Plant Archives Volume 20 No. 2, 2020 pp. 3929-3936 **e-ISSN:2581-6063 (online), ISSN:0972-5210**

BIOSYNTHESIS OF GOLD NANOPARTICALS FROM LOCAL *PROTEUS MIRABILIS* P242 STRAIN AND SOME BIOLOGICAL APPLICATION

Lubna Abdulazeem

DNA Research Center, University of Babylon, Iraq

Abstract

In recent years, the orientation of an eco-friendly pathways for the produced and synthesis of nanoparticals is of importance part in science research of nanotechnology. In this report the uses an extracellular method as a greener safety way for the synthesis (AuNPs) gold nanoparticles by using local *Proteus mirabilis* P242 strain. The addition of hydrogen tetra chloro aurate (HAuCl,,) solution directly into a cell-free extract (CFE) of *Proteus mirabilis* P242 strain resulted in to the synthesis and production of (AuNPs) gold nanoparticles at room temp. ($37R^0C$) within 24 hrs. The nanoparticles obtained were characterized many techniques include: X-ray diffraction spectroscopy (XRD) analyses, Fourier Transform Infrared (FTIR) Measurements, scanning electron microscopy (SEM) and Energy dispersive X-ray (EDX) spectroscopy. Our results indicated that *Proteus mirabilis* P242 strain can bio-synthesizes extracellular GNPs in the range of 21-27 nm. Biosynthesized AuNPs showed antibacterial activity against clinical MDR bacteria (*Staphylcoccus aureus* and *Pseudomonas aeurogenosa*), Also, antibiofilm activity of AuNPs against the same microorganism used in the antibacterial activity are studies and cytotoxic activity of AuNPs are estimation by Comet assay and showed that synthesized GNPs induced DNA damage in bacteria under tested.

***Key words*:** Gold nanopartical, Antibacterial activity, Biofilm, Genotoxic, Comet assay.

Introduction

Metal nanoparticles is very importants in the field of scientific interest due to their properties: chemical, electronic and optical. Among of them particals, (AuPNs) gold nanoparticles have received great attention in the newly years due to their properties by greatly stability, spectroscopic, electronic depending on size and optical properties (Njoki *et al.*, 2007). Gold nanoparticals widely applied in different fields and science (Saha *et al.*, 2012; Versiani *et al.*, 2016). Nanoparticles can be synthesized by Physical, chemical and biological methods. Gold nanoparticles (AuNPs) are synthesized by using methods of bottom-up procedure like methods of chemical reduction, by using the protective, stabilizing and reducing agents. Mentioned agents are always toxic and flammable (Rai *et al.*, 2011), prospect adsorb usually on the surface of nanoparticals and may have safty effects when used in biological applications (Philip, 2010). For these reasons, biological approaches for the produsd and synthesis of (AuNPs) gold nanoparticles it is the best way are used. The eco-friendly organisms are provides an easy way to get on non-toxic, non-hazardous and clean metal nanoparticles.

Among different microbes, bacterium is always been an organism of choice to produce nanoparticals due to its inherent properties to produce different types of enzymes for chemical detoxification and energy-dependent ion efflux, responsible for reduction and stabilization of metallic nanoparticles (Nishant and Mausumi, 2014), easy treatment methods, eco-friendly are ease of disposal and downstream method (Velusamy *et al.*,2016). Gold nanoparticals (AuNPs) synthesis by using various strains of bacterial such as, *Bacillus* sp., *B. clausii*, *B. amyloliquefaciens*, and *Azoarcus* sp. has been documented (Elbeshehy *et al.*, 2015).

Among the inorganic metal nanoparticles as antibacterial, gold nanoparticles are power to kill both (G-ve) Gram- negative and (G†ve) Gram- positive bacteria, and shown capability effective against multi drug-

***Author for correspondence :** E-mail: albayatilubna@yahoo.com

resistant bacteria (MDR) (Zhu, 2014). The synthesized AuNPs are strongly inhibited pathogenic biofilm formation and invasion bacteria, and supported host immune response. Moreover, these inhibitory effects are associated with the electrostatic attractions between AuNPs and their targeted cells (Yu-Han *et al.*, 2016). The toxic action of metal nanoparticles against pathogenic bacterial cells and their biofilm has been related to the reactive oxygen species (ROS) are generation and bacterial membrane disruption (Lemire *et al.*, 2013).

Nanoparticals can lead to the genotoxicity that due to the direct effects of nanoparticles with the genomic material (DNA) or indirectly by increased synthesis of (ROS) reactive oxygen species by cellular components due to their effects of nanoparticles (Donaldson *et al.*, 2010). According surface properties their are tow pathways, like, transition metals are presence via surface, mobilization of intracellular iron, or processes of lipid peroxidation. From other hands related to primary genotoxicity by nanoparticle, the size, shape, nanoparticle exposure, and the presence of mutagens found on the or with the nanoparticles. Single cell gel electrophoresis assay is the one of the commonly DNA damage assays, also called as a comet assay**.** pH are used is the basis of the comet assay work, for this, the assay can able to detect a large variations of DNA damage like incomplete excision repair sites, single- and double-strand breaks, cross-links [by decreased comet tail], alkali-labile sites [e.g., a basic sites] that by using enzymes of lesion-specific and lesions of oxidized DNA (Bowman *et al.*, 2012). Virtually any type of cell with a nucleus can be used, both cell- and tissue- can used comet assay to measured the specific DNA damage induced by any nanoparticas. Comet assay is a informative and suitable procedure for study the genotoxicity (Vandghanooni and Eskandani, 2011). Gold nanoparticles (AuNPs) stimulate in vitro cyto- and genotoxicity (Plotnikov *et al.*, 2017).

