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Magnesium Oxide Nanoparticles Produced via Biosynthesis Using *Pseudomonas aeruginosa, Klebsiella pneumoniae,* and Pathogenic *Escherichia coli*

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إنتاج جسيمات نانوية من أكسيد المغنسيوم عن طريق التخليق الحيوي باستخدام الزائفة الزنجارية والكلبسيلا الرئوية والإشريكية القولونية المسببة للأمراض

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ABSTRACT

Background: The world problem in medicine is multidrug resistance, so all researchers tend to investigate alternative antibacterial materials, for example, nanoparticles. This research aims to synthesize the MgO nanoparticles by using bacteria.

Materials and Methods: survey the ability of some bacteria collected from the Advance Microbial Lab ten isolates (*E. coli*, *Pseudomonas aerogenosa*, and *Klebsiella pneumoniae*). One isolate from each genus of bacteria was chosen for this research, and 10 ml of magnesium nitrate ($Mg(NO_3)_2.6H_2O$) was added to the culture broth of *E.coli* at 37°C in a dark shaker water bath. After 24 hours, two molars of sodium-hydroxide solution were added to the chemical reaction mixture to achieve pH 10. The solution color was changed to brown two hours after the addition of the alkaline solution, then MgO NPs were characterization by, ultraviolet -Visible spectrophotometer wave line at (405-630 nm), energy-dispersive x-ray-analysis (EDX) and scanning-electron-microscopy.

Results: The result of scanning electron microscopy found the diameters of nano were (31-35,104-109,37.43-40,22) nanometer for *E. coli*, *K. pneumoniae*, and *P. aeruginosa*. The MgO nanoparticle minimum-inhibition concentration detected was 125µg/ml, which affected both G^+ and G^- bacteria.

Conclusion: *E. coli*, *Klebsiella*, and *Pseudomonas* successfully biosynthesized MgO nanoparticles. These nanoparticles exhibited effective antibacterial activity. This suggests that these bacterial strains are capable of producing nanoparticles with potential antimicrobial properties.

Keywords: Magnesium Oxide Nanoparticles; *E. Coli; P. aeruginosa and K. Pneumonia.*

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INTRODUCTION

There is an immediate need to discover new anti-microbial medications due to the growing danger of infection and drug-resistant bacteria [1]. The green synthesis of nanoparticles is considered environmentally friendly, non-toxic and highly stable. Magnesium oxide nanoparticles were effectively produced by this method. Magnesium oxide nanoparticles are considered economically important molecules due to their physical and chemical properties and their refractive index [2]. Bacteria have exceptional abilities to reduce metal ions to their zero forms (nanoparticles) because they are the most suitable candidates for nanoparticle synthesis and the requirements of the medium culture [3].

The MgO NPs have shown great antibacterial effects against both G+ and G- bacteria, so, it can represent revolutionary antibiotic drugs in the decreasing occurrence of infection obtained by drug resistant bacterial [4]. Nanoparticles of MgO effect on microbial cells after treatment with it revealed the emergence of big pits on the surface of the membrane causing defects to the membrane structure and the cell volume decreased and compact, due to lose of cellular contents in response to treatments [5]. Other potential mechanisms related to the antimicrobial effect of nanoparticles of Mg-oxide are due to the electro-static reaction between the microbial surface and MgONPs. It has been shown that MgONPs due to their positive charge interact vigorously with a negative charge surface of the bacteria [4]. This study aimed to detect the ability of bacteria to synthesis MgO nanoparticles from local isolates and its antibacterial activity.

MATERIALS AND METHODS

The isolates were taken from the microbiology laboratory at the College of Science, University of Babylon. Three isolates were used in the biosynthesis of MgO NPs, *E.coli*, *Pseudomonas aeruginosa* and *Klebsiella pneumoniae*.

The method for biosynthesis depends on Mohanasrinivasan , Jebur. and Abd [6, 7]. The bacteria were cultured on N.B and put in an incubator at Thirty-seven degrees Celsius for twenty-four hours. The microbial cultures were then diluted with sterilized newly prepared N.B in the ratio of one to three, then one of tenth molar of magnesium-nitrate [Mg(NO3) 2 ·6H2O] was added to every culture, followed by the addition of 0.2 M NaOH, The implanted tubes were put in a water bath at 40°cellulose for fifteen to twenty minutes until the white colored precipitate settle down. At the final step implanted tubes were saved at room temperature for ten hours, after that the cultures were centrifuged at five-thousands rpm for fifteen minutes. The supernatant was carefully throw away and the precipitate was washed twice with distilled water. The NPs were then dried carefully and obtained in the powder form.

