Antimicrobial activity by diffusion method using iron oxide nanoparticles prepared from (Rose plant) extract with rust iron

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Abstract. This research succeeded in the prepared of iron oxide NPs using rose plant extract with rust iron extract at 300 °C for two hours by simple chemical method. Iron oxide NPs have been developed as an death of antimicrobial as an alternative to toxic chemical drugs to prevent negative effects on human health. Iron oxide NPs were diagnosed using X-ray diffraction (XRD) analysis, Field Emission Scanning Electron Microscopy (FE-SEM) analysis, and Photoluminescence (PL) spectrum. XRD measurements explained the small crystalline size (61 nm) with (inverse cubic) structure (wustite) for (Fe0.911O0.4) NPs at 300 °C using rose plant extract. FESEM showed the average grain size of Fe0.902O NPs (wustite) rose plant extract at 300 °C was 79.59 nm. PL spectrum determined a blue shift for the optical near band edge value was 2.75 eV for Fe0.911O NPs (wustite) at 300 °C. Iron oxide NPs were applied in antimicrobial for removal of toxic bacterial by diffusion method. The success of this work will open wide new horizons for us in solving the problem of spent iron and how to get rid of it and the manufacture of new nanoparticles in medical treatments.

Keywords: Iron oxide NPs; Rose plant extract; Rust iron extract; Antimicrobial activity.

1. Introduction

Nanoparticles (NPs) could be defined as small particles with diameter ranging less than 100 nm in dimensions, synthetic of inorganic or organic materials, having new properties as contrasted to the bulk materials [1]. Among the NPs, Iron oxide NPs are significant materials which have great future advantages compared to other materials [2] special physiochemical properties, such as low toxicity, high catalytic activity, small sizes, with different physical properties [3-5]. At the present time human activity in all areas of life is associated with chemicals and these substances have great harm to the human body, especially a number of these substances are constantly increasing in used, and it is agreed that there is no completely safe chemical substance and in return it is not. There is a chemical substance that can be considered completely harmful. Chemists in the middle ages suffered from debilitating and other diseases due to the damages of explosive and toxic materials that they worked to, and serious environment troubles began for show in a world at the beginning of the 17 century due to cinder released of the mines, in addition into fabrication of pigment and other chemicals of charcoal tar in Germany through the 18 century, which led to emergence of toxic and polluting secondary compounds for the
environment, and the steadily increasing quantities and numbers of chemical compounds manufactured in 19 century, counting steely and waste iron rust, lead batteries and petroleum, and with it amount of pollutants and harmful materials raised in environment, among these materials a focus come to iron metal which play a major element in the universal due to the varied uses in too many applications and industry. The overmuch uses of iron metal, this led to founding as thousands of tons from scrap and rubbish rusty iron in the garbage yard without any possible resolution to reuse or benefit from it till now which make the perilous environmental issue in different places in this world.In summery the iron consumed and the huge quantities of it formed a burden on the environment and became a real problem for disposal at the present time.Bio-synthesis of iron oxide NPs using plant extracts as biological components has numerous interest such as cost-effectiveness, atom economy, simplicity, benign, nontoxic, elimination of toxic and dangerous materials, easy availability and removal toxic substances for environmental remediation [6-8]. Wüstite (FeO) is a mineral form of iron (II) oxide found with meteorites and native iron. It has a gray colour with a greenish tint in reflected light. Wüstite crystallizes in the isometric-hexoctahedral crystal system in opaque to translucent metallic grains. The Literature Review of this work: In (2020) R. C. Popescu et al., The synthesized of iron oxide NPs and application in cancer treatments [9]. In (2018), Helale Kaboli Farshchi, et al., Synthesized iron oxide NPs using Rosemary extract and application in cancer treatments [10].In (2017), Sathiskumar G., et al., Synthesized iron oxide NPs using Couroupita guianensis Aubl by (chemical method). Application in vitro anticancer activity and antibacterial activity [11]. In (2018), Shraddha C., et al., Synthesized of iron oxide NPs using henna leaves extract by (chemical method). Application of iron oxide in antimicrobial [12]. In (2019), Saeed J., et al., Prepared iron oxide NPs using plant extract. Application in antimicrobial [13]. The novelty of this work that all the materials used (which it is used in the first time as the authors knowledge) considered as a waste matter without any cost mentioned to produce valuable and useful NPs materials. In addition, there are no chemical compound used in this research, neither the raw materials nor the final product. All materials used are natural without any supplement. In this work, iron oxide NPs were prepared by mixing wasted iron rust with (rose plant) extract at the (300) oC for two hours. The antimicrobial treatment using iron oxide NPs by diffusion method. The iron oxide NPs (Fe0.911O) were characterization by X-ray diffraction (XRD) using "(XRD6000 Shimadzu, Company / Japan), Filed Emission Scanning Electron Microscopy (FE-SEM) using (Tescan Mira3 FESEM-Czechia)". Also, the photo luminance (PL) by (Jobin Yvon HR800UV).

