

# Green Synthesis of $\alpha$ -Fe<sub>2</sub>O<sub>3</sub> from Ginger Extract Enhanced the Potential Antioxidant Activity Against DPPH

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## Article Info

Received  
17/08/2022

Accepted  
02/10/2022

Published  
30/12/2022

## ABSTRACT

The synthesis of nanooxides in an easy and environmentally friendly way using simple and green materials is one of the hot topics of sustainable chemistry for many pharmaceutical and medical applications. Herein, we successfully synthesized  $\alpha$ -Fe<sub>2</sub>O<sub>3</sub> NPs ( $\alpha$ -Fe<sub>2</sub>O<sub>3</sub> NPs) using ginger extract. The prepared  $\alpha$ -Fe<sub>2</sub>O<sub>3</sub> NPs were examined using ultraviolet-visible reflection spectroscopy (UV-VIS), Fourier Transform Infrared Spectroscopy (FTIR), photoluminescence spectroscopy (PL), X-ray diffraction (XRD), field emission scanning microscopy (FE-SEM), energy-dispersive X-ray (EDX) spectroscopy, and zeta potential. After well characterizations, the potency of the prepared  $\alpha$ -Fe<sub>2</sub>O<sub>3</sub> NPs to monitor some scavenging activity was explored against DPPH. The results revealed that the intensity of the PL has one peak in the UV region between (480-490) nm of the spectrum depending on the geometric shape and size of the  $\alpha$ -Fe<sub>2</sub>O<sub>3</sub> NPs. The UV-visible spectra showed a peak at 296.0 nm, which represented the  $\alpha$ -Fe<sub>2</sub>O<sub>3</sub> NPs. The EDX micrograph confirmed pure oxide and the XRD pattern showed that the  $\alpha$ -Fe<sub>2</sub>O<sub>3</sub> NPs had an average crystal size (19.3) nm. SEM images of  $\alpha$ -Fe<sub>2</sub>O<sub>3</sub> NPs revealed irregular, rod, and spherical shapes and sizes ranging from (15 to 60) nm. Furthermore, the antioxidant activity of  $\alpha$ -Fe<sub>2</sub>O<sub>3</sub> NPs against DPPH showed 51.8% free radical scavenging ability at 360  $\mu$ g/mL, which approved good evidence of the antioxidant activity of  $\alpha$ -Fe<sub>2</sub>O<sub>3</sub> NPs.

**KEYWORDS:**  $\alpha$ -Fe<sub>2</sub>O<sub>3</sub> NPs; green synthesis; ginger extract; antioxidant.

## الخلاصة

يعد تصنيع الأكاسيد النانوية بطريقة سهلة وصديقة للبيئة باستخدام مواد بسيطة وامنة أحد اهم الاهتمامات الساخنة في مجال الكيمياء المستدامة والتي تحتمل العديد من التطبيقات الصيدلانية والطبية. تم في هذه الدراسة تحضير ناجح جزيئات الحديد النانوية ( $\alpha$ -Fe<sub>2</sub>O<sub>3</sub> NPs) باستخدام مستخلص نبات الزنجبيل. تم توصيف الجسيمات النانوية المحضرة بالتحليل الطيفي للانعكاس المرئي فوق البنفسجي، مطياف فورييه للأشعة تحت الحمراء، مطيافية المعان الضوئي، حيود الأشعة السينية، الفحص المجهرى لمسح الانبعاث الميداني، مطيافية الأشعة السينية المشتتة للطاقة، وجهد زيتا. بعد التوصيف الجيد للجسيمات النانوية، تم استكشاف فاعلية الجسيمات النانوية  $\alpha$ -Fe<sub>2</sub>O<sub>3</sub> المحضرة لكسح نشاط الجذور الحرة الناتجة من المركب DPPH. أوضحت النتائج أن شدة المعان الضوئي لها قمة واحدة في منطقة الأشعة فوق البنفسجية بين (480-490) نانومتر من الطيف اعتماداً على الشكل الهندسي وحجم الجسيمات النانوية، كما وأظهرت الأطياف المرئية للأشعة فوق البنفسجية قمة واحدة أيضاً عند طول موجي 296.0 نانومتر، والتي تمثل وجود الجسيمات النانوية، في حين أكدت الصورة المجهرية لمسح الانبعاث الميداني وجود أكسيد واحد نقي وأظهر نمط حيود الأشعة السينية أن جسيمات الحديد  $\alpha$ -Fe<sub>2</sub>O<sub>3</sub> NPs لها متوسط حجم بلوري (19.3) نانومتر وكشفت صور الماسح الإلكتروني ان الاجسام النانوية لأوكسيد الحديد لها أشكال وأحجام كروية وقضيبية وغير منتظمة تتراوح من (15 إلى 60) نانومتر. علاوة على ذلك، أظهرت تلك الجسيمات النانوية المحضرة هنا نشاطاً مضاداً للأكسدة ضد الجذور الحرة المتولدة من DPPH مما يدل على قدرتها على تنظيف الجذور الحرة بنسبة 51.8 % عند 360 ميكروغرام/مل.

