

Research Article

Green Synthesis by Berberine and Biological Evaluation of Fe₂O₃ and FeS Nanoparticles

Fatima. A. Al-Hamd and Mustafa Hammadi

Department of Chemistry, College of Education for Pure Sciences, University of Diyala, IRAQ



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Abstract

This study demonstrates a green and cost-effective method for fabricating crystalline hematite Fe₂O₃ and iron sulfide FeS nanoparticles using a bioactive alkaline berberine extract. The berberine-coated nanoparticles exhibited high structural integrity and excellent colloidal stability, supported by strong negative zeta potentials of -28.2 mV Fe₂O₃ and -30.9 mV FeS. Biologically, both nanostructures demonstrated potent concentration-dependent antimicrobial activity against *S. aureus* and *Escherichia coli*: the Fe₂O₃ nanoparticles specifically targeted *S. aureus* at a minimum inhibitory concentration (MIC) of 32 µg/ml (a concentration below the MIC of 16 µg/ml). FeS NPs exhibited balanced activity against both strains at a minimum inhibitory concentration (MIC) of 64 µg/ml (lower MIC: 32 µg/ml). Furthermore, ferric oxide NPs demonstrated superior antioxidant capacity as measured by DPPH (88.4% inhibition vs. 60.5% for ferric sulfide). Importantly, in vitro assays confirmed minimal cytotoxicity (0.52% for ferric oxide and 0.89% for ferric sulfide) at therapeutic concentrations, making them promising platforms for biomedical applications.



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Corresponding Author: Fatima A. Department of Chemistry, College of Education for Pure Sciences, University of Diyala, Iraq. Email: pch.fatimahamdi@uodiyala.edu.iq



1. INTRODUCTION

The rapid global emergence of multidrug-resistant (MDR) bacterial pathogens has significantly reduced the clinical efficacy of conventional antibiotic treatments [1]. The defense mechanisms in strains such as *Staphylococcus aureus* and *Escherichia coli*, including biofilm formation and enzymatic degradation, underscore the need for nontraditional antimicrobial agents [2]. Furthermore, reactive oxygen species (ROS) is a primary factor in chronic inflammation and tissue degeneration [3]. The development of multifunctional nanoplateforms that integrate bactericidal properties with antioxidant defense mechanisms is a key focus in contemporary nanomedicine [4]. Green nanotechnology offers a sustainable, economical, and biocompatible alternative to conventional chemical synthesis methods that often use toxic reagents [5]. This approach leverages the phytochemicals in plant extracts, such as polyphenols, flavonoids, and alkaloids, as reducing and stabilizing agents [6]. The quinoline alkaloid berberine possesses intrinsic antimicrobial, anti-inflammatory, and antioxidant properties [7]. Its rigid molecular structure supports controlled crystal growth, enabling the functionalization of nanoparticle surfaces to create a bio-interface with an enhanced therapeutic index [8]. Transition metals, such as Fe₂O₃ and FeS, are increasingly being studied for nanopharmaceutical applications due to their polyvalent oxidation states, chemical stability, and high surface-to-volume ratios [9]. Comparative analyses of oxide- and sulfide-based matrices provide deep insights into how crystal lattices and anionic electronegativity influence ion release and radical-scavenging dynamics [10].

While Fe₂O₃ interfaces support efficient electron transport, the FeS network affects cell membrane permeability [11]. Therefore, this study aims to develop an environmentally friendly fabrication protocol using a berberine template to produce stable Fe₂O₃ and FeS nanoparticles using a berberine template. We present a systematic assessment of their physical and chemical properties, antibacterial activity against MDR *Staphylococcus aureus* and *Escherichia coli*, antioxidant capacity, and in vitro biosafety profiles.



