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Green Biosynthesis of Iron Oxide Nanoparticles and Testing Their Inhibitory Efficacy Against Some Pathogens

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Abstract: The biosynthesis of iron oxide (Fe_2O_3, also known as haematite) nano particles (NPs) using *Hydra helix* and *Beta vulgaris* aqueous extracts were adduced, respectively, where the extracts act as a stabiliser and reductant reagent. The crystal structure and size of particles were investigated using X-ray diffraction (XRD), while the morphology was examined using field emission scanning electron microscopy (FESEM), XRD patterns showed the synthesised nanoparticles with well-crystallised structure from *Beta vulgaris* extract with size 12 nm, while the results by using *Hydra helix* showed many peaks back to Goethite phase with 16 nm. The antibacterial and antifungal activity were examined using Staphylococcus (showed inhibition zone diameter 23 mm, 16 mm using *Hydra helix* and *Beta vulgaris*, respectively), *E. coli* (showed no inhibition) and Candida fungi (showed inhibition zone 16 mm, 11 mm using *Hydra helix and Beta vulgaris*, respectively).

Key words: Iron oxide, nanoparticles, haematite, *Beta vulgaris*, *Hydra helix*.

Introduction

Many studies have been focussed on the main objective of green nanotechnology which includes facilitating the preparation of nanotechnology-based products that are eco-friendly and safe for all existence (Ishibashi et al., 2000). The fabrication of nanoparticle using the chemical method may result in the presence of several harmful chemical species that are adsorbed on the surface and it causes a negative impact on its applications. To get rid of this problem fabrication of the nanoparticles using the green method must be developed. The green method includes the synthesis of nanoparticles using plant extract or microorganism with many advantages such as the low cost, simplicity and low scale of nano size particles. The green synthesis includes two steps: choosing a solvent medium and reducing reagent and non-toxic materials for the stability of nanoparticles (Loo et al., 2012). In this study, we illustrate the synthesis of α-Fe_2O_3 nanoparticles using the green method for antibacterial application. The α-Fe_2O_3 phase is one of the many phases for iron oxide. Various methods were utilised to prepare nanoparticles of haematite, including photolysis (Dong et al., 2015), hydrothermal (Mohanpuria et al., 2008; Velioglu et al., 1998; Wang et al., 2004), thermal decomposition (Li et al., 2007; Zhao et al., 2006), sol-gel (Basent et al., 2013; Kayania et al., 2015; Raja et al., 2015), and others. It attracted many researchers because of its characteristics such as stability, environmental and non-toxicity (Mor et al., 2007), and its applications that illustrate in several studies (Long et al., 2015; Mishra and Chun, 2015).

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Haematite has acquired attention from the researchers for its antibacterial application. To inactive bacteria, many factors play a main role such as the morphology of particles and functionalisation of the surface. These factors control the quantity release of metal ions and reactive oxygen species (Leung et al., 2012; Pal and Tak, 2007; Vatsha et al., 2013; Zawadzka et al., 2014). Previous studies have shown a detailed study about the growth of bacteria in the presence of haematite nanoparticles and other has been showing the effect of particle morphology on the eradication (Basent et al., 2013). From these encouraging results, material researches were carried out by many researchers to explore the effect of haematite nanoparticles on the properties of bacteria.

Materials and Methods

Fresh *Hydra helix* and *Beta vulgaris* leaves were collected from the local garden of Baghdad, Iraq. It was washed and crushed using mortar. $FeSO_4.H_2O$ and NaOH were supplied from Sigma-al-Aldrich Chemicals Ltd and the distilled water was purchased from the Baghdad University lab. All these materials were used to prepare α-Fe_2O_3 nanoparticles. Gentamicin sulphate (30 mg/ml) was bought from SEDICO Co and used for comparison with the biological activity of the prepared nano particles.

Microbial Synthesis of Fe O NPs

Field emission scan electron microscope (FESEM) analysis was carried out using (Tescan Mira3, France) model, X-ray diffraction carried out using (Xpert Philips, Holland) model in Sample testing laboratories in Mashhad university.

X-ray diffraction measurement was carried out using a Philips model with Cu Kα radiation (λ = 0.154 nm) and collection range of 10°-80°. The sizes of crystallites were investigated using the Scherrer equation as following:

$$D = (KI)/\beta\cos\theta$$

where K is a constant and indicates the crystallite shape and equals 0.9, D is related to the size of crystallite, λ is the wavelength of copper $K\alpha$ radiation, θ represents the angle of highest peaks intensity, while β is the FWHM of the same peak. The biological activity was studied using the agar well diffusion method (Jahangirian et al., 2013) with Mueller-Hinton media (from HKM) for bacteria (*Staphylococcus aureus*, *Escherichia coli*) and Sabouraud Dextrose Agar (from Innovating Science) for fungi (*Candida albicans*), all pathogens are provided by the microbiology laboratory in University of Baghdad College of Education for Pure Sciences.

