Egypt. J. Biophys. Biomed. Eng., **Vol., 25** No. 1 pp.65-73 (2024)

Antimicrobial Activity of Spinel and Hexaferrites‘’

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NANOMAGNETIC materials may be especially helpful in treatment of bacterial infections. In order to prevent infection and accelerate wound healing, examples include the use of NPs in antibacterial for pharmaceutical materials. This work aimed to evaluate the antimicrobial activity of Spinel and hexaferrites magnetic nanoparticles. The nanoparticles of spinel ferrites ($MgFe_2O_4$, $CoFe_2O_4$) and hexaferrites ($Ba_2Co_2Fe_{12}O_{22}$, $BaFe_{12}O_{19}$) were synthesized using the solgel and coprecipitation method respectively. The prepared samples were characterized by various techniques such as X-Ray diffraction (XRD), transmission electron microscopy (TEM), and antimicrobial activity of different concentrations of $MgFe_2O_4$, $CoFe_2O_4$, $Ba_2Co_2Fe_{12}O_{22}$ and $BaFe_{12}O_{19}$ w tested on some microorganisms of Gram-positive (Staphylococcus aureus and Bacillus subtlus), and Gram-negative (Escherichia coli) bacteria. It was found that the spinel and hexaferrites displayed different levels of antibacterial activities against all tested microorganisms. The study reported validated the dominance of M-type ($BaFe_{12}O_{19}$) as an antibacterial agent for Gram-negative and positive bacteria over $MgFe_2O_4$, $CoFe_2O_4$, and $Ba_2Co_2Fe_{12}O_{22}$. The results also suggested that the created magnetic nanoparticles might be used as antibacterial agents.

Keywords: Spinel ferrites, Cobalt ferrite, Magnesium ferrite, hexaferrites, antibacterial activity.

Introduction

The advantageous magnetic and antibacterial properties of magnetic nanoparticles have recently drawn a lot of attention in the field of biomedicine. One of the strongest magnetic materials is ferrites. (Raouf et al. 2020).These metal ferrites have been successfully demonstrated to have a high level of biocompatibility, and their antibacterial activity makes them a suitable choice for antibacterial applications in the industrial and medical areas(Sanpo et al. 2013). There are two groups of antibacterial agents: organic and inorganic (Kaviyarasu et al. 2017).Organic antibacterial compounds include medicinal plants as notable examples. An important area of research in the field of innovative antibacterial therapies is the optimization of antibacterial activity mediated by NPs.(Wang, Hu, and Shao 2017a). Their ability to be magnetic has enabled them to provide a wide range of biomedical uses, including in vivo treatment approaches and diagnostics. Based on their mode of action against bacteria, NPs can be generally classified as having either bactericidal (killing) or bacteriostatic (inhibiting growth) properties. Most MNPs have bactericidal effects against both Gram-positive and Gram-negative bacteria because of a variety of mechanisms(Rodrigues et al. 2019).The creation of nanoscale ferrites via various methods opened up new possibilities, including a multitude of biological uses (Baykal et al. 2015; Stefanescu et al. 2013).The number of studies examining NPs' possible antibacterial actions has increased as a result of their rising use in medicine. The metabolic activity of bacteria, for instance, can be altered by metal nanoparticles. Its ability to eliminate microorganisms and treat diseases is a major benefit(Chatzimitakos and Stalikas 2016).Cell walls and membranes are the main factor for mediating bacterial resistance to the external environment. In particular, maintaining

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Received 29/6/2024 ; accepted 4/8/2024
DOI :10.21608/EJBBE.2024.300137.1075

a bacterial cell wall is essential to maintaining the bacterium in its original form.(Munita and Arias 2016).Different adsorption paths for NPs and Gram-positive and Gram-negative bacteria are produced by the components of the cell membrane(Lesniak et al. 2013).NPs are more efficient against Gram-positive bacteria than Gram-negative bacteria, according to numerous studies(Wang, Hu, and Shao 2017b), because of the fact that the phospholipid and lipoprotein components of Gram-negative bacteria's cell walls create a penetration barrier that only permits macromolecules to enter. In contrast, the thin coating of peptidoglycan, teichoic acid, and many holes found in the cell wall of Gram-positive bacteria allow foreign molecules to enter and cause damage to the cell membrane and eventual cell death (Sarwar et al. 2015). A previous study indicated that MgFe2O4 nanoparticles demonstrated notable antimicrobial and antibiofilm properties against both gram-positive and gram-negative bacteria. the results suggested that MgFe2O4 nanoparticles hold promise as potential agents for antimicrobial applications and detoxification purposes(El-Khawaga et al. 2024).. Lately, there has been significant interest in the antibacterial properties of magnetic nanoparticles. However, there is limited information available regarding the antibacterial effectiveness of CFO (cobalt ferrite) specifically)(Hassanzadeh-Afruzi et al. 2022).The objective of this study is to prepare the members of spinel ferrites (MgFe2O4 and CoFe2O4) and hexaferrites (Ba2Co2Fe12O22 and BaFe12O19). Samples were characterized by differenttechniques: XRD, TEM, and antibacterial properties. The antibacterial activity of samples was tested on microorganisms (Staphylococcus aureus, Bacillus subtlus, and Escherichia coli).