2. METHOD

2.1. Preparation of *Berberis vulgaris* (Barberry) Aqueous Extract

The seeds of *B. vulgaris* were purchased from a local market in Baghdad, Iraq, and were botanically authenticated by the Department of Biology, College of Education for Pure Science, University of Diyala. To ensure reproducibility, the seeds were thoroughly cleaned by rinsing three times with distilled water to remove surface impurities and then rinsed once with deionized water. The seeds were then dried in a hot air oven at 40 °C for 24 h until a constant weight was achieved and subsequently ground into a fine powder. The aqueous extract was prepared by dissolving 25 g of the seed powder in 250 ml of deionized water (1:10 w/v). The mixture was stirred for 2 h at 50 °C and 350 rpm on a magnetic stirrer for 2 h. Finally, the mixture was filtered through Whatman No. 1 filter paper to obtain a clear filtrate [12]. This extract was stored in an amber bottle at 4 °C in the dark to prevent photodegradation and was used for the green synthesis of Fe₂O₃ and FeS nanoparticles.

2.2. Green Synthesis of Iron Oxide Nanoparticles Fe₂O₃ NPs

For the synthesis of iron oxide nanoparticles, 4 g of iron (III) chloride hexahydrate (FeCl₃ · 6H₂O, 0.59 M) was completely dissolved in 25 mL of the prepared Berberine extract. A basic solution was prepared by dissolving 1 g of sodium hydroxide (NaOH, 1.0 M) in 25 mL of the Berberine extract. The iron solution was placed on a magnetic stirrer, and the NaOH solution was added dropwise under constant stirring for 30 min at 60 °C until the pH of the mixture reached pH 7. After 15 min of ultrasonic treatment in an ultrasonic bath, the mixture was continuously stirred at 90-100 °C for 1 h. Vacuum filtration was then used to isolate the precipitate, and ash-free Whatman Grade 42 quantitative filter paper was used to retain the nanoparticles. The collected residue was thoroughly rinsed with ethanol (twice) and deionized water (three times) before being dried in an oven at 120°C for 6 hours. The dried sample was then calcined in an electric furnace at 600°C for 4 h to obtain stable hematite Fe₂O₃ nanoparticles free of carbonaceous residues.

2.3. Green Synthesis of FeS NPs

To prepare FeS nanoparticles in an environmentally friendly manner, 4 g of hydrated ferric chloride (FeCl₃·6H₂O) at 0.59 M was dissolved in 25 mL of the prepared berberine extract. A sodium sulfide solution was prepared by dissolving 3 g of 0.50 M hydrated sodium sulfide (Na₂S·9H₂O) in 25 mL of the extract. Continuous magnetic stirring at 60°C for 30 min; sodium sulfide solution was added dropwise while maintaining a pH of 7. The mixture was ultrasonicated for 15 min and then heated to 90-100 °C for about 1 h with stirring. Then, the black precipitate was isolated by vacuum filtration using ash-free quantitative filter paper (Whatman 42), successively washed with ethanol (twice) and deionized water (three times) successively to remove unreacted reactant, and then dried in an oven at 120°C for 6 h. Pure, crystalline iron sulfide nanoparticles were obtained after calcination in a furnace at 400°C for 4 hours, pure, crystalline iron sulfide nanoparticles were obtained, free of carbonaceous or papery residues.

2.4. MIC and sub-MIC levels of antibacterial activity

To assess the effectiveness of antibacterial agents against MDR and *E. coli* strains, the lowest inhibitory concentration (MIC) and sub-inhibitory thresholds (Sub-MIC) of bioactive nanoparticles Fe₂O₃ and FeS were stimulated using a standard broth microdilution assay mediated by Resazurin [13]. The nanoparticle solutions were serially diluted in 96-well microplates containing preformed Mueller-Hinton broth (MHB) to a characteristic concentration gradient. Every well will receive a standardized bacterial inoculum adjusted precisely to 5 × 10⁵ CFU/mL, according to the 0.5 McFarland turbidity standard [14]. Microbial metabolic viability was assessed by adding 10 µL of sterile resazurin solution (0.02 % w/v) to each well and incubating the plates at 37 °C for approximately 18–24 h. The MIC was the lowest concentration of the nanoparticles that prevented the resazurin color change from blue (no growth) to pink (active growth). Sub-MIC parameters were modified to half of the determined MIC (0.5×MIC) to obtain the sublethal dosage. The reliability and validity of the assay and the negative and positive controls were proved. The positive control (C+) contained the bacterial inoculum without nanoparticles to prove microbial viability, whereas the negative control (C–) contained the nanoparticles in the broth without inoculum to confirm the sterility and optical baseline of the nanoparticles.