The NPs were in the hydroxide form which was turned to oxide form by calcination of the nanoparticles at 300 degrees Celsius for four hours. The synthesized MgONPs were characterized by Ultra Violet –visible-spectroscopy, Scanning Electron Microscope, and energy dispersive x-ray analysis (EDX). In this topic, the agar-well diffusion method was used to detect bacterial response to respond positively or negatively to the magnesium oxide nanoparticles by inhibiting or resisting the aforementioned molecules, so a series of dilutions of the nanoparticles were as “500, 250, 125, 62.5, 31.25, 15.6 µg/ml” in triplicates, the MIC measured by spectrophotometer at 600 nm.

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McFarland tube 0.5 It has been prepared as a reference to estimate the growth of bacteria, microbial isolates were put in the incubator at 37°C overnight in a ten ml NB, then cultured each sample was with 1ml of the microbial suspension (10^8 colony/ml), MgONPs were prepared as six dilutions.

Statical analysis

In this research, the SPSS software was used to calculate the deviation from the mean

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 (*E.coli*, *Pseudomonas aeruginosa* and *Klebsiella Pneumoniae* bacterial isolates and extracted with a UV spectrometer and a visual analysis of the powder color. Figure (1) appeared the change in color after adding the salts.

Figure (1) Survey the bacteria that Mg O synthesis

This study aimed to find a rapid, cheap, ecologically-friendly method for producing MgO-NPs using the bacterial strain**.** The bio-synthesis of MgONPs were depicted using various methods consisting of UV-vis spectroscopy, scanning and energy dispersive X-ray-spectroscopy (SEM-EDX). [9] exhibited that microorganisms such as bacteria can change metals and their oxides to NPs. The production of NPs using bacteria has huge benefits e.g., easy to multiply, grow, handle, and downstream process for nano-biosynthesis [10]. 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 that these bacteria have genes more responsible for the production of nanoparticles than other isolates. After the addition of the alkaloid solution, a solution was created for Mg (OH)2, allowing two hours to consider turning its color into brown as seen in this**.**

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The image analysis indicated that *E.coli* MgO-NPs were approximately spherical, with an average diameter of 31-36 nm this agreed with Al-Salhie & Al-Kalifawi [11] were demonstrated that the field emission SEM image of MgO NPs, appear exhibit flakelike structure throw the aggregation of several thousand MgO NPs. The MgO nano-flakes are dense and inter-connected with each other such that no clear boundaries exist between one another. The size of nanoparticles that synthesis by *P. aeruginosa* was 32-40 nm this agreed with Wetteland[12] found the sizes of MgONPs were between 29.05 nm and 67.83nm and reported the biosynthesis and presence of MgO peaks isolated from different bacteria in the XRD-spectrum are predicted because MgONPs are hygroscopic and they can readily react with water in the atmosphere to create MgONPs. The present study also agreed with Hussan and his group [8] found the size of MgO nanoparticles synthesis by *Leuconostoc spp* was 37-70 nm. Another study by Jebur and Abd [7] found the size of MgO nanoparticles was (49,66) by using two *Streptococcus* isolates.

