Iraqi Journal of Science, 2023, Vol. 64, No. 6, pp: 2759-2773
DOI: 10.24996/ijs.2023.64.6.10

ISSN: 0067-2904

Potentials of Iron Oxide Nanoparticles (Fe_3O_4): As Antioxidant and Alternative Therapeutic Agent Against Common Multidrug-Resistant Microbial Species

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Received: 2/8/2022 Accepted: 24/9/2022 Published: 30/6/2023

Abstract

For the treatment of pathogenic bacterial infections, multidrug resistance (MDR) has become a major issue. The use of nanoparticles is a promising strategy for combating medication resistance in a variety of pathogens that cause deadly diseases. The goal of our research was to extract multidrug-resistant bacteria from wound infections and then use iron oxide nanoparticles (Fe_3O_4) as alternative therapeutic agents in vitro. Gram staining, morphological attributes evaluation, and biochemical testing were used to assess the microbes. The Kirby-Bauer disk diffusion method was used to test MDR-bacterial strains against several antibiotics; the majority of these isolates were resistant to ceftazidime, amoxicillin, Gentamicin, and tetracycline. the iron oxide nanoparticles were produced by the co-precipitation method and were confirmed by changing the color to dark black as well as the Scanning Electron Microscope (SEM) and Transmission Electron Microscope (TEM) analysis that shows the shape and average size between (29.03-56.54) nm. The highest effect of iron oxide nanoparticles (Fe_3O_4) on the growth of *Proteus.mirabilis* (*P.mirabilis*) was as it was found that the average diameter of the inhibition zone was 22.66±1.15 mm, followed by *Staphylococcus.epidermidis (S.epidermidis), Acinetobacter.baumannii* (*A.baumannii*) with the average diameter of the inhibitory zone ~~it~~ was 21.66±1.52 mm, 20.33±1.53 mm respectively, and *Candida.albicans* (*C.albicans*) was 18.33±1.15 mm at 100 μgmL^{-1} (stock). The synthesized iron oxide nanoparticles (Fe_3O_4) are used to capture rapidly microbes under the magnetic field effect. The antioxidant activity DPPH of the iron oxide nanoparticles (Fe_3O_4) showed 29.3%, 42.2%, 58.6%, 67.4%, and 74 % at a concentration (6.25, 12.5, 25, 50, 100) μgmL^{-1} respectively, it demonstrated that the scavenging percentage increase with increasing the iron oxide nanoparticles (Fe_3O_4) concentrations.

Keywords: Multidrug-resistant species; Iron oxide nanoparticles; Antimicrobial activity; Antioxidant activity.

إمكانات جزيئات أكسيد الحديد النانوية (Fe3O4): كمضاد للأكسدة وعامل علاجي بديل ضد الأنواع الميكروبية الشائعة المقاومة للأدوية المتعددة

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الخلاصه

لعلاج الالتهابات البكتيرية المسببة للأمراض، أصبحت مقاومة الأدوية المتعددة مشكلةرئيسية. يعد استخدام الجسيمات النانوية استراتيجية واعدة لمكافحة مقاومة الأدوية في مجموعة متنوعة من مسببات الأمراض التي تسبب أمراضًا مميتة. كان الهدف من بحثنا هو استخراج البكتيريا المقاومة للأدوية المتعددة من التهابات الجروح ثم استخدام جزيئات أكسيد الحديد النانوية (Fe3O4) كعوامل علاجية بديلة في المختبر. تم استخدام صبغة الجرام وتقييم الصفات المظهرية والاختبارات الكيموحيوية لتشخيص الميكروبات .تم استخدام طريقة نشر الاقراص Kirby–Bauer لاختبار السلالات البكتيرية MDR ضد عدد من المضادات الحيوية. كانت غالبية هذه العزلات مقاومة للسيفترايديم والأموكسيسيلين والجنتاميسين والتتراسيكلين. تم إنتاج جزيئات أكسيد الحديد النانوية بطريقة الترسيب المشترك وتم تأكيدها من خلال تغيير اللون إلى الأسود الداكن وكذلك تحليل المجهر الالكتروني الماسح SEMوالمجهر الالكتروني النافذ TEMالذي يوضح الشكل والحجم المتوسط بين (29.03–56.54) نانومتر. كان أعلى تأثير لجسيمات أكسيد الحديد النانوية على نمو *P.mirabilis* حيث وجد أن متوسط قطر منطقة التثبيط كان 22.66 ± 1.15 مم ، يليه *S.epidermidis* ، *A.baumannii* بمتوسط قطر المنطقة المثبطة كان 21.66 ± 1.52 مم ، 20.33 ± 1.53 مم على التوالي ، وكانت 1.15 ± 18.33 *C.albicans* مم عند 100 ميكروغرام / مل (مخزون). تم استخدام جزيئات أكسيد الحديد النانوية (Fe3O4) المركبة لالتقاط الميكروبات بوسعة تحت تأثير المجال المغناطيسي. أظهر النشاط المضاد للأكسدة DPPH لجسيمات أكسيد الحديد النانوية 29.3٪ ، 42.2٪ ، 6.58٪ ، 67.4٪ ، 74٪ عند التركيز (6.25 ، 12.5 ، 25. 50. 100) ميكروغرام / مل على التوالي ، أظهر أن نسبة الكسح تزداد مع زيادة تركيزات جزيئات أكسيد الحديد النانوية(Fe3O4) .

1. Introduction

Wound infections include surgical wound infections, acute soft tissue infections, bite wound infections, burn wound infections, and pyogenic wound infections. Wounds may take a long time to heal and may spread due to human habits. They are also aggravated in people with diabetes, obesity, or heart disease. Infectious wounds are dangerous, unsightly, odorous, and hypersensitive, giving patients pain and discomfort. *S. epidermidis, P. mirabilis, A. baumannii* and *C. albicans*. were found to be the most common isolates for wounds in a recent study [1].

Due to widespread antibiotic resistance and an increased incidence of infections caused by polymicrobial flora, wound infection control has become difficult [2]. Numerous antibiotics are becoming ineffective in managing bacterial illnesses as a result of multi-drug resistance in many bacteria. Because of overuse, self-medication, random prescribing of inappropriate medications, and prolonged antibiotic use, bacteria have developed drug resistance [3].

Furthermore, the conjugation of antibiotic resistance genes results in the emergence of resistant microorganisms [4]. Because of the rising number of antibiotic-resistant "superbugs," many diseases are becoming increasingly resistant to commonly used antibiotics. Drug-resistant germs kill 25,000 people in Europe per year, whereas MDR-bacterial infections kill 23,000 people in the United States every year. Drug resistance in bacteria has been detected in all parts of the world, according to WHO studies. Approximately 50% of *S. epidermidis*, *P. mirabilis*, and *A. baumannii* infections were resistant to most effective antibiotics, such as Gentamicin, as a result, these drug-resistant bacterial isolates have a high fatality rate [5].

