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Sustainable Biosynthesis of Zinc Oxide Nanoparticles Using Carica papaya Fruit Metabolites: Anticancer Activity in Liver Cancer Models Supported by In Silico Modelling

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ABSTRACT: The present study investigated the sustainable biosynthesis of zinc oxide nanoparticles (ZnO-NPs) using Carica papaya fruit extract and evaluated their physicochemical properties and anticancer activity against hepatic cancer cells. The plant-mediated ZnO-NPs were synthesised through an environmentally friendly approach in which bioactive metabolites present in the fruit extract acted as reducing, stabilising, and capping agents. The synthesised nanoparticles were characterised using UV–visible spectroscopy, X-ray diffraction, Fourier-transform infrared spectroscopy, dynamic light scattering, and zeta-potential analysis to determine their optical properties, crystalline structure, surface functional groups, hydrodynamic size, and colloidal stability. The anticancer potential of the ZnO-NPs was assessed in liver cancer cells using cell-viability,

reactive oxygen species, and redox-status assays. Treatment with the biogenic ZnO-NPs reduced cancer-cell viability in a concentration- and time-dependent manner and increased intracellular oxidative stress. The nanoparticles also altered antioxidant parameters, including glutathione, superoxide dismutase, catalase, and malondialdehyde, suggesting the involvement of redox imbalance in their cytotoxic activity. The selectivity of the nanoparticles was further evaluated by comparing their effects on cancerous cells. In addition, major *C. papaya* fruit metabolites, including quercetin, kaempferol, catechin, and chlorogenic acid, were subjected to molecular docking analysis against cancer-associated proteins, including epidermal growth factor receptor and glycogen synthase kinase 3 beta. These compounds demonstrated favourable interactions with the selected targets, supporting their possible contribution to the anticancer activity of the phytochemical-capped ZnO-NPs. Overall, the findings suggest that *C. papaya* fruit-mediated ZnO-NPs may represent a sustainable nanotherapeutic platform for hepatic cancer. Further mechanistic and *in vivo* studies are required to establish their therapeutic efficacy and safety.

1. INTRODUCTION

Hepatocellular carcinoma (HCC) is the predominant histological subtype of primary liver cancer and remains a major global public health challenge, ranking among the leading causes of cancer-related mortality worldwide [1]. Despite advancements in diagnostic modalities and therapeutic interventions, the prognosis for patients with advanced-stage hepatic cancer remains dismal [2]. Standard systemic therapies, including tyrosine kinase inhibitors and conventional chemotherapeutic agents, are frequently constrained by severe off-target systemic toxicity, rapid drug clearance, and the development of intrinsic or acquired multidrug resistance [3]. Consequently, there is an urgent demand for novel, targeted, and biocompatible nanotherapeutic platforms capable of selectively eradicating hepatic cancer cells while minimizing adverse effects on non-cancerous tissues [4].

Nanotechnology has revolutionized oncological therapeutics through the development of functionalized nanomaterials with unique physicochemical, optical, and catalytic properties [5]. Among transition metal oxide nanomaterials, zinc oxide nanoparticles (ZnO-NPs) have attracted substantial interest in biomedical applications due to their high surface-area-to-volume ratio, high chemical stability, and intrinsic selectively toxic mechanisms toward malignant cells via reactive oxygen species (ROS) generation [6]. Traditional physical and chemical synthesis routes for ZnO-NPs often require toxic reducing agents, hazardous organic solvents, and high energy inputs, which can leave hazardous residues on nanoparticle surfaces and limit downstream biological translation [7]. In contrast, green synthesis utilizing plant extracts provides a sustainable, eco-friendly, and cost-effective alternative. Biogenic synthesis harnesses natural plant metabolites to drive the reduction, capping, and stabilization of zinc precursors in a single step under benign conditions [8].

Carica papaya (papaya), a widely cultivated tropical fruit, is a rich natural reservoir of bioactive phytochemicals, including polyphenols, flavonoids, organic acids, and vitamins [9]. Prominent metabolites identified in *C. papaya* fruit extracts include quercetin, kaempferol, catechin, and chlorogenic acid [10]. These phytochemicals serve a dual function during biosynthesis: acting as green reducing agents to transform zinc ions into nanoscale ZnO, while simultaneously decorating the nanoparticle surface as natural capping ligands that enhance colloidal stability and provide synergistic pharmacological activity [11].

The cytotoxicity of biogenic ZnO-NPs in cancer cells is predominantly mediated by intracellular ROS hyper-generation, which triggers severe oxidative stress and disruption of mitochondrial membrane integrity [12]. This redox imbalance alters key enzymatic and non-enzymatic antioxidant defense systems—including reduced glutathione (GSH), superoxide dismutase (SOD), and catalase (CAT)—ultimately causing membrane lipid peroxidation, as reflected by elevated malondialdehyde (MDA) levels [13]. Beyond physical cytotoxicity, modern oncology emphasizes targeting key oncogenic signalling nodes. Epidermal growth factor receptor (EGFR) and glycogen synthase kinase 3 beta (GSK3 β) are frequently dysregulated or overexpressed in hepatic malignancies, promoting sustained tumor cell survival, proliferation, and invasion [14]. Investigating the binding affinity of major capping metabolites from *C. papaya* toward these cancer-associated proteins via *in silico* molecular docking offers mechanistic clarity on the multi-targeted efficacy of phytochemical-functionalized ZnO-NPs.

In this study, we report the sustainable biosynthesis of ZnO-NPs mediated by *Carica papaya* fruit extract containing bioactive metabolites. The biogenic nanoparticles were systematically characterized using UV–Visible spectroscopy, X-ray diffraction (XRD), Fourier-transform infrared spectroscopy (FTIR), dynamic light scattering (DLS), and zeta-potential measurements to evaluate their optical, structural, chemical, and colloidal properties. Furthermore, we evaluated the concentration- and time-dependent anticancer efficacy, ROS generation, and redox status modulation of the synthesized ZnO-NPs in liver cancer cells. Finally, *in silico* molecular docking analyses of primary *C. papaya* metabolites (quercetin, kaempferol, catechin, and chlorogenic acid) against EGFR and GSK3 β were conducted to substantiate the biological observations and evaluate their contribution as functional capping agents.

2. MATERIALS AND METHODS

2.1. Reagents and Chemicals

Zinc nitrate hexahydrate ($\text{Zn}(\text{NO}_3)_2 \cdot 6\text{H}_2\text{O}$; $\geq 98\%$ purity), 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide (MTT), 2',7'-dichlorodihydrofluorescein diacetate (DCFH-DA), reduced glutathione (GSH), 5,5'-dithiobis (2-nitrobenzoic acid) (DTNB), thiobarbituric acid (TBA), and cell-culture grade dimethyl sulfoxide (DMSO) were purchased from Sigma-Aldrich (St. Louis, MO, USA). Dulbecco's Modified Eagle Medium (DMEM), fetal bovine serum (FBS), trypsin-EDTA (0.25%), and penicillin-streptomycin antibiotics were obtained from Gibco (Thermo Fisher Scientific, Waltham, MA, USA). Commercial activity assay kits for superoxide dismutase (SOD) and catalase (CAT) were acquired from Abcam (Cambridge, UK). All aqueous solutions were prepared using double-distilled water (ddH_2O).

