

Green synthesis Of Magnetite Iron Oxide Nanoparticles by Using Al-Abbas's (A.S.) Hund Fruit (*Citrus medica*) var. Sarcodactylis Swingle Extract and Used in Al-'alqami River Water Treatment

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Abstract

In this study, magnetite iron oxide nanoparticles were synthesized from aqueous ferrous chloride, ferric chloride and sodium hydroxide through a simple and eco-friendly route using Al- Abbas's (A.S.) Hund Fruit extracts, which acted as a reductant and stabilizer simultaneously. Characterizations of nanoparticles were done by using UV-visible, FT-IR, XRD, and SEM methods. The ultraviolet-visible spectrum of the aqueous solution containing magnetite iron oxide nanoparticle showed an absorption peak at round 340 nm. FT-IR graph showed peaks at 519 cm^{-1} confirm the presence of Magnetite iron oxide nanoparticles in the synthesized samples. It is clear that the bioactive molecules present in the Abbas's (A.S.) Hund Fruit extract interacted with the synthesized Magnetite iron oxide nanoparticles. The diffractogram exhibits six distinct diffraction peaks at 2θ values, as the (220), (311), (400), (422), (511) and (440) crystallographic planes of the inverse spherical magnetite crystal. The average crystallite size was evaluated by diffraction line broadening (d 311) using the Debye-Scherrer equation. The average size of Fe_3O_4 crystallites obtained from nanocomposite was about 45nm. The magnetite iron oxide nanoparticles synthesized by the help of Abbas's (A.S.) Hund Fruit extracts were scanned using SEM. It reveals that an iron oxide nanoparticle seems to be spherical in morphology.

The Al-'alqami River water treatment by magnetic iron oxide nanoparticles synthesize by Abbas's (A.S.) Hund Fruit extracts led to the reduction of the number of bacteria to 80% after twelve hours of treatment whereas water treatment by magnetic iron oxide nanoparticles for 24 hours led to kill all the bacteria in water.

Keywords: Green synthesis, Magnetite Iron Oxide Nanoparticles, Al- Abbas's (A.S.) Hund Fruit, Water treatment, Al-'alqami River.

1. Introduction

Iron oxide nanoparticles play an important role in environmental remediation circles. As it removes both of organic and inorganic heavy metal pollutants from polluted water [1]. There are several chemical and physical methods available for synthesis of iron oxide nanoparticles. Those methods are used toxic or potentially hazardous as starting materials and more energy. However, it is still a big challenge to develop simple and reliable synthetic method for low-dimensional iron oxide nanostructures. The primary goal of nanotechnology is to improve simple ecofriendly method for synthesis of nanoparticles. The biomaterial such as microbes and plant extract can be used to prepare various types of nanoparticles and even nanorods. However, as some organisms are pathogens, it is risky to handle. Microorganisms need maintenance of culture and controlled conditions such as temperature, pH and other factors for growth. Sometimes, the synthesis of nanoparticles utilizing plant parts could prove advantageous over other biological processes by eliminating the elaborate process of maintaining the microbial culture. Synthesis of iron oxide nanoparticles by using plant extract has been quite limited and few works have been reported. The reports are available for synthesis of hexagonal metallic iron, amorphous iron, and α-Fe_2O_3 by using tea extract [2, 3], and iron nanoparticles by using Aqueous Sorghum bran Extracts [4]. The tea extract mediated synthesized iron nanoparticles were found to be nontoxic when compared with iron nanoparticles prepared using conventional $NaBH_4$ reduction protocols. The concentration of the tea extract in the reaction mixture plays an important role in the size and crystallinity (hexagonal metallic iron, amorphous iron, and R-Fe_2O_3) of the synthesized iron nanoparticles [3].

The finger citron (*Citrus medica* var. sarcodactylis Swingle) (FC) is a medicinal plant from the Rutaceae family. Customarily, it is more popularly named “Buddha hand citron”, “Longevity orange”, or “Five finger orange” in commercial vegetable markets [5]. This plant is of economic importance since it has been used as a traditional Chinese medicinal material and functional vegetables, and preserved as sweetmeats [6, 7]. In Oriental countries, the finger citron fruits are extensively consumed as functional vegetables in a specific diet. As a medicinal plant, the finger citron has been credited with a long list of medicinal uses. Because traditionally finger citron fruits have been considered to be beneficial to pancreas, liver and stomach functions, and people like to take finger citron fruits as adjuvant herbal medicines to treat a diversity of chronic diseases like asthma, hypertension and respiratory tract infections [6, 7, 8]. It is worth noting that the bioactive substances contained in the finger citron plays an important role in its biological activity. Moreover, diets rich in selected natural antioxidants such as the essential oils (EOs) and various extracts from finger citron are related to reduced risk of incidence of hyperlipidemia, obese and other chronic diseases has led to the revival of interest in plants-based foods [7, 8].

River Al-'alqami is a river that branches from the Euphrates River (Furat). Beside it, Abul Fadhl Al-Abbas (A.S.) was killed on Ashoora. Alqam means 'sour' in Arabic. It is told to every tree that is sour, or water that is sour. It is used very often in poetry when talking about Al-Abbas (A.S.) [9].

In Iraq the (*Citrus medica*) var. sarcodactylis Swingle called Al-Abbas's Hand fruit ,the raw fruit peel and extracts are highly regarded in Iraq for their many medicinal uses (ranging from Alzheimer’s to cancer to diabetes to ulcers to intestinal parasites) and as an insect repellent. People in Iraq believed that cultivation of this plant brings them luck and livelihood.

The present study aims to synthesize of magnetite iron oxide nanoparticles by ecofriendly method using Al-Abbas's fruit extract and used in Al-'alqami River water treatment.

2. Materials and Methods

2.1 Materials

Al- Abbas's (A.S.) Hund Fruit figure 1 were purchased from Al-Warda market for shopping and retail, Karada, Baghdad, Iraq. Ferric chloride hexa-hydrate ($FeCl_3.6H_2O$,AR), ferrous chloride tetra-hydrate ($FeCl_2.4H_2O$, AR) and sodium hydroxide (NaOH) were purchased from Sigma-Aldrich Chemicals. All solutions were freshly prepared using double-distilled water and kept in the dark to avoid any photochemical reactions.

