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RESEARCH ARTICLE

In Vitro Anticancer Efficacy of Photo-Induced Silver Nanoparticles Synthesized from Desmodium gangeticum Against MCF-7 Breast Cancer Cells

Akshaya Viswanathan[1], Thomas N[2], Harish M S[1] and Vimal S[1]*
[1]Department of Biochemistry, Saveetha Medical College and Hospitals, Saveetha Institute of Medical and Technical Sciences, Saveetha University, Chennai, Tamil Nadu, India.
[2]Department of General Medicine, Saveetha Medical College and Hospitals, Saveetha Institute of Medical and Technical Sciences, Saveetha University, Chennai, Tamil Nadu, India.

*Corresponding Author
Vimal S

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Abstract: This study presents an innovative photo-induced green synthesis strategy for the fabrication of bioactive silver nanoparticles (AgNPs) utilizing the aqueous extract of Desmodium gangeticum, a traditional Ayurvedic medicinal plant. This method integrates controlled photo-illumination, which enhances electron excitation within phytochemical constituents, thereby accelerating the reduction of Ag^+ ions and promoting the formation of homogeneous, stable, and biologically active nanoparticles. Fourier Transform Infrared (FTIR) spectroscopy confirmed the involvement of phytochemicals in nanoparticle synthesis through characteristic functional groups such as O=C=O stretching, C–H aromatic stretching, and N–O vibrations, indicating their roles in reduction, stabilization, and capping of AgNPs. The biosynthesized nanoparticles exhibited potent cytotoxic activity against MCF-7 human breast cancer cells with an IC_{50} value of 16 ± 0.2 µg/mL, demonstrating superior anticancer efficacy compared to conventionally synthesized AgNPs. Morphological and nuclear changes typical of apoptosis, including membrane blebbing and chromatin condensation, were confirmed by acridine orange/ethidium bromide (AO/EtBr) dual staining. The novelty of this study lies in the integration of photonic activation with plant-derived bio-reductants, establishing a synergistic "photo-biogenic" synthesis paradigm that enhances both structural uniformity and biological potency of AgNPs. This environmentally sustainable and scalable approach provides a promising foundation for advancing light-assisted green nanotechnology and developing next-generation nanomedicine for effective cancer therapy.

Keywords Green synthesis, silver nanoparticles (AgNPs), Desmodium gangeticum, Photo-induction, anticancer activity.

INTRODUCTION

In recent years, nanotechnology has appeared as a transformative force across various scientific disciplines, especially in medicine, where nanoparticles offer new possibilities for diagnosis and treatment [1]. Silver nanoparticles (AgNPs) have gained significant attention among the various kinds of nanoparticles that have been studied because of their remarkable biological characteristics, which include antibacterial, anti-inflammatory, antioxidant, and anticancer effects [2]. However, one of the major limitations in the widespread biomedical application of silver nanoparticles lies in their synthesis [3]. Traditional chemical methods often involve reducing agents such as sodium borohydride, hydrazine, or citrate, which can be toxic or harmful to both the environment and living systems [4]. Moreover, such processes typically require high temperatures, inert atmospheres, or prolonged reaction times. These drawbacks have prompted a significant shift toward green synthesis, which emphasizes eco-friendly, safe, and sustainable methods [5] [6].

Green synthesis methods, particularly those using plant extracts, have recently gained significant attention as a safer and more sustainable alternative [7, 8]. Numerous phytochemicals, including flavonoids, tannins, alkaloids, phenolics, and terpenoids, are abundant in plants and can act as natural stabilizing and reducing agents when nanoparticles are forming [9]. The use of plant extracts not only avoids toxic chemicals but also enhances the biological compatibility and functional properties of the resulting nanoparticles [10]. Furthermore, integrating light exposure into the synthesis process known as photo-induction can accelerate the reduction of metal ions like Ag^+ and produce nanoparticles in a faster and more controlled manner under ambient conditions. This approach minimizes the need for high-temperature processing or strong chemical reductants, aligning well with the principles of green chemistry [11, 12].