S. epidermidis is one of the wound infections, involving surgical wound infections, indwelling foreign devices, and bacteremia in immunocompromised patients, cardiac devices, and catheters [6], *S.epidermidis***,** is a coagulase-negative gram-positive cocci bacteria, that is also a catalase-positive, non-motile and facultative anaerobe, it is the most common coagulase-negative Staphylococcus species that live on the human skin [7]. It is a very hardy microbe that forms white elevated cohesive colonies about 1-2 mm in diameter and is not hemolytic on blood agar, grouped in grape-like clusters, it is grow on mannitol salt agar medium but does not ferment mannitol it is an important test to distinguish it from another staphylococcus, it is

spherical bacteria that grows easily on several growth media and varieties of varying medical importance, ability to cause disease and is present on the skin and mucous membranes in a large proportion and acts as opportunistic bacteria that occurs in damaged tissue [8].

P. mirabilis is most frequently a pathogen of Urinary tract infections (UTI), also one of the other bacteria that are present in wound infection. Gram-negative bacteria can cause bacteriuria and catheter obstruction [9], the swarming property of these bacteria appears, which helps them to spread widely in the area of injury, and the adhesion factor is one of the important virulence factors in it [10]. *P.mirabilis* strains are responsible for the majority of MDR ability to use antibiotics [11].

A. baumannii is a gram-negative bacteria, coccobacillus, non-motile, non-fermented lactose when grown on MacConkey agar and strict aerobic, catalase positive and oxidase negative [12]. Bacterial colonies developing on blood agar medium appear lead to light or white, it is not hemolytic, however, colonies on MacConkey agar appear smooth, small, and pale due to not fermenting lactose and size are 1-2 mm [13]. *A. baumannii* is an opportunistic pathogen and dangerous to humans because of the severe injuries it causes to patients who are hospitalized, including skin infections, soft tissue infections, Urinary tract infections, and Wound infections [14].

C. albicans this fungus is often grown on a sabouraud dextrose agar medium, is the only species that has the potential to form the germ tube in this test and in the presence of the stimulus that works to form it around the yeast cell, and the germ tube is crucial in the penetration process of the epithelial cell layer lining the body and tissues and access to the bloodstream [15], fungus infection as Candidiasis has been discovered in chronic and surgical wounds [16].

The routine use of antibiotics leads to the spread of antibiotic resistance, fighting against infections has become more difficult due to the emergence of different bacterial strains that are resistant to antibiotics (MDR bacteria). To overcome this, scientists are looking for new and more effective antibacterial agents. A good alternative for this purpose is nanotechnology [17]. We think that the antibacterial therapy can be improved considerably using iron oxide nanoparticles (Fe_3O_4) as Nano-vehicles for supporting the cellular growth and viability of pathogens, some anti-oxidants could be delivered externally through food and referred to as exogenous antioxidants. Lately, the development of biocompatible NPs having antioxidant properties has gained a great deal of attention [18]. Iron oxide nanoparticles (Fe_3O_4) ranging in size from 1-100 nm represent a new trend that is increasingly being developed and of interest for adoption in research related to medical applications [19]. Magnetic properties such as superparamagnetism, high magnetic susceptibility, high coercive, etc. [20,21]. The aim of this study is to diagnose MDR microbes, chemically synthesized iron oxide nanoparticles (Fe_3O_4), and study their antimicrobial and antioxidant activity against (*S.epidermidis, P.mirabilis, A.baumannii,* and *C.albicans*).

2. Materials and Methods

2.1 Materials

The media used in this study are Mannitol Salt agar, MacConkey agar, and Nutrient broth purchased from (Acumedia Company, USA) whereas Blood agar, Muller-Hinton Agar medium, Sabouraud Dextrose Agar, Tryptone soya broth and Gram stain purchased from (Himedia Company, India). Chemicals that are used here are iron (II) chloride di-hydrate ($FeCl_2 \cdot 2H_2O$, 98%), iron (III) chloride anhydrous ($FeCl_3$), NH4OH, 1,1-Diphenyl-2-picryl-hydrazyl (DPPH, MW: 394.32 g/mol) and Phosphate buffer solution (PBS) purchased from (Sigma-Aldrich Company, India). Amoxicillin, Gentamicin, Ceftazidime, Ciprofloxacin, Imipenem and Tetracycline these antibiotics used here were purchased from (Bioanalyse company, Turkey).

2.2 Collection of microbial isolates

This study included the collection of isolates from wound infections in Hospitals. The diagnosis was made based on the morphological characteristics of the isolates by growing them on culture media, and they were diagnosed with biochemical tests and a final diagnosis with the Vitek2 System. The following microbes *A.baumannii, P.mirabilis, S.epidermids* and *C.albicans* were obtained.

2.3 Morphological and microscopic examination

Microbial isolates were cultured on the following culture media: MacConkey agar, this medium was used to investigate the ability of bacteria to ferment the sugar lactose, especially for gram-negative bacteria. Blood agar, this medium was used to detect the ability of bacteria to produce the hemolysin enzyme by observing the transparent areas surrounding the colonies. Mannitol salt agar, this medium was used for the ability of bacteria to ferment mannitol. Sabouraud dextrose agar, is used for the cultivation of fungi, then stained with Gram stain [22].

2.4 Preparation of McFarland solution

The McFarland standards (0.5) were prepared according to [23]. This solution was used as a reference to altering the turbidity of bacterial suspensions so that the number of bacteria stayed within a certain range, allowing microbiological testing to be more consistent [24].

2.5 Antibiotic Sensitivity test

In this study, 6 types of antibiotics were used based on the Clinical and Laboratory Standards Institute (CLSI 2021) [25]. The diffusion method was used, the colony was taken by a sterile loop from a culture grown for 24 hours and placed in a normal tube containing 5 ml of normal saline to make a bacterial suspension and compared with the turbidity of the preparation McFarland concentration of 0.5. On Muller Hinton agar medium, 100 µL of the bacterial suspension was taken and spread by sterile cotton swab then the dishes were left to dry and an antibiotic tablet was then placed in the dish, the plates were incubated for 24 h at 37°C, then the inhibition diameters were determined [26].