2. The experimental part

2.1. Apparatus and equipment

The plants have been collected from the local market in (Baghdad / Iraq), as a preliminary work. The wasted iron specie were pruched from locally (Baghdad / Iraq). All glass ware were cleaned by distilled water (distilled water device, Company / Gallenkamp, England). In addition, all solutions in this work were prepared via distilled water. The beneficial components present in the rose plant are flavonoids, anthocyanins, terpenes and glycosides, and it has been found that the components have the most therapeutic properties in leaf plants are the phenolic compounds that are antioxidant, depression and inflammatory, and the rose leaves contain a number of vitamins such as vitamin E, D, C., A, and vitamin B3 [14].

2.2. The prepared of the (rose plant) extract.

The rose plant were washed with distilled water and then dried under sunlight for 5 days. Then the rose plant were ground using an electric grinder. The mixing 7 gram from rose plant powder with 80 ml from distilled water on magnetic stirrer at 70 °C for 1.5 hours. It observes the colour change of the solution and this is an indication of the formation of an iron oxide NPs material. The final solve are
freeze to room temperatures and filter using Whatman filter sheet. Figure 1 explains the phases of transferring the (*rose plant*) into the extract.

**Figure 1.** shows the phases of preparation the plant extract (*rose plant*), A) *rose plant*, B) *rose plant* powder, and C) *rose plant* extract.

2.3. **The prepared of wasted iron (rust) extract**

The weight of the wasted iron (rust) used to synthesis iron oxide NPs is 146.0 g. The wasted iron piece was washed with distilled water to get rid of the impurities attached to it. After that, the wasted iron piece was placed with 500 ml of distilled water in a glass flask under the sunlight for 8 days. Then the wasted iron extract was obtained in red color with a size of 250 ml and stored in sealed tubes to synthesis iron oxide nanoparticles. Figure 2 explains the phases to synthesis wasted iron (rust) extract from wasted iron specie (rust) with distilled water.

**Figure 2.** shows the steps for preparation a spent iron extract, A) iron specie, B) iron specie with distilled water before exposes for sunlight, C) spent iron extract after exposes for sunlight, and D) spent iron extract.

2.4. **The prepared of iron oxide NPs (Fe$_{0.911}$O) of (rose plant) extract and wasted iron extract (rust).**

The Prepared of iron oxide NPs from mixing 250 ml of wasted iron extract (rust) to 100 ml from rose plant extract. After that, solve were place on hotplate stirrer at 70 °C for 60 minutes. It observes the solution during synthesis, the colour from extractor reaction changed suddenly from translucent offlight pink into black, referring the formed of iron oxide NPs. The resulting solve was cooled at room temperature. For obtain the nano-powder of iron oxide solve, the taken 30 ml of iron oxide NPs solution was putted in a ceramic vine with steamed at 200 °C for 2 hours. Lastly, iron oxide NP solves were stocked in sealed serum tubes to further diagnostic. In figure 3 (A-D) explains the phases to synthesis for iron oxide NPs from rose plant extractor and wasted iron extract (rust).

**Figure 3.** The transfering phases of the wasted iron specie into extract under sunlight for 5 dayes, A) wasted iron specie, B) *rose plant* extract, C) iron oxide NPs, and D) iron oxide NPs powder.
2.5. Characterization of iron oxide NPs (Fe$_{0.911}$O) created using (rose plant) extract.

The specimen was determined by XRD analysis of via the results supplied using the joint committee on powder diffraction standards (JCPDS) card. The scale range of XRD measurements were collected between 20°–70° by use a step-by-step examination model (XRD-6000, Shimadzu) employment at 30 mA and 40 kv. The PL spectrum were resolved by a double beam spectrophotometer (Jobin Yvon HR800UV).