Preparation of Fe_2O_3 NPs

Firstly, in two separately 200 ml beakers, 20 g of each plant leaves were put in 100 ml of distilled water and heated at 40°C for 30 min. Then, the extract was filtered and freshly used. After that, 0.05 M of ferrous sulphate solution was added into two extract beakers and under continuous stirring the solution turned dark immediately. Solution of 1M NaOH was added drop wise to the mixture until pH became 9. Finally, the two mixtures were centrifuged for 15 min and washed several times with ethanol and distilled water was used to remove the impurities; the iron oxide nanoparticles NPs were dried and crushed and stored (Huang et al., 2014).

Result and Discussion

The phase transformation structure and the size of particles were investigated by XRD analysis. Figures 1a and 1b explained that the XRD patterns of α-Fe_2O_3 and α-Fe_2O_3 with α-FeOOH, respectively. As shown in Figure 1a, the results confirmed that haematite had a rhombohedral phase and the peaks showed at 2θ = 24.12, 33.14, 35.61, 40.85, 49.47, 54.08, 57.58, 62.42, 63.98 and 72.25 can be indexed to the Bragg Low reflection at planes (012), (104), (110), (113), (024), (116), (018), (214), (300) and (119). All the results are in agreement with JCPDS card no: 33-0664 with high purity. The XRD patterns of nanoparticles prepared using *Hydra helix* extract are shown in Figure 1b. The results explain a low intensity peak of haematite NPs thus indicating towards the co-existence of FeOOH with α-Fe_2O_3. The presence of FeOOH may indicate the incomplete transformation to the haematite phase.

The surface morphology of nanoparticles synthesised using *Beta vulgaris* and *Hydra helix* are shown in Figure 2a and 2b, respectively. The measurement shows a lot of agglomeration of particles and this may be back to the condition during synthesis. The FESEM results show that for *Hydra helix* extract, the particles of iron oxide are needle shaped, while for *Beta vulgaris* extract, the particles are oval shaped within a nano range and the results are in agreement with XRD measurements.

The biological activity of NPs prepared using *Hydra helix* and *Beta vulgaris* was determined with respect to gentamicin as standard, using the agar well diffusion method. The two compounds were tested and the plates were incubated at 37ºC for 24 hours, the inhibition zone

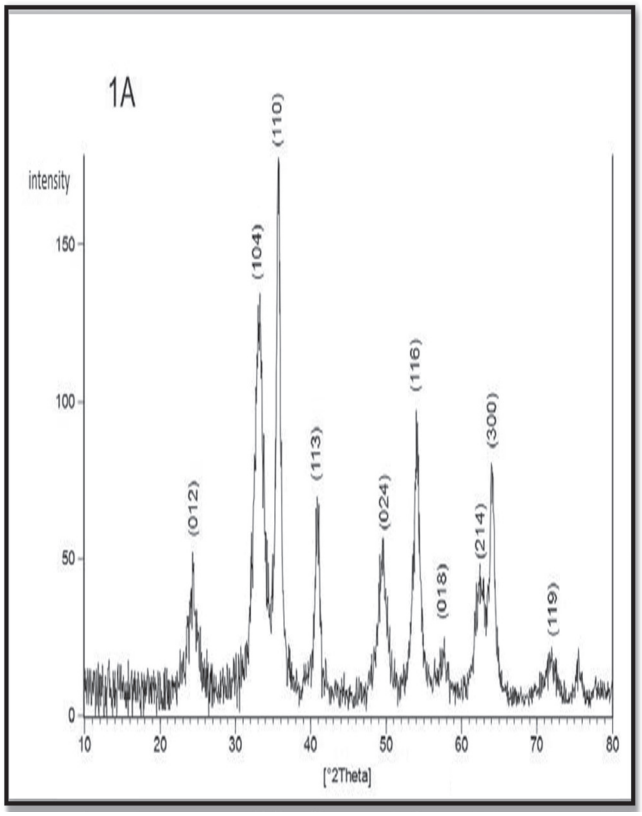

Figure 1a: XRD patterns of α-Fe_2O_3 prepared using *Beta vulgaris* extract.

