Chelonian Conservation And Biology

Vol. 19 No. 1 (2024) | https://www.acgpublishing.com/ | ISSN - 1071-8443

DOI: doi.org/10.18011/2024.01(1).309.321

STUDY OF THE BACTERIAL ACTIVITY OF SILVER NANOPARTICLES (AGNPS) PRODUCED USING CYNOPHYTA ALGA EXTRACT (*SPIRULINA PLATENSIS*).

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Abstract

The study included the production of biologically produced silver nanoparticles (AgNPs) by the algae extract *Spirulina platensis*, as well as the diagnosis of some effective chemical compounds from the blue-green algae *S.platensis* and the evaluation of the inhibitory effectiveness of silver nanoparticles (AgNPs) against some types of pathogenic bacteria that included *Escherichia coli*, *Pseudomonas aeruginosa* and *Staphylococcus aureus*.

Different concentrations of silver nanoparticles (0.5, 1, 1.5 and 2) mg/ml were prepared to study their effect on the types of bacteria studied by determining the area of diameter of bacterial growth inhibition. The silver nanoparticles showed their microbial effectiveness against two isolates of Gram-negative bacteria, *E.coli* and *P.aeruginosa* and the isolate of Gram-positive bacteria *S.aureus*, where the average diameter of the inhibition zone for the concentrations used was 12.58, 13.75 and 15.24 mm, respectively, by the diffusion well method. Bio-synthesized silver nanoparticles from the algae extract *S. platensis*, at very low concentrations, proved effective against the bacterial species studied.

The results of drug susceptibility testing using the disk method showed that all bacterial isolates were 75% resistant to the antibiotics Amoxicillin, Chloramphenicol, Ciprofloxacin, Clindamycin, Trimethoprim/Sulfamethoxazole and Tetracyclin, while 25% were sensitive to the antibiotics Imipenem and Piperacllin/Tazobactam. The results of the drug susceptibility test using the Vitec system also showed that the *E.coli* bacteria were resistant to the antibiotics Ampicillin, Piperacillin/Tazobactam, Cefazolin, Cefoxitin, Ceftazidime, Ceftriaxone, Cefepime, Imipenem, Ciprofloxacin, Levofloxacin, Trimethoprim/Sulfamethoxazole at a rate of 73.3% , As for the antibiotics Amikacin, Gentamicin, Tigecycline and Nitrofurantoin, the sensitivity rate was 26.7%. As for the *P. aeruginosa* bacteria, it was 100% resistant to all antibiotics used. The *S.aureus* bacteria resisted the antibiotics Benzylpenicillin, Oxacillin, Ciprofloxacin, Erythromycin, Clindamycin, Tetracycline, Fusidic Acid and Rifampicin by 57.1%, while the antibiotics to which it was sensitive included Moxifloxacin, Linezolid, Teicoplanin and Trimethoprim/Sulfamethoxazole by 42%. 9%.

1: Introduction

Infections of wounds and burns are among the most complex problems facing doctors who deal with serious cases at the present time. They are caused by the frequent indiscriminate use of antibiotics, including beta-lactam antibiotics, which leads to the emergence of problems with resistant strains, inefficiency of treatment, and the emergence of strains characterized by being multi-resistant to several types of antibiotics. At the same time, antibiotics make it difficult to control diseases from a medical standpoint, which led to directing research on alternative treatments (Dubey *et al*., 2013).

Human skin is the first defense barrier against the external environment, and is responsible for immune protection, control of fluid levels, and temperature regulation, as well as the natural colonization of natural and beneficial microorganisms on human skin. It also provides protection against microorganisms that cause inflammatory diseases (Abdallah, 2017).

The most common disease-causing bacterial species in burns and wounds are *E. coli*, *P.aeruginosa*, *S. aureus*, and *Klebsiella pneumonia* (Felgueiras, 2021). These types of bacteria cause danger to human life after a burn or wound occurs, because the skin area will lack protection at that time, and any type of bacteria can reach it, leading to infections or blood poisoning (Vale de macedo., 2021).

