

Research Article

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Dose-dependent cytotoxicity against lung cancer cells via green synthesized ZnFe_2O_4 /cellulose nanocomposites

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Abstract: Cancers are complicated sicknesses that happen because of many different things going wrong in cells, and as they get worse, the cells undergo many changes one after another. Nanomedicine is a new way to treat diseases like cancer. Tiny particles called nanoparticles have special properties that can help to treat diseases better than regular treatments. These particles are very small but have a lot of surface area, can carry different drugs, and can be designed to target specific areas. They can move around the body, go into cells, and release drugs slowly. Because of

these benefits, nanoparticles could be better for cancer treatment. In this continuous research, we present a simple technique for the quick and single-step synthesis of ZnFe_2O_4 /cellulose nanocomposites, employing the polymer cellulose. This method is not only cost-effective but also environment friendly. Scanning electron microscopy, powder X-ray diffraction, Fourier transform infrared spectroscopy, and the ultraviolet-visible (UV) spectrum were all used to examine the morphological, structural, and electrical properties of ZnFe_2O_4 /cellulose nanocomposites. The nanocomposite derived from UV-DRS exhibits an optical energy bandgap of 1.8 eV. The mechanical strength of the composites gradually increases as ZnFe_2O_4 is added to the cellulose polymer matrix. These findings propose a straightforward and innovative approach to produce ZnFe_2O_4 /cellulose nanocomposites that can serve as functional biomaterials. In addition, the ZnFe_2O_4 /cellulose nanocomposite exhibits decreased antioxidant activity compared to ascorbic acid. ZnFe_2O_4 /cellulose nanocomposite was found to have an IC_{50} of $49.64 \text{ g}\cdot\text{mL}^{-1}$. With an IC_{50} value of $55.91 \text{ g}\cdot\text{mL}^{-1}$, the synthesized ZnFe_2O_4 /cellulose nanocomposites demonstrate significant cytotoxicity in a dose-dependent manner against the lung cancer cell lines A549. In conclusion, nanocomposites are potential materials for usage in biomedical applications due to their affordable production and mild magnetic sensitivity.

Keywords: cellulose, ZnFe_2O_4 /cellulose nanocomposite, antioxidant activity, cytotoxicity, computational studies

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1 Introduction

The synthesis of nanomaterials has become a subject of significant research interest in recent times, owing to their unique properties such as size effect, structural characteristics, and high specific area (1). Many biologists have recognized the exceptional potential of nanoparticles in various fields, particularly pharmacology and biotechnology.

However, certain hybrid materials that exhibit toxicity have limited their use in medical applications (2). For a variety of applications, including antiviral, anticancer, targeted drug delivery, and antibacterial materials, inorganic antibacterial compounds with high thermal stability and resistance to bacterial infections have fortunately been discovered as potential options (3). Because of its strong optical transmission in the visible area, ionic makeup, and low electrical resistance, zinc ferrite (ZnFe_2O_4) has received a lot of attention recently from researchers (4). ZnFe_2O_4 has been found to exhibit antimicrobial behaviour against several pathogens (5).

The process of preparing polymer nanocomposites involves the combination of organic polymer matrices and nanoparticles, with the aim of creating a single material that possesses the desirable properties of both components (6). In recent years, the field of nanotechnology has made significant strides, leading to increased interest in the development of polymers and composites with unique characteristics for specific applications. Polymer matrices are well suited to serve as host materials for composites (7), and the addition of nanoparticles to polymers has generated significant interest due to the resulting lightweight nature, cost-effective processability, and distinctive physicochemical properties (8,9). These properties include enhanced electric/heat conductivity, improved mechanical properties such as stiffness and strength, magnetic properties, and the ability to replicate complex shapes with precision (10).

The biodegradable nanocomposites offer significant advantages, including their environment friendly nature that does not harm the environment or living organisms (11). Once cellulose-biodegradable nanocomposites have absorbed or depleted environmental pollutants – both metals and non-metals – they can be recycled and decomposed easily into the environment (12). In addition, it has been demonstrated that cellulose biodegradable nanocomposites offer outstanding promise in a number of areas, including water treatment, biomedical applications, tissue engineering, modified electrodes, and microbial fuel cells (2).

The synthesized ZnFe_2O_4 /cellulose nanocomposites were tested for antioxidant and anticancer activity. Nanocomposites of ZnFe_2O_4 and cellulose may have synergistic lethal effects on cancer cells when used to treat lung cancer. The objectives of this study were to synthesize ZnFe_2O_4 /cellulose nanocomposites with cellulose, characterize the synthesized nanocomposite, and evaluate the antioxidant and anticancer activities of the synthesized nanocomposite.

2 Experimental method

2.1 Materials required

Ferric chloride [FeCl_3] (Fisher Scientific) with a purity of 99.99%, sodium hydroxide [NaOH], zinc chloride, and cellulose powder (from wood pulp) are supplied by Byhut, Jaipur.

2.2 Preparation of active cellulose

One gram of cellulose powder was added to a 20% NaOH solution and kept as such for 3 h at room temperature. After filtering the mixture, activated cellulose ($\text{C}_6\text{H}_{10}\text{O}_5^* \text{NaOH}$) was extracted and collected.

