Determination the Inhibition activity of Magnesium Oxide Nanoparticles Synthesized by *Aspergillus niger* against some uncommon bacterial species which isolated from different sources of infection

Mohameed N. Maaroof
College of Education For pure science, University of Tikrit, Tikrit, Iraq

Abstract

The resistance of bacteria to chemically manufactured antibiotics is dangerous in human health، this has made the need for continuous detection of diverse resources ,especially from natural sources in the treatment of human diseases. The current study employs *Aspergillus niger* for the formation of of Magnesium Oxide Nanoparticles, and it involves the antimicrobial activity of the particles against some of uncommon bacterial species which include Gram positive *Aeromonas salmonicida, Kocuria rosea and Alloiococcus otitis* and Gram negative *Acinatobacter calcoaceticus, Pantoea agglomerans* and *Ochrobactrum anthropic* by agar wells diffusion method. The results were indicated that biosynthesized of MgO NPs were found as extracellular and it was appear in the range of size 40-90 nm through characterization technique such as Scanning Electron Microscopy (SEM), and assurance by UV-Visible spectroscopy for the absorbance band at 258 nm, The results revealed that MgO NPs is an effective antibacterial agent against Gram positive *Aeromonas salmonicida , Kocuria rosea and Alloiococcus otitis* and the zone inhibition diameter was at 20,19 and23 mm respectively compared with inhibition effects of ciprofloxacin antibiotics at 18, 17and 20 mm respectively. while against Gram negative *Acinatobacter calcoaceticus, Pantoea agglomerans* and *Ochrobactrum anthropic* bacteria was at 18,20,19 mm respectively, compared with inhibition effects of ciprofloxacin antibiotics at 16, 20 and 15 mm respectively.

Keywords: Nanoparticles, Biosynthesis, Aspergillus niger, Antibacterial activity, Uncommon bacteria.

تحديد الفعالية التثبيطية لجزيئات اوكسيد المغنسيوم النانوية المصنعة *Aspergillus niger* بواسطة الفطرضد بعض انواع البكتريا غير الشائعة والمعزولة من مصادر اخماج مختلفة

محمد نظير معروف

الملخص

إن مقاومة البكتيريا للمضادات الحيوية المصنعة كيميائيا تشكل خطرا على صحة الإنسان، مما جعل الحاجة إلى الكشف المستمر عن الموارد المتنوعة، وخاصة من المصادر الطبيعية في علاج امراض الانسان. اعتمدت الدراسة الحالية على تصنيع جزيئات اوكسيد المغنسيوم النانوية بواسطة الفطر *Aspergillus niger* وتحديد فعاليتها التثبيطية ضد بعض الأنواع البكتيرية غير المألوفة والمعزولة من مصادر اخماج مختلفة والتي شملت انواع موجبة لصبغة كرام وهي *Aeromonas salmonicida , Kocuria rosea , Alloiococcus otitis* اما الانواع السالبة لصبغة كرام فهي *Acinatobacter calcoaceticus, Pantoea agglomerans* و*Ochrobactrum*

Aspergillus anthropic باستخدام طريقة الانتشار في الحفر، وتميزت النتائج بقدرة الفطر *niger* على تصنيع جزيئات المغنسيوم النانوية وباحجام تتراوح بين 40-90 نانومتر من خلال الكشف عن خصائصها النانوية بواسطة تقنية المجهر الإلكتروني الماسح، والامتصاصية عند طول موجي 258 نانومتر باستخدام جهاز UV-Visible -Spectroscopy، واظهرت نتائج الفعالية التثبيطية لجزيئات اوكسيد المغنسيوم النانونية تأثيرها الفعال ضد انواع البكتريا الموجبة لصبغة كرام *Alloiococcus otitis* , *Kocuria rosea* , *Aeromonas salmonicida* باقطار تثبيط 19,20 و23 ملم على التوالي مقارنة مع التاثير التثبيطي لمضاد Ciprofloxacin والذي يعتبر 17,18 و20 نانومتر على التوالي ،بينما ضد الانواع السالبة لصبغة كرام *Acinatobacter calcoaceticus, Pantoea agglomerans* و *Ochrobactrum anthropic* فكانت اقطار التثبيط 20,18و19 نانومتر على التوالي مقارنة مع اقطار تثبيط مضاد Ciprofloxacin 20,16و15 على التوالي .