In this study, a novel bacterial strain, *Proteus mirabilis* P242 strain, was able to synthesize AuNPs as extracellular synthesis. The characterization of AuNP synthesis were investigated by XRD, FTIR and SEM-EDS, then antimicrobial, antibiofilm and genotoxic activity are study.

Material and Method

Bacteria *Proteus mirabilis* P242 strain identification by 16s RNA was obtained from hospital floor. Diagnosis in Microbiology Lab Biology Department, College of Science, University of Babylon, Iraq, and confirmed diagnosis of bacteria was done according to (MacFaddin, 2000 ; Forbes *et al.*, 2007).

Solution and media

Hydrogen tetra chloro aurate (HAuCl,,), brain heart infusion Agar and broth medium, antibiotics disk, Ethidium Bromide and other chemical solution and reagents were purchased from Merck Germany.

Biosynthesis of (AuNPs) Gold nanoparticles

Extracellular biosynthesis of AuNPs (Gold nanoparticles), taken two flasks, the supernatant of *Proteus mirabilis* P242 putting in the one (as acontrol) and the other flask containing 10^{-3}mM Hydrogen tetra chloro aurate (HAuCl,,) solution and the supernatant of *Proteus mirabilis* P242 as test solution that were incubated at room temperature on shaker during 24 hrs. After 24 hrs., purified gold nanoparticals solution in the obtained supernatant are cell free by repeated cooling centrifuge at 15,000 rpm for 20 min. using cold centrifuge. Supernatant was removed and the pellet was dissolved in deionized water (Thirumurugan *et al.*, 2012). The detection of AuNP by the UV–Vis diffuse reflectance measurements, X-Ray diffraction measurements (XRD), Fourier Transform Infrared (FTIR), Measurements the Field Emission-Scanning Electron microscopes (FE-SEM) and (EDX) measurements.

Determination the Minimum Inhibitory Concentration (MIC) and Minimum Bactericidal Concentration (MBC)

Human pathogenic bacteria, G–ve bacteria (three isolates of *Pseudomonas aeruginosa*), and G+ve bacteria (three isolates of *Staphyllococcus aureas*) that were maintained on nutrient agar slants, were incubated at 37°C overnight, and which were used to preform 0.5 McFarland. The total of 10 ml tube from nutrient broth medium was prepared, and then, each sample of bacteria was inoculated aseptically with 1 ml of the bacterial suspension (about 108 colony-forming unit/mL). Five dilutions of AuNPs were prepared (500, 250, 125, 62.5, and 31.25 ¼g/ml) in sterile deionized water and a negative control (without AuNPs) was used. Each isolate was tested performed in triplicates using multiplate count. After 37°C overnight the MIC always determined by using the spectrophotometry at wavelength 600 nm (Frey and Meyers, 2010). Wells showed that no turbidity was cultured on nutrient agar plates and incubated at 37°C overnight. Bacterial colonies growth was checked and the MBC value was recorded by shows the concentration that no growth.

Anti-Bacterial Activity of AuNPs against Multi Drug Resistant Pathogenic Bacteria

Antibacterial activity of AuNPs bio-synthesized by *Proteus mirabilis* P242 strain were used to evaluate their

ability for inhibition growth of MDR bacteria under study. Now after choosing the (MIC) and (MBC) concentrations this need always to determine the concentration by using a test that was dependence on the protocol explained in references, by using a sterile, disposal petri dishes with diameter (90 mm) containing sterile (MHA) Mueller Hinton agar medium (15 ml). Antibacterial activity of AuNPs was determined by agar well diffusion method that according to instructions of (NCCLS) National Committee for Clinical Laboratory Standard (CLSI, 2016).

Anti-Biofilm Activity of AuNPs against Pathogenic Bacteria Biofilms Formation

Bacteria isolate are incubating overnight at 37°C on blood agar plates and this plate was stored at 4°C. The bacterial biofilm formation are tested according to the Christensen *et al.*, 1985 (Christensen *et al.*, 1985). The classification of bacterial biofilm formation by multi well plate method in to three categories: Weak (BF<0.120), Moderat (0.120>BF ≤0.240), and Strong (BF >0.240) at OD. Value 630 nm. Data are documented as removing completely and incompletely in the biofilm bacterial growth with presence of GNPs and compared with the absence of AuNPs (control).

Genotoxic Activity and DNA damage by AuNPs (Comet assay)

This assay has been done according to [OxiSelect™ Comet Assay Kit (3-Well Slides) Cat. No. STA-350] used to identify DNA damage by AuNPs as a flawing (Kumari *et al.*, 2008).

Tail length = length of tail measured by ocular micrometer (µm)

DNA Tail Moment (µm) = average order to program of comet assay. Comet Assay Index = (width of head / length of tail).

