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Green synthesis of gold nanoparticles (AuNPs) using pathogenic bacteria Acinentobacter baumanii with evulation their antibacterial activity

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Abstract:

The current study concentrated on the environmentally friendly production of gold nanoparticles (AuNPs) by pathogenic bacteria, specifically *Acinetobacter baumannii*, that were isolated from blood. By monitoring the color change of the reaction mixture Tetrachloroauric acid (HAuCl4.3H2O + bacterial filtrate), the production of AuNPs was verified following isolation and molecular identification. Physicochemical tests were used to evaluate the produced AuNPs. The results showed that the particles had a UV spectroscopic peak at a wavelength of 560 nm and that their spherical size ranged from 91 to 43 nm when viewed under a scanning electron microscope Fe-SEM.The chemical components in the bacterial extract have a fundamental impact on the formation of AuNPs as a bioreducing agent, according to the infrared spectrum obtained using the FTIR technique. According to the XRD analysis, the NPs exhibit a cubic crystalline structure, and the EDX-Mapping analysis verified the presence of gold. Furthermore, the antibacterial efficacy of AuNPs against two strains of Gram-positive *Staphylococcus aureus* and Gram-negative *Echerichia coli* pathogenic bacteria was confirmed using inhibitory diameters (24 mm and 22 mm) based on the antibiotic gentamicin. with diameter (20mm) as a control at a concentration of 1000 mg/mol as the results showed that the particles have good antibacterial activity

Keywords: AuNPs,Antibacterial, *Acinetobacter baumannii*,XRD,FTIR

Introduction:

Nanotechnology is the process of developing and building nanostructured materials for a range of applications (Shah *et al*.,2021). The nanoparticles exhibit unique physical, chemical, and biological properties at the nanoscale as opposed to their counterparts at greater dimensions. NPs differ from bulk metals in their chemical and physical characteristics, such as their greater specific surface areas, lower melting temperatures, and specific magnetizations. These differences may make NPs appealing for a variety of industrial applications (Horikoshi&Serpone,2013).The synthesis of NPs is categorized into two classes, namely “top down” and “bottom-up” based on the way of NPs formation (Ijaz *et al*.,2020).There are several methods of NPs synthesis including physical,chemical and biological method.

The utilization of biological and green technologies to manufacture different NPs is becoming more and more popular among the many synthetic methods for NP preparation (Khan *et al*.,2022). Because the process can be modified by varying the culture factors, including nutrition, pH, pressure, and temperature, bacteria, yeast, and fungus are used in the environmentally friendly creation of NPs (Yadav *et al*.,2023). The intracellular and extracellular techniques are the two approaches for NP synthesis where extracellular and intracellular material produced by bacteria serves as a reducing agent when combined with Ag or Au solution to create NPs (Qamar *et al*.,2021). Over the past few decades, gold nanoparticles (AuNPs) have drawn a lot of attention and their properties have been studied in a variety of fields, including biology, physical and chemistry (Sadiq *et al*.,2024). In a recent work, Srinath and Rai demonstrated how the bacteria *Enterobacter aerogenes* produces pure AuNPs (Srinath & Rai,2015). One of the most significant nosocomial pathogens, *Acinetobacter baumannii*, can cause infections such meningitis, pneumonia, urinary tract infections, septicemia, and wound infections (Dehbanipour & Ghalavand,2022).As resistant strains and their infections increase, there is an inevitable need for a new antibacterial agent that is inexpensive, has few side effects, and is strong (Hosseini *et al*.,2016).When Rajan et al. examined the antibacterial properties of AuNPs made by *Elettaria cardamomum*, they found that the generated AuNPs were more effective against *S. aureus* than against *E. coli* and *P. aeruginosa* (Rajan *et al*.,2017). The current study addressed two objectives: firstly is the biosynthesis of AuNPs by one of the pathogenic bacterial isolates *Acinetobacter baumannii* with some physicochemical tests. The second is to verify the ability of AuNPs to inhibit bacterial cells to replace antibacterial agent.

Material and method:

1-Collection and isolation of Bacteria:

One bacterium was isolated from blood in this investigation. The polymerase chain reaction (PCR) method was used to molecularly diagnose the samples, which were taken from the Children and Maternity Hospital laboratory in Maysan, Iraq. According to (Al-Naqshbandi *et al*.,2019), the separation technique was.

