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Extracellular Biosynthesis of Cobalt Ferrite Nanoparticles By Monascus purpureus and Their Antioxidant, Anticancer and Antimicrobial Activities: Yield Enhancement by Gamma Irradiation

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Abstract

Cobalt ferrite nanoparticles were successfully synthesized using the fungus *Monascus purpureus* ATCC16436 as a potentially low-cost, eco-friendly and easy to produce method. Fourier transform infrared spectroscopy confirmed the functional groups present in the prepared samples. X-ray diffraction pattern of the synthesized nanoparticles revealed a single-phase crystalline structure. Transmission electron microscope studies showed the spherical shape with a mean particle size of 6.50 nm. Vibrating sample magnetometer analyses revealed that the synthesized nanoparticles have a superparamagnetic behavior. In addition, the antioxidant, anticancer and antimicrobial activities of the synthesized nanoparticles were evaluated. The synthesized nanoparticles exhibited antioxidant potential as compared by ascorbic acid with 50% inhibitory concentration of 100.25 µg mL\(^{-1}\). Based on the MTT assay, the synthesized nanoparticles significantly inhibited the proliferation of two different human cancer cell lines (breast and liver) and normal human melanocytes. The recorded 50% inhibitory concentrations of the respective cell lines were 45.21, 61.86 and 200.15 µg mL\(^{-1}\). The synthesized nanoparticles showed potent antibacterial and antifungal activities against all the tested plant and human microbial pathogens with minimal inhibitory concentration range 250 – 500 µg mL\(^{-1}\). Moreover, the feasibility of production enhancement of the synthesized nanoparticles using the fungal culture as affected by gamma irradiation was also adopted. Gamma irradiation at 1000 Gy dramatically intensified the yield of nanoparticles to 24.87 g L\(^{-1}\). Accordingly, these findings suggest a new and alternate approach with the excellent biotechnological potentiality for the nanoparticles production that will open up the way for the industrial manufacture of nanomaterials.

**Key words:** Cobalt Ferrite; *Monascus purpureus*; Nanoparticles; Antioxidant; Anticancer; Antimicrobial; Gamma irradiation
1. Introduction

Magnetic nanoparticles with unique material properties have gained considerable attention in the industrial and medical fields. Among these nanoparticles, cobalt ferrite nanoparticles exhibit promising characteristics of the high overload magnetization with no preferred route of magnetization and the high permeability [1]. Moreover, the ability to generate magnetic nanospheres, the good coupling efficiency and the high magnetostriction render them as a widely accepted candidate for many industrial and medical applications [2,3]. In addition, the high biocompatibility and antibacterial potential make them available for several antimicrobial applications [4]. Therefore, it was introduced in several medical and industrial applications including delivery of drugs, magnetic resonance imaging, magnetic thermo-drug delivery hyperthermia, biosensors, catalytic treatment of gases, gas detectors, and oxidation of alkanes [5,6]. Currently, preparation of the cobalt-ferrite nanoparticles is performed by several chemical and physical synthesis methods [7]. However, these methods are oftentimes carried out at elevated temperatures and pressure with toxic solvents that result in high energy demand and environmental costs [8]. Hence, there is a pressing scientific need to overcome these drawbacks by finding alternative green approaches including the microbial synthesis. Interestingly, bioactive metabolites present in the microbial cultures include many enzymes as well as various combinations of the organic and inorganic compounds that can end the reaction within few minutes in one step [9]. Moreover, the microbiologically synthesized nanoparticles are found to be reliable, biocompatible, eco-friendly, and economic [10,11]. In finding alternatives for the synthesis of cobalt-ferrite nanoparticles, the use of microorganisms especially bacteria and yeast have been developed. The recombinant mms6 protein of the bacterium *Magnetospirillum magneticum* was used for the in vitro synthesis of cobalt-ferrite nanocrystals [12]. In addition, the extracellular dissimilatory reduction of the ferric(III)-oxyhydroxides by the bacterium *Geobacter sulfurreducens* was applied for the
synthesis [8]. Moreover, the yeast *Saccharomyces cerevisiae* was used for the extracellular biosynthesis of these nanoparticles [13]. Nevertheless, there are no studies in the literature focus on the fungal production of the cobalt-ferrite nanoparticles. Since the sustainable growth of nanoparticles production requires cost effective and eco-friendly methods, fungi could be used as the most efficient biotechnology agents. Fungi are an easy, flexible, tolerant, and economic biological system that has been used extensively in large-scale production of different industrial compounds [14]. Moreover, the use of biotechnological platforms for the biosynthesis of nanomaterials is highly recommended due to its powerful tools for improvement, modification, and large-scale production [14]. Indeed, the extraordinary power of the fungi to produce a wide range of valuable nanoscale materials has until now been overlooked for manufacture on a commercial scale. In this paper, we describe the synthesis and characterization of cobalt-ferrite nanoparticles using the fungus *Monascus purpureus* ATCC16436. The antioxidant, anticancer and antimicrobial activities of the synthesized nanoparticles were also evaluated. Furthermore, the efficacy of the fungal culture on yield enhancement of the cobalt-ferrite nanoparticles as influenced by gamma irradiation was adopted.

2. Materials and methods

2.1. Fungal strain

*Monascus purpureus* ATCC16436 was obtained from Egypt Microbial Culture collection (EMCC), Ain Shams University, Cairo, Egypt, http://www.wfcc.info/ccinfo/collection/by_id/583. The fungal strain was maintained on Malt extract agar composed of (g L\(^{-1}\)): glucose 20, malt extract 20, peptone 1.0, and agar 20. Pure fungal cultures were stored as a suspension of spores and mycelium in 15% (v/v) glycerol at -4°C.
2.2. Preparation of cell-free filtrate

Fungal spores from the *M. purpureus* cultures (5 days old) were harvested separately by the flooding of the slants with sterile distilled water containing 0.1% Tween 20 and gently scraping off the spores with a sterile glass rod. After which, the spore concentration was adjusted using haemocytometer to a concentration of $10^6$ spores/mL. Potato-dextrose broth (pH 6.0, 50 mL/250 mL Erlenmeyer flask) was prepared, sterilized, cooled and then inoculated with 1 mL of the freshly prepared spore suspensions. Potato-dextrose broth composition (g L$^{-1}$): potato infusion 200, and D-glucose 20. The inoculated flasks were then incubated at 30°C for 7 days. At the end of the incubation period, the inoculated flasks were filtered through Whatman No.1 filter paper and the pH adjusted to 6, then used for the preparation of nanoparticles.