2.5. In vitro antioxidant activity (DPPH assay)

The scavenging ability of the Fe₂O₃ and FeS nanoparticles was quantitatively determined using the stable 2,2-diphenyl-1-picrylhydrazyl (DPPH) radical assay [11]. The reaction mixtures (equal volumes of the serial dilutions of nanoparticles (31.25–1000 µg/mL in analytical methanol) and a freshly prepared methanolic DPPH solution (0.1 mM)) were incubated in total darkness at 25 °C for 30 min. Ascorbic acid was used as the standard reference control, and DPPH solution without nanoparticles was used as the baseline. The DPPH radical was reduced, as indicated by a color change from purple to yellow and confirmed spectrophotometrically at 517 nm [14]. The efficiency of DPPH scavenging was calculated using the following equation:

$$\text{DPPH Scavenging Activity (\%)} = \frac{A_{\text{control}} - A_{\text{sample}}}{A_{\text{control}}} \times 100 \quad (1)$$

Where A control and A sample are the absorbances for the control and nanoparticle standard treatments, respectively.

2.6. Toxicity screening and biosafety in vitro

To evaluate the biocompatibility and establish the biosafety profiles of the green-synthesized Fe₂O₃ and FeS nanoparticles, an in vitro toxicity screening was conducted at their determined bacterial MIC [15]. Briefly, the nanoparticle treatments were incubated with cell suspensions for 1 h at 37°C to monitor potential membrane disruption and cellular damage. The mixtures were incubated and then centrifuged at 3000 rpm for 10 min, and the supernatant was separated.

The extent of cellular toxicity and biochemical damage was measured colorimetrically by measuring the supernatant absorbance at 540 nm using a UV-Vis spectrophotometer [16]. Phosphate-buffered saline (PBS) was used as the negative control (C-, representing 0% baseline toxicity) was phosphate-buffered saline (PBS), while specific lysing agents or Triton X-100 were used as the positive control (C+, representing 100% complete cellular destruction). Quantitative toxicity percentages were calculated using standard optical density formulas to verify the overall cytocompatibility of the fabricated nanocores before safe biomedical translation [11]. The hemolysis percentage was calculated as

$$\text{Hemolysis (\%)} = \frac{A_{\text{sample}} - A_{\text{negative control}}}{A_{\text{positive control}} - A_{\text{negative control}}} \times 100 \quad \dots\dots (2)$$

3. RESULTS AND DISCUSSION

3.1. FT-IR Spectroscopy of Fe₂O₃ and FeS Nanoparticles

FT-IR spectroscopy was used to investigate the surface functional groups and chemical frameworks of the green-synthesized NPs. (Figure 1) shows the FT-IR spectrum of the iron oxide Fe₂O₃ nanoparticles, where two characteristic bands at 540–580 cm⁻¹ and 440–470 cm⁻¹ are observed, suggesting the successful formation of the Fe₂O₃ phase. These bands correspond to the Fe–O stretching vibrations in the octahedral and tetrahedral environments of the Fe₂O₃ lattice [14, 15]. (Figure 2) shows the FT-IR spectrum of the FeS nanoparticles, which indicates successful phase formation through a major sharp absorption peak in the lower fingerprint region at about 450–490 cm⁻¹, specifically attributed to the characteristic Fe–S stretching vibrations in the crystalline metal-sulfide network [17]. Both spectra retained the organic signatures of the stabilizing berberine biomolecules; common peaks at 3400–3440 cm⁻¹ suggest –OH stretching of phenolics, 1620–1650 cm⁻¹ for C=C aromatic ring stretching, and 1380–1410 cm⁻¹ indicate C–N stretching of the is quinoline alkaloid skeleton, respectively [6]. The presence of these vibrational bands across the nanoparticle surfaces suggests that the plant-derived phytochemical constituents facilitate stabilization and reduction during bio-fabrication.