Nanotechnology is one of the basic fields of current science, which allows scientists to acquire wonderful innovations the size of nanoparticles, which have a diameter of less than 100 nanometers. Different methods are used to prepare these particles in the smallest possible size. These methods are classified into physical, chemical, organic, and photochemical, and produce These particles of various metals, including palladium, copper, tin, gold, and silver, have gained great interest due to their properties and applications in various fields. Most of the available methods are expensive and not environmentally friendly, in addition to the negative results that affect living organisms. Therefore, scientists prefer biological methods for producing nanoparticles. Such as using algae, bacteria, fungi and plant extracts as a reducing agent , Silver nanoparticles have been widely used in various fields such as industrial purposes, medicine, food, and health care due to their chemical and physical properties, distribution, size, shape, and high surface area (Zahoor *et al*., 2021).

2: Materials and Methods

2.1:Characterization of bacterial isolates used in the study

Isolates of *E. coli* and *P. aeruginosa* bacteria were grown on Macconkey agar medium, while *S. aureus* bacteria were grown on Mannitol Salt Agar medium, after which they were incubated at (37)°C for (24) hours to obtain colonies and they were diagnosed based on cultural diagnosis.

2.2:Charaacterization of bacterial isolates using the Vitek-2compact system

All bacterial isolates under study were diagnosed using the Vitek-2 device based on what was stated in Koneman *et al*., (2006), as the device is used for accurate diagnosis of bacterial isolates and for conducting biochemical tests and testing their sensitivity to various antibiotics, through the use of a diagnostic kit for negative and positive bacteria. For the Gram stain, each sample requires two kits, one for the ID diagnosis and the other for the AST examination, as its Reagent card contains 64 holes, Each hole contains a dried medium and a color guide in which biochemical tests for the microscopic organism are conducted, according to the instructions attached to the device's examination kit. The device records the color changes occurring as a result of the growth of the bacteria to be diagnosed, while the antibiotic susceptibility test kit contains 20 antibiotics, as there are for each antibiotic. More than one concentration, and the device records the changes occurring as a result of bacterial growth by observing turbidity, by following the following steps: -

A - *E. coli* and *P. aeruginosa* bacteria were grown on Macconkey agar medium, while *S. aureus* bacteria were grown on Manitole agar medium using the planning method and incubated at 37°C for 24 hours.

B- Put 3 ml of Normal Salin into the tubes of the device and transfer a single colony to each tube.

T- Prepare a Densichek turbidity measuring device and zero the device on the neutral salt solution, then place the tubes containing the bacterial suspension with a turbidity ranging between (0.5 - 0.63) in the device.

D- The card was taken out of the cover and then placed in the test tubes.

C - Turn on the Vitek-2 device, then place the card in the filler door, which is deflated, then close the door and press the Start filler button to pull the bacterial suspension from the tubes into the card, and the process continues for 70 seconds.

H - The test results were displayed electronically. The device calculated the results and compared them with the results stored in the device, which included a number of test measurements. Finally, the device showed the test results within 6 hours.

2.3:Antibacterial activity of silver nanoparticles Ag NPs

In this study, the bacterial species *E.coli*, *P.aeruginosa*, and S.aureu were used. In order to evaluate the effectiveness of silver nanoparticles against these species, the Agar well diffusion method was used according to the method of (Skogman *et al*., 2016):

A- Prepare Mueller Hinton agar medium, pour 25 ml into Petri dishes, each dish, and leave until set.

B- *E. coli* and *P. aeruginosa* bacteria were activated on Macconkey agar medium, while *S. aureus* bacteria were activated on Manitole agar medium, after which they were placed in the incubator for 24 hours at a temperature of 37°C.

C- The bacteria were spread on a Petri dish containing Muller Hinton agar using a sterile cotton swab.

D - Wells holes with a diameter of 8 mm were made in each dish using a sterilized Cork borer, with five holes for each dish.

C - 100 microliters of silver nanoparticle concentrations, including the following concentrations (0.5, 1, 1.5, 2) mg/ml, were added to each hole using a small volumetric pipette and with great care to avoid scattering the extract on the surface of the culture medium. The fifth hole represents the control hole as it contained On distilled water.