Desmodium gangeticum (L.) DC., commonly known in traditional Indian medicine as an ancient Ayurvedic remedy, "Shalaparni," is a perennial herb that has been utilized for generations [13]. Among its several known pharmacological characteristics are anti-inflammatory, antioxidant, antibacterial, and anticancer effects. Its aqueous extract is abundant in flavonoids, polyphenols, and other bioactive substances that have the ability to stabilize nanoparticles and actively lower silver ions [14]. Moreover, the photo-induction technique used in this synthesis offers an additional layer of control and efficiency. When light is introduced during the reaction, it helps excite the electrons in the phytochemicals,

speeding up the reduction of Ag^+ ions to metallic nanoparticles [15]. In addition to reducing the synthesis time, this can also affect the nanoparticles size, shape, and distribution all of which are crucial factors in biological applications. The synergy between the light-induced energy and the plant's phytochemicals ensures a rapid, clean, and scalable synthesis process [16].

In this study, we present a novel and green route for synthesizing silver nanoparticles using the aqueous extract of D. gangeticum under photo-induction. Light exposure acts as a catalyst to speed up the reduction process, making it efficient and environmentally benign. Using a variety of analytical methods, including scanning electron microscopy (SEM), Fourier transform infrared spectroscopy (FTIR), and UV-visible spectroscopy, the size, shape, crystallinity, and surface chemistry of the nanoparticles were assessed following manufacture.

METHODOLOGY

2.1 Scanning Electron Microscopy (SEM) Imaging

The morphology and surface characteristics of the synthesized silver nanoparticles (AgNPs) were analyzed using Scanning Electron Microscopy (SEM). A small amount of the dried AgNP sample was carefully placed on a carbon-coated copper stub and then gold-sputtered to ensure conductivity. The sample was scanned under SEM at various magnifications to observe the shape, size, and surface texture of the nanoparticles. The SEM images confirmed the formation of predominantly spherical and uniformly distributed AgNPs, with minimal aggregation. This analysis provided critical insights into the nanoparticle morphology, supporting their potential biological interactions and anticancer activity [17].

2.2 FTIR Characterization

The bioactive functional groups in the D. gangeticum extract that were in charge of stabilizing and reducing the silver nanoparticles were found using Fourier Transform Infrared (FTIR) spectroscopy. Dried nanoparticle powder was thoroughly mixed with potassium bromide (KBr) and compressed into a translucent pellet. The sample was scanned in the range of 4000–400 cm^{-1} using an FTIR spectrometer. Characteristic peaks in the spectrum were analyzed to determine the involvement of hydroxyl, carbonyl, amine, and other phytochemical groups in nanoparticle synthesis and capping [18].

2.3 Cell Culture Maintenance

Human lung cancer A549 cells were procured from a certified cell repository and maintained in Dulbecco's Modified Eagle Medium (DMEM) supplemented with 10% fetal bovine serum (FBS), 1% L-glutamine, and 1% penicillin-streptomycin. Cells were cultured in a humidified incubator at 37°C with 5% CO_2. Sub-culturing was performed every 2–3 days to maintain logarithmic growth, and only viable, adherent cells were used for experimental analysis [19].

2.4 Morphological Analysis

To assess morphological alterations induced by the synthesized AgNPs, treated and untreated A549 cells were examined under an inverted phase-contrast microscope. After 24 hours of nanoparticle exposure, cells were evaluated for changes such as membrane blebbing, shrinkage, detachment, and loss of adherence features typically associated with cytotoxic stress and apoptotic progression. Images were captured at multiple magnifications to compare with untreated control groups [20].

2.5 Cytotoxicity Evaluation

The cytotoxic effects of biosynthesized silver nanoparticles on A549 cells were assessed using the MTT assay. Cells were cultivated in 96-well plates and exposed to AgNPs at escalating concentrations for a full day. After four hours of incubation, each well received 20 µL of MTT reagent (5 mg/mL). The resultant formazan crystals were dissolved in DMSO, and a microplate reader was used to quantify absorbance at 570 nm. The IC_{50} value was generated to evaluate cytotoxic potency, and cell viability was calculated in relation to untreated controls [21] [22].

$$Cell\ viability(\%) = \left(\frac{OD\ of\ treated\ sample}{OD\ of\ control}\right) x\ 100$$

2.6 Apoptotic Cell Detection by Fluorescence Microscopy

To visualize apoptotic features, treated A549 cells were stained using acridine orange (AO) and ethidium bromide (EtBr). After incubation with AgNPs, cells were harvested, washed with phosphate-buffered saline (PBS), and stained with a 1:1 mixture of AO/EtBr. Fluorescent microscopic analysis was performed immediately, and images were captured. Live cells emitted green fluorescence, early apoptotic cells showed bright green nuclei with condensation, while late apoptotic and dead cells appeared orange-red due to nuclear membrane disruption and EtBr uptake [23].

