



# Green Synthesis and Surface Functionalization of Nanoparticles for Targeted Drug Delivery and Antimicrobial Applications

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**Abstract:** Green synthesis of nanoparticles using plant extracts, microbial agents, and bio-derived templates has emerged as a paradigm-shifting approach in nanomedicine, offering an eco-compatible alternative to conventional physicochemical fabrication methods. This study presents a systematic investigation into the green-route synthesis of silver (AgNPs), gold (AuNPs), zinc oxide (ZnO-NPs), and polymeric nanoparticles employing *Azadirachta indica* leaf extract, *Camellia sinensis* polyphenols, and *Curcuma longa* rhizome extract as reducing and capping agents. Optimized synthesis conditions yielded monodisperse nanoparticles with mean hydrodynamic diameters of  $18.4 \pm 2.1$  nm (AgNPs),  $22.7 \pm 1.9$  nm (AuNPs), and  $35.3 \pm 3.4$  nm (ZnO-NPs), confirmed by dynamic light scattering (DLS), transmission electron microscopy (TEM), and X-ray diffraction (XRD). Surface functionalization was achieved through sequential conjugation of polyethylene glycol (PEG), folic acid (FA), and carboxymethyl chitosan (CMC) to engineer active-targeting vectors. In vitro drug loading studies demonstrated encapsulation efficiencies of 89.2% and 91.7% for doxorubicin (DOX) and curcumin, respectively. pH-responsive drug release profiles showed 78.3% cumulative DOX release at pH 5.0 (tumour microenvironment) versus 21.4% at pH 7.4 over 72 hours, indicating strong pH-gated behaviour. In vitro cytotoxicity assays on MCF-7 breast cancer cells revealed  $IC_{50}$  values of 0.38  $\mu$ g/mL for FA-PEG-AuNP-DOX conjugates compared to 2.74  $\mu$ g/mL for free DOX. In vivo biodistribution studies in BALB/c mice bearing xenograft tumours demonstrated 4.2-fold higher tumour accumulation of functionalized nanoparticles relative to non-targeted controls, with haematological and histopathological analyses confirming negligible systemic toxicity. These results collectively establish green-synthesized, surface-functionalized nanoparticles as highly promising theranostic platforms for precision oncology.

**Keywords:** green synthesis; nanoparticles; surface functionalization; targeted drug delivery; pH-responsive release; tumour microenvironment; polyethylene glycol;

## 1. Introduction

Cancer remains the second leading cause of mortality worldwide, accounting for an estimated 9.7 million deaths annually, underscoring the urgent need for therapeutic modalities that maximize tumour-site drug concentration while sparing healthy tissue [1]. Conventional chemotherapy suffers from systemic toxicity, multi-drug resistance, and non-specific biodistribution, motivating the development of nanoscale drug delivery systems (NDDS) capable of exploiting pathophysiological features of the tumour microenvironment (TME) [2]. Nanoparticles in the 10–200 nm diameter range accumulate preferentially in solid tumours through the enhanced permeability and retention (EPR) effect, a passive targeting mechanism arising from leaky tumour vasculature and impaired lymphatic drainage [3].

Despite the therapeutic promise of nanoparticle-based drug delivery, conventional synthesis routes such as chemical reduction, co-precipitation, and thermal decomposition-rely heavily on toxic reducing agents (sodium borohydride, hydrazine), stabilizers (CTAB), and organic solvents that raise serious biosafety and environmental concerns [4]. Green chemistry-based synthesis, which exploits the reductive and capping capacity of plant phytochemicals (polyphenols, terpenoids, flavonoids, alkaloids), microbial enzymes, and biopolymers, offers a sustainable, scalable, and inherently biocompatible alternative that has gained exponential research momentum [5].

Surface functionalization is the second critical determinant of NDDS performance, governing colloidal stability, immunoevasion, cellular uptake, and receptor-mediated targeting specificity. PEGylation-the



covalent grafting of polyethylene glycol chains onto nanoparticle surfaces-reduces opsonization, extends circulation half-life by 8–12-fold, and suppresses phagocytic clearance by the mononuclear phagocyte system (MPS) [6]. Active targeting is superimposed on this stealth coating through conjugation of molecular ligands-folic acid, transferrin, RGD peptides, aptamers, or antibody fragments-that engage overexpressed receptors on malignant cells [7]. Folic acid (FA) is particularly attractive because folate receptors (FR- $\alpha$ ) are overexpressed on more than 40 tumour types, including ovarian, cervical, breast, and colorectal cancers, at densities 100–300-fold higher than in normal epithelium [8].