Figure 1. Al- Abbas's (A.S.) Hund Fruit.

2.2 Synthesis of Magnetite Iron oxide Nanoparticles

Magnetite iron oxide nanoparticles were synthesized by dissolving $FeCl_2.4H_2O$ and $FeCl_3.6H_2O$ (1:2 molar ratio)in 500 mL of sterile deionized water and heated at 80℃ using magnetic stirrer. After 10 minutes, 25 mL of the aqueous solution of Al- Abbas's (A.S.) Hund Fruit ethanolic extract was added to the mixture, immediately yellowish colour of the mixture changed to reddish brown colour. After 5 minutes, 100 mL aqueous solution of sodium hydroxide (26%) was added to the mixture with rate 3 ml/min for allowing the iron oxide precipitations uniformly. From the first addition of sodium hydroxide the reddish brown mixture changed to black suspended particles. The mixture was allowed to cool down to room temperature and the iron oxide nanoparticles were obtained by decantation, further dilution with sterile distilled water and centrifugation to remove heavy biomaterials of Al- Abbas's (A.S.) Hund Fruit ethanolic extract. The iron oxide nanoparticles were purified by dispersing in sterile distilled water and centrifugation for three times. The iron oxide nanoparticles after purification were dried overnight at 80℃ [10].

2.3 Characterization of magnetite iron oxide nanoparticles

Low concentration of the Magnetite (Fe_3O_4) nanoparticles was suspended in ethyl alcohol at room temperature, ultra-sonicated (Qsonica Sonicator Q500) (Tokyo, Japan) for 10 min and examined by X-ray diffractometer (Shimadzu, XRD-6000) (Tokyo, Japan) equipped with CuKαradiation source using Ni as filter at a setting of 30 kV/30mA. All XRD data were collected under the experimental conditions in the angular range $3^o \leq 2\theta \leq 50^o$.FT-IR spectra of Al- Abbas's (A.S.) Hund Fruit extract and magnetite iron oxide nanoparticles was obtained in the range of 4000-400 cm^{-1} with FT-IR spectrophotometer (IR-Prestige 21, Shimadzu) (Tokyo, Japan) using KBr pellet method. Scanning electron microscopy (SEM) analysis of the synthesized magnetite nanoparticles was done by SEM machine, Hitachi S-4500 (Tokyo, Japan). Thin films of magnetite nanoparticles were prepared on a carbon coated copper grid by just dropping a very small amount of the sample on the grid, extra solution was removed using blotting paper and then the film on the SEM grid were allowed to dry by putting it under a mercury lamp for 5 minutes. UV-vis spectroscopic studies were carried out using Shimadzu UV-1601 spectrophotometer (Tokyo, Japan) [11].

2.4 Al-'alqami River water samples collection

The figure 2 shows the Al Alqami River to the far right in blue, the Imam Al-Abbas Shrine next to the Al-'alqami River and to the left of it, , Imam Al-Hussain Shrine and the spot where Sayida Ruqai was standing to the far left in full view of the scene unfolding in front of her. Sayida Ruqiya was standing on the Tel Al-Zainabi which is a small rise where the Camp of

Imam Hussain (A.S.) and his family stood. It is 192 meters to the left of the Imam Hussain Shrine and 566 meters to the Imam Al-Abbas Shrine to the right and 895 meters to the Al-'alqami River. The water samples taken from the mosque of Imam Mahdi (A.S.) site, the Hands Monument of Imam Abbas site and near the Al-Abas Shrine. Three samples taken from each site. The turbidity was measured using Milwaukee Mi415 Turbidity Meter, Martini Instruments, Inc., USA. [12]. Total count of bacteria in the water using the method of counting dishes and that planting 1 ml of the original or diluted sample at the center of Nutrient agar the plate incubated at 35 ℃ for 48 hours**.**

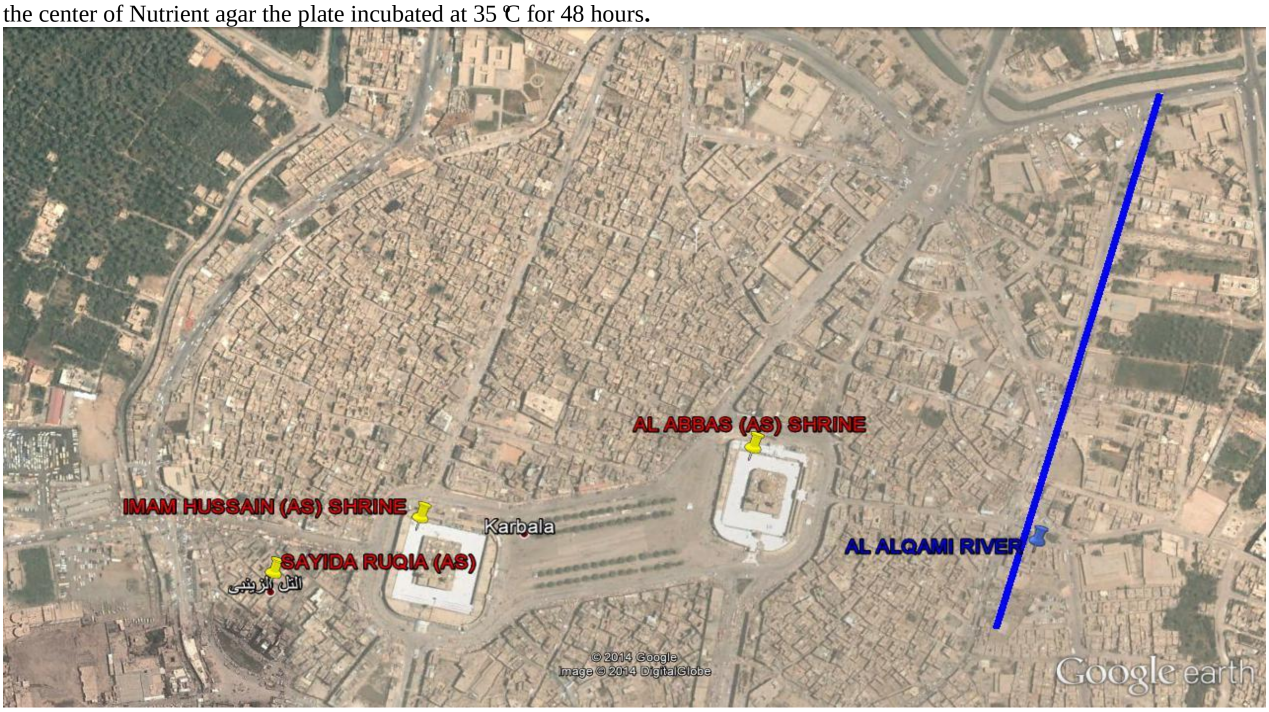

Figure 2. The Al-'alqami River, Holy Karbala, Iraq.