2.3. Synthesis of cobalt ferrite nanoparticles

Cobalt nitrate (Co(NO$_3$)$_2$.6H$_2$O) and ferric nitrate (Fe(NO$_3$)$_3$.9H$_2$O) (Sigma-Aldrich, St. Louis, MO, USA) in a 1:2 molar ratio were dissolved in deionized water and then stirred to obtain a homogeneous clear solution. This solution was then added dropwise to the prepared cell-free fungal culture (in an equal volume basis) under vigorous stirring at room temperature for several hours (4-6 h) until it turned a dark color, indicating the complete reduction process. The mixture was then heated for 5 min at 60°C to initiate the formation of cobalt-ferrite nanoparticles. After which, the mixture was then cooled and left stand at room temperature overnight where a dark precipitate started to appear, indicating the initiation of nanoparticles formation.

2.4. Separation and purification of nanoparticles
The reaction mixture including the precipitate of the reduced nanoparticles was used for separation and purification. Nanoparticles were separated by the ultra-centrifugation process at 20000 rpm for 20 min at 4°C. The collected nanoparticles were dispersed in deionized water and ethanol several times to remove the residual biological molecules. The purified nanoparticles were then dried using a hot air oven at 70°C where a fine powder was obtained. Furthermore, the powder was dispersed in ethanol and treated ultrasonically for the dispersion of the individual particles and used for characterization.

2.5. Characterization of nanoparticles

Fourier Transform Infrared (FT-IR) spectra were achieved using IRAffinity-1 spectrophotometer, Shimadzu, Japan. The spectrum was recorded at 400–4000 cm⁻¹. X-ray diffraction (XRD) pattern was recorded in the range 20° ≤ 2θ ≤ 80° through a BRUKER diffractometer (D8 DISCOVER with DAVINCI design, USA) using Cu-Kα radiation with a wavelength of 1.5406°A at 40 KV and 40 mA. Morphology and particle size distribution were determined by Transmission electron microscope (TEM) performed on a JOEL model 2100, Japan, operated at an accelerating voltage at 8000 KV by focusing on nanoparticles. The magnetic properties were measured using a vibrating sample magnetometer (VSM, 7410; Lakeshore, USA) at room temperature and at the maximum applied magnetic field of 10000 Oe. The saturation magnetization, remanence magnetization, and the coercive force were determined.

2.6. Antioxidant activity

Free radical scavenging activity of the synthesized nanoparticles was evaluated by 2,2'-diphenyl picrylhydrazyl (DPPH) radical scavenging assay [15]. The synthesized nanoparticles were dissolved in methanol to obtain the concentrations range of 5, 10, 20, 40,
80 and 160 µg mL\(^{-1}\) and then treated ultrasonically. The stock solution was prepared by dissolving 24 mg of DPPH (Sigma-Aldrich, St. Louis, MO, USA) in 100 mL of methanol and stored at -4°C. Then, 2 mL of this solution was added to 1 mL of the nanoparticles solution of different concentrations. The reaction mixture was incubated in the dark for 30 min and the absorbance was taken at 517 nm. The change in absorbance with respect to the control (containing DPPH only) is calculated as percentage scavenging activity. The same procedures were conducted in a similar manner using ascorbic acid (Sigma-Aldrich, St. Louis, MO, USA) as standard. The antioxidant concentration required to reduce the radicals by 50\% (IC\(_{50}\)) was estimated from graphic plots for each concentration using GraphPad Prism software (San Diego, CA, USA).

### 2.7. Anticancer activity

#### Propagation of cell culture

Human breast carcinoma (MCF-7), hepatocellular carcinoma (HepG-2), and normal human melanocytes (HFB-4) cell lines were obtained from The Holding Company for Biological Products & Vaccines (VACSERA, Cairo, Egypt, https://www.vacsera.com/). HepG-2 and MCF-7 were maintained in Dulbecco’s modified eagle’s medium supplemented with 10\% fetal bovine serum, 100 µg streptomycin and 100 units penicillin, while HFB-4 was maintained in Dulbecco’s modified eagle’s medium supplemented with 10\% fetal bovine serum, 100 µg streptomycin and 100 units penicillin. Culture conditions were adjusted at a temperature of 37°C in a humidified atmosphere consisting of 5\% CO\(_2\) and 95\% O\(_2\).

#### Cytotoxicity evaluation

The synthesized nanoparticles were dissolved in sterile phosphate buffer saline to a concentration range of 2.0 - 1000 µg mL\(^{-1}\). Cytotoxicity was measured against cell lines using...
the 3-(4,5-dimethyl-2-thiazolyl)-2,5-diphenyl-2H tetrazolium bromide (MTT) assay [16,17]. Cell monolayers \(10^4\) cells/well) were plated in 96-well tissue culture plate and incubated for 24 h at 37°C in a humidified incubator with 5% CO\(_2\) to allow attachment of cells to the plate except for four wells without cells as blank, before treatment with the compounds in different concentrations. Then, cells were incubated for another 48 h, washed by HEPES buffer (Lonza Bioproducts, Belgium) and 50 µL of 0.5 mg mL\(^{-1}\) MTT (Serva Electrophores, Germany) was added to each well. Cells were then incubated in the dark for 4 h for the reduction of MTT into formazan followed by addition of 50 µL of DMSO to solubilize the purple crystals of formazan. Absorbance was finally measured at 570 nm with microplate ELISA reader (BioTek, USA). The untreated cells were used as control. Control and samples were assayed in quadrates for each concentration. The relative viability of cells was expressed as follows: 

\[
\text{% Cell viability} = \left( \frac{A_{570} \text{ of treated cells}}{A_{570} \text{ of control cells}} \right) \times 100.
\]

The 50% inhibitory concentration (IC\(_{50}\)) was estimated from graphic plots of the dose–response curve for each concentration using GraphPad Prism software (San Diego, CA, USA).