2.6 Synthesis and characterization of iron oxide nanoparticles (Fe_3O_4)

The iron oxide nanoparticles (Fe_3O_4) were prepared chemically using a co-precipitation method, which was done in the University of Technology/Applied science/Biotechnology laboratory. Initially, 4.8834g of iron (II) chloride di-hydrate ($FeCl_2 \cdot 2H_2O$, 98%) and 7.2995g of iron (III) chloride anhydrous ($FeCl_3$) were dissolved into 50 mL of De-ionized (D.I) water. Next, 25 mL of NH4OH was added under steady stirring. The solution was heated at 80 °C with constant stirring for one hour. After the reaction was completed, the black precipitated nanoparticles were washed several times with D.I. water to remove impurities and collected using an external magnet [27]. Scanning electron microscopy (SEM**)** analysis used to determine the size and shape of nanoparticles were taken on Scanning Electron Microscope (Zeiss, Jena, Germany). The morphological features and particle size distribution of the iron oxide nanoparticles (Fe_3O_4) were examined using a Transmission electron microscope (Zeiss, Jena, Germany) and Image J software [28].

2.7 Detection of the antimicrobial activity of iron oxide nanoparticles (Fe_3O_4)

The test was carried out using the method of well diffusion, as Muller's medium was prepared according to the manufacturer's instructions, then left to solidify, then the microbial suspension was prepared and compared with a standard McFarland tube. Cotton swabs were used to disseminate the microorganisms, and wells were punched into the agar with a sterile well cutter. All types of microorganisms wells were poured with 100 µL of iron oxide

nanoparticles (Fe_3O_4) at different concentrations (12.5, 25, 50, 100) µgmL^{-1}, then incubated in the incubator at a temperature of 37 ° C for 24 hours. The diameter of the inhibition resulting from each concentration was recorded [29,30].

2.8 Capturing of microbial isolates by iron oxide nanoparticles (Fe_3O_4)

S. epidermids, P. mirabilis, A. baumannii, and *C. albicans* were cultured in nutrient broth media for 24 hours to reach the density at 1.5x10^8 colony-forming units (CFU) ml in a typical experiment. After that, 1 mL of bacteria was collected by centrifugation. The bacteria were dispersed in 900 µL of PBS (phosphate buffer solution) and mixed with 100 µL of iron oxide nanoparticles (Fe_3O_4) solution and incubated in darkness at 37°C for 1 hour. For one minute, an external magnetic field was applied to the combination to see if the bacteria could be separated from the solution [31].

2.9 Antioxidant activity (DPPH assay)

The antioxidant activity of iron oxide nanoparticles (Fe_3O_4) with modest changes was evaluated using DPPH radicals. Iron oxide nanoparticles (Fe_3O_4) were used at concentrations (6.25, 12.5, 25, 50, and 100 µgmL^{-1}) to look into the scavenging activity. The 750 µl of samples were blended with 750 µl of 1,1-Diphenyl-2-picryl-hydrazyl (DPPH) solution and mixed well, then 750 µl of DPPH with 750 µl of methanol was used as a negative control, 750 µl of DPPH with 750 µl of ascorbic acid was used as a positive control. For 30 minutes, the samples and controls are kept at 37 °C temperature in the water bath [32]. At 517 nm, the optical density was read. Scavenging activity is calculated by the equation formula:

$$\boldsymbol{Scavengingactivity} = \frac{\boldsymbol{ODofcontrol - ODofsample}}{\boldsymbol{ODofcontrol}} \boldsymbol{x}100\%$$

3. Results and Discussion

3.1 Microbial isolates and identification

To identify the microbial isolates of *S.epidermidis, P.mirabilis, A.baumannii,* and *C.albicans* the isolates were cultured on Maconkey agar, Gram-negative bacteria *A.baumannii, P.mirabilis* were cultured on this medium, the colonies appeared in pale color due to their inability to ferment lactose sugar, Mannitol-salt agar: Gram-positive bacteria *S.epidermids* were cultured on this medium to distinguish them from the other *Staphylococci* by their inability to ferment mannitol [33]. Sabouraud dextrose agar: *C.albicans* was grown on this medium, specific to the fungus [34]. Blood agar: was cultured on this medium, all these colonies of bacteria appeared gamma(ϒ) hemolysis this is evidence of the inability to produce hemolycin enzyme [35]. As shown in Figure (1).

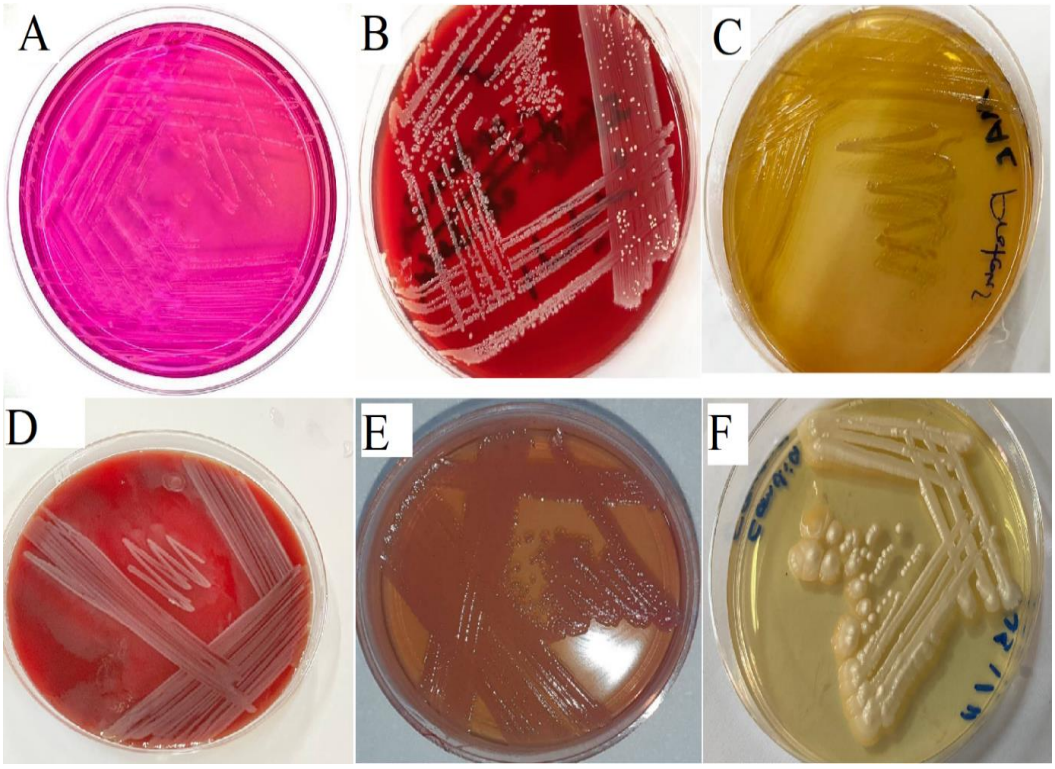

Figure 1: Clarify microbial isolates growing on culture media. **(A), (B)** *S.epidermidis* on Mannitol salt agar, Blood agar, **(C),(D)** *P.mirabilis* on MacConkey agar, Blood agar, **(E)** *A.baumannii* on MacConkey agar and **(F)** *C.albicans* on Sabouraud dextrose agar.