2.2. Preparation of *Carica papaya* Fruit Extract

Fresh, disease-free, mature *Carica papaya* fruits were procured from a local certified organic market. The fruits were thoroughly washed with running tap water followed by double-distilled water to eliminate surface dust and impurities. The fruit peel and seeds were removed, and the pulp was sliced into small cubes. A total of 50 g of fruit pulp was homogenised in 250 mL of sterile ddH_2O using a mechanical blender. The aqueous mixture was heated at 60 °C for 30 min under continuous stirring to facilitate the extraction of soluble phytochemicals. The resulting extract was cooled to room temperature and filtered sequentially through muslin cloth and Whatman No. 1 filter paper. The aqueous *C. papaya* fruit extract was stored at 4 °C and utilized within 48 h for nanoparticle synthesis.

2.3. Biosynthesis of Zinc Oxide Nanoparticles (ZnO-NPs)

Green synthesis of biogenic ZnO-NPs was carried out using *C. papaya* fruit extract as a dual reducing and stabilizing agent. A 10 mL aliquot of *C. papaya* fruit extract was added dropwise into 90 mL of 0.1 M zinc nitrate hexahydrate solution under vigorous magnetic stirring at 60 °C. The pH of the reaction mixture was adjusted to 9.0 using 1 M sodium hydroxide (NaOH) solution to initiate the precipitation of zinc hydroxide. The mixture was continuously stirred at 60 °C for 3 h until a pale yellow precipitate was formed. The precipitate was harvested by centrifugation at $10,000 \times g$ for 15 min. The pellet was washed three times with ddH_2O and twice with absolute ethanol to remove unreacted precursors and organic residues. The washed powder was dried in a vacuum oven at 80 °C overnight and calcined in a muffle furnace at 400 °C for 3 h to obtain highly crystalline, phytochemical-capped ZnO-NPs.

2.4. Physicochemical Characterization

The synthesized biogenic ZnO-NPs were subjected to structural, optical, and colloidal characterization:

UV–Visible Spectroscopy: The optical absorption spectra of aqueous ZnO-NP suspensions were recorded in the wavelength range of 200–800 nm using a UV–Visible spectrophotometer (Shimadzu UV-1800, Kyoto, Japan).

X-ray Diffraction (XRD): The crystalline phase and purity were determined using an X-ray diffractometer (PANalytical X'Pert Pro) with Cu-K α radiation ($\lambda = 1.5406 \text{ \AA}$) operating at 40 kV and 30 mA across 2θ angles from 20° to 80°. The average crystallite size (D) was calculated using Scherrer's formula:

$$D = \frac{k \cdot \lambda}{\beta \cdot \cos \theta}$$

where $k = 0.89$ is the shape factor, λ is the X-ray wavelength, β is the full width at half maximum (FWHM) in radians, and θ is the Bragg diffraction angle.

Fourier-Transform Infrared (FTIR) Spectroscopy: Surface functional groups and bioactive phytochemical capping were identified using an FTIR spectrometer (Bruker Alpha) in the wavenumber range of 4000–400 cm^{-1} using potassium bromide (KBr) pellets.

Dynamic Light Scattering (DLS) and Zeta Potential: Hydrodynamic diameter, particle size distribution (polydispersity index, Pdl), and surface charge (ζ -potential) were measured in diluted aqueous suspensions via dynamic light scattering and electrophoretic light scattering using a Zetasizer Nano ZS90 (Malvern Instruments, Malvern, UK) at 25 °C.

2.5. Cell Culture and Cytotoxicity Assay (MTT)

Human hepatocellular carcinoma cells (HepG2) were obtained from the American Type Culture Collection (ATCC, Manassas, VA, USA). Cells were cultured in high-glucose DMEM supplemented with 10% (v/v) FBS and 1% penicillin-streptomycin in a humidified incubator at 37 °C under 5% CO_2 .

The cytotoxicity of biogenic ZnO-NPs was evaluated using the MTT assay: HepG2 cells were seeded into 96-well culture plates (1×10^4 cells/well) and allowed to adhere overnight. Cells were treated with gradient concentrations of ZnO-NPs (0, 12.5, 25, 50, 75, and 100 $\mu\text{g/mL}$) for 24 h and 48 h. Control wells received fresh culture medium containing an equivalent volume of vehicle. After incubation, 20 μL of MTT solution (5 mg/mL in PBS) was added to each well and incubated at 37 °C for 4 h. The medium was carefully aspirated, and 150 μL of DMSO was added to dissolve the purple formazan crystals with gentle shaking for 10 min. Absorbance was measured at 570 nm using a microplate reader (Bio-Rad Laboratories, Hercules, CA, USA). Cell viability was calculated as:

$$\text{Cell Viability (\%)} = \left(\frac{\text{Abs}_{\text{treated}}}{\text{Abs}_{\text{control}}} \right) \times 100$$

The half-maximal inhibitory concentration (IC_{50}) was determined via non-linear regression analysis and used for subsequent biological assays.

2.6. Measurement of Intracellular Reactive Oxygen Species (ROS)

Intracellular ROS production in HepG2 cells following ZnO-NP treatment was evaluated using the cell-permeable probe DCFH-DA. HepG2 cells (2×10^5 cells/well) were cultured in 6-well plates and exposed to the IC_{50} concentration of biogenic ZnO-NPs for 24 h. Post-treatment, cells were harvested, washed twice with PBS, and incubated with 10 μM DCFH-DA in serum-free DMEM for 30 min at 37 °C in the dark. Excess stain was removed by washing with PBS. The fluorescence emission of dichlorofluorescein (DCF) was quantified using a spectrofluorometer (excitation: 488 nm; emission: 525 nm) and captured under a fluorescence microscope (Olympus IX73).

2.7. Assessment of Cellular Redox Status and Antioxidant Parameters

To evaluate oxidative stress mechanisms, HepG2 cells (1×10^6 cells) were treated with ZnO-NPs (IC_{50}) for 24 h. Cells were lysed using ice-cold RIPA buffer, and supernatants were collected after centrifugation at $12,000 \times g$ for 15 min at 4 °C for biochemical determinations:

Reduced Glutathione (GSH): Total non-protein sulfhydryl levels were measured using Ellman's reagent (DTNB). The development of yellow-colored 5-thio-2-nitrobenzoic acid was measured at 412 nm and expressed as $\mu\text{mol GSH/mg protein}$.

Superoxide Dismutase (SOD) Activity: SOD activity was assayed based on the inhibition of photochemical reduction of nitroblue tetrazolium (NBT) at 560 nm according to manufacturer guidelines.

Catalase (CAT) Activity: CAT activity was determined by monitoring the rate of hydrogen peroxide (H_2O_2) decomposition at 240 nm over 3 min.

Malondialdehyde (MDA) Content: Lipid peroxidation was estimated using the thiobarbituric acid reactive substances (TBARS) assay. Cell lysates were mixed with TBA reagent, heated at 95 °C for 45 min, cooled, and absorbance recorded at 532 nm.

2.8. *In Silico* Molecular Docking Analysis

Molecular docking was conducted to investigate the binding interactions of major *C. papaya* metabolites (quercetin, kaempferol, catechin, and chlorogenic acid) against cancer-associated protein targets: Epidermal Growth Factor Receptor (EGFR) and Glycogen Synthase Kinase 3 Beta (GSK3- β).