2.8. Antimicrobial sensitivity tests

Antibacterial activity

The synthesized nanoparticles were dissolved in different volumes of methanol then treated ultrasonically to obtain the desired concentrations of 5, 10, 20, 40, 80 and 160 µg mL\(^{-1}\). Antibacterial susceptibility assay was performed against different Gram positive and Gram negative bacterial strains (\textit{Staphylococcus aureus} ATCC6538, \textit{Escherichia coli} ATCC11229, \textit{Klebsiella pneumoniae} ATCC13883, and \textit{Pseudomonas aeruginosa} ATCC15442) using agar well diffusion assay technique [18]. Nanoparticles solution (50 µL) was applied to agar well (9 mm, diameter) in a Petri-dish containing 25 mL Muller-Hinton agar medium (composed of
Antifungal activity

Antifungal susceptibility assay was performed against three plant pathogenic fungal isolates (Aspergillus niger, Alternaria solani and Fusarium oxysporum) and Candida albicans ATCC10231. The three plant pathogenic fungal isolates are used as standard microorganisms in our Laboratory; Microbiology Research Unit, Plant Research Department, Nuclear Research Center, Egypt. Petri-dishes containing Czapek-Dox's agar medium composed of (g L\(^{-1}\)): NaNO\(_3\), 3; sucrose, 30; KH\(_2\)PO\(_4\), 1; MgSO\(_4\).7H\(_2\)O, 0.5; KCl, 0.5; FeSO\(_4\).7H\(_2\)O, 0.01; agar, 20, pH 6.0, seeded with 0.2 mL spore suspension (10\(^7\) spores/ml) of the tested fungal species were also prepared to test the antifungal activity at the representative concentrations using the agar well diffusion assay technique [18]. Meanwhile, C. albicans (10\(^7\) cell/ml) cultured in Petri-dish containing 25 mL Sabouraud's-glucose agar medium composed of (g L\(^{-1}\)): bactopeptone, 10; glucose, 20; MgSO\(_4\).7H\(_2\)O, 1; KH\(_2\)PO\(_4\), 1 and agar, 20.

Control Petri-dishes for each tested bacterial or fungal species were made by applying methanol only (50 µL) to the agar wells. All the inoculated plates were incubated overnight at 4\(^\circ\)C and then for 24 h at 35\(^\circ\)C for the tested bacterial species and C. albicans or 5 days at 30\(^\circ\)C for the tested fungal species. The resulted inhibition zones around the wells were carefully measured and the wells containing the lowest concentration of nanoparticles that still showed a zone of inhibition around were considered the minimum inhibitory concentration (MIC).

2.9. Impact of gamma irradiation on nanoparticles production
The efficacy of the *M. purpureus* cell-free culture filtrate on the production of nanoparticles was evaluated as influenced by gamma irradiation. Spore suspensions were prepared as described earlier and then transferred in vials sealed with paraffin. Spore suspensions were exposed to gamma irradiator at doses varied between 250 and 4000 Gy. Irradiation process was carried out at the Nuclear Research Center, Cairo, Egypt. The facility used was $^{60}$Co Gamma chamber (MC20, Russia) with an average dose rate of 605.726 Gy h$^{-1}$ at the time of the experiment. The irradiated-spore suspensions were then inoculated in the potato-dextrose broth (pH 6.0) and incubated at 30°C for 7 days. At the end of the incubation period, the cell-free filtrate was used for nanoparticles production as described earlier and the carefully weighted.

### 2.10. Statistical analyses

Results were expressed as the mean ± standard deviation (SD) and statistical significance was evaluated using analysis of variance (ANOVA, SPSS software version 22; IBM Corp., NY) test followed by the Least Significant Difference LSD test at 0.05 level.

### 3. Results

#### 3.1. Synthesis and characterization of cobalt ferrite nanoparticles

FTIR spectra of the synthesized nanoparticles and the cell-free fungal filtrate were recorded in the 400–4000 cm$^{-1}$ range (Fig. 1). The spectrum of the cell-free fungal filtrate showed main bands of O-H in water, C-H in CH$_2$ and in the phenyl ring, and symmetric and asymmetric vibrations of C-O and C=O bonds in COO$^-$ groups. Moreover, bands of phenols were also observed. However, the recorded spectrum of the synthesized nanoparticles showed an absorption peak observed at 416 cm$^{-1}$ due to the ferrite skeleton for the octahedral site. The intense peak at 567 cm$^{-1}$ in the FTIR spectrum is attributed to the M-O tetrahedral
site in the spinel structure. Interestingly, an absorption band at 1678 cm\(^{-1}\) appeared in the spectrum of the synthesized nanoparticles due to the formation of carboxylic acids. Fig. 1 further showed that an intense peaks at 3421 cm\(^{-1}\) due to the H-O-H stretching vibrations of the absorbed water molecules on the surface of nanoparticles.

**Fig. 2** shows the XRD pattern of the synthesized nanoparticles and **Table 1** presents the crystallographic data obtained by XRD analyses. The obtained results showed that the crystal structure of the samples was found to be an inverse cubic spinel type. Moreover, the presence of 220, 311, 222, 400, 422, 511, 440, 620, 533, 622, and 444 planes in the XRD pattern confirms the inverse cubic spinel structure of the synthesized nanoparticles with space group fd-3m. The single phase was also confirmed as no peaks corresponding to impurities were detected. The obtained results further showed that the recorded lattice parameter was 8.3806 Å. The crystallite size of the synthesized nanoparticles was calculated using the Scherrer equation from the FWHM of the most intense peak corresponding to the (311) plane as follows:

\[
D = \frac{k \lambda}{\beta \cos \theta},
\]

where D, k, \(\lambda\), \(\beta\), and \(\theta\) are crystallite size, Scherrer constant, the wavelength of the X-ray, full width half-maximum of the (311) plane, and Bragg diffraction angle, respectively. The synthesized nanoparticles exhibited a mean crystallite size of 6.73 nm.

