xIlkogretim Online - Elementary Education Online,2021; Vol 20 (Issue 1): pp.3336-3346
http://ilkogretim-online.org
doi: 10.17051/ilkonline.2021.01.377

Antibacterial, Antibiofilm and Antioxidant Activity of Gold Nanoparticals Biosynthesis of from Local Proteus mirabilis Isolate

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Abstract: The development of environmentally sustainable pathways for the synthesis of nanoparticals has been a major focus of nanotechnology research in recent years. The extracellular system for synthesis gold nanoparticles is used in this report in a greener, safer way by using local *Proteus mirabilis*. The addition of a hydrogen tetra chloro aurate (HAuCl4) solution to a Proteus mirabilis cell-free extract (CFE) resulted in the synthesis of gold nanoparticles (AuNPs) within 24 hours at room temperature (37oC). X-ray diffraction spectroscopy (XRD) analyses, Fourier Transform Infrared (FTIR) measurements, scanning electron microscopy (SEM), and energy dispersive X-ray (EDX) spectroscopy were used to characterize the nanoparticles. *Proteus mirabilis* can biosynthesize extracellular GNPs in the 28 nm range, according to our findings. Antibacterial activity of biosynthesized AuNPs against clinical MDR bacteria was discovered (*Staphylcoccus aureus* and *Pseudomonas aeurogenosa*), Also, The antibiofilm activity of AuNPs against the same microorganism used in the antibacterial activity has been investigated, the synthesized AuNPs were evaluated for their antioxidant activities (In vitro) of AuNPs from bacteria by DPPH assay.

KEY WORDS: Gold nanopartical, Antibacterial activity, Biofilm, Antioxident.

I. INTRODUCTION:

Due to their chemical, electronic, and optical properties, metal nanoparticles have occupied a field of scientific interest. Gold nanoparticles, for example, have attracted a lot of attention in recent years due to their higher stability, spectroscopic, size-related electronic, and optical properties [1]. They've been used in a variety of fields and sciences [2,3]. Physical, chemical, and biological methods can all be used to make nanoparticles. The protective, reducing, and stabilizing agents are used to synthesize gold nanoparticles (AuNPs) using bottom-up procedures such as chemical reduction methods. These agents are always toxic and flammable [4], and they have the potential to adsorb on the surface of nanoparticals, causing problems in biological applications [5]. For these reasons, biological approaches to gold nanoparticle synthesis are preferable. Environmentally friendly organisms make it simple to obtain non-toxic, non-hazardous, and clean metal nanoparticles. Because of its inherent properties to produce different types of enzymes for chemical detoxification and energy-dependent ion efflux, which are responsible for the reduction and stabilization of metallic nanoparticles, bacteria has always been a preferred organism for producing nanoparticles [6], Easy-to-handle methods, environmentally friendly disposal, and a much simpler downstream method [7]. The synthesis of gold nanoparticals (AuNPs) using *Bacillus* sp., *Bacillus clausii*, *Bacillus amyloliquefaciens*, and *Azoarcus* sp. has been reported [8-12].

Gold nanoparticles are one of the few inorganic metal nanoparticles that can kill both Gram-negative and Gram-positive bacteria, and have been shown to be effective against multidrug-resistant bacteria (MDR) [13]. Pathogenic biofilm formation and invasion bacteria were strongly inhibited by the AuNPs, which also supported the host immune response. Furthermore, these inhibitory effects are linked to the electrostatic interactions between AuNPs and the cells they target [14]. Metal nanoparticles' toxicity against pathogenic bacterial cells and biofilms has been linked to the generation of reactive oxygen species (ROS) and bacterial membrane disruption [15].

Oxidative stress is caused by an increase in reactive oxidative species and a decrease in antioxidative systems, and it can cause tissue damage and a variety of diseases in humans, including atherosclerosis, Diabetes, chronic inflammation, neurodegenerative disorders, and cardiovascular diseases are all examples of chronic diseases [16,17]. Oxidative stress is likely to play a role in cancer development as people get older. The reactive species produced during oxidative stress can damage DNA directly, making them mutagenic, It may also inhibit apoptosis and promote proliferation, invasiveness, and metastasis by suppressing apoptosis [18]. The antioxidant capacity of gold nanoparticles has also been used in a variety of applications. Free radicals cause oxidative stress, which is caused by metabolic reactions that use oxygen and disrupt the balance of pro-oxidant antioxidant reactions in living organisms [19].