2.6. Antibacterial activity from the spent iron extract with (rose plant) extract.

The microbial cultures were providing by the Microbiology laboratory of the University of Baghdad-College of Education for pure science/ Ibn al-Haitham. This test carried out by agar well diffusion methods in sterile petri-dishes with 90 mm diameter containing sterile nutrient agar medium. Nutrient agar medium was prepared by dissolving 0.5 g peptone this provides organic nitrogen; 0.3 g yeast extract these contributes vitamins, carbohydrates, nitrogen, and salts; 28 g agar this gives the mixture solidity in 1000 ml distilled water. pH was adjusted to 6.8 at 27 °C. These ingredients are combined and boiled for approximately 15 minutes to ensure they are mixed typically at 121 °C and poured into petri dishes which are covered immediately until it becomes solidified. After that, iron oxide NPs were dissolved in Dimethyl Sulphoxide (DMSO) solvent with concentration 30 mg/ml. The freshly prepared bacteria were swabbed over the surface of the petri-dishes. Wells of 8 mm diameter each were made, in the medium of bacterial and fungal for each plate using sterilized gel puncher/crok borer. Each well contains 40 μL of iron oxide NPs, and control (DMSO) for bacteria and fungi respectively at desired concentration is introduced into the well. The test samples were incubated at ±25 °C for 24 hours for bacteria and fungi followed by the gauge of diameter of inhibition zone in (mm). Percentage of inhibition zone have been measured using the following equation:

\[
Inhibition\ Zone\ (\%) = \frac{Diameter\ of\ the\ inhibition\ zone\ in\ mm}{Diameter\ of\ petriplate\ (90\ mm)} \times 100\%\]

3. Results and discussions

3.1. Synthesizing and Characterizing of iron oxide NPs from (rose plant) extract.

Iron oxide NPs prepared by the modern plant rose plant extract; the extract is mixed with spent iron extract at different reaction conditions. The factors identifying a terms of the rose plant extract to control the rate from iron oxide NPs formation as well as their filed and stability. The phytochemicals inside rose plant extract have ability to less spent iron ions in short time. In addition, rose plant extract plays an important role from reduce and stabilize factors in iron oxide NPs prepared way for simplify iron oxide NPs. The beneficial components present in the rose plant are flavonoids, anthocyanins, terpenes and glycosides, and it has been found that the components have the most therapeutic properties in leaf plants are the phenolic compounds that are antioxidant, depression and inflammatory, and the rose leaves contain a number of vitamins such as vitamin E, D, C., A, and vitamin B3, which are responsible to synthesis of iron oxide NPs.

3.2. The XRD analysis of iron oxide NPs (Fe$_{0.911}$O) prepared from (rose plant) extract with rust iron extract.

Iron oxide NPs were bio-synthesis by a chemical way from mixing the (rose plant) extract with rust iron extract at 300 °C in Figure 4. The peaks high of the crystalline (Fe$_{0.911}$O) phase (Wustite, space group Fm-3m, JCPDS no.01-079-1880) is (220) corresponding to (111), and (200) millers indices with the face centre cubic (F.C.C), as shown in Figure 4 [15-16]. The results the phases, and crystallite size of
iron oxide NPs (Fe_{0.91}O) shown in Table 1. The crystallite size (D) was determined Applied the following Scherrer’s formula [17-18].

\[ D (\text{nm}) = \frac{k \lambda}{\beta \cos \theta} \]

Where k called shape factor (0.9), \( \lambda \) the wavelength (0.15418) nm (CuK\( \alpha \)), \( \beta \) is full width at half maximum (FWHM) and \( \theta \) is diffraction angle [19].

Table 1. XRD analysis shows of the crystallite size for iron oxide NPs prepared from rust iron extract with (rose plant) extract.

| Plant extract | Materials     | Temperature (°C) | (hkl) | FWHM  | Crystallite size D (nm) |
|---------------|---------------|------------------|-------|-------|------------------------|
| Rose Plant    | Fe_{0.91}O    | 300              | (220) | 0.13  | 61                     |

Figure 4. XRD analysis shows of the iron oxide NPs prepared from rust iron extract with the (rose plant) extract, the crystalline Fe_{0.91}O phase (wustite, space group Fm-3m, JCPDS no.01-079-1880) at 300 °C.

