Original Article

# Antibacterial Effect of Iron Nanoparticles (FeNPs) on *Acinetobacter baumannii*

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## Abstract

**Background:** Excessive and incorrect use of antibiotics has led to the emergence of strains of *Acinetobacter baumannii* that have resistance to antibiotics, multidrug resistance *A. aumannii* has become a real threat to life of humans. So scientists have resorted to using nanotechnology to limit the spread of infection with these strains. **Objectives:** This study aimed to use an eco-friendly method for synthesizing iron nanoparticles FeNPs using Cinnamon extract as a reducing and stabilizing agent. Also, explore the antibacterial activity of FeNPs against *A. baumannii*. **Materials and Methods:** The Iron nanoparticles (FeNPs) are synthesized by what is called (green synthesis) the easiest and nontoxic method, as microorganisms or plant extracts are used. We discuss how to synthesize FeNPs using plant extracts, where cinnamon powder was used and prepared as a plant extract to obtain FeNPs by adding 5 mL of the extract to 100 mL of aqueous iron nitrate and conducting tests for these nanoparticles, which include (UV, SEM, XRD, and FTIR). As well as studying the effect of iron nanoparticles as an antibiotic on many strains of *A. baumannii* with multidrug resistance using different concentrations as follows (0.1 M, 0.01 M, 0.001 M, and 0.0001 M). **Results:** This study showed that UV recorded approximately 360 nm, and SEM images exhibited spherical shape FeNPs nanostructures with an average size of 12.44 nm. The maximum inhibition diameter FeNPs against *A. baumannii* isolates was recorded at 33 mm in diameter. **Conclusion:** The preparation of FeNPs with cinnamon extract is a quick, nontoxic giving significant antibacterial effect on *A. baumannii* isolates.

**Keywords:** *Acinetobacter baumannii*, green synthesis, iron nanoparticle

## Introduction

*Acinetobacter baumannii* is a gram-negative opportunistic coccobacilli, motile, strictly aerobic, and lactose non-fermenting. *Acinetobacter baumannii* strains associated with hospital-acquired infections such as pneumonia, bloodstream, abdominal, central nervous system, Burn, urinary tract, and skin and soft tissue infection.[1,2] Recently, the resistance of *A. baumannii* to antibiotics has increased in most parts of the world, which led to an increase in the number of deaths, as it included resistance to many antibiotics such as penicillin, cephalosporin, aminoglycosides, as well as Carbapenem's resistance-*A. baumannii* (CRAB). *Acinetobacter baumannii* infection is common in patients with severe infections, and is often accompanied by other bacterial or fungal infections.[3,4] Patients that are infected with Multidrug resistance *A. baumannii* (MDR-AB) have high mortality. One of the main causes of a burn patient's mortality is microbial infection by gram-negative bacteria such as *Pseudomonas aerogenusa, klebsilla* spp., and *A. baumannii*.[5] Which is related to burn unit contamination, which represents a significant health problem in the burn ward in addition, secondary infections caused by multidrug-resistance *A. baumannii* (MDR-AB) have been detected in COVID-19 patients worldwide and also had been linked with poor prognosis.[6] Therefore, we need to discover and develop new methods and treatments to reduce the spread of MDR-*A. baumannii* infection. Nanotechnology is a multidisciplinary research field, as this technology has

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undergone extensive development and has attracted attention all over the world during the past few years, and it has had an impact on different areas of human life.[7,8] Nanotechnology includes a wide spectrum of techniques, and processes that can deal with a matter at the Nanoscale falling in between 1 and 100 nm in size.[9] The nanoparticles have unique and advanced electrical, chemical, physical properties, optical, and thermal.[10] The nanoparticle fields have a very wide range of applications, such as medicine, chemistry, environment, and communication fields.[11] Metal nanoparticles have attracted the interest of researchers because of their unusual chemical and physical properties. It offers a range of potential applications in materials science, electronics, optics, medicine, biomedical sciences, and engineering.[12,13] Iron nanoparticles are nontoxic particles and with excellent dimensional stability, it have high catalytic activity, high magnetism, high thermal and electrical conductivity, high surface area, and high microwave adsorption ability.[14,15] Iron nanoparticles are involved in many scientific applications, the most important of which are the manufacture of Nanoscale antibiotics, gene therapy, drug delivery, pollution reduction, and the manufacture of biological dyes. To prepare iron nanoparticles, we will use cinnamon as an active substance to stimulate the synthesis of iron nanoparticles, as cinnamon is a native plant of Srilanka and a tropical Asian spice[16] obtained from the inner bark of many trees of the genus Cinnamon.[17] The bark and leaves are commonly used to treat various disorders and are known to act as antibacterial, antifungal, antioxidant, antidiabetic, anti-inflammatory, nematicidal, insecticidal, and anticancer effects.[18]