Proteus mirabilis, a novel bacterial strain, was able to synthesize AuNPs as an extracellular synthesis in this study. XRD, FTIR, and SEM-EDS were used to characterize AuNP synthesis, and then antimicrobial, antibiofilm, and antioxidant activity were investigated.

II. MATERIAL AND METHODS:

The bacteria *Proteus mirabilis* was identified using 16s RNA on the hospital floor. The bacteria were diagnosed in the Microbiology Lab, Biology Department, College of Science, University of Babylon, Iraq, and confirmed [20,21].

Solution and media:

Merck Germany provided hydrogen tetra chloro aurate (HAuCl4), brain heart infusion Agar and broth medium, antibiotics disk, Ethidium Bromide, and other chemical solutions and reagents.

Biosynthesis of Gold nanoparticles (AuNPs):

Extracellular biosynthesis of AuNPs (Gold nanoparticles) was performed in two flasks, one with *Proteus mirabilis* supernatant as control and the other with 103mM Hydrogen tetra chloro aurate ($HAuCl_4$) solution and Proteus mirabilis supernatant as test solution, incubated at room temperature on a shaker for 24 hours. After 24 hours, the obtained cell free supernatant of gold nanoparticle solution was purified using a cold centrifuge at 15,000 rpm for 20 minutes. The pellet was dissolved in deionized water after the supernatant was removed [22]. UV–Vis diffuse reflectance measurements, X-Ray diffraction measurements (XRD), Fourier Transform Infrared (FTIR), Field Emission-Scanning Electron microscopes (FE- SEM), and (EDX) measurements are all used to detect AuNP.

Determination the Minimum Inhibitory Concentration(MIC)

and Minimum Bactericidal Concentration (MBC)

G+ve bacteria (three isolates of *Staphylococcus aureas*) and Gve bacteria (three isolates of *Pseudomonas aeruginosa*) were incubated at 37°C overnight on nutrient agar slants, and on which 0.5 McFarland was performed. A total of 10 mL of nutrient broth medium was prepared, and each sample of bacteria was aseptically inoculated with 1 mL of the bacterial suspension (approximately 108 colony-forming unit/mL). In sterile deionized water, five dilutions of AuNPs (500, 250, 125, 62.5, and 31.25 g/ml) were prepared, as well as a negative control (without AuNPs). Multiplate counting was used to test each isolate in triplicate. The MIC is always determined using spectrophotometry at wavelength 600 nm after overnight storage at 37°C [23]. Cultured on nutrient agar plates with no turbidity and incubated at 37°C overnight, wells showed no turbidity. The growth of bacterial colonies was monitored, and the MBC value was recorded, indicating that no growth had occurred.

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

The antibacterial activity of AuNPs produced by Proteus mirabilis was tested to see if they could inhibit the growth of MDR bacteria. Following the selection of the (MIC) and (MBC) concentrations, it is necessary to confirm the concentration using a test that was carried out according to the protocol described in the references, by using sterile Mueller Hinton agar (MHA) medium in sterile Petri dishes with a diameter of 90 mm (15 ml). The antibacterial activity of AuNPs was determined using the National Committee for Clinical Laboratory Standards' agar well diffusion method (NCCLS) [24].

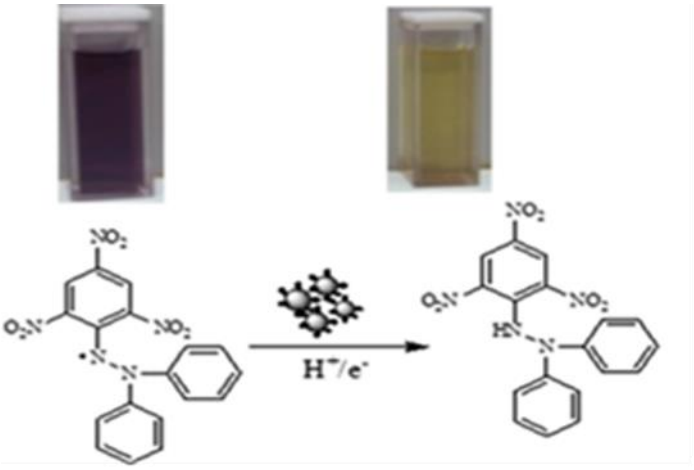
Anti-Biofilm Activity of AuNPs against Pathogenic Bacteria Biofilms Formation:

Bacteria isolates were incubated on blood agar plates overnight at 37°C and then stored at 4°C. According to Christensen et al., 1985, bacterial biofilm formation is tested [25]. The multiwell plate method classified bacterial biofilm formation into three categories: weak (BF0.120), moderate (0.120>BF0.240), and strong (BF >0.240) at OD. 630 nm is the wavelength. When GNPs are present in the biofilm, bacterial growth is reduced completely and partially, as compared to when AuNPs are not present (control).

