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Antibacterial effects of Biosynthesized Magnesium oxide (MgO) Nanoparticles produced by *Pseudomonas aerogenosa*.

A research submitted to the College of Science, Department of Biology, as part of the requirements for obtaining a Bachelor's degree in Biology, Microbiology Branch.

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بسم الله الرحمن الرحيم

" وَلَقَدْ آتَيْنَا دَاوُودَ وَسُلَيْمَانَ عِلْمًا ۖ وَقَالَا الْحَمْدُ لِلَّهِ الَّذِي فَضَّلَنَا عَلَىٰ كَثِيرٍ
مِّنْ عِبَادِهِ الْمُؤْمِنِينَ* وَوَرِثَ سُلَيْمَانُ دَاوُودَ ۖ وَقَالَ يَا أَيُّهَا النَّاسُ
عُلِّمْنَا مَنطِقَ الطَّيْرِ وَأُوتِينَا مِن كُلِّ شَيْءٍ ۖ إِنَّ هَٰذَا لَهُوَ الْفَضْلُ الْمُبِينُ"

{ سورة النمل، الآيات 15، 16}

صدق الله العلي العظيم

(الشكر والاهداء)

الى صاحب السيرة العطرة، والفكر المستنير

فلقد كان له الفضل الأوّل في بلوغي التعليم العالي

(والدي الحبيب)، أطال الله في عُمره.

الى من وضعتني على طريق الحياة، وجعلتني رابطة الجأش،

ورعيتني حتى صرت كبيرة

(أمي الغالية)،التي وهبتني كل شيء

الى إخوتي؛ من كان لهم بالغ الأثر في كثير من العقبات والصعاب.

الى جميع أساتذتي الكرام؛ ممن لم يتوانوا في مد يد العون لي

أهدي اليكم بحثي.....

❖ Abstract:

The world problem in medical is multidrug resistance ,So all researchers tend to investigate alternative antibacterial materials example nanoparticles .The MgO nanoparticles synthesis by using the bacteria was the aimed of this research . survey the ability of some bacteria collected from advance microbiology (6 *E. coli* , 3 isolate *Pseudomonas aerogenosa* and 3 isolates *Klebsiella Pneumoniae*). The *Pseudomonas aerogenosa* was chosen to this research after estimate Uv.by using spectrophotometers at wave line at(405-630) .The isolate number 3 was chosen. The results of scanning electron microscopy found the diameter of nano was (32-40) nanometer .the MgO nanoparticles had antibacterial effect on Gram negative and gram positive bacteria .

الخلاصة :

البكتريا المقاومة للعديد من المضادات الحيوية تعتبر مشكلة عالمية ولازال العديد من الباحثين يبحث في استخدام بدائل للمضادات الحيوية ومنها استخدام الدقائق النانووية الصديقة للبيئة .فهدفت الدراسة البحثية الى استخدام البكتريا في تصنيع دقائق اوكسيد المغنسيوم كمضاد بكتيري . تم التحري عن قابلية البكتريا على الانتاج باستخدام (6 عزلات من بكتريا الاشريشيا القولونية و3 عزلات من الزوائف الزنجارية و3 من بكتريا الكلبسيلا) وتم التحري على انتاج الدقائق النانووية لاوكسيد المغنسيوم باستخدام سبكتروفوتوميتر با طوال موجية بين 405 و630 وتم اختيار عزلة رقم 3 حيث تم تنميتها في لتر من المرق المغد ي وبعدها تم تصنيع الدقائق النانووية وبعدها تم تحديد الخصائص النانووية باستخدام المجهر الالكتروني وكانت الاقطار بين 32-40 نانونومتر كما درست الفعالية التثبيطية ضد بعض البكتريا الموجبة والساالبة لصبغة غرام .

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Chapter One

Introduction

(1.1) Introduction:

Nanotechnology is a modern field of sciences to research phenomena at atomic, molecular, and macromolecular scales, delivered by coupling of nanotechnology and biotechnology. The field of nanotechnology included the synthesis, characterization, design, production, and application of structure, by controlling size and shape at the nanometer scale as well as applications of nanoparticles (Ranjithkumar *et al*., 2013).

