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Inhibitory Effect of Silver nanoparticles on Some Bacterial Species that cause Otitis Media

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ABSTRACT:

The biological preparation of nanoparticles is a modern, economical and inexpensive method, including the manufacture of silver nanoparticles from lemon peels, which were determined through the color change of the mixture of silver nitrate and lemon extract, and UV tests, which determined _441 nm, while the FTIR examination, whose bands ranged between 443.64-3458.48. As for the SEM, it The dimensions of the nanoparticles ranged from 31.94 to 49.83 nanometers.

The results showed that the concentrations of AgNPs silver nanoparticles, which included (100, 75, 50, and 25)%, had an inhibitory effect on multiple antibiotic-resistant bacterial species by measuring the diameters of inhibition caused by the nanoparticle concentrations against *Pseudomonas aeruginosa* 17 mm, *E. coli* 20 mm, *Staphylococcus aureus* 21 mm, *Klebsiella pneumonia* 18 mm

Keywords: Pathogenic bacteria , Nanoparticles, Silver Nitrate .

التأثير التثبيطي لجسيمات الفضة النانوية على بعض الانواع البكتيرية المسببة لالتهاب الاذن الوسطى

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مستخلص

التحضير الحيوي لجسيمات النانوية طريقة حديثة واقتصادية وغير مكلفة اقتصاديا من ضمنها تصنيع جسيمات الفضة النانوية من قشور الليمون التي حددت من خلال التغير اللوني لمزيج نترات الفضة ومستخلص الليمون وفحوصات uv الحزمة التي حددت _441 نانومتر بينما فحص FTIR والتي تراوحت حزمها مابين 443.64-3458.48 اما SEM فقد اظهر ابعاد احجام الجسيمات النانوية تراوحت مابين 31.94 الى 49.83 نانومتر

بينت النتائج ان تراكيز الفضة النانوية *AgNPs* التي شملت (100,75,50,25)٪ ذات تاثير تثبيطي للانواع البكتيرية المتعددة المقاومة للمضادات الحيوية من خلال قياس الاقطار التثبيطية التي احدثتها تراكيز الجسيمات النانوية تجاه *Psedomonas aeruginosa* 17 ملم ، *E.coli* 20 ملم، *Staphylococcus aureus* 21 ملم، *Klebsiella pneumonia* 18 ملم .

الكلمات المفتاحية: بكتريا، جسيمات نانوية، نترات الفضة .

Introduction

Nanotechnology is a scientific field where the development includes the manufacture and application of nanoparticles that have unique physical and chemical properties (Gurunathan *et al*, 2009 (the size of these nanoparticles ranges from 0.1 nanometers to 1000 nanometers in at least one of their dimensions) Seid *et al*, 2022 (the particles vary Nanoparticles have higher surface energy than ordinary materials, as well as surface atoms in huge numbers, and they have fewer defects (Le *et al*, 2021). Metal NPs have the properties of catalysts and antimicrobial activity. Among these metal nanoparticles are silver nanoparticles (Bhattarai *et al*, 2018; Ashraf *et al*, 2016), which is used in several diverse applications due to its small size Nanoparticles are manufactured by physical and chemical methods to this day, but they produce by-products that are harmful to the environment. Therefore, there is a constant need to search for new methods that are simple, fast, consume less energy, and are environmentally friendly. Therefore, scientists focus on the green manufacturing of nanoparticles from plant extracts (Niluxsshun *et al*, 2021).

Which is an economical and quick method (Vankar *et al*, 2012). Citrus peels, including lemons, are considered a cheap fruit, commercially available, environmentally safe, and a rich source of flavones, polymethoxylated flavones, and carboxylic acids such as ascorbic acid and citric acid, which are very rare in other plants that can These materials are used as reducing agents that have a role in the manufacturing of nanoparticles (Mohammed *et al*, 2022; Niluxsshun *et al*, 2021).

The current study aimed to determine the effectiveness of silver nanoparticles manufactured from lemon peels against multi-antibiotic-resistant bacterial isolates isolated from patients with otitis media.

Materials and methods

Collecting and Diagnosis of Bacterial Samples

The study samples were collected from patients visiting Salah al-Din General Hospital who had symptoms of middle ear infection for the period from October 16, 2022 until May 2023, after the symptoms were diagnosed by the specialist doctor. The samples were collected using a cotton swab by

inserting and rotating the sterile cotton swab in the ear. The medium was then placed in media transport until it was transported to the laboratory to conduct biochemical diagnostic tests for the bacteria after culturing them on culture media: blood culture, MacConkey, and menthol saline, according to Forbes, 2007.