DPPH radical assay:

The activity of silver nanoparticals synthesis from cumin seed extract was determined using the DPPH (1,1-diphenyl,2-picryl-hydrazil) free radical scavenging procedure described by [26]. The radical scavenging activity of samples against the stable DPPH radical was determined spectrophotometrically using an ELISA reader using different concentrations (12.5, 25, 50, and 100 g/ml) of silver nanoparticals. When DPPH reduction was measured at 517 nm, the colorimetric changed (from deep violet to light yellow) (Fig. 1). As a positive control, ascorbic acid was used (as a reference). The percentage inhibition was calculated using the equation below:

$$\text{Inhibition\%} = \frac{\text{Absorbance of -ve control} - \text{Absorbance of sample}}{\text{Absorbance of -ve control}} \times 100$$



DPPH• **DPPH-H**

Fig. (1): Scavenging of DPPH• to DPPH-H by AgNPs, plausible mechanism.

III. RESULTS AND DISCUSSION:

The ability of *Proteus mirabilis* to synthesize AuNPs is consistent with many studies on microorganism biosynthesis of AuNPs. *Pseudomonas aeruginosa* [27] *Lactobacillus* [28] *Arthrobacter* genus [29] Chloroaurate ions are reduced to AuNPs. Color chanching and the appearance of purple color from pale yellow color (Fig. 2) studies confirmed the formation of nanoparticles [30]. The important reasons for the synthesis of biological metal nanoparticles that rely on NADH- and NADH-dependent enzymes, and the reduction appears to begin with the transfer of an electron from NADH to a NADH-dependent reductase as an electron carrier. The gold nanoparticles were detected using XRD, and the peak of The XRD spectrum revealed four intense peaks at positions (38), (45), (66), and (83), which correspond to the positions (38), (45), (66), and (83), respectively, in the spectrum (Fig.3)[30]. The presence of various chemical groups, one of which is an amide, was also confirmed by FTIR spectrum, which indicated the presence of AuNPs. The –COO- could also be present due to amino acid residues that indicate protein co-existence with AuNPs in the wavelength range of 4000–400 cm, as seen in the spectra 1900 and 3700 (Fig.4) [31]. Scanning Electron Microscopy – Field Emission Nanoparticles have a size of 28 nm, according to (Fig. 5). AuNPs biosynthesis by *E. coli* was found to be 11.8 and 130 nm in another study [31].