2.3 Preparation of ZnFe_2O_4 /cellulose nanocomposites

In a beaker containing 100 mL of deionized water, precise amounts of FeCl_3 (0.6 g) and ZnCl_2 (1 g) were dissolved. To maintain a pH of 12, 0.2 M NaOH solution was then added gradually to the mixture. This resulted in the formation of a yellowish-brown precipitate.

To this yellowish-brown precipitate, at a temperature of 60°C , 1 g of active cellulose was added, and the mixture was rapidly agitated for 3 h. Following the stirring process, the composite was subjected to centrifugation and washed twice with distilled water and ethanol. Finally, it was dried at 60°C for 48 h (Figure 1).

2.4 Characterization and testing

X-ray diffraction (XRD) was used to analyse the crystal phase of ZnFe_2O_4 /cellulose nanocomposites, as well as their purity. The XRD instrument (DW-XRD-2700 A) was used on ZnFe_2O_4 /cellulose nanocomposites using $\text{CuK}\alpha$ radiation (origin of X-rays employed for the detection, measurement, and visualization of the molecular crystalline arrangement), which was supplied within a 2θ range that ranged from 10 to 60° . The FESEM and EDX instruments (by EVO MA15/18 and 51N1000-EDS System), were made accessible by the Centre for Nanoscience and Nanotechnology at Jamia Milia Islamia in New Delhi, were used to investigate

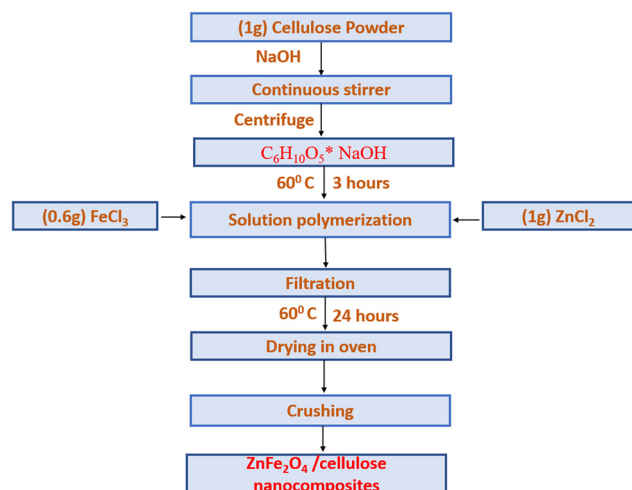


Figure 1: Flowchart illustrating the sequential stages involved in synthesizing the ZnFe_2O_4 /cellulose nanocomposite.

the surface morphology. The functional groups that were found in the nanocomposite were investigated by means of Fourier transform infrared (FTIR) spectroscopy (Agilent Cary 630, at Sharda University), which covered a frequency range of $4,000\text{--}400\text{ cm}^{-1}$. The two-probe method was utilized to determine the conductivity of a ZnFe_2O_4 /cellulose nanocomposite by utilizing a CH instrument with the model number CHI604D. In addition, a UV-1800 double-beam spectrophotometer at Sharda University was utilized to analyse the optical properties of the nanocomposite.

2.5 Methods that involve computational processes

The Gaussian 09 W program was employed to carry out density functional theory (DFT) calculations using the B3LPY hybrid functional. This hybrid functional contains both the BLPY correlation functional and Becke's three-parameter exchange functional. The 6-31G(d) basis set was utilized, and the Berny method was employed to optimize the geometry.

2.6 Antioxidant activity

ZnFe_2O_4 /cellulose nanocomposite was used in varied concentrations, from 15 to $120\text{ g}\cdot\text{mL}^{-1}$, to assess its capacity to scavenge DPPH radicals. Using 95% ethanol, a 0.2 mM DPPH (2,2-diphenyl-1-picrylhydrazyl) radical solution was prepared. In addition to a control experiment utilizing only DPPH, different ZnFe_2O_4 /cellulose nanocomposite liquid

sample concentrations were coupled with 1 mL of DPPH (0.2 mM). Then, after an hour, the mixture's decreasing absorbance % was used to calculate the radical concentration. The absorbance change was measured at 517 nm . Ascorbic acid served as a positive control. To calculate the radical scavenging activity, Eq. 1 is used.

Sample absorbance is the absorbance in the presence of antioxidants like ZnFe_2O_4 /cellulose nanocomposite or ascorbic acid, while control absorbance is the absorbance when antioxidants are not present.

2.7 Anticancer potential of ZnFe_2O_4 /cellulose nanocomposites

The National Centre for Cell Science in Pune, India, made the human lung cancer cell line A549 available to researchers. The cells were subcultured in DMEM (Invitrogen, USA) with 10% foetal bovine serum and 1X antibiotic solution while maintaining a temperature of 37°C , a humid atmosphere, and 5% carbon dioxide. The MTT assay was used to gauge the cytotoxic effects of ZnFe_2O_4 /cellulose nanocomposites. The 96-well culture plate was filled with about 1×10^{-5} cells per well. Nanocomposites of ZnFe_2O_4 and cellulose were added to A549 cells and incubated for 24 h. The control cultures were administered DMSO. Twenty litres of MTT solution was added to each well after incubation, and each was then given a further 4 h of incubation. The absorbance of the crystals at 575 nm was assessed using an ELISA reader after the addition of $200\text{ }\mu\text{L}$ of DMSO. The experiment was carried out in triplicates.

