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Trends in Multidisciplinary Research
2026, Vol. 1, No. 1, pp. 6–10
DOI: 10.54646/TMDR.2026.02
www.tmdr.co.in

REVIEW

Green synthesis of Fe and Zn nanoparticles with anticancer activity

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Received: 26 December 2025; **Accepted:** 05 January 2026; **Published:** 24 January 2026

Background: The conventional production of nanoparticles is based on the use of toxic chemicals, which restricts their safety and clinical use. Green synthesis is an alternative, biomedical, and sustainable method that utilizes plant extracts for biocompatibility.

Materials and methods: Nanoparticles were prepared under alkaline conditions and studied using UV-VIS, XRD, FTIR, FE-SEM, EDX, and zeta potential, whereby the crystal structure, surface chemistry, morphology, elemental composition, and stability were determined. MTT assay was done in the cancer cell lines to determine the dose-dependent antiproliferative activity.

Results: Fe nanoparticles proved to be the most efficient anticancer with a 50 concentration (IC 50) of about 40 μg/which leads to a high decrease in cancer cell viability. The cytotoxicity of FeZn nanoparticles was moderate (IC 50 ≈164 μg/mL), but Zn nanoparticles were of lesser activity.

Conclusion: Fe and Fe Zn nanoparticles synthesized in a green way using Calotropis procera exhibited good anticancer properties. This study supports the importance of plant-based production as environmentally friendly and scalable ways to achieve the production of innovative nanoparticle-based cancer therapeutics.

Keywords: *Calotropis procera*, green synthesis, Fe–Zn nanoparticles, cytotoxicity, MTT assay, anticancer activity

Introduction

Medical research is widely expanding, and nanoparticles have proved to be effective when incorporated with other pharmaceutical compounds. Nanoparticle production involves robust chemical processes, which are actually harmful to the ecosystem. Hence environment environment-friendly alternative approaches of synthesizing nanoparticles from plants and microorganisms are on the rise (1).

Even though microorganisms are also used for green synthesis of nanoparticles, plant-based synthesis increases the rate of synthesis and is more biocompatible. The microstructure of the materials easily penetrates blood vessels, tissues, and cell junctions, thereby creating and enhancing the overall therapeutic efficacy of a drug (2). Various metals like silver, gold, zinc (Zn), and iron (Fe) are used in the phytochemical nanoparticles. Among these, Zn and Fe have been widely studied by many researchers due to their stability and pharmacological properties, such as anticancer activities (3). These nanoparticles are well reported in various studies, which are linked to the reduced cytotoxicity of cancer cells by reducing the ROS(reactive oxygen species) generation and also increasing the apoptosis, and thereby reducing the DNA damage (4).

Calotropis procera (C procera) is a wild plant species from the Apocynaceae family, found from West Africa to Southeast Asia. It is often referred to as milkweed because of the unique white, sticky latex that oozes from all parts of the plant, making it a fascinating topic for phytochemical research. Literature indicates that C. procera contains active phytoconstituents like cardiac glycosides, flavonoids, and triterpenes, which suggest its potential biological activities (5).

In this regard, the current study aims to prepare Fe, Zn, and Fe-Zn nanoparticles through a plant-based method using C. procera leaf extract and to estimate their anticancer efficacy.

Materials and methods

The Fresh Calotropis procera leaves were meticulously washed, air-dried, and subsequently blended to produce an aqueous extract. After that, separate solutions of iron (III) chloride and zinc acetate were made and added to the extract at an alkaline pH. We carefully adjusted the reaction conditions to make it easier for Fe, Zn, and Fe-Zn nanoparticles to form and grow.

Methods for characterization

Using UV–Visible spectroscopy, we found that CP-FeNPs had a distinct absorption band at about 250nm (**Figure 1**), CP-ZnNPs had a wide absorption band at about 370nm (**Figure 2**), which is consistent with the formation of ZnO, and CP-Fe-ZnNPs had combination peaks of absorption between 310 and 320nm (**Figure 3**). The X-ray diffraction patterns showed that CP-Zn NPs had exact wurtzite-phase ZnO reflections, while CP-FeNPs and CP-Fe-Zn NPs had wider diffraction signals, which means that the material is either very amorphous or not well crystallized.