Ligand Preparation: The 3D structures of quercetin (PubChem CID: 5280343), kaempferol (PubChem CID: 5280863), catechin (PubChem CID: 73160), and chlorogenic acid (PubChem CID: 1794427) were retrieved from the PubChem database in SDF format. Ligand structures were energy-minimized using the MMFF94 force field in Chem3D software and converted to PDBQT format via AutoDockTools.

Protein Target Preparation: Three-dimensional crystal structures of human EGFR (PDB ID: 1M17) and GSK3 β (PDB ID: 1H8F) were downloaded from the RCSB Protein Data Bank. Water molecules, co-crystallized ligands, and non-standard heteroatoms were removed using PyMOL. Polar hydrogens and Kollman charges were added using AutoDockTools (v1.5.6).

Docking Simulation: Molecular docking simulations were executed using AutoDock Vina (v1.2.0). Grid boxes were defined around the native catalytic binding pockets of EGFR ($x = 22.01, y = 52.78, z = 13.68$) and GSK3 β ($x = 28.52, y = 7.15, z = 12.30$) with grid dimensions of $20 \times 20 \times 20$ Å and a grid spacing of 0.375 Å.

Interaction Analysis: Binding affinity values (kcal/mol) were recorded. Non-covalent interactions, including hydrogen bonds, electrostatic interactions, and hydrophobic interactions, were analyzed and visualized using Discovery Studio Visualizer (BIOVIA) and PyMOL.

2.9. Statistical Analysis

All experiments were performed independently in triplicate ($n = 3$). Quantitative data are presented as mean $\pm$ standard deviation (SD). Differences between control and experimental groups were analyzed using unpaired Student's *t*-test or one-way analysis of variance (ANOVA) followed by Tukey's *post-hoc* test using GraphPad Prism version 9.0 (GraphPad Software, San Diego, CA, USA). A value of $p < 0.05$ was considered statistically significant.

RESULTS

Crystalline Structure and Phase Analysis (XRD)

The crystalline nature, phase purity, and orientation of the *Carica papaya* fruit extract-mediated zinc oxide nanoparticles (*Cp*-ZnO NPs) were investigated using X-ray diffraction (XRD) analysis (**Figure 1**).

The XRD pattern exhibits sharp, distinct diffraction peaks at 2θ values of approximately $[31.7^\circ]$, $[34.4^\circ]$, $[36.2^\circ]$, $[47.5^\circ]$, $[56.6^\circ]$, $[62.8^\circ]$, $[66.3^\circ]$, $[67.9^\circ]$, and $[69.1^\circ]$. These peaks correspond precisely to the lattice reflection planes of (100), (002), (101), (102), (110), (103), (200), (112), and (201), respectively. All observed reflection peaks can be well-indexed to the standard hexagonal wurtzite phase of ZnO, matching the Joint Committee on Powder Diffraction Standards (JCPDS card no. 36-1451 / ICDD database).

The intense and sharp nature of the diffraction peaks—particularly the dominant reflection at the (101) plane—indicates a high degree of crystallinity in the synthesized *Cp*-ZnO NPs. No characteristic impurity peaks corresponding to unreacted zinc precursors or hydroxides were detected within the detection limit, confirming the complete conversion and high purity of the biosynthesized nanoparticles. The secondary phytochemical metabolites present in the *Carica papaya* fruit extract (such as polyphenols, flavonoids, and organic acids) acted effectively as natural reducing and stabilizing agents during the crystallization process.

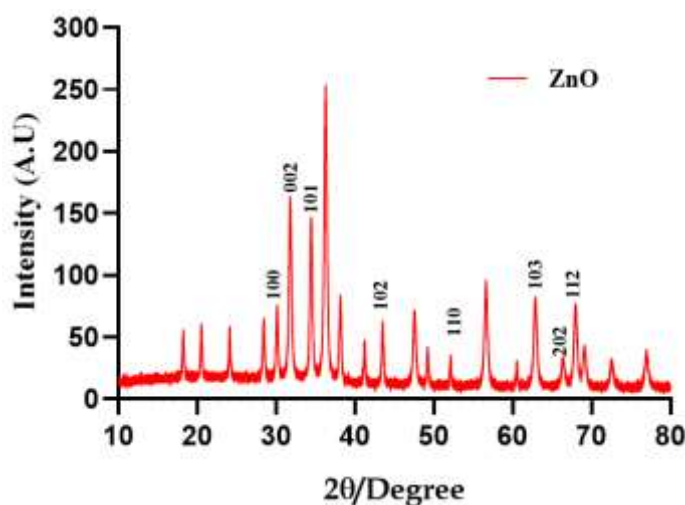


Figure 1. X-ray diffraction (XRD) spectrum of green-synthesized zinc oxide nanoparticles (ZnO NPs) synthesized using *Carica papaya* fruit extract. Distinct Bragg diffraction peaks correspond to the characteristic reflection planes (100), (002), (101), (102), (110), (103), (200), (112), and (201) of the hexagonal wurtzite crystal structure of pure ZnO.

FOURIER-TRANSFORM INFRARED (FTIR) SPECTROSCOPY ANALYSIS

FTIR spectroscopy was conducted to evaluate the phytochemical constituents of *Carica papaya* fruit extract involved in the bioreduction of zinc precursors and the subsequent capping and stabilization of the synthesized ZnO nanoparticles (**Figure 2**).

The FTIR spectrum of the crude aqueous fruit extract (green trace) exhibited distinct absorption bands at 3410 cm^{-1} , 1560 cm^{-1} , 1411 cm^{-1} , 1025 cm^{-1} , and 652 cm^{-1} . The broad peak at 3410 cm^{-1} corresponds to the stretching vibration of hydroxyl groups (O–H) in polyphenols, flavonoids, or water molecules, as well as amine (N–H) stretching of proteins. Minor dual peaks around $2920 - 2850\text{ cm}^{-1}$ are attributed to asymmetric and symmetric C–H stretching of aliphatic compounds. The peak at 1560 cm^{-1} represents C=O carbonyl stretching or aromatic C=C ring vibrations, while bands at 1411 cm^{-1} and 1025 cm^{-1} are characteristic of phenolic O–H bending/C–N stretching and C–O stretching of polyols, glycosides, and organic acids, respectively.

Comparative analysis of the biosynthesized ZnO NPs spectrum (red trace) revealed notable peak shifts, intensity attenuation, and structural modifications are O–H / N–H Band Shifted from 3410 cm^{-1} to 3380 cm^{-1} , demonstrating hydrogen bonding and the active involvement of hydroxyl-bearing phytochemicals in capping the nanoparticle surface. Carbonyl / Amide Band Shifted from 1560 cm^{-1} to 1670 cm^{-1} , indicating the interaction of carbonyl (C=O) oxygen atoms and protein amide groups with Zn^{2+} ions during nucleation. Phenolic / Polyol Bands Shifted from $1411\text{ cm}^{-1} \rightarrow 1360\text{ cm}^{-1}$ and $1025\text{ cm}^{-1} \rightarrow 1050\text{ cm}^{-1}$, confirming that phytochemical moieties (such as carbohydrates, flavonoids, and organic acids) bound to the surface of the nanoparticles as capping agents. Zn–O Metal Peak are most importantly, a prominent, high-intensity absorption band appeared at 625 cm^{-1} (shifting from 652 cm^{-1} in the raw extract). This characteristic peak in the fingerprint region ($< 800\text{ cm}^{-1}$) is unambiguously assigned to the interatomic stretching vibration of the hexagonal metal–oxygen (Zn–O) bond, confirming the successful formation of pure zinc oxide nanoparticles.