H - The dishes were incubated at a temperature of 37°C for 24 hours, after which it was observed that there was a transparent halo around each hole, and this represents the diameter of the inhibition zone. The diameter of the inhibition zone was then measured using a ruler by taking the average of two perpendicular diameters measured in millimeters, and the above-mentioned steps were repeated. Twice, then the average damping diameters were taken.

2.4: Antibiotic sensitivity test

Disc diffusion methods were used on Mueller Hinton agar medium, and according to what was mentioned by (Josephine *et al*., 2003) and my agencies:

A - A bacterial suspension was prepared from the bacterial isolates whose sensitivity to antibiotics was to be tested. A small number of pure colonies, 24 hours old, were transferred to 3 ml of physiological saline solution, and compared with a standard constant turbidity solution.

B- Spread an amount of the bacterial suspension on the surface of the dishes containing Mueller Hinton Agar medium using a cotton swab, then leave the dishes for 15 minutes at laboratory temperature.

T - The antibiotic tablets were transferred using sterile forceps, 8 tablets were placed in each dish, and these dishes were incubated at a temperature of 37°C for 24 hours.

D - The results were read by measuring the zones of inhibition formed around the antibiotic tablets and interpreted as whether the bacteria were resistant or sensitive.

3: Results and Discussion

3.1:Characterization of bacterial isolates used in the study The results of culture diagnosis of *E.coli* bacteria showed bright pink colonies with a mucous consistency, which is a distinctive characteristic of them when grown on Macconkey agar medium (Figure 1), as it was noted that they were able to ferment the sugar lactose (Riedel *et al*., 2020).

Figure (1) *Escherichia coli* bacteria on Macconkey agar medium.

The results of culture diagnosis of *P.aeruginosa* bacteria on Macconkey agar medium showed pale-coloured colonies as in Figure (2) on this medium due to their inability to ferment the sugar lactose (Tille, 2014).

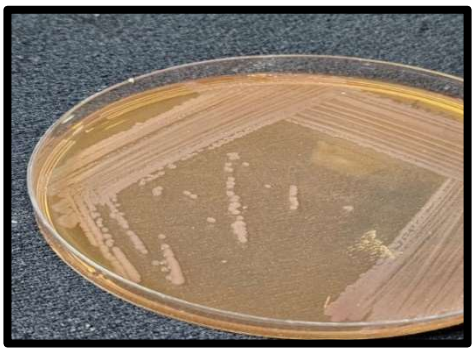

Figure (2) *Pseudomonas aeruginosa* bacteria on Macconkey agar medium .

The results of culture diagnosis of *S.aureus* bacteria also showed yellow, circular colonies of medium size when grown on Manitole salt agar medium, as it was observed that they were able to ferment mantole sugar and change the color of the medium (Figure 3) from pink to yellow (Rasigade and Vandenesch, 2014).), and although manthol medium is considered a differentiation medium to distinguish the *S.aureus* bacteria that produce the enzyme Coagulase from other types of bacteria that do not produce it, some bacterial species belonging to the CONS group have the ability to ferment mantol sugar and change the color of the medium (Simor *et al*., 2001).

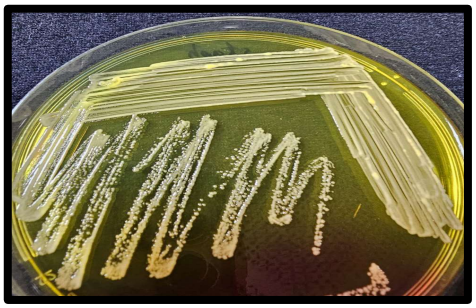

Figure (3) *Staphylococcus aureus* bacteria on Manitole salt agar medium.