## INTRODUCTION

Metal oxide-based NPs are synthesized to modify the properties of their corresponding metal-based NPs. In the presence of oxygen, iron (Fe) NPs instantly oxidize to iron oxide ( $\alpha$ -Fe<sub>2</sub>O<sub>3</sub>), increasing their reactivity compared to iron NPs. Metal oxide NPs are made primarily for their increased reactivity and efficiency [1]. Because of

their superior physicochemical properties when compared to bulk counterparts, iron oxide NPs have gained popularity in recent years. Because of their chemical stability and low cost among transition metal oxides, iron oxide NPs have piqued the interest of researchers over the last decade. Iron oxide can be found in nature in a variety of forms, including FeO,  $\alpha$ -Fe<sub>2</sub>O<sub>3</sub>,  $\beta$ -Fe<sub>2</sub>O<sub>3</sub>,  $\gamma$ -Fe<sub>2</sub>O<sub>3</sub>, and

$\text{Fe}_3\text{O}_4$  [2]. Because of its unique electrical, optical, and magnetic properties, interest in the synthesis of iron oxide NPs has grown over the last few decades. Iron is, indeed, the central focus of modern materials science [3]. Iron oxide NPs (IO NPs) are non-toxic [4] and biocompatible with superior magnetic properties that allow wide-ranging applications in biomedicine such as hyperthermia, photothermal therapy, biosensors, and physiotherapy applications [5]. They can also be used for magnetic particle imaging (MPI), magnetic resonance imaging (MRI), drug administration with precision, proteins, antibodies, and nucleic acids, tissue repair, and separation of biomolecules [6], and antimicrobial agents [7]. Furthermore, magnetic NPs have the ability to control biomolecule motion and transport, as well as generate heat in an external magnetic field to kill tumor cells. The biocompatibility of  $\alpha\text{-Fe}_2\text{O}_3$  and  $\gamma\text{-Fe}_2\text{O}_3$  is a significant advantage, and ferumoxytol (Feraheme) was approved by the US Food and Drug Administration in 2009 for the treatment of patients with chronic kidney disease who are iron deficient [8]. In general, it absorbs photons at wavelengths as long as 600 nm in general and collects more than 40% of the solar light spectrum. Its color is red because of these abilities. Its narrow bandgap of about 2.0-2.2 eV makes it a promising photocatalysis candidate. [9].

Recently, there has been a general trend toward the use of NPs in biomedical applications. Among the interesting properties of superparamagnetic IO NPs, they are low toxic and therefore they are used in many fields in diagnostic, therapeutic, or prognostic fields, including hyperthermia, drug delivery, magnetic resonance imaging, and cell separation. Zinc oxide, silver, gold, and core-shell silver-gold NPs have previously showed anti-diabetic properties. Furthermore, Sharifi *et al.* discovered that these superparamagnetic IO NPs suppress the vast majority of risk genes involved in the development of T2DM in human primary adipocytes [10]. These NPs showed significant antioxidant activity [11]. Antioxidants are substances in the body that fight free radicals and prevent or slow down cell damage caused by free radicals and unstable molecules. Antioxidants can control autoxidation by interrupting free radical propagation or inhibiting free radical formation, reducing oxidative stress, improving immune function, and increasing healthy longevity. Antioxidants are found in many foods, including

fruits and vegetables, and are also available as dietary supplements [12,13].