3.2 Microscopic examination

Gram stain was used to distinguish the shapes, colors, and sizes of cells under the microscope. Gram-positive bacteria of *S. epidermidis* looked purple in color and formed like clusters under the microscope [6]. *P. mirabilis* bacteria had a pink color and were Gram-negative bacteria, but *A. baumannii* bacteria had a rod shape and were Gram-negative bacteria, as shown in Figure (2). Gram-positive bacteria stained with the test dye crystal violet appear purple. This is due to the thick peptidoglycan coating of the bacterial cell wall that it retains. Gram-negative bacteria, are unable to maintain a violet dye. The anti-dye (safranin or fuchsine) is absorbed and turns red or pink due to the fact their peptidoglycan layer is drastically thinner and trapped among an internal membrane and a bacterial outer membrane [36].

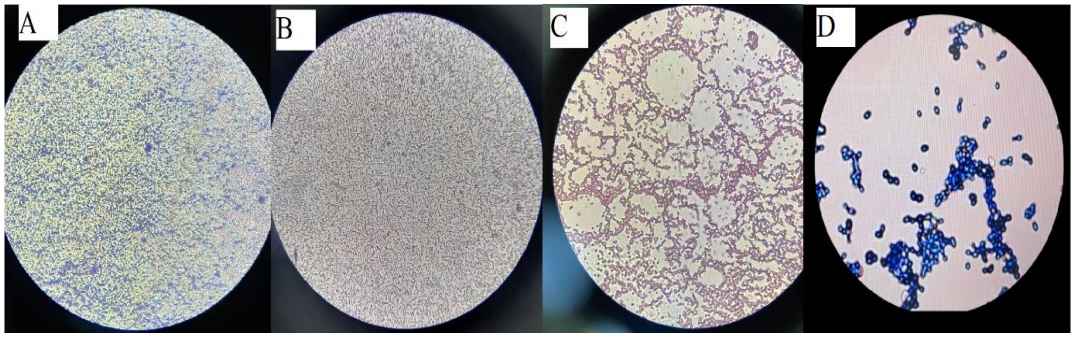

Figure 2: Show microorganisms under a microscope. **A:** *S.epidermids* , **B:** *P.mirabilis* , **C:** *A.baumannii* and **D:** *C.albicans.*

3.3 Biochemical tests

Biochemical tests were carried out, such as *S. epidermidis* gram-positive bacteria, *P. mirabilis*, and *A. baumannii*, which appeared gram-negative bacteria. In terms of the ability of

gram-negative bacteria *P. mirabilis* and *A. baumannii* to ferment lactose, they were not fermentable when grew on macConkey agar medium, and there was no hemolysis of these bacterial isolates when they grew on the medium of blood agar, and *S .epidermidis* bacteria it was not fermented mannitol when grown on mannitol salt agar medium, other tests showed negative results for both coagulase test and oxidase test, whereas positive result for Catalase test, growth test at 44 temperature was positive for *A. baumannii* bacteria and motility test was positive for *P. mirabilis* bacteria [37,38] as shown in Table (1).

Table 1: Biochemical test of isolated bacteria in this study

Test	*S. epidermids*	*A, baumanni*	*P. mirabilis*
Gram stain	**positive +**	**negative -**	**negative -**
MacConkey agar	**no growth**	**non lactose fermenter**	**non lactose fermenter**
Blood agar	**non haemolytic -**	**non haemolytic -**	**non haemolytic -**
Mannitol salt agar	**non ferment mannitol -**		
Shape	**cocci(grape-like clusters)**	**coccobacillus**	**rod-shaped**
Coagulase	**negative -**	**negative -**	**negative -**
Catalase	**positive +**	**positive +**	**positive +**
Oxidase	**negative -**	**negative -**	**negative -**
Growth at 44 C	**negative -**	**positive +**	**negative -**
Motility	**negative -**	**negative -**	**positive +**

3.4 Antibiotic sensitivity test of bacteria

Sensitivity was studied by the disk diffusion method to determine the extent of resistance of bacterial isolates to antibiotics and by comparing it with the standard tables mentioned in a previous study (CLSI,2021). The results showed that *S. epidermidis* was resistant to Ceftazidime, Amoxicillin and Gentamicin at a percentage of with an inhibition diameter of (11,12,13) mm. While it was sensitive to Imipenem, Ciprofloxacin, and Tetracycline with an inhibition diameter of (38, 35, and 20) mm respectively [39]. As for *P.mirabilis* bacteria, it showed resistance to Gentamicin and Tetracycline at a percentage of with an inhibition diameter of (10, 7)mm. While it was sensitive to Amoxicillin, Ceftazidime, Ciprofloxacin and Imipenem with an inhibition diameter of (26, 24, 38, 22) mm respectively [40]. *A. baumannii* bacteria, resistant to Gentamicin with an inhibition diameter of (12) mm. While it was sensitive to Tetracycline, Ceftazidime, Amoxicillin, Ciprofloxacin and Imipenem and with an inhibition diameter of (32, 24, 20, 32, 38) mm. [41] *A. baumannii* has a high rate of resistance to most aminoglycoside antibiotics, and there is no correlation between biofilm development and antibiotic resistance [42]. As shown in ~~the~~ Figure (3).

