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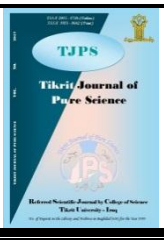

Antibacterial effect of biosynthesis silver nanoparticles on *Pseudomonas aeruginosa*

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ABSTRACT

The current study indicated possibility determining the inhibitory activity of silver nanoparticles which synthesis by the Cinnamomum zeylanicum bark against 5 pathogenic bacterial isolates of to Multidrugs resistant *Pseudomonas aeruginosa* collected from hospitals (Azadi Education, Kirkuk General, Tuz General), Silver nanoparticles showed their inhibitory effect against bacteria by the Well diffusion method and The Diameter of inhibition zones ranged between (14-23)mm As for the growth inhibition assaysilver nanoparticles showed activity against the bacterial species at concentrations of (25 ,50, 75, 100)%, respectively through the absence of colony growth on the surface of the culture media at these concentrations Therefore it is possible to use nanoparticles synthesis from plants for some minerals due to their effective inhibitory properties that they have against bacteria.

Introduction

Pseudomonas aeruginosa is one of the most prevalent bacterial species and its ability to cause infections among patients with wounds and burns helped by its virulence factors including the formation of biofilms and the production of toxins that cause extensive tissue damage as it plays a role in the invasion and colonization of the affected area of the human host body in addition to its ability to survive and reproduce in moist environments that contain small amounts of nutrients [1][2] The increasing of multidrug resistant bacteria has led many researchers to attempt to develop new effective antibacterial materials that are low-cost and do not cause bacterial resistance[3]

Advances in nanotechnology have allowed the synthesis of nanoparticles that are a modern alternative to the problem of bacterial resistance to antibiotics and seem to be the solution to the problem multidrug resistance of bacteria which is increasing become a dilemma that has caused a public health crisis [4]

The researchers emphasized that NPS has antibacterial properties against bacteria fungi and other microbes[5] Moreover The development of synthesis processes has become a major focus for researchers as one of the most observed technologies is the biosynthesis of metallic nanoparticles using living organisms. Among them plants have many advantages when compared to chemical and microbial synthesis due to the ease of production no complicated process for cell development and maintenance or the use of dangerous chemicals or requires high cost and energy purification processes[6]

Silver nanoparticles synthesis from plants represent a promising source as an antibacterial agent due to their mechanism of action that targets multiple sites in bacteria. Silver nanoparticles have a high medicinal value due to their unique antibacterial properties [7] and topical creams to prevent wound infections[8]

Aim of the study Determining the inhibitory activity of silver nanoparticles against multi-drug resistance *Pseudomonas aeruginosa*

Materials and Methods

Collection and isolation of the bacterial isolates

Bacterial isolates were collected from (Azadi Education - Kirkuk General - Tuz General)from the period of (1\8\2020 to1\11\2020) isolated and identified by using VITEK2.

Preparation and Synthesis silver nanoparticles

AgNPs silver nanoparticles were obtained by synthesizing from bark extract

it prepared from 1 ml of prepared cinnamon bark extract was added to 50 ml of aqueous silver nitrate solution and placed in a 200rpm incubator at room temperature for 1-8 hours to produce silver particles. The solution initially appeared as a pale yellow and then changed to a dark brown color[9]

Then Concentrations (100,75,50,25) were prepared for the solution of these particles and it was used in preparing the concentrations of sterile distilled water by taking (25,50,7 5) ml of the silver nanoparticles and adding to it (25,50,75) ml of sterile distilled water respectively As for 100 it is the concentration that was taken from storage without dilution [10]

Inhibitory activity of silver nanoparticles against bacteria

The inhibitory activity of silver nanoparticles against 5 bacterial isolates of Pseudomonas aeruginosa tested by the Well diffusion method and the growth inhibition assay after fixing the number of the bacterial species under study.

Agar diffusion assay

Bacterial samples were cultured in nutrient broth and incubated for 24 hours at 37°C. The number was then fixed by comparison with McFarland's standard 1×10^{-8} cell Then the bacterial samples were spread by a sterile cotton swab on the medium 6 ml diameter pits were made and then 100 µl of different concentrations of silver nanoparticles 25% 50% 75% 100% were transferred to the pits. In addition to transferring 100 µl of cinnamon extract (control sample) to one of these pits, the plates were incubated for 24 hours at a temperature of 37 °C after which the diameters of the zone inhibition that appeared around the pits were measured in mm using a ruler[11]

growth inhibition assay

This experiment was conducted by transferring 120 µl of the confirmed pathogenic bacteria (1×10^{8}) to the pits of the Microtiter Plate Wells (which contains 96 holes) and adding 80 µl of silver AgNPs concentrations in those pits (25,50, 75, 100)% Then the plate incubated at 37°C for 24 hours, then the optical density (OD) was measured using a Microplate Spectrophotometer (Biotech µQuant™ USA). The growth results of the isolates under study were observed [12]

