

Green synthesis of iron nanoparticles using *Vitis vinifera*'s tannin and its application in antibacterial activity / apoptotic capacity versus cancer cells

Roslina Binti Jamil

Universiti Sains Malaysia

SUHASHNE SELVAM

suhashne6@gmail.com

Universiti Sains Malaysia

Balqis Sofea Abu Hisham

Universiti Sains Malaysia

Joo Shun Tan

Universiti Sains Malaysia

Danesh Thangeswaran

Universiti Sains Malaysia

Venugopal Balakrishnan

Universiti Sains Malaysia

Zhang Jin Ng

Universiti Sains Malaysia

M. Hazwan Hussin

Universiti Sains Malaysia

Nicolas Brosse

University of Lorraine: Universite de Lorraine

Thangamani Arumugam

Karpagam University

Pandian Bothi Raja

Universiti Sains Malaysia <https://orcid.org/0000-0003-4274-158X>

Research Article

Keywords: green synthesis, iron nanoparticles, *Vitis vinifera*'s tannin, antibacterial agent

Posted Date: January 5th, 2026

DOI: <https://doi.org/10.21203/rs.3.rs-8381309/v1>

Abstract

Biosynthesized iron nanoparticles (Fe-NPs) have emerged as sustainable alternatives to conventional nanomaterials, offering enhanced biocompatibility and multifunctionality. In this study, Fe-NPs were successfully synthesized using tannin-rich *Vitis vinifera* pomace extract, which acted as a natural reducing and stabilizing agent. Comprehensive characterization using UV-Vis spectroscopy, FTIR, TEM, FESEM, and EDX confirmed the formation of predominantly spherical Fe-NPs with particle sizes ranging from 3 to 95 nm. Phytochemical constituents, particularly hydroxyl and aromatic groups, were identified as key contributors to nanoparticle nucleation and stabilization. The synthesized Fe-NPs exhibited potent antibacterial activity, demonstrating higher efficacy against *Escherichia coli* than *Staphylococcus aureus*, with antibacterial potencies of 1425.88 AU/mL and 628.32 AU/mL, respectively. Additionally, cytotoxicity assessment using the MTT assay revealed a dose-dependent antiproliferative effect on HeLa cervical cancer cells, yielding an IC₅₀ value of 49.36 ppm. These findings highlight the potential application of *Vitis vinifera*-derived Fe-NPs as effective antimicrobial and anticancer agents for future biomedical and environmental use.

1. Introduction

Nanotechnology and the development of nanoparticles (NPs) have progressed rapidly in recent years, contributing significantly across various product sectors, including medicine, agriculture, and environmental science. Nanoparticles are extremely small materials, typically ranging from 1 to 100 nanometers (10^{-9} m) in size. They occupy the scale between molecular and macroscopic dimensions, allowing them to exhibit unique physicochemical properties. Despite their small size, NPs are structurally composed of three layers: (i) a surface layer, which can be functionalized with a wide array of molecules, metal ions, surfactants, or polymers; (ii) a shell layer that is chemically distinct from the core; and (iii) a central core, which defines the nanoparticle's composition and functionality [1, 2]. Nanoparticles can be synthesized from a broad range of materials, including metals, metal oxides, ceramics, and polymers.

Among all types, metal-based nanoparticles are extensively studied due to their tunable properties. Iron, in particular, is an essential trace element involved in critical biological processes such as oxygen transport, DNA synthesis, and enzymatic function [3]. Moreover, iron is the only naturally occurring element that possesses magnetic properties, making iron nanoparticles (Fe-NPs) highly versatile for various applications. These include environmental remediation, drug delivery, imaging, biosensing, and antimicrobial activity against pathogens like bacteria and fungi [4].

Fe-NPs can be synthesized through physical, chemical, and green (biological) approaches. Chemical methods commonly utilize potent reducing agents such as sodium borohydride (NaBH_4), sodium dodecyl sulfate ($\text{NaC}_{12}\text{H}_{25}\text{SO}_4$), and hydrazine hydrate ($\text{N}_2\text{H}_4\cdot\text{H}_2\text{O}$) [5]. While these techniques yield uniform and high-purity nanoparticles, they often generate hazardous by-products and require high energy input, contributing to environmental concerns. Physical methods such as pulsed laser ablation,

spark discharge, and gamma irradiation also produce high-quality nanoparticles, but demand sophisticated and expensive equipment. In contrast, green synthesis using plant extracts, bacteria, or fungi has emerged as a sustainable, cost-effective, and environmentally benign alternative [6].

Plant-based synthesis offers several advantages, including simplicity, biocompatibility, and scalability. Reports indicate that Fe-NPs synthesized using plant extracts exhibit excellent stability, are biocompatible, and are environmentally friendly [7]. *Vitis vinifera* L. (grape), a member of the Vitaceae family, is rich in natural compounds such as polyphenols, flavonoids, terpenoids, alkaloids, phenolic acids, and proteins that serve as reducing and stabilizing agents in nanoparticle synthesis [8, 9]. Among these compounds, tannins are particularly valuable. These biodegradable and non-toxic polyphenols are known for their ability to chelate metal ions and act as antioxidants [10]. Their ortho-dihydroxyl and galloyl groups facilitate the reduction of Fe^{3+} to Fe^{2+} and help stabilize the nanoparticles during synthesis [11]. Tannins are also known for their roles in plant defense, UV protection, and metabolic regulation, and have found applications in medicine, the food and beverage industry, tanning, water purification, and coatings [10, 11]. During Fe-NP biosynthesis, tannins act both as reducing and capping agents. Their phenolic groups donate electrons to reduce Fe^{3+} ions, while the hydroxyl moieties form stable complexes with the nanoparticles [7, 10].

In this context, the current study evaluates the synthesis of Fe-NPs using tannin-rich *Vitis vinifera* extract and investigates their antibacterial and anticancer activities. The antimicrobial efficacy of Fe-NPs has been extensively documented, with promising effects against both Gram-negative (*Escherichia coli*) and Gram-positive (*Staphylococcus aureus*) bacteria. The nanoscale size of Fe-NPs allows for effective penetration of microbial cell walls, leading to membrane disruption, oxidative stress, and eventual cell death [12]. Fe-NPs also exhibit photothermal properties, whereby they absorb light and convert it into heat for therapeutic applications. When directed at bacteria or cancer cells, this localized heating can selectively kill pathogens without damaging surrounding tissues.

The antibacterial activity of Fe-NPs in this study was evaluated using the agar well diffusion assay, a standard technique in microbiology for assessing the inhibitory effect of test compounds. The size of the inhibition zone surrounding the well reflects the potency of the nanoparticles [13]. Their effectiveness against *E. coli* and *S. aureus* was assessed to demonstrate their broad-spectrum antimicrobial potential.

