *Staphylococcus aureus* using the disk diffusion method shown in **Table 1** and **Figure 7**. The results demonstrate that the zone of inhibition (ZOI) varies depending on the bacterial species and nanoparticle concentration. Different concentrations of the biosynthesized Mn-doped ZnO nanoparticles (50, 100, and 150 µg/mL) were tested and compared with the control. It was observed that the antibacterial effect increased with increasing nanoparticle concentration. Among the tested strains, *S. aureus* exhibited greater variation in response across all concentrations compared to *E. coli*. Additionally, each sample showed noticeable differences in the size of the inhibition zones. At the highest concentration (150 µg/mL), Mn-doped ZnO nanoparticles produced the largest zone of inhibition against *S. aureus*, whereas the lowest concentration (50 µg/mL) resulted in the smallest inhibition zone against *S. aureus*. These findings confirm a clear concentration-dependent antibacterial effect. The standard antibiotic streptomycin produced inhibition zones ranging from 6–9 mm, while the control, DMSO, showed negligible antibacterial activity (<5 mm). The enhanced antibacterial activity can be attributed to the interaction between Mn-doped ZnO nanoparticles and the negatively charged bacterial cell membrane. This interaction alters the membrane potential and disrupts cellular integrity. The presence of positively charged Zn²⁺ and Mn²⁺ ions contribute to changes in membrane permeability and structural damage.

Table 1. ZOI (mm) of ZnO NPs and Mn-doped ZnO nanoparticles against selected bacterial strains at different concentrations

Nanomaterials	<i>E. coli</i> 50 ppm	<i>E. coli</i> 100 ppm	<i>E. coli</i> 150 ppm	<i>S. aureus</i> 50 ppm	<i>S. aureus</i> 100 ppm	<i>S. aureus</i> 150 ppm
Mn-doped ZnO nanoparticles	6	8	9	6	9	10
ZnO nanoparticles	6	7	9	5	7	9
Streptomycin	6	7	8	6	8	9

Furthermore, the generation of reactive oxygen species (ROS), such as hydrogen peroxide (H₂O₂), hydroxyl radicals (•OH), and singlet oxygen, plays a crucial role in bacterial cell destruction [55][56]. These reactive species induce oxidative stress, leading to membrane damage, leakage of cellular contents, and eventual cell death. Overall, the results indicate that

green-synthesized Mn-doped ZnO nanoparticles exhibit significant antibacterial activity and can serve as effective antimicrobial agents against pathogenic bacterial strains [56, 57].