Nanoparticles—particles having one or more dimensions of the order of 100 nm or less (Kato 2011). There are a large number of physical, and chemical, methods available to synthesize different types of nanoparticles (Liu *et al.*, 2011). Although physical and chemical methods are more popular in the synthesis of nanoparticles, the use of toxic chemicals greatly limits their biomedical applications, in particular in clinical fields. Therefore, the development of reliable, nontoxic, and eco-friendly methods for the synthesis of nanoparticles is of utmost importance to expand their biomedical applications, emphasis has been placed on biological synthesis systems and exploited for the synthesis of nanoparticles to provide a safer alternative to physical and chemical methods(Musarrat *et al*., 2011).

The biological method of synthesizing nanoparticles uses biological agents such as plants, algae, fungi, actinomycetes, yeast, bacteria and viruses used to produce the nanoparticles, (Duhan *et al.*, 2017). Nanoparticles produced by a biogenic enzymatic process are far superior, in several ways, to those particles produced by chemical methods. Despite that the latter methods are able to produce large quantities of nanoparticles with a defined size and shape in a relatively short time, they are complicated, costly, and inefficient and produce hazardous toxic wastes that are harmful, not only to the environment but also to human health. The "biogenic" approach is further supported by the fact that the majority of the bacteria inhabit ambient conditions of varying temperature, pH, and pressure. The particles generated by these processes have higher catalytic reactivity (Uzair *et al.*, 2020).

Bacteria have exceptional abilities to reduce metal ions to their zero forms (Case of nanoparticles) are perhaps the most suitable candidates for nanoparticles the synthesis is attributed to the ease of handling and requirements of the medium culture (Ruttkay-Nedecky

et al., 2017). The biosynthesis of metal NPs by microbes it is one of the functions of heavy metal toxicity resistance mechanisms, where toxic substances are heavy minerals are converted into non-toxic types and deposited in the form of mineral groups the nanoscale dimension and the specific shape (Narayanan and Sakthivel, 2010).

Magnesium oxide nanoparticles exhibit antimicrobial activity against pathogenic bacteria, making them a suitable therapeutic alternative to using antibiotics (Krishnamoorthy *et al.*, 2012).

Aim of study:

The current study aimed to investigate the effect of MgO NPs produced by bacteria on MDR bacteria as an antibacterial.

2- Literature Review:

2.1 Nanotechnology:

Nanotechnology is a field of science that studies the properties design, manipulation, devices at the nan scale level (10-9 m). production, and applications of structures and European and other International Committees have defined NPs, as particles of matter in which at least one of their phases has one dimension (length, width, or thickness) within the range of 1 to 100 nanometers (nm) (Khan *et al.*,2020).

Nano materials are structural components that range between 1-1000 Nanometers and they are composed of subgroups with a Size range of 1-100 nanometer particles, these particles are called nanoparticles. Nanoparticles are found In Various shapes and structures such as conical, spiral, flat, Hollow, etc. and they exhibit physical properties which are more Extraordinary than their bulk form and offer them unique Mechanical strength, increased stability, and several more. They are being widely used Nanotechnology is a modern field of sciences to research phenomena at Atomic, molecular, and macromolecular scales.

Nano biotechnology includes biological principles with physical and chemical procedures to supply Nano-sized particles with specific functions and represents an economic alternative to chemical and physical methods of nanoparticle formation. Nanoparticles exhibit new and improved properties based on the specific characteristics such as size, and morphology (Singh et al,. 2020).

2.1.1 Characteristics of MgO (NPs):

Magnesium oxide nanoparticles possess properties that are beneficial in multiple ways. MgO (NPs)are grabbing more Attention than various other metal oxide nanoparticles that Are widely being used throughout various fields because they are promising structural materials in biological Implants due to their high strength-to-weight ratio, low Density, good functionality, recycling activity, nontoxic, and hygroscopic nature; these characteristics of MgO (NPs) increases its utility manifolds and they also have various other properties such as high melting point, cost-effective production, Biodegradability, and biocompatibility.