الكلمات المفتاحية :الجزيئات النانوية ،البناء الحيوي ،*Aspergillus niger* ،الفعالية التثبيطية للبكتريا و البكتريا الغير شائعة .

Introduction

The metallic nanoparticles are the most promising material as antibacterial activity, and it gain the current interest in research due to the growing microbial resistance against antibiotics and the developing of the resistant strain (1). Nanotechnology is a field that is developing day by day, making an impact in all spheres of human life and creating a growing sense of excitement in the life sciences especially biomedical devices and biotechnology (2). At the nanoscale level, materials have distinct chemical, physical, optical, magnetic and electrical properties due to their large surface area to volume ratio (3). one of the most important aspects of nanotechnology is the synthesis of nanoparticles (NPs), which form the essence of the nanomaterials (4). Nanoparticles exhibit new pr0perties based 0n specific characteristics such as size, distribution, and morphology (5). Nowadays, nanoparticles are used in many fields including manufacturing and materials, the environment, energy and electronics and in medicine. MDR is a growing problem in the treatment of infectious diseases due to the widespread use of broad spectrum antibiotics has resulted in produced antibiotic resistance for many human bacterial pathogens (6). Many researchers have attempted to correlate the biological activity of inorganic antibacterial agents with the size of the constituent particles (7, 8). In particular, inorganic oxide nanomaterials like Cao, Zno and Mgo have shown potential as effective alternatives in addressing some of these challenges (9). Mgo NPs have the advantage of non-toxicity, high thermal stability, biocompatible, low cost, and have considerable potential as an antibacterial agent. Magnesiume plays several vital roles in human biology (10). The mechanism of metal oxide nanoparticle acti0n 0n bacteria is c0mplicated and n0t fully underst00d. It has been rep0rted that the antibacterial activity of Mgo nanoparticles is attributed to the production of reactive oxygen species (RoS) which induce lipid peroxidation in bacteria (11). Several studies have shown that smaller particles have greater antibacterial activity due to higher reactive surface area (12). Biological methods for nanoparticle synthesis using microorganisms, enzymes, and plants or plant extracts have been suggested as possible ecofriendly alternatives to chemical and physical methods (13, 14). Therefore this study aimed to synthesize Mgo NPs by biological method and characterize the synthesized NPs by utilizing UV-vis, TEM. Besides, their antibacterial activity against

some uncommon bacteria were tested. (Gram positive *Aeromonas salmonicida, Kocuria rosea and Alloiococcus otitis* and Gram negative *Acinatobacter calcoaceticus, Pantoea agglomerans* and *ochrobactrum anthropic*) used well diffusion method.

Materials and methods

Biosynthesis of Mgo nanoparticles

The Mgo NPs were prepared using method 0f (15). with some modification. The synthesis of Mgo NPs was carried with *Aspergillus. niger* ATCC 16404, the active culture of the isolate was inoculated into Potato dextrose broth (PDB) and the flasks were incubated at 28 °C ±2 , 150 rpm for 3 days. After incubation, the fungal filtrate was obtained by passing through Whatman No.1filterpaper. The collected supernatant was added to deionized water treated with 1% 0f mM MgCL2 and further incubated with shaker incubator at 150 rpm for 96 hrs at 28 °C. Conical flasks with either fungal filtrate or MgCL2 served as positive and negative control respectively.

UV-vis spectra analysis

The UV-Visible spectra of Mgo nanoparticles were characterized UV VIS spectrophotometer measured using a Systronics UV double-beam spectrophotometer. This is a simple method that give information about particle concentration and size, and size/size distribution, the shift of absorbance relay on the size (diameters) and shape of particles. The scanning range for the samples was 200-600 nm at a scan speed 0f 480 nm/min. The spectrophotometer was equipped with "UVWinlab" software to record and analyze data. Base line correction of the spectrophotometer was carried out by using a blank reference (16).

Scanning electron microscope (SEM)

A scanning electron microscope was (Cam Scan-3200 LV SEM machine) used to record the micrograph images, characterize mean particle size and morphology of synthesized Mgo NPs. A thin layer of gold was coated in an auto fine coater to make the samples conductive, after that the material was subjected to analysis by SEM machine was operated at a vacuum (14 , 17).