2-Moleculer identification of bacteria:

Numerous methods have used a universal primer (Table 1) to ascertain the DNA sequence of the material (Macrogen/Korea).Genomic DNA was isolated from isolates using a DNA kit (Presto' Mini g DNA Bacteria Kit, Geneaid, Taiwan) (Hassan *et al*., 2022). The isolated strain was genotypically identified using 16s rRNA sequencing analysis, and the outcomes were compared with NCBI GenBank (Ibrahim *et al*., 2019).

Table (1) Primer kite that used in study

No	Gene	Primers Sequence	Product size Annaling temp.
1	16 S rRNA	F- AGAGTTTGATCMTGGCTCAG	1500 bp 56 C
		R- TACGGYTACCTTGTTACGACTT	

3-Biosynthesis of AuNPs:

For the creation of NPs, the centrifuged supernatant was utilized. In order to create AuNPs, 50 ml of hydrogen tetrachloroaurate (HAuCl4) at concentrations of 2 mM was mixed with 100 ml of supernatant, and the mixture was then incubated at 37 °C. The same experimental conditions were used to incubate a cell control that did not contain the gold salt.

4-Study physical characterization of the bio-synthesized AuNPs:

The biosynthesis nanoparticles' physicochemical characteristics were investigated by a series of tests, including FTIR, UV, Fe-SEM, EDX-mapping, and XRD.

5-Testing anti-bacterial activity of bio-synthesized AuNPs:

Gram-positive *S.aureus* and gram-negative *E.coli* were both tested for antibacterial activity using the disc difusion method.These bacteria were separated from harmful bacteria that VITIk-2 detected at the Child and Martinty Hospital in Misan, Iraq.Pure bacterial colonies were cultured for 24 hours at 37°C on nutrient agar, and the turbidity was brought down to the (0.5) McFarland standard using D.W. sterile distilled water.Following equal swabbing of each species of bacteria onto MHA plates, AuNPa discs were added. Plates were then incubated. Using gentamicin at a concentration of 1000 mg/mol as a reference, petri dishes were assessed for the inhibitory zone measure in millimeters (mm) following incubation for 24 hours at 37°C (Malathi & Palani,2019)

Results and Discussion:

1-Molecular identifecation of bacteria

The polymerase chain reaction approach showed that the primers amplified the gene sequence after the DNA pathogenic bacteria isolate from blood were extracted, and that the amplified bands emerged between 1000 and 1500 pb Fig 1

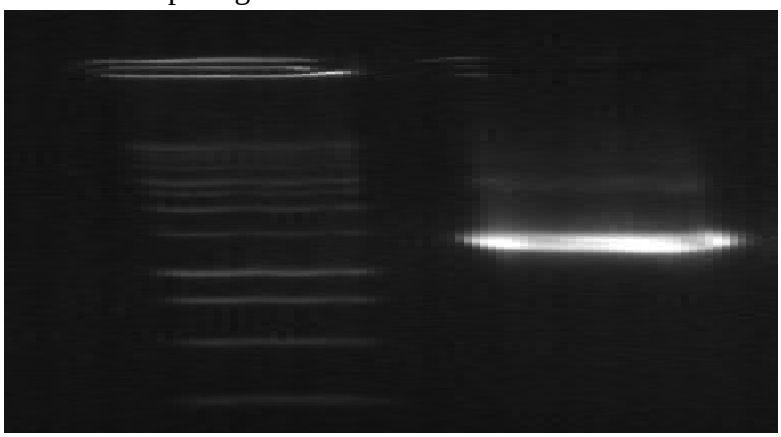


Fig (1) Electrophoresis on agarose gel of the PCR product using primers

2-Biosynthesis of AuNPs:

Observing the color change of *A. baumannii* supernatant treated with (HAuCl4) solution served as the initial confirmation of the biosynthesis of AuNPs. Following 48 hours of incubation at 37ºC, a time-dependent color change was seen in the supernatant, as seen in Fig (2). The supernatant's color changed from pale yellow to dark red with time, primarily as a result of the AuNPs surface plasmon (SPR) vibrations being excited. This indicates that the bacteria and its protein alone were responsible for the biosynthesis of AuNPs (Srivastava & Mukhopadhya,2013).