**Fig. 3a** shows the TEM images of the synthesized nanoparticles. Morphological analyses revealed that the nanoparticles were almost spherical in shape and not uniformly arranged. The SAED pattern consists of five distinct rings (**Fig. 3b**), indexed to be the 220, 311, 400, 511, and 440 lattice planes of the synthesized nanoparticles which was confirmed by the XRD. The particle size distribution of the synthesized nanoparticles was taken along the diameter of the particles (**Fig. 3c**) which was 3–15 nm with the mean particle size of 6.50±0.08 nm.
M-H curve of the synthesized nanoparticles powder prepared by the *M. Purpureus* culture was recorded using vibrating sample magnetometer (Fig. 4). The recorded values of saturation magnetization was 46.04 emu g\(^{-1}\), very low retintivity of 4.39 emu g\(^{-1}\) and coercive field of 411.6 Oe in the M-H curve indicated their superparamagnetic nature at room temperature. This means the prepared nanoparticles readily displayed magnetization when subjected to a magnetic field.

### 3.2. DPPH free radical scavenging activity

Data presented in Table 2 clearly showed that the synthesized nanoparticles inhibited the DPPH free radicals in a dose-dependant manner, as the concentration of nanoparticles increases the scavenging activity also increased. It is also evident from the results that the synthesized nanoparticles exhibited moderate antioxidant activity at different concentrations as compared by ascorbic acid (Table 2). The least inhibitory concentration of the synthesized nanoparticles was at 3.9 µg mL\(^{-1}\) and that of ascorbic acid was at 2.00 µg mL\(^{-1}\). The obtained results further showed that the recorded IC\(_{50}\) value of the synthesized nanoparticles was 100.25 µg mL\(^{-1}\). However, the recorded IC\(_{50}\) value of the ascorbic acid was 25.31 µg mL\(^{-1}\).

### 3.3. Cytotoxicity studies

Results presented in Table 3 indicated that the synthesized nanoparticles were active against both the malignant MCF-7 and HepG-2 cancer cell lines as well as the nonmalignant Hfb-4 cell line. The least concentration of nanoparticles necessary to inhibit the proliferation of the tested cell lines was found to vary from cell line to another (Table 3). The obtained results showed that the least inhibitory activity of the synthesized nanoparticles was 2.00 µg mL\(^{-1}\) against MCF-7, and HepG-2 cells and was 3.90 µg mL\(^{-1}\) against Hfb-4 cells. The obtained data further indicated that the synthesized nanoparticles caused concentration-dependent cell
death where the increase in concentration led to a significant decrease in cell proliferation.

Moreover, the recorded IC$_{50}$ values were found to be 61.86 µg mL$^{-1}$ against HEp-2 and 45.21 µg mL$^{-1}$ against MCF-7. While the IC$_{50}$ for the nonmalignant Hfb-4 was 200 µg mL$^{-1}$.

3.4. Antimicrobial activity

Data from Table 4 indicated that the synthesized nanoparticles showed a broad spectrum of antibacterial activity, where it inhibited the growth of all the tested bacterial species. The recorded results also clearly showed that the MIC of the *E. coli*, *Staph. aureus* and *P. aeruginosa* was 250 µg mL$^{-1}$ while for *K. pneumoniae* it was 500 µg mL$^{-1}$.

Regarding the antifungal activity of the synthesized nanoparticles, the growth of the tested fungal species was significantly inhibited (Table 5). From the obtained results it is obvious that the MIC of the tested fungal species was at 250 µg mL$^{-1}$ where the inhibition zones of growth around the agar well observed were 14.53, 10.53 and 11.76 mm for *A. niger*, *A. solani*, and *F. oxysporum*, respectively. However, *C. albicans* was the most sensitive towards the nanoparticles where the inhibition zones of growth around the agar well of 9.53 at a concentration of 250 µg mL$^{-1}$.

3.5. Impact of gamma irradiation on production of cobalt ferrite nanoparticles

Results presented in Table 6 illustrated that the cell growth and production of nanoparticles as affected by gamma irradiation was found to be dose-related. A significant reduction in the obtained dry biomass was recorded after receiving higher doses at 2000 and 4000 Gy. Regarding the effect of irradiation on the nanoparticles production, 1000 Gy was the most effective gamma radiation dose, where significant differences in the nanoparticles yield were observed. The highest yield of the synthesized nanoparticles obtained was 24.87 g L$^{-1}$ culture filtrate; which was approximately twice that obtained using their respective control.
treatments (non-irradiated cultures). The obtained data further showed that increasing the radiation dose in the range of 2000–4000 Gy resulted in a lower yield of nanoparticles.