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

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

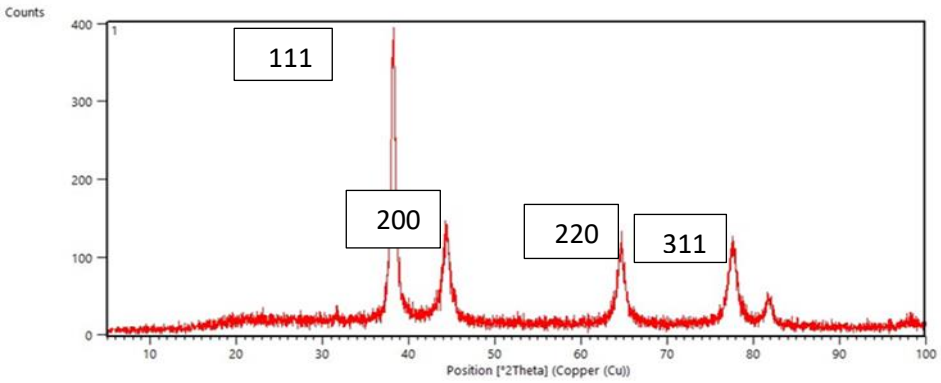
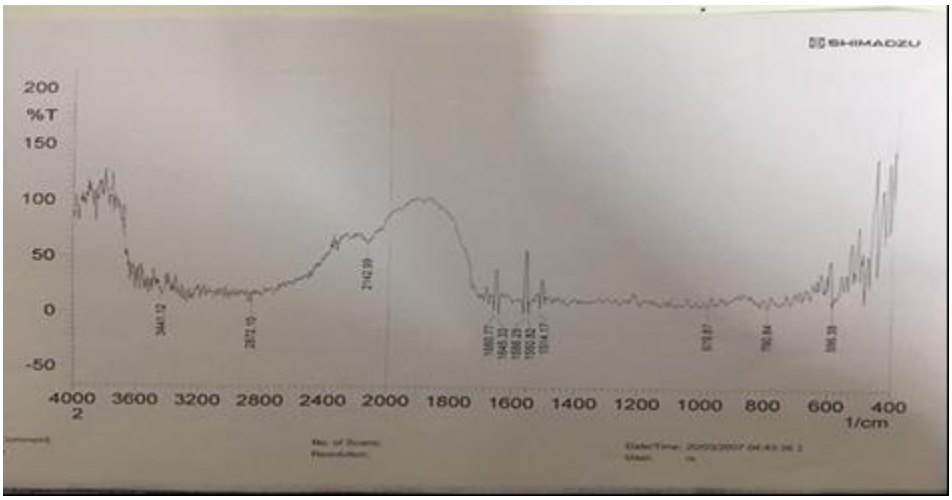
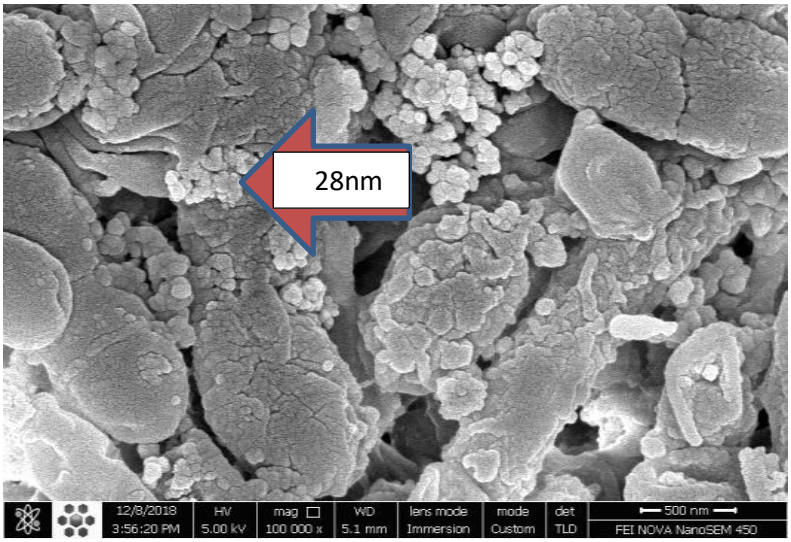


Figure (2): XRD of AuNPs synthesis by *Proteus mirabilis*



Figure(3): FT-IR of AuNPs synthesis by *Proteus mirabilis*



Figure(4): FE-SEM of AuNPs synthesis by *Proteus mirabilis* with 28nm in size and nanorodes in shapes

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

Lists of antibiotic susceptibility testing were done utilizing accordance 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 includes antibiotic sensitivity testing for the bacteria under investigation, with Cefotaxime, Amoxicillin, Bacitracin, Aztreonam, Ampicilin, and Sulphamethoxazole being the six antibiotics used to evaluate the susceptibility of the isolated bacteria, as in Table (1).

Table (1): Antibiotic sensitivity of bacterial isolate from different sources

Bacteiral Isolate	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

The results showed that two isolates of *P.aeruginosa* and three isolates of *Staph*. *aureus* were 100% resistant to the six types of antibiotics tested, whereas only one sample of *P.aeruginosa* was 33.33 percent resistant to Cefotaxime and Sulphamethoxazole showed in table (1). Because they resist more than one type of antibiotic, *P.aeruginosa* and *Staph.aureus* were classified as multidrug resistant (MDR) in this study. MDR was defined as acquired non-susceptibility to at least one agent in three or more antimicrobial categories, Bacterial multidrug resistance This can be caused by the activity of multidrug efflux pumps, each of which may pump out many drug types, or by the accumulation of genes coding for drug resistance to a single agent on resistance (R) plasmids or transposons [32].

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

Biosynthesis GNPs were tested against three multidrug (G-ve) Gram-negative *P. aeruginosa* strains and three multidrug (G-ve) Gram-positive *Staph. aureus* strains. After incubation, spectrophotometry at wavelength (600 nm) was used to determine the MIC and MBC concentrations for nanoparticles produced using the broth micro dilution technique and spectrophotometry at wavelength (600 nm).