## Materials and Methods

### Bacterial isolates and identification

In the current study 455 clinical specimens (wounds, urine, burns, blood, and sputum) were collected from hospitals of different Iraqi provinces including: Baghdad Medical City (the martyr Ghazi Al-Hariri hospital for surgical specialties, Baghdad teaching hospital and Child care Hospital), Tikrit–Salahaddin General Hospital, Kirkuk–Azadi Hospital at (August 2022 until March 2023) all specimens were cultured by streaking on Nutrient agar, MacConkey agar, and *A. baumannii* Hichrome agar, and incubated at 37°C for 24 h, then subculture in same ager were done to colony purified. Gram stain and group of biochemical tests were done According to Gram stain and a group of biochemical tests were done to confirm the diagnosis of *A. baumannii*.[19]

### Antibiotic susceptibility test of *Acinetobacter baumannii*

Antibiotic sensitivity testing was done according to Kirby–Bauer Method.[20] The inhibition diameter was adjusted to the reading of NCLSI guidelines.[21] In sensitivity testing was using 13 bacterial antibiotics, including Piperacillin, Ciprofloxacin, Levofloxacin, Gentamicin, Amikacin, Imipenem, Azithromycin, Amoxicillin, Clavulanic Acid, Cefepime, Cefotaxime, Ampicillin, Chloramphenicol, and Ceftazidime.[22]

### Cinnamon extract preparation

To prepare the plant extract of cinnamon, 10 g of commercially prepared ground cinnamon were weighed and placed in a beaker containing 100 mL of sterile distilled water. It was heated on a heat plate to 80°C to obtain the effective substance of cinnamon, after which it was left to cool at room temperature. It was then filtered through Whatman-1 filter paper and placed in a clean and sterilized bottle and stored in the refrigerator at 4°C until use and depending on the method.[23]

### Green synthesis preparation of Fe nanoparticles

Iron nanoparticles (0.1 M) were synthesized by Green synthesis. By added Fe(NO3)3.9H2O (4.04 g) into a beaker containing 100 mL sterile distilled water and heated to (45°C–60°C) on a heat plate, Then 5 mL of cinnamon extract was added to the solution with stirring for 30 min until the color change from yellow to brown occurred. It is iron nanoparticles at a concentration of (0.1 M). Left the solution to cool at room temperature, placed at a clean and sterile dark glass vial, and kept at room temperature.[24]

### Characterization of Iron nanoparticles (FeNPs)

This study describes an eco-friendly method for supporting FeNPs using Cinnamon extract as reducing and stabilizing agent without using any toxic reagents. The structural, morphological, and physicochemical properties of the nanocatalyst were determined by various analytical techniques including FTIR, SEM, XRD, and UV studies.

### Inhibition determination of Iron nanoparticles (FeNPs) on *Acinetobacter baumannii in vitro*

Antibacterial activity of the prepared aqueous nanoparticles was conducted using wells-diffusion method on Molar Hinton ager cultured with *A. baumannii* by cotton swab After comparing the bacterial suspension with McFarland, then 100 µL of FeNPs were added to each hole depending on serial logarithmic dilution method. And incubating for 24 h at 37°C, the inhibition diameters were measured.[25]

### Ethical approval

The research was carried out in accordance with the ethical guidelines. The objective of this study was verbally conveyed to the participants. Prior to collecting any samples, ethical approval was obtained and patients were selected for inclusion in the study. The goal and process of the survey were effectively communicated by the researcher to the patients, accompanied by standardized instructions

and guidance for the completion of the questionnaire. The study design, patient information, and permission form underwent a thorough evaluation and received approval from the local Ethics Committee 4053 on October 19, 2022.

# Results

## Bacterial isolates results

In this study, the results revealed that a total number of *A. baumannii* was included in 50 of the 455 clinical samples tested. The samples were taken from various clinical sources, including wounds, urine, blood, otitis, septum, and burns[26] as shown in Table 1.

## Susceptibility test results

Susceptibility testing was conducted on isolates of *A. baumannii* using 13 different antibiotics, they were classified as having multidrug resistance isolates according to the results[27] shown in Table 2.

