Synthesis of iron oxide (β-Fe$_2$O$_3$) nanoparticles from Iraqi grapes extract and its biomedical application

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Abstract. A synthesis of iron oxide (β-Fe$_2$O$_3$) nanoparticles (NPs) made using a simple chemical method from a mixture of iron (III) chloride (FeCl$_3$) solution and Iraqi grape extract has applications in the biomedical field. Iraqi grape extract was used to reduce iron (III) chloride salt to iron oxide (β-Fe$_2$O$_3$) NPs. The green synthesis method was cheap, non-toxic, safe, and eco-friendly. The iron oxide (β-Fe$_2$O$_3$) NPs were diagnosed using x-ray diffraction (XRD), Fourier transform infrared spectrophotometry (FT-IR), ultraviolet spectrophotometry (UV-VIS), and scanning electron microscopy (SEM). The UV-VIS spectrophotometry analysis showed the energy gap (E$_g$) was 2.9 eV. The peak of strong absorption at 526 cm$^{-1}$ indicated that a Fe-O vibration band was reported on the FT-IR spectrum. The XRD showed the highest peaks at 102 and 222, with average crystallize sizes between 29–37 nm. Besides, XRD spectrum analysis revealed a cubic structure. The surface morphology of the sample, which was identified using the SEM analysis, found the average grain size was from 49 to 50 nm with a cubic shape. After investigating the inhibition of zones, the synthesized (β-Fe$_2$O$_3$) NPs showed antibacterial activity of 18 mm for positive-gram aureus staphylococcus bacteria and 19 mm for negative-gram Escherichia coli bacteria.

1. Introduction
The most significant elements of nanotechnology in structural masses are nanoparticles NPs [1]. NPs are microscopic atomic or partial groupings that consist of a specific number of atoms, ranging from diameters of 1-100 nm, that are made from materials that are either organic or inorganic in nature. They have novel properties when compared to manufactured and bulk materials [2], [3]. Metal NPs have gained significant importance among the various NPs; due to their extremely high aspect ratio, their nature is unique. Iron oxide NPs have become quite vital due to their environmentally friendly nature [4], catalytic activity [5], low-cost [6], non-toxicity[7], and biocompatibility [8], [9]. Iron oxide NPs are also useful because of their availability, robustness, and high surface area. Additionally, they have wide applications in industry, including biosensors, magnetic storage, biotechnology, biomedicine, catalysis, thin films, drug delivery, and hyperthermia [10], [11]. Green synthesis is a growing technique and has been adopted by many researchers due to its cost-effectiveness, safety, eco-friendliness, non-toxicity and cleanliness [12]. There have been many successful attempts in preparing iron oxide NPs and taking these oxides in many applications from natural sources during recent years.

Venkateswarlu et al. [5] used plantain peel extract to synthesise iron oxide NPs of 30–50 nm using a sol-gel method. They fostered these NPs for removal of a toxic metal in wastewater applications.
Luo et al. [7] produced iron oxide NPs with a chemical synthesis technique using grape leaves for biomolecular application and proved that grape extract works as a stabilizer and reducer of nano-sized particles. Pattanagak et al. [13] prepared iron oxide NPs of 50–100 nm in diameter by applying coffee extract via a simple chemical method. They used a precursor made from a mixture of 0.001 M of an aqueous solution of iron Ferric (III) chloride (FeCl$_3$) in triple-distilled water with 25 g of coffee extract in 5–10 ml of deionized water. Yew et al. [14] reported that they managed to synthesis a highly pure phase of the prepared k. alvarezi/iiron oxide NPs from kappahycus alvaerzi extract. Sathishkumar et al. [15] revealed that iron oxide NPs under 80 nm were polydispersed with a spherical shape when synthesized by a chemical method using Couroupita guianensis Aubl. They utilized these NPs in antibacterial and vitro anticancer activity applications. Jafarirad et al. used a co-precipitation method to prepare semi-spherical iron oxide NPs from the extract of tea leaves for biological applications [16].

Using the grapes extract as a capping and reducing agent, the green synthesis of iron oxide NPs might open a new window towards gaining cheaper, cleaner, and more eco-friendly methods of producing NPs. Moreover, as plant materials are readily available, green synthesis has achieved more dedicated interest from industries today due to its significant pharmacological applications. Nanotechnology has been expected to be a key factor in the biomedical sector because it allows the prevention and fight against diseases using materials based on an atomic scale. Recently, iron oxide NPs have been proven to exhibit remarkable biostatic and biocidal actions to gram-positive and gram-negative bacteria [17].

