

Article

Hematite Nanoparticles Synthesized by Green Route: Characterization, Anticancer and Antioxidant Activities

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Abstract: Recently, attention has shifted towards the green synthesis of nanoparticles using plant extracts rich in phytochemicals like phenols and flavonoids, offering an alternative method that avoids harmful chemicals and enables large-scale, low-cost production. This study introduces a straightforward and eco-friendly approach to synthesizing hematite α - Fe_2O_3 nanoparticles utilizing an aqueous extract of *Musa paradisiaca*. The variation in the calcination temperature resulted in the formation of nanoparticles presented as Fe_2O_3 (1), Fe_2O_3 (2), and Fe_2O_3 (3), obtained at 650, 750, and 900 °C for 4 h, respectively. This variation allowed for an investigation into the impact of different reaction temperatures on the structural and optical properties of the nanoparticles. Structural analysis was conducted using X-ray diffraction (XRD), while scanning electron microscopy (SEM) and transmission electron microscopy (TEM) were employed to examine morphology. Optical properties were assessed via UV-vis spectroscopy, revealing a reduction in the energy band gap (from 2.5 to 1.87 eV), attributed to an increase in crystallite size resulting from longer calcination temperatures (650–900 °C). A biological assay was carried out to evaluate the antioxidant and anticancer potentials of the nanoparticles. Both Fe_2O_3 (1) and Fe_2O_3 (2) with IC_{50} values of 46.84 and 46.14 $\mu\text{g/mL}$, respectively, showed similar antioxidant potentials, while peel extract exhibited the least activity with an IC_{50} of 79.26 $\mu\text{g/mL}$. The nanoparticles, peels, and 5-FU (used as standard) showed a stronger inhibitory effect on the Human Embryonic Kidney (HEK) 293 cells compared to the HeLa cells. This implies that the HEK 293 cells might be more susceptible to the drug samples and a lower concentration might even be sufficient to achieve the inhibition of normal cell proliferation. These results indicate a better therapeutic window with a lesser inhibitory effect compared to standard drugs used as controls.

Keywords: hematite; green synthesis; biological assay; anticancer; antioxidant; morphology



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1. Introduction

In recent years, the use of different parts of plants, such as leaves, fruits, seeds, bark, and peels, in the synthesis of nanoparticles (known as biogenic, green, or phyto-mediated synthesis) has continued to grow and attract research interests. This interest is spurred by the several advantages that green synthesis has over physical and chemical

processes. For example, using natural materials eliminates the need for harmful chemicals and harsh reaction conditions [1,2]. The biochemical approach offers flexible benefits over the chemical approach, including lower cytotoxicity, eco-friendly nature, reduced environmental footprint during synthesis, and minimal or no harmful waste production [3]. Therefore, it has emerged as a good alternative for the preparation of nanomaterials, specifically when they are meant for environmental or biomedical use. Several applications are made possible by the exceptional features of metal oxide nanoparticles, including ZnO, TiO₂, MgO, and CuO. In different processes, including oxidation, reduction, and photocatalysis, they operate as catalysts or catalyst supports [4]. For example, metal oxide nanoparticles are widely used in environmental remediation to purify water by breaking down organic contaminants and removing heavy metals. TiO₂ nanoparticles are particularly used for the photocatalytic destruction of contaminants [5]. They are also useful in biological applications for antibacterial coatings, imaging, drug delivery, and biosensing because of their biocompatibility and reactivity [6]. Moreover, their employment in gas sensors, biosensors, and chemical sensors is supported by their sensitivity to gases and biomolecules [7]. Additionally, their unique optical and electrical properties make them suitable for optoelectronics and photovoltaics, including solar cells, LEDs, and smart windows [8].

Due to the synthesis conditions, the major challenge associated with biogenic synthesis is to prepare nanoparticles with well-defined morphology [9,10]. This is critical because, within the nanometric domain, both the shape and size of nanoparticles are important. A reduction in particle size with an increase in mixing speed has been reported for iron nanoparticles prepared using an extract of *Prunus avium stem* [11]. Similarly, the extract of *Hibiscus rosa sinensis* flower has been used to produce size-reduced iron oxide nanoparticles [12].

Metal oxide nanoparticles have benefited immensely from green synthesis compared to other nanomaterial compounds since they do not suffer corrosion and are relatively stable in the air. Iron oxide is one of the important metal oxides that is widely studied due to its magnetic properties, biocompatibility, and easy surface functionalization [13,14]. It exists in different forms. It has different forms, including magnetite, maghemite, hematite, and wurtzite [15]. Due to its magnetic and electronic properties, iron oxide nanoparticles obtained from *Abutilon indicum* leaf extract have been reported to potentially serve as a therapeutic agent for a wide range of human diseases [16]. The notable biomedical properties of green-synthesized iron oxide nanoparticles using *A. indicum* and *Nicotiana glauca* leaf extract and their promising applications in the field of medicine have been reported [16,17]. Magnetite (Fe₃O₄), maghemite (γ-Fe₂O₃), and hematite (α-Fe₂O₃) are the main forms that are widely studied due to their fascinating features and unique physicochemical properties [15,18]. These properties are further improved when iron oxides are reduced to the nanoscale due to quantum size phenomena, thus resulting in particles with outstanding chemical stability, optical, magnetic, structural, and electrical properties [19,20].

Several studies have described the advancement of iron oxide nanoparticles in a wide range of applications such as catalysis [21], magnetic recording media [22], lithium-ion batteries [23], and biomedical [24,25]. The use of iron oxide nanoparticles for biomedical applications has garnered a lot of interest specifically in the fields of antimicrobial [26,27], anticancer [28,29], antidiabetic [30], antitumor [31], antioxidant [32,33], and wound-dressing applications [34,35]. For the broad biomedical application of these nanoparticles, crystal size control and shape modifications are very important because these features define the chemical and physical properties of the nanoparticles [36].