2.5 Determination the suitable amounts of MIONPS.

The test was down to determine the amount of magnetite iron oxide nanoparticles capable to killing the largest number of bacteria during the treatment of contaminated water experimentally by *E. coli*. The following concentrations were used 5 µg, 10 µg, 15 µg, 20 µg, 25 µg, 50 µg, 75µg and 1000µg / mL [13]. After that the magnetite iron oxide nanoparticles particles are separated from solution by magnet bar.

2.6 Efficacy of iron oxide nanoparticles.

The synthesized nanoparticles were evaluated for their efficiency to treat raw water collected from Al-'alqami River. Initial and final concentration of *E.coli* was measured by Plate Count Method as per the Standard Method for Water and Wastewater Analysis [14]. A dose of 1 gL^{-1} of nanoparticles was added to Al-'alqami River water. The solution was stirred at 160 rpm at 37 ℃ for 12 and 24 hours. After treatment, the nanocomposites were magnetically separated and the supernatant was filtered through Whatman® Grade GF/C filter paper and the filtrate was analyzed for bacteria. The experiment was done in triplicates.

3. Results

The UV Visible spectrum of magnetite iron oxide nanoparticles in the aqueous Al- Abbas's (A.S.) Hund Fruit extract is shown in Figure 2. The absorption peaks at wavelengths of 340 nm indicate the formation of magnetite iron nanoparticles.

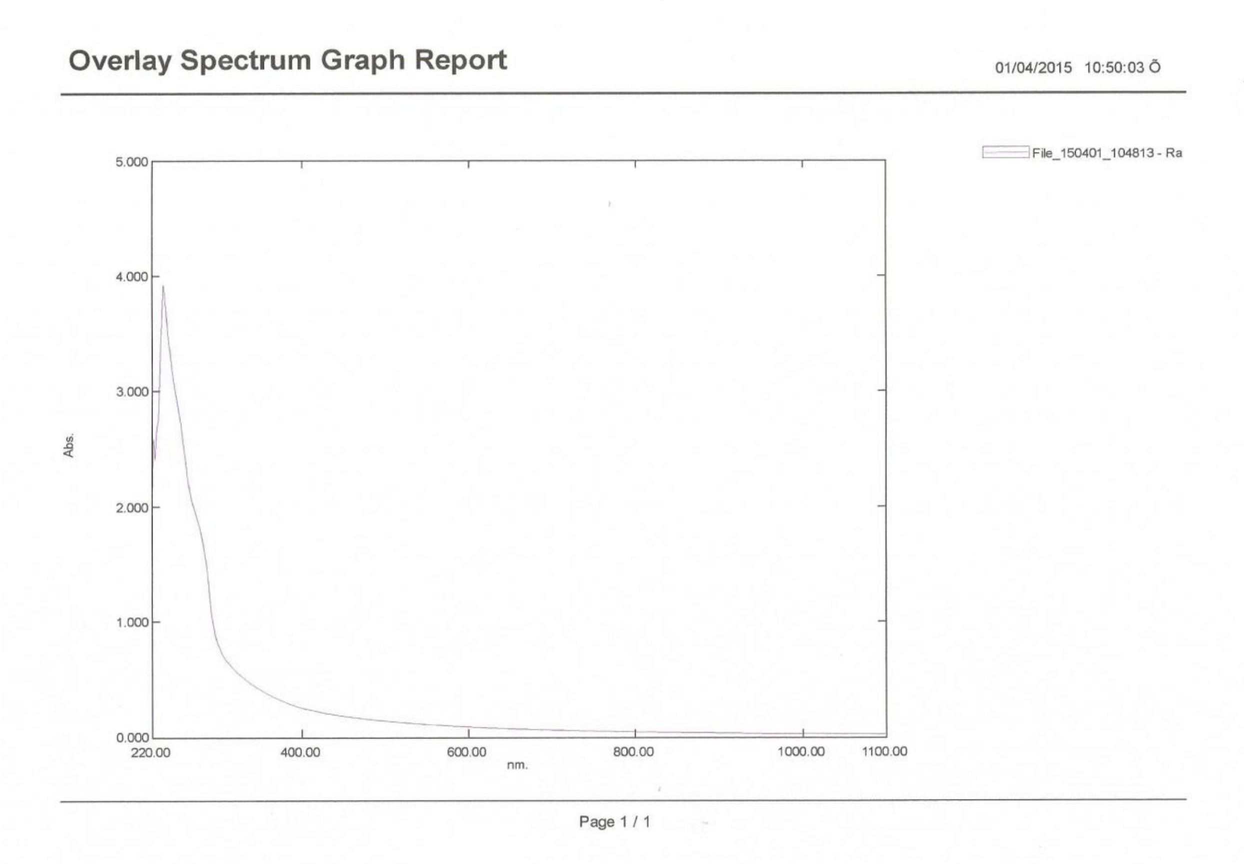

Figure 3. UV-visible absorption spectra of Magnetite iron oxide nanoparticles synthesized by Al- Abbas's (A.S.) Hund Fruit extract.

The FTIR spectra for Magnetite Iron oxide nanoparticles after treatment with the Al- Abbas's (A.S.) Hund Fruit extract is shown in Figure 3. The peaks at 519 cm^{-1} confirm the presence of nanoparticles in the synthesized samples. The magnetite iron oxide nanoparticles vibration comes around 1676 cm^{-1}, which confirms the hydrogen bonding between oxygen molecule present in Iron oxide nanoparticles. From the FT-IR data, it is clear that the bioactive molecules present in the Al- Abbas's (A.S.) Hund Fruit extract interacted with the synthesized Magnetite iron oxide nanoparticles.