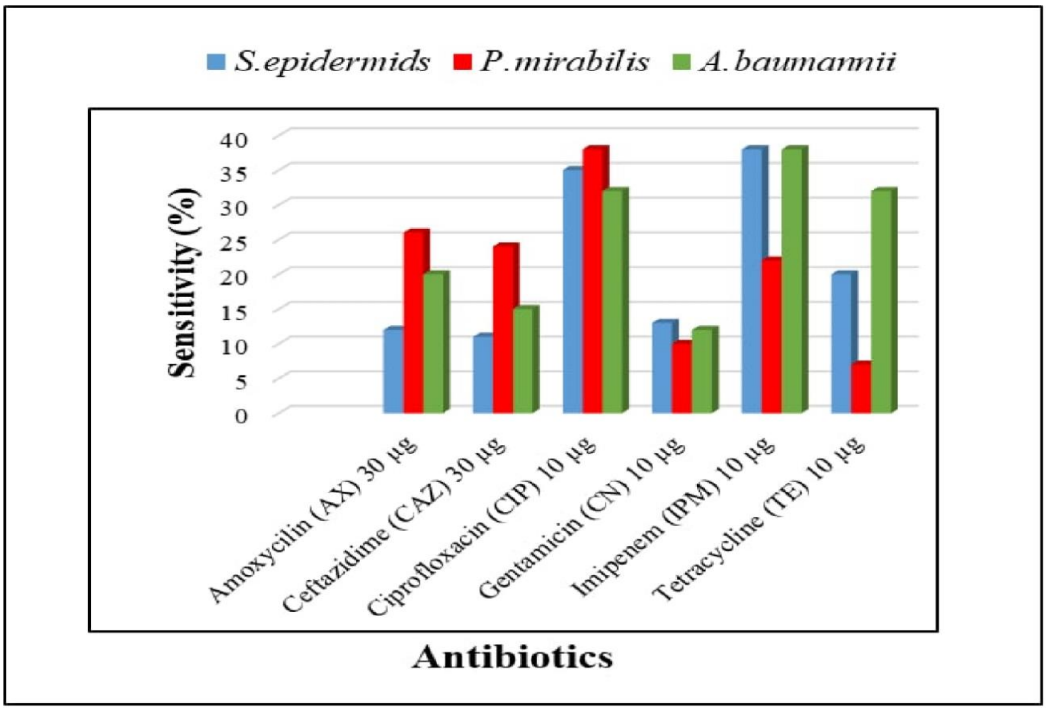

Figure 3: Antibiotic sensitivity test of *S.epidermids*, *P.mirabilis*, and *A.baumannii*.

3.5 Synthesis and characterization of iron oxide nanoparticles (Fe_3O_4)

Synthesis of iron oxide nanoparticles (Fe_3O_4): The results showed the iron oxide nanoparticles (Fe_3O_4) were successfully synthesized and had a dark black color as shown in Figure (4B) with ferromagnetic properties, which was in agreement with [43], who synthesized iron oxide nanoparticles (Fe_3O_4) by a chemical technique. The produced nanoparticles were strongly attracted by an external magnet as illustrated in Figure (4A).

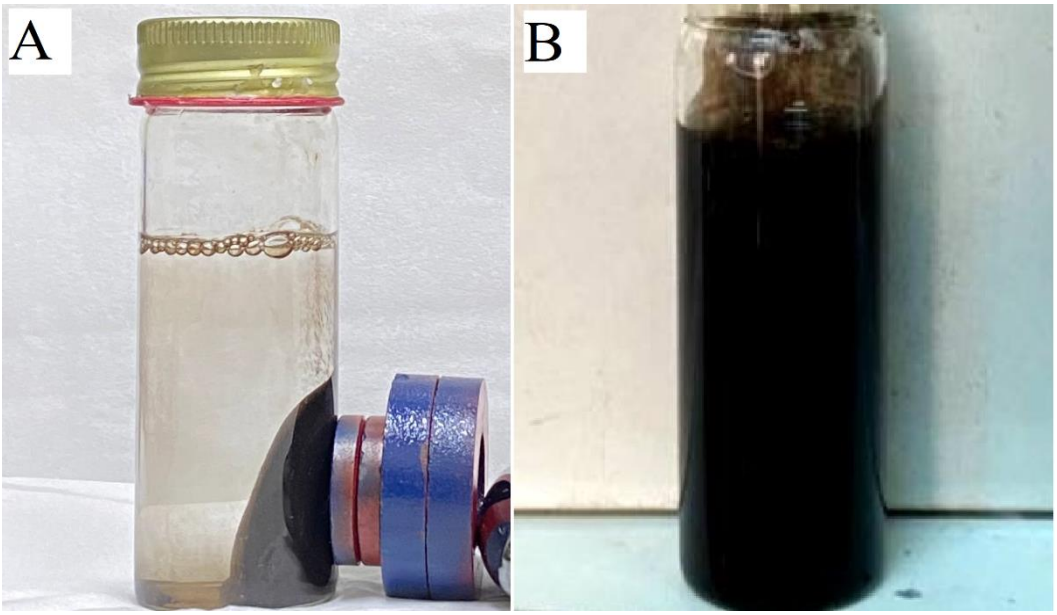

Figure 4: A: Separated iron oxide nanoparticles (Fe_3O_4) using an external magnet**, B:** iron oxide nanoparticles (Fe_3O_4) without an external magnet.

3.6 Characterization of iron oxide nanoparticles (Fe_3O_4)

Scanning electron microscopy (SEM) and Transmission electron microscopy (TEM) is used to analyze the morphology of the materials produced. Figure (5) shows SEM images of iron

oxide nanoparticles (Fe_3O_4), which appear to be made up of small particles. Iron oxide nanoparticles (Fe_3O_4) synthesized by the co-precipitation method indicated the mean size of particles was (29.03-56.54) nm. Because the properties of Nano-crystal are significantly dependent on the dimension of nanoparticles, controlling the monodisperse size is critical [44]. Transmission electron microscopy (TEM) indicates that the iron oxide nanoparticles (Fe_3O_4) are spherical with an average mean size of 28.69 nm by using the Image J software, as shown in Figure (6) [45].

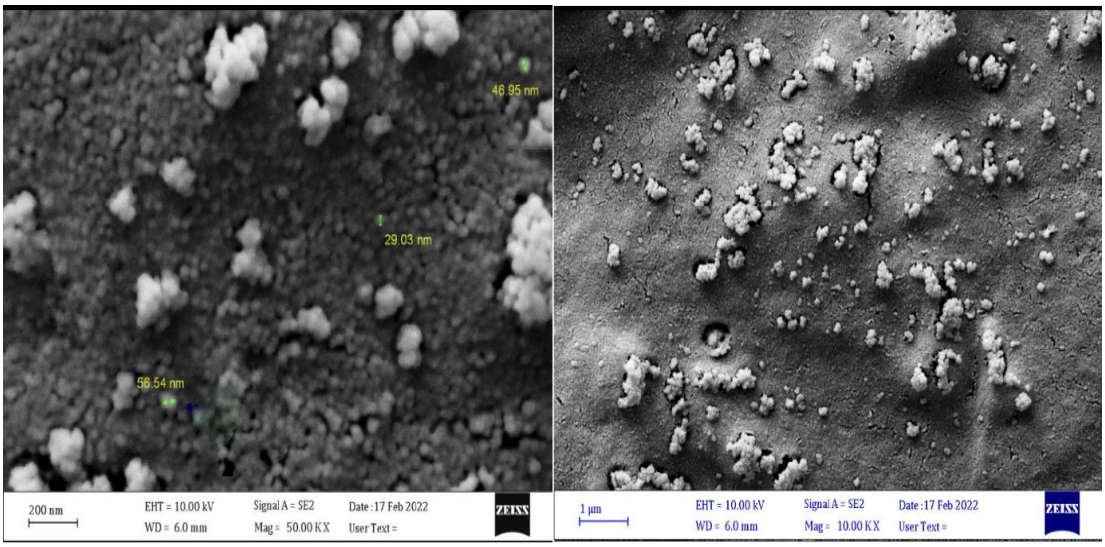

Figure 5: SEM images of iron oxide nanoparticles (Fe_3O_4) size and top view.