Antioxidant activity(%)

$$= \frac{\text{Control absorbance} - \text{Sample absorbance}}{\text{Control absorbance}} \times 100 \quad (1)$$

3 Results and discussion of nanocomposites

3.1 Spectrum of ZnFe_2O_4 /cellulose nanocomposites in the UV-visible (UV/V) range

As shown in Figure 2(a), the UV/V spectra of the ZnFe_2O_4 /cellulose nanocomposites were examined in the $200\text{--}800\text{ nm}$ range. Radiation within this range was found to be absorbed by the material, exhibiting an absorbance peak at 472 nm . The visible spectrum revealed the ferrite's absorption edge, which

was caused by the excitation of electrons from the spinel-type oxide nanoparticles' Fe-3d state from O-2p.

A Tauc plot was produced using Eq. 2, and an optical band gap of the nanocomposites was determined (13).

$$(ah\nu) = A(h\nu - E_g)^n \quad (2)$$

where A is the proportionality constant, the energy gap is E_g , n serves as an index used for defining the optical absorption process, and the energy of the incoming photon is represented by $h\nu$, all contribute to the absorption coefficient (14). Figure 2(b) represents the Tauc plot of $(ah\nu)^2$ against $h\nu$ (in eV) for a ZnFe_2O_4 /cellulose nanocomposite. The band gap energy was discovered to be 1.8 eV, which is within the range of previously recorded values.

3.2 XRD

The XRD patterns for ZnFe_2O_4 nanoparticles, cellulose, and the ZnFe_2O_4 /cellulose nanocomposite are shown in Figure 3. The measurements were made between 15° and 60° 2θ angles. Peaks at 2θ values of 29.9 , 35.2 , 42.9 , 53.1 , and 56.4 correspond to the (111), (311), (400), (422), and (511) reflection planes, respectively (15). These peaks confirm the presence of a spinel cubic structure. Scherrer equation (Eq. 3) is used to determine the crystallite size (16,17).

$$D = \frac{k\lambda}{\beta \cos \theta} \quad (3)$$

From Figure 3(c), the successful formation of a nanocomposite system consisting of ZnFe_2O_4 /cellulose nanocomposites can be easily observed. The ZnFe_2O_4 nanoparticles

physically combine with the cellulose polymer without any chemical interactions, thereby not altering the physical characteristics of cellulose. Crystallite size for the ZnFe_2O_4 /cellulose nanocomposite was calculated using the Scherrer equation and was found to be around 25.5 nm.

3.3 FE-scanning electron microscopy (SEM) coupled with EDX spectroscopy

The physical properties of the samples are being altered by changes in their morphology and microstructures, which indicate the development of interfacial interactions within their composite. Figure 4(a)–(h) depicts the distinct morphologies of the cellulose polymer, ZnFe_2O_4 nanoparticles, and ZnFe_2O_4 /cellulose nanocomposite. As demonstrated in Figure 4(a), the cellulose FE-SEM images show a smooth surface with cross-sectional fibres that are 10–20 m in size. The spherical shape ZnFe_2O_4 nanoparticles is shown in Figure 4(b). The green method utilized in the preparation of ZnFe_2O_4 nanoparticles produced spherical structures resembling the aggregation of several thousand nanoparticles, as observed in the FE-SEM images. The composite's surface, as revealed by Figure 4(c), exhibits gaps and cylindrical aggregates consisting of homogenous ZnFe_2O_4 nanosheets that are distributed among the cellulose fibres. This distribution suggests that the material possesses high porosity and resistivity. The divergent expansion of the composite is caused by the existence of gaps. When zinc ferrite is introduced to the cellulose matrix fibres, the intermolecular hydrogen connections between the cellulose chains are disrupted (18,19). That is

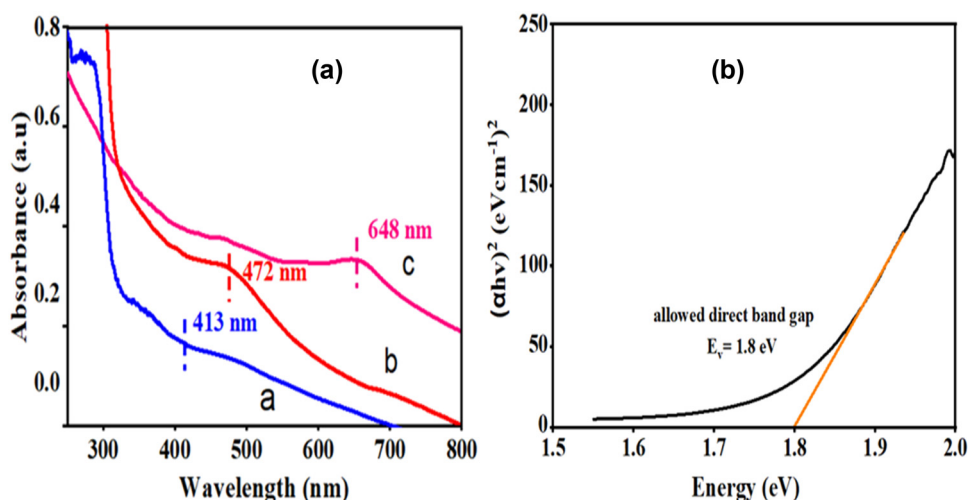


Figure 2: (a) UV spectrum of ((a) ZnFe_2O_4 nanoparticles, (b) ZnFe_2O_4 /cellulose nanocomposite, and (c) cellulose) and (b) Tauc Plot of ZnFe_2O_4 /cellulose nanocomposite.