Material and method

Synthesis of Spinel and hexaferrites

Synthesis of spinel ferrite ($MgFe_2O_4$, $CoFe_2O_4$):

Spinel structure (MFe_2O_4), where M=Mg and Co magnetic nanoparticles was prepared by sol-gel method and named as the following: magnesium ferrite ($MgFe_2O_4$) and cobalt ferrite ($CoFe_2O_4$). .Briefly, 2 mole of iron nitrate [Fe $(NO_3)3.9H_2O$, M.w = 404 g/mole] and 1 mole of magnesium nitrate [$MgNO_3.6H_2O$, M.w = 256.41 g/mole] or cobalt nitrate [Co $(NO_3)_2.6H_2O$, M.w = 291.03 g/mole] [Sigma -Aldrich (St. Louis, MO, USA)] was added to a solution of distilled water and citric acid as a hydrolysis catalyst. The mixture was kept under stirring at 37 °C until complete hydrolysis. Ammonia was added to the above solution as a gel catalyst. The obtained gel was dried at 100 °C/24 hrs. and calcined at 600 °C / 3hrs to remove the toxic nitrate. Finally, $MgFe_2O_4$, and $CoFe_2O_4$ powders were deagglomerated in a mortar agate.

Synthesis of hexaferrite ($Ba_2Co_2Fe_{12}O_{22}$, $BaFe_{12}O_{19}$)

Y-type Barium cobalt ferrite ($Ba_2Co_2Fe_{12}O_{22}$) and M-type barium ferrite ($BaFe_{12}O_{19}$) magnetic nanoparticles were prepared by co-precipitation method. Briefly a mixture of iron (III) nitrate nonahydrate, Fe $(NO_3)_3.9H_2O$, cobalt nitrate and barium chloride with the Ba^{2+}: Co^{3+}:Fe^{3+} molar ratios 1:2:12 ($Ba_2Co_2Fe_{12}O_{22}$) and 1:0:12 ratios ($BaFe_{12}O_{19}$) was dissolved in deionized water. The produced solution was treated with citric acid / ammonia solution to form a precipitate at pH 10. The produce solutions kept under stirring /100 °C until evaporates all water. Dried at 100 °C for 24 hrs. The formed precursor powders were pre-annealing temperature at 1200 °C for 6 hrs in static air atmosphere. The obtained samples were characterized by XRD. The antibacterial activity of the prepared samples [$Ba_2Co_2Fe_{12}O_{22}$, $BaFe_{12}O_{19}$, $MgFe_2O_4$, $CoFe_2O_4$] were done using various microorganisms such as Staphylococcus aureus (MTCC741), Bacillus subtlus (MTCC1789) as G^+ bacterium , and Escherichia coli (MTCC1698) G^- bacterium obtained from the faculty of science (Boyes) – at Alazhar University – Cairo – Egypt, using agar diffusion method (Abou Hammad et al. 2023; Al-esnawy et al. 2021)58S-BG/CH and STRS-loaded 58S-BG/CH beads (0% Sm, 10% Sm, 20% Sm, 30% Sm, and 40% Sm . The media used for this test have the following compositions (g/L) nutrients agar medium: - D-glucose 5.0, peptone 5.0, meat extract 5.0, NaCl 5.0, and agar 20. The PH was adjusted to 7 used for the growth of microorganism strains.