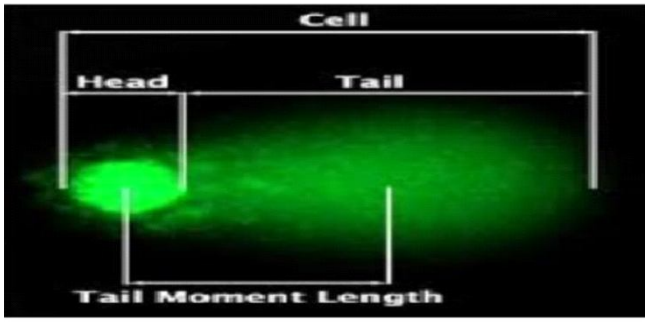

Fig. 1: A typical comet showing analysis cell with head and Schematic representation of the comet assay was shown in (Fig. 1).

(Kumari *et al.*, 2008)

Comet length, Tail length and Tail moment was used as the measure of DNA damage Fig (3-6).

Classes of damage = according to comet assay index.

1-2 (Low damage)

2-3 (Medium damage)

Up 3 (High damage)

Results and Discussion

The ability of *Proteus mirabilis* P242 to synthesis the AuNPs this agree with many studies on biosynthesis of AuNPs by microorganisms *Pseudomonas* (Husseiny *et al.*, 2007) *Lactobacillus* (Nair and Pradeep, 2002) by reduction of chloroaurate ions into AuNPs. The formation of nanoparticle was confirmed faristly from color chanching and purple color are appearance from the pale yellow color (Fig. 2) studies (Mohamed and Sherif, 2012). The important reasons of biological metal nanoparticles synthesis that depend on NADH-and NADH depending enzymes, and the reduction reaction to be started and appears by transfer the electron from the NADH by NADH-dependent reductase as electron carrier. XRD was used to detect the gold nanoparticles and the peak of The XRD spectrum resulted in four intense peaks in the spectrum in position (38), (45), (66) and (83), (Fig. 3) these agree with) Mohamed and Sherif, 2012). Also confirmed the AuNPs by FTIR spectrum to indicates the presence of various chemical groups, one of which is an amide. The –COO- possibly also presence due to amino acid residues that indicate co-exists of protein with the AuNPs wavelength range of 4000 – 400 cm found the spectra concerned 1900 and 3700 (Fig. 4) this agree with (Honary *et al.*, 2012). The Field Emission – Scanning Electron Microscopy. (Fig. 5) indicated that the size of nanoparticles are between 21.07 - 27.28 nm. In another study found AuNPs that biosynthesis by *E. coli* was 11.8 and 130 nm (Honary *et al.*, 2012).

-A - **-B-** **-C-**

Fig. 2: AuNPs biosynthesis– **A-** Nutriant broth with bacteria (negative control), **B-** Positive results color change AuNPs formation, **C-** Precipitate AuNPs from supernatants *Proteus mirabilis* P242

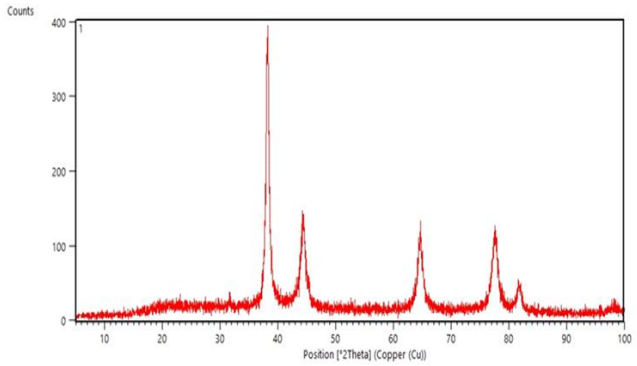

Fig. 2: XRD of AuNPs synthesis by *Proteus mirabilis* P242

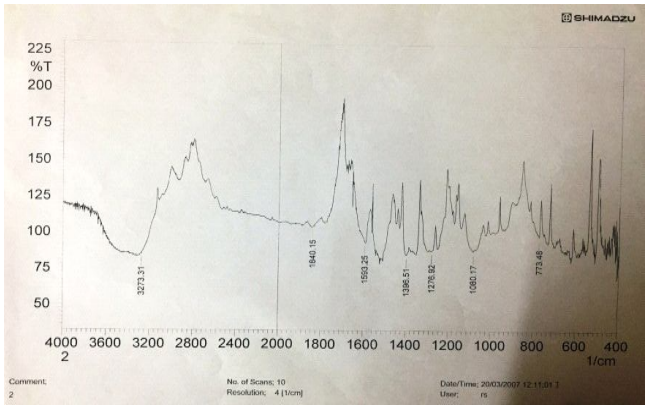

Fig. 3: FT-IR of AuNPs synthesis by *Proteus mirabilis* P242

Antibiotic Sensitivity of *Pseudomonas aeruginosa* and *Staphyllococcus aureas* as a Multi-Drug Resistance

Lists of antibiotic susceptibility testing were done according to the Clinical Laboratory Standards Institute (CLSI), the United States Food and Drug Administration (FDA) and the European Committee on Antimicrobial Susceptibility Testing (EUCAST). This study included antibiotic sensitivity for bacteria under study an six types of antibiotics were selected to test of the susceptibility of the isolated bacteria which are Cefotaxime, Amoxicillin, Bacitracin, Aztreonam, Ampicilin and Sulphamethoxazole, as in table 1.