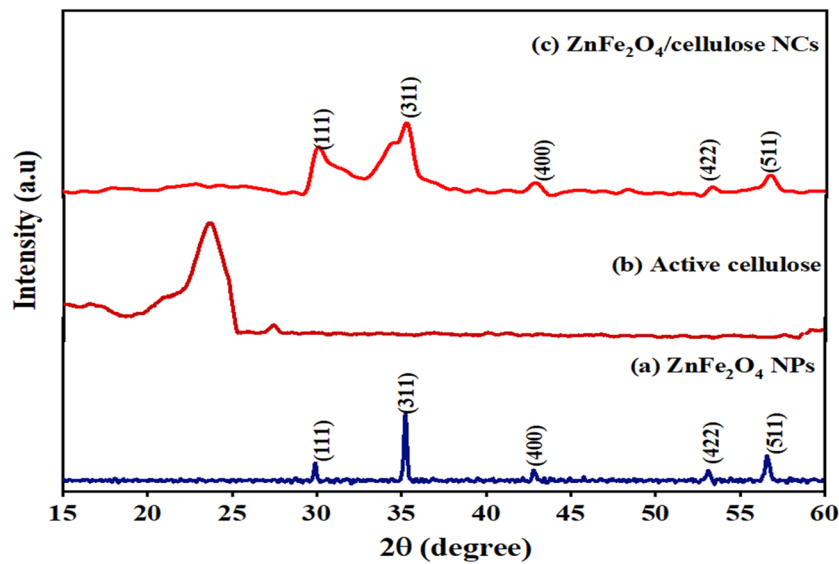


Figure 3: XRD pattern of (a) ZnFe_2O_4 nanoparticles, (b) cellulose, and (c) ZnFe_2O_4 /cellulose nanocomposite (20).

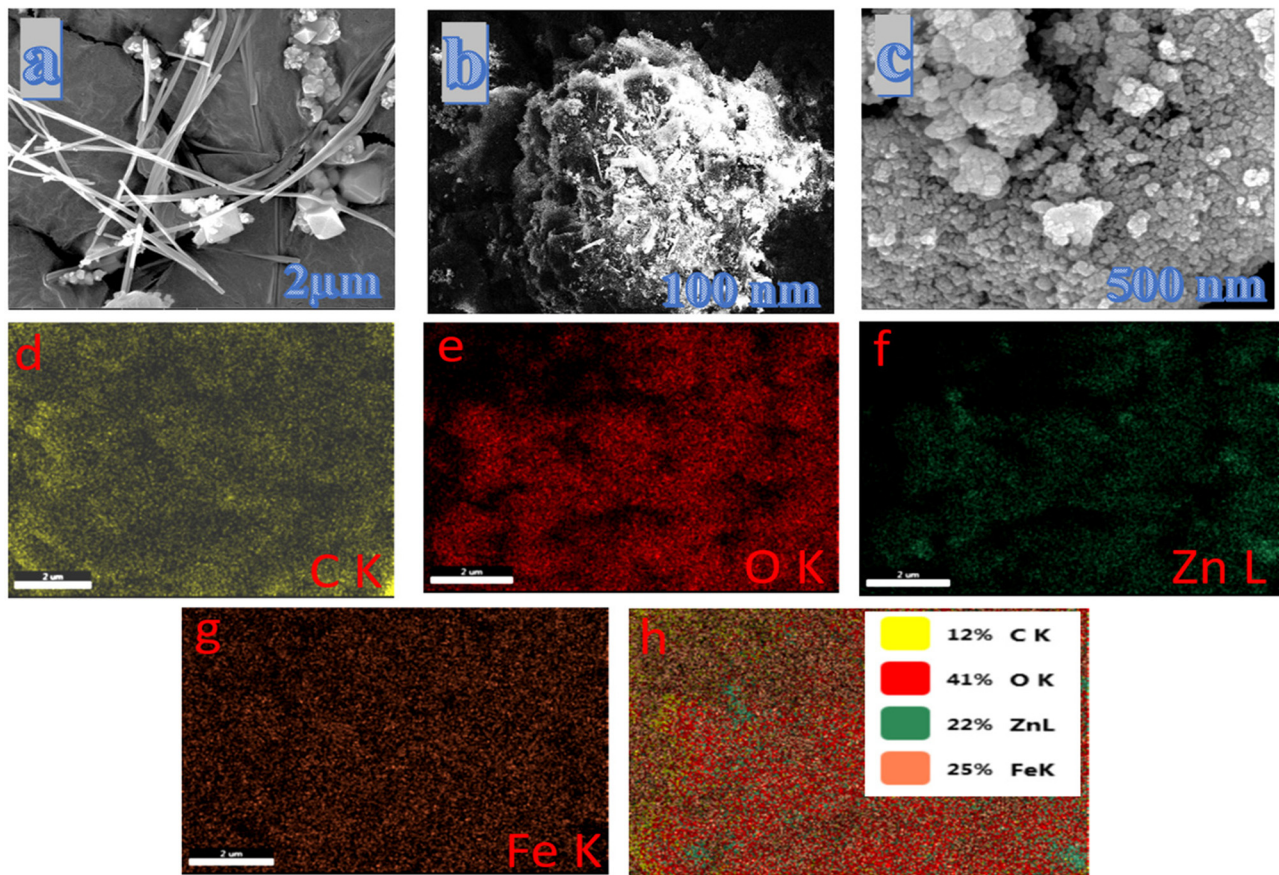


Figure 4: FESEM images of (a) cellulose, (b) ZnFe_2O_4 nanoparticles, (c) ZnFe_2O_4 /cellulose nanocomposites, and (d)–(h) elemental mapping of ZnFe_2O_4 /cellulose NCs.