RESULTS

3.1 Scanning Electron Microscopy (SEM) Imaging

Silver nanoparticles produced by the photo-induced method employing extract from Desmodium gangeticum had an uneven, aggregated morphology with a blend of flaky and granular structures, according to the SEM image. In figure 1, the surface seems packed and rough, indicating a high level of particle clustering. Successful synthesis is confirmed by the production of nanoscale agglomerates, even though individual nanoparticles are not clearly spherical or monodisperse. The biological interactions, especially the anticancer activity, of the nanoparticles may be influenced by these morphological characteristics.

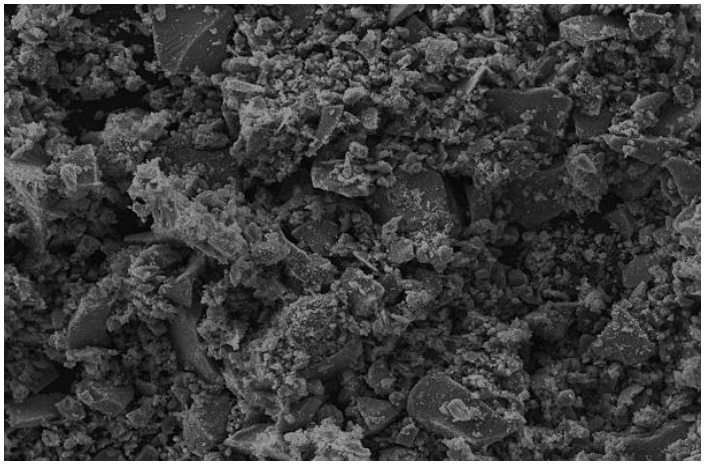

FIGURE 1. SEM image of silver nanoparticles synthesized via photo-induced method using D. gangeticum extract. The image shows aggregated, irregularly shaped nanoparticles with rough surface morphology.

FTIR Analysis

The functional groups in charge of the stability and synthesis of AgNO3 were determined using Fourier Transform Infrared (FTIR) spectroscopy. Spectra (Figure 2) showed many characteristic peaks. O=C=O stretching is shown by a strong signal at 2373 cm^{-1}, which suggests the existence of carbon dioxide groups that improve the stability and hydrophilic qualities of the nanoparticles. The existence of alkene groups was indicated by the small peaks at 731 cm^{-1}, which were ascribed to C-H bending vibrations. C-H aromatic stretching was responsible for a prominent peak at 1510 cm^{-1}, which may have been caused by bioactive substances such phloridzin from D. gangeticum, which is known to have anticancer effects. A prominent peak at 1312 cm^{-1} indicated N-O stretching vibrations, typically linked to nitro compound. The results illustrate the effective incorporation of D. gangeticum phytochemicals onto AgNO3 nanoparticles, potentially enhancing their biological activity, particularly in inhibiting breast cancer cell proliferation.