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

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

From Table (2), It is obvious that the MIC concentrations of bio GNPs production from Proteus mirabilis of 31.25 and 62.5 g/ml were effective against the sixth MDR bacterium in the same way, indicating and reflecting that this is a typical dose for bacteria inhibition. The MBC concentration, on the other hand, was confined to between 250 and 500 g/ml of bio AuNPs as a bactericidal concentration for all isolates in the investigation. The findings of this investigation revealed that AuNPs had a significant inhibitory impact on bacteria isolates due to their ability to engage with the bacterial cell wall and tear it, disrupting bacterial metabolism by affecting bacterial DNA and interacting with mitochondria and other organelles [33-35].

AuNPs as Antibacterial Activity:

After choosing the MIC and MBC concentrations, the antibacterial activity of AuNPs bio-synthesized from *Proteus mirabilis* was tested to see if they could limit the growth of various clinical bacteria classified as MDR. All of the prepared plates were incubated at 37°C for 24 hours before the widths of the inhibitory zones were measured. The inhibition of bacterial growth was measured in millimeters (mm) as zone diameters at three equidistant places taken from the center of the inhibition zone, and the average value was obtained for all observations, as in table (3):

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

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

Table (3) reveals that the biggest inhibition zone of *Proteus mirabilis* AuNPs in Gram negative bacteria was 19 mm in *P.aeruginosa* with a MIC of 62.5 g/ml and that there were significant differences (P0.05) in AuNPs utilized against three strains of *P. aeruginosa*, While the biggest inhibitory zone of AuNPs on Gram positive bacteria *S.aureus* was 15.8 mm with a MIC of 62.5 g/ml, there were no significant changes (P0.05) in AuNPs utilized against three *S.aureus* strains. AuNPs' antibacterial action is due to a variety of mechanisms. The major mechanism is thought to be connected to oxidative stress caused by ROS (it was

previously reported that replacing each Au_2 ion resulted in the release of one free electron) [36,37]. ROS (reactive oxygen species) include hydroxyl radicals, superoxide radicals, hydrogen peroxide, and single oxygen, all of which may damage DNA and proteins in bacteria [38]. Furthermore, electrostatic interactions between bacterial cell membranes or cell membrane proteins and nanoparticles can cause physical damage, resulting in bacterial cell death [37]. Other research has found that nanoparticles' modest size may contribute to their antibacterial properties [39,40]. *E. coli* was disrupted by nanoparticles of tiny diameters ranging from 10 to 80 nm penetrating into *E. coli* membranes, according to Lee et al., 2008. Another study found that AuNPs had a strong antibacterial impact against Gram-negative bacteria like *E. coli* and Gram-positive bacteria like *E. coli*. *Bacillus* sp. The antibacterial action of single AuNPs might be attributed to bacteria absorption of these nanoparticals modifying their surface modifying agents and rearranging of these nanoparticals inside the cytoplasm, according to this study [41].

Antibiofilm Activity of AuNPs by Multi-well Plate Count Method:

The same 6-isolates were utilized in this investigation, all of which are MDR bacteria, three of which are Gram negative bacteria (*P. aeruginosa*) and three of which are Gram positive bacteria (*Staph. aureus*) and were examined for biofilm production, In the multi-well plate approach, all bacteria studied were able to create biofilm in the form of a film that bordered the walls and bottoms of wells. The results showed that *P. aeruginosa* and *Staph. aureus* developed a robust biofilm, Tables (4).

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

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

Some bacterial isolates were prevented from forming biofilm by AuNPs from *Proteus mirabilis*, while others were inhibited but not prevented from forming biofilm by AuNPs from *Proteus mirabilis* in a dose-dependent manner. Because metallic nanoparticles have been found to diminish exo-polysaccharide production, which consequently inhibits biofilm development, the anti-biofilm action of AuNPs might be attributable to suppression of exo-polysaccharide production [48]. In bacterial-host cell interactions and biofilm development, exo-polysaccharide and cell surface hydrophobicity play critical roles. Previous research has shown that the hydrophobicity of the cell surface helps to reduce biofilm development in a variety of bacteria, including Candida sp. [42]. This study demonstrates that treatment with AuNPs decreases the hydrophobicity index of the bacteria under investigation, preventing biofilm formation. The influence of bacterial biofilm development in the presence of gold nanoparticles was demonstrated in vitro. Beyond the kind of bacteria, nanoparticle, nanoparticle size, and nanoparticle concentrations, differences were detected.