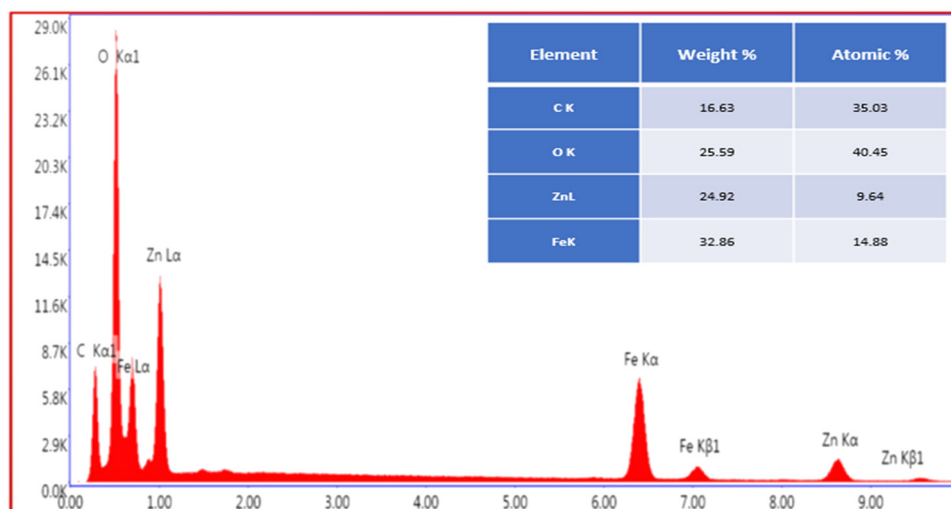


Figure 5: Energy-dispersive X-ray (EDX) spectroscopy of ZnFe_2O_4 /cellulose nanocomposites.

why compared to the typical long cellulose fibres, the cellulose fibres in the composite are significantly deteriorated.

The examined ZnFe_2O_4 /cellulose nanocomposite sample had undergone platinum (Pt) coating through ion sputtering for EDX as shown in Figure 5. The C peak is visible as a component of cellulose. In addition, peaks of the Zn, Fe, and O, which are associated with ZnFe_2O_4 nanoparticles encapsulating the cellulose surface, were also observed. In addition, the percentages of the elements Zn, Fe, O, and C in the elemental mapping's EDX spectra (in Figure 4(d)–(h)) reveal that a highly pure composite was created without any leftover byproducts.

3.4 FTIR analysis

Figure 6 depicts the FTIR spectra of synthesized ZnFe_2O_4 /cellulose nanocomposite, cellulose, and their comparative spectra with ZnFe_2O_4 nanoparticles. The peak positions of the ZnFe_2O_4 /cellulose nanocomposite in Figure 6c are 3,371.13, 1,659.4, 1,156.03, 1,044.2, and 656.4 cm^{-1} . The presence of a peak at 3,371.13 cm^{-1} suggests the existence of an alkane group in the cellulose spectrum, which is caused by C–H stretching. The presence of ketone is demonstrated by displaying C=C stretching at 1659.4 cm^{-1} . The bending vibrations of the C–H and O–H groups may be seen in the pattern at 1,156.03 cm^{-1} . The C–O and C–N stretching bands at 1,044.2 cm^{-1} indicate the presence of secondary alcohol and aromatic amine, respectively. The weak bands at 656.4 cm^{-1} can be due to the Zn–O and Fe–O vibrations representing the octahedral and tetrahedral modes of ZnFe_2O_4 , respectively (21). The presence of these peaks

shows a good interaction between the spinel ZnFe_2O_4 and the cellulose matrix.

3.5 Dynamic light scattering (DLS)

Figure 7 displays the DLS spectrum of the ZnFe_2O_4 /cellulose nanocomposites. It is evident from the spectrum that the synthesized adsorbent is around 100 nm, with the highest concentration falling around 113 nm range as determined by intensity. The various parameters coming out from DLS

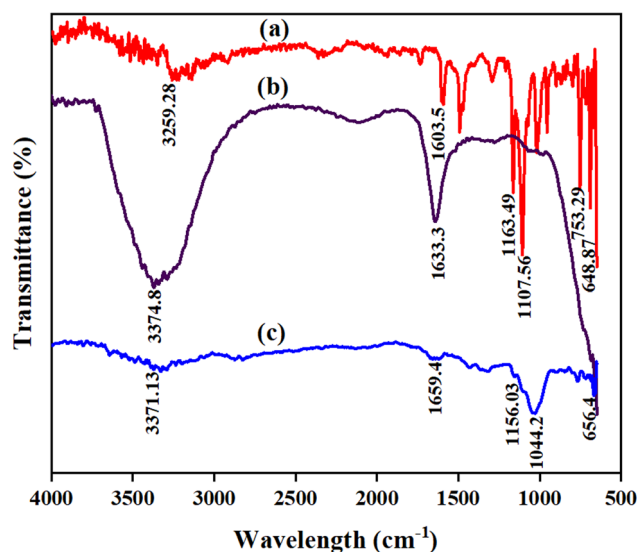


Figure 6: FTIR spectral analysis of (a) ZnFe_2O_4 nanoparticles, (b) cellulose, and (c) ZnFe_2O_4 /cellulose nanocomposites.