These MgO (NPs)have a wide range of applications in Industries and biomedical for bone regeneration; as a Medicine for anti-bactericidal and antimicrobial inhibition, they are also used in cryoinjury and apart from this they Are also employed in the sorption of uranium ions, catalysis, Lithium-ion batteries, and toxic waste curtain (Yang,W ; Peters,J.I. 2008),(Tang,Z.X 2014)(Haldorai,Y ; shim,J.J 2014)]. These nanoparticles are highly corrosive in nature, thus Their use in the field of automotive and aerospace was Inhibited, but techniques such as plating, conversion Coating, etc. have brought the metal back into the race [(Gnedenkov, 2016)]. Metal oxide nanoparticles play a vital role in Environmental remediation by treating wastewater, Industrial and domestic waste, soil sediments as well as atmospheric pollution [(Khin, M.M;Nair,A.S 2012)]. MgO (NPs) possess numerous properties such as they act as Anti-biofilm agents; they also exhibit self-cleaning activity Which helps them degrade methyl violet dye as well as removal of phosphorus from wastewater which is a reason For inh(Chimenos,J 2003) (Mahdavi,S 2013)]. Magnesium hydroxide and magnesium oxide nanoparticles possess excellent Luminescence for photonic applications, because of its Unique thermal properties biodegradability activity, and Non-toxic nature. The purified form magnesium oxide nano powder is used to improve mechanical and Fabrication characteristics of aluminum as an alloy; they Are also used in because of their enormous properties manufacturing of missiles and dietary Supplements such as Light weight and edibility[(Ramanujam,K 2014) (Anilkumar,M.R 2015)].

2.2 Nano biosynthesis by bacteria:

The chemical and physical methods used for the synthesis of nanoparticles make a large number of hazardous byproducts and hence are the major concern for environmental contamination. Most of the techniques Are expensive, as well as inefficient in materials and energy use. Hence, there is an ever-growing need to develop clean, nontoxic, and environmentally benign synthetic procedures. Consequently, researchers have used biological synthesis, Nanotechnology is emerging as a rapidly growing field with its application in science and technology for the purpose of manufacturing new materials at the nanoscale level. Bionanotechnology has emerged as an integration between biotechnology and nanotechnology for developing biosynthetic and eco-friendly technology for the synthesis of nanomaterials and nanoparticles. The biosynthesis of metal NPs by microbes is

one of the functions of resistance to heavy metal toxicity, where toxic substances are present Heavy metals are converted into nontoxic species the negatively charged cell wall interacts with the positive charge Metal Ions. Enzymes within the cell wall reduce metal ions to Nanoparticles, or through the use of flow proteins that transport metal ions By proton motive force, chemical contrast gradients, or ATP hydrolysis (Nies, 2003).

Among the microorganisms, bacteria have received the most attention in bio-synthesis of nanoparticles due to its growing success, ease of handling and genetic modification, bacteria are exposed to a concentration of increasingly hard heavy metal ions in their surroundings to combat these stressful conditions, bacteria have developed many means of defense processes, for example, intracellular isolation, flow pumps, and fluctuation in concentration metal ions and extracellular precipitation. These defense mechanisms constitute the basis for the synthesis of shape and size nanoparticles by bacteria (Joginder a`*et al.*, 2020).

In the case of the intracellular structure, the nanoparticles are produced inside Bacterial cells via reductive pathways of their cell wall and apparatus accumulation in the space around the cell. The nanoparticles It is produced extracellularly when soluble enzymes are reduced or the cell wall is reduced The secreted enzymes are extracted from the outside of the cell and are involved in the Metal ion reduction process (Tiquia-Arashiro and Rodrigues, 2016). There are several ways to detect the synthesis of nanoparticles by bacteria species either morphologic by changing the color from yellow to brown becoming colloidal dark brown, as it happened when using Pseudomonas sp. Is a gram-negative bacteria belonging to the Family Pseudomonadaceae. That is a rod-shaped Singly, in pairs, or in short Chains, when AgNP formation, or by using the study of the Characterization of AgNPs such as UV–visible spectrophotometer (UV–vis), Field emission-transmission electron microscopy(FE-TEM), X-ray diffraction (XRD), Fourier transform-infrared (FT-IR) spectroscopy and Particle size distribution (DLS) (Hina Singh 2018).Where, silver nanoparticles (AgNPs) have potential applications in the fields of medical nano-engineering and pharmaceutical for the development of therapeutic agents, chronic disease diagnostics and biosensors. AgNPs are known to have antimicrobial efficacy against bacteria, viruses and eukaryotic micro-organisms Because of its antibacterial properties