Characterization techniques

The powder of the prepared samples MgFe2O4, $CoFe_2O_4$,$Ba_2Co_2Fe_{12}O_{22}$ and $BaFe_{12}O_{19}$ were investigated by different techniques. Identification of the crystalline nature of the prepared magnetic nanoparticles was carried out using X-ray powder diffractometer model (a BRUCKER axes Germany D8 advance and CuKα radiation (١،٥٤٠٦ Å) with a typical scanning range of ٢ from 0° to 60° and scan rate of 2.min^{-1}).). The internal structure of the prepared samples was examined by transmission electron microscope (TEM) using a JEM 1230 electron microscope (Jeol, Tokyo, Japan). The antibacterial activity of the samples was tested on microorganisms (Staphylococcus aureus, Bacillus subtlus, and Escherichia coli).

TABLE 1. Chemicalcomposition of spinel and hexaferrites.

$MgNO_3.6H_2O$ 1MOLE	$Co(NO_3)_2.6H_2O$ 1 mole	$Bacl_2.2H_2O$ 1 mole	$Fe(NO_3)_3.9H_2O$ 2 mole	Sample
Spinel ferrites				
2.033 g	0	0	6.408 g	$MgFe_2O_4$
0	2.308 g	0	6.408 g	$CoFe_2O_4$
Hexaferrites				
0	0.582 g	0.244 g	4.848 g	$Ba_2Co_2Fe_{12}O_{22}$
0	0	0.244 g	4.848 g	$BaFe_{12}O_{19}$

Anti-bacterial activity

Several pathogenic microorganisms, such as Staphylococcus aureus (MTCC741), Bacillus subtlus (MTCC1789) as a G+ bacterium, and Escherichia coli (MTCC1698) as a G- bacterium, were obtained from the faculty of science (Boyes) at Alazhar University in Cairo, Egypt, and were used to test the antibacterial activity of the prepared samples using the agar diffusion method. The following compositions of the media (g/L) of the nutrient's agar medium were used for this test: - Agar 20, D-glucose 5.0, peptone 5.0, meat extract 5.0, and NaCl 5.0. To facilitate the growth of microorganism strains, the pH was changed to 7.

Antibacterial assay

The antibacterial efficiency of agar supported $MgFe_2O_4$, $CoFe_2O_4$, $Ba_2Co_2Fe_{12}O_{22}$ and $BaFe_{12}O_{19}$ against G- bacterium (E. coli) and G+ bacterium (S. aureus and Bacillus subtlus) by in-vitro study and agar diffusion method was examined. The samples of $MgFe_2O_4$, $CoFe_2O_4$, $Ba_2Co_2Fe_{12}O_{22}$ and $BaFe_{12}O_{19}$ were formed into discs with a diameter of 10 mm, UV sterilized for two hours, and then placed over the agar surface plates inoculated with the test microorganisms (nutrient agar media). The Petri dishes were then kept in the refrigerator for an hour to allow the antibacterial ions to diffuse uniformly, and then incubated at 37 ºC for a duration of 24 hours. We conducted three rounds of this test. The appearance of a clearing inhibition zone around the sample is an indication of the antibacterial activity of $MgFe_2O_4$, $CoFe_2O_4$,$Ba_2Co_2Fe_{12}O_{22}$ and $BaFe_{12}O_{19}$.*R.*

Results and discussions

X-ray diffraction (XRD)

The XRD pattern of the magnetic $MgFe_2O_4$ and $CoFe_2O_4$ nanoparticles is displayed in Fig. (1 a&b). These results coincide with the standard JCPDS card No. (01- 088-1936)(Iqbal et al. 2021). The principal characteristic peaks of $MgFe_2O_4$ in fig. (1.a) were revealed at 30.14º, 35.5º, 43.14º, 57.072º, and 62.66º, corresponding to the (220), (311), (400), (511), and (440) planes of the crystal lattice, respectively. Based on standard JCPDS card No. 01-072-1174, the results are in agreement (Al Yaqoob et al. 2019). Figure (1.b) displays the primary characteristic peaks of $CoFe_2O_4$, which are located at 2θ 30.08 º, 35.43 º, 43.05 º, 53.44, 56.97 º, and 62.58. According to the order of the sources, (220), (311), (400), (422), (511), and (440). The JCPDS card no. 00-022-1086 standard and these results agree. The significant degree of spinel structure crystallinity in $MgFe_2O_4$ and $CoFe_2O_4$ nanoparticles is confirmed by strong and sharp peaks. The Fig.(1.c&d) shows the XRD patterns of barium ferrite $BaFe_{12}O_{19}$ and barium cobalt hexaferrite $Ba_2Co_2Fe_{12}O_{22}$ which were made using the co-precipitation method. It has been verified that upon calcination at 1200 °C, $Ba_2Co_2Fe_{12}O_{22}$ and $BaFe_{12}O_{19}$ hexaferrite phases are produced. The high crystallinity of barium hexaferrite $Ba_2Co_2Fe_{12}O_{22}$ is confirmed by strong and sharp peaks that were seen at 30.4º, 32º, 35.8º, 41.1º, and 63.4º at 2θ, which correspond to (110), (10 13), (119), (02 10), (220). The JCPDS card no.00-044-0206 provides a precise reference for the peaks obtained in the XRD pattern of $Ba_2Co_2Fe_{12}O_{22}$hexaferrite(Gao et al. 2022)and the dielectric and magnetic properties were tuned by changing sintering temperature (Ta. $BaFe_{12}O_{19}$ hexaferrite phase was observed at 2θ at 32.1º, 34º, 37º, 55º, and 63º, which correspond to 107, 114, 203, 217, and 220, respectively, and was associated with the standard (JCPDS card no. 00-039-1433)(Mosleh et al. 2014).