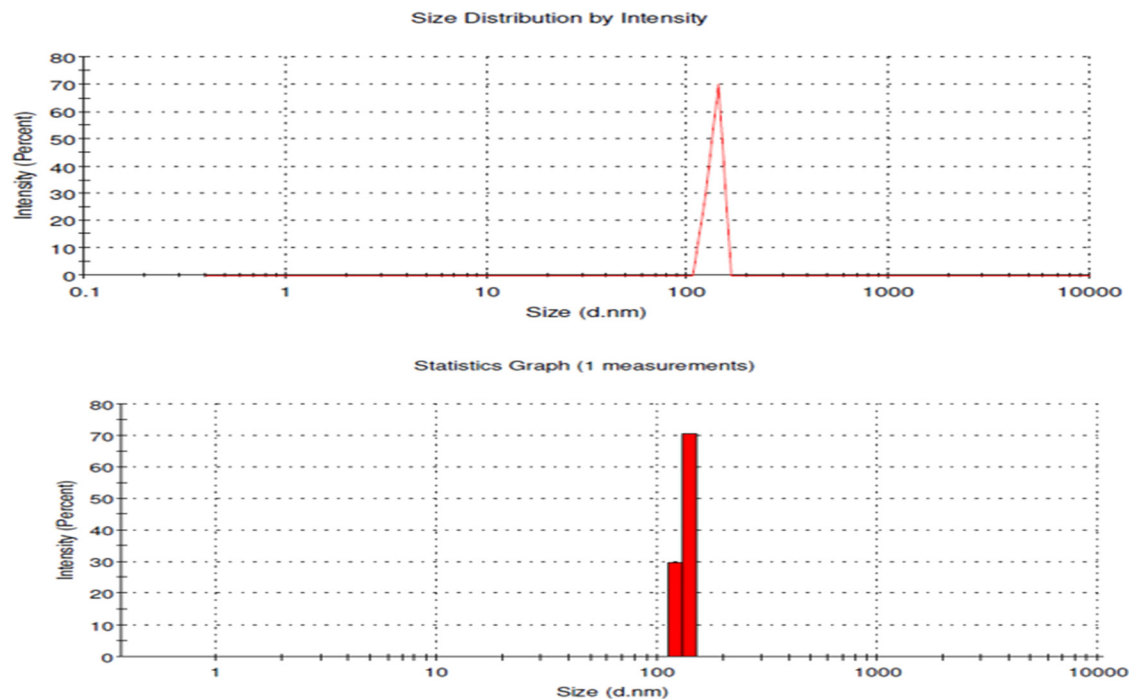


Figure 7: Intensity-based size distribution analysis report using DLS for ZnFe₂O₄/cellulose nanocomposites.

Table 1: Characterization of ZnFe₂O₄/cellulose nanocomposites by DLS

Solvent	Viscosity	RI	PDI	Z-average
Water	0.88	1.33	1.00	1,401

studies are presented in Table 1. This discrepancy can be attributed to the fact that DLS measures the Brownian motion of particles, resulting in a size distribution that reflects the average hydrodynamic size of the nanocomposite collection (18).

Furthermore, the DLS analysis revealed that nanocomposites tended to aggregate in the aqueous suspension, leading to the measurement of cluster sizes rather than individual nanocomposite sizes.

3.6 Zeta potential

The zeta potential (Figure 8) is a measure of the electrostatic force that opposes the attraction between charged particles in a dispersion (22). When it comes to the dispersion of ZnFe₂O₄/

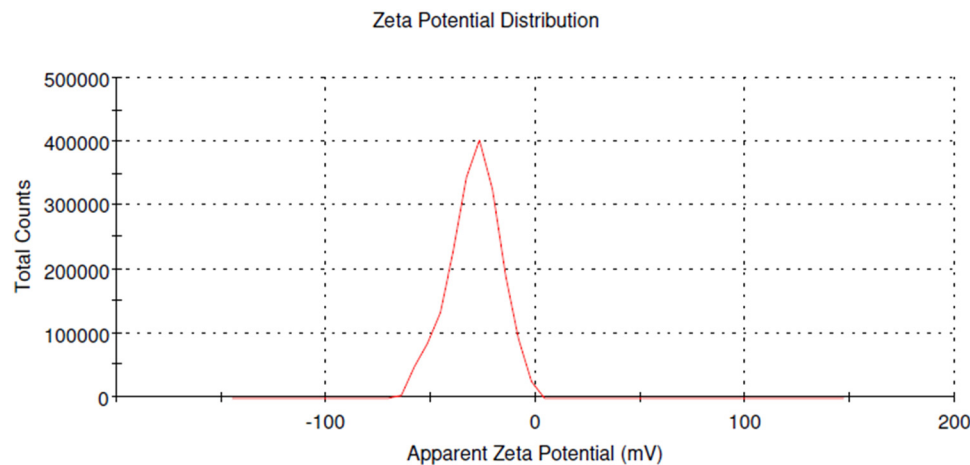


Figure 8: Zeta potential for ZnFe₂O₄/cellulose nanocomposites.