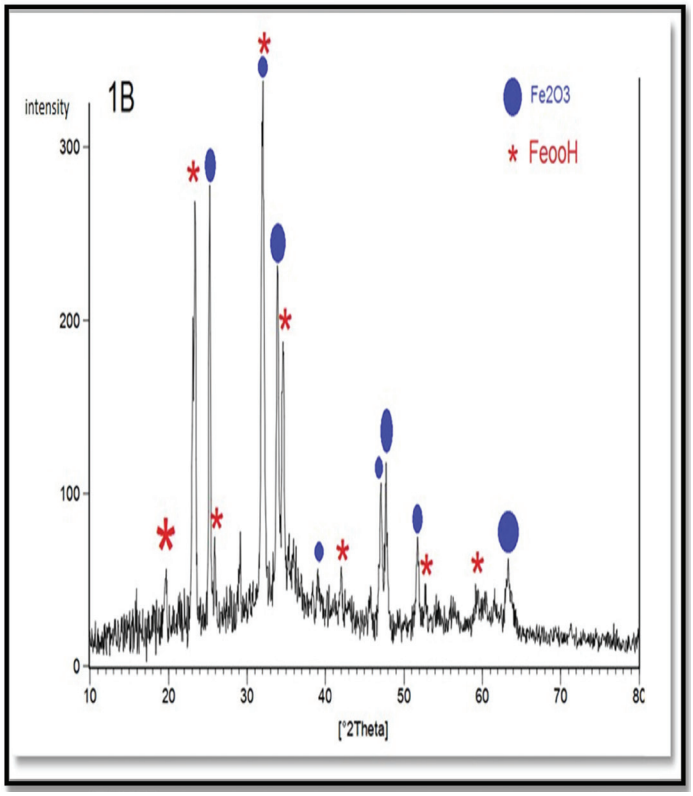

Figure 1b: XRD patterns of α-Fe_2O_3 prepared using *Hydra helix* extract.

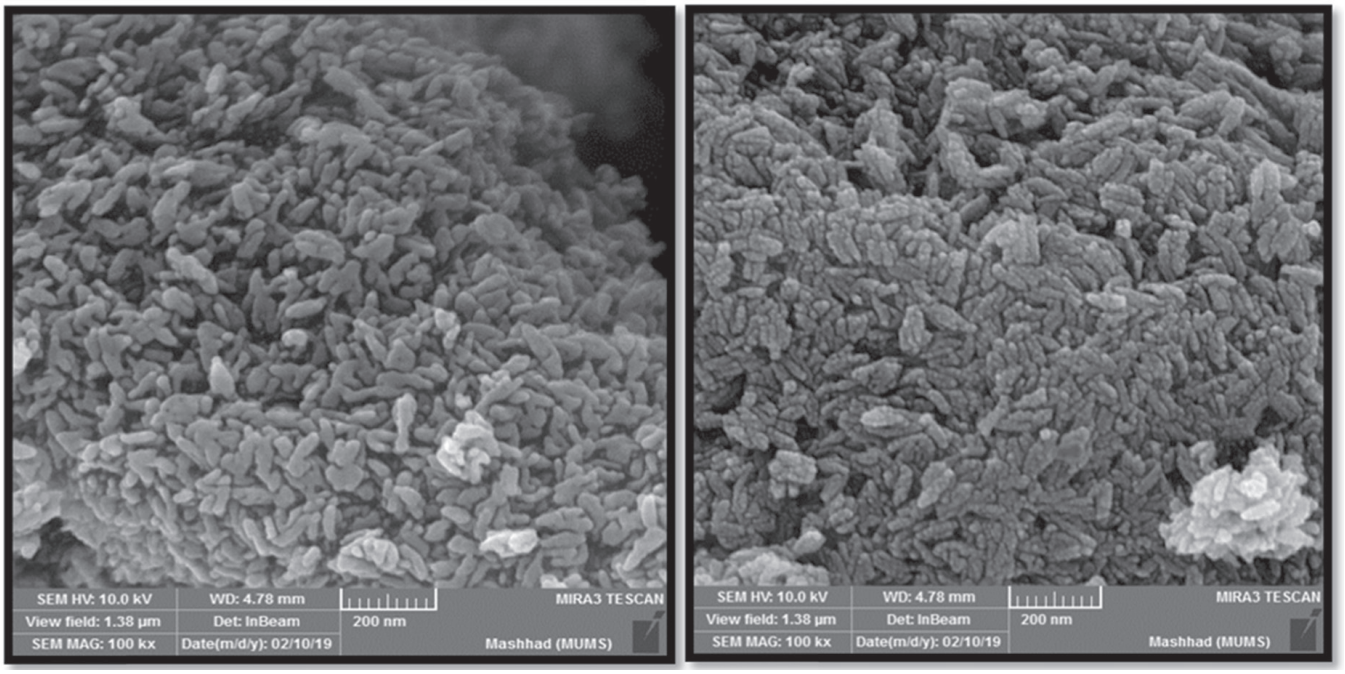

Figure 2a: FESEM image of α-Fe_2O_3 prepared using *Beta vulgaris* extract.