Furthermore, Fe-NPs have shown potential as anticancer agents, primarily due to their ability to induce reactive oxygen species (ROS), damage mitochondrial function, and promote apoptosis in malignant cells. Cancer, one of the leading causes of global morbidity and mortality, arises from uncontrolled cell proliferation and impaired apoptosis. Recent studies suggest that Fe-NPs exhibit cytotoxic effects against various cancer cell lines, including HeLa cervical cancer cells, due to their oxidative and apoptotic effects in tumor microenvironments [13].

2. Materials and methods

2.1. Extraction of grape tannin from *Vitis vinifera* pomace

Tannins were extracted from *Vitis vinifera* pomace, which was obtained from crushed white grapes sourced from the Champagne region. The pomace was immersed in an aqueous solution containing 2.5% sodium carbonate (Na_2CO_3) and 2.5% sodium sulfite (Na_2SO_3) and incubated at 80°C for 4 hours. After the extraction process, the mixture was filtered, and the resulting extract was spray-dried to obtain a concentrated tannin-rich powder. This extract was subsequently utilized as a natural reducing agent for the synthesis of Fe-NPs [13].

2.2. Green synthesis of iron nanoparticles

A 25 mL tannin solution (1000 ppm) was heated at 80°C for 5 minutes and subsequently filtered twice using Whatman No. 1 filter paper to obtain a clear extract. An equal volume (25mL) of ferric chloride hexahydrate ($\text{FeCl}_3 \cdot 6\text{H}_2\text{O}$) solution (2000 ppm) (R&M, Malaysia) was then mixed with the tannin extract at a 1:1 ratio. The mixture was stirred at room temperature for 2 hours using a magnetic stirrer, followed by centrifugation at 6000 rpm for 20 minutes. The supernatant was discarded, and the resulting Fe-NPs collected from the pellet were used for further characterization.

2.3. Physicochemical and structural characterization of iron nanoparticles

2.3.1. Ultraviolet-Visible absorption spectroscopy

The preliminary confirmation of Fe-NP formation was conducted using a UV-2600 Shimadzu UV-Vis spectrophotometer following 2 hours of stirring. The absorbance spectra of the Fe-NP samples were recorded at regular time intervals from 0 minutes to 48 hours within the

wavelength range of 200–700 nm to identify characteristic surface plasmon resonance peaks indicative of nanoparticle synthesis.

2.3.2. Transmission electron microscope (TEM)

The morphological features, size, and distribution of the synthesized Fe-NPs were examined using a Hitachi HT7830 transmission electron microscope (TEM). A freshly prepared suspension of Fe-NPs was carefully dropped onto a lacey carbon film supported by a copper grid. The sample was then allowed to air-dry at room temperature to ensure even particle distribution. After drying, the grid was mounted onto the TEM stage, and high-resolution images were captured to visualize the nanoparticles. Particle size and shape were analyzed based on the obtained micrographs, providing insights into their nanoscale structural characteristics.

2.3.3. Field emission scanning electron microscopy (FE-SEM) coupled with energy dispersive X-ray (EDX)

The morphology of the synthesized nanoparticles was examined using a Hitachi Regulus 8220 series Field Emission Scanning Electron Microscope (FE-SEM). Prior to analysis, a small amount of the nanoparticle suspension was dropped onto a microscope slide covered with aluminum foil and dried in an oven at 50–60°C overnight. To address the presence of residual oily tannins, the dried sample was cleaned using a Zone SEM II carbon cleaner to ensure surface clarity. Elemental composition was confirmed using an Oxford Instruments 100 mm windowless Energy Dispersive X-ray (EDX) detector integrated with the FE-SEM system.

2.3.4. Fourier Transform Infrared (FT-IR) spectroscopy

FT-IR analysis was performed using a Perkin Elmer System 2000 FT-ATR spectrometer to investigate the functional groups involved in the synthesis of Fe-NPs. Spectral data were collected in the range of 4000 to 600 cm⁻¹. The ATR crystal was thoroughly cleaned with ethanol before each measurement to eliminate any potential contaminants. A small amount of each sample—tannin extract (before synthesis) and the synthesized Fe-NPs (after synthesis)—was carefully deposited onto the crystal surface for direct measurement. This analysis was conducted to detect functional groups such as hydroxyl (O–H), carbon–carbon double bonds (C = C), and metal–oxygen (Fe–O) vibrations. The spectral comparison between pre- and post-synthesis samples enabled identification of chemical shifts and reductions in band intensity, indicative of the functional groups' involvement in nanoparticle formation and stabilization.

2.4. Antibacterial activity assessment

The antibacterial activity of the synthesized Fe-NPs was evaluated against *E. coli* and *S. aureus*. All materials and equipment were sterilized prior to use, and procedures were conducted under aseptic conditions in a biosafety cabinet. Bacterial cultures were prepared by inoculating 1 mL of each bacterial suspension into 10 mL of Brain Heart Infusion (BHI) broth, followed by incubation at 37°C for 24 hours. After incubation, 100 µL of the overnight culture was diluted with 900 µL of sterile distilled water and mixed thoroughly. The agar well diffusion method was employed to evaluate the antimicrobial activity. Mueller-Hinton agar plates were swabbed uniformly with the diluted bacterial suspensions using sterile cotton swabs. Three wells were created in each plate and 100 µL of the Fe-NPs suspension was introduced into each well. The plates were incubated at 37°C for 24 hours. After incubation, zones of inhibition around each well were measured (in mm).

To quantify the antibacterial activity, the following equation was used:

$$\text{Antibacterial activity} = \frac{L_z - L_s}{V}$$

where L_z represents the clear zone area (mm²), L_s represents the well area (mm²) and V represents the volume of the sample (mL). Streptomycin was used as a positive control to validate the antibacterial assay.

2.5. Cytotoxicity test

The cytotoxic potential of the synthesized Fe-NPs was evaluated against the human cervical carcinoma (HeLa) cell line. The assay aimed to determine the 50% inhibitory concentration (IC_{50}) using the [3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide] (MTT) assay. MTT reagent (Sigma Aldrich, USA) was prepared at a final concentration of 5 mg/mL in phosphate-buffered saline (PBS). The solution was sonicated to ensure full dissolution, filtered through a 0.22 μ m membrane for sterilization, and stored at -20°C until use. The reagent remained stable for up to 6 months under these conditions. HeLa cells were cultured in a T75 flask containing complete medium composed of Dulbecco's Modified Eagle Medium (DMEM), supplemented with 15% fetal bovine serum (FBS) and 1% Penicillin-Streptomycin (PenStrep). Upon reaching full confluency, the culture medium was discarded, and cells were washed with PBS. To detach the cells, 1 mL of trypsin was added, followed by incubation at 37°C for 5 minutes. Gentle tapping was used to facilitate detachment. The trypsin was neutralized with 9 mL of complete medium.