Transmission electron microscope (TEM)

The shape and size of silver nanoparticles were determined by TEM. For TEM, a drop of Mgo NPs suspension was placed on a carbon-coated copper grid then the grid was dried by Whatman paper No. 1 for 30 minutes. The carbon-coated grid was set on Phillps CM10 TEM sample holder. The average size and shape of Ag NPs was determined at magnification of 130.000×, 80 kV Modified procedure from (18)

Antimicrobial activity of synthesized Mgo nanoparticles

All pathogenic uncommom bacteria were multidrug resistant; they have obtained from out and in patients admitted in Tikrit General Hospital. The collected clinical strains were isolated from urine and sputum specimens. identification of pathogenic bacterial isolates according to the standard methods which recommended by (19,20). Confirmed by VITEK 2 System. The antibacterial efficacy of the Mgo NPs was investigated by agar well diffusion assay(21). against various types of multidrug resistant bacteria isolated from urine samples. The tested uncommon bacteria included (Gram positive *Aeromonas salmonicida , Kocuria rosea and Alloiococcus otitis* and Gram negative *Acinatobacter calcoaceticus, Pantoea agglomerans* and *0chr0bactrum anthropic*) Approximately 20ml of Mueller Hinton agar media was poured in sterilized petri dishes. Fresh overnight cultures of pathogenic bacterial isolates were adjusted 1.5×10^8 cells/ml, matching with 0.5 McFarland as

mentioned previously . Inoculums (100µl) were applied on the surface of the Muller Hinton agar plates and spread by using swabbed onto the plates. Agar wells of 5 mm diameter were prepared with the sterilized cork borer. Three wells were bored, one well containing the extract alone, control positive and the other well loaded with the synthesized Mgo NPs. The well added of Mgo- NPs 100 µg/ml and 0.50 mg/ml Ciprofloxacin antibiotics used as positive control against bacterial isolates. Then the plates were incubated at 37˚C for 24 hrs, where upon inhibitory activity was observed as a zone of clearing around the wells. The diameter of the clearing zones was measured in mm using the ruler scale. Pathogenic bacterium and compared with the standard antibiotic (22, 23).

Results and Discussion

Biosynthesis of Mgo nanoparticles:

The biosynthesis of Mgo NPs was conducted by cultivation of *Aspergillus niger* on potato dextrose broth media enrichments with ZnCL2 at 28 °C ±2with agitation at 150 rpm for 72 hrs., The Mgo NPs extracellular synthesis by cultivati0n of *Aspergillus niger* ATCC 16404 mycelia biomass, on optimal media enrichment with $MgCL_2$, The quantity of Mgo NPs was at 56 mg/100 ml which were collected after centrfuge and drying on 60 °C. In the biosynthesis of metal oxide nanoparticle by a fungus, the enzymes in metabolic pathway are action in reduce a metal to its metallic solid nanoparticles through the catalytic effects (24, 15). Many questions remain unanswered about the resistance mechanism of fungal strains towards many inhibitors such as heavy metals. Although fungi possess many properties that influence metal toxicity, the mechanisms involved in metal tolerance are highly dependent on the metabolic and nutritional status of the organism (25)

Characterization of Mgo NPs

The prepared of Mgo NPs was characterized using UV-VIS spectroscopy. Fig.1. and the absorption was appearing as a band at 258 nm. These results were agreement with the UV-visible spectrum for Mgo NPs at 260 nm in the results 0f (26). The optical properties of the Mgo NPs were studied in detail by means of the UV–vis absorption spectra in the wavelength range of 200–600 nm at room temperature.