Results and Discussion

The current study After silver nanoparticles were synthesized using C. zeylanicum bark extract, the Silver nanoparticles showed an inhibitory activity at different concentrations of 100, 75, 50 and 25% against the 5 isolates of *P. aeruginosa* as figure 1 and 2 by the two methods of etching. On the Muller Hinton agar medium which the bacteria were grown it was noted that the inhibitory diameter at 100% concentration ranged from 18-25 mm as Table 1.

This result agree with results several previous studies [13] showed that silver nanoparticles synthesized from the bark appeared an inhibition zone of 24 mm against *P. aeruginosa* also similar approach with [14] showed that the inhibitory activity of silver nanoparticles against bacteria is about 21 mm which is in agreement with the results of our current study [15] also determined the inhibitory diameter of silver nanoparticles at about 14 m which is the result of a comparison with the current study where the inhibitory diameter increases with the increase in concentrations used against bacteria. Bacteria as well as researcher [16] determined the inhibitory diameter of about 16 mm against Pseudomonas aeruginosa which is a result consistent with the diameters of the current study. The current study also agreed with the findings of [17] Which determined the damping diameter is about 17 mm. Figure 4-3 Effect of silver nanoparticles by etching diffusion method on *P. aeruginosa*.

the inhibitory ability of silver nanoparticles against Pseudomonas bacteria was determined by the growth inhibiton assy method, where the effectiveness of concentrations of silver nanoparticles 25% 50% 75% 100% in inhibiting the bacteria was determined by determining the growth of isolates (The control sample) for each type compared with the isolates treated with concentrations of silver nanoparticles on agar nutrient medium where the current study showed no bacterial growth at the concentration 50% 75% and 100% which is the result of agreement between the results [10] which showed the absence of growth of Pseudomonas aeruginosa bacteria At these concentrations of silver nanoparticles growth appeared at a concentration of 25% and the results of this study match what was reached by the researcher[18] as well as with the researcher[19] who confirmed each researcher separately that there is no growth of these bacteria at 50% .

AgNPs have physical chemical and biological properties that are different from silver ions as AgNPs can concentrate on the bacterial cell wall after sticking to the cell wall can penetrate the cell membrane and nanoparticles enter into the bacteria There is an antibacterial effect that depends on the size of the nanoparticles that is the smaller nanoparticles It has a large surface area in contact with bacterial cells and can reach the cytoplasm more than large nanoparticles. This action will lead to physical changes in the bacterial membrane such as membrane damage which can lead to leakage of cellular contents and death of bacteria[20] [21] also the positive charge of silver nanoparticles gives it the ability to attract towards the negatively charged cell membrane of bacteria and thus AgNP facilitates binding to bacterial cell membranes [22]

AgNPs that enter the bacterial cell can affect cellular structures and biomolecules such as proteins, lipids and nucleic acids and lead to their disfunctions and eventual death of the bacteria. In particular the interaction of AgNP with ribosomes leads to their denaturation, which leads to the inhibition of translation and protein synthesis. It is also expected that AgNPs interact effectively with carboxyl and

thiol groups, inhibiting the biological functions of intracellular proteins and leading to death [23]

