Antibacterial potential of synthesized iron oxide nanoparticles against uropathogens (*Escherichia coli* and *Staphylococcus aureus*)

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Abstract

Background : The biosynthesis of metal nanoparticles is an important broad and developing field of research in many fields, especially medical fields. **Material and methods:** in current study, iron oxide nanoparticles (Fe_3O_4-NPs) were synthesized from aqueous ferric chloride in a clear, simple, biosynthetic way using the aqueous extract of *Curcuma longa* powder, which is considered at the same time a reductant and a stabilizer as well. The prepared samples were characterized using several techniques: UV-Visible, TEM, SEM, and FT-IR. Through the color change, the formation of Fe_3O_4-NPs is evident through the appearance of the characteristic black color of the solution. Formation of Fe from *C. Longa* was also determined by UV spectroscopy where surface plasmon absorption was observed at 223-303 nm. Well-dispersed Fe_3O_4-NPs with isotropic and anisotropic morphology are seen for 20 mL of *C. longa* water extract with a size of 40 nm in the TEM images. The functional groups present in the NPs were mainly –OH and –COOH identified using FT-IR which makes them hydrophilic and hence the NPs do not need any functional modification. This study included bacterial isolates obtained from urine samples of patients suffering from urinary tract infections (UTIs), and then the antimicrobial activity of Fe_3O_4-NPs was evaluated against Gram-positive (*Staphylococcus aureus*) and Gram-negative (*Escherichia coli*) bacteria, and it was found that the zone of inhibition was 10.5 mm for (*E. coli*) and 17.6 mm for (*Staphylococcus aureus*). Thus naturally stabilized Fe_3O_4-NPs with herbal properties can be used in various biological applications.

Keywords: Green synthesis, *Curcuma longa*, Iron Oxide Nanoparticles (Fe_3O_4), urinary tract infections (UTIs)

Introduction

A urinary tract infection (UTI) often happens when a pathogenic microbe attaches to and multiplies in a portion of the urinary system due to a breakdown in the host's defense mechanisms. There are many host defenses, including normal urination, mucosal barrier, anatomical structures as well as urine properties and systemic immune competence. Although fungi and viruses can also infect the urinary system, bacteria are the primary cause of UTIs [1]. Urinary tract infections can affect multiple anatomic sites. They fall into two categories: lower urinary tract infections, which affect the bladder, urethra, and vagina, and upper urinary tract infections, which affect the kidneys and ureters. The upward movement of germs through the urethra and reproductive system to the bladder, ureters, and one or both kidneys causes bacterial urinary tract infections [2,3].

Numerous types of bacterial strains become resistant to antibiotics as a result of the frequent and careless use of antibiotics, and thus treating the simplest types of bacterial infections have become very difficult. This is because novel bacterial strains that are extremely resistant to antibiotics are starting to appear. Therefore, scientists are always searching for new resistant and antibacterial sources that are more effective to overcome this phenomenon. Moreover, nanotechnology is considered a good alternative for this purpose [4,5,6,7].

We believe that the use of nanotechnology can significantly improve antibacterial therapy using iron oxide nanoparticles (Fe_3O_4) to support cellular growth, and through food some antioxidants can be delivered exogenously and are referred to as exogenous antioxidants. Recently, there has been a lot of interest in the creation of biocompatible nanocomposites with antioxidant capabilities and prospective applications in the medical domain [8,9,10,11]. Iron oxide nanoparticles, which range in size from 1 to 100 nm, are a relatively new invention that is gaining a lot of attention and showing promise for use in numerous medical application studies [12,13,14,15]. Characteristics of magnetism, including strong coercivity, high magnetic susceptibility, and super-magnetism [16]. The primary objective of this research is to investigate the antibacterial activities of chemically produced iron oxide nanoparticles (Fe_3O_4) against bacterial strains such as *Escherichia coli* and *Staphylococcus aureus*.

Materials and methods

Materials

The phosphate buffer solution (PBS) and iron (II) chloride ($FeCl_2 \cdot 4H_2O$), which were acquired from Sigma-Aldrich Company in India, are the chemicals utilized in this experiment. The suppliers of the culture medium included Himedia Company in India for Gram stain and blood agar, and Accumedia Company in the USA for MacConkey agar, Mannitol salt agar, and nutritional broth.