The cell suspension was centrifuged at 1200 rpm for 8 minutes. The resulting cell pellet was resuspended in 1 mL of fresh complete medium. Cell counting was performed using the trypan blue exclusion method, mixing 20 μ L of cell suspension with 20 μ L of 0.4% trypan blue in a 1:1 ratio. Cells were seeded at a density of 10,000 cells per 100 μ L per well in a clear, flat-bottom 96-well plate. To prevent edge effects, the outermost wells were not used for treatment. The plate was incubated overnight at 37°C in a CO_2 incubator to allow for cell attachment. Following overnight incubation, the wells were examined to confirm at least 70% confluency. Serial dilutions of Fe-NPs were prepared in complete medium in separate microcentrifuge tubes. Each concentration was tested in six replicates by adding 100 μ L of the respective dilution into designated wells. The treated cells were incubated for 24 hours at 37°C . After treatment, 10 μ L of MTT solution was added to each well, and the plate was wrapped in aluminum foil to protect it from light. After 4 hours of incubation, the medium was carefully removed, and 100 μ L of DMSO was added to dissolve the formazan crystals. The plate was incubated for an additional 30 minutes, and absorbance was measured at 570 nm using a microplate reader. Cell viability was calculated by normalizing the absorbance values of treated samples to the untreated control. The IC_{50} value, representing the concentration of Fe-NPs that inhibited 50% of cell viability, was determined by plotting a dose-response curve using GraphPad Prism software.

3. Result and discussion

3.1. Synthesis of iron nanoparticles

Fe-NPs were successfully synthesized using tannin-rich leaf extract from *Vitis vinifera* as a natural reducing and stabilizing agent. The visual transformation from light brown to black (Fig. 1) confirmed the reduction of Fe^{3+} ions to elemental Fe^0 nanoparticles, attributed to surface plasmon resonance (SPR), a hallmark indicator of metallic nanoparticle formation [14]. Polyphenolic compounds present in tannins act as reducing agents, donating electrons to reduce Fe^{3+} to Fe^0 , while simultaneously stabilizing the nanoparticles by binding through hydroxyl and carbonyl groups [15]. This dual functionality supports the

green synthesis route as an effective, eco-friendly method for nanoparticle fabrication without the use of toxic chemicals or high-energy inputs.

3.2. Ultraviolet–Visible (UV-Vis) spectroscopic analysis

UV-Vis spectroscopy was utilized to confirm the formation and monitor the optical behavior of Fe-NPs synthesized using *Vitis vinifera* tannin extract. This technique is particularly valuable for nanoparticle characterization due to the distinctive surface plasmon resonance (SPR) exhibited by metal nanoparticles when exposed to light [16]. Absorbance spectra were recorded over a wavelength range of 200–700 nm at specific time intervals from 0 minutes to 48 hours to assess the progression of nanoparticle formation. As illustrated in Fig. 2, the UV-Vis spectra of the Fe-NP samples revealed a characteristic absorbance band within the 270–320 nm range, with a prominent peak centered around 287 nm. This peak corresponds to the SPR of Fe-NPs and serves as an optical indicator of successful Fe^{3+} reduction to Fe^0 . A time-dependent increase in absorbance was observed from 0 to 6 hours, indicating ongoing nucleation and growth of nanoparticles. The highest absorbance occurred at 2 hours, suggesting this as the point of maximum nanoparticle yield and dispersion.

Notably, the absorbance intensity plateaued and began to decline after 6 hours, with a marked decrease at 24 and 48 hours. This reduction is likely due to nanoparticle aggregation or sedimentation, which decreases the population of optically active, well-dispersed nanoparticles

[16]. Aggregated particles exhibit diminished SPR intensity due to reduced surface area and changes in electron oscillation behavior. Additionally, prolonged exposure to ambient conditions may induce surface oxidation, altering the electronic structure and further reducing absorbance. The estimated particle size, inferred from the SPR peak characteristics, is in the range of 10–90 nm. This estimation will be further confirmed and validated through high-resolution imaging and measurements obtained from TEM and FE-SEM.

3.3. Fourier transform infrared spectroscopy (FTIR) analysis

FTIR analysis was conducted to identify the functional groups in the tannin extract involved in the synthesis and stabilization of Fe-NPs. Figure 3 presents the comparative FTIR spectra of the pure tannin extract (red line) and the synthesized Fe-NPs (black line), with spectral ranges spanning from 4000 to 500 cm^{-1} . A broad and intense absorption band in the range of 3400–3200 cm^{-1} , observed in both spectra, is attributed to O–H stretching vibrations of hydrogen-bonded polyphenols. In the Fe-NPs spectrum, this band appears at 3404.78 cm^{-1} , indicating the presence of hydroxyl groups, which are essential in reducing Fe^{3+} to Fe^0 and stabilizing the nanoparticles through surface adsorption. The distinct peak at 1573.22 cm^{-1} in the Fe-NP spectrum corresponds to C = C stretching vibrations of aromatic rings, suggesting the involvement of aromatic polyphenols in the reduction process [17]. The presence of this peak, along with a band at 1406.64 cm^{-1} (C–C stretching), further supports the idea that these aromatic structures contribute electron density for Fe^{3+} reduction.

Additionally, a noticeable absorption band at 1120.62 cm^{-1} is assigned to C–H stretching vibrations in the presence of alkyl halides or polysaccharide structures, possibly originating from the plant matrix or tannin derivatives. Importantly, a weak but distinct band at 621.41 cm^{-1} is observed only in the Fe-NP spectrum and is attributed to Fe–O stretching vibrations, confirming the formation of iron oxide species such as Fe_2O_3 and Fe_3O_4 at the nanoparticle surface. This metal–oxygen vibration is a key indicator of the successful binding of iron ions into nanoparticulate form [7]. Compared to the original tannin spectrum, the decreased intensity and minor shifts in the O–H, C = C, and C–H bands after nanoparticle synthesis suggest that these functional groups were actively involved in the redox and capping processes. The interaction of hydroxyl and aromatic moieties with iron ions not only enabled reduction but also imparted colloidal stability by forming a capping layer around the nanoparticles. FTIR analysis confirms that the polyphenolic compounds in *Vitis vinifera* tannin acted as both reducing agents (via O–H and C = C functionalities) and stabilizers, forming strong Fe–O interactions that anchored the metal ions into nanoparticle structures [12].

3.4. Transmission electron microscopy (TEM)

Transmission Electron Microscopy (TEM) was employed to assess the morphological characteristics, particle size distribution, and surface features of the synthesized Fe-NPs [16]. The TEM micrograph in Fig. 4, captured at 200k magnification, clearly illustrates the successful formation of Fe-NPs. The nanoparticles appear predominantly spherical in shape, although some display slight variations toward ellipsoidal or irregular circular forms, likely due to natural variability during biosynthesis. As indicated in the image, individual nanoparticles exhibited diameters of approximately 34.5 nm and 31.4 nm, confirming the nanoscale dimensions and good dispersity of the particles. The overall particle size distribution, based on TEM analysis, ranged from 3 to 35 nm, which falls well within the standard nanomaterial range of 1–100 nm, supporting the efficiency of the green synthesis method using *Vitis vinifera* tannin extract.