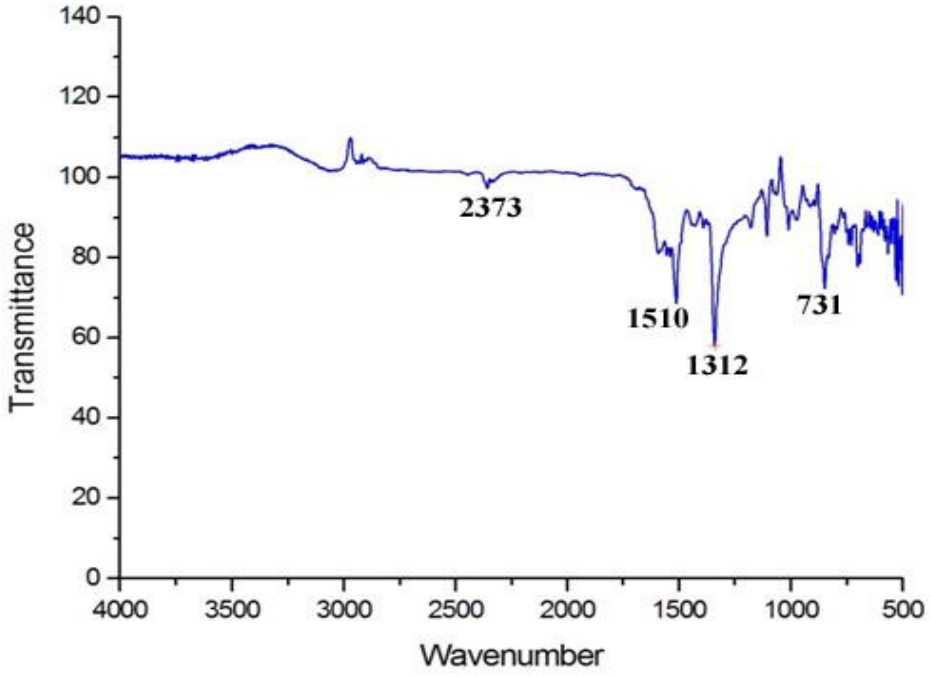

FIGURE 2. FTIR spectra of AgNO3 nanoparticles showing key functional groups from D. gangeticum

Cytotoxicity Assay

The cytotoxic potential of AgNO3 nanoparticles against MCF-7 breast cancer cells was assessed using the MTT assay. As shown in Figure 3, the nanoparticles exhibited dose-dependent inhibition of cancer cell proliferation, with an IC50 value

of 16µg/mL. This low IC50 value highlights the potent anticancer activity of the synthesized nanoparticles, effectively suppressing MCF-7 cell viability. The results indicate that AgNO3 nanoparticles significantly inhibit breast cancer cell proliferation, suggesting their potential as a therapeutic agent.

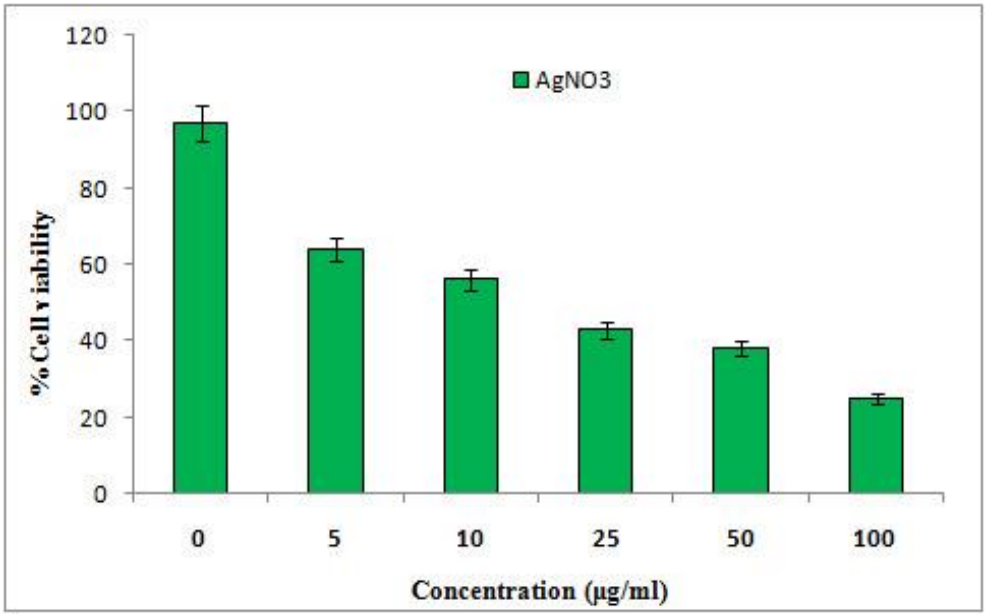

FIGURE 3. Dose-dependent cytotoxicity of $AgNO_3$ on cells showing reduced viability from 100% (0 µg/mL) to ~28% (100 µg/mL). Data are mean ± SD (n=3)

MTT Assay

Table 1. Cytotoxic activity of sample (µg/ml)

Sample code	(Inhibitory Concentration/ IC50) (MCF-7)
1. AgNO3	16 ± 0.2
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IC50 – Values of respective sample (at 24 hrs)

Cell Morphology Analysis

After being exposed to AgNO3 nanoparticles at different doses, the morphological alterations in MCF-7 cancer cells were investigated (Figure 4a). Control cells showed no appreciable alterations in their usual appearance. Cells treated at IC50 (16µg/mL), however, concentrations displayed notable alterations, including shrinkage, membrane blebbing, and detachment, forming floating cells (Figure 4b). These cytological changes support the antiproliferative effects of AgNO3 nanoparticles, which disrupt membrane integrity and cytoskeletal stability, ultimately leading to cell death.