Isolation and Identification of Microorganisms

From hospitalized UTI patients' urine samples have been collected and cultivated on selective culture media, the disease-causing bacteria (*Escherichia coli* and *Staphylococcus aureus*) were identified based on their phenotypic traits through several biochemical tests and by using the Vitek2 system for the final diagnosis of the isolates. The identification of bacterial morphology was

determined using gram stain and colony morphology after cultivation on the following culture media MacConkey agar and Mannitol salt agar [17,18].

Extraction of *Curcuma longa* Solution

In a 200 ml beaker, 10 g of *C. longa* powder was dissolved in 100 ml of deionized (D.I.) water, and the mixture was agitated for four hours at 70 °C [19]**.** The *C. longa* solution was then extracted as shown in Figure 1.

Figure 1: *Curcuma longa* extract.

Synthesis of (Fe_3O_4-NPs) from ($FeCl_2.4H_2O$).

Firstly, Fe_3O_4-NPs were prepared, the synthesis part was carried in the laboratories of the University of Baghdad. First, 2.7 g of Iron (II) Chloride ($FeCl_2 \cdot 4H_2O$) was dissolved in 100 mL of deionized water (D.I.). Then 50 ml of *C. longa* solution was added with continuous stirring. The resulting solution was heated at 80 °C; the stirring was continued for about three hours as shown in Figure 2. After the reaction was completed, the solution was placed in a centrifuge to obtain a precipitate. The precipitate was washed several times with D.I. and then the precipitated black nanoparticles were dried [20]. Secondly, tests were conducted to characterize the composition of the nanocomposite, in order to determine the nanoparticles' dimensions and formation, as well as to study the morphological characteristics of the iron oxide nanoparticles (Fe_3O_4).

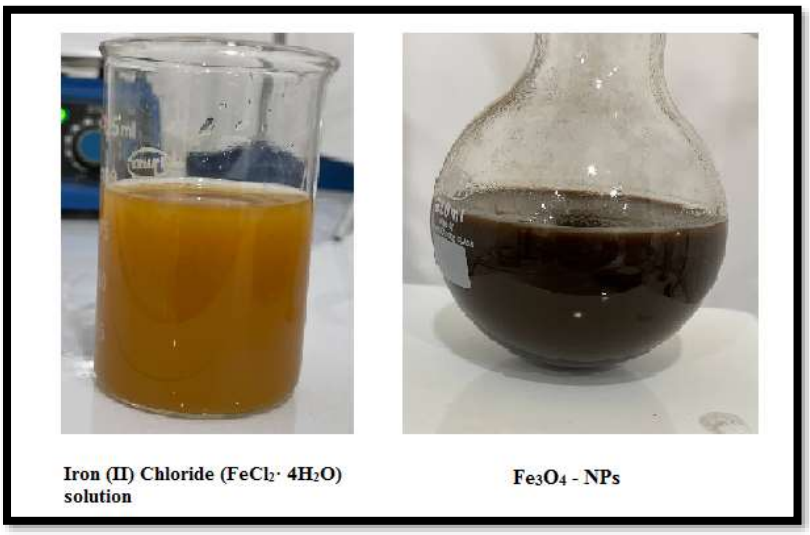

Figure 2: Synthesis of (Fe_3O_4 - NPs)

Measurement of Fe_3O_4-NPs' Antibacterial Activity

The well diffusion method was used to detect the biological activity of the prepared nanoparticles. Following the manufacturer's instructions. Iron oxide nanoparticles (Fe_3O_4) were used at concentration 100 µg mL^{-1}, at a temperature of 37 °C and incubated for 24 hours. The resulting diameter of inhibition was then recorded [21,22]
Reference drug – Gentamicin 100 µg mL^{-1}.

Results and Discussion

Synthesis and Characterization of Fe_3O_4-NPs

The outcomes demonstrated the successful synthesis of iron oxide nanoparticles (Fe_3O_4), which possessed ferromagnetic characteristics and a dark black hue as depicted in Figure (3). The obtained results were consistent with those of a previous study conducted by Win et al. [23].