The results was analyzing sensitivity pattern of 2- isolates of *P.aeruginosa* and 3- isolate of *Staph. aureus* were resistance of 100% to the six type of antibiotics are used, and only one isolate of *P.aeruginosa* is resistance of 33.33% with Cefotaxime and Sulphamethoxazole showed in table 1. *P.aeruginosa* and *Staph.aureus* in this study was multidrug resistance (MDR) because resist more than one type of antibiotic, MDR was defined as acquired non-susceptibility to at least one agent in three or more antimicrobial categories, multidrug resistance in bacteria this may be occured by the action of multidrug efflux pumps which can pump out of cell more than one type of drug and/or by the resistance (R) plasmids genes and/or transposons of genes are accumulation in each coding for drug resistance the specific agent (Handuz, 2019).

Determination Minimal Inhibitory Concentration (MIC) and Minimal Bacteriocedal Concentration (MBC) Test

The antibacterial activity of biosynthesis AuNPs were tested against three multi drug (G-ve) Gram-negative *P. aeruginosa*, and three multi drug (G†ve) Gram-positive *Staph. aureus*. Focused to determine the MIC and MBC concentration for nanoparticles prepeared by using method of broth micro dilution and then after incubation the spectrophotometry with wavelength (600 nm).

From Table 2, it's clear that the MIC concentration 31.25 and 62.5 µg/ml of bio GNPs synthesis from Proteus mirabilis P242 was found effective on the sixth (MDR) multi drug resistance bacteria at the same manner that indicate and reflect that this con. is a typical concentration for bacterial inhibition. On the other hand, the MBC concentration limited between 250 and 500 µg/ml of bio AuNPs were recorded as the concentration of bactericidal in all isolate under recent study. The results of this study showed that the AuNPs have considerable inhibitory effect on bacteria isolates, because the capacity of AuNPs to inter acted with the bacterial cell wall and ruptured him, disturbing the metabolism of bacteria by effecting on bacterial DNA and interaction with mitochondria and other organelles of bacteria (Khan *et al.*,2019).

AuNPs as Antibacterial Activity

Antibacterial activity of AuNPs bio-synthesized from

Table 1: Antibiotic sensitivity of bacterial isolate from different sources

Bacteiral strain	CTX-30 µg/ml	AMC-30 µg/ml	B-10 µg/ml	ATM-15 µg/ml	AM-10 µg/ml	SXT-25 µg/ml
P.aeruginosa	I	R	R	R	R	I
P.aeruginosa	R	R	R	R	R	R
P.aeruginosa	R	R	R	R	R	R
Staph.aureus	R	R	R	R	R	R
Staph.aureus	R	R	R	R	R	R
Staph.aureus	R	R	R	R	R	R

I= Intermediate R= Resistant S= Sensitive

CTX = Cefotaxime, AMC = Amoxicillin, B = Bacitracin, ATM = Aztreonam, AM = Ampicilin, SXT = Sulphamethoxazole

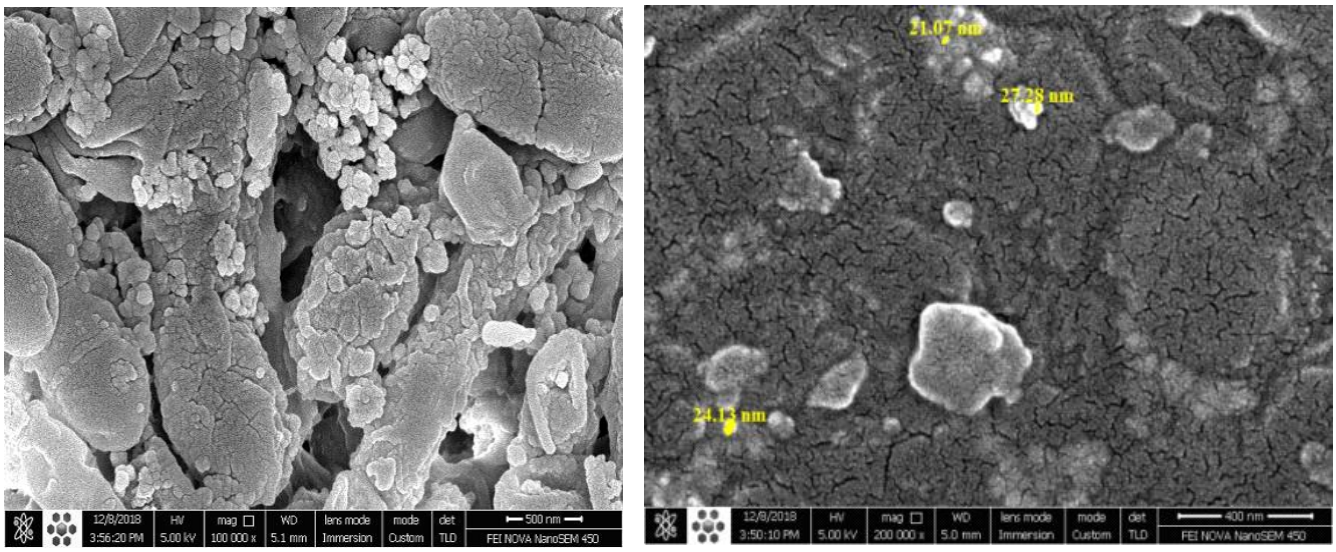

Fig. 4: FE-SEM of AuNPs synthesis by *Proteus mirabilis* P242 with 21.07 - 27.28 nm in size and nanorodes in shapes

Table 2: Minimum Inhibitory Concentration and the Minimum Bacteriocidal Concentration (µg/ml) of AuNPs