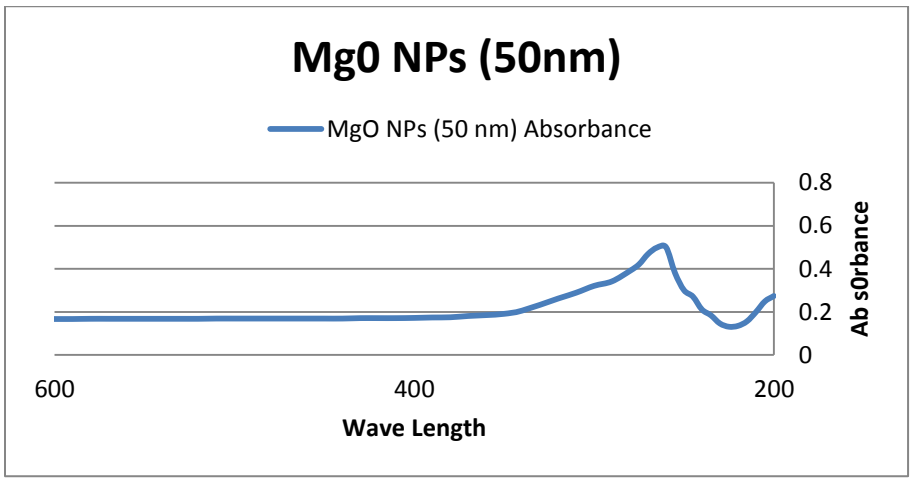


Fig. (1):- UV-Vis spectrophotometry of Mgo NPs.produced by *Aspergillus niger*

The determine of purity and the size range of Mgo NPs biosynthesized with *A. niger* used Scanning electron microscope analysis was appeared the size range from 47.35 to 98.46 nm (Fig.3). These results were agreement with the results obtained by (27). the biosynthesis of nanoparticles were induced the microbial cells to produced the biological agents that secrete a large amount of enzymes, which are capable of hydrolyzing metals, and produced the metals ions (28).

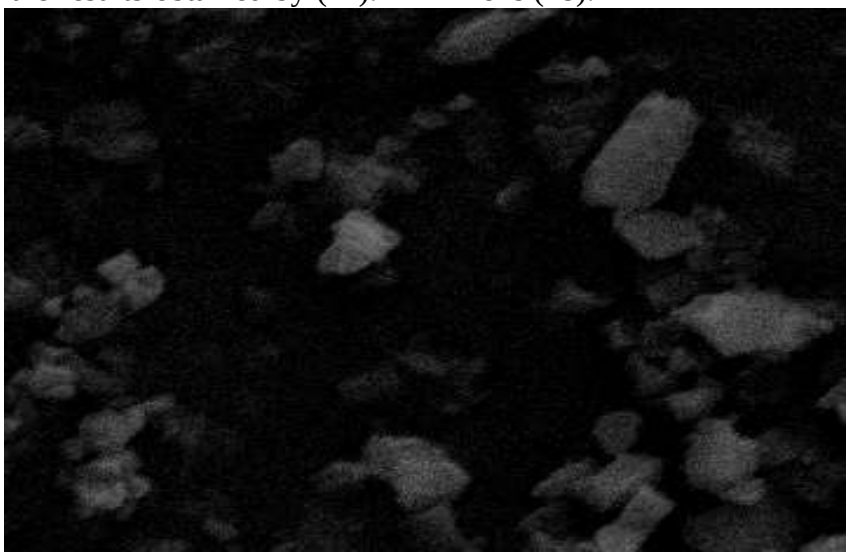
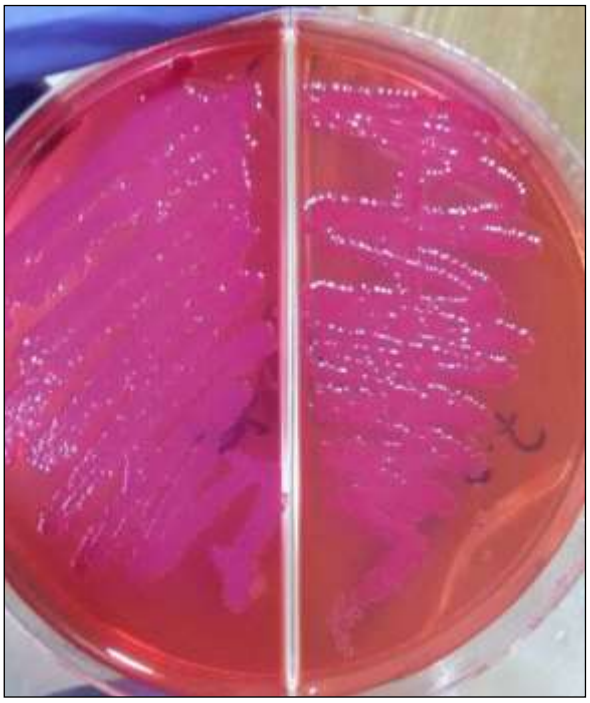