4. Discussion

The present study aimed to develop a cost-effective preparation method for the well-known cobalt ferrite nanoparticles. Thus, culture filtrate of the fungus *Monascus purpureus* ATCC16436 was successfully applied for the preparation process. The synthesized nanoparticles in the present study were separated, purified and characterized by various techniques. FT-IR spectra of the fungal filtrate showed main bands of O-H in water, C-H in CH$_2$, and in the phenyl ring, and symmetric and asymmetric vibrations of C-O and C=O bonds in COO$^-$ groups as well as bands of phenols [19,20]. However, the spectrum of the synthesized nanoparticles showed two absorption peaks at 416 and 567 cm$^{-1}$. The first peak is attributed to the ferrite skeleton for the octahedral site and the presence of the second peak is consistent with the M-O tetrahedral site in the spinel structure [7]. Position of these peaks is slightly varied due to the differences in the grain sizes of the samples and synthesis methods [7,21]. Spectrum of the synthesized nanoparticles further showed strong stretching and bending vibrations of the absorbed water molecules on the surface of nanoparticles suggesting the polar nature of the surface. Similarly, the strong stretching modes of the OH groups was mainly due to the more polar character of nanoparticles surface [13]. By comparing the FTIR spectra of the fungal culture and synthesized nanoparticles, the remarkable features were the disappearance of all the functional groups in the spectrum of the fungal culture indicating the role of the fungal culture in the reduction process. Moreover, the formation of peak located at 1678 cm$^{-1}$ in the spectrum of the synthesized nanoparticles due to formation of carboxylic acid of the lactone ring in the fungal culture. In accordance with our results, *Monascus purpureus* culture was applied in the synthesis of silver [19] and
gold nanoparticles [20]. The authors attributed the formation of silver nanoparticles to the potential redox systems in the *M. purpureus* culture that is characterized by the presence of a complex mixture of three types of pigments (red, orange, and yellow) of polyketide origin [19]. In addition, the reduction of gold chloride into gold nanoparticles was attributed to the presence of lactone ring in the culture and formation of carboxylic acids [20]. The authors further stated that the existence of many redox functional groups and several hetero atoms in the azaphilone form of the culture is responsible for the synthesis of gold nanoparticles. Similarly, the reduction of silver nitrate to silver nanoparticles by bacteria was performed by a certain NADH dependent reductase [22]. Accordingly, we suggested that the high reducing power of the *M. purpureus* culture filtrate could be responsible for the reduction of the iron and cobalt nitrate salts and precipitation of the cobalt ferrite nanocrystals.

XRD analyses of the synthesized nanoparticles confirmed the inverse cubic spinel structure with space group fd-3m which is in agreement with the JCPDS standard cards No. 22-1086 [7]. Moreover, the lattice parameter of 8.38060 Å was in a good agreement with the literature [7,23]. TEM analyses of the synthesized nanoparticles showed the almost spherical shape and not uniformly arranged nanoparticles with the mean particle size of 6.50 nm. This is due to the high crystallinity of the synthesized nanoparticles, which was confirmed by the XRD. The observed small difference in particles size calculated by the Scherrer equation may be due to the different times of the nanoparticles synthesis [13]. In literature, the average particle size of the cobalt ferrite nanoparticles prepared by the *Saccharomyces cerevisiae* culture was 7.23 nm [13]. Also, the crystallite size of the cobalt ferrite nanoparticles produced by the magnetotactic bacterium *Geobacter sulfurreducens* was 8.00 nm [8]. The obtained low lattice strain in the present study could be due to the fact that there is no any constraint during the size reduction and/or formation of a compound in the natural synthesis (biosynthetic reaction) procedures that are generally found with other chemical and physical
techniques [13]. In addition, the microbial reduction can produce nanoparticles of well-defined size (typically tens of nanometers) and crystallographic morphology [24]. VSM analyses of the synthesized nanoparticles indicated the superparamagnetic nature at room temperature. This behavior could be attributed to the fact that cobalt ions in the synthesized nanoparticles have a strong preference for the octahedral site. Thus, there will be far fewer A-B magnetic ion pairs than the B-B pairs which provide only weak magnetic coupling [13]. It is worthy here to mention that the synthesized cobalt ferrite nanoparticles dispersed in an aqueous solution showed excellent stability for four months where there is no precipitations observed. Moreover, the obtained magnetic behavior makes the synthesized cobalt ferrite nanoparticles promising candidates for several medicinal applications especially the use in hyperthermia treatment and the use as magnetic fluid.

Upon testing the antioxidant activity of the synthesized cobalt ferrite nanoparticles, results clearly indicated that the promising antioxidant activity at different concentrations as compared by ascorbic acid. In literature, many of the metal nanoparticles have been reported for the free radicals scavenging ability that enabled them to act as antioxidants [25]. The antioxidant property of the metal nanoparticles is mainly due to the neutralization of free-radical character of the DPPH by transfer of electrons. Moreover, the antioxidant activity of metal nanoparticles could be attributed to the high surface to volume ratio [26]. The present study further showed that the recorded IC$_{50}$ of the synthesized nanoparticles was 100.25 µg mL$^{-1}$. In partial accordance with our results, ZnFe$_2$O$_4$ and CuFe$_2$O$_4$ nanoparticles at a concentration of 125 µg mL$^{-1}$ showed scavenging activities of 30.57% and 28.69%, respectively [27]. However, in another study, the IC$_{50}$ value of the prepared cobalt ferrite nanoparticles was 6.8 mg mL$^{-1}$ [28]. In literature, studies on the antioxidant potentials of the ferrite nanoparticles are in rare [27]. Generally, the biocompatibility of a biomaterial is limited by its oxidative stress that results in serious problems such as inflammation and
chronic diseases. Hence, the recorded antioxidant activity of our study is promising in terms of the concentration and provides a lead in the exploration of the cobalt ferrite nanoparticles as a new source of antioxidants.