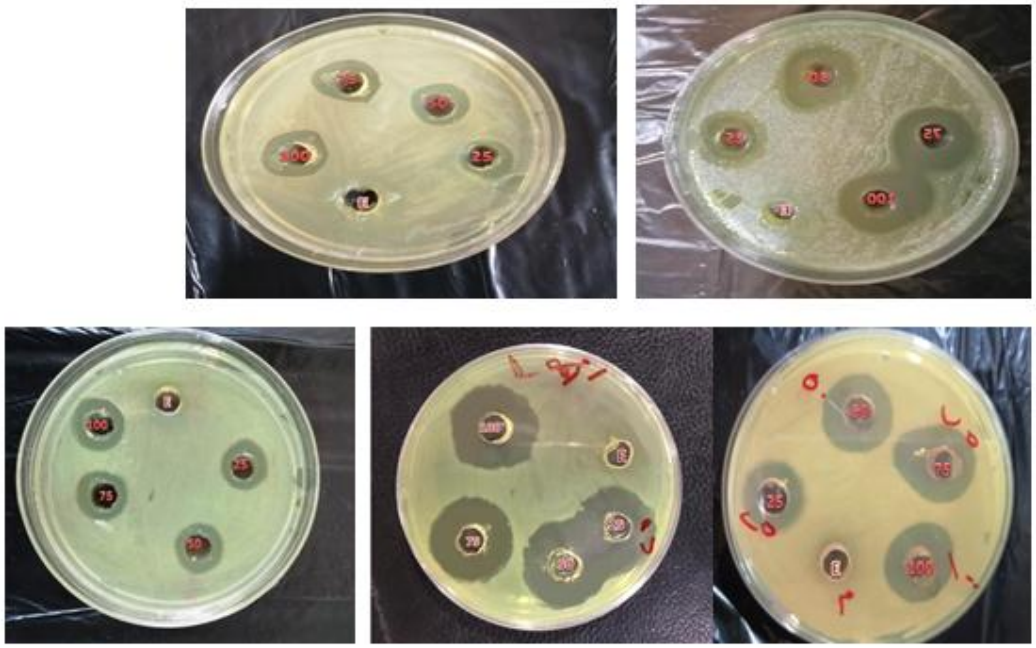

Fig. 1: shows that the inhibition of silver nanoparticles against Pseudomonas aeruginosa by well-diffusion method.

Table 1 shows the inhibition diameters of silver nanoparticles by diffusion method on Ps. aeruginosa

%100	%75	%50	%25	Bacterial number
18	16.8	15.2	14	1*Ps. aeruginosa*
20	18	16	14	2 *Ps. aeruginosa*
20	16	15.4	13	3 *Ps. aeruginosa*
21	19	16	12	4 *Ps. aeruginosa*
23	21	19.3	18	5 *Ps. aeruginosa*

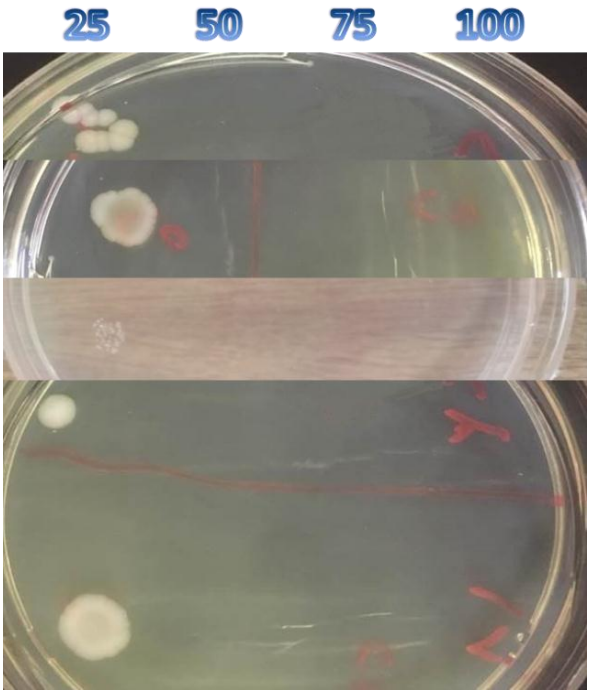

Fig. 2: Inhibitory activity of silver nanoparticles by growth inhibiton assy against P. aeruginosa by growth inhibition method

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التأثير المضاد لجسيمات الفضة النانوية ضد بكتريا الزائفة الزنجارية

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الملخص

أشارت الدراسة الحالية إلى إمكانية تحديد النشاط التثبيطي لجسيمات الفضة النانوية التي يتم تصنيعها بواسطة لحاء نبات القرفة (الدارسين) Cinnamomum zeylanicum اتجاه 5 عزلات بكتيرية ممرضة من(بكتريا الزوائف الزنجارية) Pseudomonas aeruginosa المقاومة للأدوية المتعددة والتي تم جمعها من المستشفيات (مستشفى ازادي التعليمي – مستشفى كركوك العام – مستشفى طوز العام) وأظهرت جزيئات الفضة تأثيرها التثبيطي ضد البكتيريا بطريقة الانتشار الجيد وتراوح قطر مناطق التثبيط بين (23–14) ملم أما بالنسبة لمقايسة تثبيط النمو فقد أظهرت جزيئات الفضة فعالية ضد الأنواع البكتيرية بتركيزات (25،50 ، 75 ، 100)٪ على التوالي من خلال عدم وجود نمو المستعمرة على سطح وسط الزرع في هذه التراكيز لذلك من الممكن استخدام تركيب الجسيمات النانوية من النباتات لبعض المعادن نظرًا لخصائصها المثبطة الفعالة ضد البكتيريا