DPPH assay:

Using a reliable DPPH free radical scavenging experiment, the scavenging activity of AuNPs synthesized from Gram negative bacteria was assessed. The results in Fig. (5) show that the higher the concentration of AuNPs produced by Proteus mirabilis, the higher the proportion of free radicals scavenged. The AuNPs had the maximum antioxidant activity (80.63 percent at 100 g/ml) at the concentrations employed (12.5, 25, 50, and 100 g/ml), whereas the AuNPs at concentrations (50, 25, and 12.5 g/ml) had antioxidant activity (54.90, 34.04, and 29.24 percent, respectively).

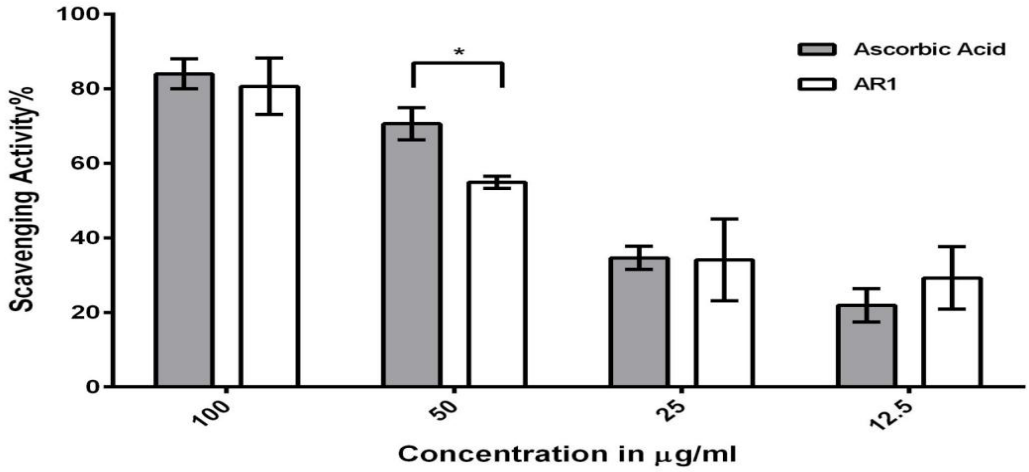


Figure (5): DPPH free radical scavenging activity of AuNPs synthesis from *Proteus mirabilis* comparing with ascorbic acid as a positive control

The foregoing findings were compared to ascorbic acid's free radical scavenging activity as a positive antioxidant, which, according to Fig (5). Except at doses of 50g/ml, when there were significant variations (P0.05) compared to control, the AuNPs synthesized from *Proteus mirabilis* showed essentially the same pattern of ascorbic acid free radical scavenging activity.

These findings suggested that AuNPs' DPPH free radical scavenging activity was dosage dependent, with greater AuNP concentrations resulting in increased antioxidant activity. Because of the delocalization of the free electron, the DPPH (1, 1-diphenyl, 1,2-picryl-hydrazyl) is a persistent radical with a maximum absorption wavelength of 517 nm, and it has been widely employed to assess the antioxidant activity of many compounds, including plant extracts [43]. The extract's antioxidants might work together in a synergistic way. These bio-compounds are adsorbed onto the surface of AuNPs during the production process. Given their large surface area to volume ratio, these AuNPs appear to have a strong proclivity for interacting with and reducing DPPH. An antioxidant component on the surface of AuNPs solution donates an electron to DPPH [44].

Other research suggests that biomolecules such as flavonoids, tannins, and other phenolic chemicals can participate in the creation of AuNPs from aqueous fruit extract, as well as the production of new links between metallic nanoparticles and functional groups of biomolecules present in the extract. These compounds serve as a capping agent when gold nanoparticles are formed, and they also aid in the stability of nanoparticles under physiological conditions [44].

IV. CONCLUSION:

The current study discovered that gold nanoparticles may be made in a simple and environmentally benign manner utilizing *Proteus mirabilis* bacteria. These bacteria-mediated nanoparticles might be employed as an antibacterial, antibiofilm, and antioxidant agent. AuNPs manufactured with bacteria exhibit a large antibacterial and antibiofilm impact against MDR and pathogenic bacteria isolates under research, with a diameter range of 28 nm. This benefit, together with the fact that AuNPs have no known toxicity, suggests that AuNPs might be used instead of antibiotics to treat MDR harmful microorganisms. As a result, it may be utilized in large-scale manufacturing and for targeted medication administration to treat vascular dysfunction caused by hypertension, diabetes, or atherosclerosis.

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