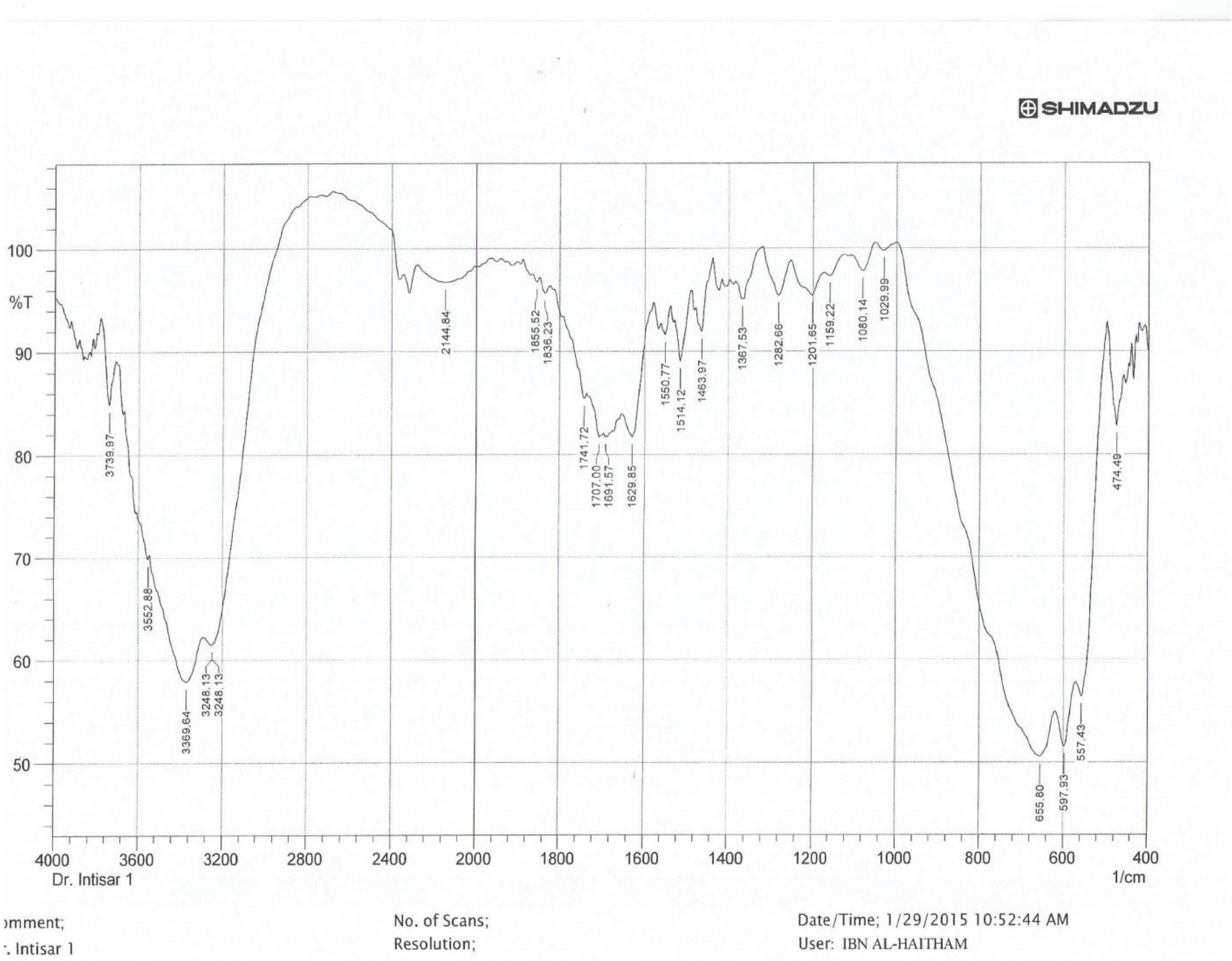

Figure 4. FTIR graph of Magnetite iron oxide nanoparticles synthesized by Al- Abbas's (A.S.) Hund Fruit extract.

The XRD pattern of the Magnetite Iron oxide nanoparticles (MIONPs) is shown in figure 4. The diffractogram exhibits six distinct diffraction peaks at 2θ values of 30.3°, 35.5°, 43.2°, 53.5°, 62.7° and 57° which correspond to the (220), (311), (400), (422), (511), and (440) crystallographic planes of the inverse spherical magnetite crystal. They matched the diffraction peaks for pure magnetite (Fe_3O_4) from the reference database (JCPDS File No.19-629). The average crystallite size was evaluated by diffraction line broadening (d 311) using the Debye - Scherrer equation. The average size of Fe_3O_4 crystallites obtained from nanocomposite was about 45nm. Although the Debye-Scherrer formula always tends to underestimate the real crystallite size [15]. The micrographs of scanning electron microscope of the magnetite iron oxide nanoparticles synthesized by Al- Abbas's (A.S.) Hund Fruit extract is shown in figure 5, the morphology of the particles was observed to be spherical.