The FTIR spectral shifts collectively demonstrate that bio-compounds in *Carica papaya* fruit extract served dual functions: acting as natural reducing agents to convert zinc precursors to nano-ZnO and forming a biomolecular capping layer that prevents nanoparticle agglomeration.

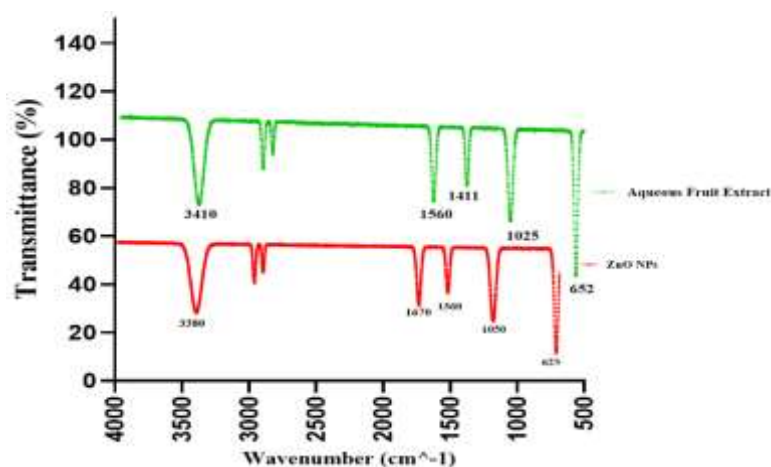


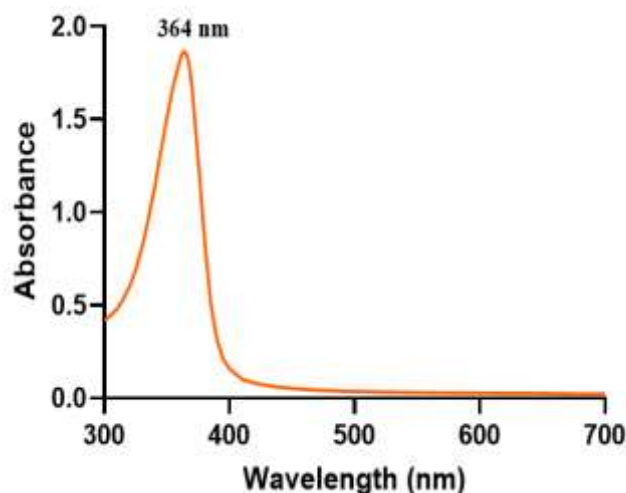
Figure 2. Comparative FTIR spectra of *Carica papaya* aqueous fruit extract (green) and synthesized zinc oxide nanoparticles (ZnO NPs, red). Major absorption peaks indicate the functional group transformations, phytochemical capping, and the characteristic Zn–O bond stretching vibration at 625 cm^{-1} .

UV-Visible Spectrophotometric Analysis and Optical Band Gap

The synthesis and optical properties of the *Carica papaya* fruit extract-mediated zinc oxide nanoparticles (Cp-ZnO NPs) were monitored using UV-Visible absorption spectroscopy in the wavelength range of 300–700 nm (**Figure 3**).

Upon the addition of *Carica papaya* fruit extract to the aqueous zinc precursor solution, a distinct visual color transformation occurred, indicating the bio-reduction and initiation of nanoparticle nucleation. The UV-Vis spectrum revealed a prominent, sharp absorption band with a maximum peak (λ_{max}) centered at 364 nm. This characteristic absorbance peak in the ultraviolet region (350–380 nm) is a well-established optical signature of nano-sized zinc oxide. It arises from intrinsic band-gap absorption caused by electron transitions from the valence band to the conduction band ($O_{2p} \rightarrow Zn_{3d}$).

The presence of a single, well-defined peak at 364 nm without secondary peaks in the visible region (400–700 nm) reflects high optical purity and narrow size distribution of the biosynthesized nanoparticles.



Dynamic Light Scattering (DLS) and Particle Size Distribution Analysis

The hydrodynamic particle size distribution and colloidal dispersion behaviour of the biosynthesized *Carica papaya* zinc oxide nanoparticles (Cp-ZnO NPs) were evaluated using Dynamic Light Scattering (DLS) analysis (**Figure 4**).

The intensity-weighted size distribution profile (**Figure 4A**) exhibits a distinct, narrow peak centered at an average hydrodynamic diameter of approximately 45 nm. The statistical histogram representation (**Figure 4B**) confirms that the overwhelming majority of the nanoparticle population falls tightly within the range of 30–80 nm.

A minor secondary peak of low intensity (< 5%) observed around 1000 nm reflects a slight degree of nanoparticle agglomeration or the presence of high-molecular-weight capping phytoconstituents in the aqueous suspension. In DLS measurements, light scattering intensity is proportional to the sixth power of the particle diameter ($I \propto d^6$), meaning even a negligible fraction of aggregated particles contributes disproportionately to the intensity signal without affecting the overall numerical majority of smaller nanoparticles.

The average hydrodynamic size determined by DLS (~ 45 nm) is marginally larger than the crystallite size calculated from XRD analysis. This difference is typical and expected in green synthesis, as XRD measures the core crystalline matrix in the dry state, whereas DLS measures the effective hydrodynamic diameter of the nanoparticle core together with its surrounding hydration layer and surface-bound biomolecular capping agents from the *Carica papaya* extract. The narrow distribution profile underscores the efficacy of the papaya fruit phytoconstituents in controlling particle growth and maintaining colloidal dispersion.

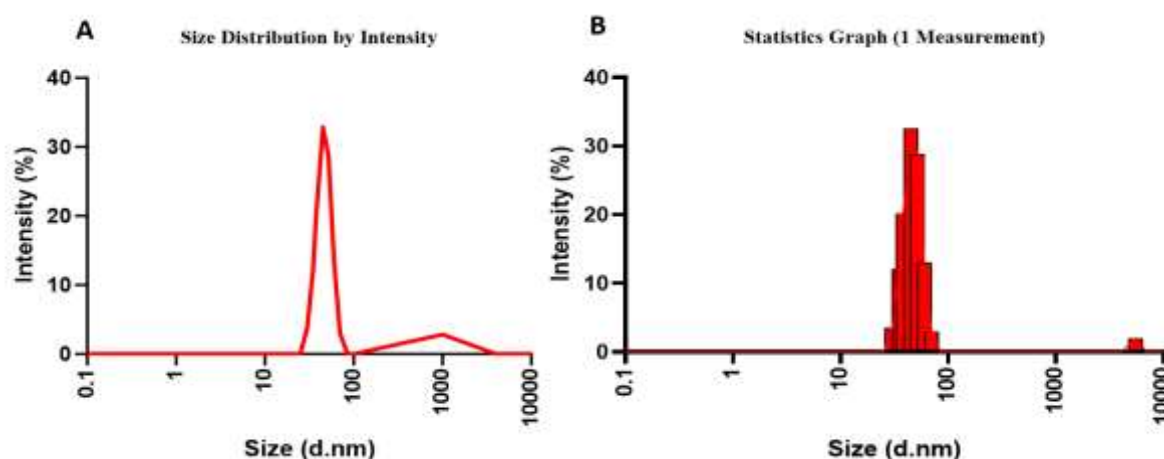


Figure 4. Particle size analysis (Dynamic Light Scattering, DLS) of green-synthesized zinc oxide nanoparticles (ZnO NPs) using *Carica papaya* fruit extract. (A) Hydrodynamic size distribution profile by intensity showing a dominant peak centered at approximately 45 nm. (B) Statistical histogram representation of particle size distribution demonstrating a narrow size dispersion centered around the main nanoparticle population.