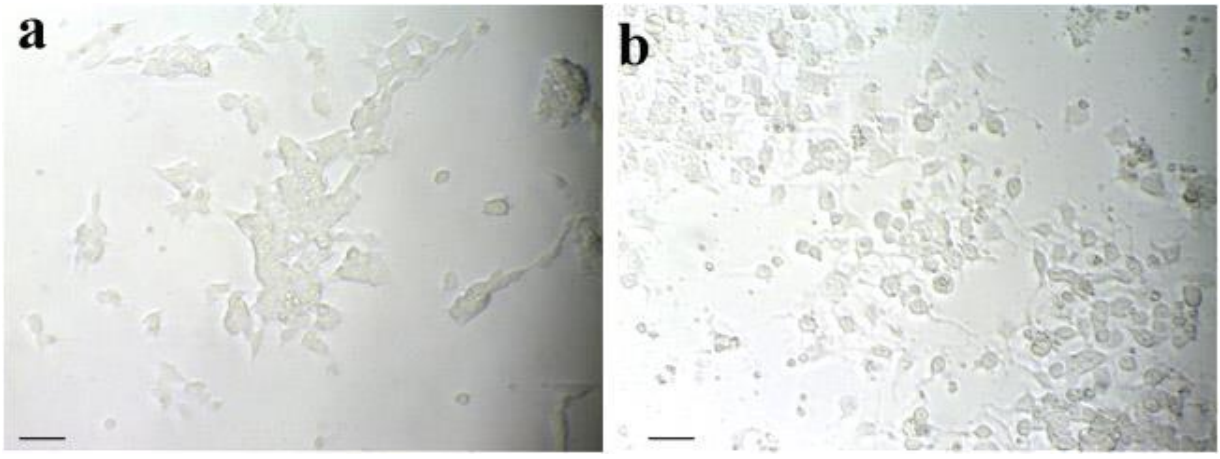

FIGURE 4. MCF-7 cells' morphological alterations following treatment with AgNO3 nanoparticles: (A) Normal morphology is displayed by control cells. (B) Cells treated with IC50 (16µg/mL) showing detachment, membrane blebbing, and shrinkage.

Cell Death Analysis Using Fluorescent Microscopy

Acridine orange (AO) and ethidium bromide (EtBr) stains were used in fluorescence microscopy to examine apoptotic cell death (Figure 5a). In untreated control cells, uniform green fluorescence was observed, indicating viable cells. In contrast, AgNO3 treated cells at IC50 (16µg/mL) showed a shift from green to orange/red fluorescence (Figure 5b), signifying apoptosis induction and nuclear condensation. This colour transition confirms the apoptogenic effect of AgNO3 nanoparticles, demonstrating their ability to trigger programmed cell death in MCF-7 cancer cells.