3.2: Antibacterial activity of silver nanoparticles Ag NPs by well diffusion method

The results of the statistical analysis in Table (1) showed that there were significant and non-significant differences between the bacterial species and the concentration. The highest value of the inhibition diameter rates was recorded at the concentration of 2 mg/ml for all bacterial species, as it reached 17.33 mm. The lowest value of the inhibition diameter rates was recorded at the concentration. 0.5 mg/ml for all bacterial species, and it reached 10.77 mm. The statistical results also showed that the bacterial species with the most inhibition effect was *S.aureus*, as its inhibition diameter rates reached 15.24 mm, and the lowest inhibition diameter rates were recorded in *E.coli*, which amounted to 12.58 mm, as shown. In Table (1) and Figure (4).

Bacterial species	**The concentration used is mg/ml**					**Average**
	control	**0.5**	**1**	**1.5**	**2**	
E.coli	0.0±0.0	10.67±0.67	11.67±0.88	13±0.58	15±0.58	12.58
P. aeruginosa	0.0±0.0	10.33±0.33	12.33±0.33	14.67±0.88	17.67±0.33	13.75
S.aureus	0.0±0.0	11.33±0.33	14.0±1.00	16.33±1.45	19.33±0.88	15.24
Average	0.0±0.0	10.77	12.66	14.66	17.33	

Figure (4) Antibacterial activity against *E.coli*, *Pseudomonas aeruginosa* and S*taphylococcus aureus* for different concentrations of silver nanoparticles manufactured using *Spirulina platensis* algae extract.

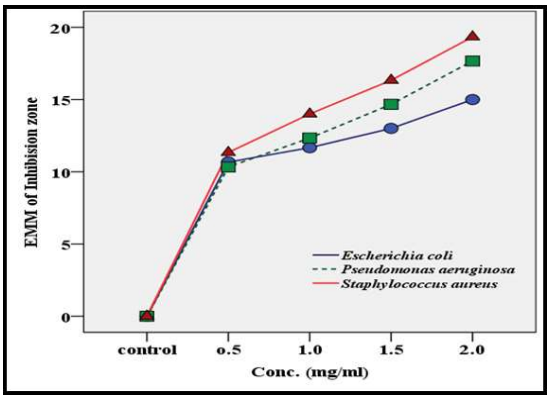

Figure (5) Estimation of the average diameters of inhibition for bacterial isolates with different concentrations of silver nanoparticles manufactured using the algae extract *Spirulina platensis*.

The results of the study showed the effectiveness of silver nanoparticles produced by the algae extract *S. platensis*, their inhibitory effectiveness on bacteria at concentrations of 0.5, 1, 1.5, and 2 mg/ml, as shown in Figure (4). Table (1) shows that the average diameter of inhibition for *E.coli* bacteria was 12.58 mm and for *P.aeruginosa* bacteria was 13.75 mm, while for *S.aureus* bacteria it was 15.24 mm. The results also showed that there was no inhibitory activity for the control solution (non-ionized distilled water).

The results of the current study showed that the inhibitory effectiveness of silver nanoparticles against Gram-positive bacteria *S.aureus* is higher compared to Gram-negative bacteria *E.coli* and *P.aeruginosa*, and this is consistent with the findings of several researchers, including Mohammed (2019); Mohammadi *et al*.,(2019); Aritonang *et al*.,(2019); Rautela *et al*.,(2019). The differential sensitivity to silver nanoparticles between Gram-positive and Gram-negative bacteria may be attributed to structural differences between the cell walls (Kedziora *et al*., 2018), or to diversity in resistance mechanisms and the presence and strength of a silver-resistant plasmid containing the *Sil* gene (Sutterlin). ,2017).

Silver nanoparticles have the ability to release Ag+ ions, and these ions interact with the thiol group in bacterial proteins, affecting the role of DNA and destroying bacteria. They also have the ability to reach the bacterial cell wall and work to inactivate some enzymes in it and produce hydrogen peroxide (H2O2). (Patra and Beak, 2017). Nanoparticles with large surface area provide the best contact with microorganisms, and therefore these particles are able to penetrate the cell membrane or attach to the surface of bacteria based on their size. In addition, it was found to be highly toxic to bacterial strains and its antibacterial efficiency increased by reducing the particle size (Rai *et al*., 2009; Agnihotri *et al*., 2014).