Table 2: Characterization of ZnFe₂O₄/cellulose nanocomposites by zeta potential

Sample	Mean zeta potential (mV)	Zeta deviation	Conductivity
ZnFe ₂ O ₄ /cellulose nanocomposites	−10.4	8.85	0.043

cellulose nanocomposites, higher zeta potential values are associated with a decreased tendency to flocculate. From Table 2, a negative zeta potential value suggests that there is a strong electrostatic force of repulsion among individual particles, which leads to good stability of the nanosuspension.

3.7 Computational processes

DFT calculations were used to look at the chemical and physical interactions between the molecules of cellulose and ZnFe₂O₄. In addition, optimized geometries and ground-state energies, such as the total energy, the energy of HOMO symbolizing the compounds' electron-donating properties, the energy of LUMO symbolizing electron-acceptor properties, the energy gap (E_g), and the dipole moment (μ), were calculated using Gaussian 09 and the b3lyp/6-31 g method (15).

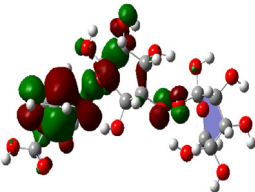
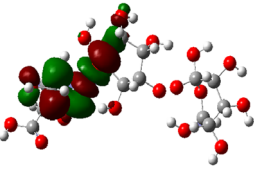
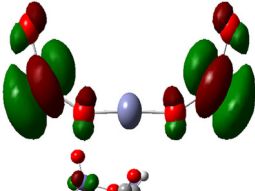
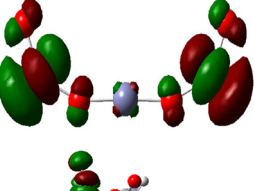
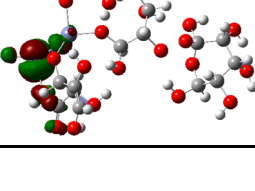
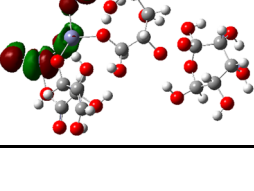
The DFT method is employed with the b3lyp/6-31 g basis set to study the physical and chemical interactions between the molecules of ZnFe₂O₄/cellulose nanocomposites. In addition, the optimized geometries and ground-state energies (HOMO), LUMO, E_g , and μ were determined by using

Gaussian (R) 09. The gap between HOMO and LUMO and the energy of HOMO are the characteristics of the molecular chemical stability (23). The energy gap (HOMO-LUMO) is the parameter to determine the molecular electrical property, and hence, it is the electron conductivity measurement (24). Tables 3 and 4 show the molecular structure and optimized geometry of ZnFe₂O₄/cellulose nanocomposites, respectively. The calculated HOMO, LUMO, and band gap of the ZnFe₂O₄/cellulose nanocomposites are −5.9652, −4.2850, and 1.680 eV, respectively (Table 4). The data reflect that the band gap energy calculated experimentally as shown in the Tauc plot (Figure 2b) is in sync with the computational value for

Table 4: The DFT calculation of ZnFe₂O₄/cellulose nanocomposites at various parameter

Orbitals	Cellulose	ZnFe ₂ O ₄	ZnFe ₂ O ₄ /cellulose
HOMO (eV)	−6.688	−5.445	−5.965
LUMO (eV)	−2.982	−4.963	−4.285
Energy gap [ΔE (eV)]	3.70	0.48	1.68
Dipole moment [μ (Debye)]	8.2971	4.8670	11.0462

Table 3: Optimized geometrical structure and energy gap

Compound	HOMO	LUMO
Cellulose nanocomposites		
ZnFe ₂ O ₄		
ZnFe ₂ O ₄ /cellulose nanocomposites		

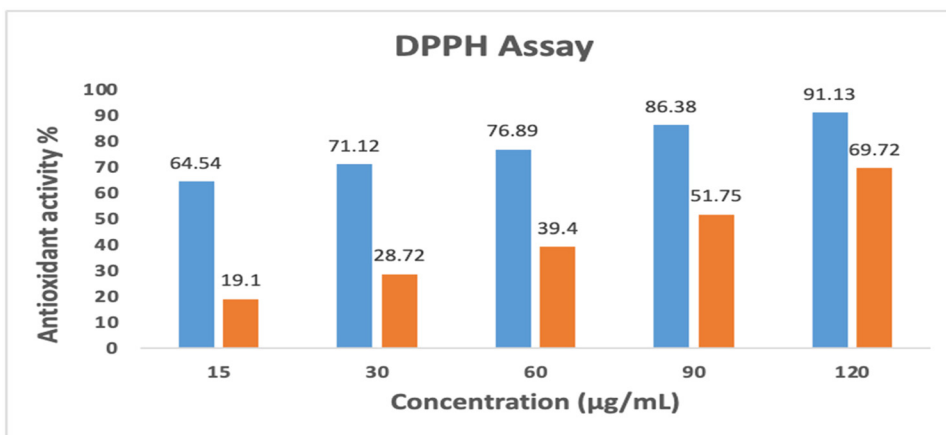


Figure 9: Effectiveness of ascorbic acid (a positive control) and ZnFe₂O₄/cellulose nanocomposites as antioxidants against DPPH.