## UV-Assay

UV–visible absorption spectrum of FeNPs was recorded in the wavelengths ranging region from (200–800 nm). The results of UV showed that two peaks of the FeNPs were centered exhibit was recorded at approximately 360,386 nm. These results are consistent with the study.[28]

**Table 1: The number and percentage of positively diagnosed *Acinetobacter baumannii***

| Type of sample | Frequency | Percent (%) |
|---|---|---|
| Wound | 20 | 40 |
| Burn | 6 | 12 |
| Urine | 10 | 20 |
| Sputum | 2 | 4 |
| Blood | 11 | 22 |
| Otitis | 1 | 2 |

**Table 2: The number and percentage of MDR- *Acinetobacter baumannii***

| Antibiotics | Frequency | Percent (%) |
|---|---|---|
| Ampicillin | 47 | 94 |
| Piperacillin | 45 | 90 |
| Amoxicillin-Clavulanic acid | 46 | 92 |
| Gentamycin | 50 | 100 |
| Azithromycin | 47 | 74 |
| Amikacin | 41 | 82 |
| Chloramphenicol | 28 | 56 |
| Cefotaxime | 33 | 66 |
| Cefepime | 30 | 60 |
| Ceftazidime | 43 | 86 |
| Levofloxacin | 30 | 60 |
| Ciprofloxacin | 31 | 62 |
| Imipenem | 23 | 46 |

## Scanning electron microscope (SEM)

SEM images of FeNPs it is evident that the sample consists of a number quantity of the nanoparticles that exhibit spherical shape FeNP nanostructures. The average size of the obtained sample is approximately 12.44 nm, indicating that homogeneous nanoparticles can be synthesized and rather good size distribution. It is within the normal range of nanoparticle size 1–100 nm, as shown in Figures 1 and 2.

## FTIR measurement of FeNPs

The results confirmed the presence of iron nanoparticles (FeNPs) at (1383.01, 1618.33, 1639.55, and 3375.54 $cm^{-1}$), these results suggest that As(V) has been successfully adsorbed on to FeNPs and formed both monodentate and bidentate complexes which are almost consistent with the study, showed in Figure 3.[29,30]

## XRD

The crystalline phases of the FeNPs before and after As(V) adsorption were also analyzed by (XRD) assay in the range of $2\theta = 10.190°–79.990°$, especially at $2\theta = 35.7004°$ in the as-prepared FeNPs, which indicates the presence of zero-valent Iron. It agrees with the study [Figure 4].[31,32]

## The inhibitory activity of iron nanoparticles (FeNPs) on *Acinetobacter baumannii*

After performed an antibiotic sensitivity test for 13 antibiotics using the disc diffusion Kirby–Bauer Method as mentioned in NCLSI.[21] Which showed that the bacterium possesses multidrug resistance (MDR). A 10 out of 50 isolates of *A. baumannii* were randomly selected and the inhibitory effectiveness of FeNPs was tested. The results showed an inhibitory response by the isolates of *A. baumannii* to the FeNPs. We note that the inhibitory effect of iron nanoparticles on *A. baumannii* was at the following concentrations (the original concentration of 0.1 M of FeNPs and the concentration of 0.01 M) with a very small inhibition ratio of 0.001 M consternation as shown in Table 3.

# Discussion

Recently, MDR-*A. baumannii* isolates became more likely to be resistant a wide number of antibiotics than other bacteria related to hospital infections. Significant resistance to carbapenems antibiotics has become part of the epidemiological problem of MDR-*A. baumannii* isolates. Nosocomial infections with *A. baumannii*, an opportunistic pathogen, have increased. The treatment of these bacteria, especially the MDR and XDR strains and the broad-spectrum beta-lactamase strains of *A. baumannii*, is a major concern.[33,34] *A. baumannii* It has emerged as a cause of infection in ICUs. MDR *A.*