The presence of this antibacterial activity indicates and confirms the existence of compounds that have the ability to inhibit bacteria, and this is consistent with research that indicated the ability of algae to produce compounds produced by effective secondary metabolism that have the ability to control bacterial growth (Faulkner, 2002), including alkaloids. They are natural compounds that

are produced as secondary metabolites in microorganisms. They are believed to have a role in defending the cells of the organisms that produce them. They are distinguished by their therapeutic efficiency and their importance from a medical standpoint, as the compounds bind to the DNA of microbes, which gives them a high inhibitory ability against many Gram-positive and Gram-negative bacteria (Kandhasamy). and Arunachalam, 2008).

3.3:Investigation of the sensitivity and resistance of bacterial isolates using the discs method

One of the most dangerous problems facing the world from a medical standpoint is that bacterial species are resistant to antibiotics, which has resulted in difficulty in choosing the appropriate treatment for patients, in addition to the indiscriminate use of antibiotics without conducting a sensitivity test, which in turn makes the bacteria able to adapt and thus increase their resistance. For antibiotics used for treatment, the results of the current study showed that there was a difference between bacterial species in their resistance to antibiotics, as the results of drug sensitivity testing using the tablet method showed that all bacterial isolates were resistant to the antibiotics Amoxicillin, Chloramphenicol, Ciprofloxacin, Clindamycin, Trimethoprim/Sulfamethoxazole and Tetracyclin by 75%. %, as for the antibiotics Imipenem and Piperacllin/Tazobactam, it was sensitive to them by 25%.

The bacterial isolates resisted the group of beta-lactam antibiotics represented by Amoxicillin. The beta-lactam antibiotics interfere in the process of manufacturing the peptidoglycan layer and thus inhibit the process of forming the cell wall of the bacteria. The bacteria resist the beta-lactam antibiotics by secreting beta-lactamase, which works to break the beta-lactam ring in the group of cephalosporins and penicillins. (Kolar *et al*., 2010). As for the antibiotic Chloramphenicol, all isolates were resistant to it.

As for the antibiotic Tetracyclin, all isolates were resistant to this antibiotic. This antibiotic inhibits the protein synthesis process by binding the antigen to the S30 ribosomal unit after it enters the bacterial cell and completely hinders the translation process, which leads to the inhibition of protein synthesis (Gales *et al*., 2005). Resistance is achieved. This antibiotic is administered by bacteria in several ways, the most important of which is changing the ribosome binding site by means of a soluble protein or by enzymatic inhibition (Nester *et al*., 2001).

As for the antibiotic Ciprofloxacin, all isolates showed resistance to it, as it belongs to the family of fluoroquiolones that target the DNA gyrase and topoismerase enzymes, and the emergence of resistance to these antibiotics is due to the occurrence of mutations in the topoismerase enzyme, which reduces the efficiency of antibiotic binding and the high expression of endogenous efflux pumps (Foster, 2017). The results also showed that all bacterial isolates were sensitive to the antibiotics Piperacllin/Tazobactam and Imipenem. As for Clindamycin, it works to inhibit bacterial protein synthesis by binding to the 23S ribosomal unit of the 50S subunit of the bacterial ribosome. This disrupts the ribosome assembly and translation process, halting bacterial growth (Dallo *et al*., 2023).

All isolates are resistant to the antibiotic Trimethoprim/Sulfamethoxazole. This antibiotic inhibits bacterial growth because it inhibits the synthesis of dihydrofolic acid. Bacteria can resist this antibiotic by modifying the target site on which the antibiotic acts (Fraser *et al*., 2010).

Figure (6) Antibiotic susceptibility test for bacterial isolates on culture media

A - *Escherichia coli* **B-** *Pseudomonas aeruginosa* **C-** *Staphylococcus aureus*

3.4:Investigation of the sensitivity of isolates to antibiotics using the Vitek-2compact system and the AST test

For the purpose of ascertaining and knowing the minimum inhibitory concentration of the antibiotics used and knowing the resistance and sensitivity of the isolates to the rest of the types of antibiotics that were not initially available in the initial susceptibility examination, so they were detected using the Vitek Card device of AST.