Morphology of $MgFe_2O_4$, $CoFe_2O_4$, $Ba_2Co_2Fe_{12}O_{22}$ and $BaFe_{12}O_{19}$

The morphology of the prepared spinel structure ($MgFe_2O_4$ and $CoFe_2O_4$) and hexaferrites ($Ba_2Co_2Fe_{12}O_{22}$ and $BaFe_{12}O_{19}$) magnetic nanoparticles were shown in fig.(2). where $MgFe_2O_4$ appeared a spherical shape (diameter is approximately 190 nm), but other samples revealed in irregular shapes for $CoFe_2O_4$ (size 140 nm^3) , $Ba_2Co_2Fe_{12}O_{22}$ (size 120 nm^3) and $BaFe_{12}O_{19}$ (size 160 nm^3).The agglomeration of smaller particles with large ones is seen.

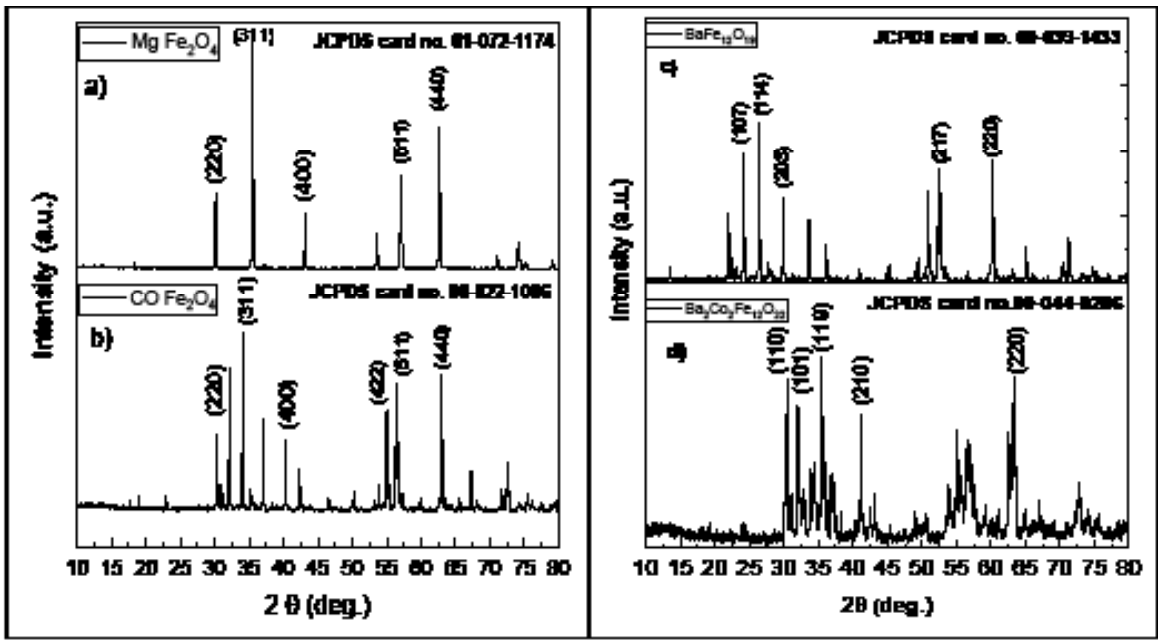

Fig .1. XRD patterns of the Prepared samples of a) MgFe2O4 , b) CoFe2O4, c) BaFe12O19, and d)Ba2Co2Fe12O22.