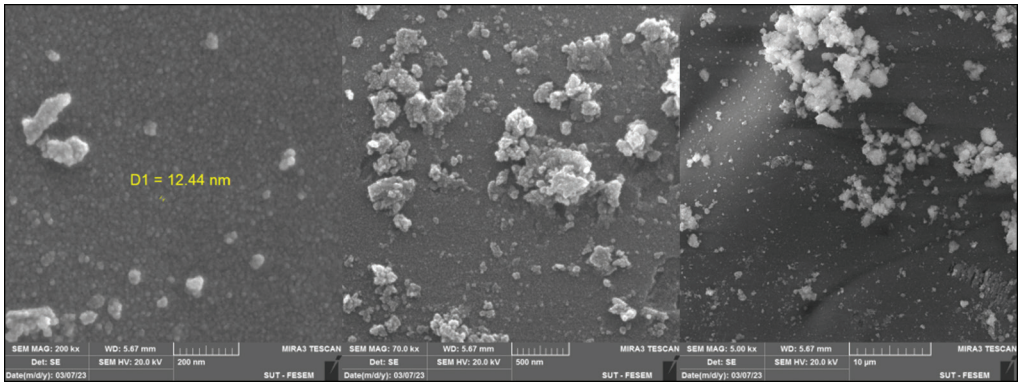


**Figure 1:** SEM images of FeNPs

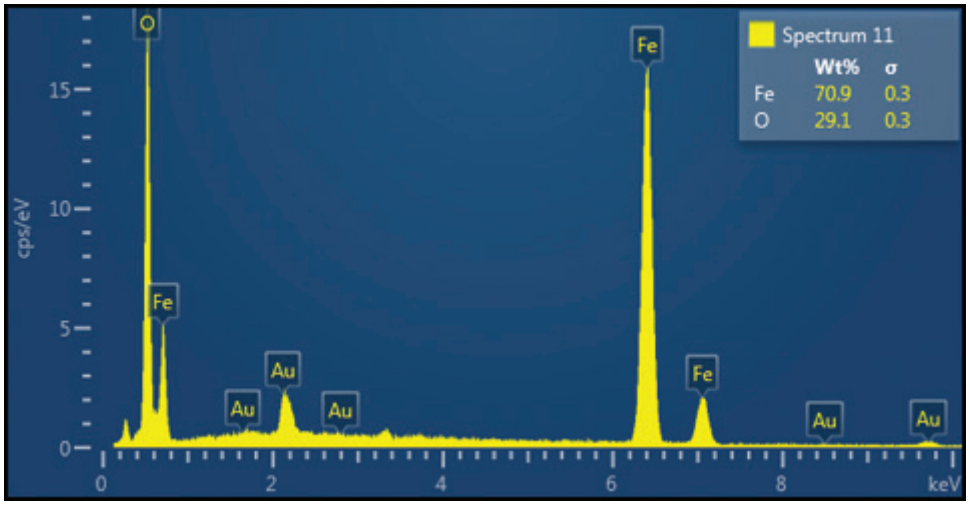


**Figure 2:** EDX of iron nanoparticles (FeNPs)

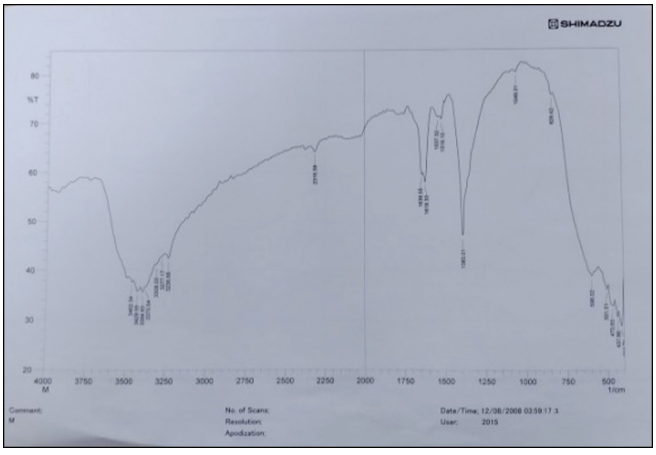


**Figure 3:** FTIR image of FeNPs

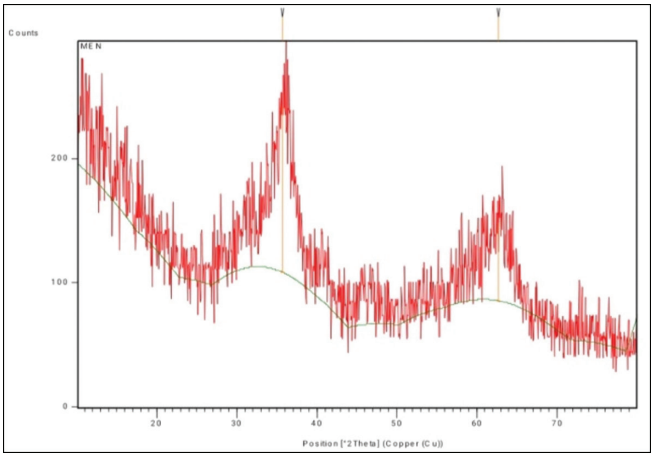


**Figure 4:** XRD assay of FeNPs

*baumannii* strains have become one of the most important and dangerous pathogens, especially in intensive care units, and are associated with outbreaks of infection.[35] In our study, there are 455 samples were collected in this study, 50 of which were positive bacterial isolates of gram-negative *A. baumannii*. Which were classified as nosocomial epidemic and fatal. *Acinetobacter baumannii* appeared to be a rod-shaped bacteria. That grows well on MacConkey agar. Although formally classified as non-fermenting lactose, it is often partially fermented when grown on McConkey agar. The growth and purity of *A. baumannii* cultures were determined by the shape of their colonies on McConkey agar and blood agar. In McConkey agar, colonies that are pale in color and are not lactose-fermented