3.5. *Field emission scanning electron microscopy (FESEM) and energy-dispersive x-ray (EDX)*

analysis

The morphology and elemental composition of the *Vitis vinifera* tannin-mediated Fe-NPs were further characterized using FESEM coupled with EDX analysis. As illustrated in Fig. 5, the FESEM micrograph obtained at 100,000× magnification revealed the presence of well-dispersed Fe- NPs with diverse morphologies. The particles predominantly exhibited spherical and near-circular shapes, with sizes ranging approximately from 20 to 95 nm. The high-resolution imaging confirmed the nanoscale nature and surface structure of the synthesized Fe NPs, suggesting effective reduction and capping by the polyphenolic components of the tannin extract. The associated EDX spectrum, also shown in Fig. 5, provides elemental confirmation of the nanoparticle composition. A prominent Fe peak in the range of 0.6–1.0 keV was observed, which corresponds to the characteristic energy region for iron and supports the presence of Fe as the major constituent. This peak may also reflect surface plasmon resonance

behavior, commonly seen in metallic nanoparticles [18]. Quantitative EDX analysis (Table 1) revealed that the Fe-NPs consisted primarily of 58.03% Fe, confirming efficient synthesis of iron-based nanostructures.

Table 1
Quantitative analysis of heavy metals in Fe-NPs

Elements	C	O	Cl	Fe
Weight %	6.43	34.17	1.37	58.03

Additional elements detected included 34.17% O, 6.43% C, and 1.37% Cl. The oxygen and carbon content are attributed to the organic matrix of the *Vitis vinifera* tannin extract, specifically the polyphenolic groups involved in the reduction and stabilization of nanoparticles. The chlorine detected likely originated from the iron precursor, FeCl₃·6H₂O, used during synthesis. These additional elements are expected in green synthesis routes and indicate the presence of bio-organic compounds adsorbed onto the nanoparticle surfaces. Overall, the SEM and EDX results confirm the successful biosynthesis of Fe-NPs with nanoscale dimensions, well-defined morphology, and a high iron content. The presence of bioorganic and precursor-derived elements further supports the involvement of plant-based functional groups in nanoparticle formation and surface stabilization.

3.6. Antibacterial activities

The antibacterial efficacy of the synthesized Fe-NPs was evaluated against two clinically relevant bacterial pathogens, *E. coli* and *S. aureus*, using the agar well diffusion method [19]. After 24 hours of incubation, clear inhibition zones were observed around wells containing Fe NPs, indicating their ability to suppress bacterial growth (Fig. 6a, b). Quantitative analysis of the inhibition zones showed that Fe-NPs exhibited stronger antibacterial activity against *E. coli* compared to *S. aureus*. The average inhibition activity was recorded at 1425.88 AU/mL for *E. coli* and 628.32 AU/mL for *S. aureus* (Table 2).

Table 2
Inhibitory activity of synthesize Fe-NPs and Streptomycin on *E. coli* and *S. aureus* by well diffusion assay

Pathogens	Average inhibitory activity (AU/mL)	
	Synthesize Fe-NPs	Streptomycin
<i>E. coli</i>	1425.88	5510.58
<i>S. aureus</i>	628.32	5222.90

This suggests that Gram-negative bacteria (*E. coli*) were more susceptible to Fe-NPs, possibly due to differences in cell wall structure that allow easier penetration or disruption by nanoparticles. When compared to the standard antibiotic streptomycin (Fig. 6c, d), Fe-NPs exhibited a lower inhibition potential. Streptomycin produced significantly larger zones of inhibition, with 5510.58 AU/mL for *E. coli*

and 5222.90 AU/mL for *S. aureus*. Nonetheless, the results clearly demonstrate that Fe-NPs possess notable antimicrobial activity, particularly against *E. coli*, validating their potential as an alternative antimicrobial agent. The inhibitory effect of Fe-NPs may be attributed to their small particle size and high surface area, which facilitate interaction with bacterial membranes, leading to oxidative stress, protein denaturation, or disruption of metabolic pathways [20]. These findings support the application of green-synthesized Fe-NPs in antibacterial formulations, although their activity remains lower than conventional antibiotics and may be further optimized through formulation strategies.

3.7. Cytotoxicity evaluation of Fe-NPs on HeLa cells

A cytotoxicity assay was performed to evaluate the potential anticancer activity of the synthesized Fe-NPs against human cervical cancer (HeLa) cells. The test aimed to determine the biocompatibility and therapeutic relevance of Fe-NPs derived from *Vitis vinifera* tannin extract. The results revealed a clear dose-dependent reduction in cell viability, indicating increasing cytotoxic effects with higher nanoparticle concentrations. As shown in Fig. 7, the percentage of viable HeLa cells decreased progressively with increasing Fe-NP concentrations on a logarithmic scale. The calculated IC_{50} value was 49.36 ppm, suggesting that at this concentration, 50% of the cancer cells were inhibited. This value reflects moderate cytotoxic potency and demonstrates that Fe-NPs have a measurable inhibitory effect on HeLa cell proliferation. The observed decline in cell viability can be attributed to possible mechanisms such as oxidative stress induction, mitochondrial dysfunction, and disruption of cellular membranes—common pathways through which metallic nanoparticles exert anticancer effects. The sigmoid shape of the dose–response curve further confirms the dose-dependent cytotoxic behavior of the Fe-NPs. These findings highlight the potential anticancer properties of green-synthesized Fe-NPs and suggest their possible application in cancer therapeutics, particularly in formulations targeting solid tumours such as cervical carcinoma [21]. However, further investigations, including apoptosis assays and in vivo studies, would be required to confirm the mechanism of action and clinical relevance.

4. Conclusion

This study demonstrated the successful green synthesis of Fe-NPs using tannin-rich extracts from *Vitis vinifera* pomace, highlighting an eco-friendly, biocompatible, and sustainable alternative to conventional chemical synthesis methods. The phytochemicals present in the extract, particularly polyphenolic compounds, played a pivotal role as both reducing and stabilizing agents during nanoparticle formation. Comprehensive characterization through UV Vis, FTIR, TEM, FESEM, and EDX confirmed the formation of predominantly spherical nanoparticles with sizes ranging from 3 to 95 nm and the presence of bioorganic functional groups on their surface. The synthesized Fe-NPs with a concentration of 2000 ppm exhibited potent antibacterial activity, with greater efficacy against *E. coli* than *S. aureus*, indicating their suitability as alternative antimicrobial agents. Moreover, cytotoxicity evaluation against HeLa cancer cells revealed a dose-dependent antiproliferative effect, with an IC_{50} value of 49.36 ppm, supporting their potential as anticancer agents. These dual bioactivities—antibacterial and anticancer—demonstrate the promising multifunctional applications of *Vitis vinifera*-derived Fe NPs in biomedical and pharmaceutical

fields. Further investigations, particularly in vivo studies and mechanistic analyses, are warranted to advance these nanoparticles toward clinical and therapeutic applications.