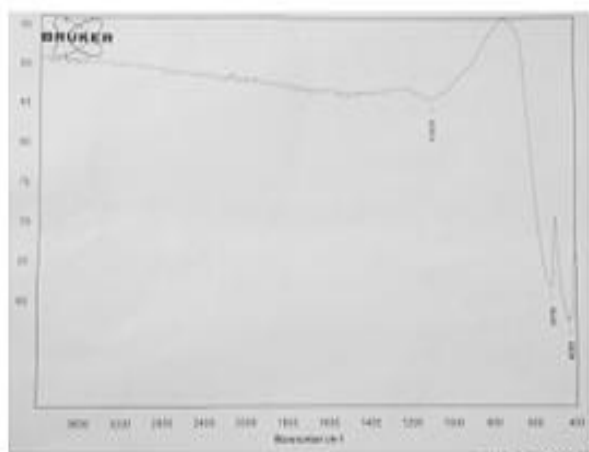


Figure (1). FT-IR analysis of berberine-mediated Fe₂O₃

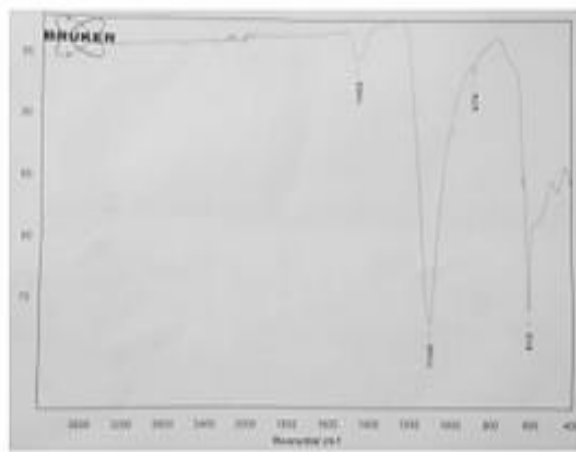


Figure (2). FT-IR analysis of berberine-mediated FeS

3.2. Structural Analysis of Nanoparticles Fe₂O₃ and FeS by XRD

The crystalline phase purity and structural parameters of the biogenic nanoparticles were determined by X-ray diffraction (XRD). The XRD diffractogram of the iron oxide Fe₂O₃ nanoparticles (Figure 3) was consistent with the standard entry number 96-154-6384, indicating a pure trigonal (hexagonal axes) hematite phase Fe₂O₃, space group R-3c with lattice dimensions a = 5.0300. Å and c = 13.7500 Å [18]. On the contrary, the XRD profile of FeS nanoparticles (Figure 4) was consistent with the standard entry number 96-900-7653, indicating a pure hexagonal Troilite phase FeS, space group P-62c with lattice constants a = 5.8610 Å and c = 11.5770 Å [19]. Neither sample showed any additional peaks attributable to unreacted chemical precursors or impurities, indicating high phase purity. The average crystallite sizes (D) of trigonal Fe₂O₃ and hexagonal Troilite FeS nanoparticles were calculated as 41.17 and 44.13 nm, respectively, using the Debye-Scherrer formula [20, 21]. The obtained nanoscale crystallite sizes suggest the efficient spatial confinement of crystal growth by the bioactive compounds in the BER extract, which act as effective capping and stabilizing agents in the green synthesis protocol [22].