3.3. The FE-SEM images of iron oxide NPs (Fe0.911O) prepared from (rose plant) extract with rust iron extract.

The FE-SEM images shows the size distribution and the surface morphology of eco-friendly iron oxide NPs created from the (rose plant) extract with rust iron extract at 300 °C. The grain average size is from (71.59) nm with the morphology is (nano-Slices) structure of the Fe_{0.91}O NPs (Wustite) at 300 °C, as shown in Figure 5 (A-B).
3.4. The PL spectrum of iron oxide NPs ($\text{Fe}_{0.911}\text{O}$) prepared from (rose plant) extract with rust iron extract.

The PL spectrum shows the near band edge of eco-friendly iron oxide NPs created from the (rose plant) extract with rust iron extract at (300)$^\circ$C by a chemical way. The near band edge is (2.65) nm with the excitation band is 325 nm of $\text{Fe}_{0.911}\text{ONPs}$ (Wustite, the near wavelength (469) nm at 300 $^\circ$C, as shown in Figure 6.

3.5. Antibacterial and antifungal activity of iron oxide ($\text{Fe}_{0.911}\text{O}$) NPs using rose plant extract.

The efficacy of anti-bacterial and antifungal activity in terms inhibition of zones (mm) has been measured against four slander bacterial isolates: gram-positive bacteria (B. subtilis and S. aureus), gram negative (K. pneumonia) and (candida). The bio-synthesis of iron oxide NPs showed the good effectiveness of inhibition percentage, but bio-synthesis of iron oxide NPs prepared from rose plant extract were more effective than rose plant only extract. The antibacterial activity of iron oxide NPs is chiefly due to the release of iron ions all have attached to the bacterial cell wall due to electrostatic attraction. Furthermore, the metal ions are not only interacting with the surface of membrane but can
also penetrate inside the bacteria. NPs may react with the thiol group (-SH) in the cell wall of the bacteria and not allow the transport of nutrients through the cell wall. The protein decreases inside the cell, eventually causing the cellular death. Or the other reason is that the smaller particle size of the synthesized metals NPs connected with (linked with) the large band gap and consequently, more available oxygen will lead to the generation of a higher concentration of reactive oxygen species (ROS) to enhance antimicrobial activities. However, the activity depended on large surface area to volume, concentration of iron oxide NPs, crystalline structure, and particle shape. The results of iron oxide NPs (Fe$_{0.911}$O) prepared using (rose plant) extract using, dose give good result against (k. pneumonia).

It notes the highest rate of destruction of negative and positive bacteria of both types when using iron oxide (Fe$_{0.911}$O) NPs, as shown in Figure 7 and in the Table 2. The disk-diffusion agar method tests the effectiveness of antibiotics on a specific microorganism. An agar plate is first spread with bacteria, then paper disks of antibiotics are placed atop of it. The bacteria are then allowed to grow on the agar media and then observed for growth and effect of the antibiotic on it.

![Figure 7. Antibacterial and antifungal activity (gram-nagative and gram-postive) of A. rose plant extract only, B. Fe0.911Ousing rose plant extract, C. rust iron extract.](image)

| Com | Staphylococcus aureus | Bacillus subtilis | Klebsiella pneumoniae | Candida albicans |
|-----|----------------------|------------------|----------------------|-----------------|
| A   | -                    | 30               | 32                   | -               |
| B   | 20                   | 38               | 45                   | 23              |
| C   | 25                   | 10               | 8                    | -               |

Table 2. results inhibition of zone of material iron oxide NPs, againts bacteria and fungi at 30 (mg/ml) concentrations.
4. Conclusion
This work succeeded in preparing iron oxide nanoparticles using natural extracts, and this success will open new and broad horizons for us in solving the problem of spent iron and how to get rid of it and the manufacture of new nanoparticles in medical treatments. Antibacterial of iron oxide NPs were certain by investigating the inhibition of zones which were from 45 mm to gram-negative bacteria (klebsiella pneumoniae) and 38 mm to gram-positive bacteria (Staphylococcus aureus and Bacillus), but were results are 23 mm to candida activity. Also, this work succeeded in studying the effective effect of bandaging of bacteria, and therefore excellent results were obtained in killing and destroying bacteria. Thus, the ability of these NPs to preserve human life from diseases caused by these bacteria was proven.

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