The phenomenal advancement of nanotechnology in recent years has ushered in a new approach to the treatment of diseases such as tumors. Studies show that cancer claims more lives than the combined statistics of death from malaria, tuberculosis, and AIDS [37]. Hence, the growing research activities on the use of nanoparticles to overcome these challenges offer unique alternatives to the treatment process. Nanoparticles attack cancerous cells and exhibit a higher degree of harm compared to normal cells. Their cytotoxic effects are associated with the reactive oxygen species (ROS), activation of caspase-3, permeabilization of the mitochondrial outer membrane, and cleavage of DNA [38,39]. Apoptosis can be triggered through internal or extrinsic pathways, and nanomaterials can induce apoptosis through various mechanisms [40]. Similarly, nanoparticles possess good antioxidant properties, and this is attributed to their increased surface-to-volume ratio [41,42]. Antioxidants are compounds that inhibit the oxidation of molecules, playing a crucial role in safeguarding cells from harm caused by unstable free radicals in biological contexts. They disrupt the chain reactions initiated by free radicals, halting their oxidation process. By doing so, antioxidants prevent oxidative damage, lowering the risk of diseases, cancer, and aging [43,44].

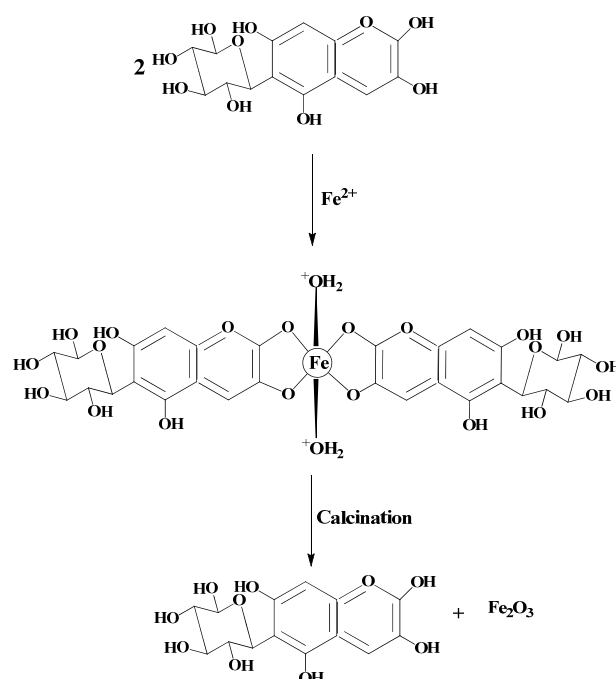
Musa paradisiaca, commonly known as plantain, is an herbaceous plant that is extensively cultivated in Southeast Asia and the western Pacific regions. The inflorescence of the plant, also known as the flower cluster, is a staple vegetable in the southern parts of Asia, particularly in regions of southern India, Malaysia, and Taiwan, and is also widely used in various African countries. In traditional medicine, plantain has been used to treat dysentery, menorrhagia, and diabetes [45]. According to Arun et al. [46], plantain is considered to be a potent antidiabetic plant. Plantain inflorescence was also reported to be a potential source of phytochemicals with promising health benefits. The qualitative phytochemical analysis of *Musa paradisiaca* extract confirmed the presence of biomolecules such as flavonoids, phenols, tannins, quinones, flavonoids, saponins, and phytosterols [47].

The objective of the current study is to examine the potential and efficacy of Fe_2O_3 NPs synthesized using an extract of plantain peels as an anticancer and antioxidant agent. While the green synthesis of Fe_2O_3 nanoparticles (NPs) is indeed a well-explored area, our study introduces a novel approach utilizing a unique plant extract that has not been reported previously for Fe_2O_3 NPs synthesis. Furthermore, we systematically correlate the synthesis conditions with the resulting morphology/size distribution and their impact on the anticancer and antioxidant efficiency, which has not been extensively addressed in previous reports. Thus, the importance of our study lies not only in the green synthesis of Fe_2O_3 NPs but also in providing a deeper understanding of how reaction temperature influences their functional properties, thereby contributing new insights to the design of sustainable nanomaterials for anticancer and antioxidant applications.

2. Results and Discussion

2.1. Synthesis Process

The synthesis process involves iron(III) salt (Fe^{3+}) as a precursor and plant extract as the source of $-\text{COOH}$, $-\text{OH}$, CHO , and CN . Sodium hydroxide solution helps drive the reaction in the forward direction. pH is directly related to the stability of nanoparticles. The change in pH can alter the double-layer properties that can directly influence the zeta potential of the system, because each type of nanoparticles is stable near the isoelectric point. In the current synthesis reaction, the ferric nitrate breaks down into Fe^{3+} and $(3\text{NO}_3)^-$, then the phyto-components present in the leaf extract of *Musa paradisiaca* induce the formation of Fe_2O_3 NPs [48]. The formation of the Fe_2O_3 nanoparticles proceeded according to the general mechanism given in Scheme 1 [49].



Scheme 1. Mechanism for the synthesis of α - Fe_2O_3 NPs using *Musa paradisiaca* leaf extract.

Similar to the formation process of metal oxides, thermal energy is needed to remove the water, oxidize the metal, and improve the crystallinity. The increase in crystallinity has been reported to increase the kinetic energy of the electrons and reduce the band gap energy [50,51]. Calcination temperature and the duration of the calcination process determine the amount of energy imparted on the nanoparticles. A higher calcination temperature and longer reaction time imply more thermal energy being absorbed by the nanoparticles. The elevated thermal energy causes Fe_2O_3 nanoparticles to lose their surface energy. Hence, thermal energy functions as a driving force, influencing the nanoparticle surface's interaction with adjacent particles.

From a thermodynamic perspective, when two particles come into contact, atomic transport occurs between them during calcination. The phenomenon is widely recognized as the Ostwald ripening process. These particles interact, reducing their boundary energy, and grain boundary migration takes place, moving from one particle to its neighboring particles through surface diffusion [52]. Throughout this process, one particle rotates towards another particle to align their crystal planes. Eventually, this process results in the formation of a new, larger particle. These new particles are denser than their initial counterparts [52]. Consequently, Fe_2O_3 nanoparticles subjected to higher calcination temperatures and longer reaction times exhibit increased X-ray density.