Fourier transform infrared spectroscopy detected the characteristic vibrational bands of hydroxyl (O–H), carbonyl (C=O), and ether (C–O) functional groups within the nanoparticles. Field-emission scanning electron microscopy revealed the morphological distinctions among the Fe, Zn, and Fe-Zn nanoparticles.

We used the MTT assay to test the nanoparticles' ability to kill cancer cells in vitro. To find out how toxic the cells were, dose-dependent IC50 values were calculated.

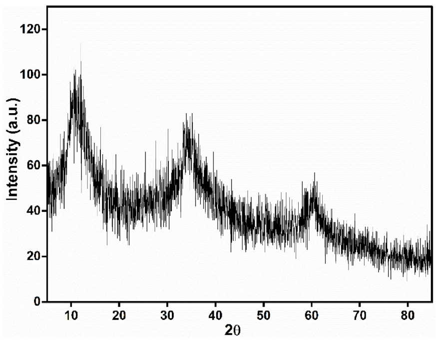

FIGURE 1 | UV–Visible Absorption Spectrum of CP-FeNPs.

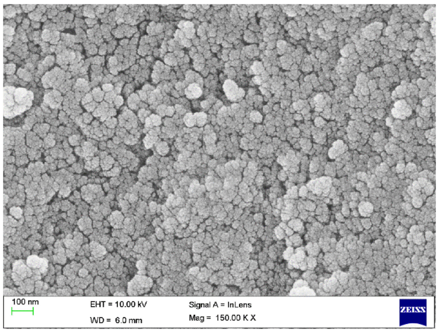

FIGURE 2 | UV–Visible Absorption Spectrum of CP-ZnNPs.

Findings

UV-visible spectroscopy

The synthesis of iron, zinc, and bimetal nanoparticles was confirmed by UV-visible spectroscopy. Fe had peaks at 250 nm, and Zn had peaks at about 370 nm.

X-ray diffraction (XRD) examination

The X-ray diffraction analysis showed the crystalline features of the nanoparticles. There were peaks in different diffraction patterns (**Figure 4**). Zinc nanoparticles had sharp peaks. The ZNO had a wurtzite structure (**Figures 5** and **6**).

FTIR

FTIR showed that the nanoparticles had hydroxyl (–OH), carbonyl (C=O), and ether (C–O) functional groups.

Field-emission scanning electron microscopy (FESEM)

FESEM analysis showed Fe nanoparticles as irregular clusters aggregated together with rough surfaces. Zn

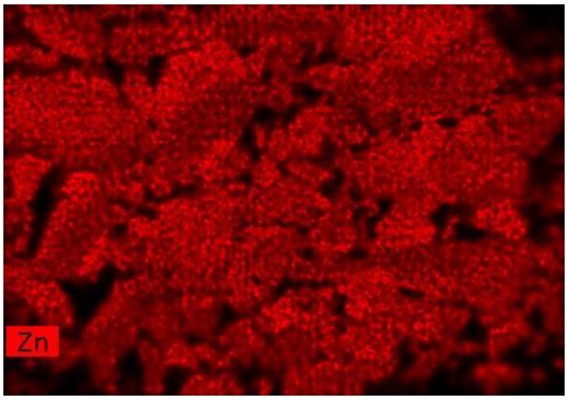

FIGURE 3 | UV–Visible Absorption Spectrum of CP-Fe-ZnNPs.