Antibiotic Susceptibility Testing

A test was conducted for the sensitivity of bacteria to 7 types of antibiotics using the disk diffusion method (Kirby-Bauer) (Cockerill, 2010). Single colonies were transferred to tubes containing 5 ml of normal saline, and the turbidity of the solution was adjusted with the turbidity of the McFarland tube, at a concentration of 0.5, and using sterile cotton swabs. Plates of Hinton-Muller agar medium were inoculated with the bacterial suspension, then the plates were left for a few minutes, after which we placed the antibiotic tablets on the surface of the medium using sterile forceps, which included the antibiotics Ciprofloxacin (CIP-10mg), Imipenem (IPM -10mg), Erythromycin (E -10mg) (AM). -25mg) Amoxicillin (AMC -30mg) Vancomycin (vn 10mg), Azthromicin AZM, Ampicillin. Then the plates were incubated for 24 hours, and the growth inhibition zone was measured using a ruler, then at a temperature of 37°C, the results were compared with standard tables (CLSI, 2017).

Preparation of nanomaterial

Collect and prepare lemon peel extract

The nanoextract was prepared according to what was stated by Niluxsshun *et al*, 2021, with some modifications. Fresh lemon fruits were brought from local markets, and after washing them well with water and peeling them, an amount of 20 grams of peels cut into small pieces was weighed and placed in a glass beaker containing 100 ml of sterile distilled, deionized water and heated. For five minutes using a hot plate at a temperature of 60°C and let it cool. After that, we filter the solution using medical gauze and filter it with filter paper and keep it in the refrigerator until use.

Biosynthesis of silver nanoparticles

10 ml of extracted and sterilized lemon peel extract are gradually transferred through a 0.22 miliporefilter into 90 ml of 1 mM silver nitrate with continuous stirring of the mixture and

incubate in an incubator shaker for 24 hours.

Characterization tests for nanoparticles

The characterization done according to (Humza *et al*,2018)

1. UV-Visible spectrophotometer. The spectrophotometer was used to determine the absorbance of silver nanoparticles. 2 ml of the previously prepared solution of secondary silver particles was transferred after filtration to a spectrophotometer device with a wavelength of (200-800) nanometers for the purpose of Know the absorption package.

2. Fourier Transmission Infrared Spectroscopy (FTIR) Infrared spectroscopy (FTIR) was used to determine the functional groups present in silver nanoparticles. 125 ml of secondary silver particles were dried using an electric oven at a temperature of 45°C in glass Petri dishes, where a thick layer was formed and the layer was removed. Using a sterile spoon, place the resulting powder in a special tablet in the form of tablets, and then place the tablet in the device.

3. Scanning electron microscopy (SEM): The scanning electron microscope was used to determine the size of the silver nanoparticles. 50 ml of the secondary silver particles were dried in a convection oven at 45 degrees Celsius, and a thick layer was formed. The formed layer was removed with a marked spoon by placing the formed powder in the device's special tube. Covering it with a special layer of gold and examining the model.

Testing the inhibitory activity of Nano-extract

The method of diffusion by etching was used to determine the effectiveness of the prepared Nano concentrates of 25, 50, 75, and 100% against multi-antibiotic resistant bacterial isolates using a cork drill. Drilling with a diameter of 6 mm was made in the surface of the medium of Muller hinton agar after. Spreading of the confirmed bacterial isolates (1 We measure the inhibition zones around the holes using a ruler (Saleh *et al*, 2021).

Results and discussion

Biosynthesis of nanoparticles

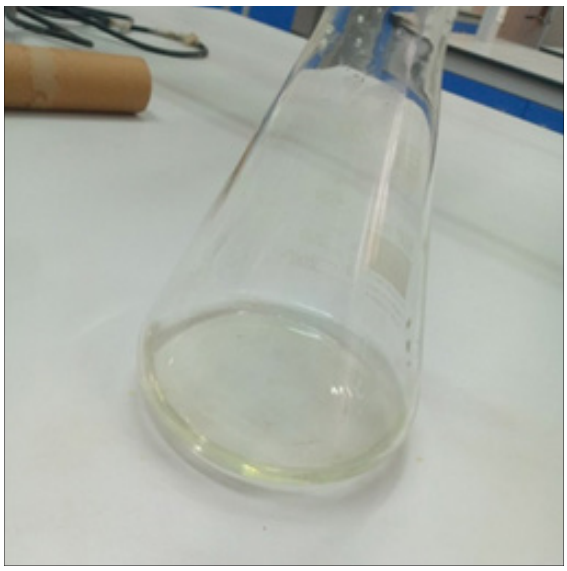
Several environmentally friendly methods have been used to synthesis nanoparticles because they have many unique advantages such as simplicity in preparation, low cost, antibacterial activity, and effective antioxidants. Many

scientists are focusing on the green manufacturing of nanoparticles from plant extracts (Bindhu and. Umadevi, 2015, Huma *et al* 2020,)

Citrus fruit peel is a rich source of flavones and several polymethoxylated flavones, which are very rare in other plants, and these materials can be used as reducing agents in the synthesis of AgNPs.