To the best of the authors' knowledge, there is a lack of reports, studies, and a gap of knowledge about production of iron oxide NPs via Iraqi grape extract for biomedical application, encouraging us to be engaging in this study. In this study, and for the first time, we managed to synthesise iron oxide (β-Fe$_3$O$_4$) NPs from Iraqi grapes extract using a simple chemical method for biomedical application. We used the grape extract as a stabilizing and reducing agent. We applied the produced iron oxide (β-Fe$_3$O$_4$) NPs against gram-positive (S. aureus) and gram-negative (E. coli) bacteria to show their activity.

2. Materials and Methods

2.1 Materials Used

The raw materials were 99% iron (III) chloride (FeCl$_3$) from Sigma-Aldrich, grapes purchased from the local market (in Baghdad, Iraq), and Borosil glassware. All solutions were prepared in deionized water without further purification.

2.2 Grapes Extract Preparation

The grapes extract was prepared by cleaning, washing the grapes and removing their inner seeds. The fresh grapes were dried in an oven at 100 °C for 3 hours, then the dried grapes were ground to powder using an electrical grinder. An amount of 10 g grape powder was mixed with 200 mL of distilled water for 2 hours at 80°C using a magnetic stirrer. The mixture was cooled to room temperature, filtered through a cotton species, and then stored in a refrigerator to isolate the grape extract from impurities. The supernatant fluid was collected and then stored at room temperature in a tube of a hermetic-capped falcon for further fresh usage (not exceeding 2 to 3 days).

2.3 Iron oxide (β-Fe$_3$O$_4$) NPs Preparation

Synthesis of iron oxide (β-Fe$_3$O$_4$) NPs occurred after mixing of 16.2 g of iron (III) chloride (FeCl$_3$) in 100 mL of distilled water with 200 mL of prepared grape aqueous solution and putting it on a magnetic stirrer at 80°C for 30 minutes. The colour of the solution changed from purple to black, indicating the formation of iron oxide NPs. The solution was cooled to room temperature and then thermally treated in an oven at 200°C for 2 hours to produce nanopowder. We diluted the nanopowder with distilled water and took a few drops and deposited them on clean glass substrates to perform further characterization testing of the NPs. Figure 1 shows a flowchart of (β-Fe$_3$O$_4$) NPs.
2.4 Characterization

2.4.1 UV-Visible spectrophotometer analysis of (β-Fe\textsubscript{2}O\textsubscript{3}) NPs

The UV-VIS transmission of the iron oxide (β-Fe\textsubscript{2}O\textsubscript{3}) NPs was diagnosed using a double-beam UV-VIS spectrophotometer (UV-1800, Shimadzu) between the range of 200 to 900 nm.

2.4.2 FT-IR and XRD Analysis of (β-Fe\textsubscript{2}O\textsubscript{3}) NPs

FT-IR and XRD analyses were conducted on the ready (β-Fe\textsubscript{2}O\textsubscript{3}) powder. An advanced x-ray diffractometer (Bruker AXS D8) was used to provide the XRD patterns with a 2θ (10º–80º) scanning angle, Cu Kα radiation, λ=1.5406 Å operated at 40 kV and 30 mA to find the crystalline size using Scherrer’s equation [18-19]:

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

where \( \beta \) is in radian is the full width at half maximum (FWHM), D is the size of crystallite in (nm), K constant = 0.94, and \( \theta \) = Bragg’s angle. For characterizing the NPs with FT-IR analysis, the type of target used for the device was the KBr pellet technique with a spectrum of 400-2000 cm\(^{-1}\).

2.4.3 SEM analysis of (β-Fe\textsubscript{2}O\textsubscript{3}) NPs

SEM was conducted using the Tescan Mira3 FESEM (Czechia) in Mashhad, Iran. The analysis revealed characteristics of the microstructure such as particle size and morphology at (10, 30) KX magnifications and accelerating voltage of 15.00 kV.

2.5 Antibacterial activity

Antibacterial activity of the synthesized β-Fe\textsubscript{2}O\textsubscript{3} was tested using the well diffusion technique against E. coli. New bacterial pathogens of 24-hour cultures on agar plates were prepared. For the antibacterial assay, standardized inoculums (McFarland No. 0.5) were used. The (β-Fe\textsubscript{2}O\textsubscript{3}) powder was dissolved in sterile distilled water and sonicated. The (β-Fe\textsubscript{2}O\textsubscript{3}) solution, having 30 mg/mL concentration, was loaded in two wells on plates and incubated for 24 hours at 37° C before the inhibition zone was calculated [15].