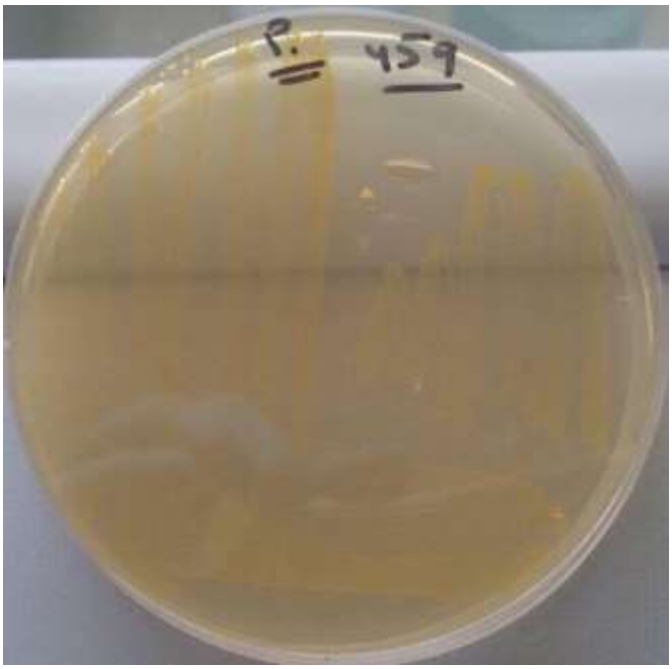
Fig. (2):- The SEM images of the of Mgo NPs.

Isolation and Identification of bacterial isolates

The isolated uncommon of bacteria from urine and sputum source, which were identified according to the microscopic, macroscopic and biochemical tests and the results confirmed by VITEK-2 SYSTEM. The most frequently isolated bacteria were (Gram positive *Aeromonas salmonicida, Kocuria rosea and Alloiococcus otitis* and Gram negative *Acinatobacter calcoaceticus, Pantoea agglomerans* and *ochrobactrum anthropic*). The isolates were cultured on Blood, MacConkey and Mannitol Salt agar plates and incubated at 37°C for 24 hours. They were identified according to colony characteristics and microscopic examination of stained smear that demonstrate microbial shape, structure, agreement, gram stain reaction, and biochemical tests like indol (I), methyl red (M.R), vogas proskauer (V.P), citrate utilization, oxidase (C), motility test, catalase, coagulase, and urease production; according to (29) as in table (2). Then the identificatin was confirmed by using VITEK 02 compact system as recommended by Biomerieux. Our results were compared with the resource reported by (30) and (19).



aeromonas anthropico ***Citrobacter freundii***



Pantoea agglomeranis ***Kokorea rosea***

Fig. (3):- Showing uncommon bacteria isolates which growth in different culture media