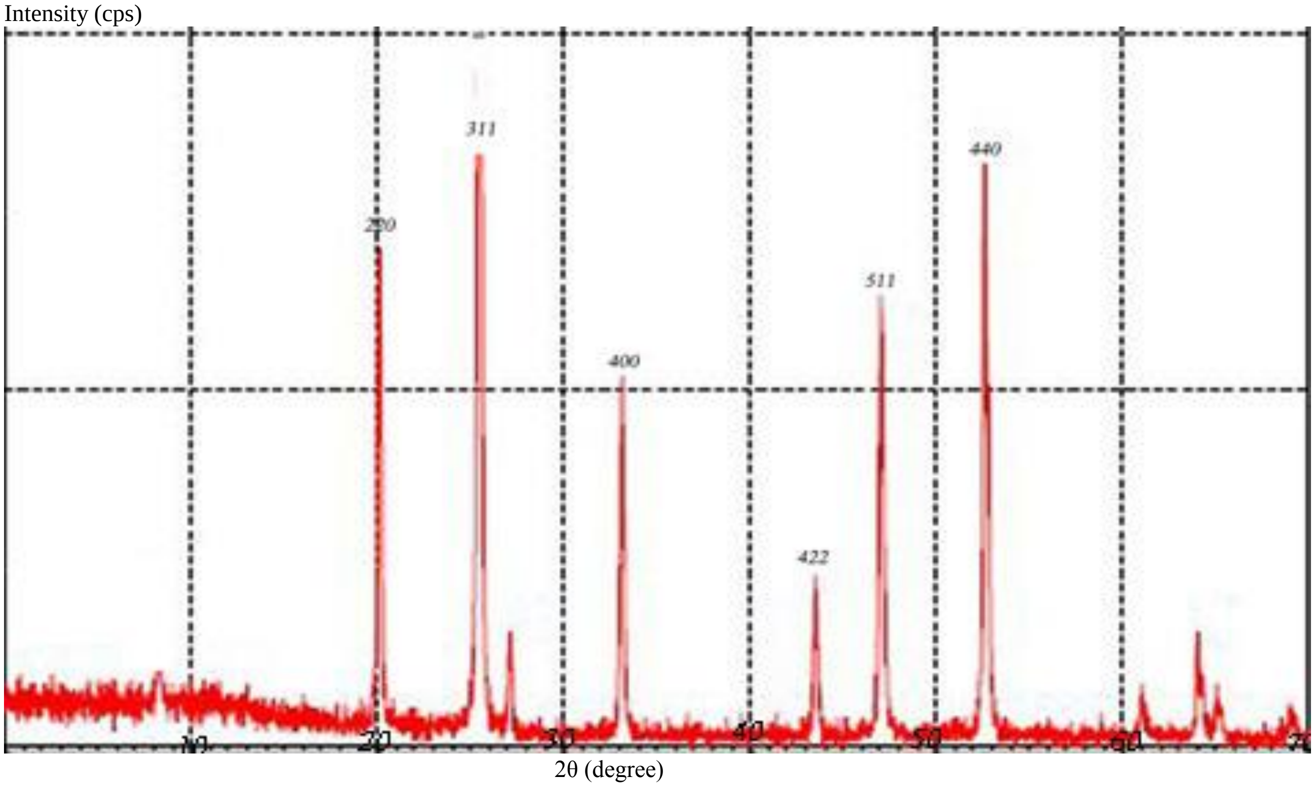

Figure 5. XRD patternof the Magnetite iron oxide nanoparticles synthesized by Al- Abbas's (A.S.) Hund Fruit extract.

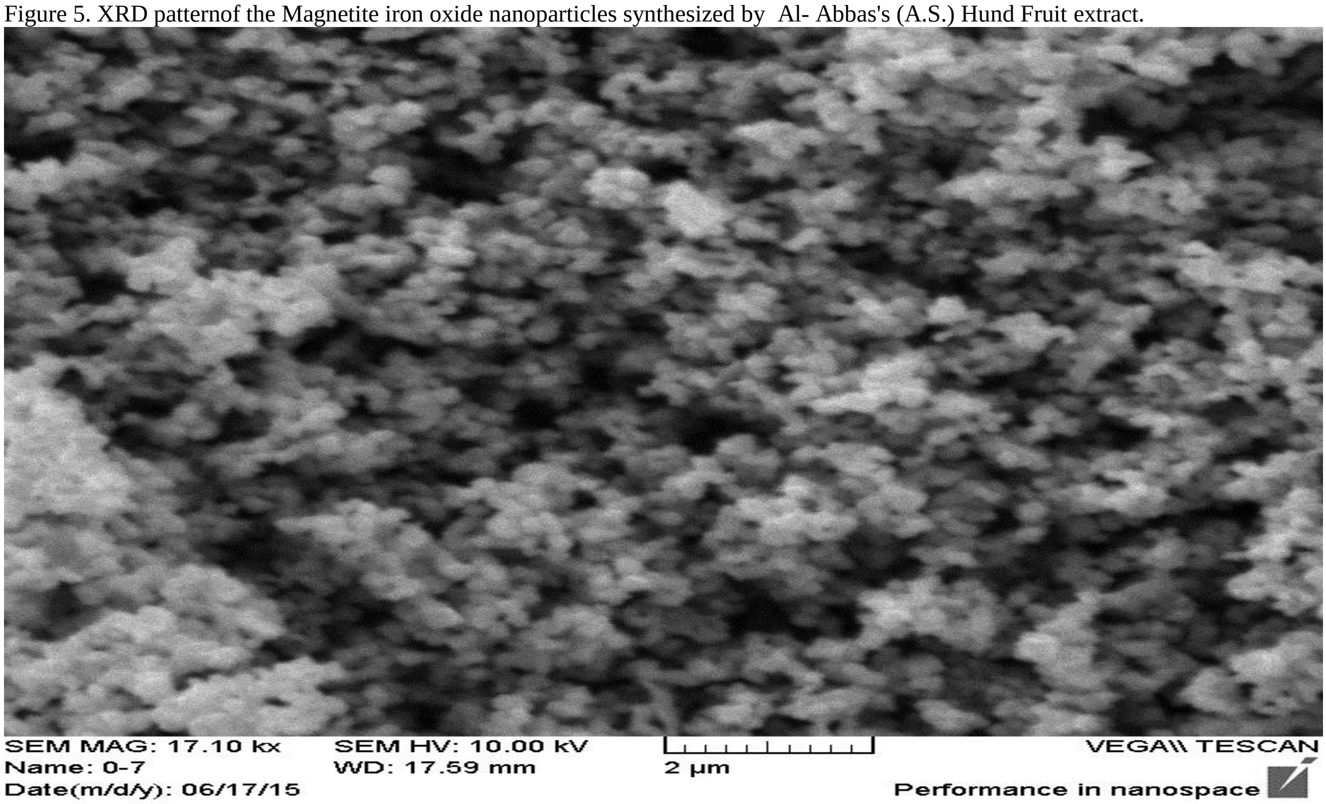

Figure 6. SEM image of Magnetite Iron oxide nanoparticles synthesized by Al- Abbas's (A.S.) Hund Fruit extract.

The effect of Magnetite iron oxide nanoparticles (MIONPs) concentration and time of exposure on *E. coli* survival are shown in Table (1) and figures (7-9). The higher reduction percentage of *E. coli* survival was 100% by using 1mg/ml of MIONPs and after 24 hours of exposure, whereas the lowest reduction percentage of *E. coli* survival was 5% by using 5μg/ml of MIONPs and after 12 hours of exposure.

Table 1: Effect of MIONPs concentration and time of exposure on *E. coli* survival.