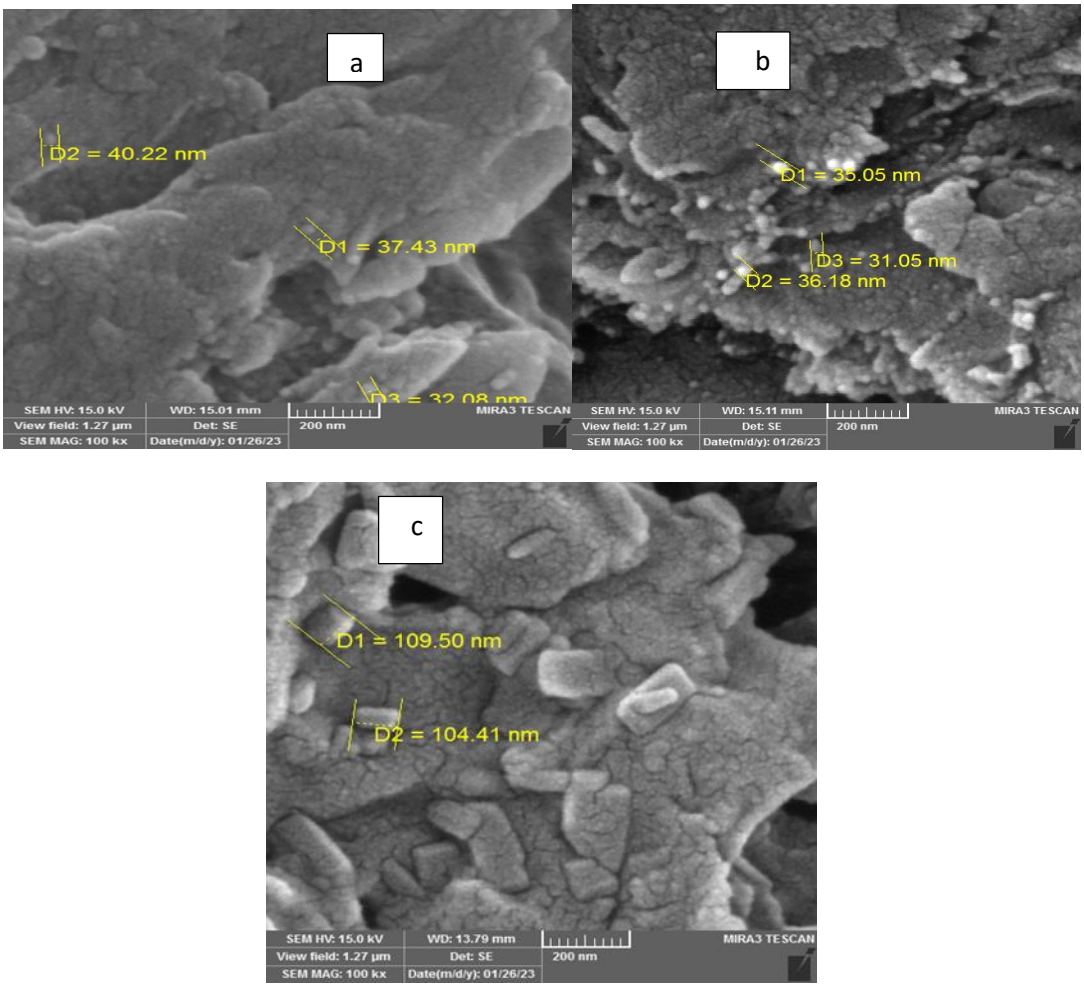

Figure (2) Scanning electron microscopy morphology of MgO NPs synthesized by a- *E.coli* b- *Pseudomonas aerogenosa* c- *Klebsiella pneumoniae*

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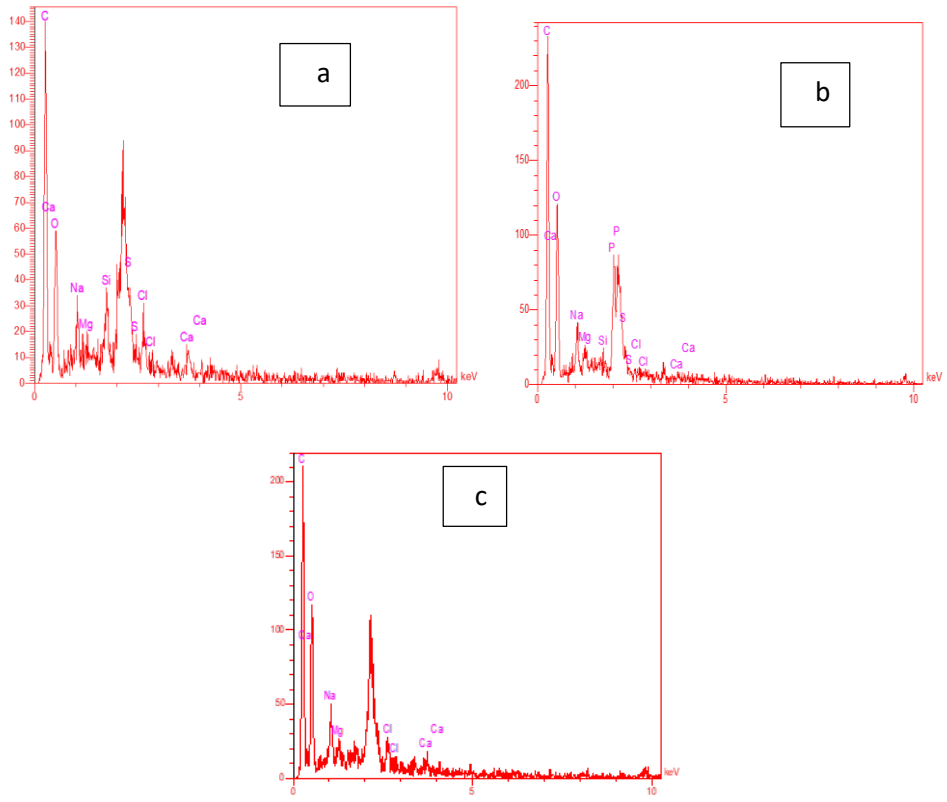