Figure 3: Characterization of (Fe_3O_4 - NPs)

Characterization of (Fe_3O_4-NPs)

The Precise scanning electron microscope (SEM) analysis is widely used to identify the surface morphology of the bio-synthesized nanoparticles. SEM images showed the production of iron oxide nanoparticles (Fe_3O_4) as presented in Figure 4. It can be observed from the SEM images that FeO nanoparticles had spherical morphology with little aggregation, and the particles were within the size range of 30 to 55 nm. Since the dimensions of the nanoparticles greatly influence the properties of the nanocrystals, and controlling the monodispersed size is also essential [24]. Morphology and size characterization of Fe_3O_4-NPs were also examined by microscopic analysis using transmission electron microscopy (TEM). Diameter measurements with TEM were performed using Image J software as depicted in Figure 5. TEM images showed that iron oxide nanoparticles were spherical and had an average size of 40 nm. Similar findings were reported by Mendes et al. [25] who have synthesized spherical shaped FeO NPs with different sizes.

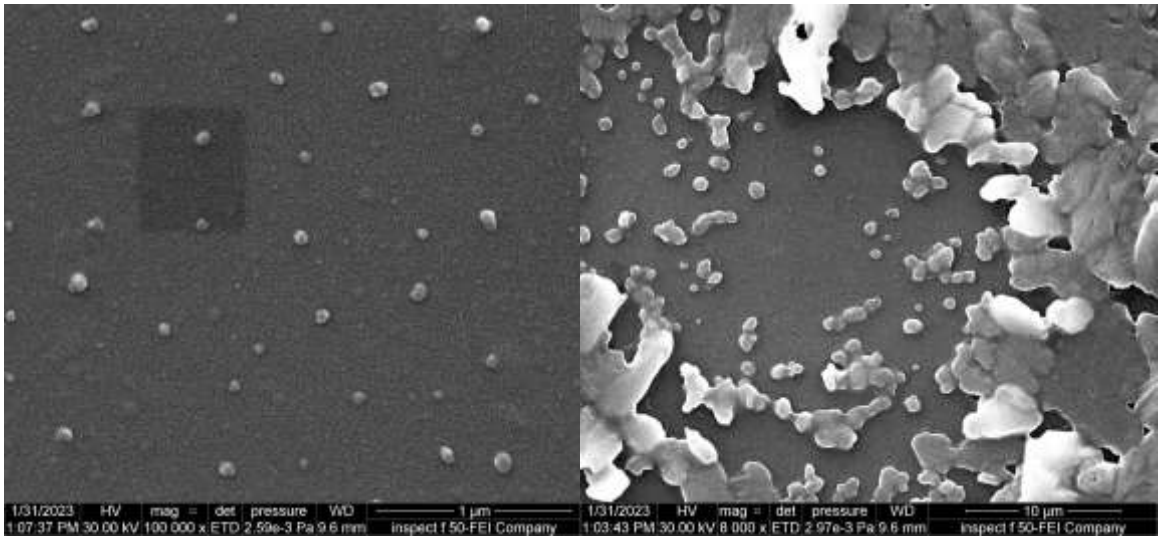

Figure 4: SEM images of iron oxide nanoparticles (Fe_3O_4) size and top view.