The color change is the first correct step in the formation of silver nanoparticles from lemon peels, as the change occurred from colorless to dark brown, as shown in Figure 1, which is a result that agreed with what was found.

Kadhim *et al* 2023 The study showed, through the first step, that a color change occurred in the mixture of plant extract and silver nitrate to dark brown. This indicates the formation of nanoparticles due to the reduction of metal ions of silver. The color change occurs over time due to the surface plasmon resonance of SNPs (Zilberberg *et al*, 2015).



Figure(1):Represents the Color Change of the Nanomaterial

1_UV-spectrophotometer examination: The peak absorbance of the nano model is represented by the 441 nm band, as shown in Figure (2), which was formed after mixing lemon extract and silver nitrate extract and an incubation period of no less than 18 hours, This band is an ideal model that was obtained by surface plasmon resonance, which is A similar result to what

was found by Shareef and his group (2023) found that the band that determined the formation of nanoparticles made from lemon peels was 440 nm. Likewise, Jahan, and her group (2021) reiterated that the absorption band was formed at 445 nm from the fabrication of lemon peels and silver.

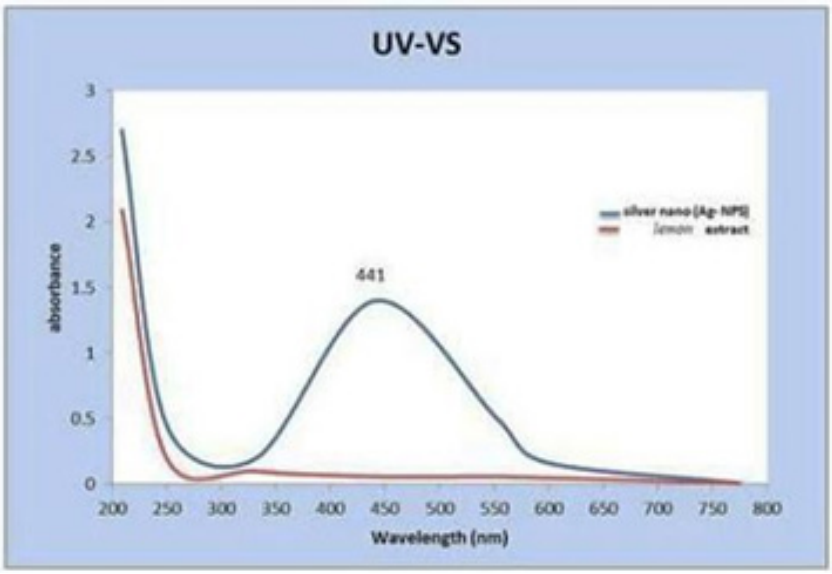


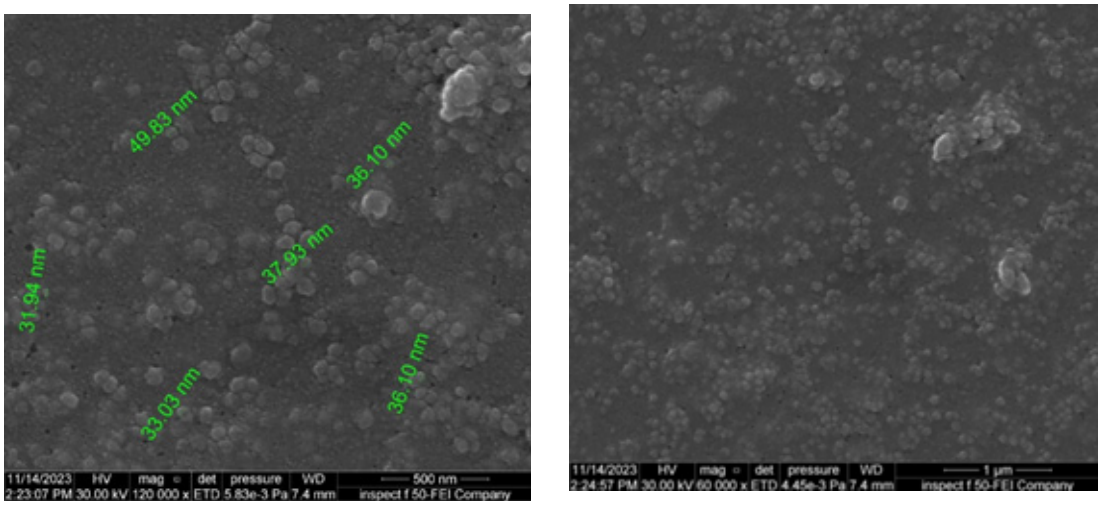
Figure (2): UV-spectrophotometer examination