Figure (3) displays the EDX spectrum, which exhibits a prominent peak corresponding to Mg and O. Additional C, N, Na, Ca, and P peaks were discovered. A peak was obtained at 1.2 keV for magnesium *a -E.coli- b-Pseudomonas aeruginosa* c- *Klebsiella pneumoniae*

Antibacterial activity was detected by using a minimum inhibition concentration 125µg/ml as , the results appeared different inhibition dimeters by using MgO nanoparticles that synthesis by (*E.coli- Pseudomonas aeruginosa , Klebsiella pneumoniae*) against four bacterial genus in table (1). the results appeared different inhibition dimeters by using MgO nanoparticles that synthesis by (*E.coli- Pseudomonas aeruginosa , Klebsiella pneumoniae*) against four bacterial genus 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 smaller NPs are interacted more widely with the bacterial cells and more often than larger NPs can reach the cytoplasm that has an antibacterial influence [13,14] said that the small size of magnesium oxide nanoparticles has a greater effect on Gram-positive *(S. aureus)* and Gram-negative bacteria *(E. coli)*

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Table (1) Antibacterial activity of MgO nanoparticles synthesis by *E.coli* ,*P. aeruginosa and K. pneumoniae* (Mean and Standard Deviation of Minimum Inhibition concentration mm)

MgO / Bacterial	*E .coli* M±SD mm	*P.aeruginosa* M±SD mm	*K. pneumoniae* M±SD mm
E .coli	7.6667±2.08167	8.0000±2	12.0000±2.6
P.aeruginosa	11.0000±1	10.6667±0.5	9.6667±0.57
K. pneumoniae	9.0000±1	9.6667±0.5	6.6667±2
Streptococcus pyogenes	9.0000±1	10.3333±1	8.6667±0.55

CONCLUSION

The ability of *E.coli*, *Klebsiella* and *Pseudomonas* to biosynthesis of MgO nanoparticles and the extraction nanoparticles have antibacterial activity.

Acknowledgments:

I would like to express our gratitude to the microbiology laboratory at the College of Science, University of Babylon, for providing the bacterial isolates used in this research. Special thanks to the staff for their support and assistance throughout the study.

Conflict of interests:

The study was conducted independently, and no financial or personal interests have influenced the results or interpretations presented.

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الخلاصة

المقدمة:

إن مشكلة العالم في الطب هي مقاومة الأدوية المتعددة، لذا يميل جميع الباحثين إلى التحقيق في مواد مضادة للبكتيريا بديلة مثل الجسيمات النانوية. يهدف هذا البحث إلى تصنيع جسيمات نانوية من أكسيد المغنيسيوم باستخدام البكتيريا.

المواد والطرق:

تم دراسة قدرة بكتيريا *E. coli* ,*P. aeruginosa* و *Klebsiella pneumoniae* التي تم جمعها من عشرة عزلات من مختبر الاحياء المجهرية المتقدم. تم اختيار عزلة واحدة من كل جنس من البكتيريا لهذا البحث، وتم إضافة 10 مل من نترات المغنيسيوم ($Mg(NO_3)_2.6H_2O$) الى مرق ثقافة البكتيريا عند 37 درجة مئوية في حمام مائي داكن. بعد 24 ساعة، تمت إضافة مولين من محلول هيدروكسيد الصوديوم إلى خليط التفاعل الكيميائي لتحقيق درجة حموضة 10. تم تغيير لون المحلول إلى البني بعد ساعتين من إضافة المحلول القلوي، ثم تم توصيف جزيئات أكسيد المغنيسيوم النانوية باستخدام خط الموجات فوق البنفسجية – المرئية عند (405–630)نانومتر، وتحليل الأشعة السينية المشتتة للطاقة (EDX)، والمجهر الإلكتروني الماسح.

النتائج:

أظهرت نتائج المجهر الإلكتروني الماسح أن أقطار النانو كانت (31–104،35–37.43،109–22،40) نانومتر لـ *E. coli* و *K. pneumoniae* و*P. aeruginosa* . تم تحديد التركيز الأدنى المثبط لجسيمات نانو أكسيد المغنيسيوم والذي هو 125 ميكروغرام/مل، والذي أثر على كل من البكتيريا G + و G –.

الاستنتاج:

قدرة *E. coli* و *Klebsiella* و *Pseudomonas* على التخليق الحيوي لجسيمات نانوية من أكسيد المغنيسيوم. وقد أظهرت هذه الجسيمات النانوية نشاطًا مضادًا للبكتيريا.

الكلمات المفتاحية: جسيمات أكسيد المغنيسيوم النانوية، الإشريكية القولونية، الزائفة الزنجارية، والبكتيريا المسببة للالتهاب الرئوي.

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