Declarations

Acknowledgment

The authors gratefully acknowledge the School of Chemical Sciences, the School of Industrial Technology, and INFORMM, Universiti Sains Malaysia, Gelugor, Penang, for their help and support.

Funding Declaration

The authors would like to acknowledge the Ministry of Higher Education Malaysia (MOHE) for funding the Fundamental Research Grant Scheme (FRGS) code: FRGS/1/2021/STG05/USM/02/4.

Conflict of Interests

The authors hereby declare that there is no conflict of interests regarding the publication of this research paper entitled “Green synthesis of iron nanoparticles using *Vitis vinifera*'s tannin and its application in antibacterial activity / apoptotic capacity versus cancer cells”.

Ethical Approval

Not Applicable.

Consent to Participate

Not Applicable.

Consent to Publish

Not Applicable.

Authors Contributions

Roslina Binti Jamil - Investigation, Analysing data, Writing-original draft

Suhashne A/p Selvam - Technical assistance

Balqis Sofea Abu Hisham - Technical assistance

Joo Shun Tan - Technical assistance and investigation in antimicrobial studies.

Danesh Thangeswaran - Technical assistance and investigation in anticancer studies.

Venugopal A/I Balakrishnan - Technical assistance and investigation in anticancer studies.

Zhang Jin Ng - Technical assistance and investigation in antimicrobial studies.

M. Hazwan Hussin - Technical assistance and investigation in tannin extraction and characterisation.

Nicolas Brosse - Technical assistance and investigation in tannin extraction and characterisation.

Thangamani Arumugam - Analysing data, writing and editing.

Pandian Bothi Raja - Funding acquisition, Methodology, Supervision, Validation, Writing- review & editing.

All authors read and approved the manuscript.

Availability of data and materials

The authors declare that the data supporting the findings of this study are available within this paper and any raw data files be needed in another format they are available from the corresponding author upon reasonable request.

References

1. Saif, S., Tahir, A., & Chen, Y. (2016). Green Synthesis of Iron Nanoparticles and Their Environmental Applications and Implications, 1–26. <https://doi.org/10.3390/nano6110209>
2. Khan, I., Saeed, K., & Khan, I. (2019). Properties, applications and toxicities. *Arabian Journal of Chemistry*, 12, 908–931. <https://doi.org/10.1016/j.arabjc.2017.05.011>
3. Abbaspour, N., Hurrell, R., & Kelishadi, R. (2014). Review on iron and its importance for human health, 3–11.
4. Kaur, K., & Sidhu, A. K. (2021). Green Synthesis: An Eco-friendly Route for the Synthesis of Iron Oxide Nanoparticles, 3 <https://doi.org/10.3389/fnano.2021.655062>
5. Xu, W., Yang, T., Liu, S., Du, L., Chen, Q., Li, X., & Dong, J. (2022). Insights into the Synthesis, types and application of iron Nanoparticles : The overlooked significance of environmental effects, 158 <https://doi.org/10.1016/j.envint.2021.106980>
6. Buarki, F., Abuhassan, H., Al Hannan, F., & Henari, F. Z. (2022). Green Synthesis of Iron Oxide Nanoparticles Using Hibiscus rosa sinensis Flowers and Their Antibacterial Activity, (2022).
7. Shigwenya, E., Patrick, M., Kareru, G., Gachui, E., Augustine, M., Nyabola, O., Indire, S., Jared, W., & Nyang, O. (2019). Biosynthesis of iron nanoparticles using Ageratum conyzoides extracts, their antimicrobial and photocatalytic activity, SN Applied Sciences <https://doi.org/10.1007/s42452-019-0511-7>
8. Makarov, V. V., Love, A. J., Sinitsyna, O. V., Makarova, S. S., & Yaminsky, I. V. (2014). *Green Nanotechnologies: Synthesis of Metal Nanoparticles Using Plants*, 6 35–44.
9. Prathiksha, Hegde, K., & Review on, A. (2022). *Vitis vinifera* L.: The Grape, 74 142–145. <https://doi.org/10.47583/ijpsrr.2022.v74i01.023>

10. Zorrilla, R. H. J. L. R. C. (2010). Tannin biosynthesis of iron oxide nanoparticles, 453–459. <https://doi.org/10.1007/s00339-010-5903-x>
11. Cuong, D. X., Hoan, N. X., & Dong, D. H. (2019). *We are IntechOpen, the world ' s leading publisher of Open Access books Built by scientists, for scientists TOP 1% Tannins*. Extraction from Plants.
12. Hashim, N., Paramasivam, M., Shun, J., Kernain, D., Hussin, M. H., Brosse, N., Gambier, F., & Bothi, P. (2020). Green mode synthesis of silver nanoparticles using *Vitis vinifera* ' s tannin and screening its antimicrobial activity / apoptotic potential versus cancer cells. *Materials Today Communications*, 25, 101511. <https://doi.org/10.1016/j.mtcomm.2020.101511>
13. Hossain, L., Lim, L. Y., Hammer, K., Hettiarachchi, D., & Locher, C. (2022). *A Review of Commonly Used Methodologies for Assessing*. the Antibacterial Activity of Honey and Honey Products.
14. Jadoun, S., Arif, R., Kumari, N., Rajesh, J., & Meena, K. (2020). Green synthesis of nanoparticles using plant extracts: a review. *Environmental Chemistry Letters*. <https://doi.org/10.1007/s10311-020-01074-x>
15. Durán, N., Marcato, P. D., Alves, O. L., De, G. I. H., & Esposito, E. (2005). Mechanistic aspects of biosynthesis of silver nanoparticles by several *Fusarium oxysporum* strains, 7 1–7. <https://doi.org/10.1186/1477-3155-3-8>
16. Malatesta, M. (2021). Transmission Electron Microscopy as a Powerful Tool to Investigate the Interaction of Nanoparticles with Subcellular Structures.
17. Ahmad, M., Ali, A., & Patel, R. (2021). Facile one-pot green synthesis of Ag e Fe bimetallic nanoparticles and their catalytic capability for 4- nitrophenol reduction. *Journal of Materials Research and Technology*, 12, 455–470. <https://doi.org/10.1016/j.jmrt.2021.02.063>
18. Yao, Y., Bhargava, A., & Robinson, R. D. (2021). Fe Cations Control the Plasmon Evolution in CuFeS 2 Nanocrystals, <https://doi.org/10.1021/acs.chemmater.0c03829>
19. Jin, Z., Abu, M., Keong, C., Phapugrangkul, P., & Shun, J. (2020). Archives of Oral Biology Isolation and characterization of *Enterococcus faecium* DSM 20477 with ability to secrete antimicrobial substance for the inhibition of oral pathogen *Streptococcus mutans* UKMCC 1019. *Archives of Oral Biology*, 110, 104617. <https://doi.org/10.1016/j.archoralbio.2019.104617>
20. Batir-marin, D., Boev, M., Cioanca, O., Lungu, I., Marin, G., Burlec, A. F., Mitran, A., Mircea, C., & Hancianu, M. (2025). Exploring Oxidative Stress Mechanisms of Nanoparticles Using Zebrafish (*Danio rerio*): Toxicological and Pharmaceutical Insights, 1–22.
21. Ying, S., Guan, Z., Ofoegbu, P. C., & Clubb, P. (2022). Environmental Technology & Innovation Green synthesis of nanoparticles: Current developments and limitations. *Environmental Technology & Innovation*, 26, 102336. <https://doi.org/10.1016/j.eti.2022.102336>