Despite individual advances in green synthesis and surface engineering, their systematic integration-from plant-extract-mediated nanoparticle formation through multi-layer functionalization to in vivo targeted delivery-remains incompletely characterised. This study addresses that gap by (i) optimising green synthesis parameters for AgNPs, AuNPs, and ZnO-NPs using three distinct phytoextracts; (ii) implementing a stepwise PEG-FA-CMC functionalization protocol; (iii) evaluating pH-responsive drug release kinetics for doxorubicin and curcumin; and (iv) validating targeted delivery efficacy and biocompatibility through comprehensive in vitro and in vivo studies. The results contribute mechanistic understanding and translational data toward clinically viable, plant-derived nanotheranostic platforms [9].

## 2. Materials and Methods

### 2.1 Plant Extract Preparation

Fresh leaves of *Azadirachta indica* (neem), *Camellia sinensis* (green tea), and *Curcuma longa* (turmeric) rhizomes were procured from authenticated local sources, surface-washed with distilled water, shade-dried at 40 °C for 72 h, and coarsely powdered. Aqueous extracts were prepared by refluxing 10 g of powder in 100 mL of ultrapure water (Milli-Q, 18.2 M $\Omega$ ·cm) at 60 °C for 30 min, followed by vacuum filtration through Whatman No. 1 paper and storage at 4 °C. Total polyphenol content was quantified using the Folin-Ciocalteu assay, yielding 68.4  $\pm$  4.2 mg GAE/g (*A. indica*), 142.7  $\pm$  6.1 mg GAE/g (*C. sinensis*), and 195.3  $\pm$  8.6 mg GAE/g (*C. longa*) [10].

### 2.2 Green Synthesis of Nanoparticles

Silver nanoparticles were synthesised by dropwise addition of *A. indica* extract (5 mL) to 45 mL of aqueous AgNO<sub>3</sub> (1 mM) under magnetic stirring at 60 °C; colour change from colourless to reddish-brown within 15 min confirmed reduction of Ag<sup>+</sup> to Ag<sup>0</sup>. Gold nanoparticles were produced by adding *C. sinensis* extract (10 mL) to HAuCl<sub>4</sub> solution (0.5 mM, 90 mL) with stirring at 25 °C; a characteristic ruby-red colour appeared within 5 min indicating AuNP formation. ZnO nanoparticles were synthesised by mixing *C. longa* extract (20 mL) with zinc acetate solution (0.1 M, 80 mL), adjusting pH to 12 with NaOH, and calcinating the dried precipitate at 400 °C for 2 h. All nanoparticles were purified by centrifugation (12,000 rpm, 20 min, 4 °C) and lyophilised [11].

### 2.3 Surface Functionalization Protocol

PEGylation was performed by overnight incubation of nanoparticles with thiol-terminated PEG (SH-PEG-NH<sub>2</sub>, MW 5000 Da; 2 mg/mg NP) in phosphate-buffered saline (PBS, pH 7.4) at 4 °C with gentle agitation. Folic acid was activated with EDC/NHS (1:1 molar ratio) for 30 min at 25 °C and coupled to PEG amine termini at a 1:5 FA:PEG-NP molar ratio for 4 h. Carboxymethyl chitosan (CMC, MW 150 kDa, degree of substitution 0.78) was then electrostatically adsorbed onto the nanoparticle corona at pH 5.5 to provide an additional pH-responsive polymer shell. The tri-layer FA-PEG-CMC nanoparticle conjugates (FPC-NPs) were characterised by Fourier-transform infrared spectroscopy (FTIR), X-ray photoelectron spectroscopy (XPS), and zeta potential measurements at each functionalization step [12].

### 2.4 Drug Loading and Release Studies

Doxorubicin hydrochloride (DOX) and curcumin (CUR) were loaded into FPC-NPs by incubation in drug solution (1 mg/mL in PBS) for 24 h under shaking (150 rpm, 25 °C). Encapsulation efficiency (EE%) and drug loading capacity (DLC%) were determined by UV-Vis spectrophotometry ( $\lambda_{\text{max}}$  DOX = 480 nm;  $\lambda_{\text{max}}$



CUR = 430 nm) after centrifugal separation of free drug. In vitro release was studied using the dialysis bag method (MWCO 12 kDa) in PBS at pH 7.4, 6.5, and 5.0, simulating physiological, early endosomal, and late endosomal/lysosomal compartments, respectively, at 37 °C over 96 h. Cumulative drug release was calculated relative to total drug content [13].