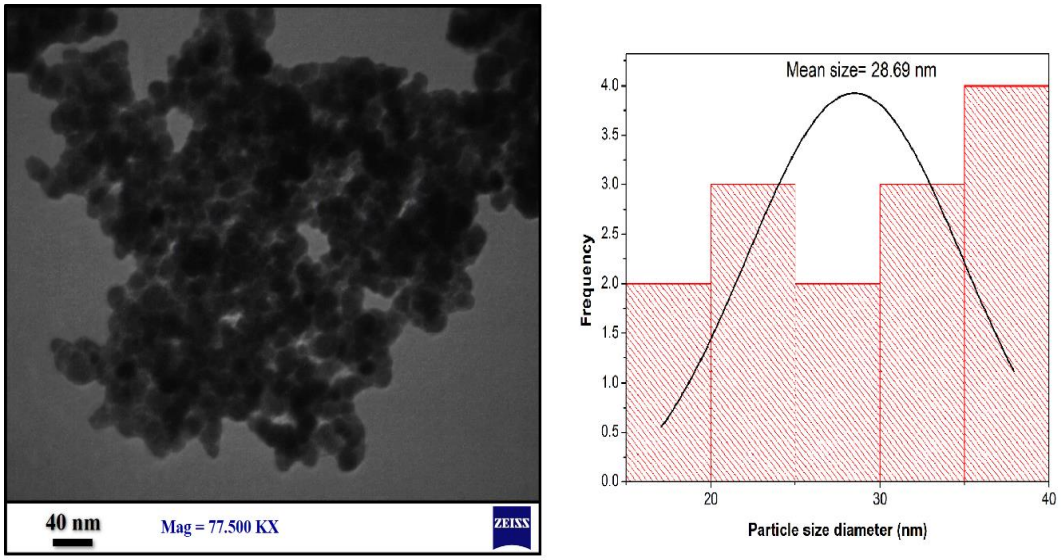

Figure 6: TEM image and histogram of the particle size distribution of iron oxide nanoparticles (Fe_3O_4).

3.7 Antimicrobial activity of iron oxide nanoparticles (Fe_3O_4) against microbial isolates

Results of studying the effect of iron oxide nanoparticles (Fe_3O_4) by agar well diffusion method appeared that they have a significant inhibitory effect. The highest effect of iron oxide nanoparticles (Fe_3O_4) on the growth of *P.mirabilis* was as it was found that an average diameter of the inhibition zone was 22.6 mm, followed by *S.epidermidis*, *A.baumannii* with the average diameter of the inhibitory zone it was 21.6 mm, 20.3 mm respectively and *C.albicans* was 18.3 mm at 100 µgmL^{-1} (stock). A concentration of 12.5 µgmL^{-1} was the lowest inhibitory

concentration, and an inhibitory diameter of at least 11.6 mm against *P. mirabilis*. As shown in Table (2) and Figure (7) [46,47]. It is obvious that as concentrations rise, the rate of inhibition rises as well. Iron oxide nanoparticles (Fe_3O_4) showed an antibacterial effect on Gram +ve and Gram –ve bacteria indicating that those nanoparticles are effective antibacterial agents. The presence of reactive oxygen species (ROS) produced by various nanoparticles is the primary cause of bactericidal activity. The chemical reaction among chemical material in the iron oxide nanoparticles (Fe_3O_4) and the outer bilayer of bacteria, as well as the reason for antibacterial of iron oxide nanoparticles (Fe_3O_4). The great efficiency of iron oxide nanoparticles can be attributed to their high biocompatibility and low toxicity to humans, in addition to their superparamagnetic properties. The iron oxide nanoparticles (Fe_3O_4) have a positive charge, while the microorganisms have a negative charge this is the mechanism of action, which forms electromagnetic gravitation between the iron oxide nanoparticles (Fe_3O_4) and the microorganisms, when the interaction takes place, the microbes are oxidized & depart this life immediately [48].

Table 2: Antimicrobial activity of Iron oxide nanoparticles (Fe_3O_4) against some pathogenic microbes

Microorganisms name	Inhibition zone diameters in (mm)			
	12.5 µgmL^{-1}	25 µgmL^{-1}	50 µgmL^{-1}	100 µgmL^{-1}
S. epidermids	12.66±0.57	15±1	19.33±1.15	21.66±1.52
P. mirabilis	11.66±0.57	13.33±1.15	19±1	22.66±1.15
A. baumannii	16±1.73	16.33±1.15	19.33±1.53	20.33±1.53
C. albicans	13.66±0.58	14.66±0.58	17.66±1.15	18.33±1.15