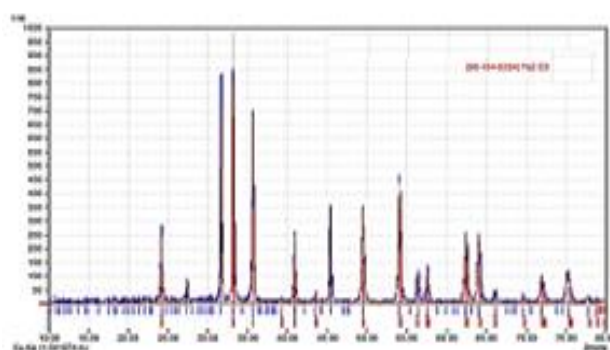
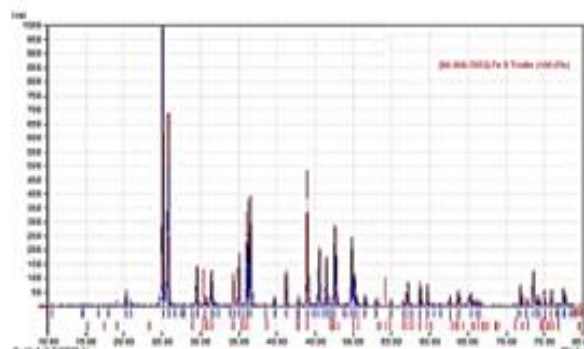
Figure (3). XRD pattern of berberine-capped Fe_2O_3 

Figure (4). XRD pattern of berberine-capped FeS

3.3. EDX elemental analysis of Fe_2O_3 and FeS NPS

The elemental composition and chemical purity of the green-synthesized NPs were assessed using energy-dispersive X-ray spectroscopy. The EDX plot of the Fe_2O_3 nanoparticles (Figure 5) shows peaks of iron and oxygen, which is consistent with the formation of Fe_2O_3 nanoparticles [21]. (Figure 6) shows the spectra of the iron sulfide nanoparticles. The distinct signals corresponding to iron and sulfur confirm the successful formation of FeS nanoparticles [22]. The absence of impurity peaks in both spectra indicates the high purity of the NPs. Weak signals for C and N were observed in both samples. The presence of these bioactive organic compounds supports the FT-IR results, suggesting that quinoline alkaloids and phenolic macromolecules from the berberine-rich extract bind to the nanoparticle surfaces, forming stable coating shells [23].

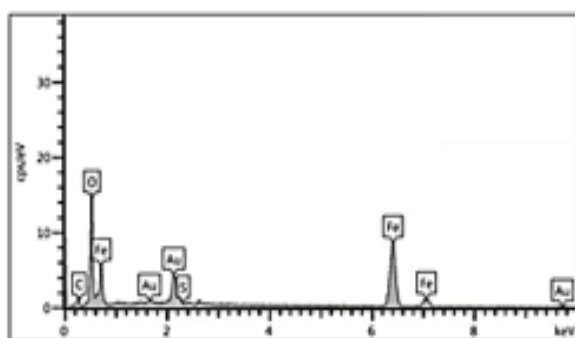
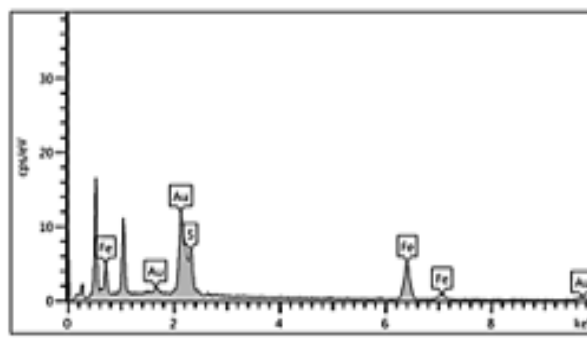
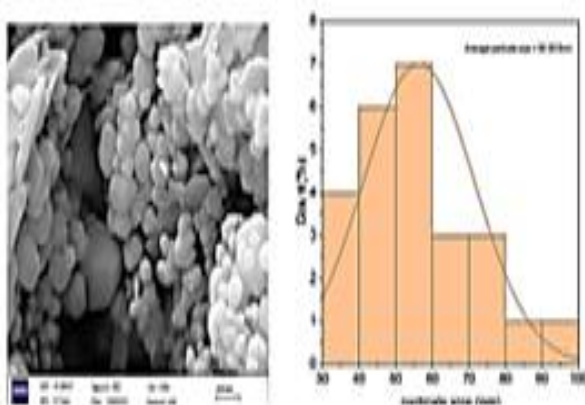
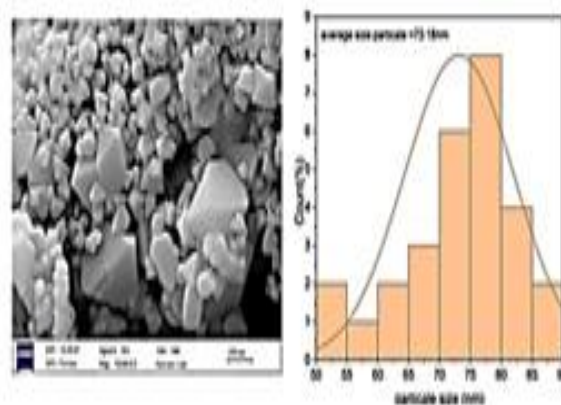
Figure (5). EDX spectrum of berberine-mediated Fe_2O_3 