3. Results and Discussions

UV-VIS spectra analysis at wavelengths of 200–900 nm was achieved to study the transmission of wavelengths and the energy gap of green synthesized β-Fe\textsubscript{2}O\textsubscript{3}. Figure 2 shows the optical transmittance spectra for the (β-Fe\textsubscript{2}O\textsubscript{3}) NPs, which were prepared by a simple chemical method using grapes extract, starting from 300 nm. According to Figure 3, the value of the energy gap was 2.9 eV for the (β-Fe\textsubscript{2}O\textsubscript{3}) NPs [20]. Table 1 shows the values of the energy gap of the (β-Fe\textsubscript{2}O\textsubscript{3}) NPs.
Figure 2. UV-VIS transmission spectra of (β-Fe₂O₃) nanoparticles as-prepared.

Figure 3. Energy band gap of (β-Fe₂O₃) nanoparticles as-prepared.

Table 1. Optical measurements of (β-Fe₂O₃) NPs by a simple chemical method using the grape extract.

| Plant extract | Material   | Energy gap (nm) |
|---------------|------------|-----------------|
| Grape         | β-Fe₂O₃    | 2.9             |

The XRD diffraction patterns of the synthesized (β-Fe₂O₃) NPs' films were created by a simple chemical method at 80º C for 2 hours using the grape extract. The crystalline phase of the contagious (β-Fe₂O₃) NPs was examined by x-ray diffraction (XRD-6000). Figure 4 displays the XRD patterns of the (β-Fe₂O₃) NPs deposited on the glass substrate. It is possible to apply the plant extracts as stabilizing and reducing agents to get pure (β-Fe₂O₃) NPs. This might be due to the phytochemical responsible for the quick reduction of iron (III) chloride (FeCl₃) to β-Fe₂O₃ NPs in an environmentally and eco-friendly single step. As shown in Figure 4, (β-Fe₂O₃) NPs were noticed at 20 deg. = 15.9º, 20.7º, 30.9º, 32.4º, 37.5º, 38.8º, 42.3º, 43.36º, and 57.8, corresponding to each Bragg reflection 102, 211, 113, 222, 116, 119, 207, 201,
and 309. It could be indexed as a (cubic) structure of (β-Fe₂O₃) NPs per standard JCPD (00-040-1139) data. It confirmed the phase formation for a single crystallite of cubic β-Fe₂O₃. The crystalline size of iron oxide (β-Fe₂O₃) was from 29 to 34 nm. Table 2 shows the value of the crystalline size of (β-Fe₂O₃) NPs.

![Image of XRD patterns](image)

**Figure 4.** Patterns of XRD for (β-Fe₂O₃) NPs.

**Table 2.** Results of XRD for (β-Fe₂O₃) NPs.

| Plant Extract | Material  | Crystallite size D (nm) |
|---------------|-----------|-------------------------|
| Grape         | β-Fe₂O₃   | 34                      |
|               |           | 29                      |

The FT-IR spectroscopy proved the formation of nanoparticles, as shown in Figure 5, which shows the FT-IR spectra of the (β-Fe₂O₃) NPs. The peaks of strong absorption at 526 cm⁻¹ were assigned to the band vibrations of Fe-O. The transition at 915 cm⁻¹ was confirmed to O-H bend. The bands at 1444 cm⁻¹ were due to C-H bending, or scissoring, in CH₃ groups or aromatic C–C stretching vibrations. The broad absorption peak band at 1634 cm⁻¹ was attributed to the bending fluctuations and stretching of the -C=C-stretch and water molecules [21], [22]. Table 3 shows the values of the absorption bands.
Figure 5. ($\beta$-$\text{Fe}_2\text{O}_3$) FT-IR spectrum.