Concentration of MIOPNs*	Reduction percentage	
	12h.	24h.
5 µg /ml	5	9
10 µg /ml	12	15
15 µg /ml	18	30
20 µg /ml	23	36
25 µg /ml	31	51
50 µg /ml	55	71
75 µg/ml	78	86
1 mg/ml	93	100

MIOPNs*= Magnetite Iron Oxide Nanoparticles.

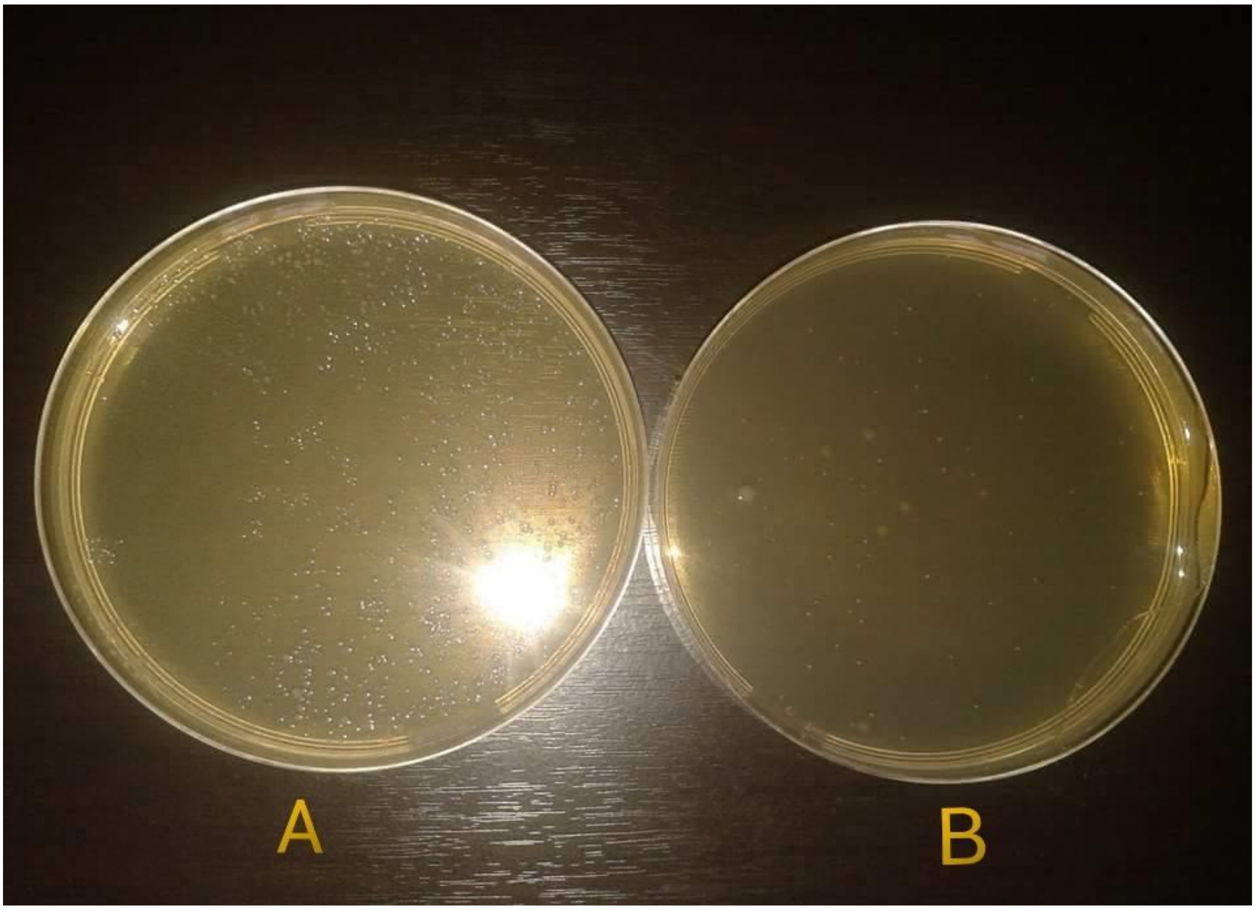

Figure 7. Water treatment by 25 µg /ml MIOPNs. A-before treatment, B-After treatment.