2_ FTIR examination: The functional groups that have a role in reducing silver ions to silver nanoparticles_441 nm were identified by FTIR examination, whose bands ranged between 443.64-3458.48, as shown in Figure 3

These are similar results to what Alaallah and her group 2023 obtained through FTIR examination, as they noticed that the bands of 3429 cm-1 represent the (–N-H) bond for amides (II) and 1630 cm-1 is related to amide I, representing the carbonyl group, as for Kharat and Mendhulkar. (2016) found that the band 1635.64 cm-1 indicates the presence of a carbonyl group linked to an amide bond.

Thus, the manufactured nanoparticles are surrounded by proteins and metabolites such as terpenoids that contain hydroxyl functional groups. Through the FTIR results, we can confirm that the proteins The resulting carbonyl groups of amino acid residues have a stronger ability to bind metals and this suggests that proteins can act as a capping agent and prevent agglomeration, thus stabilizing the nanoparticles (Niraimathi et, 2013).

SEM examination: The results of our study showed that the dimensions of the nanoparticle sizes ranged from 31.94 to 49.83 nanometers, as shown in Figure 4, which is similar to what was found by Abdelrazik 2023 found that the particle size of nano is 32 nanometers. Saleh 2021 also found that the particle size of silver is 7.55 - 24.4 nanometers, which is also close to the results of Hamzah and his group 2018.



Figure(4): SEM scan

Inhibitory Activity of Silver nanoparticles against Antibiotic-Resistant Bacteria

The result showed the trend of some bacterial species isolated from patients with otitis media that are resistant to antibiotics, as shown in Table (1) and Figure (5) and (6) that The approved concentrations compare with some types of antibiotics discs are effective against the bacteria Pseudomonas aeruginosa, by measuring the diameters of inhibition that were determined around the etched areas on Muller-Hinton medium, which showed that the diameter of inhibition at 100% concentration was approximately 17 mm, while for E. coli and Klebsilla bacteria, the inhibitory diameter was determined to be 20 and 18 mm. Respectively, it is a similar result to the study conducted by Reenaa and his group in 2017, when it was found that the inhibitory diameter of silver nanoparticles manufactured from lemon peels created an inhibitory effect of 21 mm against *Pseudomonas aeruginosa* bacteria, while it was determined to be 20 mm against *E. coli*

bacteria, while (Baker & Nagend 2016) noted that the inhibitory effectiveness of silver nanoparticles against these bacteria was approximately 21 mm.

As for Kadhim and his group (2023), they noted that the inhibitory diameter of nanosilver made from lemon peels against E.coli was 18 mm, while Alaallah and his group (2023) determined the inhibitory diameter of 22 mm against *E.coli* bacteria and *Staph.aureus* bacteria, and 25 mm for nanosilver made from lemon extract as well. Alwhibi and his group (2018) observed that the inhibitory diameter of silver nanoparticles against *K. pneumoniae* and *S. aureus* bacteria was 25 and 18 mm, respectively, which are similar results to our current study.

Silver nanoparticles (AgNPS) can enter the bacterial cell through the cell membrane and interact with cellular structures and biomolecules such as proteins, lipids, and nucleic acids, which can lead to leakage of cellular contents and death of bacteria (Khalandi et al, 2017), Silver ions can also bind to the protein amino (-NH2) and thiol (-SH) groups of the cell membrane and may result from the stimulation of ROS, leading to inhibition of respiratory chain enzymes, and thus cell death (Niluxsshun et al, 2021) as well as interaction with enzymes. Bacterial and DNA, and destabilize the ribosome, leading to cell death. (Patil *et al*, 2017).