**Figure 5:** Antibacterial activity of different concentrations of FeNPs produced using Cinnamon extract against *Acinetobacter baumannii*

**Table 3: FeNPs inhibition diameters on *Acinetobacter baumannii* isolates**

| No. | 0.1 M standard consternation | 0.01 M consternation | 0.001 M consternation | 0.0001 M consternation |
|---|---|---|---|---|
| 1 | 25 mm | – | – | – |
| 2 | 27 mm | 17 mm | – | – |
| 3 | 32 mm | 25 mm | 12 mm | – |
| 4 | 33 mm | 21 mm | 19 mm | – |
| 5 | 25 mm | – | – | – |
| 6 | 20 mm | 14 mm | 10 mm | – |
| 7 | 25 mm | – | – | – |
| 8 | 18 mm | 15 mm | – | – |
| 9 | 23 mm | 16 mm | 13 mm | – |
| 10 | 21 mm | 14 mm | 11 mm | – |

are formed. In the blood agar are formed non-hemolytic colonies.[36] Light pink color on MaCconkey agar.[37] And on the blood agar appeared a light gray color. knowing that isolates of *A. baumannii* bacteria have the ability to grow at 44°C which is a physiological characteristic that distinguishes *A. baumannii* from other species of genus *Acinetobacter*.[38] The antibiotic susceptibility pattern of *A. baumannii* isolates was examined by Kirby–Bauer disc diffusion assay, and the results showed that the majority of clinically isolated *A. baumannii* 94% were resistant to ampicillin and about 90%, 92%, 82%, and 74% were resistant to piperaciilin and Amoxicillin-Clavulanic acid, amikacin, and azithromycin, respectively, and 66%, 60%, and 86% were resistant to Cefotaxime, Cefepime, and Ceftazidime. Whereas, 60% and 62% Percentage of resistance to levofloxacin and ciprofloxacin, respectively. As well as, the minimum resistance was recorded for each of, 56% for chloramphenicol and 46% for imipenem, while the maximum resistance was 100% for gentamicin.[27] Therefore, alternative methods were resorted to limit its spread and combat it. Nanotechnology is a growing field of science with the most vibrant and conspicuous applications. Green synthesis methods of metal nanoparticles are now mentioned in literature and practiced by researchers as well to find out new and emerging applications of nanoparticles. The biological method involves the synthesis of nanoparticles by living organisms specifically plants, bacteria, fungi, and algae. Synthesis of FeNPs by plants refers to green nanotechnology.[39] Cinnamon was used as an active substance for the preparation of FeNPs, and tested their antibacterial effect on *A. baumannii* as it was exposed to Iron nanoparticles (FeNPs) with different concentrations after conducting the required tests for the FeNPs, incubating them for 24 h, and observing the effect of the nanoparticles on them [Figure 5]. As well as UV recorded approximately 360 and 386 nm. SEM images exhibited spherical shape FeNPs nanostructures with the size of the obtained sample is approximately 12.44 nm, while the FTIR measurements were at 1383.01, 1618.33, 1639.55, and 3375.54 $cm^{-1}$ confirming the presence of iron nanoparticles. As well as in the XRD examination, it was found that there is no diffraction of the FeNPs, which was shown by the measurements at the range 2θ = 10.190°–79.990°, and 2θ = 35.7004°. These results confirmed that FeNPS were composites with many functional groups on the surface of Fe spheres.[40,41] When measured the inhibition dimeter of FeNPs, the maximum inhibition diameter FeNPs against *A. baumannii* isolates was recorded 33 mm in diameter. While the minimum inhibition diameter of FeNPs on *A. baumannii* had recorded 10 mm in 0.001 M consternation.

## Conclusion

In this study, FeNPs have been biologically synthesized and characterized by UV, SEM, XRD, and FTIR. Through the tests conducted on green synthesized FeNps, it was determined that it is within the nanoscale range. The FeNPs obtained have small particle sizes with a regular spherical shape. The antibacterial experiment indicated that the FeNPs exhibited excellent antibacterial properties against *A. baumannii* compared to antibiotics.

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Nil.

### Conflicts of interest

There are no conflicts of interest.

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