2.3 Applications of MgO Nanoparticles:

Applications of nanoparticles on cellular activity and medicinal, examined the cytotoxicity of several metals oxide nanoparticles including MgO NPs on human cardiac microvascular endothelial cells, that MgO NPs possess less cytotoxic effects against human astrocytoma U87 cells when compared to ZnO and TiO2 NPs(Sun et al., 2011

MgO nanoparticles have demonstrated antibacterial properties against a variety of harmful Gram-positive and Gram-negative pathogens. As a result, it may be an effective antibiotic facial treatment. Deep pits formation was detected on the surface of the bacterial cell membrane after treatment with MgO nanoparticles, indicating membrane damage. MgO NPs are used in the rest of consistent bone regeneration (Di et al. 2012). The cytotoxicity of

MgO NPs against human umbilical vein endothelial cells were examined and Have been shown to interact strongly with a negatively charged bacterial surface due to their positive charge, many research groups have identified the alkalizing effect of MgO-NPs as a critical role in the antibacterial effect of MgO nanoparticles.

2.4.1 Antimicrobial Activity of MgO NP:

Magnesium oxide nanoparticles exhibit strong antimicrobial activity Against pathogenic bacteria, making it a suitable therapeutic alternative For the use of antibiotics (Krishnamurthy *et al*., 2012) .It has an average size (20 nm) and has great potential as antimicrobials Found that bacterial cells after treatment with MgO nanoparticles Revealed the appearance of deep pits on the surface of the membrane Which indicates damage to the membrane structure. Becomes cell size Shorter and more compact, indicating leakage of cellular contents in Response to treatment (He et al., 2016; Nguyen *et al*., 2018).

MgO nanoparticles showed excellent antibacterial effects against MgO Various pathogenic microorganisms of both gram-positive and gram-negative groups. Thus, it can be an effective antibiotic treatment in Increased prevalence of infections caused by drug-resistant bacteria (Khan *Et al*., 2020). The nanoparticles are attributed to the production of reactive oxygen species (ROS) that induce lipid peroxidation in bacteria (Tang and Lv, 2014).

A number of studies indicate that the bactericidal effect of MgO is Generally attributed to the generation of reactive oxygen species (ROS), The properties of MgO NPs may be as small as 20 nm and cannot be direct Compared to 150 nm (Nguyen *et al*., 2018) Anaerobically if nitrates are available. *P. aeruginosa* strains carry the biosynthetic genes to produce An extracellular Polysaccharide known as alginate. Alginate Is often called "mucoid exopolysaccharide" or "glycocalyx," and overproduction is responsible for the mucoid colony phenotype.

3-Materials and methods:.

3.1: Materials:

3.1.1: Instrument and Equipment:

The equipment used in the current study were listed in the table (3-1) below .

Table (3.1) : Instruments and Equipment in this study ,Table (3.1) : Materials

N0	No Instruments	Company	Origin
1	Autoclave	Tripod	UK
2	Bench centrifuge	Memmert	Germany
3	Burner	Amal	Turky
4	Cane tube	Bausch and lomb	USA
5	Digital camera	Sony	Jaban
6	DNA extraction tube 100 µl	Eppendorf	Germany
7	Enzyme – linked immunosorbent assay system	Biotech	USA
8	Eppendorf tube	Eppendrof	Germany
9	Hood	Bio lab	Korea
10	Horizontal gel	Bio-Red	Italy
11	Incubator	Selecta	Spain
12	Latex Gloves	Broche	Malaysia
13	Light microscope	Olympus	Japan
14	Micropipettes size (5-50 µl 100-1000 µl ,0.5-10 µl)	Eppendrof	Germany
15	Oven	Olympus	Japan
16	Slide	Sail Brand	Chin
17	Transfer Swab	AL hanof factory	Jorden
18	Water bath	Gallen kamp	Germany
19	X-ray powder diffraction (XRD) (6000)	Broker	Germany

3.2:Methods:

3.2.1 :- **Preparation of culture media:**

The culture media were prepared according to the manufacture company And sterilized by Autoclave (Mcfaddin, 2000).

3.2.2 : Müller-Hinton agar:

Müller-Hinton agar medium was ready to be given to the manufacturer and used for antimicrobial susceptibility testing (Forbes et al., 2007).

3.2.3 : Nutrient agar medium :

It was used for cultivation of the bacterial isolates when it was necessary .

3.2.4 : Nutrient broth medium:

This medium was used to grow and preserve the bacterial isolates.