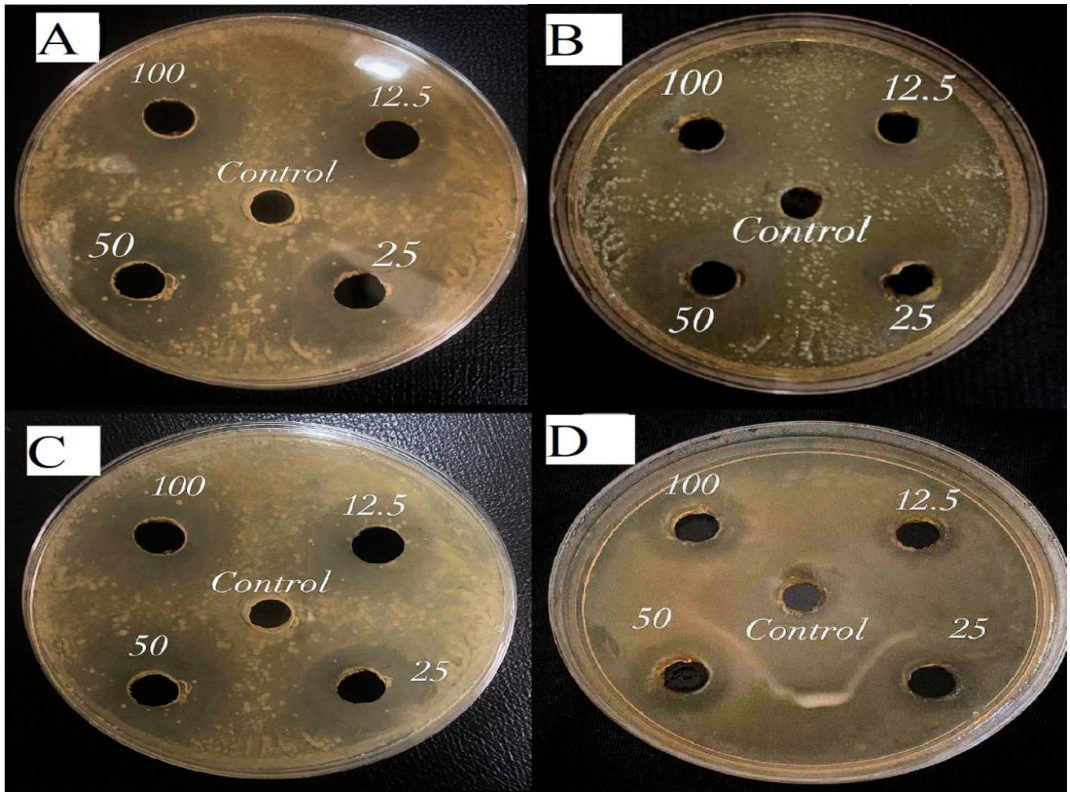

Figure 7: Inhibitory effect of iron oxide nanoparticles (Fe_3O_4) against microbial isolates **A:** *S. epidermids* , **B:** *P. mirabilis* , **C:** *A. baumannii* , **D:** *C. albicans*

3.8 Capturing of microbial isolates by iron oxide nanoparticles (Fe_3O_4)

Magnetic response of iron oxide nanoparticles (Fe_3O_4) is confirmed via their migration in a matter of minutes toward a magnet placed near the solution, and the capture efficiency of microbial isolates before and after trapping was evaluated by iron oxide nanoparticles (Fe_3O_4). Figure (8) shows the capturing of *S.epidermidis, P.mirabilis, A.baumannii,* and *C.albicans* by magnetic IONPs dissolved in PBS solution. Iron oxide nanoparticles (Fe_3O_4) are very prominent in biomedicine; doesn't just for their inherent antimicrobial properties, but also ~~for~~ their super-paramagnetic nature [49]. Iron ions generate-oxygen radicals by converting hydrogen peroxide to the extra reactive hydroxyl-radical through Fenton re-action. Hydroxyl radicals generated via those iron ions can de-polymerize polysaccharides; due to DNA strand breakage, inactivated enzymes and initiate lipid-peroxidation. Moreover, magnetic nanoparticles bound to the cell membrane or cell membrane proteins; by electrostatic interactions is any other possible mechanism that could disrupt bacterial function and cause their death [50].

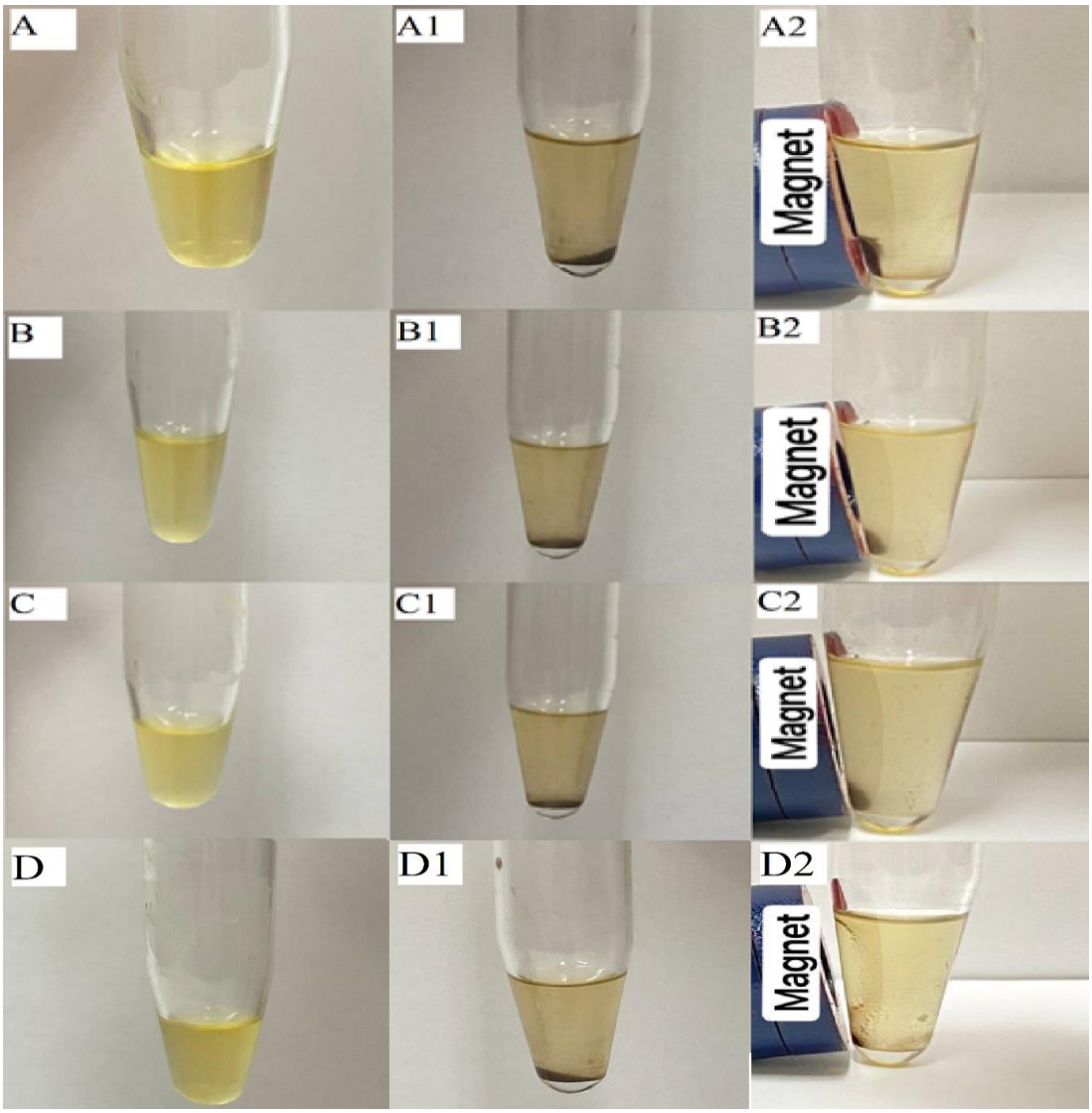

Figure 8: Magnetic capture of microbial isolates incubated for 1 hour at 37° C. **A:** *S. epidermidis,* **B:** *P. mirabilis,* **C:** A. *baumannii* and **D:** *C. albicans* without Fe_3O_4 NPs, **A1:** *S. epidermidis,* **B1:** *P. mirabilis,* **C1:** A. *baumannii* and **D1:** *C. albicans* with Fe_3O_4 NPs and captured **A2:** S. *epidermidis,* **B2:** *P. mirabilis.* **C2:** *A. baumannii* **D2:** *C. albicans* using an external magnetic field in 1 minute.