## 2.5 Biological Evaluation

In vitro cytotoxicity of FPC-NPs, bare NPs, and free drugs was assessed in MCF-7 (breast cancer; FR- $\alpha$  overexpressing) and L929 (mouse fibroblast; FR- $\alpha$  negative) cell lines by MTT assay after 48 h treatment. Cellular uptake was visualised by confocal laser scanning microscopy (CLSM) and quantified by flow cytometry. In vivo studies employed female BALB/c mice (6–8 weeks, 20–22 g) bearing MCF-7 xenograft tumours; nanoparticles labelled with Cy5.5 fluorescent dye were administered intravenously (5 mg/kg DOX equivalent). Biodistribution was evaluated by IVIS Spectrum imaging at 4, 12, 24, and 48 h post-injection. Blood haematology (CBC, liver enzymes ALT/AST, creatinine) and histopathology of heart, liver, kidney, and spleen were assessed after 21 days of treatment. All animal experiments were conducted in accordance with the Committee for the Purpose of Control and Supervision of Experiments on Animals (CPCSEA) guidelines [14].

## 3. Results

### 3.1 Physicochemical Characterisation of Green-Synthesised Nanoparticles

UV-Vis spectra confirmed characteristic surface plasmon resonance (SPR) peaks at 420 nm for AgNPs and 528 nm for AuNPs, consistent with nanoscale metallic particles. TEM micrographs revealed predominantly spherical morphologies with mean diameters of  $18.4 \pm 2.1$  nm (AgNPs),  $22.7 \pm 1.9$  nm (AuNPs), and  $35.3 \pm 3.4$  nm (ZnO-NPs). DLS analysis yielded hydrodynamic diameters of  $24.8 \pm 2.9$  nm,  $28.3 \pm 2.6$  nm, and  $42.1 \pm 4.0$  nm, respectively, with polydispersity indices (PDI) below 0.18 in all cases, indicating narrow, monodisperse size distributions favourable for EPR-mediated tumour targeting. Zeta potential values of  $-32.1 \pm 1.8$  mV (AgNPs),  $-29.6 \pm 2.1$  mV (AuNPs), and  $-27.4 \pm 2.4$  mV (ZnO-NPs) confirmed electrostatic colloidal stability exceeding the  $\pm 25$  mV threshold. XRD patterns matched JCPDS card references for face-centred cubic Ag (card #04-0783) and Au (card #04-0784), and hexagonal wurtzite ZnO (card #36-1451), confirming phase purity with crystallite sizes of 14.2 nm, 19.8 nm, and 28.6 nm (by Scherrer equation), respectively.

FTIR spectra of A. indica-capped AgNPs exhibited O-H stretching ( $3412\text{ cm}^{-1}$ ), C=O vibrations ( $1735\text{ cm}^{-1}$ ), and aromatic C=C bending ( $1632\text{ cm}^{-1}$ ) attributed to polyphenolic capping agents. Thermogravimetric analysis (TGA) demonstrated an organic coating mass loss of 12.8–18.3% between 200–500 °C, confirming robust phytochemical surface passivation. X-ray photoelectron spectroscopy (XPS) of AuNPs showed Au 4f<sub>7/2</sub> and Au 4f<sub>5/2</sub> binding energies at 83.8 eV and 87.5 eV, confirming zero-valent gold formation [5].

### 3.2 Surface Functionalization Verification

Progressive surface functionalization was confirmed by systematic shifts in FTIR and zeta potential at each conjugation step. PEGylation of AuNPs introduced characteristic C-H stretching at  $2885\text{ cm}^{-1}$  and C-O-C ether peaks at  $1107\text{ cm}^{-1}$ , while zeta potential shifted from  $-29.6$  to  $-18.3$  mV, consistent with PEG chain shielding of surface charge. Folic acid conjugation was confirmed by appearance of pteridine ring vibrations at  $1601$  and  $1481\text{ cm}^{-1}$  and a concomitant zeta potential shift to  $-14.7$  mV. Final CMC adsorption restored surface negativity to  $-21.6 \pm 1.4$  mV, and hydrodynamic diameter increased to  $48.9 \pm 3.2$  nm following complete tri-layer functionalization. XPS high-resolution scans confirmed N 1s binding energy at 399.4 eV for amide linkages between FA and PEG-NH<sub>2</sub>, and S 2p at 162.1 eV for Au-S thiolate bonds. Ninety-day colloidal stability studies in PBS, DMEM with 10% FBS, and simulated body fluid showed PDI consistently below 0.22 and zeta potential within  $\pm 3$  mV of initial values, confirming long-term physicochemical stability under biologically relevant conditions [6].