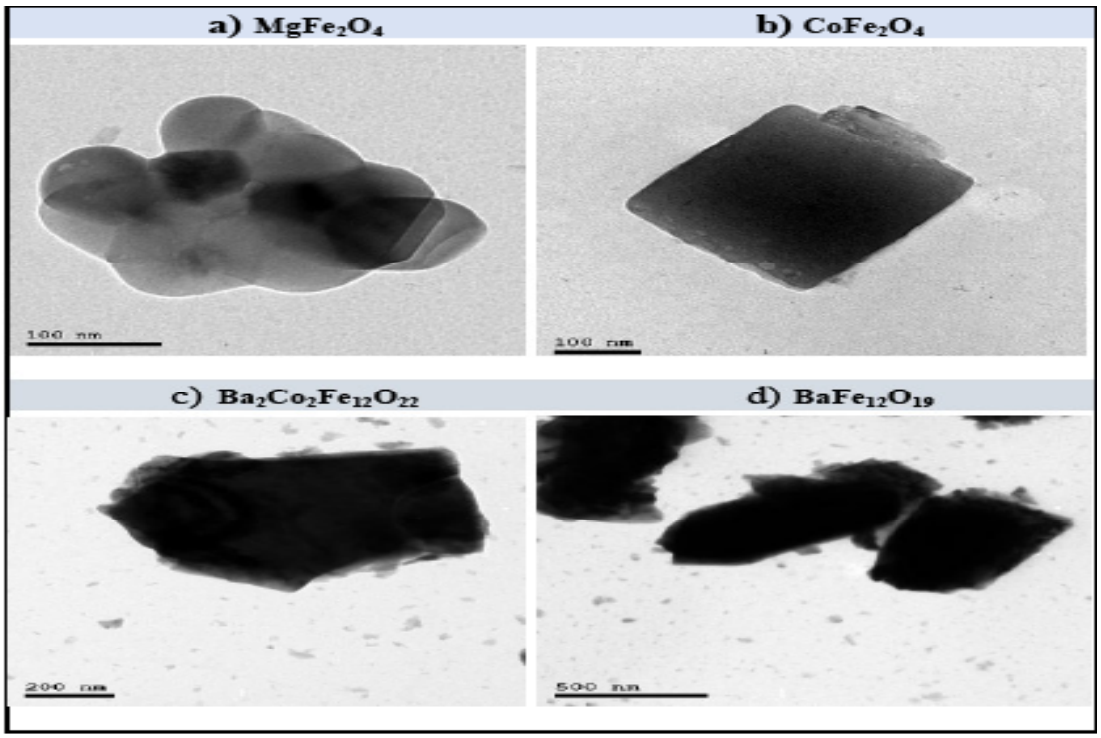

Fig.2. Transimission electron microscope imagesof a) MgFe2O4 b) CoFe2O4,c)Ba2Co2Fe12O22,,and d) BaFe12O19

Bacterial sensitivity of $MgFe_2O_4$, and $CoFe_2O_4$ (MNPs)

$MgFe_2O_4$, $CoFe_2O_4$ magnetic nanoparticles were tested against three different bacterial strains: G+ (Escherichia coli) (MTCC1698) and G- (Staphylococcus aureus (MTCC741) and Bacillus subtlus (MTCC1789)). Table (2) shows the diameters of the cleaning inhibition zones of the prepared samples at 25, 50, 100, 200, and 300 ug/mL. In summary, when exposed to Staphylococcus aureus, the diameters of the inhibition zones of $MgFe_2O_4$ and $CoFe_2O_4$ were 18.3 and 20 mm at 300 ppm. Following their exposure to Bacillus subtlus, their measurements were 18.67 and 18.7 mm. Lastly, when exposed to Escherichia coli, they show inhibition zones of 16.33 and 15.67 mm, respectively. Increases in Mg and Co concentrations led to an increase in the clearing inhibition zone. The findings revealed that the tested spinel ferrite magnetic nanoparticles released magnesium and cobalt ions, which may have contributed to the antibacterial activity of all derivatives of the spinel structure magnetic nanoparticles ($MgFe_2O_4$ and $CoFe_2O_4$) as shown in fig.3.These findings demonstrated that $MgFe_2O_4$ and $CoFe_2O_4$ have more antibacterial action against G+ bacteria (S. aureus and B. subtlus) than against G-bacteria (E. coli). These findings confirmed that in contrast to the single peptidoglycan membrane of the G+ bacterium, the cell wall of the G-bacteria is complex, consisting of internal and exterior membranes composed of phospholipid molecules, lipopolysaccharides, peptidoglycan, and lipoproteins.