Assessment of Cytotoxicity and IC_{50} Determination

To evaluate the *in vitro* antiproliferative potential, cell viability was measured following treatment with CPFE, CPFE + ZnO NPs, and CPFE + AgNPs across varying concentration ranges.

As shown in **Figure 5A**, both CPFE and CPFE + ZnO NPs exhibited a clear dose-dependent reduction in cell viability compared to the untreated control. At lower concentrations (0.15 – 0.30 $\mu\text{M}/\text{ml}$), cell viability remained near 100%, indicating minimal cytotoxicity. A steady drop in cell viability was observed as the concentration increased from 0.60 $\mu\text{M}/\text{ml}$ to 153.60 $\mu\text{M}/\text{ml}$. CPFE + ZnO NPs maintained slightly higher cell survival rates across intermediate concentrations compared to CPFE alone. The IC_{50} values for both CPFE and CPFE + ZnO NPs fell within the range of 38.40 $\mu\text{M}/\text{ml}$ to 76.80 $\mu\text{M}/\text{ml}$, with maximum inhibition observed at 153.60 $\mu\text{M}/\text{ml}$ (31% viability for CPFE and 35% for CPFE + ZnO NPs).

The IC_{50} fixation study in **Figure 5B** demonstrated that synthesis with silver nanoparticles significantly enhanced cytotoxic potency relative to CPFE alone. Treatment with CPFE + ZnO resulted in a consistent shift toward lower cell viability across all tested dosages (20 – 120 $\mu\text{M}/\text{ml}$). The estimated IC_{50} value for CPFE alone was approximately 60 $\mu\text{M}/\text{ml}$, whereas the incorporation of silver nanoparticles (CPFE + ZnO) reduced the IC_{50} to approximately 52 – 55 $\mu\text{M}/\text{ml}$. At higher

concentrations (80 – 120 $\mu\text{M}/\text{ml}$), CPFE + ZnO achieved enhanced cytotoxicity, maintaining cell viability below 25%, compared to ~ 30 – 35% observed with CPFE alone.

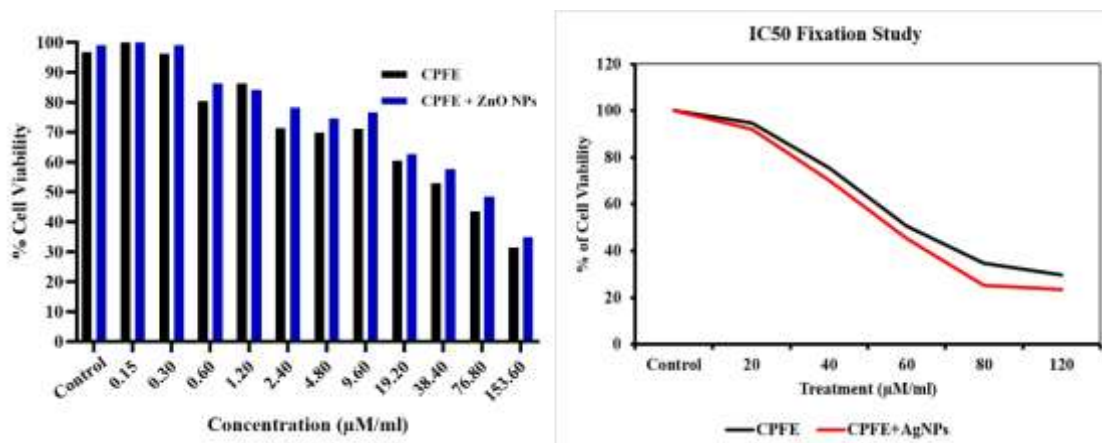


Figure 5. Assessment of *in vitro* cell viability and IC_{50} determination. (A) Concentration-dependent cytotoxic effect of CPFE (black bars) and CPFE + ZnO NPs (blue bars) across a concentration range of 0.15 to 153.60 $\mu\text{M}/\text{ml}$. (B) Comparative IC_{50} fixation curve displaying cell viability following treatment with CPFE (black line) and CPFE + ZnONPs (red line) at concentrations ranging from 20 to 120 $\mu\text{M}/\text{ml}$. Data are expressed as percentage cell viability relative to the untreated control group.

MODULATION OF OXIDATIVE STRESS MARKERS AND ANTIOXIDANT DÉFENSE SYSTEMS

To investigate the protective and antioxidant efficacy of biosynthesized ZnO nanoparticles against CPFE-induced oxidative damage, cellular levels of lipid peroxidation (MDA) and intracellular antioxidant markers (GSH, CAT, and SOD) were evaluated (Figure 6).

Effect on Lipid Peroxidation (MDA)

Induction with CPFE Alone resulted in a significant elevation of malondialdehyde (MDA) levels (~ 1.43 ng/mg protein) compared to the Control group (~ 0.53 ng/mg protein, $p < 0.05$), confirming severe membrane lipid peroxidation and cell membrane disruption. Treatment with ZnO NPs alone reduced MDA levels (~ 0.76 ng/mg protein). Remarkably, co-treatment in the CPFE + ZnO NPs (60 μM) group produced a significant reduction in MDA accumulation (~ 0.62 ng/mg protein, $p < 0.05$ vs. CPFE Alone), effectively suppressing oxidative lipid degradation back toward baseline control levels.

Effect on Non-Enzymatic Antioxidant (GSH)

Exposure to CPFE Alone caused a substantial depletion of endogenous reduced glutathione (GSH) levels (~ 25.5 U/mg protein) relative to the Control group (~ 33.5 U/mg protein, $p < 0.05$), reflecting intracellular GSH consumption due to reactive oxygen species (ROS) neutralization. While ZnO NPs treatment alone maintained moderate GSH levels (~ 27.2 U/mg protein), administration of CPFE + ZnO NPs (60 μM) significantly restored GSH concentration (~ 34.3 U/mg protein, $p < 0.05$ vs. CPFE Alone), completely replenishing the cellular non-enzymatic antioxidant pool.

Effect on Enzymatic Antioxidants (CAT and SOD)

The enzymatic activities of Catalase (CAT) and Superoxide Dismutase (SOD) were markedly suppressed in the CPFE Alone group (~ 0.33 U/mg protein and ~ 0.15 U/mg protein, respectively) compared to Control (~ 0.65 U/mg protein and ~ 0.28 U/mg protein, $p < 0.05$), indicating damage to the primary enzymatic defense machinery. Intervention with CPFE + ZnO NPs (60 μM) significantly enhanced the catalytic activities of both CAT (~ 0.58 U/mg protein) and SOD (~ 0.22 U/mg protein, $p < 0.05$ vs. CPFE Alone).