Figures

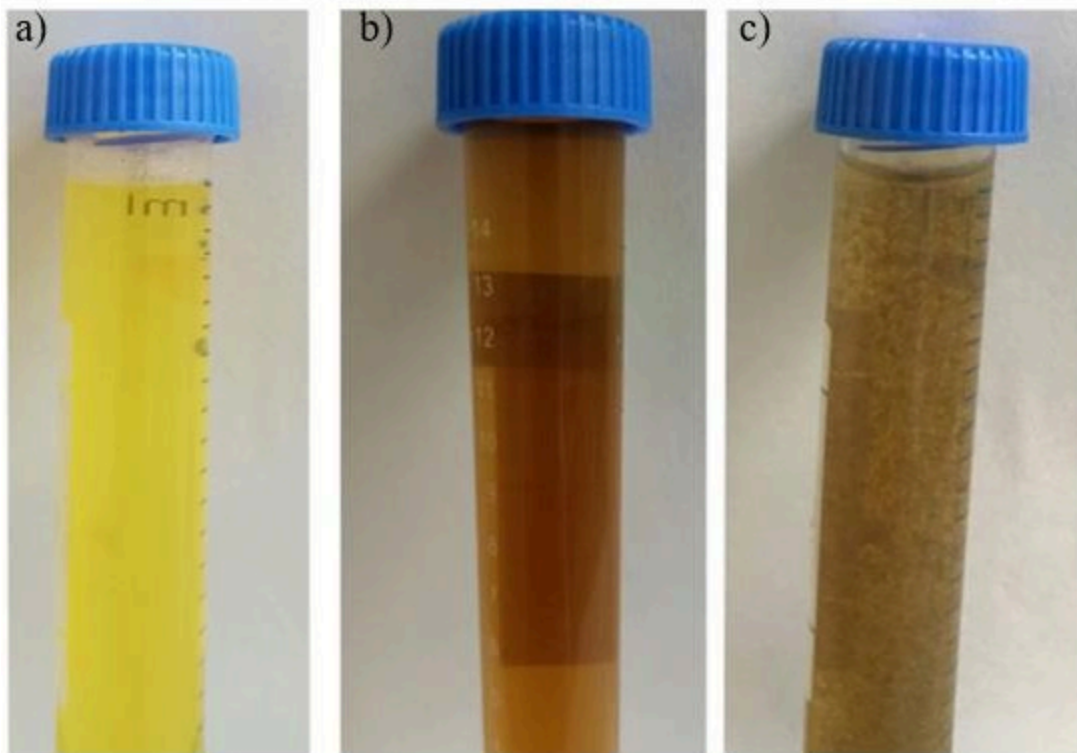


Figure 1

Visual representation of (a) iron chloride hydrate ($\text{FeCl}_3 \cdot 6\text{H}_2\text{O}$) (b) tannin leaf extract (c) Fe-NPs.