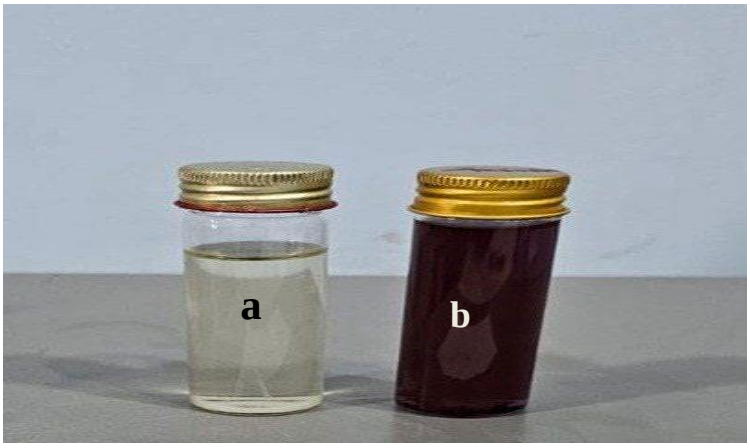


Fig (2) color chang of supernatant a) without salt b) with salt for AuNPs produced by *A. baumanni*

3-Study physical characterization of the bio-synthesized AuNPs:

The peaks at 560 nm wavelength confirmed the production of AuNPs synthesized from *A. baumannii* after incubation for 24 h Fig (**3**), as the study (Srinath & Rai,2015) showed the surface plasmon resonance (SPR) of AuNPs usually ranges from 510 to 560 nm. where the UV spectrum of AuNPs synthesized from *Enterobacter aerogenes* filtrate was determined at a peak of 540 nm

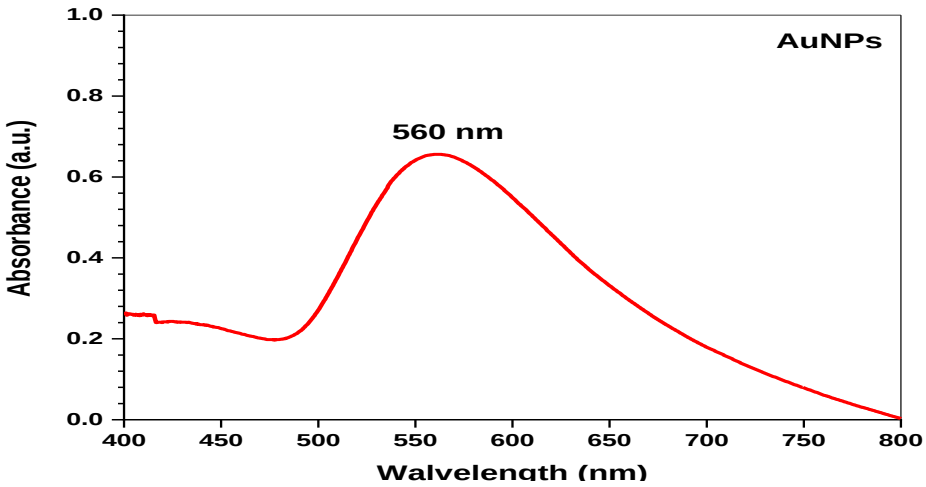


Figure (3) UV-Vis spectroscopy of AuNPs synthesized by *A. baumannii*

Here, FTIR spectroscopy was used to find the bands and functional groups that provide NPs with a unique identity. The table (2) shows the presence of fuctional groups at the indicated peaks. Fig (4) showed FTIR peaks of AuNPs. These findings might point to the role that proteins and other molecules play in the stability of nanoparticles and the bioreduction process (Sirisha *et al*.,2017) (Alfryyan *et al*., 2022) revealed these results may the contribution of proteins and other molecules in the bio-reduction process and the stability of NPs.