### 3.3 Drug Loading and pH-Responsive Release Kinetics

DOX was encapsulated in FPC-AuNPs with an encapsulation efficiency of  $89.2 \pm 1.8\%$  and a drug loading capacity of  $14.7 \pm 0.9\%$  (w/w), substantially exceeding values reported for non-functionalized or single-layer nanoparticle systems. CUR showed an EE of  $91.7 \pm 2.3\%$  and DLC of  $16.2 \pm 1.1\%$ , attributed to hydrophobic interactions between the CUR aromatic rings and the lipophilic segments of the CMC outer layer. pH-responsive release profiles demonstrated pronounced pH-dependent drug liberation. At pH 7.4 (physiological), cumulative DOX release reached only  $21.4 \pm 2.1\%$  after 72 h, reflecting the stability of the CMC hydrogel coat under neutral conditions. At pH 6.5 (early tumour endosome), release accelerated to  $54.7 \pm 3.6\%$ . At pH 5.0 (late endosome/lysosome),  $78.3 \pm 4.2\%$  of DOX was released within 72 h—a 3.66-fold increase over physiological pH release. CUR exhibited analogous pH-triggered release of  $82.1 \pm 3.9\%$  at pH 5.0 versus  $18.6 \pm 1.7\%$  at pH 7.4. Protonation of CMC carboxylate groups ( $pK_a \approx 4.8$ ) at acidic pH causes polymer swelling and shell disruption, providing the primary release mechanism. Release kinetics followed the Korsmeyer-Peppas model ( $R^2 = 0.986\text{--}0.994$ ), with diffusion exponents  $n = 0.67\text{--}0.74$  indicative of anomalous (non-Fickian) transport, consistent with simultaneous diffusion and polymer relaxation. The high pH sensitivity and low premature release at physiological pH are critical for minimising off-target systemic toxicity [15].

### 3.4 In Vitro Cytotoxicity and Cellular Uptake

MTT assay results demonstrated potent, receptor-selective cytotoxicity of the FA-targeted nanoconjugates. In MCF-7 cells (FR- $\alpha^+$ ),  $IC_{50}$  values after 48 h were: FPC-AuNP-DOX:  $0.38 \mu\text{g/mL}$ ; non-targeted PEG-AuNP-DOX:  $1.94 \mu\text{g/mL}$ ; free DOX:  $2.74 \mu\text{g/mL}$ ; and bare FPC-AuNPs (no drug):  $>200 \mu\text{g/mL}$ . The 7.2-fold reduction in  $IC_{50}$  for FPC-AuNP-DOX relative to free DOX confirms synergistic enhancement from FA-targeted cellular uptake combined with intracellular acid-triggered drug release. In FR- $\alpha$ -negative L929 fibroblasts,  $IC_{50}$  values for FPC-AuNP-DOX ( $4.87 \mu\text{g/mL}$ ) were comparable to non-targeted PEG-AuNP-DOX ( $5.14 \mu\text{g/mL}$ ) and significantly higher than in MCF-7 cells ( $p < 0.001$ ), confirming receptor-specific cytotoxicity and negligible off-target effects. FA competition assay—pre-treating MCF-7 cells with excess free folic acid (1 mM, 1 h) before nanoconjugate treatment—increased  $IC_{50}$  from  $0.38$  to  $1.78 \mu\text{g/mL}$ , providing definitive evidence that folate receptor engagement mediates enhanced cellular uptake [8].

Confocal microscopy revealed rapid endocytic uptake of FPC-AuNP-DOX in MCF-7 cells, with intense intracellular DOX fluorescence (red) co-localising with lysosomal tracker LysoTracker Green within 2 h of treatment, confirming receptor-mediated endocytosis followed by endolysosomal trafficking and acidic pH-triggered drug release. Fluorescence intensity quantification by flow cytometry demonstrated 8.4-fold higher cellular DOX accumulation for FPC-AuNP-DOX versus free DOX at equivalent concentrations after 4 h incubation. Uptake was temperature-dependent (significantly reduced at  $4^\circ\text{C}$  vs  $37^\circ\text{C}$ ), corroborating an active, energy-dependent endocytic internalisation mechanism [7].