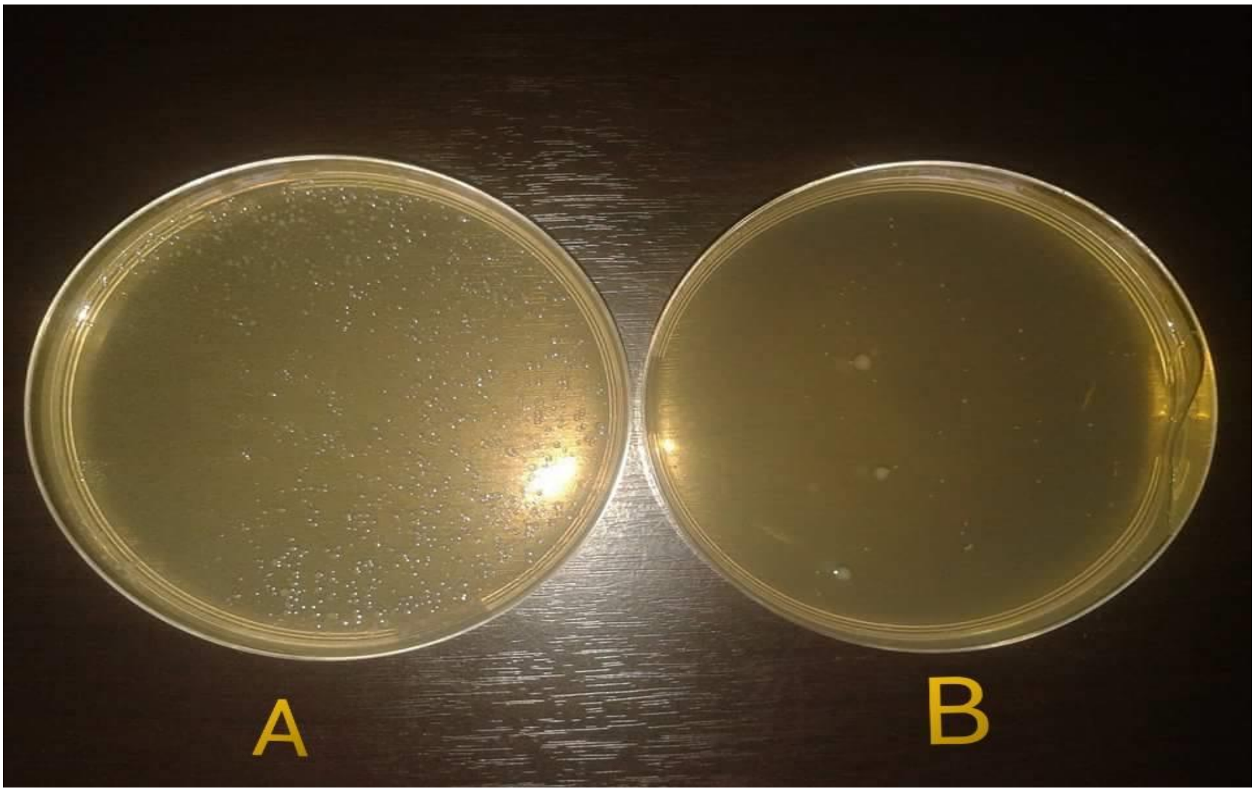

Figure 8.Water treatment by 50 µg /ml MIOPNs. A-before treatment, B-After treatment.

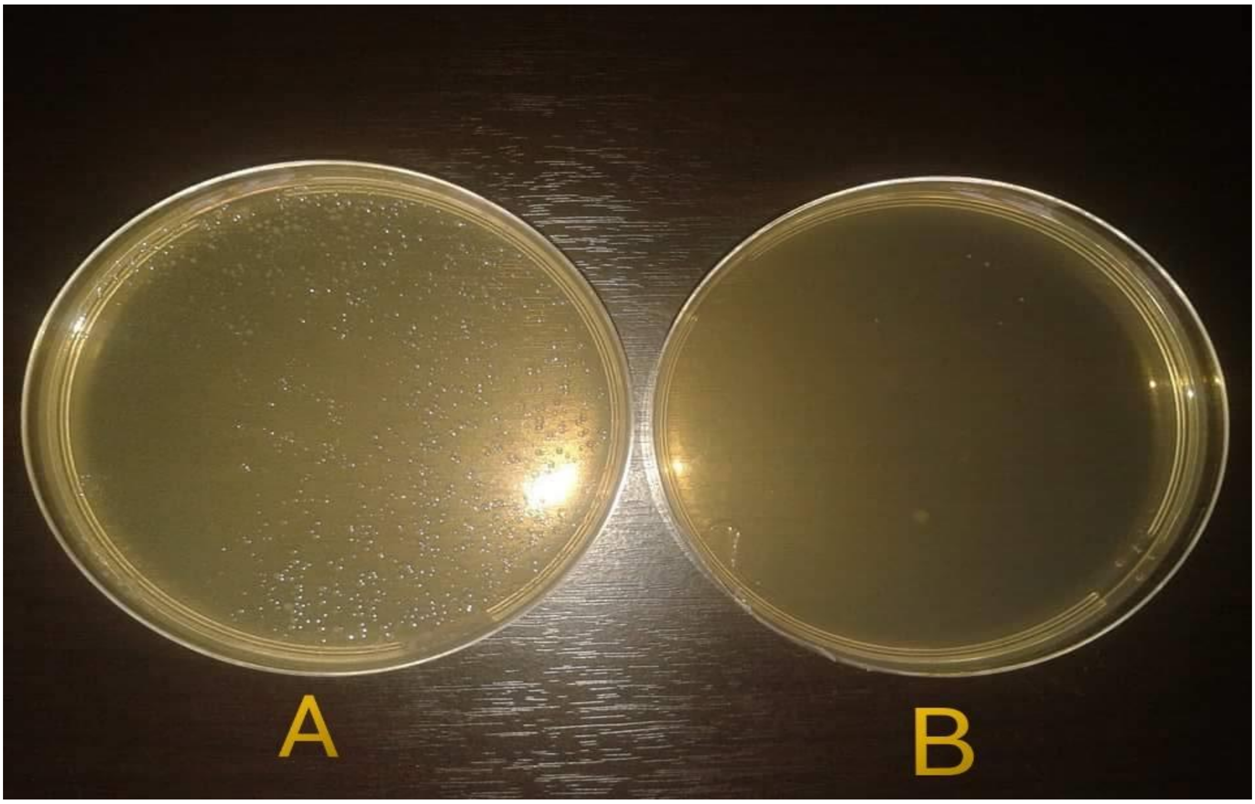

Figure 9.Water treatment by 75 µg /ml MIOPNs. A-before treatment, B-After treatment.

The results of Al-'alqami River samples examination shows, the lowest turbidity and number of colony forming units were 40 NTU and 4×10^4 respectively at the mosque of Imam Mahdi (A.S.) location. Whereas the highest turbidity and number of colony forming units were 60 NTU and 8×10^7 respectively at The Hands Monument of Imam Abbas (A.S.) (Table 2).

Table 2: The sample collection from.

Sampling sites	Turbidity/NTU	Mean CFU/100ml
Mosque of Imam Mahdi (A.S.)	40	4×10^4
Hands Monument of Imam Abbas	60	8×10^7
Al-Abbas's Shrine	12	2×10^3

The Al-'alqami River water treatment by magnetic iron oxide nanoparticles (MIOPNs) synthesis by Al- Abbas's (A.S.) Hund Fruit extracts led to the reduction of the number of bacteria to 80% after twelve hours of treatment either water treatment by magnetic iron oxide for 24 hours led to kill all the bacteria in water (Table 3) and figure 10.

Table 3: Effect of magnetite iron oxide nanoparticles on microbes in Al-'alqami River water samples.

Location of Samples	CFU/100ml before treatment	CFU/100ml after 12 hrs. treatment	CFU/100ml after 24 hrs. treatment
Mosque of Imam Mahdi (A.S.)	4×10^4	1×10^3	0
Hands Monument of Imam Abbas	8×10^7	3×10^4	0
Al-Abbas's Shrine	2×10^3	1×10^2	0

* The recorded value is mean value of 3 replicates.

Figure 10. The Treatment of Al-'alqami River water by Magnetite Iron Oxide nanoparticles (MIONPs). A- Al-'alqami River water. B- Al Al-'alqami River water treated by (MIONPs), C- Separation of (MIONPs) by Magnetic bar.