In the present study, the synthesized nanoparticles caused concentration-dependent cell death and the recorded IC$_{50}$ values were 61.86, 45.21 and 200 $\mu$g mL$^{-1}$ against HEp-2, MCF-7 and Hfb-4, respectively. In concurrence with our results, a significant decrease in the cell viability of the breast cell lines (MCF-7) up on exposure to 1 mg mL$^{-1}$ of the synthesized nanoparticles and the relative cell viability was 63% [28]. Moreover, the lowest cell viability (17% and 20%) was observed at 500 $\mu$g mL$^{-1}$ of CuFe$_2$O$_4$ and ZnFe$_2$O$_4$ nanoparticles, respectively [27]. Surprisingly, our results indicated that the two types of malignant cells (MCF-7 and HepG2) seemed to be more sensitive to the synthesized cobalt ferrite nanoparticles than the nonmalignant Hfb-4 cells. Similarly, at a concentration lower than 200 $\mu$M, cobalt ferrite nanoparticles did not show any toxicity against seven types of cancer cells [29]. The authors further showed that exposure of cancer cells to concentrations above 200 $\mu$M showed different toxic activities among the tested cell types. Up on exposure to nanoparticles, a high linear correlation was observed between the concentration of generated reactive oxygen species and the toxic effect of these nanoparticles [30]. Nanoparticles not only disrupt normal cellular function, but also affect membrane integrity, inducing various apoptotic signaling genes of mammalian cells, leading to programmed cell death [31]. Moreover, cell cycle arrest or apoptosis may result from the increase in the concentrations of hydrogen peroxide and ROS in tumor cells. Generally, magnetic nanoparticles with a mean size range of 10 - 100 nm are promising candidates for biomedical applications [32]. This is due to high permeability in solution, the increased surface area for attachment, and easy infiltration into cells [33]. This study further demonstrated that the synthesized nanoparticles showed a broad spectrum of antibacterial and antifungal activity, where it inhibited the growth of all the tested bacterial
and fungal species. In accordance with our results, the antimicrobial activity of the synthesized nanoparticles against several bacterial and fungal species was well reported [7,9,29,34]. However, the concentration of the synthesized nanoparticles used in the present study is much lower than that used in literature (1-10 mg mL\(^{-1}\)) indicating the better characteristics of our nanoparticles. The difference in the antimicrobial behavior could be attributed to the vary in the size of the applied nanoparticles. In particular, nanoparticles with smaller sizes showed high efficacy in inhibiting bacterial growth due to the increased production of reactive oxygen species [35]. Similarly, the antibacterial activity was found to increase by decreasing the particle size [36]. This would allow a higher level of penetration of free radicals such as peroxide ions and superoxide radical, causing cell death at lower concentrations [37]. Nanoparticles with the smaller size can interact quickly with the cell wall and membrane causing leakage of genetic materials, proteins, and minerals that finally result in cell death [12].

The present results showed that the cell growth and production of cobalt ferrite nanoparticles as affected by gamma irradiation was found to be dose-related. Gamma radiation dose of 1000 Gy was the optimum dose, where significant differences in the nanoparticles yield were observed. The highest yield of the synthesized nanoparticles obtained was 24.87 g L\(^{-1}\); which was approximately twice that obtained using their respective control treatments (non-irradiated cultures). The enhancement in production of the nanoparticles is mainly due to the fact that gamma irradiation may cause some mutations to the genes of cells through the DNA repair mechanisms that results in the overproduction of the bioactive metabolites responsible for the reduction process [38]. Again, it is a clear evidence for the responsibility of the fungal culture in the reduction process and formation of the nanoparticles. In literature, gamma irradiation at specific doses was successfully applied for the production enhancement of many industrial fungal metabolites. For example, a gamma radiation dose of 750 Gy was
used for enhancing the production process of the immunosuppressant mycophenolic acid by *Penicillium roqueforti* strains [39,40]. Also, exposure of spores of *Aspergillus fumigatus* and *Alternaria tenuissima* to a gamma radiation dose of 1000 Gy significantly increased the production titer of the anticancer drug paclitaxel [41,42]. Interestingly, following gamma radiation, the enhanced yield of the synthesized nanoparticles obtained in this study was much greater than that obtained in previous studies using bacteria [8,12].

5. Conclusion

Cobalt ferrite nanoparticles were successfully synthesized using *Monascus purpureus* cell-free culture filtrate. The nanoparticles exhibited a superparamagnetic nature according to the VSM analysis. Using the DPPH assay, the synthesized nanoparticles exhibited promising antioxidant activity as compared by ascorbic acid as a standard. Moreover, the MTT cytotoxicity assay showed their concentration dependent activity against human breast and liver cancer cell lines. The synthesized nanoparticles further showed potent antimicrobial activities against all the tested plant and human pathogens. Consequently, the synthesized cobalt ferrite nanoparticles can be better explored in the near future for various biomedical, industrial and agricultural applications. Moreover, the high yield of the cobalt ferrite nanoparticles achieved in the present study could open up the way for the manufacture of nanoparticles at an industrial scale using a cost-effective and eco-friendly methodology. Current work is in progress to scale up the production of cobalt ferrite nanoparticles and study the mechanism of their antimicrobial and anticancer activities.

**Compliance with ethical standards**

**Conflict of interest** The authors declare that they have no conflicts of interest.
**Ethical approval** This article does not contain any studies with human participants or animals performed by the authors.

**References**


[38] A.A. Ismaiel, A.S. Ahmed, E.R. El-Sayed, Optimization of submerged fermentation conditions for immunosuppressant mycophenolic acid production by *Penicillium roqueforti* isolated from blue-molded cheeses: enhanced production by ultraviolet and


Figures Legends:

**Fig. 1.** FT-IR spectra of the *Monascus purpureus* cell-free culture filtrate (dotted line) and the synthesized cobalt ferrite nanoparticles (solid line).

**Fig. 2.** X-ray diffraction pattern (Cu Ka-radiation) of the synthesized cobalt ferrite nanoparticles by *Monascus purpureus* cell-free filtrate at room temperature.

**Fig. 3.** TEM analyses of the synthesized cobalt ferrite nanoparticles by *Monascus purpureus* cell-free culture filtrate; (A) TEM micrograph, (B) SAED pattern and (C) Particle size distribution histogram.

**Fig. 4.** The magnetic hystress curve of the cobalt ferrite nanoparticles synthesized by the *Monascus purpureus* cell-free culture filtrate.
Table 1: Crystallographic data of the synthesized cobalt-ferrite nanoparticles obtained from XRD analysis.