2.2. Microstructural and Morphological Properties

The purity and crystallinity of the obtained nanoparticles were ascertained by powder X-ray diffraction (XRD) measurement. Figure 1a presents the diffraction patterns of the nanoparticles at different calcination temperatures of 650, 750, and 900 °C, respectively. All the patterns show the diffraction peaks that correspond well to the (012), (104), (110), (113), (024), (116), (214), and (300) planes of the rhombohedral structure of α - Fe_2O_3 phase (JCPDS card No: 79-0007) [53]. Irrespective of the calcination temperature, they all belong to the same crystal structure and space group of R-3c. The lattice constants are $a = 5.0285 \text{ \AA}$ and $c = 13.7360 \text{ \AA}$ [54]. The sample calcined at temperatures below 650 °C showed poor crystallinity (Figure 1b) with no distinct peak observed. The (110), (111), and (221) peaks of α - $\text{FeO}(\text{OH})$ could be observed, indicating that this hydroxide is the intermediate product,

and at a temperature above 650 °C, the FeOOH is converted to α -Fe₂O₃ hematite phase. This implies that the samples calcined below 650 °C were amorphous with no crystalline growth. There are no peaks that are associated with other crystalline phases of Fe₂O₃, including the β -Fe₂O₃, ϵ -Fe₂O₃, and γ -Fe₂O₃, or oxides such as Fe₃O₄ (magnetite) and FeO(OH) in the diffraction patterns, indicating the high purity of the α -Fe₂O₃ nanoparticles.

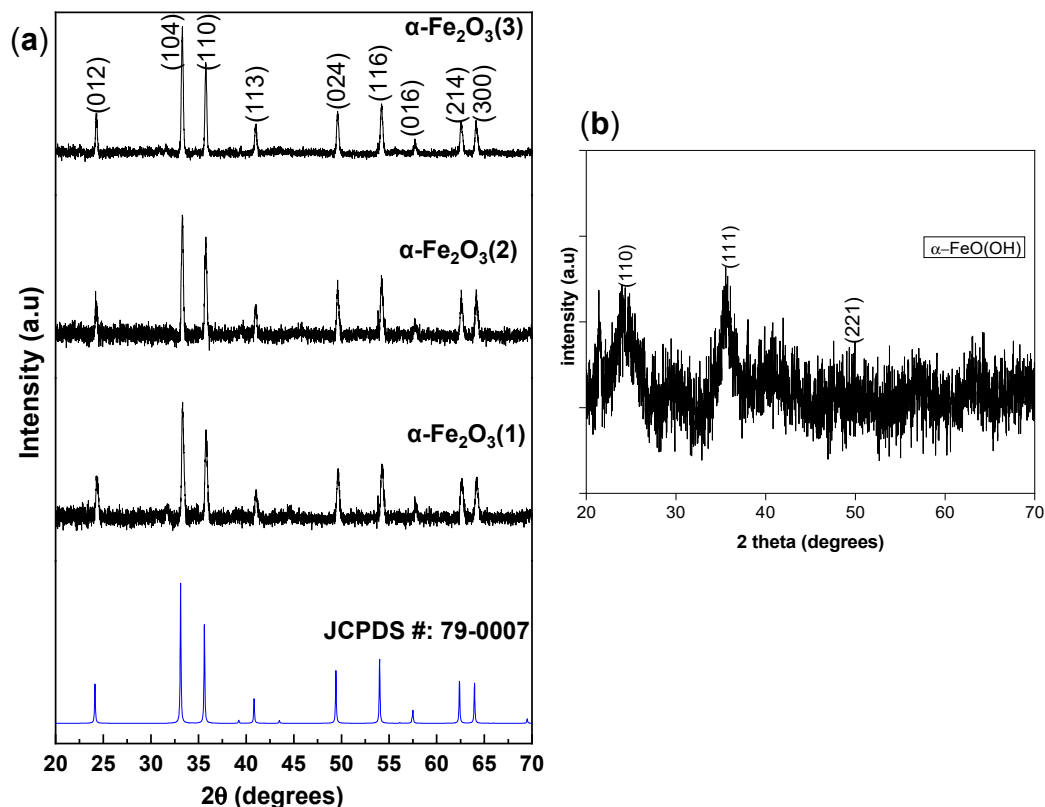


Figure 1. XRD pattern of the green synthesized (a) Fe₂O₃ (1), Fe₂O₃ (2), Fe₂O₃ (3), and (b) α -FeO(OH) NPs using extract from *Musa paradisiaca* peels.

The average crystallite size (*D*) of the Fe₂O₃ NPs was estimated using Scherrer's Equation (1) [55]:

$$D = \frac{K\lambda}{\beta \cos \theta} \quad (1)$$

Here, λ represents the wavelength of the X-ray (1.5406 Å), *K* is a shape factor represented as 0.9, θ is the diffraction angle in degrees, and β represents the full width at half maxima (FWHM) of the peaks of interest [43]. In the current study, the two most intense peaks of (104) and (110) were considered for the estimation of the crystallite size to give 25.20, 30.50, and 38.30 nm for the Fe₂O₃ (1), Fe₂O₃ (2), and Fe₂O₃ (3) NPs, respectively. This confirms that higher particle growth is associated with calcination time.

SEM was used to explore the morphological characteristics, and Figure 2a–c illustrates the morphology of the synthesized Fe₂O₃ (1), Fe₂O₃ (2), and Fe₂O₃ (3) samples, respectively. The figures reveal spherical shapes with some observable agglomeration of grains with irregular shapes as the calcination time increased. The formation of the powder seems to have occurred through the coalescence of grains, possibly accounting for the irregular spherical shapes and aggregated particles observed in the final grains of the samples calcinated at 900 °C. Similar observations are made in previous studies involving the hydrothermal synthesis of α -Fe₂O₃ [56,57].