Figure (6). EDX spectra of berberine-mediated FeS

3.4. SEM Morphological Analysis of Fe_2O_3 and FeS Nanoparticles

SEM was employed to characterize the surface morphology and topography of the bio-synthesized nanoparticles, as illustrated in (Figures 7 and 8). The SEM images reveal that the iron oxide Fe_2O_3 nanoparticles exhibit a characteristic spherical shape with an average grain diameter of 56.36 nm. The FeS nanoparticles exhibited a denser and more granular morphology with an average particle size of 73.18 nm. A natural tendency toward spatial aggregation, driven by inherent magnetic dipole interactions between particles and high surface energy constraints [24, 25]. The observed surface irregularities are consistent with the presence of a bio-organic capping layer originating from the biosynthesis of the berberine-rich extract used during biosynthesis.

Figure (7). SEM analysis of berberine-mediated Fe_2O_3 

Figure(8) SEM analysis of berberine-mediated FeS

3.5. Stability analysis of the zeta potential of Fe₂O₃ and FeS nanoparticles

The surface charge and colloidal stability of the green-processed nanomaterials were evaluated by zeta potential analysis (Figures 9 and 10). The bio-based Fe₂O₃ nanoparticles exhibited a negative surface charge of -28.2 mV [26], and the FeS NPs exhibited a more pronounced negative potential of -30.9 mV [27]. These large negative values suggest strong electrostatic repulsion between the dispersed nuclei, potentially preventing agglomeration and flocculation in the aqueous medium. This negative profile indicates that the successful coating of the nanoparticles with the berberine extract's hydroxyl and amine functional groups of the berberine extract provides a stable protective layer on their surface [28].