In this research, IO NPs were synthesized by a green method using ginger plant extract. The resulting NPs were well characterized by UV, FTIR, X-ray diffraction, SEM, AFM, and zeta potential. The antioxidant potential against DPPH has been investigated compared to that of vit C.

## MATERIALS AND METHODS

### Chemicals and reagents

Iron Sulfate heptahydrate ( $\text{FeSO}_4 \cdot 7\text{H}_2\text{O}$ ) (BDH), Sodium hydroxide (NaOH) Sigma-Aldrich It was received from the chemical materials store at the College of Science, Mustansiriyah University. The extract was made in the lab with dried ginger and all solutions were made with deionized water. To study the antioxidant activity, 2,2-diphenyl-1-picrylhydrazyl (DPPH) was used against Vit C as a standard antioxidant.

### Preparation of the extract

The dried ginger was purchased from the local market, washed well with distilled water several times, dried, ground, and kept in an airtight container afterward before analysis. A weight of 10 g of ginger rhizome was mixed with 200 ml of deionized water and boiled at 80 °C for 15 minutes until a yellow solution remained. After being cooled to room temperature, the solution was filtered to obtain a clear yellow plant extract solution. This plant extract was used as a reducing agent. The filter extract was further used for the green synthesis of  $\alpha\text{-Fe}_2\text{O}_3$  NPs.

### Green Synthesis of $\text{Fe}_2\text{O}_3$ NPs

A weight of 3 g of Iron Sulfate heptahydrate ( $\text{FeSO}_4 \cdot 7\text{H}_2\text{O}$ ) was dissolved in 300 mL deionized water for a final concentration of 0.1 M. To achieve a homogeneous solution, the solution was agitated at room temperature for 5 minutes. Then, 20 ml of rhizome extract was added with stirring until the solution turned yellow. After 30 minutes of stirring, 1 M NaOH was dropped wise until the blue solution turned into grey. The solution was centrifuged at 7500 rpm for 10 minutes and the supernatant was rinsed multiple times with water. A black-reddish  $\alpha\text{-Fe}_2\text{O}_3$  NPs were collected and dried at 95°C for 4 hours. The dried  $\alpha\text{-Fe}_2\text{O}_3$  NPs were calcined at 400°C for 4 hours to remove any evaporable impurities. The calcined samples were cooled to room temperature and kept for future research [14].

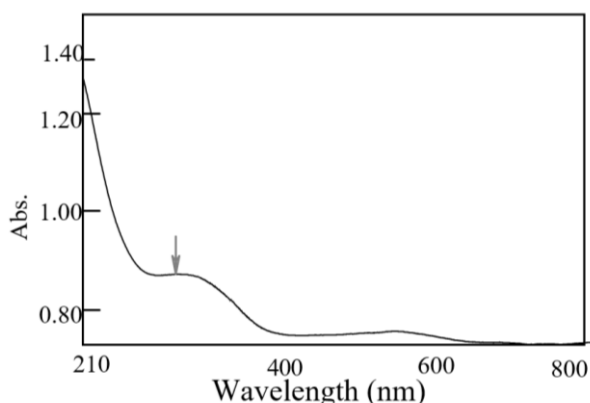
### Characterization of $\alpha$ -Fe<sub>2</sub>O<sub>3</sub> NPs

For optical studies, UV-vis 1800 spectrophotometer (Shimadzu, Japan) with a wavelength range from 210 to 800 nm. Poly(l-methyl) (PL) was used for UV- analysis to provide valuable information about the purity and quality of the material.  $\alpha$ -Fe<sub>2</sub>O<sub>3</sub> NPs were further characterized using FTIR spectroscopy, XRD, AFM, EDX, and SEM and zeta potentia ( $\zeta$ -potential).

## RESULTS AND DISCUSSION

### UV-visible spectroscopy analysis

UV-vis spectroscopy was used to confirm the synthesis of  $\alpha$ -Fe<sub>2</sub>O<sub>3</sub> NPs. Due to the surface plasmon resonance (SPR) effect, the conducting electrons begin to oscillate at a specific wavelength range, as shown in Figure 1. The presence of  $\alpha$ -Fe<sub>2</sub>O<sub>3</sub> NPs demonstrated by the presence of a peak at 296.0 nm, which represents the UV-visible spectra of prepared  $\alpha$ -Fe<sub>2</sub>O<sub>3</sub> NPs. This result was consistent with the findings of Paulson, E and Jothibas, M [15].