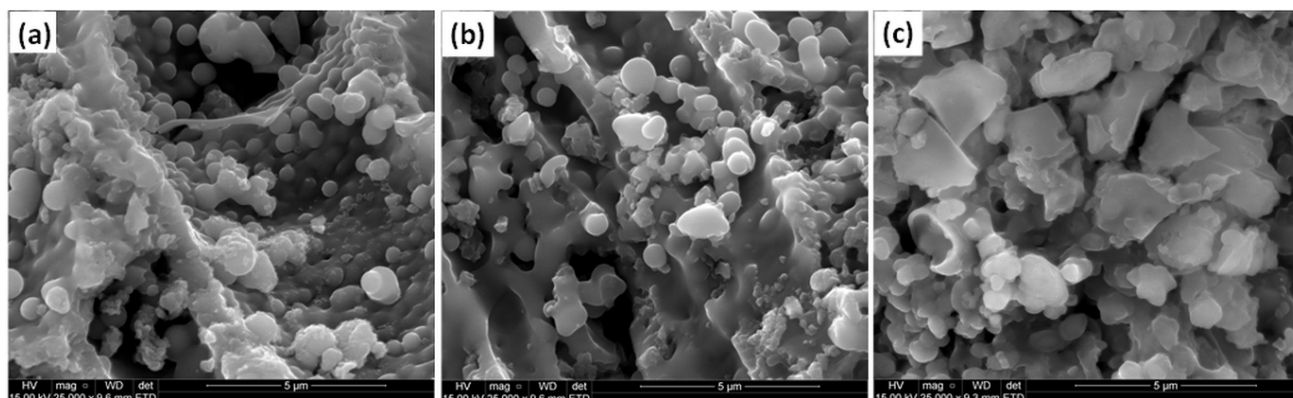


Figure 2. SEM images of the green synthesized (a) Fe_2O_3 (1), (b) Fe_2O_3 (2), (c) Fe_2O_3 (3), mediated with the extract of *Musa paradisiaca* peels.

TEM was further used to examine the particle size and morphology of the biosynthesized nanoparticles (Figure 3). A quasi-spherically shaped nanoparticles with an average diameter of 20.28, 28.10, and 36.33 nm could be observed in samples Fe_2O_3 (1), Fe_2O_3 (2), and Fe_2O_3 (3), respectively. Similar morphologies were earlier reported for single-crystalline hematite $\alpha\text{-Fe}_2\text{O}_3$ via the hydrothermal method [58,59]. Figure 4 shows the corresponding high-resolution TEM (HRTEM), and in the inset of Figure 4 is the fast Fourier transform (FFT), which shows it to be $\alpha\text{-Fe}_2\text{O}_3$. The distinct, well-defined crystalline patterns reveal two different interplanar spacings of 0.25 and 0.27 nm, corresponding to the (1 1 0) and (1 0 4) planes of $\alpha\text{-Fe}_2\text{O}_3$, respectively.

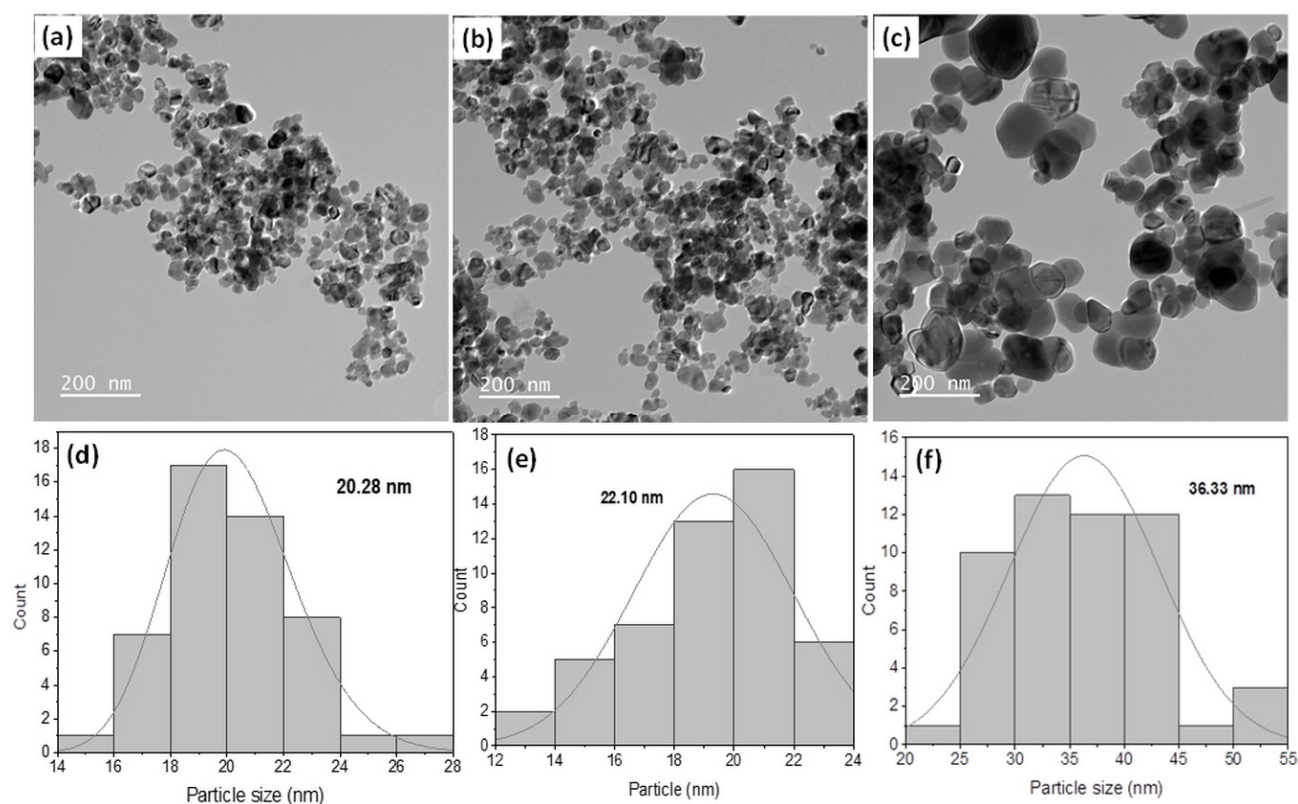


Figure 3. TEM images of the green synthesized (a) Fe_2O_3 (1), (b) Fe_2O_3 (2), (c) Fe_2O_3 (3), and their respective particle size distribution histogram (d–f).

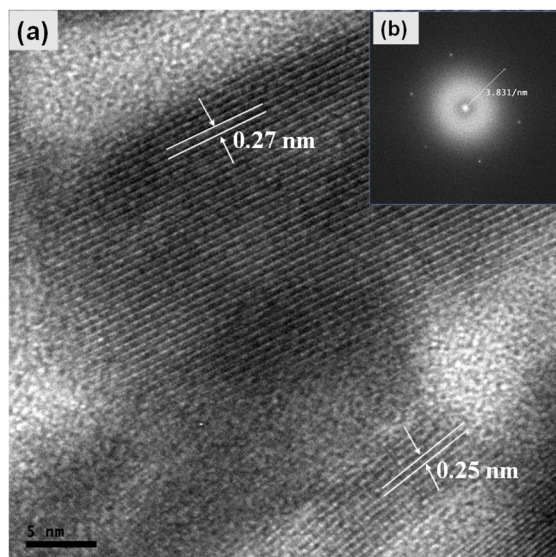


Figure 4. (a) HRTEM images and (b) Fast Fourier transform (FFT) patterns of Fe_2O_3 nanoparticles.