3.3 : Bacterial synthesis of MgO NPs:

The MgO NPs were manufactured from 86 isolates and then Uvspectrophotometer Examined at the wavelengths (405 , 450 ,490 , 630) nm, and the isolates pseudomonas Aerugenosa that gave The highest reading were selected, according to the work method Below (Mohanasrinivasan et al., 2018):

1- The bacteria were grown in N.B and incubated at 37 °C for 24 hr.2- The bacterial Cultures were then further diluted with sterilized uninoculatedN.B in the ratio 1:3. Post dilution.

2- 0.1 M magnesium nitrate [Mg(NO3)2 ·6H2O] was added to each Diluted culture, followed by drop wise addition of 0.2 M NaOH, to delay the Process of Transformation.

3- bThe cultures were then kept in a water bath at 40 °C for 15–20 min for the White colored Precipitate to settle down

4- The cultures were finally incubated undisturbed at room temperature for 10Hr.

5- Post 10 hr. of incubation, the cultures were centrifuged at 5000 RPM for 15Min.

6- The supernatant was carefully discarded and the pellet was washed twice With distilled Water.

7- The nanoparticles were then carefully dried and obtained in the powder form.

8- The obtained nanoparticles were in the hydroxide form which were converted To oxide Form by calcinating the nanoparticles at 300 °C for 4hr.

4-Result and Discussion:

The Results and Discussion

The Biosynthesis test of MgO NPs was conducted under different testing protocols on all isolates to find out the highest productive and purest of isolates. They were evaluated on (6 E.coli , 2 isolate Pseudomonas aerogenosa and 3 isolates Klebsiella Pneumoniae bacterial isolates and extracted with a UV spectrometer and a visual analysis of the powder color. Figure (1,2) Appeared the change in colour after adding the salts.

Figure (1) Survey the bacteria that Mg O synthesis

Figure (2) change in colour after adding the salt

All isolates were give positive for MgONPs production. The results agreed with (Ali *et al.*,2020) the appeared of a white precipitate below the tube it means positive result.

In the present study, it had tried to explore a rapid, cost-effective, eco-friendly method for fabricating MgO-NPs using the bacterial strain**.** The biogenically synthesized MgO-NPs were characterized using various techniques consisting of UVvis spectroscopy, X-Ray diffraction (XRD), scanning and energy dispersive X-Ray spectroscopy (SEM-EDX). Eid *et al*.,(2020) exhibited that microorganisms such as bacteria can reduce metal and their oxides to NPs. The synthesis of NPs using bacteria has numerous advantages e.g., easy to multiply, grow, handle, and downstream process for nano biosynthesis (Samak *et al*.,2020). The greatest absorption at 400 wavelengths was determined for all isolates after examination in

all spectrophotometers of the UV. One of the isolates was chosen, the greatest and purest productivity of other tested isolates. This might be because these bacteria have genes that are more responsible for the production of nanoparticles than other isolates. After the addition of the alkaline solution, a solution was created for Mg (OH)2, allowing two hours to consider turning its color into brown as seen in this**.**

Table (1) The survey on the greatest isolate that give nanoparticles

Wave length	**405**	**450**	**490**	**630**
1: E.coli	1.531	1.407	1.340	1.131
2:	1.133	0.989	0.915	0.717
3:	1.551	1.412	1.345	1.140
4:	1.224	1.108	1.037	0.810
5:	1.460	1.337	1.256	1.024
6:	1.806	1.659	1.579	1.354
1: *P.aerugenosa*	1.061	0.947	0.874	0.656
2:	1.156	1.074	1.025	0.862
3:	1.396	1.239	1.152	0.887
1: *K.pnumoniae*	1.610	1.472	1.392	1.152
2:	1.292	1.183	1.112	0.893

Figure (3) displays an FE-SEM image showing the surface morphology in MgO nanoparticles The image analysis indicated that *Pseudomonas aerogenosa* MgO-NPs were approximately spherical, with an average diameter of 32-40 nm (Al-Salhie & Al-Kalifawi 2020) were demonstrated that the field emission scanning electron microscopy image of MgO NPs,which exhibit flakes-like structure due to the aggregation of several thousand MgO NPs. The MgO nanoflakes are dense and interconnected with each other such. that no clear boundaries exist between one another. The sizes of MgONPs were between 29.05 nm and 67.83nm.