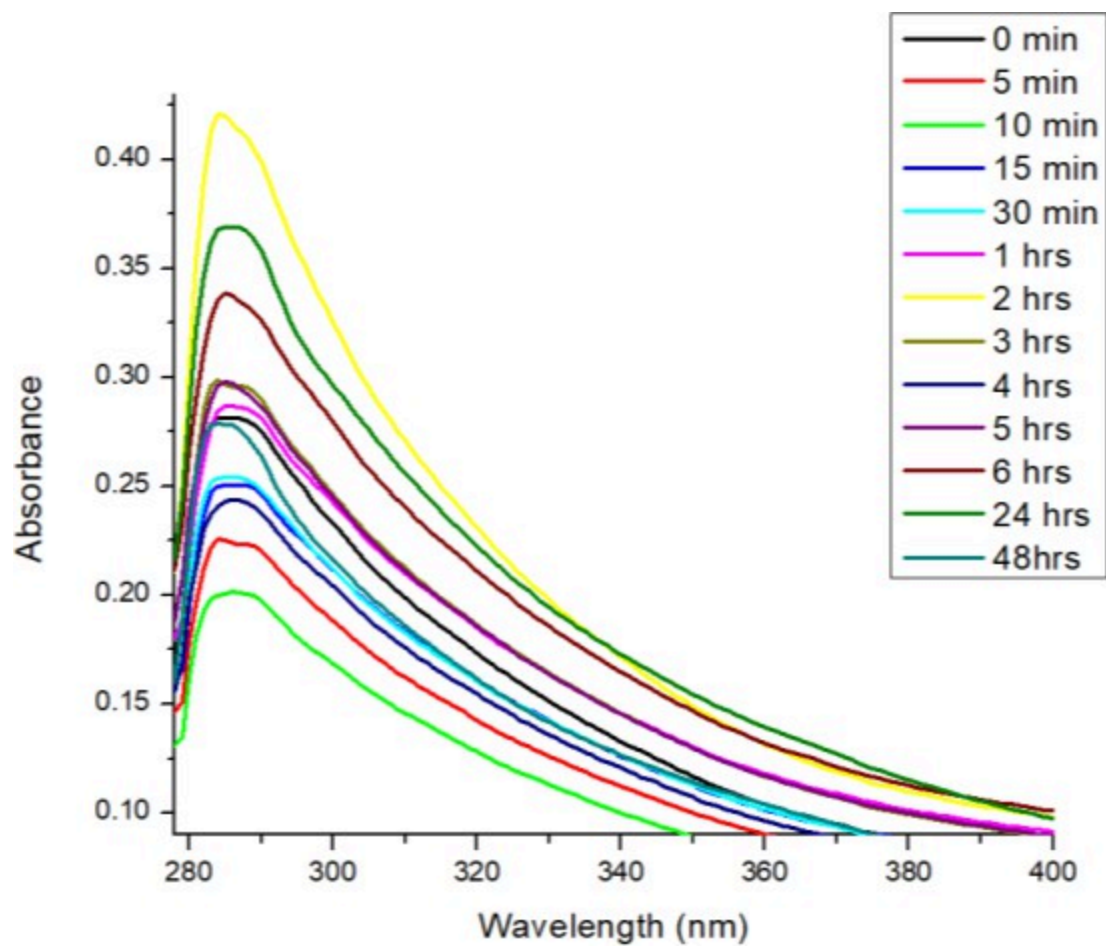


Figure 2

UV-Vis spectra of synthesised Fe-NPs at different times

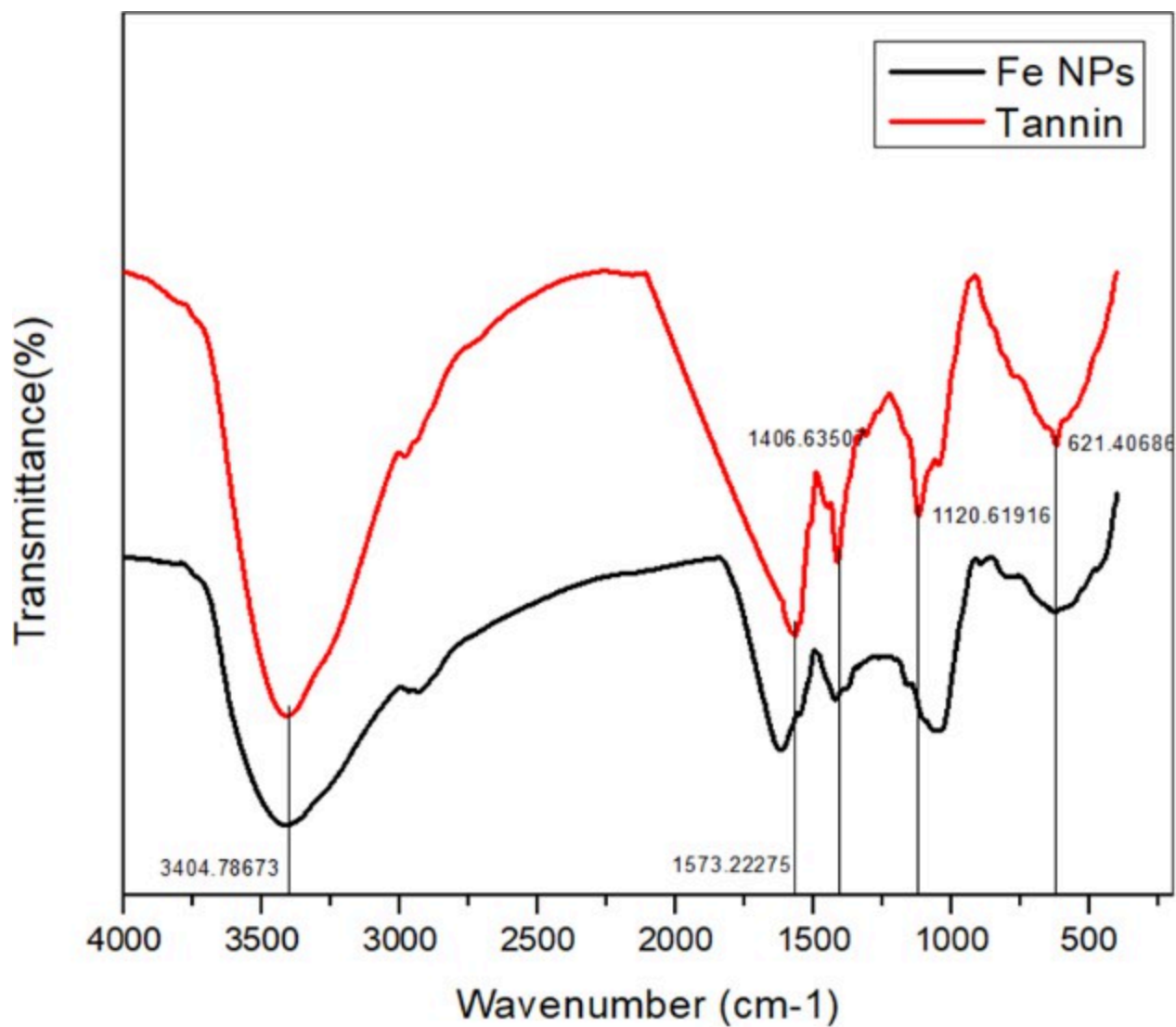


Figure 3

FT-IR spectra of Fe-NPs and grape tannin



Figure 4

TEM images of biosynthesized Fe-NPs at 200 k magnification

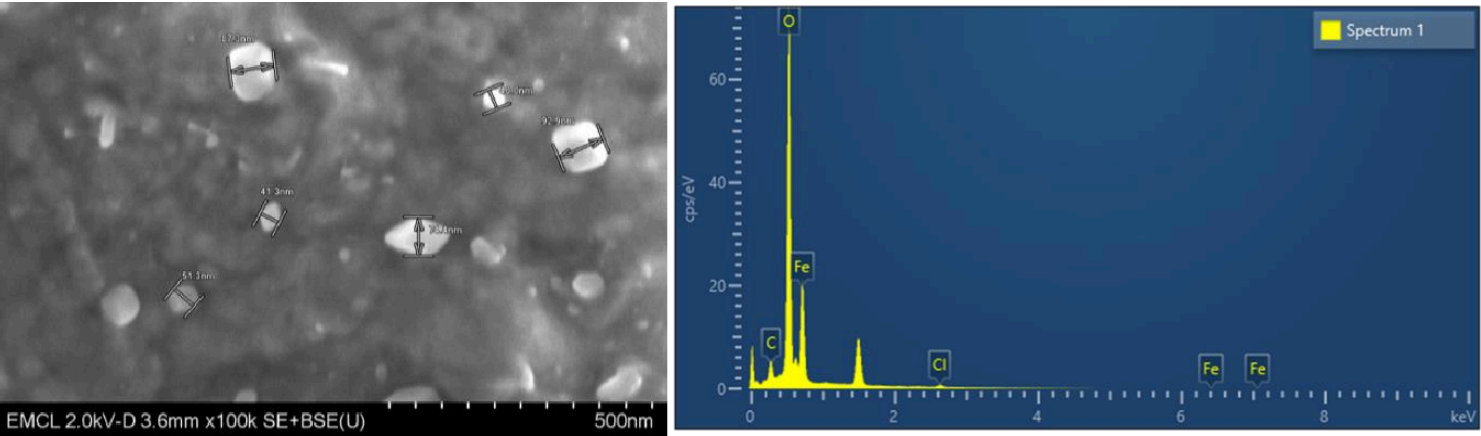


Figure 5

SEM images of grape tannin mediated Fe-NPs and EDX elemental analysis