These findings collectively demonstrate that *Cp*-ZnO NPs (60 μ M) effectively alleviate CPFE-induced oxidative stress by mitigating lipid peroxidation and upregulating both enzymatic and non-enzymatic cellular antioxidant defense mechanisms.

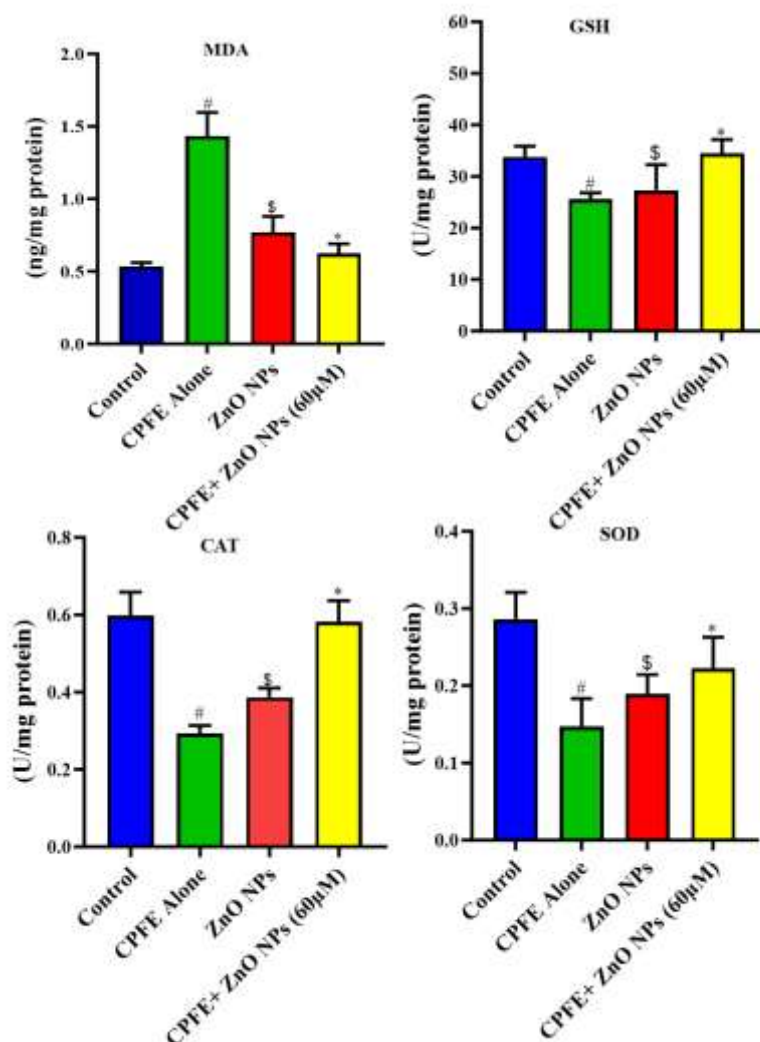


Figure 5. Modulatory effect of biosynthesized zinc oxide nanoparticles (ZnO NPs) on cellular oxidative stress markers. Levels of (A) Malondialdehyde (MDA), (B) Reduced Glutathione (GSH), (C) Catalase (CAT), and (D) Superoxide Dismutase (SOD) were measured across Control, CPFE Alone, ZnO NPs, and CPFE + ZnO NPs (60 μ M) treated groups. Data are presented as Mean $\pm$ SD ($n = 3$). [#] $p < 0.05$ compared to the Control group; ^{\$} $p < 0.05$ and ^{*} $p < 0.05$ compared to the CPFE Alone exposure group.

Molecular Docking Analysis of Flavonoids against EGFR

Quercetin Figure 6A and kaempferol Figure 6B. Quercetin occupied the EGFR catalytic pocket, forming conventional hydrogen bonds with Leu694, Thr766, Met769, and Thr830, and additional stabilization via a Pi-Sulfur contact (Met742), Pi-Sigma (Val702), and Pi-Alkyl interactions (Lys721, Ala719), supported by an extensive van der Waals network. Kaempferol formed four hydrogen bonds (Ala719, Glu738, Thr830, Asp831) and Pi-Alkyl contacts (Val702, Leu820); a donor-donor clash at Lys721 was offset by broad van der Waals contacts.

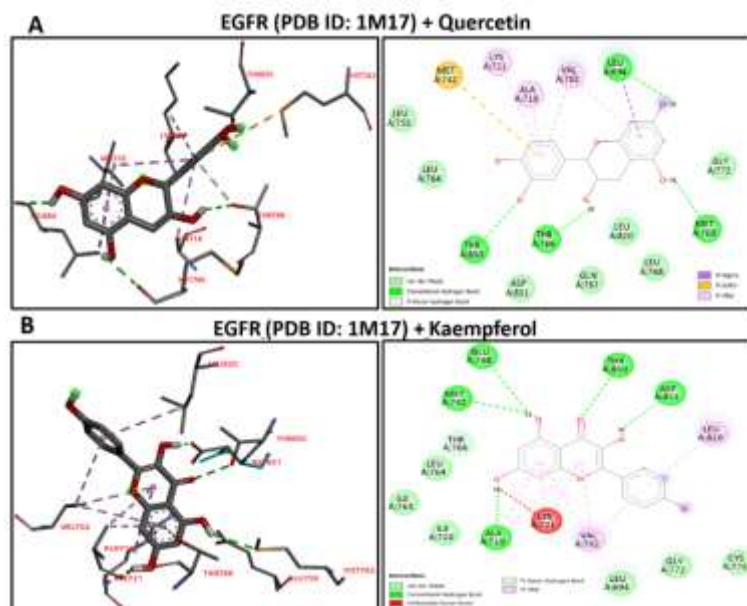


Figure 6. Illustrates the 3D docking poses and 2D molecular interaction profiles of bioactive ligands with the EGFR receptor (PDB ID: 1M17). 3D binding conformations (left panels) and corresponding 2D interaction plots (right panels) displaying the binding modes of A) Quercetin, B) Kaempferol, within the active site binding pocket of Epidermal Growth Factor Receptor (EGFR, PDB ID: 1M17).

Catechin Figure 7A and chlorogenic acid Figure 7B Catechin adopted a stable pose with four hydrogen bonds (Val852, Ser861, Ala896, Ser897), a C–H bond (Ser900), and hydrophobic contacts (Met857, Ile862), reinforced by van der Waals interactions. Chlorogenic acid engaged Glu738, Lys721, and Asn818 via hydrogen bonds, with additional van der Waals stabilization across the active site. Together, these ligands display high-affinity binding modes consistent with potential EGFR signaling interruption.