3.9 Antioxidant activity using DPPH radicals scavenging assay

When dissolved in organic solvents, DPPH is a free radical that is strong at room temperature and creates a darkish violet color. The UV saw absorption curve acquired for the synthesized samples as exemplified in Figure (9) it could be visible that the height depth of DPPH constantly dropped, and the lower ~~in~~ absorbance throughout 517 nm changed into used to calculate the unfastened radical scavenging percentage. Figure (9) shows the antioxidant activity of iron oxide nanoparticles (Fe_3O_4) exhibit potential free radical scavenging action. Ascorbic acid was used as a positive control in the same concentration range. The DPPH scavenging activity of iron oxide nanoparticles (Fe_3O_4) at five different concentrations (6.25, 12.5, 25, 50, 100 µg/mL) was 29.3%, 42.2%, 58.6%, 67.4%, 74 % respectively, it becomes referred to that this effect will increase with increase the concentration of iron oxide nanoparticles (Fe_3O_4) however it stays decrease as compared to ascorbic acid [51]. The presence of bioactive components in the extract with potent antioxidant activity can be attributed to the nanoparticles' radical scavenging capabilities, as a result, the surface functionalized iron oxide nanoparticles have considerable free radical scavenging activity. Because of their high cyto-compatibility and antioxidant activity, the produced nanoparticles could be a prospective candidate for a variety of biomedical applications. Surface functionalization with bio-entities can be employed to alter nanoparticle features as regards chemical functionality and applications [32].

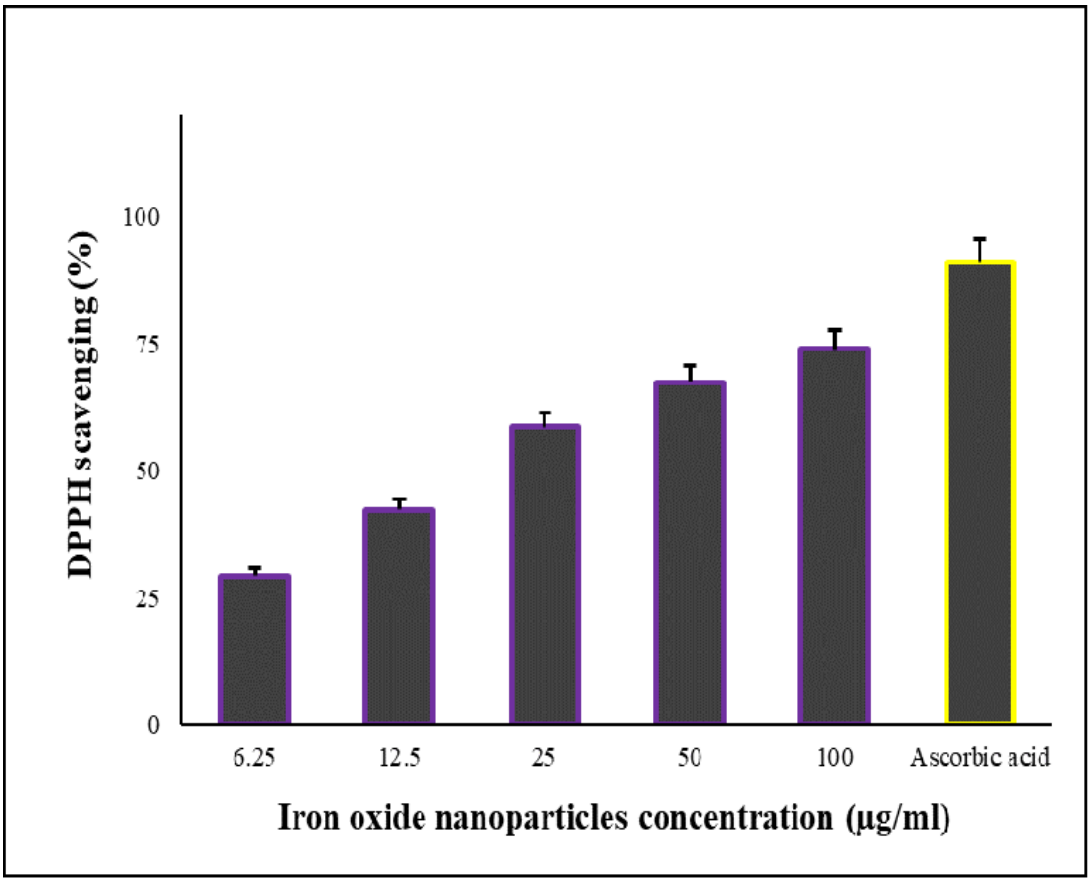

Figure 9: Antioxidant activity of iron oxide nanoparticles (Fe_3O_4) by using DPPH assay, Ascorbic acid represents the positive control.

4. Conclusions

The most commonly isolated microbes from septic wound infections were *S. epidermidis, P. mirabilis, A. baumannii,* and *C. albicans*. These isolates had a high resistance to ceftazidime, amoxicillin, Gentamicin, and tetracycline. Because physiological activities occur at the

nanoscale, the association of iron oxide nanoparticles (Fe_3O_4) is expected to alleviate numerous biological and healthcare difficulties. The activity of the NPs is effective against common multidrug-resistant (MDR) species, and this is a pressing need as antibiotic abuse continues to promote the emergence of resistant strains around the world. Our research indicates the successful production of iron oxide nanoparticles (Fe_3O_4), as well as their use in antimicrobial efficacy against MDR-bacterial isolates from wound infections and antioxidants. We can conclude that the iron oxide nanoparticles (Fe_3O_4) have superparamagnetic and very effective antimicrobial agents.

Conflict of Interest

The authors declare that there are no conflicts of interest regarding the publication of this manuscript.

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