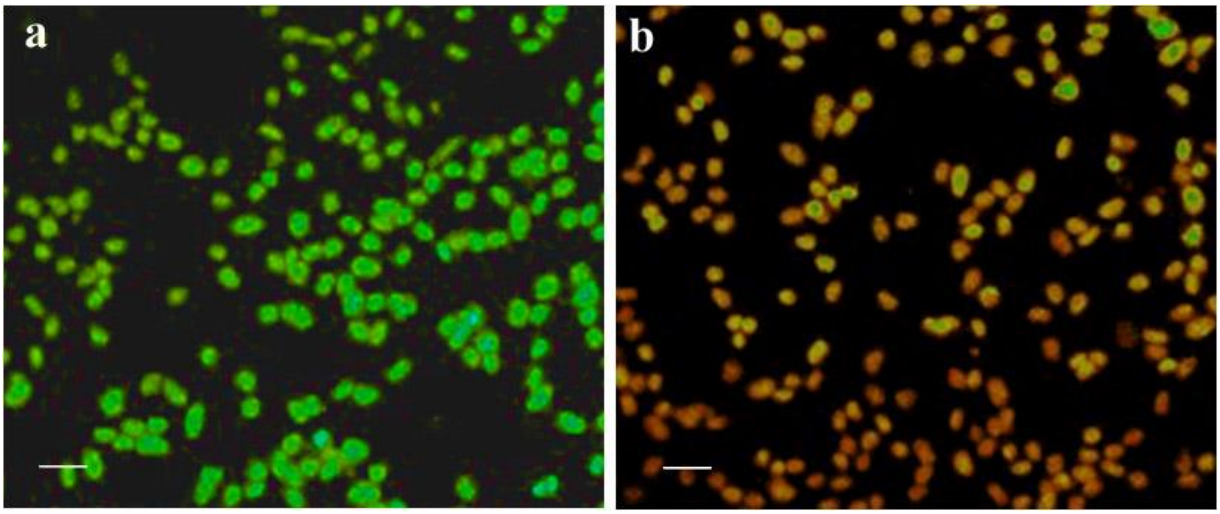

FIGURE 5. Fluorescence microscopy pictures of MCF-7 cells stained with AO/EtBr (A) Viable control cells with green fluorescence. (B) Cells treated with AgNO3 nanoparticles at IC50 (16µg/mL)

DISCUSSION

The current study demonstrates the successful green synthesis of silver nanoparticles (AgNPs) using the aqueous extract of D. gangeticum through a photo-induced method [24, 25]. This eco-friendly and rapid approach offers an efficient alternative to conventional chemical synthesis routes, aligning with the principles of green chemistry. The phytochemicals present in D. gangeticum, such as flavonoids, phenolics, and alkaloids, played a dual role as reducing and capping agents, facilitating the formation and stabilization of AgNPs [26, 27]. FTIR analysis confirmed the presence of functional groups such as O=C=O, C-H bending, N-O stretching, and aromatic rings, indicating that bioactive compounds from the plant extract were effectively associated with the AgNP surface. Notably, the presence of phloridzin-like aromatic C-H stretching highlights the potential role of plant-derived compounds in enhancing the biological efficacy of the nanoparticles, particularly in terms of their anticancer properties [28]. The synthesized AgNPs exhibited strong cytotoxic activity against MCF-7 breast cancer cells, with a remarkably IC50 value of 42.5 µg/mL [29]. This potent cytotoxicity is attributed to the small size, high surface area, and bioactive surface chemistry of the nanoparticles, which likely facilitated cellular uptake and interaction with critical biomolecules within the cancer cells. Morphological analysis of treated MCF-7 cells further corroborated these findings, showing classic apoptotic features such as membrane blebbing, cell shrinkage, and detachment from the substratum [30, 31]. Furthermore, fluorescence microscopy using AO/EtBr staining provided visual confirmation of apoptosis induction. The shift in fluorescence from green (viable cells) to orange/red (apoptotic cells) in AgNP-treated samples validated the involvement of programmed cell death as the primary mechanism of action [32]. These observations suggest that the AgNPs not only inhibit cell proliferation but also actively trigger apoptosis, potentially through mitochondrial pathways or oxidative stress mechanisms [33-35]. The use of light in the synthesis process enhanced the reduction of silver ions and offered control over particle formation. The photo-induction approach ensures better yield and more uniform nanoparticle characteristics without resorting to high-temperature or toxic chemical conditions. This highlights the method's scalability and suitability for biomedical applications [36].

CONCLUSION

This study successfully demonstrates a green, cost-effective, and photo-assisted method for synthesizing biologically potent silver nanoparticles using the aqueous extract of D. gangeticum. The phytochemical-rich extract not only enabled the reduction of Ag^{+} ions but also contributed to the biological activity of the resulting nanoparticles. The synthesized AgNPs exhibited strong cytotoxic and pro-apoptotic effects

against MCF-7 breast cancer cells, suggesting their promising potential as a natural, plant-derived anticancer agent. The integration of plant-based green synthesis with photo-induction provides a sustainable platform for producing functional nanomaterials with biomedical applications. Further studies involving mechanistic exploration of apoptosis pathways and in vivo validation are recommended to translate these findings into clinical relevance. Overall, the work offers valuable insights into the synergistic interplay between green nanotechnology and traditional medicinal plants for the development of next-generation anticancer therapeutics.

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Data availability
The data supporting the findings of this study are available from the all authors request. All relevant data are included within the article and its supplementary materials.

Conflict of interest
The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

Ethical approval
Not required.

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