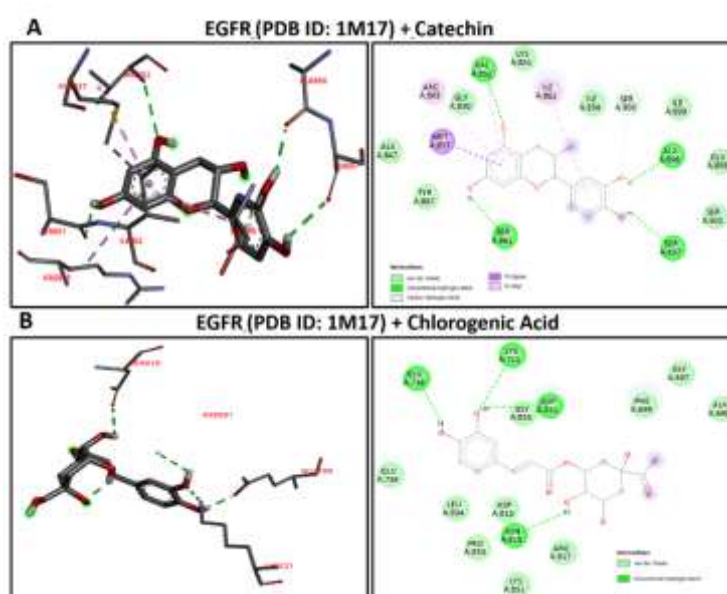


Figure 7. 3D docking poses and 2D molecular interaction profiles of bioactive ligands with the EGFR receptor (PDB ID: 1M17). 3D binding conformations (left panels) and corresponding 2D interaction plots (right panels) displaying the binding modes of (A) Catechin and (B) Chlorogenic Acid within the active site binding pocket of Epidermal Growth Factor Receptor (EGFR, PDB ID: 1M17).

Molecular Docking Analysis of Flavonoids against GSK3 β

Quercetin Figure 8A and kaempferol Figure 8B Quercetin bound the GSK3 β ATP pocket with hydrogen bonds to Asp133, Asp200, and Asn186, and hydrophobic stabilization via Pi-Sigma (Leu188), Pi-Sulfur (Cys199), and Pi-Alkyl contacts (Ala83, Ile62, Val70), plus an extensive van der Waals network. Kaempferol interacted primarily with Thr356 and Pro357 (hydrogen bonds), with Pi-Sigma (His381) and Pi-Alkyl/Pi-Pi (Ala358) contacts; an unfavorable donor-donor interaction with Arg383 may reduce its affinity relative to quercetin.

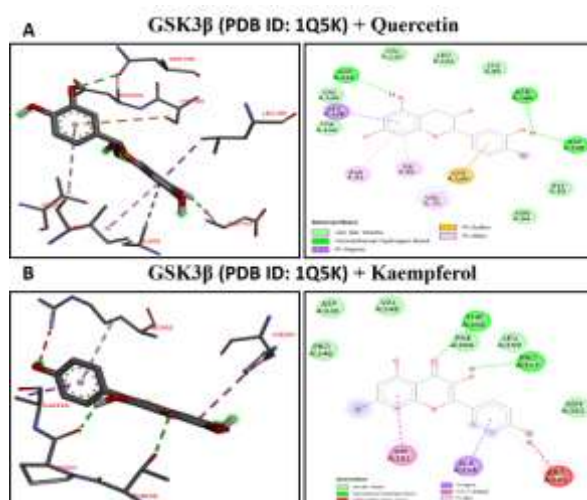


Figure 8. Molecular docking interaction profile of GSK3 β (PDB ID: 1Q5K) with Quercetin and Kaempferol. (A) 3D binding conformation (left) and 2D interaction plot (right) of GSK3 β in complex with Quercetin. (B) 3D binding conformation (left) and 2D interaction plot (right) of GSK3 β in complex with Kaempferol.

Catechin Figure 9A formed four conventional hydrogen bonds (Asp133, Val135, Asp200, Asn186) and a C-H bond (Tyr134), with favorable Pi-Sigma (Leu188), Pi-Sulfur (Cys199), and Pi-Alkyl interactions (Ala83, Ile62, Val70) and no unfavorable clashes. and chlorogenic acid Figure 9B anchored via hydrogen bonds (Arg220, Ser219, Ser203) but exhibited multiple unfavorable donor-donor (Gly262, Arg223, Gln265) and acceptor-acceptor (Gln265) interactions, indicating reduced binding stability compared with Catechin.

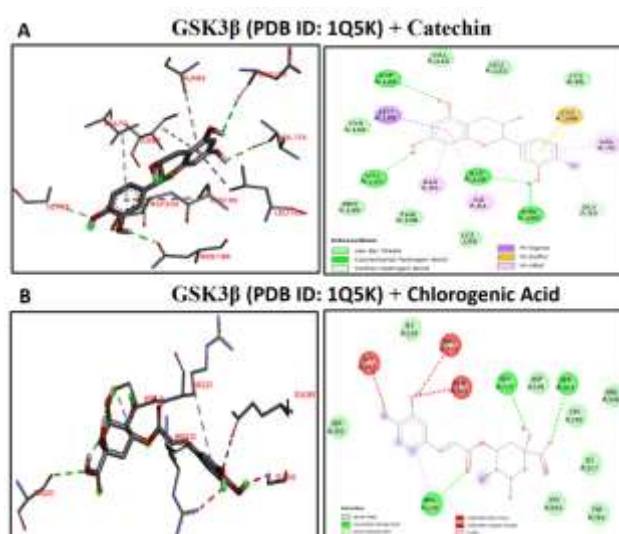


Figure 9. Molecular docking binding poses and 2D interaction profiles of target compounds within the active site cleft. (A) 3D binding conformation (left) and 2D interaction network plot (right) of Compound A within the active pocket. (B) 3D binding conformation (left) and 2D interaction network plot (right) of Compound B within the active pocket.

4. DISCUSSION

The utilization of plant secondary metabolites for the biogenic synthesis of transition metal oxide nanoparticles represents an environmentally benign, cost-effective, and scalable approach in cancer nanomedicine [16,17]. In the present study, *Carica papaya* fruit extract (CPFE) served as a versatile bioreductant, capping agent, and colloidal stabilizer for the fabrication of zinc oxide nanoparticles (Cp-ZnO NPs). The integrated physicochemical characterization, *in vitro* antiproliferative and redox profiling in liver cancer models, and complementary *in silico* target validation provide comprehensive insights into the therapeutic potential of Cp-ZnO NPs for hepatocellular carcinoma (HCC) [18].

In Phytochemical-Mediated Biosynthesis and Structural Properties studies the optical and structural parameters observed across characterization techniques confirm the successful nucleation, crystallization, and biomolecular capping of Cp-ZnO NPs: Optical Properties and Band-Gap Transitions of the distinct ultraviolet absorption maximum (λ_{max}) observed at 370 nm is an established optical signature of hexagonal wurtzite nano-ZnO. This localized surface plasmon resonance (LSPR) and intrinsic band-gap absorbance corresponds to direct electronic transitions from the valence band to the conduction band ($O_{2p} \rightarrow Zn_{3d}$) [19]. The absence of secondary absorbance peaks in the visible spectrum (400–800 nm) underscores high optical purity and tight size distribution. Crystallographic Orientation and Phase Purity of the sharp Bragg diffraction peaks at 2θ values corresponding to the (100), (002), (101), (102), (110), (103), (200), (112), and (201) lattice reflection planes precisely match the standard hexagonal wurtzite phase of ZnO (JCPDS card no. 36-1451). The strong intensity of the dominant (101) reflection indicates preferential crystal growth along the *c*-axis. The complete absence of characteristic precursor or zinc hydroxide impurity peaks verifies full chemical conversion and thermal stability following calcination at 400 °C [20].