Bacterial sensitivity of Ba2Co2Fe12O22 andBa-Fe12O19 (MNPs)

Fig.4. shows the antimicrobial activity of different concentrations (25,50,100,200,and 300 µg/mL) of $Ba_2Co_2Fe_{12}O_{22}$ (Y-type) , and $BaFe_{12}O_{19}$ (M-type) on some microorganisms Staphylococcus aureus (MTCC741), and Bacillus subtlus (MTCC1789)}, and G- bacterium (Escherichia coli) (MTCC1698) appearance of a clearing zone around sample's discs confirmed that M-type hexaferrite enhanced the antimicrobial activity compared to Y-type hexaferrite alginates Staphylococcus aureus, Bacillus subtlus, and Escherichia coli. It was noticed, with an increases in the concentrations of the M-type and Y-type Hexaferrites, the clearing zone was increased [Table.2]. This effect may be explained by the release of heavy metals like iron from M-type freely than the complex structure of Y-type hexaferrite.

Fig.5. showed that the different samples were tested against different microorganisms. final results suggested that the best sample in terms of antibacterial activity is M-type ($BaFe_{12}O_{19}$) sample compared to $Ba_2Co_2Fe_{12}O_{22,}$ $CoFe_2O_4$,and $MgFe_2O_4$.

TABLE 2. Bacterial sensitivity in (mm) of bafe12o19, ba2co2fa12o22, cofe2o4, and mgfe2o4 against tested microorganisms.

	Concentration					Sig.
Elements	300ug/mL	200ug/mL	100ug/mL	50ug/mL	25 ug/mL	(P-value)
S. aureus						
$BaFe_{12}O_{19}$	23.	19.67	16.3	13.67	0	9.053
$Ba_2Co_2Fe_{12}O_{22}$	21.	18.0	14.6	0	0	8.53
Co Fe_2O_4	20	17.3	14.3	0	0	0.000001
Mg Fe_2O_4	18.3	15.7	12.3	0	0	0.000001
B. subtlus						
$BaFe_{12}O_{19}$	22.	18.67	16.33	12.67	0	2.39
$Ba_2Co_2Fe_{12}O_{22}$	19.	16.67	13.3	11.33	0	5.45
Co Fe_2O_4	18.7	16.3	13.7	11.67	0	0.000001
Mg Fe_2O_4	18.67	16.3	13.67	10.3	0	0.000001
E. coli						
$BaFe_{12}O_{19}$	23.	20.8	17.6	13.67	0	7.11
$Ba_2Co_2Fe_{12}O_{22}$		16.33			0	1.51
Co Fe_2O_4	15.6	12.67	0	0	0	0.000001
Mg Fe_2O_4	16.3	14.33	12.33	9.67	0	0.000001

P-value <0.05was significant.