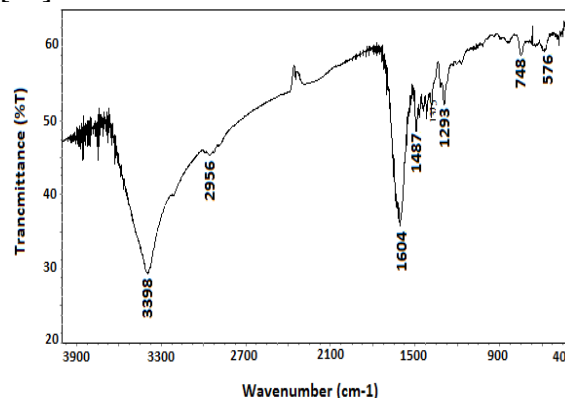


**Figure 1.** UV-vis spectrum of  $\alpha$ -Fe<sub>2</sub>O<sub>3</sub> NPs synthesized by Zingiber extract.

### FTIR spectroscopy

FTIR spectrum of  $\alpha$ -Fe<sub>2</sub>O<sub>3</sub> NPs synthesized by Zingiber is shown in Figure 2. Several peaks at 3398 cm<sup>-1</sup> appeared due to the a broad-OH of phenolic compounds. A strong peak at 1604 cm<sup>-1</sup> indicated the involvement of amide C=O extension, in the reduction process. Also, the presence of a peak at 2956 cm<sup>-1</sup> assigned for O = C = O stretching of carbon dioxide. The strong peaks at 1487 and 1293 cm<sup>-1</sup> showed the alkane's CH bending and the sulfide group' S=O stretching,

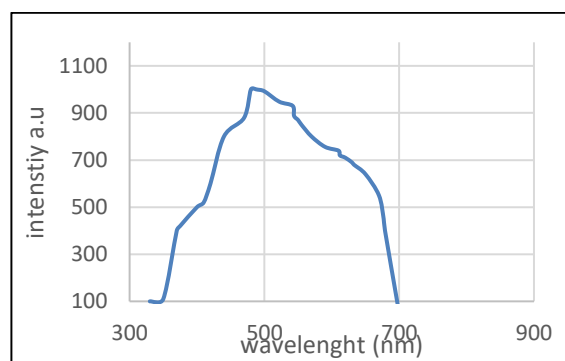
respectively. Peaks at 748 and 576 cm<sup>-1</sup> verified the presence of NPs. These peaks were due to the organic compounds of ginger extract that play an important role in reducing and stabilizing  $\alpha$ -Fe<sub>2</sub>O<sub>3</sub> NPs, this result was in agreement with Abdulah *et al* [16].



**Figure 2.** FTIR spectrum of  $\alpha$ -Fe<sub>2</sub>O<sub>3</sub> NPs.

### Photoluminescence (PL) spectroscopy

For crystalline quality, the photoluminescence (PL) technique was used. All spectra were composed of one emanation peak, Figure 3. A peak in the region (480-490 nm) indicated the emission peak of the photoluminescence analysis of  $\alpha$ -Fe<sub>2</sub>O<sub>3</sub> at room temperature and the formation of iron oxide NPs [17].



**Figure 3.** PL spectra of the  $\alpha$ -Fe<sub>2</sub>O<sub>3</sub> NPs prepared from Zingiber extract.

Energy gap values of  $\alpha$ -Fe<sub>2</sub>O<sub>3</sub> NPs measured according to the equation [18]:

$$E_g \text{ (eV)} = 1240/x \quad (1)$$

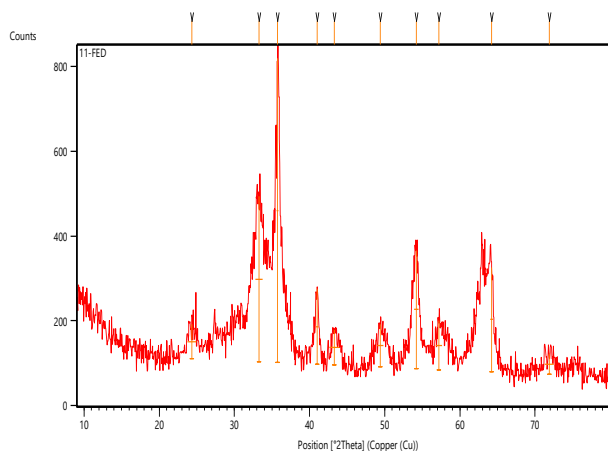
Where  $E_g$  is the energy gap, and  $x$  is the maximum wavelength represents the value of the energy gap (2.5 eV) at the wavelength (485 nm).