2.3. Optical Characterization

To explore the impact of calcination time on the optical characteristics of the Fe_2O_3 nanoparticles, UV-vis spectroscopy analysis was conducted at room temperature within the 200–900 nm range for all calcined samples (Figure 5). The optical band gap values for these samples, processed at various calcination times, were calculated from the absorption spectra utilizing the Tauc Equation (2) [60].

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

where h is Planck's constant (6.626×10^{-34} Js), n is an exponential factor for the electronic transition ($n = 1/2$, for indirect band, $n = 2$, for direct band), and α is the absorption coefficient.

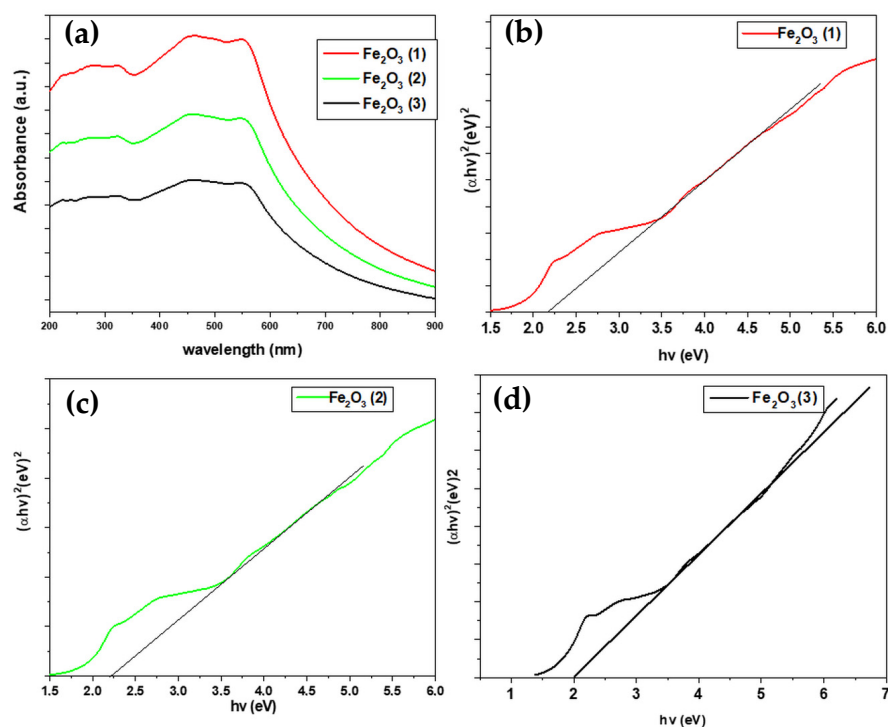


Figure 5. (a) overlapped UV-vis spectra of the green synthesized Fe_2O_3 (1), Fe_2O_3 (2), Fe_2O_3 (3), and their respective Tauc plot (b–d).

α -Fe₂O₃ is a direct band-gap-semiconducting material; hence, a plot of the values of $(\alpha h\nu)^2$ versus photon energy ($h\nu$) is presented in Figure 5b–d for Fe₂O₃ (1), Fe₂O₃ (2), and Fe₂O₃ (3), respectively. The optical band gap at different calcination times was deduced by the linear extrapolation of $(\alpha h\nu)^2$ versus $h\nu$ to the energy axis [61]. A decrease in the optical band gap from 2.5 eV in Fe₂O₃ (1) to 2.0 eV in Fe₂O₃ (3) was observed as the calcination temperature increased. This reduction in the energy band gap with temperature increase can be attributed to the increase in particle size and the improvement in crystallinity, as confirmed by XRD analysis. The rationale behind this phenomenon lies in the larger particle size, which leads to a higher number of atoms constituting a particle. Consequently, the valence and conduction electrons become more strongly attracted to the ions at the core of the particles, thereby causing a decrease in the band gap of the particle.

2.4. Antioxidant and Anticancer Studies

The antioxidant activity of green-synthesized Fe₂O₃ nanoparticles and peels of *Musa paradisiaca* was evaluated using the DPPH radical inhibition assay. The result of the percentage inhibition is presented in Table 1 and Figure 6. DPPH is a well-known nitrogen-centered radical with a deep purple color [62,63]. Antioxidants donate electrons or hydrogen atoms to the radical to neutralize the DPPH, forming a yellow-colored non-radical DPPH [62,64,65]. As observed in this study, the nanoparticles and peels demonstrated a concentration-dependent inhibition of DPPH radicals. The potency of the nanoparticles and extracts was measured based on the concentration of the sample that inhibited 50% of the radical (IC₅₀). Ascorbic acid, used as a standard, with an IC₅₀ value of 30.17 µg/mL showed the best antioxidant activity, while Fe₂O₃ (1) and Fe₂O₃ (2) with IC₅₀ values of 46.84 and 46.14 µg/mL, respectively, showed similar antioxidant potentials. The least antioxidant activity was observed for the peel extract, which had an IC₅₀ value of 79.26 µg/mL. Previous studies show that *Musa paradisiaca* peels are rich in extractable polyphenols and tannins that are effective as radical scavengers [66]. It was demonstrated that the peel is a good antioxidant, with its polar components contributing to the observed effect [67]. Overall observation in this study agrees with other studies on *Musa paradisiaca* peel as a potent antioxidant source [66,67]. Reports are only available for biosynthesized zinc oxide nanoparticles from plantain peel, and the results demonstrate the biological potential of the nanoparticles [68]. Antioxidants from plants are good reducing and stabilizing agents, able to enhance metal oxide nanoparticle synthesis from metal salts as precursors [69]. The high polyphenolic and tannins previously reported in *Musa paradisiaca* peel could favor the bio-fabrication of the iron oxide nanoparticles, with subsequent improved DPPH radical inhibition observed in this study.

Table 1. Antioxidant activity (%) of green synthesized Fe₂O₃ nanoparticles and plantain peel waste.