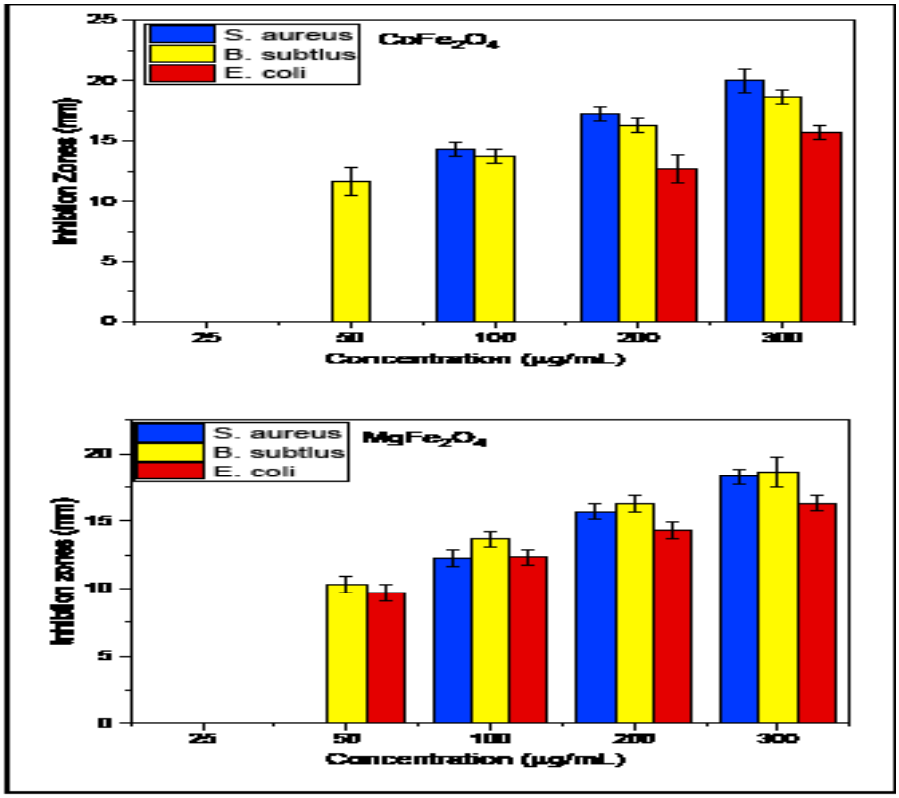

Fig. 3. Inhibition zone in mm of MgFe2O4, and CoFe2O4 (MNPs) against tested microorganisms.

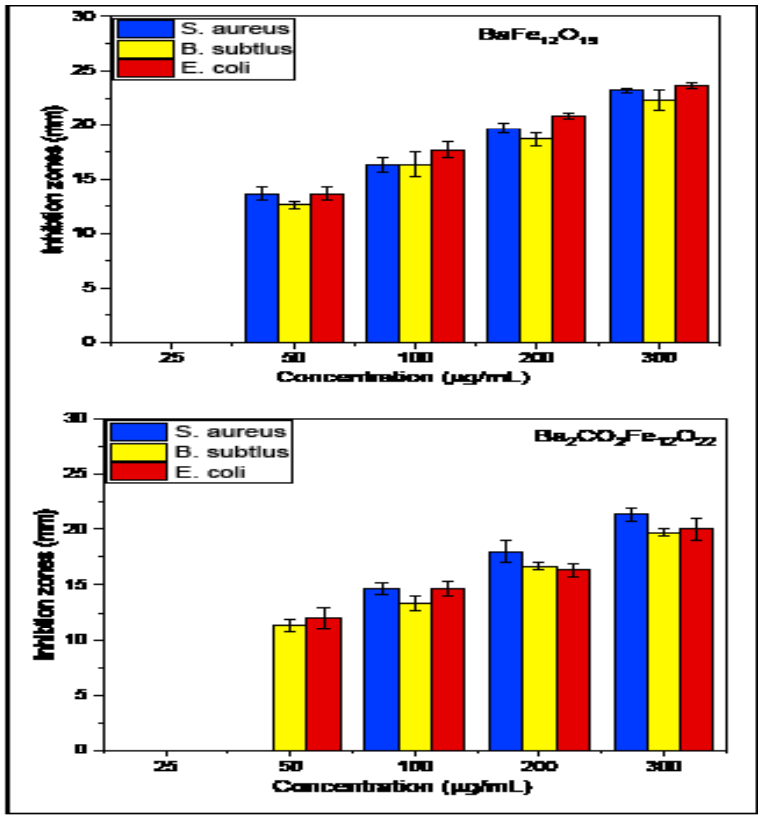

Fig.4. Inhibition zone in mm of Ba2Co2Fe12O22 and BaFe12O19 against tested microorganisms.