### X-ray diffraction (XRD)

X-ray crystallographic study was performed to confirm the crystalline structure of  $\alpha$ -Fe<sub>2</sub>O<sub>3</sub> NPs after calcination at 400 °C. The analysis was carried out by a Shimadzu-7000 Powder X-Ray diffractometer with Cu-K $\alpha$  ( $\lambda=1.54$  Å) radiation in a range from 10 to 70 Å°. The Debye-Sherrer equation was used to calculate the particle size [19].

$$D = K \cdot \lambda / \beta \cdot \cos(\theta) \quad (2)$$

Where D is the size of the crystallite, K=0.9 Sherrer constant,  $\lambda$  is the X-ray wavelength,  $\beta$  is the full width half maximum (FWHM) and  $\theta$  is the Bragg diffraction angle. XRD patterns of  $\alpha$ -Fe<sub>2</sub>O<sub>3</sub> NPs are observed at  $2\theta= 24.36^\circ, 33.25^\circ, 35.76^\circ, 41.02^\circ, 43.27^\circ, 49.43^\circ, 54.19^\circ, 57.22^\circ$  and  $64.22^\circ$ . The corresponding reflecting planes are (012), (104), (110), (113), (116), (018), (214), (300), respectively. The formation of  $\alpha$ -Fe<sub>2</sub>O<sub>3</sub> NPs indexed of JCPDS No. 33-0664. The analysis also revealed that the synthesized NPs were crystalline. The average crystal size was (19.3) nm, which was calculated using Debye-Sherrer equation.



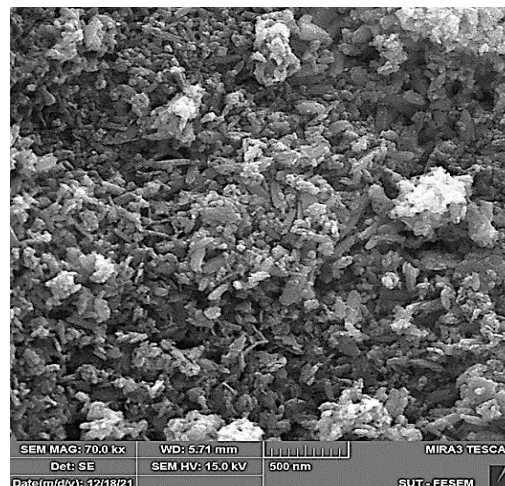
**Figure 4.** X-ray diffraction pattern of  $\alpha$ -Fe<sub>2</sub>O<sub>3</sub> NPs powder.

The sharp diffraction peaks indicated the crystallinity of the preparation material, see Figure 4. Particles size was calculated to be (19.3) nm, in agreement with Huang, Wei, et al [20], and Samrot, Antony V, et al [21].

### Scanning electron microscopy (SEM)

The morphology, shape, and size of the synthesized green NPs were effectively illustrated using SEM micrographs. SEM scans of  $\alpha$ -Fe<sub>2</sub>O<sub>3</sub> NPs indicated that they were spherical, rod-shaped, and irregular in form, with sizes ranging from 15 to 60 nm,

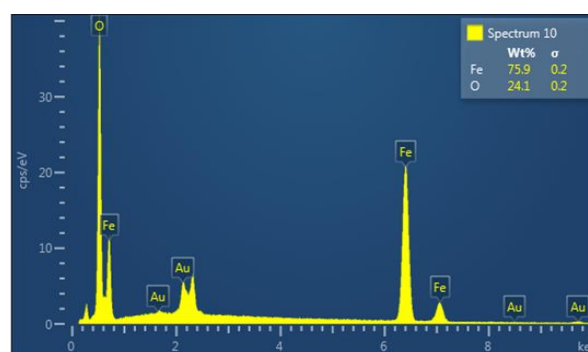
Figure 5. This size range also corroborated the particle size determined by the XRD spectra in Figure 4. The aggregation in the SEM images was most likely caused by electrostatic contact between the NPS surface layers [22]. A similar observation was reported earlier by Saqib, Saddam, *et al* [23].