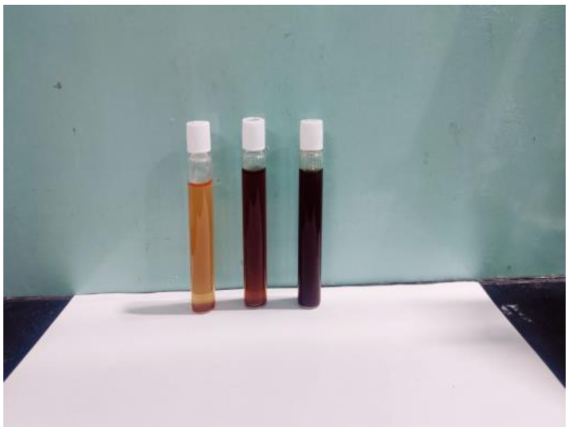

FIGURE 4 | X-Ray Diffraction Pattern of CP-FeNPs.

nanoparticles showed a quasi-spherical morphology. Fe-Zn showed mixed features.

In vitro cytotoxicity (MTT assay)

The MTT assay demonstrated cytotoxic activity with Fe nanoparticles exhibiting an IC50 value of 40.07 μg/mL. Fe–Zn nanoparticles showed cytotoxic activity with an IC50 value of approximately 164 μg/mL. Zn nanoparticles exhibited comparatively lower cytotoxic activity under the experimental conditions.

Discussion

Nanotechnology uses the technology to produce small materials having interatomic characteristics in structure with a size range of 1–100 nm. Their characteristics are unique to them that which serves as a connection between the materials used and their molecular structures. Hence, they are widely used in drug discovery and identification and in various other approaches (6, 7).

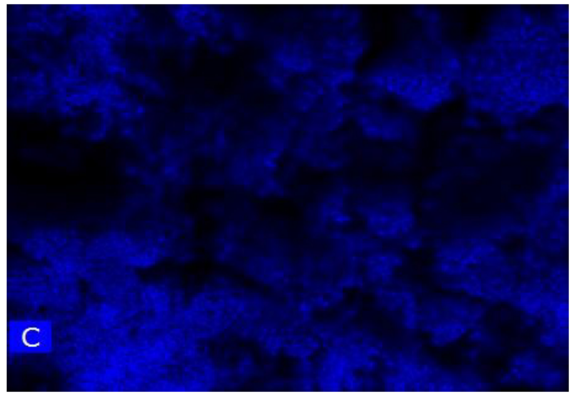

FIGURE 5 | X-Ray Diffraction Pattern of CP-ZnNPs.

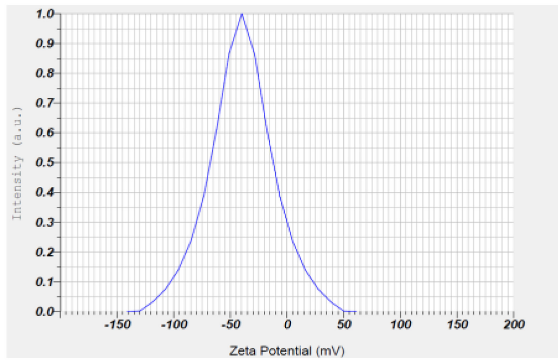

FIGURE 6 | X-Ray Diffraction Pattern of CP-Fe-ZnNPs.

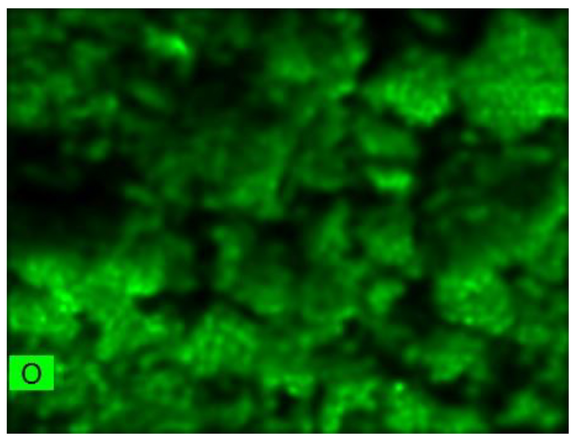

FIGURE 7 | XRD pattern highlighting crystalline ZnO phase in CP-ZnNPs.