***Proteus mirabilis* P242 AuNPs**		
MBC	**MIC**	**Bacterial Isolate**
500	31.25	*P. aeruginosa*
250	31.25	*P. aeruginosa*
500	31.25	*P. aeruginosa*
500	62.5	*Staph. aureus*
500	62.5	*Staph. aureus*
500	31.25	*Staph. aureus*

Proteus mirabilis P242 were used to evaluate their ability for inhibition growth of some clinical bacteria characterized as a MDR after choosing the MIC and MBC concentrations. All prepared plates were incubated at 37°C for 24 hr., and then measured the diameters in each inhibition zones. We can measured the inhibition of bacterial growth by (mm) as diameters of zone at 3- points are equidistant that measured from the center in each inhibition zone, in all observation, then the average value was taken, as in table 3:

Table 3: Antimicrobial Activity of (MIC) Minimum Inhibitory Concentration (MIC Values in µg/ml) of *Proteus mirabilis* P242 AuNPs against *P. aeruginosa* and *Staph. aureus*

Mean(mm)±Std	**Concentration µg/ml**	**Bacterial Isolate**
1.00000±18	31.25	*P. aeruginosa*
7.637±18.8	62.5	*P. aeruginosa*
1.0000±19	62.5	*P. aeruginosa*
LSD= 1.943		
1.00000±13	31.25	*Staph. aureus*
0.76376±15.8333	62.5	*Staph. aureus*
2.08167±13.3333	31.25	*Staph. aureus*

Table 3 show largest inhibition zone of Proteus mirabilis P242 AuNPs in Gram negative bacteria was 19 mm in P.aeruginosa with MIC concentration 62.5 µg/ml and shows significant differences (P=0.05) in AuNPs were used against three strain of *P. aeruginosa*, while show largest inhibition zone of AuNPs on Gram positive bacteria *S. aureus* was 15.8 mm with MIC concentration 62.5 µg/ml and shows no significant differences (P=0.05) in AuNPs were used against three strain of S.aureus. The antibacterial activity of AuNPs beyond to the many mechanisms. The chief mechanism are suggest to generation of oxidative stress by action of ROS (that replacement each ion of Au^{2}† are results when released one of free electron) (Sathyanarayanan *et al.*, 2013). Many type of ROS, these includes hydroxyl radicals, superoxide radicals, single oxygen, and hydrogen peroxide that lead to chemical destroy and damage to DNA and proteins in bacteria cells (Lee *et al.*, 2008). Also, interactions of electrostatic between cell membranes in bacteria or proteins in cell membrane and nanoparticles may lead to the physical damage, then finally leads to death of bacterial cell (Sathyanarayanan *et al.*, 2013). Other studies showed that the nanoparticles with a small size may be lead to their antibacterial activites (Mahmoudi *et al.*, 2011). Lee *et al.*, 2008 reported that disruption of *E. coli* because penetration of nanoparticles with small sizes ranging from 10 to 80?nm into *E. coli* membranes. Similarly, another study showed that AuNPs as excellent antibacterial effect against (G-ve) Gram-negative bacteria *E. coli* and (G†ve) Gram-positive bacteria Bacillus. This study also demonstrated that the antibacterial activity of nanoparticals could be due to the uptake of single AuNPs by bacteria changing their surface modifications agents and rearrangement of these nanoparticals inside

cytoplasm (Zhou *et al.*, 2012).

Antibiofilm Activity of AuNPs by Multi-well Plate Count Method

In this study the same 6-isolates was used all of them are MDR bacteria, three of them are Gram negative bacteria (*P. aeruginosa*) and other three are Gram positive bacteria (*Staph. aureus*) and tested to producing biofilm, all tested microorganisms showed their ability to form biofilm in the form of film lined the wall and bottom of wells in the multi-well plate method. The results appeared strong biofilm formed by *P. aeruginosa* and *Staph. aureus*, (Tables 4).

Table 4: Antibiofilm Activity of (MIC Value in µg/ml) of *Proteus mirabilis* P242 AuNPs Towered *P. aeruginosa* and *Staph. aureus.*

Biofilm formation with AuNPs	Biofilm formation with out GNPs	Bacterial Isolate
Non	Strong	*P. aeruginosa*
Non	Strong	*P. aeruginosa*
Non	Strong	*P. aeruginosa*
Non	Moderate	*Staph. aureus*
Non	Strong	*Staph. aureus*
Moderate	Strong	*Staph. aureus*

The AuNPs from *Proteus mirabilis* P242 were prevented the formation of biofilm in some bacterial isolates while some isolates is inhibited but not preventing the production of biofilm in a dose depending manner. Anti-biofilm effects of AuNPs results by inhibition of exo-polysaccharide formation and shown that metallic nanoparticles reduced exo-polysaccharide formation, then

Table 5: The criteria of DNA in *P.aeruginosa* and *Staph. aureus* exposed to GNPs according to comet assay, Data represent the Mean(µm)± SD.