Test Samples	Sample Concentrations (µg/mL)						IC ₅₀ (µg/mL)
	1.56	3.13	6.25	12.5	25	50	
Ascorbic acid	4.62 ± 0.022	14.16 ± 0.030	28.95 ± 0.021	42.04 ± 0.020	55.51 ± 0.052	63.50 ± 0.044	30.17
Fe ₂ O ₃ (1)	4.82 ± 0.042	8.44 ± 0.023	17.48 ± 0.041	28.80 ± 0.025	36.56 ± 0.051	50.63 ± 0.310	46.84
Fe ₂ O ₃ (2)	3.95 ± 0.039	8.36 ± 0.074	16.63 ± 0.052	26.30 ± 0.055	35.80 ± 0.031	49.63 ± 0.640	46.14
Fe ₂ O ₃ (3)	4.11 ± 0.026	9.46 ± 0.033	18.43 ± 0.034	28.20 ± 0.055	38.49 ± 0.034	50.23 ± 0.065	47.04
Plantain peels	2.21 ± 0.018	3.34 ± 0.040	10.52 ± 0.024	18.29 ± 0.033	24.09 ± 0.074	30.11 ± 0.047	79.26

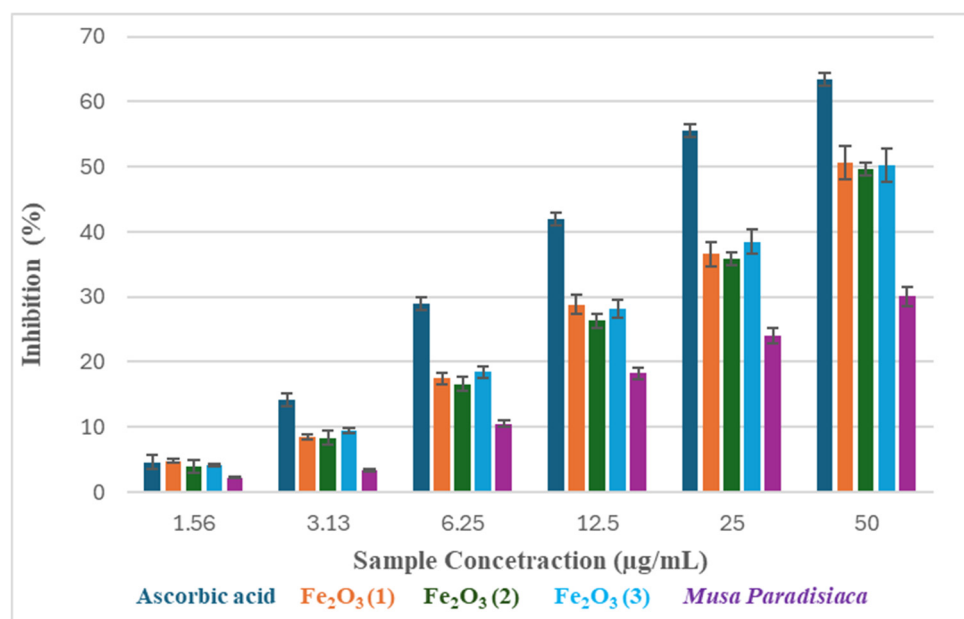
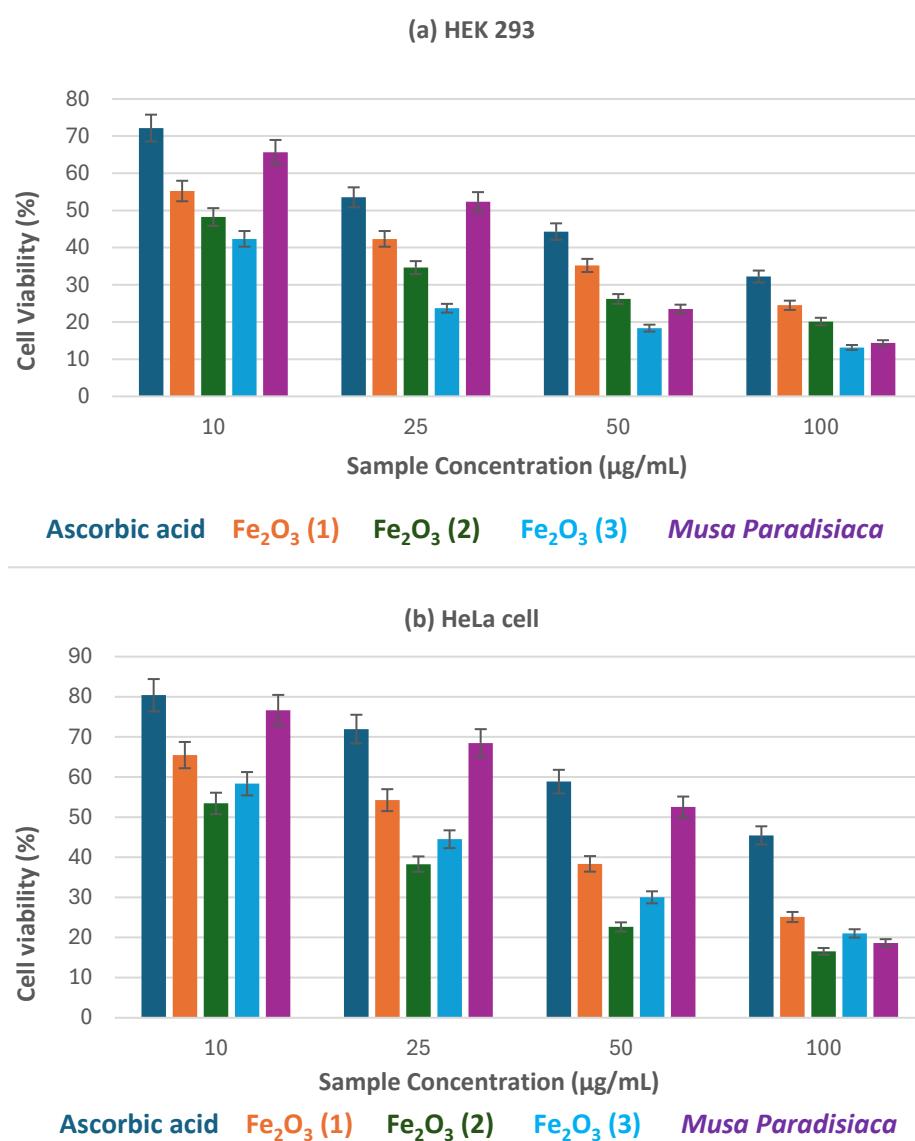


Figure 6. Antioxidant activity (IC₅₀) of green-synthesized Fe₂O₃ nanoparticles and Musa paradisiaca peel waste.