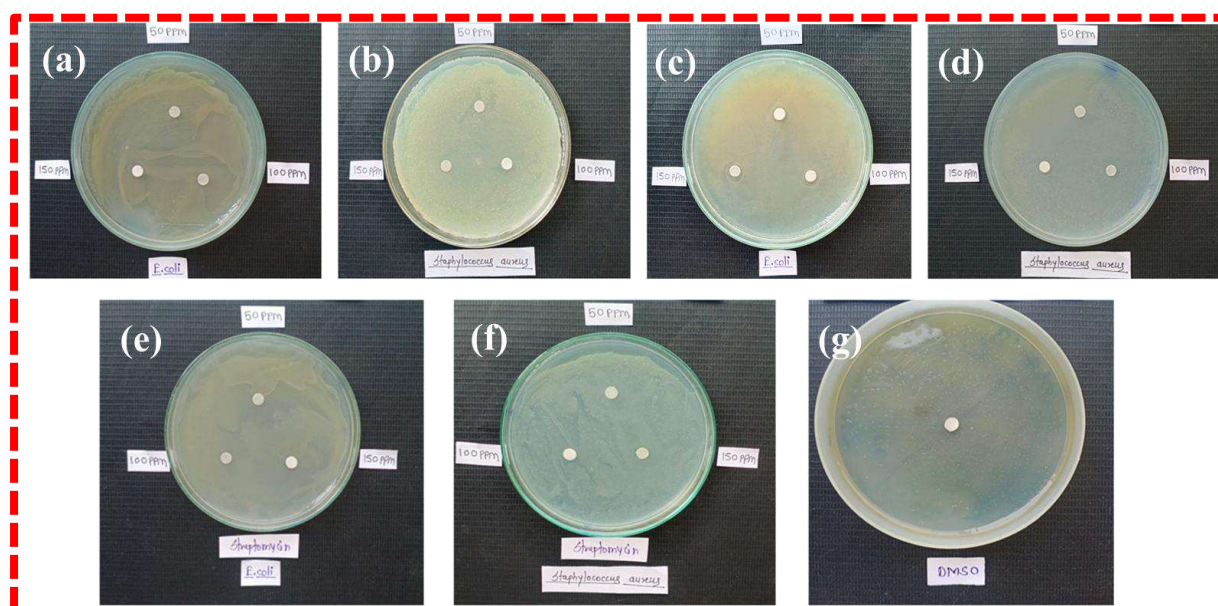


Fig. 7. Growth inhibition of ZnO nanoparticles (a and b) and Mn-doped ZnO(c and d) against *Escherichia coli* and *Staphylococcus aureus* by the disk diffusion method with streptomycin (positive control) (e and f) and DMSO (negative control) (g).

3.5 Cytotoxicity (anticancer activity) of Mn-doped ZnO nanoparticles

The anticancer efficacy of the biosynthesized pure ZnO and Mn-doped ZnO nanoparticles was systematically investigated using the MCF-7 human breast cancer cell line as an in vitro model. Cytotoxicity was quantitatively assessed by determining the percentage of cell inhibition across a range of concentrations shown in **Figure 8**, and the corresponding IC_{50} values were calculated to compare their effectiveness with a standard chemotherapeutic agent. The IC_{50} value obtained for pure ZnO nanoparticles was 107.5 $\mu\text{g/mL}$, whereas Mn-doped ZnO nanoparticles exhibited a slightly higher IC_{50} value of 124.2 $\mu\text{g/mL}$, suggesting comparatively lower cytotoxic potency. In contrast, the reference drug 5-fluorouracil demonstrated a much lower IC_{50} value (39.33 $\mu\text{g/mL}$), confirming its superior anticancer efficiency under identical experimental conditions. To further understand the dose-dependent response, both nanoparticle systems were evaluated at concentrations of 20, 40, 60, 80, and 100 $\mu\text{g/mL}$. A progressive decline in cell viability was observed with increasing concentration, indicating a clear concentration-dependent cytotoxic effect. In the case of pure ZnO nanoparticles, cell viability decreased from 95.34% at 20 $\mu\text{g/mL}$ to 52.77% at 100 $\mu\text{g/mL}$.

Similarly, Mn-doped ZnO nanoparticles reduced cell viability from 93.79% to 57.35% over the same concentration range. Although both materials exhibited significant cytotoxic behavior, pure ZnO nanoparticles consistently showed slightly higher inhibition efficiency compared to Mn-doped ZnO at equivalent doses. The enhanced anticancer activity of ZnO nanoparticles can be attributed to their ability to generate reactive oxygen species (ROS), induce oxidative stress, and trigger apoptotic pathways in cancer cells. The incorporation of manganese into the ZnO lattice appears to modify the surface and electronic properties, which may influence ROS generation and cellular interactions, ultimately leading to a marginal reduction in cytotoxic performance compared to undoped ZnO. In addition to the quantitative data, morphological observations (as shown in **Figure 9**) support these findings, where treated MCF-7 cells exhibit visible structural alterations such as cell shrinkage, membrane damage, and loss of cellular integrity, particularly at higher nanoparticle concentrations. These changes are characteristic of apoptosis-mediated cell death. The obtained results confirmed that both pure and Mn-doped ZnO nanoparticles possess significant anticancer potential, with their activity strongly dependent on concentration. While Mn doping introduces subtle changes in biological response, pure ZnO nanoparticles demonstrate comparatively better efficacy against MCF-7 cells. These findings highlight the promise of ZnO-based nanomaterials as potential candidates for anticancer applications, although further mechanistic and in vivo studies are necessary to fully establish their therapeutic potential.