ZnFe₂O₄/cellulose nanocomposites. The difference in dipole moment values is also due to the change in the energy values of the band gap between HOMO and LUMO (25).

3.8 Antioxidant activity

Free radicals are the primary contributor to oxidative stress and other health problems (26). The common dangerous free radical DPPH has been shown to have negative impacts on human health. According to (27), the ZnFe₂O₄/cellulose nanocomposites' antioxidant properties are most

likely caused by the transfer of electrons from this nanocomposite's exceptionally dense oxygen atom to an extra electron on the nitrogen atom, which decreases the intensity of the $n \rightarrow \pi^*$ transition at 517 nm and removes the violet colour that is typical of DPPH. The antioxidant activity of ZnFe₂O₄/cellulose nanocomposites and ascorbic acid, which served as the positive control in this study, was assessed using the DPPH scavenging assay, as shown in Figure 9. According to the results, ZnFe₂O₄/cellulose nanocomposites had much less antioxidant activity than ascorbic acid, with an IC₅₀ of 41.60 µg·mL⁻¹ as opposed to 49.64 µg·mL⁻¹ for ZnFe₂O₄/cellulose nanocomposites. The results provide strong evidence in favour of the application of ZnFe₂O₄/cellulose-

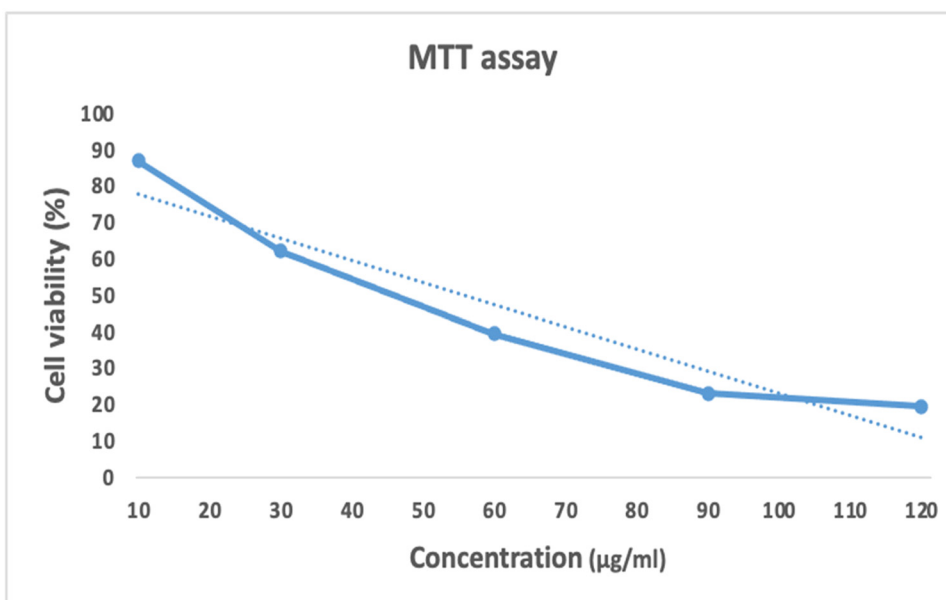


Figure 10: *In-vitro* cytotoxicity of ZnFe₂O₄/cellulose nanocomposites was performed by testing the viability of A549 cell lines at different concentrations.

based nanocomposite as beneficial natural antioxidants for protecting health against various types of oxidative stress connected to degenerative diseases.

3.9 *In-vitro* studies on A549 cells

A549 cells were used to examine the viability of ZnFe₂O₄/cellulose nanocomposites, and the results are presented in Figure 10. After incubation for 24 h, the synthesized ZnFe₂O₄/cellulose nanocomposites were used in the current study at varied concentrations, including 10, 30, 60, 90, and 120 µg·mL⁻¹. After 24 h, there was a 50% reduction in cell growth at 55.91 µg·mL⁻¹ in lung cancer cell lines. According to the results of cell viability using MTT assay, it suggests that the ZnFe₂O₄/cellulose nanocomposites are cytotoxic for cancer cells in a dose-dependent manner.

In contrast, the aforementioned results demonstrate that cell viability decreased as ZnFe₂O₄/cellulose nanocomposites concentration increased. These results clearly show the enhanced effectiveness of the ZnFe₂O₄/cellulose nanocomposites against lung cancer cells.

4 Conclusion

A facile and eco-friendly method was used to synthesize ZnFe₂O₄ nanoparticles, which was incorporated with cellulose into the formation of ZnFe₂O₄/cellulose nanocomposites. Various analytical techniques, including UV, FTIR, X-ray, FE-SEM, EDX, DLS, Zeta potential, and computational methods, were employed to confirm the successful coupling of cellulose to ZnFe₂O₄ nanoparticles into ZnFe₂O₄/cellulose nanocomposites. Furthermore, computational calculations using DFT/b3lyp/6-31g(d) basis sets showed the reactivity and stability of ZnFe₂O₄/cellulose nanocomposites. The biological activities were also assessed by our nanocomposite's ability to prevent the growth of lung cancer cells. The cytotoxicity and antioxidant capabilities of our nanocomposite were studied in A549 cell lines, and intriguingly, we observed a remarkable dose-dependent reduction in cell viability against the cell lines.

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Conflict of interest: The authors declare no conflict of interest.

Data availability statement: Not applicable.

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