<table>
<thead>
<tr>
<th>Crystallographic data</th>
<th>XRD data</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Formula</strong></td>
<td>CoFe$_2$O$_4$</td>
</tr>
<tr>
<td><strong>Crystal system</strong></td>
<td>Cubic</td>
</tr>
<tr>
<td><strong>Space group (n)</strong></td>
<td>fd-3m (227)</td>
</tr>
<tr>
<td><strong>Lattice parameter (nm)</strong></td>
<td>0.838060</td>
</tr>
<tr>
<td><strong>Cell volume, (Å$^3$)</strong></td>
<td>588.61</td>
</tr>
<tr>
<td><strong>Mean crystallite size (nm)</strong></td>
<td>8.73</td>
</tr>
<tr>
<td><strong>Temperature (°C)</strong></td>
<td>25</td>
</tr>
<tr>
<td><strong>Wavelength [CuKa] (nm)</strong></td>
<td>0.15406</td>
</tr>
<tr>
<td><strong>Monochromator</strong></td>
<td>Graphite</td>
</tr>
<tr>
<td><strong>Measuring range (°)</strong></td>
<td>20$\leq\theta$$\leq$80</td>
</tr>
<tr>
<td><strong>Step (°2θ)</strong></td>
<td>0.011</td>
</tr>
<tr>
<td><strong>Integration time (s)</strong></td>
<td>30</td>
</tr>
</tbody>
</table>
Table 2: DPPH free radical scavenging activity of the synthesized cobalt ferrite nanoparticles and ascorbic acid as standard.

<table>
<thead>
<tr>
<th>Nanoparticles conc. (µg mL⁻¹)</th>
<th>Free radical scavenging activity (%)</th>
<th>Ascorbic acid</th>
<th>Cobalt ferrite nanoparticles</th>
</tr>
</thead>
<tbody>
<tr>
<td>0.00 (C)</td>
<td>00.00±0.00⁹</td>
<td>00.00±0.00⁹</td>
<td></td>
</tr>
<tr>
<td>2.00</td>
<td>05.37±0.35⁸</td>
<td>00.00±0.00⁹</td>
<td></td>
</tr>
<tr>
<td>3.90</td>
<td>17.51±1.03⁸</td>
<td>03.22±1.27⁷</td>
<td></td>
</tr>
<tr>
<td>7.80</td>
<td>39.83±1.88⁷</td>
<td>19.69±2.05⁶</td>
<td></td>
</tr>
<tr>
<td>15.60</td>
<td>47.51±2.79⁶</td>
<td>31.83±4.77⁵</td>
<td></td>
</tr>
<tr>
<td>31.30</td>
<td>68.44±4.71⁵</td>
<td>43.51±2.68⁴</td>
<td></td>
</tr>
<tr>
<td>62.50</td>
<td>72.91±3.68⁴</td>
<td>58.37±5.71³</td>
<td></td>
</tr>
<tr>
<td>125</td>
<td>90.74±8.04⁴</td>
<td>78.61±3.68⁴</td>
<td></td>
</tr>
<tr>
<td>250</td>
<td>97.52±7.31³</td>
<td>89.31±5.71²</td>
<td></td>
</tr>
<tr>
<td>500</td>
<td>100.00±0.00²</td>
<td>94.28±3.79¹</td>
<td></td>
</tr>
<tr>
<td>1000</td>
<td>100.00±0.00¹</td>
<td>99.57±4.62⁰</td>
<td></td>
</tr>
<tr>
<td>IC₅₀ (µg mL⁻¹)</td>
<td>25.31</td>
<td>100.25</td>
<td></td>
</tr>
</tbody>
</table>

DPPH scavenging assay was used for measuring the antioxidant activities of cobalt ferrite nanoparticles at 517 nm using DPPH solution under the conditions described in Materials and Methods. Calculated mean is for triplicate measurements from two independent experiments ± SD. *⁸ means with different superscripts in the same column are considered statistically different (LSD test, P ≤ 0.05).
Table 3: Anticancer activity of the synthesized cobalt ferrite nanoparticles against Hepatocellular carcinoma (HepG-2), human breast carcinoma (MCF-7) and normal human melanocytes (Hfb-4).

<table>
<thead>
<tr>
<th>Nanoparticles conc. (µg mL⁻¹)</th>
<th>Cell viability (%)</th>
<th></th>
<th></th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>HepG-2 (Liver)</td>
<td>MCF-7 (Breast)</td>
<td>Hfb-4 (Normal)</td>
<td></td>
</tr>
<tr>
<td>0.00 (C)</td>
<td>100.00±0.00ᵃ</td>
<td>100.00±0.00ᵃ</td>
<td>100.00±0.00ᵃ</td>
<td></td>
</tr>
<tr>
<td>2.00</td>
<td>99.76±7.23ᵇ</td>
<td>96.78±0.00ᵇ</td>
<td>100.00±0.00ᵇ</td>
<td></td>
</tr>
<tr>
<td>3.90</td>
<td>92.31±3.62ᵇ</td>
<td>75.62±1.97ᵇ</td>
<td>98.51±3.17ᵇ</td>
<td></td>
</tr>
<tr>
<td>7.80</td>
<td>82.38±1.85ᶜ</td>
<td>67.51±4.32ᶜ</td>
<td>82.21±8.11ᶜ</td>
<td></td>
</tr>
<tr>
<td>15.60</td>
<td>79.31±4.65ᵈ</td>
<td>66.86±1.79ᵈ</td>
<td>78.25±4.32ᵈ</td>
<td></td>
</tr>
<tr>
<td>31.30</td>
<td>75.04±3.17ᵈ</td>
<td>57.04±2.88ᵈ</td>
<td>76.31±6.19ᵈ</td>
<td></td>
</tr>
<tr>
<td>62.50</td>
<td>50.52±2.57ᵉ</td>
<td>36.34±6.17ᵉ</td>
<td>65.07±2.57ᵉ</td>
<td></td>
</tr>
<tr>
<td>125.00</td>
<td>45.39±1.65ᶠ</td>
<td>35.21±5.21ᶠ</td>
<td>53.94±3.17ᶠ</td>
<td></td>
</tr>
<tr>
<td>250.00</td>
<td>36.44±1.65ᵍ</td>
<td>29.53±2.73ᵍ</td>
<td>41.53±1.65ᵍ</td>
<td></td>
</tr>
<tr>
<td>500.00</td>
<td>31.21±1.09ᵇ</td>
<td>28.33±6.17ᵇ</td>
<td>35.77±6.17ᵇ</td>
<td></td>
</tr>
<tr>
<td>1000</td>
<td>02.08±1.09ᵍ</td>
<td>03.11±0.11ᵍ</td>
<td>31.98±6.17ᵍ</td>
<td></td>
</tr>
<tr>
<td>IC₅₀ (µg mL⁻¹)</td>
<td>61.86</td>
<td>45.21</td>
<td>200.15</td>
<td></td>
</tr>
</tbody>
</table>