**Figure 5.** SEM analysis of  $\alpha$ -Fe<sub>2</sub>O<sub>3</sub> NPs.

### Energy dispersive-X-ray (EDX)

The initial composition of  $\alpha$ -Fe<sub>2</sub>O<sub>3</sub> NPs was determined by EDX Figure 6. The weight percentage was 24.1% and 75.9% for oxygen and iron, respectively. The presence of iron and oxygen peaks suggested that iron oxide NPs were formed, a similar observation reported earlier by Ali, Musrat *et al* [24].



**Figure 6.** EDX spectrum of  $\alpha$ -Fe<sub>2</sub>O<sub>3</sub> O NPs.

Table 1 showed that the practical EDX values were compatible with the calculated values, obtained by x-ray mapping.

**Table 1.** Practical and Calculated ratio of  $\alpha$ -Fe<sub>2</sub>O<sub>3</sub>.

Experimental formula	Fe%	O%
$\alpha$ -Fe <sub>2</sub> O <sub>3</sub>	75.86 (70.1)	24.14 (30.1)

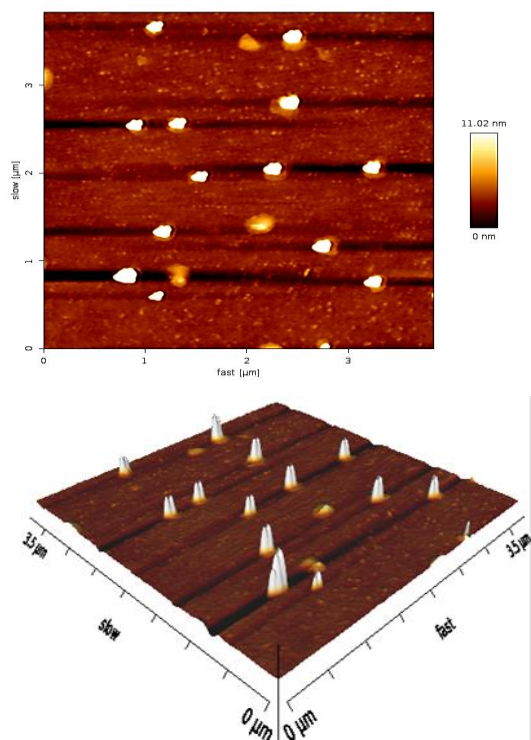
### Atomic Force Microscopy (AFM)

AFM (spm-AA300 contact mode spectrometer, Angstrom) was used to characterize the size and morphology of  $\alpha$ -Fe<sub>2</sub>O<sub>3</sub> NPs. The origin of the surface morphology of the irregularly shaped particle sizes and the size distribution broadens of  $\alpha$ -Fe<sub>2</sub>O<sub>3</sub> NPs synthesized by ginger extract are shown in Figure 7.

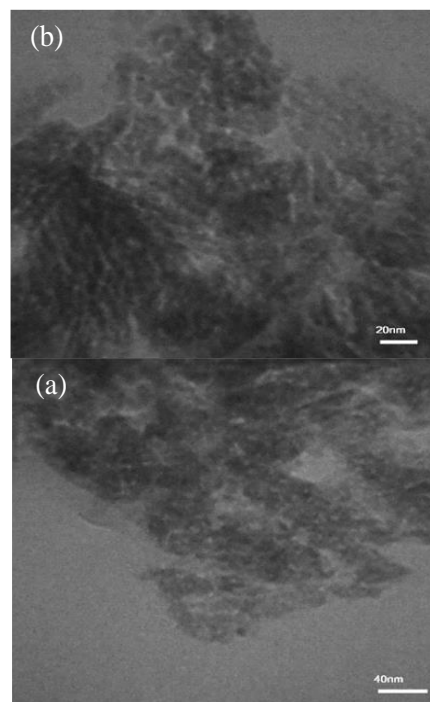
The images confirmed the uniform distribution of  $\alpha$ -Fe<sub>2</sub>O<sub>3</sub> NPS, as did the majority of NPs (aged for 2 weeks) were approximately 30–100 nm. The size obtained in this study almost agreed with other studies by Jin Ying *et al* [25].