UV visible spectroscopy measured the absorption peaks to confirm the synthesis of nanoparticles. Fe nanoparticles had an absorption at around 250nm. Zn nanoparticles had an absorption band at around 370nm. The phytochemicals produced by C procera showed the combined absorption properties of Fe-Zn nanoparticles. This confirms the efficient production of the bimetals and also the stabilisation (8, 9).

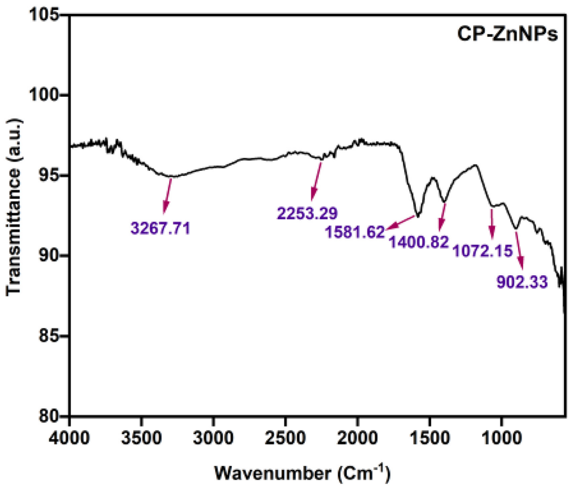

FIGURE 8 | Comparative XRD pattern showing amorphous characteristics of CP-FeNPs and CP-Fe-ZnNPs.

Xray diffraction analysis showed differences in the nature of the particles. Zn showed sharp and defined peaks, whereas ZnO exhibited the hexagonal structure. This confirms the crystallinity of the nanoparticle. Whereas Fe and Fe-Zn nanoparticles didn't show sharp peaks but broadened patterns, which reflect their poor crystalline structures. Whenever green synthesis is used, there is a common peak broadening because of phytochemical capping which limits the growth of the crystals (10).

Our product of nanoparticles from C procera showed restricted crystallite growth and has stabilized the amorphous state of the nanoparticles. The low crystalline nature increases the reactivity of the surface in the nanoparticles causing it to be more available for further attachment (**Figures 7** and **8**). Similar type of growth of nanoparticles is seen in various other studies indicating their bio availability for anticancer potential (10, 11).

FTIR data revealed that there are hydroxyl, carbonyl, and ether functional group features on nanoparticle surfaces, which are the result of plant-phytochemicals, natural reducing and capping agents. These biomimetic surface coatings increase the stability of nanoparticles, reduce aggregation and increase biocompatibility, and maximize their biomedical relevant uses (7, 10).

Analysis by FESEM showed that there were well-defined morphological differences between the nanoparticles. Fe nanoparticles were observed to be irregularly shaped, aggregated, and rough; Zn nanoparticles were observed to be quasi-spherical, and relatively discrete in their distribution; and Fe-Zn nanoparticles were observed to have mixed morphologies, consisting of zinc-like globular structures within an iron matrix. The agglomeration witnessed is typical of green-synthesized nanoparticles and is due to surface capping by organic materials and not synthetic inefficiency (8, 12).

The MTT test showed considerable cytotoxicity, with Fe nanoparticles showing the lowest 50 centimetres of concentration (IC 50) (40.07 μg/mL of Zn), which resulted in the highest anticancer activity compared with the Fe-Zn nanoparticles. The increased activity of Fe nanoparticles can be linked to oxidative stress caused by reactive oxygen species, which results in apoptosis (13–15).

This research paper indicates that the green-synthesized Fe and Fe-Zn nanoparticles have potential applications in the field of anticancer. The results support the idea of plant-mediated synthesis as a green approach to increasing nanoparticles' bioactivity and promote the idea of future mechanistic and in vivo studies to enable the effect of brain to green nanotechnology-based cancer treatment.

Conflict of interest

The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

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