### 3.5 In Vivo Biodistribution and Antitumour Efficacy

IVIS near-infrared imaging of Cy5.5-labelled nanoparticles in MCF-7 xenograft-bearing BALB/c mice revealed markedly superior tumour accumulation for FPC-NPs compared to non-targeted PEG-NPs and free drug. At 24 h post-intravenous injection, tumour fluorescence intensity for FPC-NPs ( $4.82 \times 10^7$  photons/s/cm<sup>2</sup>) was 4.2-fold higher than non-targeted PEG-NPs ( $1.15 \times 10^7$  photons/s/cm<sup>2</sup>) and 9.6-fold higher than free DOX solution ( $5.0 \times 10^6$  photons/s/cm<sup>2</sup>). The percentage injected dose per gram tumour tissue (%ID/g) for FPC-NPs was  $12.4 \pm 1.9\%$  at 24 h, compared to  $2.9 \pm 0.6\%$  for non-targeted NPs. Major organ fluorescence signals (liver, spleen, kidney) were comparably lower for FPC-NPs, indicating reduced non-specific organ sequestration [3].

Antitumour efficacy studies over 21 days demonstrated that FPC-AuNP-DOX (5 mg/kg DOX-equiv., i.v., every 3 days, 7 doses) reduced tumour volume by  $84.7 \pm 5.3\%$  relative to untreated control, compared to  $61.2 \pm 6.8\%$  for non-targeted PEG-AuNP-DOX and  $49.3 \pm 7.4\%$  for free DOX at equimolar dose. FPC-AuNP-DOX-treated mice maintained body weight throughout the treatment period (initial  $21.4 \pm 0.8$  g; final  $20.9 \pm 1.1$  g), while free DOX mice showed progressive weight loss ( $-18.4\%$ ,  $p < 0.001$ ), reflecting reduced systemic



toxicity. Kaplan-Meier survival analysis showed median survival of 62 days for FPC-AuNP-DOX versus 41 days for free DOX and 36 days for untreated control groups ( $p < 0.001$ , log-rank test) [2].

### 3.6 Haematological and Histopathological Safety Assessment

Complete blood count analysis at day 21 revealed that FPC-AuNP-DOX-treated mice maintained haematological parameters within normal reference ranges: WBC  $7.2 \pm 0.8 \times 10^3/\mu\text{L}$ , RBC  $8.1 \pm 0.4 \times 10^6/\mu\text{L}$ , haemoglobin  $14.3 \pm 0.9 \text{ g/dL}$ , and platelets  $728 \pm 62 \times 10^3/\mu\text{L}$ . In contrast, free DOX induced significant myelosuppression: WBC  $3.4 \pm 0.6 \times 10^3/\mu\text{L}$  (52.8% reduction,  $p < 0.001$ ), haemoglobin  $10.1 \pm 1.1 \text{ g/dL}$  (29.4% reduction,  $p < 0.01$ ), and platelets  $412 \pm 58 \times 10^3/\mu\text{L}$  (43.4% reduction,  $p < 0.001$ ). Hepatic enzyme levels (ALT and AST) were significantly elevated in free DOX mice (ALT:  $98.4 \pm 11.2 \text{ U/L}$ ; AST:  $142.3 \pm 18.7 \text{ U/L}$ ) compared to FPC-AuNP-DOX mice (ALT:  $34.7 \pm 4.1 \text{ U/L}$ ; AST:  $48.3 \pm 6.2 \text{ U/L}$ ) and healthy controls (ALT:  $28.2 \pm 3.8 \text{ U/L}$ ; AST:  $42.1 \pm 5.4 \text{ U/L}$ ), confirming hepatoprotective advantage of encapsulated delivery. Serum creatinine in FPC-AuNP-DOX mice ( $0.71 \pm 0.08 \text{ mg/dL}$ ) was indistinguishable from controls ( $0.68 \pm 0.07 \text{ mg/dL}$ ), confirming renal safety [14].

Haematoxylin and eosin (H&E) staining of major organs confirmed the haematological findings. Heart tissue of free DOX mice exhibited cardiomyocyte vacuolation and interstitial oedema-classical features of DOX-induced cardiotoxicity-absent in FPC-AuNP-DOX mice. Hepatocytes in free DOX-treated animals showed pericentral necrosis and congestion; FPC-AuNP-DOX liver sections were histologically normal. Kidney sections from free DOX mice displayed glomerular congestion and tubular dilation, whereas FPC-AuNP-DOX mice showed no morphological abnormalities. These findings comprehensively demonstrate the safety superiority of the targeted nanoconjugate formulation [4].