### Transmission Electron Microscopy (TEM)

Imaging by TEM was useful for confirming the size and shape of the green synthesized produced iron oxide NPs as shown in Figure 8 at different scale bars (a and b). There were clusters of NPs dispersed during the formation of the hematite. The particle sizes of hematite  $\alpha$ -Fe<sub>2</sub>O<sub>3</sub> NPs were (20–40 nm) with an average size of 25 nm, which confirmed the results predicted by XRD analysis using Debye-Scherrer formula. This result agreed with the study of Pallela, Panduranga Naga Ummey *et al* [26], where iron oxides NPS were synthesized by *Sida cordifolia* plant extract.



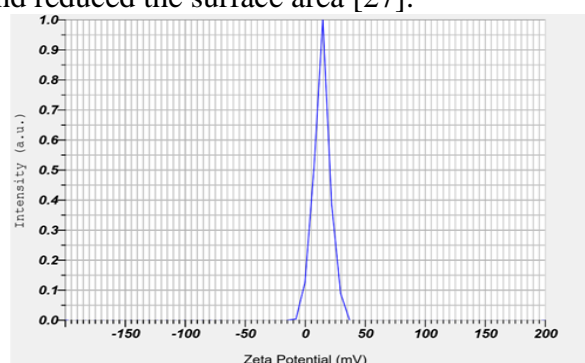
**Figure 7.** AFM microscope shows the distribution and roughness of  $\alpha$ -Fe<sub>2</sub>O<sub>3</sub> NPs.



**Figure 8.** TEM images of the green synthesized iron oxide NPs. a) IO NPs at a scale bar 40nm, b) IONPs at a scale bar 20nm.

### Zeta potential analysis ( $\zeta$ -potential)

The zeta potential ( $\zeta$ -potential) analysis was important for studying the net charge on the surface of NPs and calculating the charge to understand their stability. The potential value of  $\alpha$ -Fe<sub>2</sub>O<sub>3</sub> NPS was observed to be 13.8 mV (see Figure 9) which indicated high stability of NPs. The presence of  $\zeta$ -potential values within the positive range only demonstrated that the surface of the particles prepared from  $\alpha$ -Fe<sub>2</sub>O<sub>3</sub> within the nanocomposite was homogeneous (i.e a charged surface of one type). Although the positive charge value was small, the prepared particles were agglomerated and reduced the surface area [27].

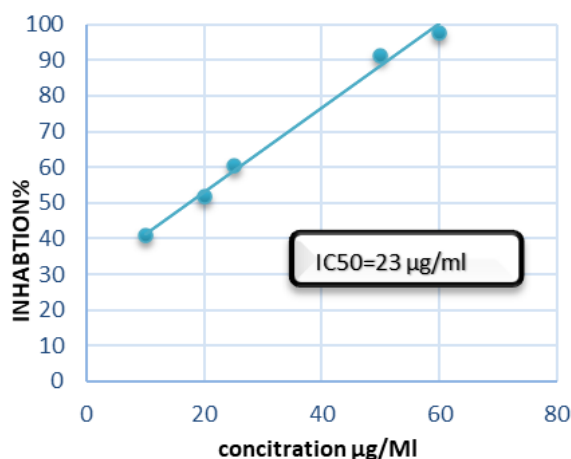


**Figure 9.** zeta potential for  $\alpha$ -Fe<sub>2</sub>O<sub>3</sub> NPs.

## Antioxidant Activity

Dietary antioxidants are the first line of defense against free radicals [13]. IC<sub>50</sub> value assists the estimation of the material amount able to inhibit 50% of DPPH. The lower the IC<sub>50</sub> value, the greater the scavenging potency. The present results showed the scavenging activity for dried ginger compared with Vitamin C as a standard.

Vitamin C exhibited powerful antiradical activity against DPPH with IC<sub>50</sub>=23  $\mu$ g/mL, Figure 10 and Table 2.