The results in Table 2 and Figure 7 provide the inhibitory efficacies of the iron oxide nanoparticles on cell growth and proliferation of HEK 293 (Human Embryonic Kidney cells) and HeLa (Cervical Cancer) cell lines. As observed in the result, variation in the IC₅₀ values across the samples and cell lines reflects their behavior with different cell lines. In addition, the results from the study show that the viability of the cell lines decreases with increasing concentration of the samples, indicating a concentration-dependent efficacy of the samples. General observation showed that the iron oxide nanoparticles, peels, and 5-FU might have a stronger inhibitory effect on the HEK 293 cells compared to the HeLa cells. By implication, the HEK 293 cells might be more susceptible to the drug samples, and a lower concentration might even be sufficient to achieve the inhibition of normal cell proliferation. Furthermore, Fe₂O₃ (2) and Fe₂O₃ (3), on the other hand, showed better efficacy against the HeLa cell line than HEK 293, implying that subtle variations in the preparation or composition of the nanoparticles can significantly influence the biological response, and consequently, the anticancer efficacy. Asimeng et al. [70] reported a concentration-dependent inhibition of HeLa cell proliferation after treatment with iron oxide nanoparticles synthesized from maize leaves. It was observed from the study that better anticancer efficacies were observed for particles with smaller sizes. The effectiveness of the smaller size particles against cancer cells could be attributed to the ability of the nanoparticles to penetrate pores more effectively due to their ability to go through the blood–brain barrier [71]. The same nanoparticles synthesized using Terminalia catappa showed similar inhibition of HEK 293 and HeLa cell lines with IC₅₀ values of 44.12 and 34.94 µg, respectively [72]. Overall observation in this study is the cytotoxic effects of the nanoparticles and *Musa paradisiaca* peel on the normal cell (HEK 293); however, modification of the bio-fabrication methods might result in better observation. Although nanoparticles are known for their various biomedical applications, toxicity to normal cell lines such as HEK-293 has been reported [73]. This toxicity is associated with nanoparticle size, dose, agglomeration, and the route of administration [73]. Based on the IC₅₀ values recorded in this study, it is clear that the nanoparticles and *Musa paradisiaca* peel extracts were more toxic to HEK-293 cells compared to HeLa cells, whereas the Fe₂O₃ demonstrated better selective toxicity by inhibiting the cancer cell line more than the normal cell line, thus indicating a better therapeutic window [59,69,74].

Table 2. Anticancer activity of green synthesized Fe₂O₃ (1), Fe₂O₃ (2), and Fe₂O₃ (3) NPs and plantain peel waste.

Cell Lines	Test Samples	Sample Concentration (µg/mL)				IC ₅₀ (µg/mL)
		10	25	50	100	
HEK 293	5-FU	72.14 ± 0.062	53.54 ± 0.033	44.30 ± 0.061	32.23 ± 0.054	47.64
	Fe ₂ O ₃ (1)	55.23 ± 0.041	42.34 ± 0.052	35.22 ± 0.062	24.51 ± 0.055	11.93
	Fe ₂ O ₃ (2)	48.24 ± 0.014	34.64 ± 0.024	26.21 ± 0.061	20.10 ± 0.046	17.03
	Fe ₂ O ₃ (3)	42.34 ± 0.015	23.72 ± 0.056	18.35 ± 0.032	13.15 ± 0.054	48.40
	Plantain peel	65.65 ± 0.043	52.33 ± 0.023	23.52 ± 0.036	14.40 ± 0.060	26.68
HeLa	5-FU	80.40 ± 0.20	71.94 ± 0.060	58.85 ± 0.32	45.45 ± 0.040	83.39
	Fe ₂ O ₃ (1)	65.45 ± 0.012	54.24 ± 0.052	38.34 ± 0.041	25.11 ± 0.061	36.56
	Fe ₂ O ₃ (2)	53.43 ± 0.042	38.28 ± 0.032	22.64 ± 0.054	16.54 ± 0.015	0.84
	Fe ₂ O ₃ (3)	58.32 ± 0.062	44.51 ± 0.021	30.02 ± 0.015	21.02 ± 0.055	16.63
	Plantain peel	76.63 ± 0.032	68.47 ± 0.071	52.52 ± 0.028	18.62 ± 0.071	52.49

**Figure 7.** Anticancer activity (IC₅₀) of green synthesized Fe₂O₃ (1), Fe₂O₃ (2), and Fe₂O₃ (3) NPs and Musa paradisiaca peel waste on (a) HEK and (b) Hela Cell line.

Fe₂O₃ nanoparticles can cause antitumor effects in several ways, such as by producing reactive oxygen species (ROS), upsetting the cellular redox equilibrium, and causing mito-

chondrial malfunction, which in turn triggers apoptosis [75,76]. Furthermore, ferroptosis, a controlled type of cell death marked by iron-dependent lipid peroxidation, may be involved [77]. Given the intrinsic iron content of Fe_2O_3 nanoparticles, it is plausible that when cancer cells internalize them, intracellular iron levels rise, causing lipid ROS buildup and Fenton-type reactions that may lead to ferroptosis [78]. Ferroptosis may contribute to the initiation of oxidative stress and mitochondrial impairment, thereby activating apoptotic pathways [79,80].

3. Materials and Methods

3.1. Chemicals

High-purity (99%) ferric nitrate nonahydrate $\text{Fe}(\text{NO}_3)_3 \cdot 9 \text{H}_2\text{O}$ and sodium hydroxide (NaOH) were purchased from Merck, Riyadh, Saudi Arabia, and used as received. Ethanol was procured from ACE Chemicals, Riyadh, Saudi Arabia.