Tail moment	Tail length	Comet length	
0.17205±0.6707	1.73205±6.200	3.6055±52.6200	**Control**
3.000±36.3665	3.000±63.5600	3.60555±247.38	**AuNPs on** *P. aeruginosa*
4.000±22.7887	6.000±36.3200	4.000±160.841	**AuNPs on** *Staph. aureus*

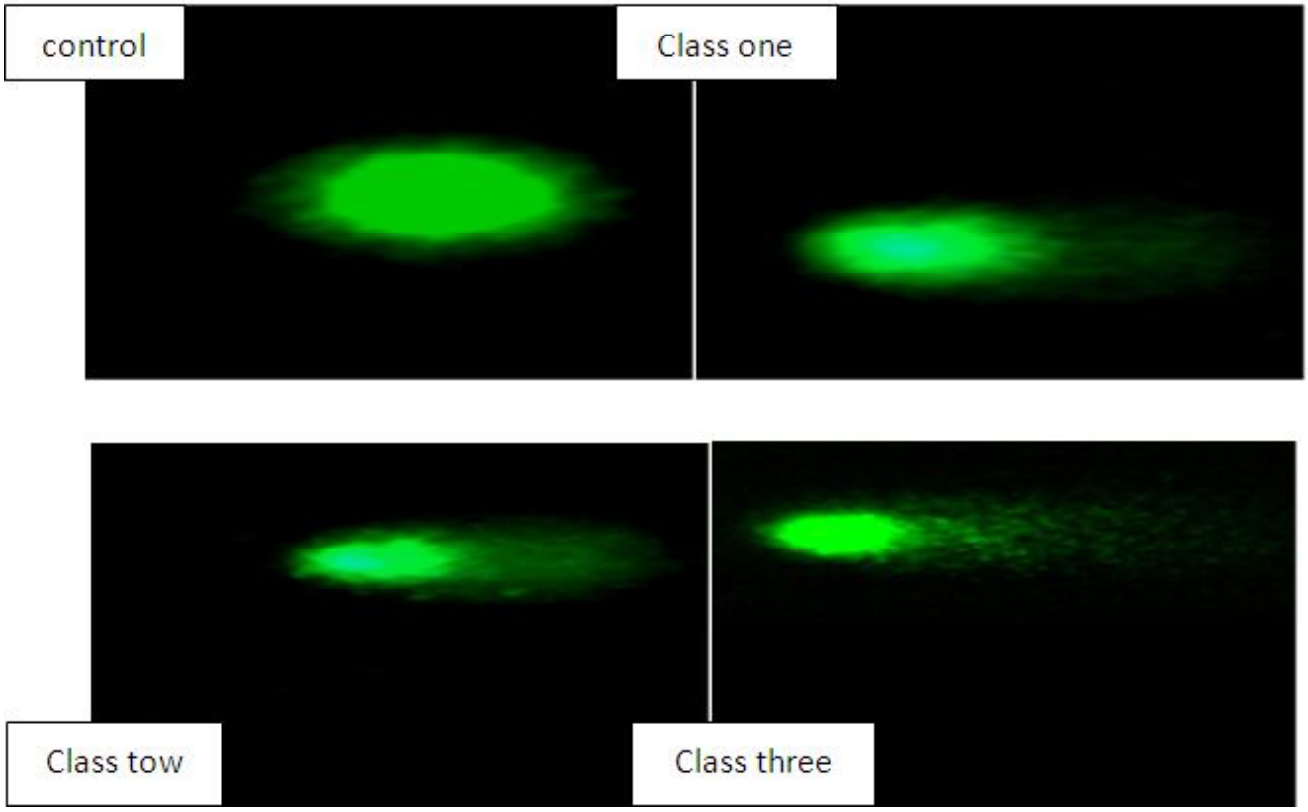

Fig. 5: Classes of DNA damage in bacteria treated with GNPs according to comet assays. Class one: low damage, Class two: medium damage and Class three: high damage(40X).

limits biofilm synthesis (Kalishwaralal *et al.*, 2010). Exo-polysaccharide and hydrophobicity in cell surface have an important performance in interactions of biofilm formation and bacterium-host cell. Previous studies confirmed that the hydrophobicity of cell surface helps in the reduction of formation of biofilm in several microorganisms that include *Candida* sp. (Borghi *et al.*, 2011). This study shows when treatment the bacteria with AuNPs the index of hydrophobicity in bacterial under study are reducesed, that leads to the inhibition of biofilm formation. In vitro studies have results showed the effect of biofilm of bacteria production in the presence of AuNPs. Were observed differences beyond to the bacterial type, nanoparticle type, nanoparticle size and nanoparticle concentrations.

Genotoxicity Assessment by Comet assay

In this study, present alkaline comet assay with (G-ve) Gram negative bacteria *P. aeruginosa* and (G†ve) Gram positive bacteria *Staph. aureus* to investigate the degree of DNA damage generated upon exposure to 0. 1 mg/ml of AuNPs synthesis from *Proteus mirabilis* P242. A demonstration of DNA damage induced by the AuNPs would further support the role of oxidative stress in the mechanism of antibacterial activity. According to pictures were obtained from comet assay technique, the DNA damage can be classified into three classes of damage that are class one: low damage, class two: medium damage and class three: high damage in bacteria treated with AuNPs, (Fig. 5):

In this table, the DNA damage markers were show significant differences between *P. aeruginosa* and *Staph. aureus* exposed to AuNPs synthesis by *Proteus mirabilis* P242 according to statistical analysis at ($p<0.05$). Results of statistical analysis showed that AuNPs synthesis by *Proteus mirabilis* P242 have effect on the DNA of the tested bacteria, the DNA damage appear more in *P. aeruginosa* treated with AuNPs as compared with *Staph. aureus* that may be due to the different in type of cell wall between (G-ve) Gram negative and (G†ve) Gram positive. Another probability might be a genotoxic mechanism that beyond to the electrical charge found in particle surface (Perde-Schrepler *et al.*, 2019). The AuNPs immediately catalyze the cracking of hydrogen peroxide radicals, then caused the formation of hydroxyl radicals that in turn caused excessive oxidation damage to the cell (He *et al.*, 2013).