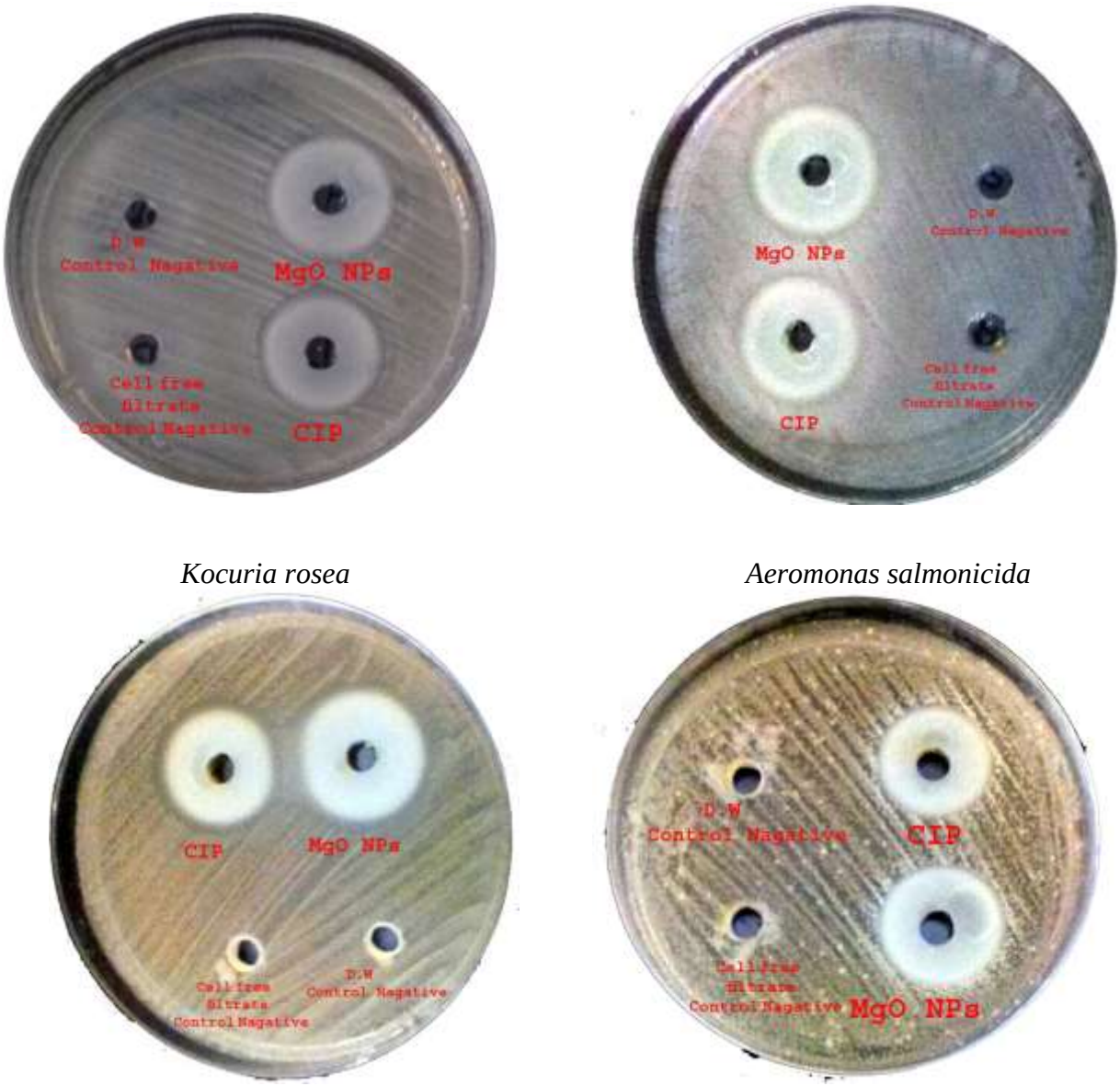
Antibacterial activities of Mgo NPs

Antibacterial activity of 1.0 mg from Mgo NPs was tested against uncommon bacteria gram positive *Aeromonas salmonicida, Kocuria rosea and Alloiococcus otitis* and gram negative *Acinatobacter calcoaceticus, Pantoea agglomerans* and *ochrobactrum anthropic* bacteria. The results were indicated that highly inhibition activity of Mgo NPs against these bacterial pathogens and the zone inhibition diameter (ZID) against against Gram positive *Aeromonas salmonicida, Kocuria rosea and Alloiococcus otitis* and the zone inhibition diameter was at 20,19 and 23 mm respectively compared with inhibition effects of ciprofloxacin antibiotics at 18, 17 and 20 mm respectively. while against Gram negative *Acinatobacter calcoaceticus, Pantoea agglomerans* and *ochrobactrum anthropic* bacteria was at 18,20,19 mm respectively, compared with inhibition effects of ciprofloxacin antibi0tics at 16 , 20 and 15 mm respectively. Also The results demonstrated a slightly higher antibacterial activity against Gram positive bacteria than Gram negative bacteria these results were agreement with results founds by (26). Whom found that the amount of Mgo NPs which killed bacteria was strongly dependent on particle size, (31) said the gram positive bacteria more sensitive to oxides nanoparticles than the gram negative bacteria, The inhibition efficacy of Mgo NPs was tested against different pathogens (32)

Table (1):- Antibacterial activity of Mgo NPs against some uncommon bacteria species

Microorganism Species	Zone of inhibition(mm)		Cell-free filtrate
	Mgo NPs 1.0mg/ml	CIP 0.5 mg/ml	
Aeromonas salmonicida	**20**	**18**	**0**
Kocuria rosea	**19**	**17**	**0**
Alloiococcus otitis	**20**	**16**	**0**
Acinatobacter calcoaceticus	**18**	**16**	**0**
Pantoea agglomerans	**20**	**20**	**0**
ochrobactrum anthropic	**19**	**15**	**0**

The antibacterial activity effect of Mgo NPs on cell membrane integrity, which affects the permeability of membranes where nanoparticles enter and induce stress in bacterial cells, subsequently resulting in the inhibition of cell growth and eventually in cell death (7, 33).



Kocuria rosea *Aeromonas salmonicida*

Acinatobacter calcoaceticus *chrobactrum anthropico*

Fig. (4):- Antibacterial activity of Mgo NPs used well diffusion test

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