Table (1) Effect of nanoparticle concentrations on bacterial species

Antibiotics							AgNPs concentrations				Bacteria
E	AM	AZM	IPM	VN	CIP	AMC	100%	75%	50%	25%	
R	R	R	R	R	R	R	17	16	15	14	*Psedomonas aeruginosa*
R	R	R	S	R	R	R	20	19	18	17	*E.coli*
R	R	R	S	R	R	R	21	20	19	18	*Staphylococcus aureus*
R	R	S	S	R	R	R	18	17	16	15	*Klebsiella pneumonia*

R=resistance

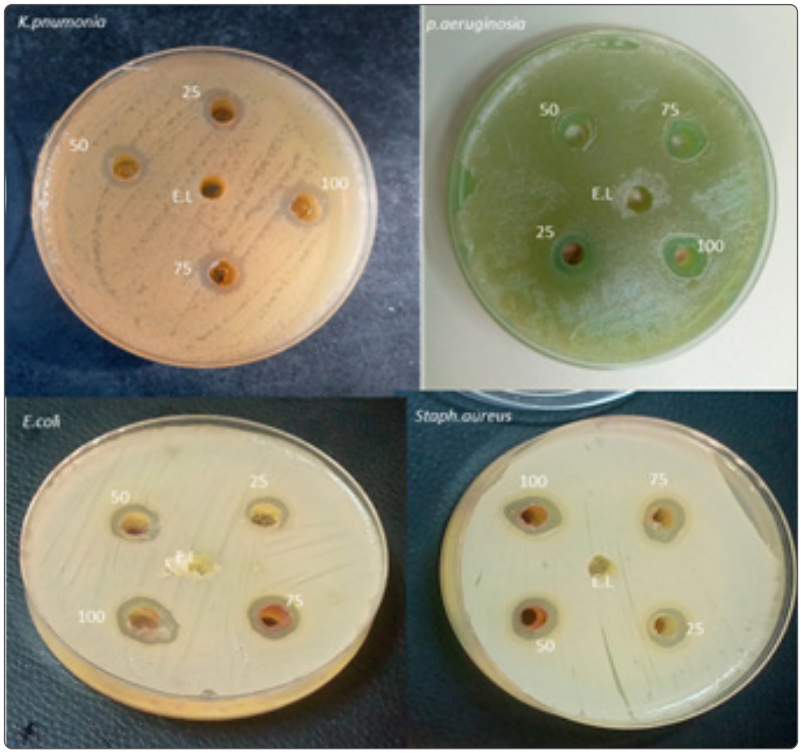


Figure (5): The effect of nanoparticles on specific bacterial species. EL = represents lemon peel extract

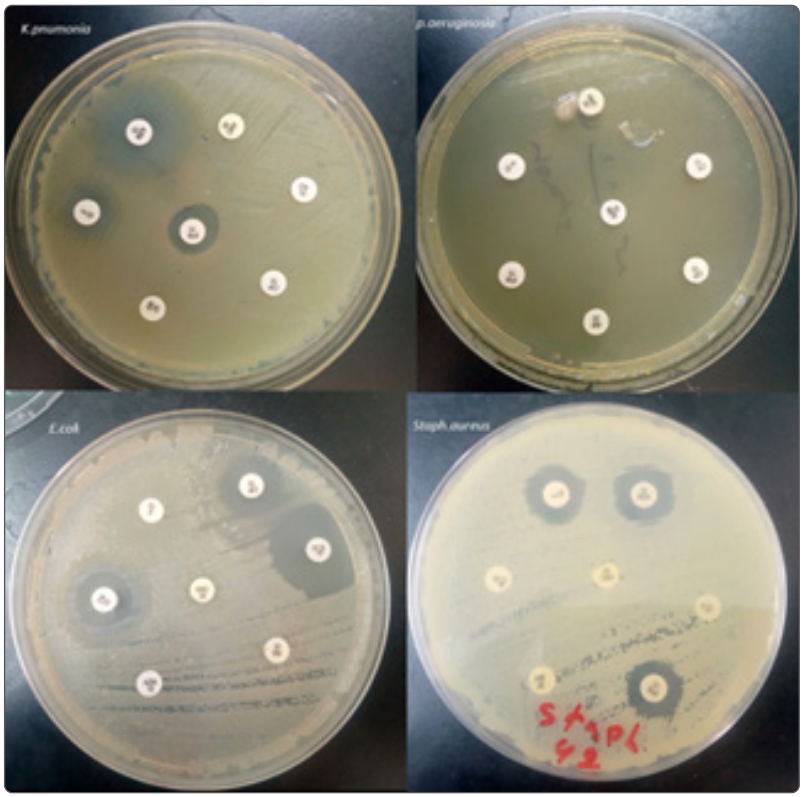


Figure (5): Antibiotic resistance

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