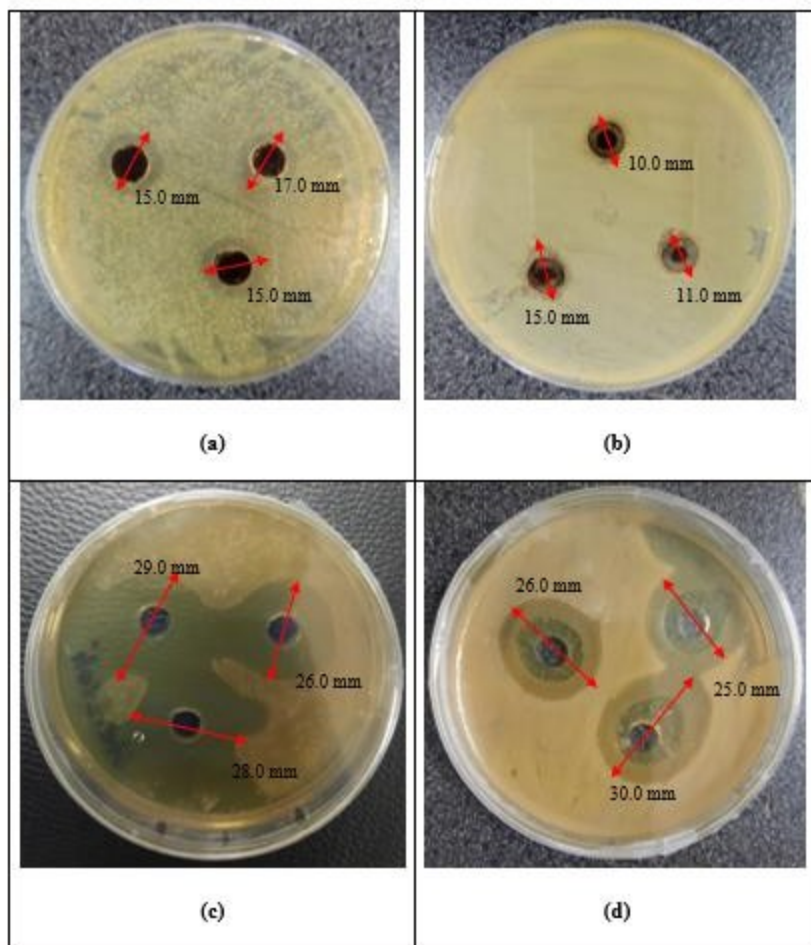


Figure 6

Inhibition zone of (a) *E. coli* (b) *S. aureus* with synthesized Fe-NPs and (c) *E. coli* (b) *S. aureus* with standard antibiotic, Streptomycin.

Cytotoxicity evaluation of iron nanoparticles on Hela cell li

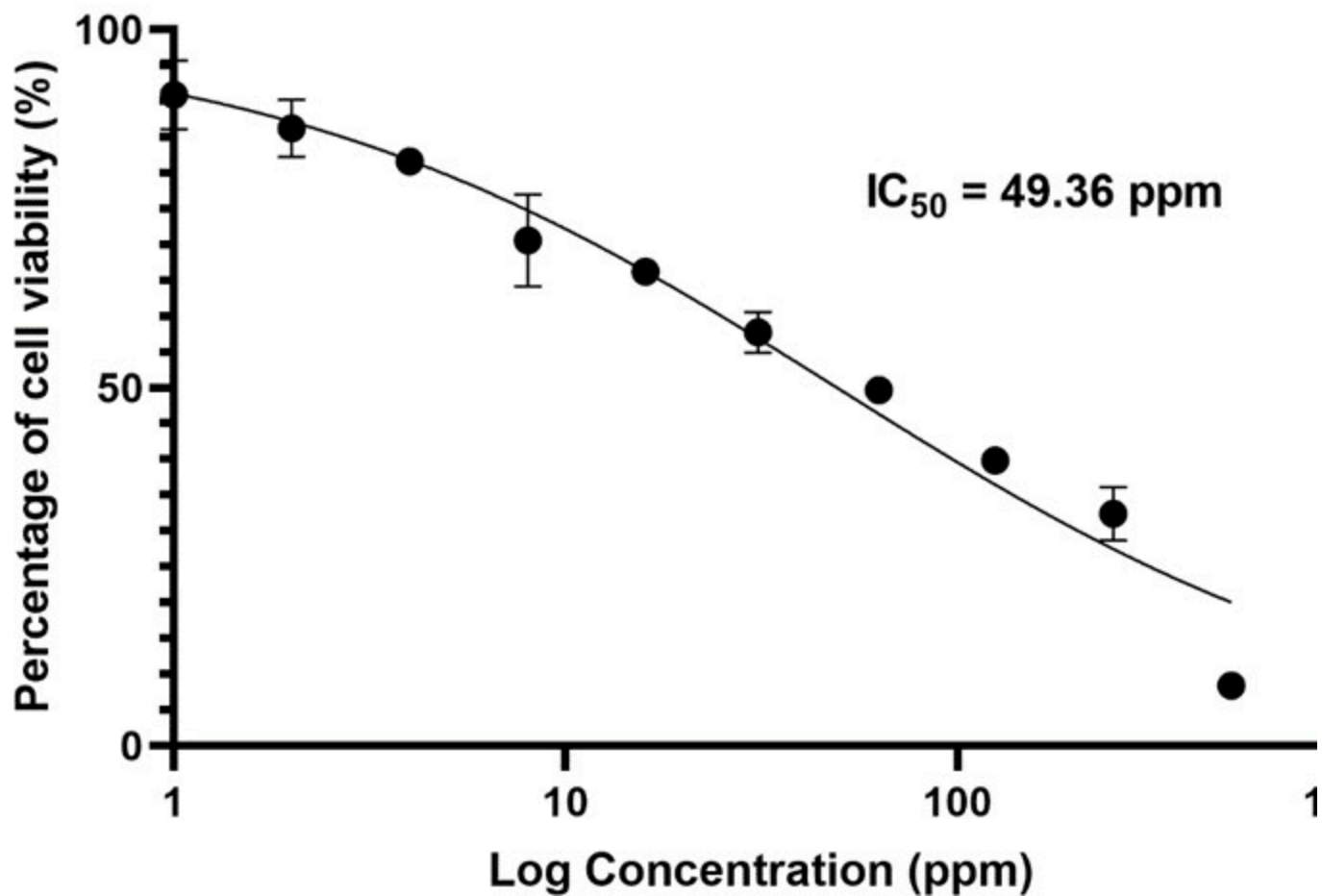


Figure 7

Cell proliferation results of Fe-NPs in HeLa cell lines