3.2. Collection and Preparation of Aqueous Plant Extract

Musa paradisiaca (plantain) was sourced locally from a farm and properly identified. The peels were removed, properly cleaned with water, and then chopped into smaller bits. An electric blender (TOSCANA U 2500 W 2-in-1 Electric Blender Multi-Function, China) was used to blend about 500 g in 1000 mL of distilled water. The obtained pulp was filtered via a muslin cloth and was kept for a few minutes to allow the settling of the sediments, and the supernatant obtained was then filtered through a Whatman No 1 filter paper with a pore size of 25 μm .

3.3. Synthesis of $\alpha\text{-Fe}_2\text{O}_3$ Nanoparticles

A 100 mL aqueous solution of ferric nitrate nonahydrate $\text{Fe}(\text{NO}_3)_3 \cdot 9 \text{H}_2\text{O}$ was heated to 80 $^\circ\text{C}$, and 50 mL of the extract of *Musa paradisiaca* was added dropwise. The pH of the solution was adjusted to 8 with a few drops of a solution of sodium hydroxide (NaOH) and stirred for 30 min. The product was poured into a Teflon-lined stainless-steel reactor and placed in a muffle furnace with the temperature maintained at 150 $^\circ\text{C}$ for 6 h [81]. The obtained product was centrifuged at 4300 rpm for 20 min, followed by thorough rinsing with distilled water and finally with ethanol. The crude precipitate was divided into 3 portions, which were transferred to different crucibles and calcinated at 650, 750, and 900 $^\circ\text{C}$ for 4 h to afford samples denoted as Fe_2O_3 (1), Fe_2O_3 (2), and Fe_2O_3 (3).

3.4. Characterization

All sample characterizations were carried out at room temperature and ambient conditions. No control over pressure or humidity was necessary except if stated otherwise. Ultraviolet-visible spectroscopy (UV-Vis, Merck Prove 300, by Merck KGaA, Darmstadt, Germany) was used for optical characteristics, X-ray diffraction (a Bruker D8 Advance X-ray diffractometer, from Bruker AXS GmbH, Karlsruhe, Germany) was used for crystallographic information and phase information. The external morphology was studied using a field emission scanning electron microscope (FESEM, FEI Inspect F50 produced in Eindhoven, The Netherlands) and transmission electron microscope (TEM, FEI Tecnai G2 20 S-Twin produced in Eindhoven, The Netherlands) for internal morphology, high-resolution imaging, and selected area diffraction.

3.5. Biological Activity

3.5.1. Antioxidant Activity Measurement Using DPPH Scavenging Assay

The antioxidant activity of nanoparticles has been extensively measured using the DPPH scavenging assay [82,83]. Scavenging of (1,1-diphenyl-2-picrylhydrazyle) (DPPH)

was utilized to assess the free radical scavenging activity of the green synthesized Fe_2O_3 nanoparticles. Six different concentrations (1.56–50 $\mu\text{g}/\text{mL}$) of the nanoparticles were prepared and to these different concentrations was added 1 mL of 0.1 mM DPPH in 100 percent ethanol. The solutions were analyzed after a 30 min incubation period at room temperature in the dark. The measurement was conducted by sampling 250 μL of each solution into a 96-well microplate in triplicates and measuring the absorbance at 517 nm in comparison to a blank. Ascorbic acid and plantain peels were used as positive and negative controls. The percentage of free radical scavenging activity of the DPPH was estimated using Equation (3) [84]:

$$\text{DPPH scavenging activity (\%)} = \frac{A_o - A_t}{A_o} \quad (3)$$

where A_o represents the absorbance of the control at 30 min and A_t is the absorbance of Fe_2O_3 (1), Fe_2O_3 (2), and Fe_2O_3 (3).

3.5.2. Cytotoxicity Activity Measurement Using MTT Assay of the Nanoparticles

Human embryonic kidney 293 (HEK 293) and human cervical carcinoma (HeLa) cells were obtained from the ATCC in Manassas, USA. These cell lines were cultured in 25 cm^2 tissue culture flasks using EMEM (Lonza BioWhittaker, Verviers, Belgium) supplemented with 10% fetal bovine serum, 100 U/mL penicillin, and 100 mg/mL streptomycin. To assess cell viability in HeLa and HEK 293 cell lines, the MTT assay was conducted in 96-well plates, seeding 2.5×10^2 cells per well in 100 mL of EMEM. The cells were incubated at 37 °C overnight, and the media were replaced. Subsequently, samples at different concentrations (10, 25, 50, and 100 μg) were added. After another overnight incubation at 37 °C, the medium was replaced, and the samples were added again at varying concentrations. The prepared solutions were then incubated for 48 h at 37 °C, followed by the MTT assay. Additionally, 5-fluorouracil (5FU) was used as a standard alongside untreated cells. In the assay, 50% of the MTT reagent (5 mg/mL in PBS) was replaced and incubated for 4 h at 37 °C. Afterwards, 100 mL of DMSO was used to dissolve the insoluble formazan crystals, and the absorbance was measured at 570 nm using a Mindray MR-96A microplate reader (Vacutec, Hamburg, Germany), with DMSO as the blank. The entire test was performed in triplicate.

4. Conclusions

Pure single-phase hematite $\alpha\text{-Fe}_2\text{O}_3$ nanoparticles were successfully synthesized by an environmentally friendly route using the waste peels of *Musa paradisiaca*. The calcination temperature was varied, and the goal of determining the optimal conditions for obtaining high-quality nanoparticles while conserving energy was achieved. Reaction temperature affected the structure, size, and optical properties of the nanoparticles. The particles' crystallinity was found to improve with calcination temperature. Furthermore, calcinating the powder at higher temperature facilitated boundary migration and the movement of one particle to its neighboring particles through surface diffusion impurity. Anticancer and antioxidant assays of the green synthesized nanoparticles showed that they display a concentration-dependent efficiency. Both Fe_2O_3 (1) and Fe_2O_3 (2) demonstrated comparable antioxidant potentials with IC_{50} values of 46.84 and 46.14 $\mu\text{g}/\text{mL}$, respectively, while the peel extract showed the lowest activity with an IC_{50} of 79.26 $\mu\text{g}/\text{mL}$. The nanoparticles, peels, and 5-FU (used as a standard) exhibited a stronger inhibitory effect on HEK 293 cells compared to HeLa cells. This suggests that HEK 293 cells may be more susceptible to the drug samples, and a lower concentration might suffice to inhibit normal cell proliferation, thus indicating a better therapeutic window.

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