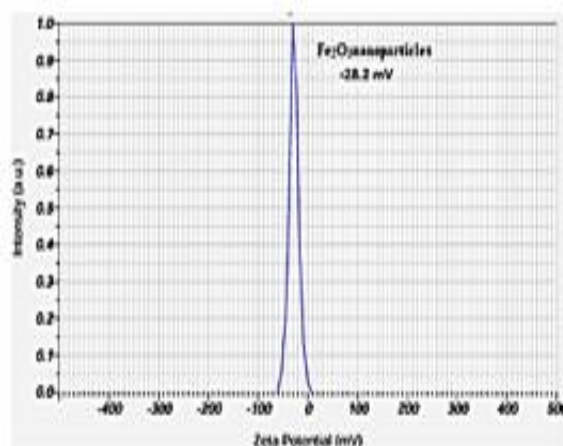


Figure (9). Zeta potential of berberine-mediated Fe₂O₃

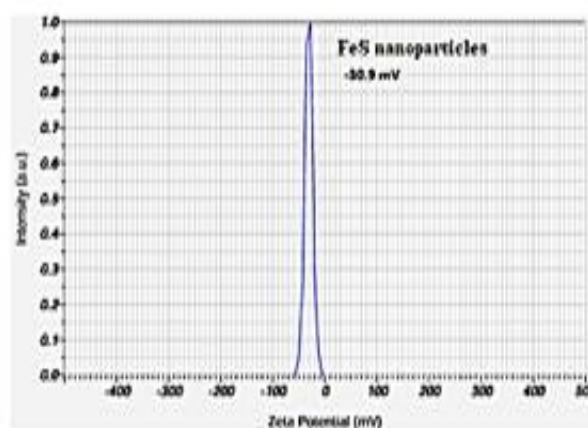


Figure (10). Zeta potential of berberine-mediated FeS

3.6 Evaluation of antibacterial and sub-MICs

Determined quantitatively using a resazurin-based micro-calibration plate method, the antibacterial activity of bio-nanoparticles Fe₂O₃ and FeS (Table 1). Broth microdilution assays proved that the green-processed Fe₂O₃ nanoparticles were more active against *S. aureus*, with the lowest inhibitory concentration of 32 µg/ml (Sub-MIC: 16 µg/ml), which required a higher threshold of 256 µg/ml (Sub-MIC: 128 µg/ml) to inhibit *E. coli* [13]. In addition, the bioactive FeS nanoparticles showed homogeneous antibacterial activity, yielding identical MIC values of 64 µg/ml (below the sub-MIC of 32 µg/ml) against both strains [14]. This variation in sensitivity is related to the structure of the cell wall. The outer lipopolysaccharide membrane of Gram-negative Escherichia coli acts as a selective barrier that restricts the flow of nanoparticles, while the porous peptidoglycan matrix of Gram-positive Staphylococcus aureus allows for clearer physical penetration [18]. Mechanistically, the synergistic interface between the metal cores and the bioactive Berberine coating shell triggers membrane depolarization, lipid peroxidation, and intracellular ROS generation, culminating in systemic oxidative stress and cell death [24, 25]. The bound phytochemical matrix acts as a localized biochemical pressure, effectively down-regulating bacterial virulence and preventing biofilm formation [26].

Table (1). Antibacterial MIC and Sub-MIC parameters of biogenic nanoparticles against *S. aureus* and *E. coli*.

Nono-compound	<i>S. aureus</i>		<i>E. coli</i>	
	MIC	Sub-MIC	MIC	Sub-MIC
FeS nanoparticles	64 µg/ml	32 µg/ml	64 µg/ml	32 µg/ml
Fe ₂ O ₃ nanoparticles	32 µg/ml	16 µg/ml	256 µg/ml	128 µg/ml

3.7. Antioxidant Activity of Fe₂O₃ and FeS Nanoparticles

The antioxidant profiles of the biogenic nanoparticles were quantitatively assessed at three concentrations (High, MIC, and Low) using DPPH radical scavenging analysis (Table 2). Both nanomaterials exhibited concentration-dependent responses; however, Fe₂O₃ nanoparticles demonstrated significantly higher scavenging performance than their FeS counterparts. Fe₂O₃ nanoparticles at the highest concentration achieved an inhibition rate of 88.4% (Absorbance: 0.204), retaining 86.6% at the MIC level [19]. In comparison, FeS NPs exhibited lower antioxidant activity, reaching a maximum of 60.5% (absorbance: 0.694) at high concentrations and decreasing to 32.6% at the lowest dilution [30]. It is hypothesized that the improved radical scavenging capacity of the Fe₂O₃ nanocores is driven by efficient electron transfer kinetics between the shifting iron oxidation states (Fe²⁺/Fe³⁺) and free radicals [27]. Furthermore, this performance may be enhanced by the berberine extract's synergistic capping layer, where bound phenolic and alkaloid macromolecules potentially act as efficient hydrogen atom donors [11, 23]. These results, which are based specifically on DPPH assays, provide insights into the antioxidant potential of the synthesized nanoparticles but are not broadly generalizable to all oxidative stress models.

Table (2). Radical scavenging capacity of Fe₂O₃ and FeS nanoparticles.