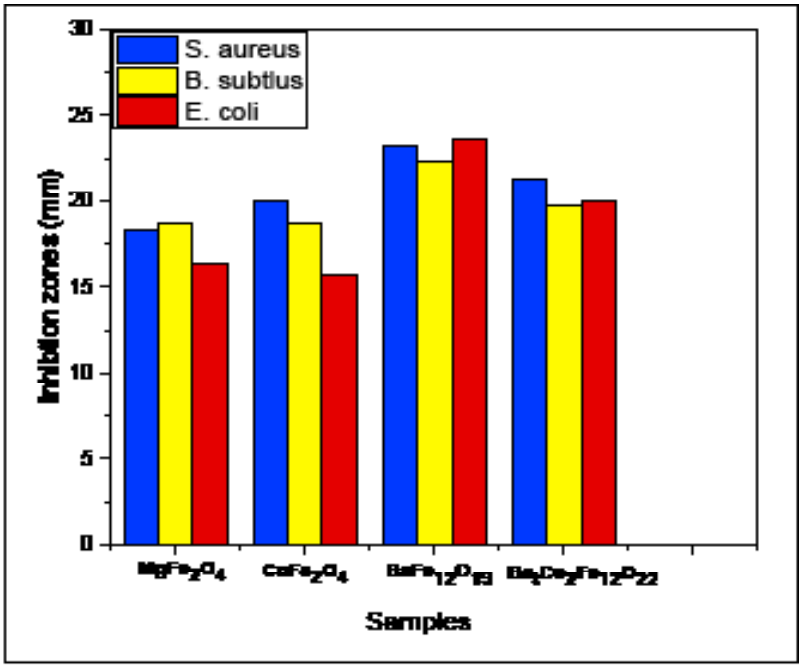

Fig. 5. Inhibition zone in mm of Ba2Co2Fe12O22 and BaFe12O19, CoFe2O4 ,and MgFe2O4 (MNPs) against tested microorganisms at concentration 300 ug/mL.

Conclusion

The magnetic nanoparticles ($MgFe_2O_4$, $CoFe_2O_4$) and ($Ba_2Co_2Fe_{12}O_{22}$,$BaFe_{12}O_{19}$) were prepared via sole-gel and coprecipitation methods respectively. The crystalline nature of the internal structure was investigated by different instruments: (XRD), (TEM), and the antimicrobial activity of magnetic nanoparticles was tested on S. aureus, B. subtlus, and E. coli using the agar diffusion method. XRD results showed that spinel ferrites of $MgFe_2O_4$, $CoFe_2O_4$, $Ba_2Co_2Fe_{12}O_{22}$, and $BaFe_{12}O_{19}$ are in crystalline nature. Finally, $MgFe_2O_4$, and $CoFe_2O_4$ possessed anti-bacterial activity on G^+ bacterium (S. aureus, and B. subtlus) higher than that on G- bacterium (E. coli). The presented study confirmed the superiority of ($BaFe_{12}O_{19}$) as an antibacterial agent for Gram-negative and Gram-positive bacteria compared to $MgFe_2O_4$, $CoFe_2O_4$, and $Ba_2Co_2Fe_{12}O_{22}$. Results showed that spinel and hexaferrites can be used for medical applications with the simultaneous ability to be antimicrobial agents.

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"النشاط المضاد للميكروبات للسبينيل والهكسافيرايت"

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قد تكون المواد النانوية المغناطيسية مفيدة بشكل خاص في علاج الالتهابات البكتيرية. ومن أجل منع العدوى وتسريع التئام الجروح، تشمل الأمثلة استخدام الجسيمات النانوية في المواد الصيدلانية المضادة للبكتيريا. يهدف هذا العمل إلى تقييم النشاط المضاد للميكروبات لجسيمات النانو المغناطيسية السبينيل والهكسافيرايت. تم تصنيع الجسيمات النانوية من فيريت السبينيل ($MgFe_2O_4$ و$CoFe_2O_4$) والهكسافيرايت ($Ba_2Co_2Fe_{12}O_{22}$ و $BaFe_{12}O_{19}$) باستخدام طريقة السولجيل والترسيب المشترك على التوالي. تميزت العينات المحضرة بتقنيات مختلفة مثل حيود الأشعة السينية (XRD)، والمجهر الإلكتروني النافذ (TEM)، والنشاط المضاد للميكروبات لتركيزات مختلفة من $MgFe_2O_4$ و $CoFe_2O_4$ و $Ba_2Co_2Fe_{12}O_{22}$ و $BaFe_{12}O_{19}$ التي تم اختبارها على بعض الكائنات الحية الدقيقة إيجابية الجرام (Staphylococcus aureus و Bacillus subtlus) والبكتيريا سلبية الجرام (Escherichia coli). وقد وجد أن السبينيل والهكسافيرايت أظهرا مستويات مختلفة من النشاط المضاد للبكتيريا ضد جميع الكائنات الحية الدقيقة المختبرة. أفادت الدراسة بإثبات هيمنة النوع M ($BaFe_{12}O_{19}$) كعامل مضاد للبكتيريا للبكتيريا سلبية الجرام وإيجابية الجرام على $MgFe_2O_4$ و $CoFe_2O_4$ و $Ba_2Co_2Fe_{12}O_{22}$. كما اقترحت النتائج أن الجسيمات النانوية المغناطيسية التي تم إنشاؤها يمكن استخدامها كعوامل مضادة للبكتيريا.