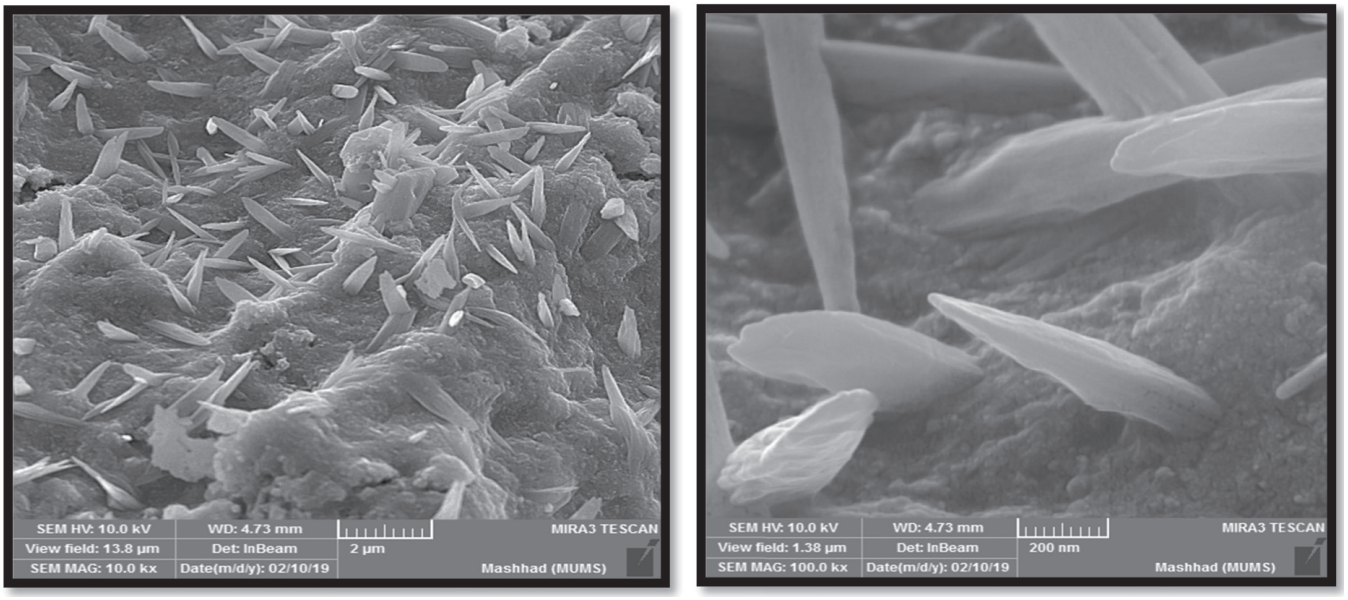

Figure 2b: FESEM images of α-Fe_2O_3 prepared using *Hydra helix* extract.

was measured in mm. Iron oxide NPs were evaluated for their antibacterial activity against different pathogen bacterial strains (*Staphylococcus aureus, Escherichia coli*) and fungi (*Candida albicans*); the inhibition zones caused by these compounds are determined and listed in Table 1.

The results in Table 1 indicate that iron oxide NPs prepared using *Hydra helix* have higher biological activity than those prepared using *Beta vulgaris*, but it's still less than the standard (gentamicin). Extracts from both plants did not show any biological activity against Gram-negative (*Escherichia coli*) may be due to the presence of a double membrane surrounding the cell membrane in Gram-negative bacteria.

Conclusion

The haematite NPs were successfully synthesised using the green method by *Beta vulgaris* and *Hydra helix* extract with biological activity application. The structure, crystallite size and morphology were investigated using XRD and SEM. The results appear incompletely transformed goethite to haematite by using *Hydra helix*. The nano particles prepared showed biological activity against *S. aureus* and *C. albicans* but no such activity was reported against *E. coli*.

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Table 1: Biological activity for the prepared compound with gentamicin as standard

Compounds	*Inhibition zone diameter (mm) for prepared compounds*		
	Gram positive	Gram negative	Fungi
	Staphylococcus aureus	*Escherichia coli*	*Candida albicans*
NPs using *Hydra helix*	23	-	16
NPs using *Beta vulgaris*	16	-	11
Gentamicin	36	27	15

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