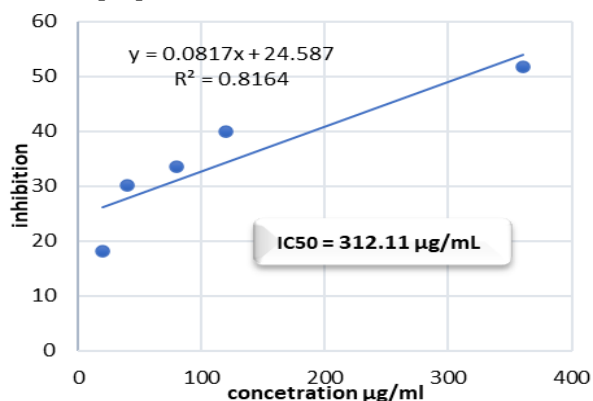


**Figure 10.** Vitamin C antiradical inhibition plot against DPPH.

**Table 2.** Vitamin C inhibitory percentage on DPPH.

Concentration $\mu$ g/mL	Inhibition %
10	40.8
20	51.66
25	60.6
50	91.3
60	97.6

The scavenging power of  $\alpha$ -Fe<sub>2</sub>O<sub>3</sub> NPS showed good inhibitory characteristics against DPPH with IC<sub>50</sub> = 312.11  $\mu$ g/mL, Figure 11. The inhibition activity of  $\alpha$ -Fe<sub>2</sub>O<sub>3</sub> NPs was close to that of vitamin C. By increasing the concentration of NPs, the antioxidant activity was increased [28].



**Figure 11.**  $\alpha$ -Fe<sub>2</sub>O<sub>3</sub> NPs antiradical inhibition plot against DPPH.

Results showed that the highest scavenging % was at 360 ppm of  $\alpha$ -Fe<sub>2</sub>O<sub>3</sub> NPs with 51.8% (see Table 3), indicated good antioxidant activity. This result was in agreement with Ahmad *et al* [29,30]. This IC<sub>50</sub> value of the prepared  $\alpha$ -Fe<sub>2</sub>O<sub>3</sub> NPs was lower than that previously prepared from the natural biowaste precursor, namely Psidium guajava leaves (579.9  $\mu$ g/ml) [31], which indicated stronger inhibitory effect. However, This IC<sub>50</sub> value was higher than that of  $\alpha$ -Fe<sub>2</sub>O<sub>3</sub> NPs prepared by Centaurea alba extract (287  $\mu$ g/mL) Jian Xiu *et al* [31].

**Table 3.** The  $\alpha$ -Fe<sub>2</sub>O<sub>3</sub> NPS inhibitory percentage against DPPH.

Concentration $\mu$ g/mL	Inhibition %
10	12
20	18.1
40	30.2
80	33.5
120	40
360	51.8

## CONCLUSIONS

We have demonstrated here successful use of a natural and low-costed reducing agent to prepare metallic oxide nanostructures, the ginger plant extract. The green synthesis of NPs here helped in avoiding organic solvents and hazardous and toxic waste. The Uv-vis results confirmed the surface plasmon resonance of the biosynthetic iron oxide. The biocombined NPs using ginger extract demonstrated good catalytic activity. The potential value of  $\alpha$ -Fe<sub>2</sub>O<sub>3</sub> NPS (13.8 mV) indicated the stability of these NPs. Furthermore, the current study provided good evidence for the antioxidant activity of the green-synthesized  $\alpha$ -Fe<sub>2</sub>O<sub>3</sub> NPs against DPPH.

## ACKNOWLEDGMENT

The authors would like to thank Mustansiriyah University in Baghdad, Iraq ([www.uomustansiriyah.edu.iq](http://www.uomustansiriyah.edu.iq)) for their assistance with this project.

**Disclosure and conflicts of interest:** The authors declare that they have no conflicts of interest.

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## How to Cite

D. H. Hilo, A. H. Ismail, and Z. S. Al-Garawi, "Green Synthesis of  $\alpha$ -Fe<sub>2</sub>O<sub>3</sub> from Ginger Extract Enhanced the Potential Antioxidant Activity Against DPPH", *Al-Mustansiriyah Journal of Science*, vol. 33, no. 4, pp. 64–71, Dec. 2022.