4. Discussion

Citrus medica L. var. sarcodactylis belonging to the family Rutaceae has originated in India and has been worldwide spread to other regions following the paths of civilization [16]. It is known as fingered citron or as fo-shou (Buddha's hand) in China [17]. In Iraq this type of trinj is rare and it is known in Diyala city and some parts of north of Iraq as Al-Abbas's Hand fruit and used in folk medicine as tonic, antispasmodic, antiemetic, expectorant and inhaler. People in Iraq believe that cultivation of this plant brings them luck and livelihood. Recent studies reported that *C. medica* L. var . sarcodactylis constituted coumarin compounds, p-coumaric acids, steroids, triterpenoids, limonin, nomilin, etc [18, 19]. The major volatile components in the peel oil from the Japanese fingered citron were limonene and γ- terpinene [20].

Al-'alqami is the name of the river on whose bank was the last scene of the life of Al-Abbas (A.S.). This river was gathered around by big numbers of soldiers whose mission was to prevent the camp of Imam Al-Hussein (A.S.) from water. By his giant determination and unmatched heroism, Al-Abbas could attack that army and occupy the river to carry water to the camp of his brother more than once. In the last time, he was martyred there. Hence, he was called 'Battal Al- -'alqami -the hero of Al-'alqami'[9]. Today this river still running and its holy to Shia Muslims, they are performs ablution using its water and they are demanding needs from God by dip their hands in it.

The UV Visible spectrum of magnetite iron oxide nanoparticles (MIONPs) in the aqueous Al- Abbas's Hand fruit extract is shows the absorption peaks at wavelengths of 340 nm which indicated the formation of magnetite iron nanoparticles. This finding is in agreement with [21, 22]. The FTIR spectra for Magnetite Iron oxide nanoparticles after treatment with the Al-Abbas's Hand fruit extract showed the peaks at 519 cm^{-1} This confirms the presence of nanoparticles in the synthesized samples. The hydroxyl, sulphate and aldehyde group present in the Al- Abbas's Hand fruit extract are apparently involved in the bioreduction and stabilization of magnetite Fe_3O_4 -NPs. The results are in agreement with observation of several studies

[23, 24]. The results of the XRD pattern of the Magnetite Iron oxide nanoparticles (MIONPs), exhibits six distinct diffraction peaks at 2θ values. The diffraction angles of different peaks are corresponds to Fe_3O_4 nanoparticles. The X-ray power diffraction (XRD) results of nanoparticles confirmed that the synthesized product was a magnetite (Fe_3O_4). The results are in agreement with several studies dealing with green synthesis of MIONPs [25, 26]. The average size of Fe3O4 crystallites obtained from nanocomposite was about 45nm. The micrographs of scanning electron microscope of the magnetite iron oxide nanoparticles synthesized by Al- Abbas's Hand fruit extract is shown in figure (8), the morphology of the particles was observed to be spherical. The results are in agreement with observation from SEM images [27].

After that we conducted tests that particles are produced were magnetite iron oxide nanoparticles (MIONPs) resulting from the reduction of iron ions mediated by ascorbic acid (vitamin C) found in large quantities in citrus, particularly in the Al- Abbas's Hand fruit these finding are in agreement with several studies [28, 28, 30].

The effect of Magnetite iron oxide nanoparticles (MIONPs) on *E. coli* survival showed that the higher effect was 100% by using 1mg/ml of MIONPs and after 24 hours of exposure, whereas the lowest effect was 5% by using 5μg/ml of MIONPs and after 12 hours of exposure these finding is in agreement with [31, 32].

The high level of turbidity and bacteria in the Hands Monument of Imam Abbas (A.S.) site compared to others is attributed to the higher polluted materials discharged into the river from Iraqi and foreign visitors in this site.

The Al-'alqami River water treatment by magnetic iron oxide nanoparticles (MIOPNs) synthesis by Al- Abbas's Hand fruit extracts led to the reduction of the number of bacteria to 80% after twelve hours of treatment either water treatment by magnetic iron oxide for 24 hours led to kill all the bacteria in water

The bacteria are used the iron oxide as a source of iron ions. The main mechanism by which these particles showed antibacterial activity might be via oxidative stress generated by ROS [31]. ROS, including superoxide radicals (O_2^-), hydroxyl radicals (^-OH), hydrogen peroxide (H_2O_2), and singlet oxygen ($^1O_{2)}$, can cause damage to proteins and DNA in bacteria. In the present study, metal oxide (FeO) could be the source that created ROS leading to the inhibition of most of the pathogenic bacteria found in Tigris River water samples. A similar process was also described by Kim *et al*., (2007) in which Fe_2^+ reacted with oxygen to create hydrogen peroxide (H_2O_2). This H_2O_2 consequently reacted with ferrous irons via the Fenton reaction and produced hydroxyl radicals which are known to damage biological macromolecules. Some authors have demonstrated that the small size of nanoparticles can also contribute to bactericidal effects. Lee *et al*., (2008) reported that the inactivation of *Escherichia coli* by iron nanoparticles could be because of the penetration of the small particles (sizes ranging from 10-80 nm) into *E. coli* membranes. Nano scale iron could then react with intracellular oxygen, leading to oxidative stress and eventually causing disruption of the cell membrane.

Studies on nanoparticles have also shown that antibacterial activity increased with decreasing particle size due to the higher surface area to volume ratio. Furthermore it shows better bactericidal activity in Gram-positive bacteria as compared to Gram-negative bacteria [27, 31, 32, 33].

For these reasons Magnetic Iron Oxide Nanoparticles (MIOPNs) synthesized by Al- Abbas's Hand fruit extracts was efficient in water treatment its kill all bacteria exists in water beside the ability of this material to absorbed some heavy metal, minerals, dyes and suspended materials such as clay, algi, fungi conidia etc, therefor cooperation in water purification.

5. Conclusions

Application of Magnetite Iron Oxide nanoparticle in water treatment showed potential effect against bacteria existed in Al-'alqami River water. It is suggested that these may be used in future at large scale water purification. In particular, the use of magnetite nanoparticles as adsorbents in water treatment provides a convenient approach for separating and removing the contaminants by applying external magnetic fields.

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