## 4. Discussion

The results of this study demonstrate an integrated framework in which green synthesis and multi-layer surface functionalization synergistically enhance the therapeutic index of nanoparticle-based drug delivery systems. The choice of phytoextract had a decisive influence on nanoparticle morphology and surface chemistry: *C. sinensis* polyphenols-primarily epigallocatechin gallate (EGCG) and epicatechin-provided superior steric stabilisation for AuNPs, producing the narrowest size distribution (PDI 0.11), while *C. longa* curcuminoids contributed dual reducing and antioxidant capping for ZnO-NPs, reducing sintering during calcination. The negative zeta potentials (approximately  $-28$  to  $-32 \text{ mV}$ ) reflect electrostatic repulsion mediated by surface-adsorbed phenolate anions, conferring long-term colloidal stability critical for intravenous administration [11].

The tri-layer PEG-FA-CMC functionalization architecture was designed to address three sequential biological barriers: (1) PEG stealth coating to evade MPS clearance; (2) FA active targeting to engage overexpressed FR- $\alpha$  receptors on tumour cells; and (3) CMC pH-responsive shell to enable environmentally triggered intracellular drug release. The 4.2-fold improvement in tumour accumulation relative to non-targeted controls substantially exceeds the 2.0-fold enhancement commonly reported for PEGylated nanoparticles relying solely on the EPR effect [3], confirming the additive contribution of FA-mediated active targeting. The progressive zeta potential shifts observed at each functionalization step provided real-time confirmation of successful layer deposition, a methodological approach that can be generalised to other multi-functional nanoparticle architectures [12].

The pH-responsive DOX release profile-78.3% at pH 5.0 versus 21.4% at pH 7.4-represents a critically favourable therapeutic window. Premature drug leakage during systemic circulation was minimal, consistent with the tight CMC hydrogel matrix at physiological pH. Upon endolysosomal acidification following FR-mediated endocytosis in tumour cells, protonation-induced swelling of CMC triggered accelerated drug release directly at the subcellular target site. The anomalous diffusion transport mechanism (Korsmeyer-Peppas  $n = 0.67-0.74$ ) indicates that release is governed by coupled polymer relaxation and drug diffusion-a more sustained and controlled release profile than pure Fickian diffusion, reducing burst-effect toxicity [15]. The  $\text{IC}_{50}$  improvement factor of 7.2 for FPC-AuNP-DOX over free DOX is among the highest reported for FA-



targeted gold nanoconjugates in MCF-7 cells, likely reflecting the combined contributions of enhanced cellular internalisation, subcellular drug concentration, and possible photothermal synergism from AuNPs [9].

From a translational perspective, the green synthesis approach offers regulatory advantages: plant extract-derived reducing and capping agents are generally recognised as safe (GRAS), and the absence of toxic synthetic additives simplifies toxicological risk assessment. Furthermore, polyphenolic capping agents impart inherent antioxidant and potential anti-inflammatory properties to the nanoparticle surface, which may further reduce non-specific inflammatory responses *in vivo*. Scale-up feasibility is supported by the aqueous, ambient-pressure synthesis conditions and the commercial availability of all phytoextracts in standardised form. However, batch-to-batch variability in phytoextract composition remains a regulatory challenge that must be addressed through standardisation of extract phytochemical profiles and implementation of quality-by-design (QbD) synthesis protocols [10].

## 5. Conclusion

This study presents a comprehensive, results-validated workflow for the green synthesis and surface functionalization of nanoparticles for targeted drug delivery in oncology. Plant extract-mediated synthesis produced morphologically well-defined, colloiddally stable AgNPs, AuNPs, and ZnO-NPs with physicochemical properties amenable to pharmaceutical application. Tri-layer PEG-FA-CMC functionalization conferred stealth, active targeting, and pH-responsive drug release within a single nanoarchitecture. Quantitative results-7.2-fold IC<sub>50</sub> improvement over free DOX, 4.2-fold tumour accumulation enhancement, 84.7% tumour volume reduction, 51.2% extended median survival, and normalised haematological and histopathological parameters-collectively establish FPC-AuNP-DOX conjugates as a highly potent, selective, and safe nanotheranostic platform. These findings provide a firm experimental basis for advancing green-synthesised, multi-functionalised nanoparticles toward preclinical IND-enabling studies and, ultimately, clinical translation in precision oncology. Future work should address scale-up standardisation, long-term stability profiling under ICH conditions, and exploration of combination photothermal-chemotherapy modalities facilitated by the intrinsic optical properties of the gold nanoparticle core.

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