References

Borghi, E., R. Sciota, C. Biassoni, D. Cirasola, L. Cappelletti, L. Vizzini, P. Boracchi and G. Morace (2011). Cell surface hydrophobicity: a predictor of biofilm production in *Candida* isolates. *J. Med. Microbiol*. **60**, 689-690.

Bowman, L., V. Castranova and M. Ding (2012) .Single cell gel electrophoresis assay (comet assay) for evaluating nanoparticles-induced DNA damage in cells. *Methods Mol Biol* **906**, pp: 415–422.

CLSI. Performance Standards for Antimicrobial Susceptibility Testing. 26th ed. CLSI Supplement M100S. Wayne, PA: Clinical and Laboratory Standards Institute; 2016.

Christensen, G. D., W. A. Simpson, J. J. Younger, L. M. Baddour, F. F. Barrett and D. M. Melton (1985). Adherence of coagulase-negative staphylococci to plastic tissue culture plates: A quantitative model for the adherence of staphylococci to medical devices. *J Clin Microbiol*; **22**: pp: 996-1006.

Donaldson, K., C. A. Poland and R. P. F. Schins (2010) Possible genotoxic mechanisms of nanoparticles: criteria for improved test strategies. *Nanotoxicology* **4**: PP:414–420.

Elbeshehy, E. K., A. M. Elazzazy and G. Aggelis (2015). Silver nanoparticles synthesis mediated by new isolates of *Bacillus* spp., nanoparticle characterization and their activity against Bean Yellow Mosaic Virus and human pathogens Front. *Microbiol.*, **6**

Elbeshehy, X., A. F. Radovic-Moreno, J. Wu, R. Langer and J. Shi (2014). Nanomedicine in the management of microbial infection – Overview and perspectives. *Nano Today*. Vol. **9(4)**, PP.:478–98.

Forbes, B. A., F. S. Daniel and S. W. Alice (2007) Bailey and Scott's Diagnostic Microbiology. 12th ed., Mosby Elsevier Company, USA.

Frey, F. M. and R. Meyers (2010). Antibacterial activity of traditional medicial plants used by Haudenosaunee peoples of New York State. *J Int Soc Complement Med Res*; **10**: PP:64.

Handuz, W. A. (2019). Microbiological and Immunological Study of Patient with Acute Tonsillitis. M. S. C. Thesis College of Science, University of Babylon, Iraq.

He, W., Y. T. Zhou, W. G. Wamer, X. Hu, WuX. , Z. Zheng, M. D. Boudreau and J. J. Yin (2013). Intrinsic catalytic activity of Au nanoparticles with respect to hydrogen peroxide decomposition and superoxide scavenging. *Biomaterials*, **34**: 765-773.

Honary, S., E. Gharaei-Fathabad, Z. K. Paji and M. Eslamifar (2012). A Novel Biological Synthesis of Gold Nanoparticale by Enterobacteriaceae Family. Mazandaran Uni. Of Med. Sci School of Fharm., Sari, Iran. *Tropical Journal of Pharmaceutical Research*; **11(6)**: pp:887 – 891.

Husseiny, M., M. Abd El-Aziz, Y. Badr and M. A. Mahmoud (2007). Biosynthesis of gold nanoparticles using *Pseudomonas aeruginosa*, Spectrochimica Acta A, *Mol. Biomol. Spect*. **67**: pp: 1003-1006.

Kalishwaralal, K., S. Barath, S. ManiKanth, R. Pandian, V. Deepak and S. Gurunathan (2010). Silver nanoparticles impede the biofilm formation by *Pseudomonas aeruginosa*

and *Staphylococcus epidermidis*, *Colloids Surf. B* **79**: 340-344.

Khan, F., P. Manivasagan, J. W. Lee, D. T. N. Pham, J. Oh and Y. M. Kim (2019). Biofilm Inhibition, Attenuation of Virulence and Motility Properties in *Pseudomonas aeruginosa* PAO1. *Mar. Drugs* **17(3)**: 208-227.

Kumari, S., R. P. Rastogi, K. L. Singh, S. P. Singh and R. P. Sinha (2008). DNA damage: detection strategies. *Excli Journal*, **7**: pp: 44-62.

Lee, C., J. Y. Kim, W. I. Lee, K. L. Nelson, J. Yoon and D. L. Sedlak (2008). Bactericidal Effect of Zero-Valent Iron Nanoparticles on *Escherichia coli*. *Environmental science & technology*, **42(13)**: 4927-4933.

Lemire, J. A., J. J. Harrison and R. J. Turner (2013). Antimicrobial activity of metals: mechanisms, molecular targets and applications. *Nat Rev Microbiol*. **11(6)**: PP:371–84.

MacFaddin, J. F. (2000). Biochemical Tests for Identification of Medical Bacteria. 3rd.ed. Lippinocott Williams and Wilkins, USA..pp: 555-565.