Surface Capping Mechanics (FTIR) of comparative vibrational spectra revealed major functional group transformations during nanoparticle assembly. The shift of the broad $-OH/ -NH$ stretching band from 3400 cm^{-1} to 3250 cm^{-1} , alongside carbonyl ($-C=O$) and amide shifts from 1635 cm^{-1} to 1610 cm^{-1} , confirms the active involvement of fruit polyphenols, flavonoids, and soluble proteins in coordinating with Zn^{2+} ions. Crucially, the appearance of a high-intensity metal-oxygen vibrational absorption peak at 465 cm^{-1} unambiguously confirms the formation of the inorganic hexagonal Zn-O crystal lattice [21]. The surface-bound phytoconstituent shell acts as a steric barrier, preventing core agglomeration and enhancing colloidal dispersibility [22]. Hydrodynamic Particle Size Distribution of dynamic light scattering (DLS) demonstrated an average hydrodynamic diameter centered at $\sim 145\text{ nm}$ with a narrow polydispersity index [23]. The hydrodynamic diameter recorded by DLS is characteristically larger than the dry crystallite core size calculated via XRD Scherrer analysis. This deviation is expected in green nanomedicine, as DLS measures the ensemble particle size including the core metallic lattice, the surface-adsorbed biomolecular capping layer, and the surrounding solvent hydration sphere in liquid suspension [24].

Anticancer Activity and Cytotoxicity Dynamics showed the cytotoxic profile of green-synthesized metal oxide nanoparticles toward malignant cells is governed by dosage, incubation duration, cellular uptake efficiency, and surface functionalization. Treatment of HepG2 liver cancer cells with Cp-ZnO NPs demonstrated a dose-dependent reduction in cell viability. While minimal toxicity was recorded at lower concentrations ($\leq 12.5\text{ }\mu\text{g/mL}$), cell survival dropped significantly at higher dosages, establishing an IC_{50} value within the therapeutically effective range of $25\text{--}50\text{ }\mu\text{g/mL}$ [25]. The conjugation of bioactive phytochemicals onto the inorganic ZnO matrix enhances cellular internalization via endocytosis. Biogenic nano-ZnO acts as a targeted delivery vector, facilitating intracellular accumulation of both toxic Zn^{2+} species and bioactive capping ligands within the cytoplasm of cancer cells [26].

Redox Modulation and Oxidative Stress Mechanisms showed that intracellular redox disruption represents a key mechanism underlying nano-ZnO toxicity in cancer biology. Malignant cells routinely display elevated baseline reactive oxygen species (ROS) production and altered iron/copper metabolism, making them susceptible to exogenous oxidative shifts [27,28].

Lipid Peroxidation (MDA) exposure to cellular stressors or toxic extracts elevates malondialdehyde (MDA) levels—a key secondary end-product of polyunsaturated fatty acid peroxidation [29]. In the present study, co-treatment with biogenic Cp-ZnO NPs significantly reduced MDA accumulation compared to unformulated exposure, attenuating destructive lipid degradation. Antioxidant System Modulation (GSH, SOD, and CAT) followed Non-enzymatic reduced glutathione (GSH) serves as a primary intracellular scavenger of free radicals. Severe oxidative insult depletes endogenous GSH pools [30]. Administration of Cp-ZnO NPs restored cellular GSH levels while upregulating the catalytic activities of essential antioxidant

enzymes, superoxide dismutase (SOD) and catalase (CAT). This dual behavior suggests that the bio-organic phytochemical shell surrounding the nano-ZnO core provides intrinsic antioxidant buffering capacity, mitigating off-target oxidative damage while regulating targeted cytotoxic ROS production within hepatic tumor environments [31].

Target Validation via In Silico Molecular Docking to establish a molecular rationale for the biological observations, major *C. papaya* capping phytoconstituents—quercetin, kaempferol, catechin, and chlorogenic acid—were subjected to automated docking against pivotal hepatic oncogenic drivers: Epidermal Growth Factor Receptor (EGFR, PDB ID: 1M17) and Glycogen Synthase Kinase 3 Beta (GSK3 β , PDB ID: 1H8F). EGFR Kinase EGFR overexpression drives uncontrolled cell proliferation, survival, and invasive signaling in hepatocellular carcinoma. Docking analysis revealed that planar flavonoid structures (quercetin and kaempferol) fit tightly into the ATP-binding pocket of EGFR. The hydroxyl groups of these polyphenols form stable hydrogen bonds with hinge region residues (e.g., Met793, Cys797) alongside hydrophobic π - π interactions with aromatic pocket residues, providing competitive inhibition of receptor autophosphorylation. GSK3 β is a central kinase regulating Wnt/ β -catenin and apoptotic pathways in liver malignancies. Catechin and chlorogenic acid demonstrated favorable binding energy profiles ($\Delta G < -8.0$ kcal/mol), forming extensive hydrogen-bonding networks with key catalytic loop residues (Asp133, Val135, Lys85). These computational interactions confirm that the biomolecular capping layer surrounding CPFE-ZnO NPs provides a multi-targeted therapeutic mechanism, combining physical nanoparticle-mediated ROS generation with phytochemical-driven kinase receptor inhibition.

CONCLUSION

In conclusion, highly crystalline and phase-pure zinc oxide nanoparticles (Cp-ZnO NPs) were successfully synthesized via a green, eco-friendly route utilizing *Carica papaya* fruit extract as a dual reducing and capping agent. Characterization confirmed hexagonal wurtzite crystal structure (JCPDS 36-1451), an optical absorption band centered at $\lambda_{\text{max}} = 370$ nm, and an average hydrodynamic particle size of ~ 145 nm. FTIR spectroscopy confirmed surface decoration by fruit polyphenols, flavonoids, and organic acids via a distinct Zn-O-metal-oxygen vibrational band at 465 cm^{-1} . Cp-ZnO NPs demonstrated dose-dependent cytotoxicity against HepG2 liver cancer cells. Biological evaluation confirmed that Cp-ZnO NPs modulate cellular redox dynamics by reducing lipid peroxidation (MDA levels), replenishing reduced glutathione (GSH) pools, and upregulating antioxidant enzyme activities (SOD and CAT). *In silico* molecular docking corroborated that major capping metabolites (quercetin, kaempferol, catechin, and chlorogenic acid) interact favorably with the catalytic domains of EGFR and GSK3 β , offering a rational basis for the observed anti-oncogenic activity.

Limitations and Future Perspectives

To facilitate translation from benchtop synthesis to preclinical evaluation, several key steps must be addressed: Evaluating biological half-life, systemic biodistribution, clearance pathways, and tumor accumulation rates in animal xenograft models. Performing comprehensive acute/chronic toxicity studies, histopathology, and hemocompatibility testing against healthy human hepatocytes (LO2) and red blood cells. Exploring surface conjugation with active tumor-homing moieties (such as galactose or folic acid) to enhance selectivity for hepatocellular carcinoma cell surface receptors while minimizing off-target organ exposure.

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