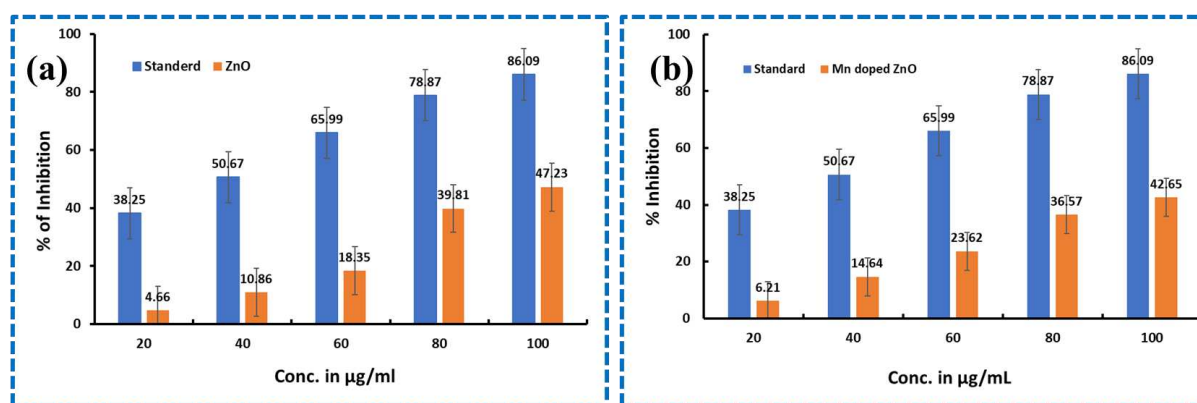


Fig. 8. Cell inhibition of MCF-7 cells with different doses of (a) pure ZnO and (b) Mn-ZnO nanoparticles.

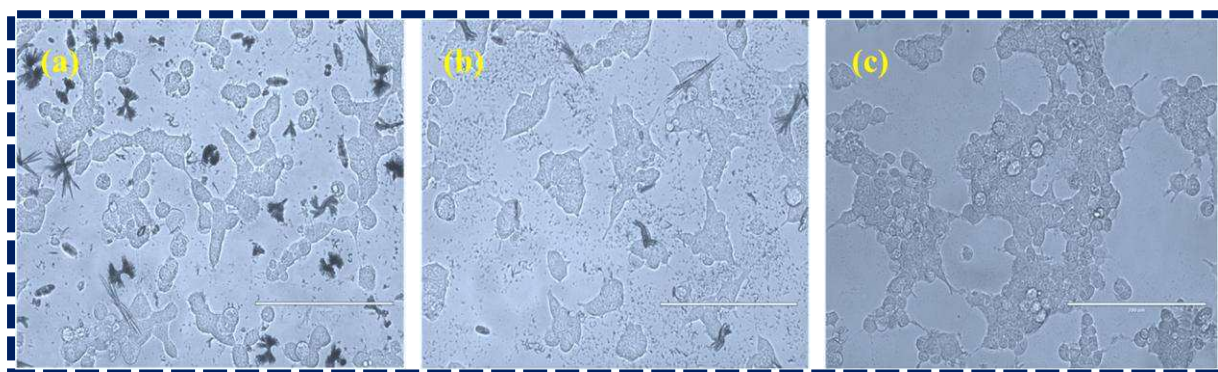


Fig. 9. Cell inhibition of MCF-7 cells with different doses of (a) pure ZnO and (b) Mn-ZnO nanoparticles with respect to (c) standard.

4. Conclusion

In this study, a facile, green, and rapid method was presented for the preparation of pure ZnO and Mn-doped ZnO nanoparticles using the cow dung extract. TEM and SEM images indicated spherical nanoparticles with sizes of about 34–46 nm. The nanoparticle characterization results showed that Mn doping decreased the size of zinc oxide particles. The antibacterial activity study showed that Mn-doped ZnO nanoparticles had a greater effect on *S. aureus* and *E. coli* bacteria, due to the doping in the zinc oxide structure. Anticancer activity of biosynthesized pure ZnO and Mn-doped ZnO nanoparticles was evaluated against MCF7. Results revealed that pure ZnO and Mn-doped ZnO nanoparticles exhibited anticancer activity against MCF7 cell lines, with an IC_{50} of 107.5 and 124.2 $\mu\text{g/mL}$ respectively. Furthermore, Mn-doped ZnO nanoparticles exhibited strong antibacterial activity against both Gram-positive and Gram-negative bacterial strains. Based on these findings, we can propose the utilization of our biosynthesized nanoparticles for biological applications. According to the promising results, the prepared eco-friendly and cost-effective Mn-doped ZnO nanoparticles may be used in the biomedical fields as a smart and encouraging agent.

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CRedit authorship contribution statement

Priyanka P. Patil: Methodology, Writing – original draft. **Ganesh S. Kamble:** Validation and Revision, Supervision, Resource. **Sneha R. Bhosale:** Resources. **Rajesh M. Jorgewad:** Resources. **Babasaheb D. Bhosale:** Supervision, Project administration.

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Data availability

Original data were generated and analysed in this study. All data are finds within the manuscript.

Ethics, Consent to Participate, and Consent to Publish declarations: not applicable.

Conflicts of interest

There is no actual or potential conflict of interest in relation to this article.

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