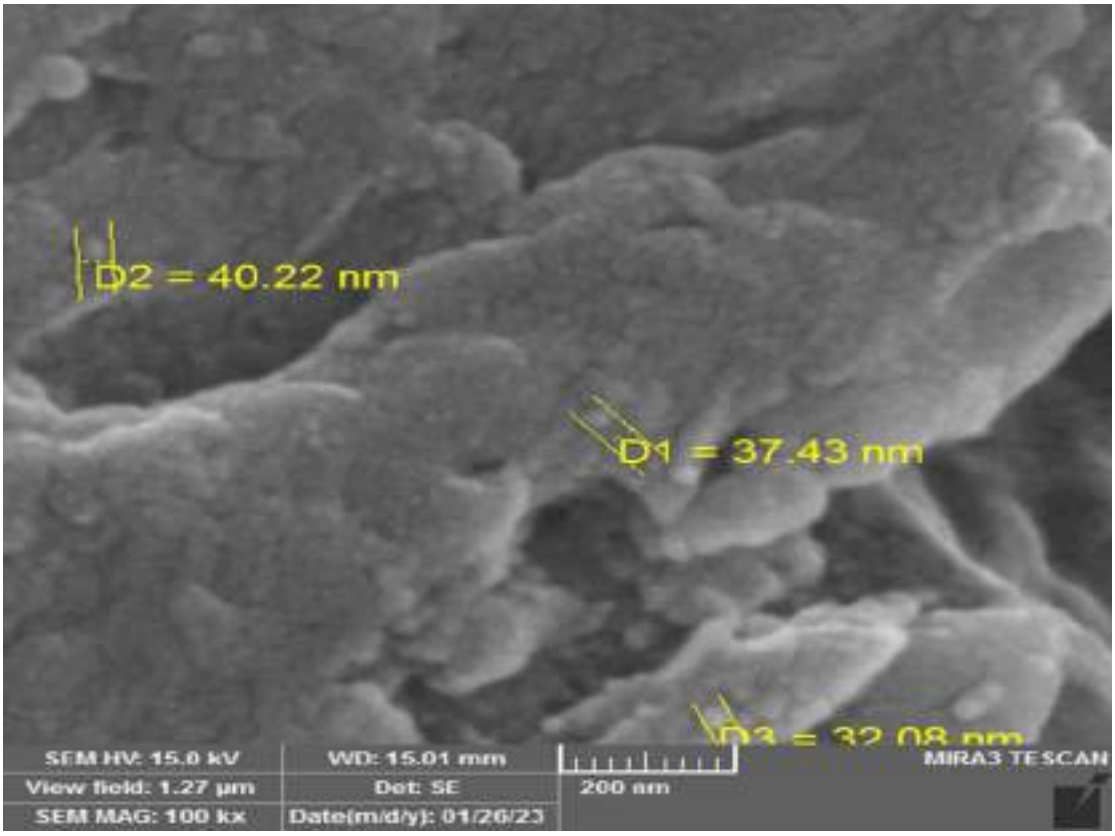

Figure (3)The surface morphology of MgO NPs synthesized by Pseudomonas aerogenosa -SEM

Wetteland *et al.*, (2016) reported The biosynthesis and presence of MgO peaks isolated from different bacteria in the XRD spectrum are expected because MgO NPs are hygroscopic and they can readily react with water in the atmosphere to form MgONPs. The size distribution of MgO NPs was normal and narrow, with an average of 23 ± 5 nm, in agreement with the previous study. The zeta potential and electrical mobility of MgO in water were 32.31 ± 4.1 mV and 1.68 ± 0.22 (×10−4 cm2 V−1 s−1), similar to what was reported in previous literature (Brown & Salt.,1956; Wetteland *et al.*, 2016).