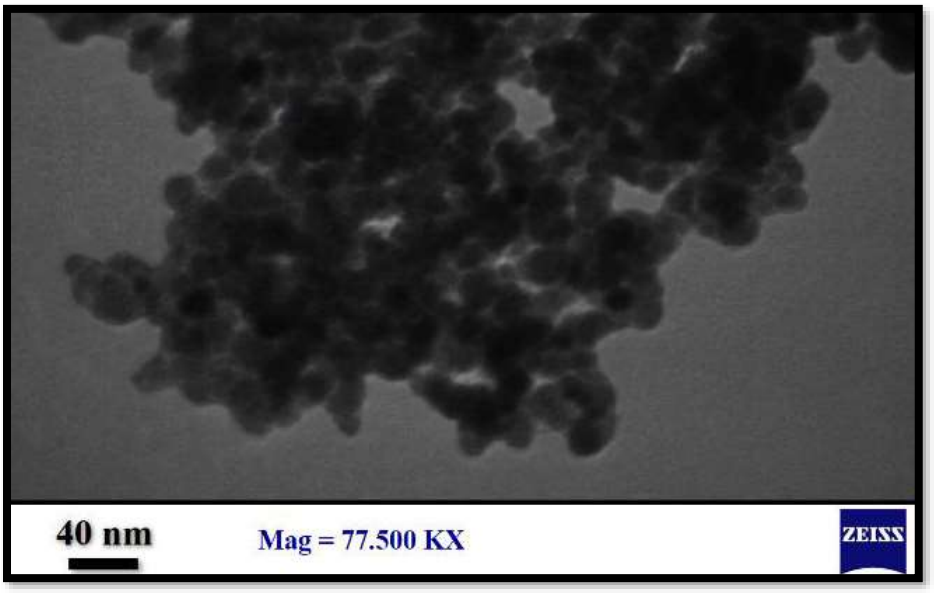

Figure 5: TEM image of (Fe_3O_4-NPs)

FT-IR Analysis of (Fe_3O_4-NPs)

FT-IR analysis was performed to identify the biomolecules responsible for the reduction of iron oxide nanoparticles present in *Curcuma longa* extract. Based on the current results, FT-IR analysis gave clear stretching vibrations at 3400.5 cm^{-1},1625.99 cm^{-1} and 626.87 cm^{-1} in the region of 400 - 4000 cm^{-1} (Fig. 6). These peaks represent the following bonding in the sample confirms the reducing agent role in the formation of Fe_3O_4 –NPs. The broad peak around 3400.5 cm^{-1} showed the OH stretching bond vibration which was due to the water adsorption on the surface of Fe_3O_4 –NPs [26], while 1625.99 cm^{-1} corresponds to the stretching of the C=O bond to the phytochemicals present in the plant extract. The present findings seem to be consistent with a previous study which found that the presence of bioactive compounds may be responsible for the capping, reduction and conversion of Fe^+ to Fe-O nanoparticles [27]. In addition, a broad short peak appears at 626.87 cm^{-1}

corresponds to the inorganic stretching of Fe–O–Fe and indicates the formation of iron oxide nanoparticles [28].

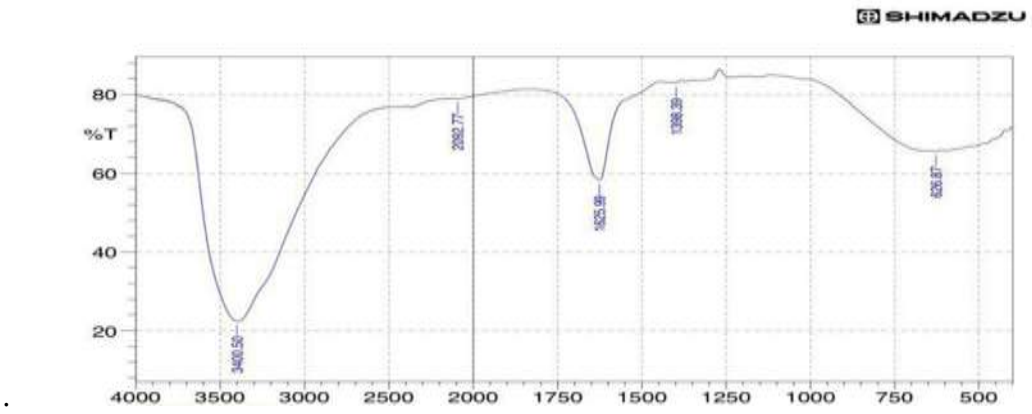

Figure 6: FT-IR analysis of (Fe_3O_4-NPs)

UV-Visible Spectrophotometer of (Fe_3O_4-NPs)

UV-Visible spectrum of iron oxide nanoparticles is shown in Figure 7. As is widely known, metal oxide NPs have significant absorption bands between 200 and 800 nm at room temperature. In the present study, UV-visible spectra of the synthesized Fe_3O_4-NPs showed a characteristic absorption peak between 223 and 303 nm. It was reported earlier that the UV-spectral absorbance around 250-260 nm is a unique characteristic feature of Fe_3O_4-NPs [29]. According to the obtained data, the absorption maxima of synthesized iron oxide nanoparticles are similar to those observed in other studies which used *Ficus carica* and *Skimmia laureola* extracts to produce iron nanoparticles [30-31].