The results shown in Table (2) of the results of testing the sensitivity and resistance of bacterial isolates to antibiotics showed that *E.coli* bacteria were resistant to the antibiotics Ampicillin, Piperacillin/Tazobactam, Cefazolin, Cefoxitin, Ceftazidime, Ceftriaxone, Cefepime, Imipenem, Ciprofloxacin, Levofloxacin, Trimethoprim/Sulfamethoxazole. At a rate of 73.3%, as for the antibiotics Amikacin, Gentamicin, Tigecycline and Nitrofurantoin, it was sensitive to it at a rate of 26.7%. As for the *P. aeruginosa* bacteria, it was 100% resistant to all antibiotics used. Including the antibiotic Amikacin, which belongs to the group of aminoglycosides. Bacteria resist this antibiotic through several mechanisms, the most important of which is the occurrence of a chromosomal mutation in the gene responsible for protein synthesis, or modification of the antigen and secretion of a group of enzymes specific to the antigen molecule, or by reducing the cell's permeability to the antibiotic (Levinson, 2014). *S.aureus* bacteria resisted the antibiotics Benzylpenicillin, Oxacillin, Ciprofloxacin, Rifampicin, Erythromycin, Clindamycin, Tetracycline and Fusidic Acid by 57.1%, The latter inhibits protein synthesis by binding to the elongation factor EF-G-GDP, which participates in the protein translation process and thus inhibits the process of peptide transfer and ribosome disassembly. *S.aureus* bacteria resist this antibiotic when a mutation occurs in the Fus A gene. Some mutations can also lead to to a high level of resistance to antibiotics (Fernandes, 2016). As for the antibiotics to which she was sensitive, they included Moxifloxacin, Linezolid, Teicoplanin, and Trimethoprim/Sulfamethoxazole, at a rate of 42.9%.

Table (2) Percentage of resistance and sensitivity of bacterial isolates to antibiotics used in the AST test.

Bacterial species	Sensitive		Resistant		Total	
	Count	(%)	Count	(%)	Count	% within Bacteria
E.coli	4	26.7%	11	73.3%	15	100.0%
P. aeruginosa	0	0.0%	9	100.0%	9	100.0%
S. aureus	6	42.9%	8	57.1%	14	100.0%
	10	26.3%	28	73.7%	38	100.0%

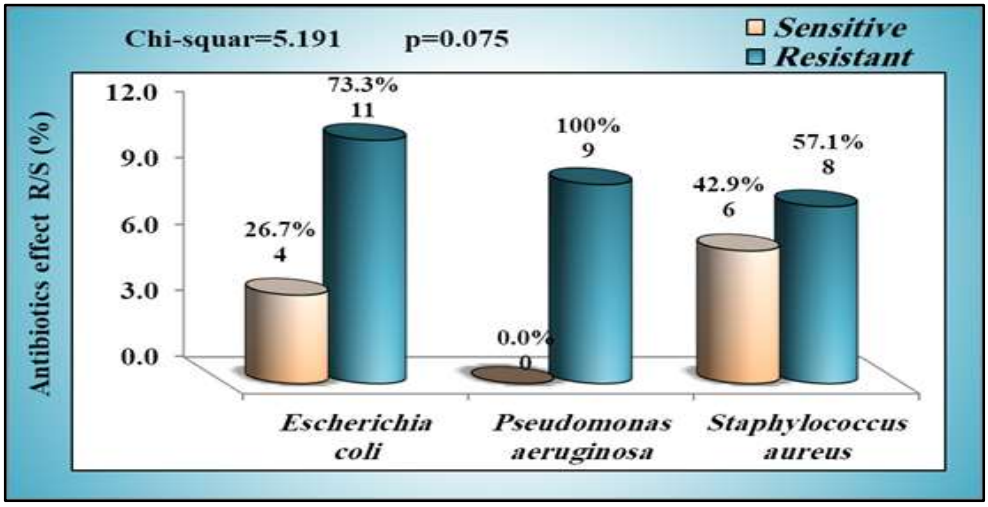

Figure (7) Percentage of resistance and sensitivity of bacterial isolates to the antibiotics used in the AST test.

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