MTT-based assay was used for measuring the cytotoxic activities of the synthesized cobalt ferrite nanoparticles at 570 nm using MTT solution under the conditions described in Materials and Methods, calculated mean is for triplicate measurements from two independent experiments ± SD. ᵉ⁾ means with different superscripts in the same column are considered statistically different (LSD test, P ≤0.05).
Table 4: Antibacterial activity of the synthesized cobalt ferrite nanoparticles against different gram positive and gram negative pathogenic bacterial strains.

<table>
<thead>
<tr>
<th>Nanoparticles conc. (µg mL⁻¹)</th>
<th>Diameter of inhibition zone (mm)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>E. coli</td>
</tr>
<tr>
<td>0.00 (C)</td>
<td>0.00&lt;sup&gt;d&lt;/sup&gt;</td>
</tr>
<tr>
<td>100</td>
<td>0.00&lt;sup&gt;d&lt;/sup&gt;</td>
</tr>
<tr>
<td>250</td>
<td>10.61±0.21&lt;sup&gt;c&lt;/sup&gt;</td>
</tr>
<tr>
<td>500</td>
<td>15.73±0.89&lt;sup&gt;b&lt;/sup&gt;</td>
</tr>
<tr>
<td>1000</td>
<td>25.78±0.74&lt;sup&gt;a&lt;/sup&gt;</td>
</tr>
</tbody>
</table>

Calculated mean is for triplicate measurements from two independent experiments ± SD. Means with different superscripts in the same column are considered statistically different (LSD test, P ≤ 0.05).
Table 5: Antifungal activity of the synthesized cobalt ferrite nanoparticles against different plant pathogenic fungi and *C. albicans*.

<table>
<thead>
<tr>
<th>Nanoparticles conc. (µg mL⁻¹)</th>
<th>Diameter of inhibition zone (mm)</th>
<th>A. niger</th>
<th>A. solani</th>
<th>F. oxysporum</th>
<th>C. albicans</th>
</tr>
</thead>
<tbody>
<tr>
<td>0.00 (C)</td>
<td></td>
<td>0.00²</td>
<td>0.00²</td>
<td>0.00²</td>
<td>0.00⁴</td>
</tr>
<tr>
<td>100</td>
<td></td>
<td>0.00⁶</td>
<td>0.00³</td>
<td>0.00³</td>
<td>0.00³</td>
</tr>
<tr>
<td>250</td>
<td></td>
<td>0.00³</td>
<td>0.00³</td>
<td>0.00³</td>
<td>0.00⁴</td>
</tr>
<tr>
<td>500</td>
<td></td>
<td>14.53±0.69⁵</td>
<td>10.53±0.69⁵</td>
<td>11.76±0.77⁶</td>
<td>18.52±0.43⁵</td>
</tr>
<tr>
<td>1000</td>
<td></td>
<td>26.08±0.57⁴</td>
<td>16.08±0.57⁴</td>
<td>18.54±0.93⁴</td>
<td>33.55±0.71⁴</td>
</tr>
</tbody>
</table>

Calculated mean is for triplicate measurements from two independent experiments ± SD, a-d means with different superscripts in the same column are considered statistically different (LSD test, *P* ≤ 0.05).
Table 6: Influence of $^{60}$Co gamma irradiation at various doses on dry biomass and yield of the cobalt ferrite nanoparticles.

<table>
<thead>
<tr>
<th>Dose (Gy)</th>
<th>Dry biomass (g L$^{-1}$)</th>
<th>Nanoparticles Yield (g L$^{-1}$)</th>
</tr>
</thead>
<tbody>
<tr>
<td>0.00 (C)</td>
<td>8.54±0.44$^a$</td>
<td>12.01±1.03$^d$</td>
</tr>
<tr>
<td>250</td>
<td>8.47±0.61$^a$</td>
<td>12.05±1.24$^c$</td>
</tr>
<tr>
<td>500</td>
<td>6.98±0.23$^b$</td>
<td>18.13±0.97$^b$</td>
</tr>
<tr>
<td>1000</td>
<td>5.74±0.55$^c$</td>
<td>24.87±1.05$^a$</td>
</tr>
<tr>
<td>2000</td>
<td>1.67±0.11$^d$</td>
<td>8.97±0.88$^d$</td>
</tr>
<tr>
<td>4000</td>
<td>0.00$^e$</td>
<td>0.00$^e$</td>
</tr>
</tbody>
</table>

Cultures were carried out at 30°C for 7 days using an inoculum size of 1 mL/50 mL medium. Calculated mean is for triplicate measurements from two independent experiments ± SD, $^a$-$^e$ means with different superscripts in the same column are considered statistically different (LSD test, $P \leq 0.05$).
Highlights

- Cobalt ferrite nanoparticles were synthesized using culture filtrate of the fungus *Monascus purpureus* as a potentially low-cost, eco-friendly and easy to produce method.
- The synthesized nanoparticles showed promising antioxidant, anticancer and antimicrobial activities.
- Gamma irradiation at specific doses enhanced the synthesized nanoparticles production process.
- The successful production of cobalt ferrite nanoparticles in this study at high yields could open up the way for the industrial manufacture of nanoparticles.
Conflict of interest The authors declare that they have no conflicts of interest.