Table 3. FT-IR absorption bands and possible assignment in the typical FT-IR spectrum of ($\beta$-$\text{Fe}_2\text{O}_3$) NPs as-prepared by a simple chemical method using a grape extract.

| Plant Extract | Material   | Bands (cm$^{-1}$) |
|---------------|------------|-------------------|
| Grapes        | $\beta$-$\text{Fe}_2\text{O}_3$ | 526               |
|               |            | 915               |
|               |            | 1444              |
|               |            | 1634              |

SEM images of the iron oxide ($\beta$-$\text{Fe}_2\text{O}_3$) NPs that were synthesized at 200ºC for 2 hours are shown in Figure 7. The ($\beta$-$\text{Fe}_2\text{O}_3$) particle size was different between 49 to 50 nm[4]. Besides, XRD spectrum analysis appeared a Cubic structure.

Figure 6. SEM images of ($\beta$-$\text{Fe}_2\text{O}_3$) particles using grape extract at 200 ºC for 2 hours at a) 10 Kx magnification. b) 30 Kx magnification.
3.1 Antibacterial Activity
The agar well diffusion technique was applied to study the grape extract stabilizer antibacterial activity of (β-Fe₂O₃) NPs. Figure 8 reveals the bacteria zones of inhibition. The pathogenic gram-positive bacteria, Staphylococcus aureus, and the gram-negative bacteria, Escherichia coli, were adopted to investigate the antibacterial effect of (β-Fe₂O₃) NPs because they cause foodborne illnesses [14]. Two different substances were used to kill bacteria by injecting them with 30 mg/mL of β-Fe₂O₃ NPs (2) and DI water (C). In Figure 8, the percentage of destruction on bacteria using (β-Fe₂O₃) NPs was 18 mm for gram-positive bacteria Escherichia coli and 19 mm for gram-negative bacteria Staphylococcus aureus. The (β-Fe₂O₃) NPs were highly effective in gram-negative bacteria Escherichia coli when compared to gram-positive bacteria Staphylococcus aureus. The (β-Fe₂O₃) NPs effectiveness in mechanical bacteria interaction was because the bacterial plasma membrane was negatively charged via the attraction of electromagnetism that bound with positively charged metal (β-Fe₂O₃) NPs [9]. This killed the microbes directly because the membrane oxidized when exposed to iron oxide (β-Fe₂O₃) NPs as a result of gravity. This attraction mechanism was identical to the binding of the negatively charged lipid membrane of vesicles to positively charged antimicrobial peptides [18-23]. Generally, the (β-Fe₂O₃) NPs released ions that reacted with the (-SH) thiol proteins groups in the bacterial cells that lead to the cell membrane and cell lysis [26], [27]. The antibacterial activity mechanism of (β-Fe₂O₃) NPs that damaged the DNA and proteins in the bacteria might be due to stress oxidation caused by the reaction of oxygen species that involved the radicals of singlet oxygen (¹O₂), hydrogen peroxide (H₂O₂), hydroxyl (-OH), and superoxide (O₂⁻). Table 4 illustrates the results of the inhibition zone of (β-Fe₂O₃) NPs against two types of bacteria at 30 (mg/mL) concentrations.

![Figure 7. Antibacterial activity of (β-Fe₂O₃) NPs against gram-positive (S. aureus) and gram-negative (E. coli) (2) β-Fe₂O₃, and (C) DI water.](image)

| Material       | Plant extract | Gram-positive (+) | Gram-negative (-) | Percentage of inhibition zone (%) | Control |
|----------------|---------------|-------------------|-------------------|-----------------------------------|---------|
| β-Fe₂O₃        | Grape         | S. aureus         | E. coli           | S. aureus                        | E. coli | 18 mm | 19 mm | 19.5 | 21.5 | 0                   |
4. Conclusions

In this paper, iron oxide (β-Fe₂O₃) NPs were created using grape extract and a simple chemical method at 200º C for 2 hours. Green synthesis succeeded in reducing and converting iron (III) chloride (FeCl₃) to iron oxide (β-Fe₂O₃) NPs. This method proved to be environmentally friendly, inexpensive, socially acceptable, safe, and economically available. XRD analysis showed the crystalline sizes of (β-Fe₂O₃) NPs were 34 nm and 29 nm. UV-VIS spectrophotometry explained that the value of the energy gap of (β-Fe₂O₃) NPs was 2.9 eV. The strong band of (β-Fe₂O₃) NPs at 526 cm⁻¹ referred to Fe-O within the visible area. The iron oxide (β-Fe₂O₃) NPs were used to eliminate both gram-positive (S. aureus) and gram-negative (E. coli) bacteria. When compared to other approaches, (β-Fe₂O₃) NPs prepared by using grape extract have the smallest size, the best crystalline quality, and the best value against bacteria application.

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