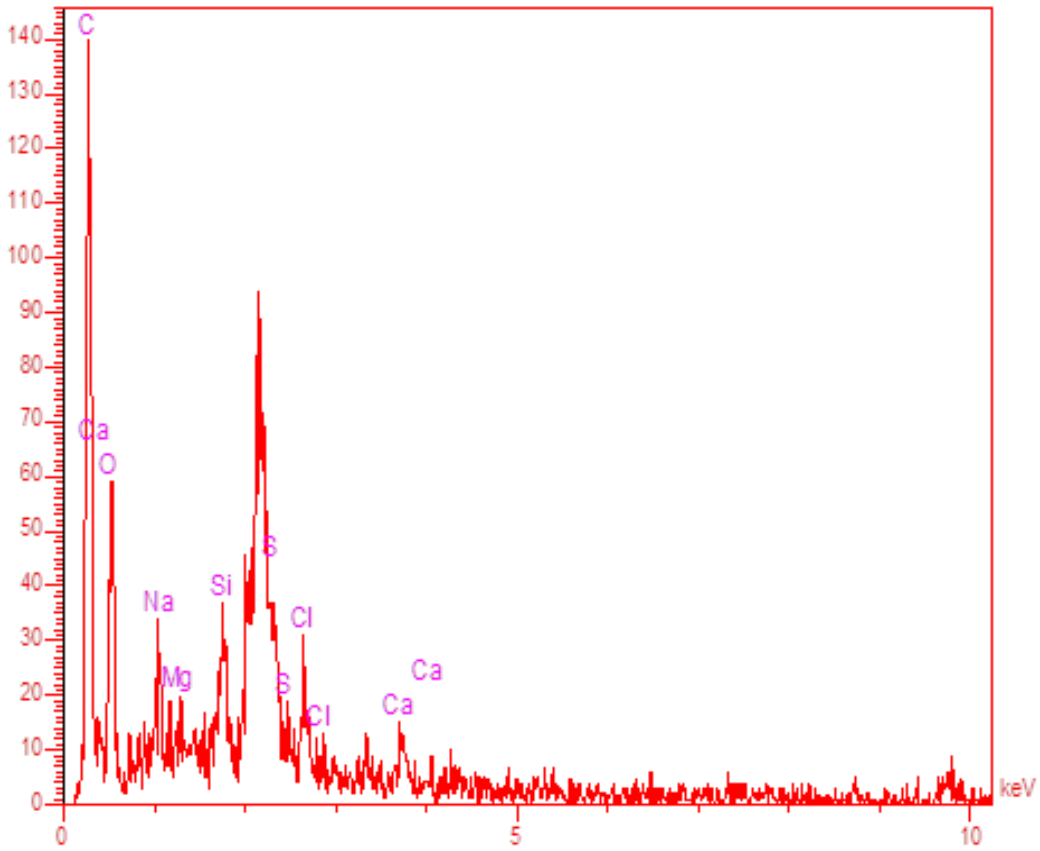

figure (4)displays the EDX spectrum, which exhibits a prominent peak corresponding to Mg and O. Additional C, N, Na, Ca, and P peaks were discovered.A peak was obtained at 1.2 keV for magnesium.

The energy of the X-rays will be measured and evaluated so that the chemical elements in the sample are accurately and quantitatively informational (figure 4).

Several antibacterial activities as in table (2)features of metal oxides, including particle size, mixture concentration, and powder of surface properties, were examined and active oxygen and metal oxide particles were developed (Saratale *et al.*, 2018). Smaller NPs interacted more widely with the bacterial cells and more often than larger NPs can reach the cytoplasm that has an antibacterial influence (Thi and Yen., 2019). This result agrees with(Tang and Bin-Feng 2014) were said that small-sized MgO nanoparticles had better antibacterial activities towards both gram-positive and gram-negative bacteria.

Table (2)the diameter of antibacterial inhibition extracted by Pseudomonas aerogenosa

Bacteria	No	Mean	Std. Deviation	Std. Error Mean	Sig
E.coli	3	7.6667	2.08167	1.20185	0.024
Pseudomonas	3	11.0000	1.00000	.57735	0.003
Klebsiella	3	9.0000	1.00000	.57735	0.004
Streptococcus	3	9.0000	1.00000	.57735	0.004

Conclusions:

-The ability of *Pseudomonas aerogenosa* to synthesis MgO nanoparticles

-The characterization of nanoparticles was confirmed by Uv visible ,XRD

-The image of SEM showing the surface morphology in MgO nanoparticles The image analysis indicated that *Pseudomonas aerogenosa* MgO-NPs were approximately spherical, with an average diameter of 32-40 nm

- the MgO has antibacterial activity

Recommendations:

-Study the effect of MgO on other bacteria

-Study the effect of MgO on fungi

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