Magdolenova, Z., M. Drlickova, K. Henjum, E. Runden-Pran, J. Tulinska, D. Bilanicova, G. Pojana, A. Kazimirova, M. Barancokova, M. Kuricova, A. Liskova, M. Staruchova, F. Ciampor, I. Vavral, Y. Lorenzo, A. Collins, A. Rinna, L. Fjellsbo, K. Volkovova, A. Marcomini, M. Amiry-Moghaddam and M. Dusinska (2014). Coating-dependent induction of cytotoxicity and genotoxicity of iron oxide nanoparticles. *Nanotoxicology* **1**: pp:44-56.

Mahmoudi, M., S. Sant, B. Wang, S. Laurent and T. Sen (2011). Super paramagnetic iron oxide nanoparticles (SPIONs): development, surface modification and applications in chemotherapy. *Advanced Drug Delivery Reviews*, **63**: no. 1-2, pp: 24–46.

Mohamed, A. K. A. and A. A. M. Sherif (2012). The dimensional hematological alterations induced in blood of rats in vivo by intraperitoneal administration of gold nanoparticles. *J. Nanomed Nanotechol*. **3**: pp:1-4.

Nair, B. and T. Pradeep (2002) Coalescence of nanoclusters and formation of submicron crystallites assisted by *Lactobacillus* strains. *Cryst Growth Des*. **2**: pp: 293–298.

Nishant, S. and M. Mausumi (2014). Biosynthesis and Characterization of Gold Nanoparticles Using *Zooglea ramigera* and Assessment of Its Antibacterial Property. *Journal of Cluster Science*. Available from URL: http://www.springer.com/chemistry-/catalysis/journal/10876

Njoki, P.N., I. I. S. Lim, D. Mott, H. Y. Park, B.Khan, S. Mishra, R. Sujakumar, J. Luo and C. J. Zhong (2007). Size correlation of optical and spectroscopic properties for gold nanoparticles. *J. Phys. Chem. C*, **111**: pp.: 14664-14669.

Perde-Schrepler, M., A. Florea, L. Brie, P. Virag, E. Fischer-Fodor, A. Vâlcan, E. Gurzău, C. Lisencu and A. Maniu (2019). Size-Dependent Cytotoxicity and Genotoxicity of Silver Nanoparticles in Cochlear Cells In Vitro. *Journal of Nanomaterials*. Volume. 12.

Philip D. (2010). Rapid green synthesis of spherical gold nanoparticles using *Mangifera indica* leaf Spectrochim. Acta A, **77**: pp.: 807-810.

Plotnikov, E., S. Zhuravkov, A. Gapeyev, V. Plotnikov, I. Martemianova and D. Martemianov (2017). Comparative Study of Genotoxicity of Silver and Gold Nanoparticles Prepared by the Electric Spark Dispersion Method. *Journal of Applied Pharmaceutical Science.* **7(07)**, pp: 035-039.

Rai, M., A. Gade and A. Yadav (2011). Biogenic nanoparticles: an introduction to what they are, how they are synthesized and their applications Metal Nanoparticles in Microbiology, Springer, Berlin Heidelber, pp.: 1-14.

Saha, K., S. S. Agasti, C. Kim, X. Li and V. M. Rotello (2012). *Gold nanoparticles in chemical and biological sensing Chem. Rev.*, **112**: pp.: 2739-2779.

Sathyanarayanan, M. B., R. Balachandranath, Y. G. Srinivasulu, S. K. Kannaiyan and G. Sybbiahdoss (2013). The effect of gold and Iron-Oxide Nanoparticales on biofilm –Forming Pathogens. ISRN Microbiology Articale ID 272086, 5 -11.

Thirumurugan, A., S. Ramachandran, T. Neethu Anns, G. J. Lin and G. Rajagomathi (2012). Biological synthesis of gold nanoparticles by *Bacillus subtilis* and evaluation of increased antimicrobial activity against clinical isolates. *Ind. Kor J. Chem. Eng*. **29(12)**: PP: 1761–1765.

Vandghanooni, S. and M. Eskandani (2011). Comet Assay: A Method to Evaluate Genotoxicity of Nano-Drug Delivery System. BioImpacts; **1(2)**: pp: 87-97.

Versiani, A. F., L. M. Andrade, E. M. Martins, S. Scalzo, J. M. Geraldo, C. R. Chaves, D. C. Ferreira, M. Ladeira, S. Guatimosim, L. O. Ladeira and F. G. da Fonseca (2016).*Gold nanoparticles and their applications in biomedicine Future Virol.*, **11**: pp.: 293-309.

Velusamy, P., G. V. Kumar, V. Jeyanthi, J. Das and R. Pachaiappan (2016). Bio-inspired green nanoparticles: synthesis, mechanism, and antibacterial application Toxicol. Res., **32**: pp.: 95.

Yamamoto, O. (2001). Influence of particle size on the antibacterial— activity of zinc oxide. *Int. J. Inorg. Mater*. **3**: 643- 646.

Yu-Han, Q., J. Li, Y. Zhang, Y. Wang, L. Liu and M. Li (2016). Inhibition of gold nanoparticles (AuNPs) on pathogenic biofilm formation and invasion to host cells. *A nature research J. Scientific Reports.* **(6)**: Article number: 26667.s

Zhou, Y., Y. Kong, S. Kundu, J. D. Cirill and H. Liang (2012). Antibacterial activities of gold and silver nanoparticles against *Escherichia coli* and *Bacillus* Calmette-Guérin. *Journal of Nano-biotechnology*, **10**: 19.