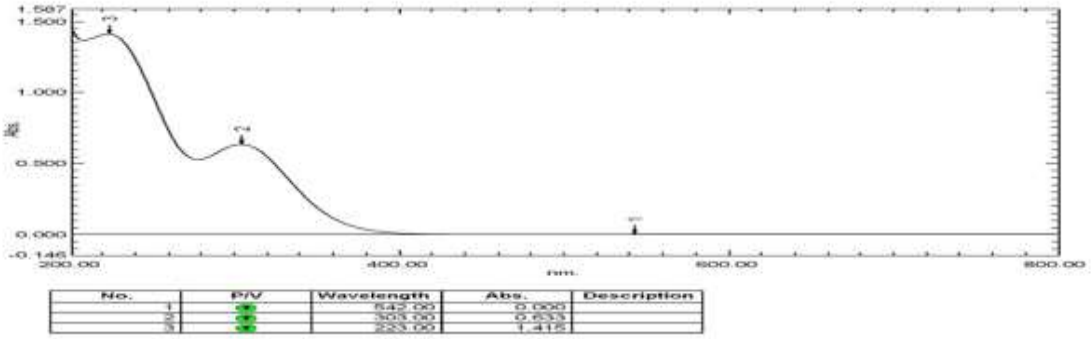

Figure 7: UV-visible spectrophotometer of (Fe_3O_4-NPs)

Antimicrobial Activity of (Fe3O4-NPs) Against Microbial Isolates

The agar well-diffusion method was used to evaluate the antibacterial activity of Fe_3O_4-NPs at a concentration of 100 µg mL^{-1} against Gram-negative (*Escherichia coli*) and Gram-positive (*Staphylococcus aureus*) bacteria as demonstrated in Figure 8. According to the obtained data, the antibacterial property of the bio-synthesized Fe_3O_4-NPs revealed that the nanoparticles have moderate antibacterial activity in comparison to the used antibiotic. The result also showed higher antibacterial activity against *S. aureus*, whereas moderate activity was revealed against *E. coli*. The inhibitory activities in culture media of the Fe_3O_4 -NPs reported in Table. 1. Although, the

antibacterial activities of several bio-synthesized iron oxide nanoparticles have been reported, the bacteria inhibition mechanism of the nanoparticles has yet to be elucidated. However, some theories have suggested that the nanoparticles invade the cell membrane and denature the enzyme of bacteria, and damage bacterial DNA, which in turn induce cell death (32,33). It has been reported that bio-synthesized nanoparticles show better antimicrobial activity compared to chemically synthesized nanoparticles, because various plant extracts that were used for the synthesis of nanoparticles have antimicrobial properties [23].

Table 1: Antimicrobial Activity of the Synthesized Iron Oxide Nanoparticles by *Curcuma longa* Aqueous Extract.

Pathogenic Bacteria	Zone of Inhibition in mm	
	Fe_3O_4-NPs (100 µg mL^{-1})	Gentamicin (100 µg mL^{-1})
E. coli	10.5	13.6
S. aureus	17.6	18.8

Concluding Remarks

The most common microbes isolated from samples of patients with urinary tract infections were (*Escherichia coli*) and (*Staphylococcus aureus*). Since physiological activities occur at the nanoscale, the attachment of iron oxide (Fe_3O_4) nanoparticles is expected to alleviate many biological and healthcare difficulties. It's known that the uncontrolled use of antibiotics might fuel the growth of resistant strains around the world. The synthesis of NPs is considered a powerful technology due to their potency against common multidrug-resistant (MDR) species. Our findings demonstrated the successful synthesis of iron oxide (Fe_3O_4) nanoparticles and their application for antibacterial activity against bacterial isolates that are resistant to multiple drugs and antioxidants. We may conclude that superparamagnetic and very potent antibacterial agents are present in iron oxide nanoparticles (Fe_3O_4).

E. Coli Staphylococcus spp.

Conflict of Interest

The research's publishing is free of conflicts of interest, according to the authors.

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