Nono-compound	A (High)	A (MIC)	A (Low)	%Inhib (High)	%Inhib (MIC)	%Inhib (Low)
FeS nanoparticles	0.694	0.939	1.183	60.5	46.5	32.6
Fe ₂ O ₃ nanoparticles	0.204	0.236	0.416	88.4	86.6	76.3
C+	0.122					
C-	1.756					

3.8. In vitro toxicity evaluation

To confirm the biosafety of the green-synthesized nanomaterials, an in vitro toxicity assay was conducted at their respective bacterial MICs (Table 3). Both biogenic architectures exhibited negligible toxicity, well below standard cytotoxicity thresholds, establishing an outstanding biocompatibility profile. FeS NPs show low toxicity of 0.89% at a MIC of 64 µg/mL. Fe₂O₃ nanoparticles show an even lower toxicity of 0.52% at an effective MIC of 256 µg/mL. This green synthesis method, which uses berberine-rich plant extract, effectively protects cellular components from oxidative damage and exhibits significant metal-ion chelation properties [11, 23]. These minimal toxicity values indicate that both Fe₂O₃ and FeS NPs are safe at bactericidal concentrations, validating their suitability for biomedical applications and drug delivery [26].

Table (3). Biosafety and toxicity profiles of nanoparticles synthesized at MIC levels

Nano-Compound	Concentration (MIC)	Absorbance	Toxicity	Interpretation
FeS nanoparticles	64 µg/ml	0.017	0.89%	Non-toxic
Fe ₂ O ₃ nanoparticles	256 µg/ml	0.010	0.52%	Non-toxic
C+		1.892	100%	Completely toxic
C-		0	0%	Non-toxic

4. CONCLUSION

This study confirms a green synthesis method for the preparation of iron oxide Fe₂O₃ and iron sulfide FeS nanoparticles. This method was eco-friendly, cost-effective, and efficient, using the bioactive phytochemicals of BER. Characterization verified the formation of highly stable nanocrystalline structures. Zeta potential determinations indicated excellent colloidal and electro-steric stability of the organic layers, with negative surface charges of -28.2 and -30.9 mV for Fe₂O₃ and FeS NPs, respectively. Biologically, the green-synthesized nanomaterials exhibited concentration-dependent antibacterial activity against gram-positive (*S. aureus*) and gram-negative (*E. coli*) pathogens, as determined by resazurin-based microtiter assays. Interestingly, Fe₂O₃ nanoparticles were more potent against *S. aureus* (MIC of 32 µg/mL), whereas the FeS nanoparticles were more balanced and evenly bactericidal (MIC of 64 µg/mL) against both test strains.

Furthermore, the Fe₂O₃ configurations demonstrated excellent antioxidant and radical-scavenging properties, with an inhibition efficiency of 88.4% at the highest tested concentration, representing a substantial improvement over the FeS counterparts (60.5%). Importantly, in vitro toxicity screens demonstrated that both fabricated nanostructures have negligible toxicity profiles (well below 1%) at their therapeutic antimicrobial concentrations, establishing a safe biocompatibility threshold. Taken together, these promising findings indicate that berberine-mediated green synthesis offers a sustainable pathway for fabricating metal chalcogenides and generating highly potent, biocompatible, and multifunctional nanoplateforms for advanced biomedical, pharmaceutical, and targeted drug-delivery applications.

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BIOGRAPHIES OF THE AUTHORS

	Dr. Mustafa Abdul Majeed Hameed Hammadi is a Professor at the College of Education for Pure Sciences, University of Diyala, Iraq. He earned his B.Sc. in Chemistry from the University of Baghdad, his M.Sc. from the University of Technology (Iraq), and his Ph.D. in Nanoscale Inorganic Chemistry from Saint Petersburg State University, Russia. His research primarily focuses on the synthesis, characterization, and biological applications of inorganic nanomaterials. He has authored numerous research papers published in peer-reviewed national and international journals and conference proceedings. He can be contacted by email: mustafa.hameed@uodiyala.edu.iq
	Fatima. A. Al-Hamdi is a Master's student at the Department of Chemistry, College of Education for Pure Science, University of Diyala, Iraq. He received a B.Sc. in Chemistry from the College of Education for Pure Sciences, Ibn Al-Haytham University of Baghdad. His research interests include nanoscale inorganic materials, their characterization, and biological effectiveness studies. He can be contacted via email. pch.fatimahamdi@uodiyala.edu.iq