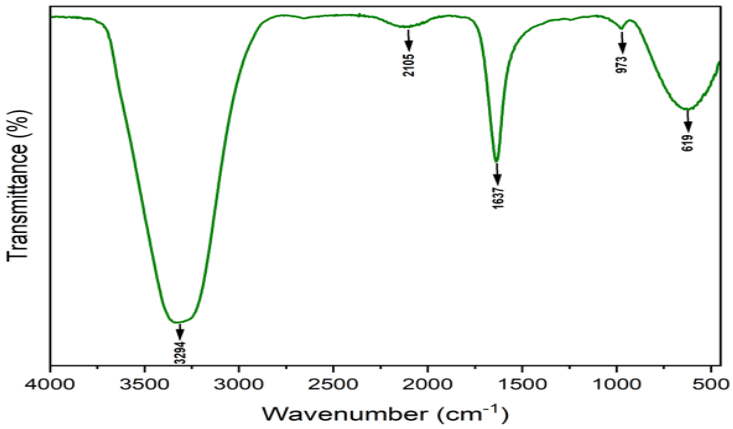


Fig (4) FTIR analysis of AuNPs produced by *A. baumannii*

Table (2) The varios bands and Functional groups of AuNPs

AuNPs wavenumber(cm^{-1})	Bands	Functional group
3294	N-H	Aliphatic primary amine
2105	N=C=S	Isothiocyanate
1637	C=C	Alkene

Fig (5) shows Fe-SEM images of AuNPs synthesized from *A. baumannii* filtrate with a spherical shape and a scale bar of 500 nm and different sizes ranging from (43-91nm) and an average of 68nm Fig (6).The outcomes of a prior work demonstrated the successful synthesis of AuNPs with a spherical morphology, where the bio-synthesised AuNPs are homogeneous, uniform, and well-dispersed. Additionally, a propensity for aggregation is noted for artificial NPs while their size were ranged 10-50nm (Chang *et al*.,2021)

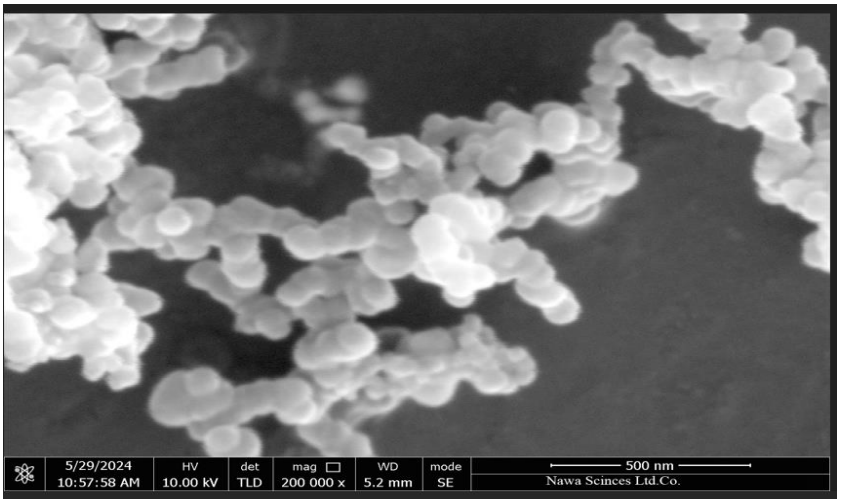


Fig (5) Fe-SEM image of AuNPs Produced by *A. baumannii*

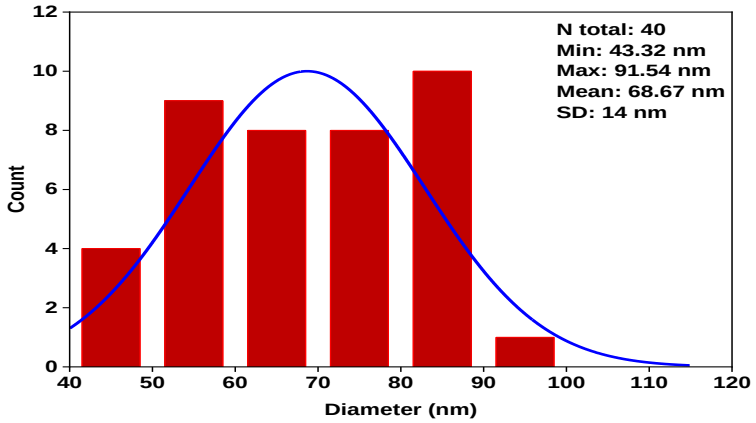


Fig (6) The histogram of the size distribution of AuNPs Produced by *A. baumannii*

Fig (7) shows the XRD spectrum of AuNPs created from the filtrate *A. baumannii* four Bragg reflections (111) (200) (220) (311) at a value of 2 theta for angles (38) (44) (64) (77) respectively, referring to JCPDS data file No. 00-004-0784 as a standard reference for AuNPs, indicating that they are particles.The crystalline nature tends to have a cubic structure and the average particle size using the Debye-Schwarr equation was 22.6 nm ,Table (3).This is consistent with (Sunderam *et al*.,2019)

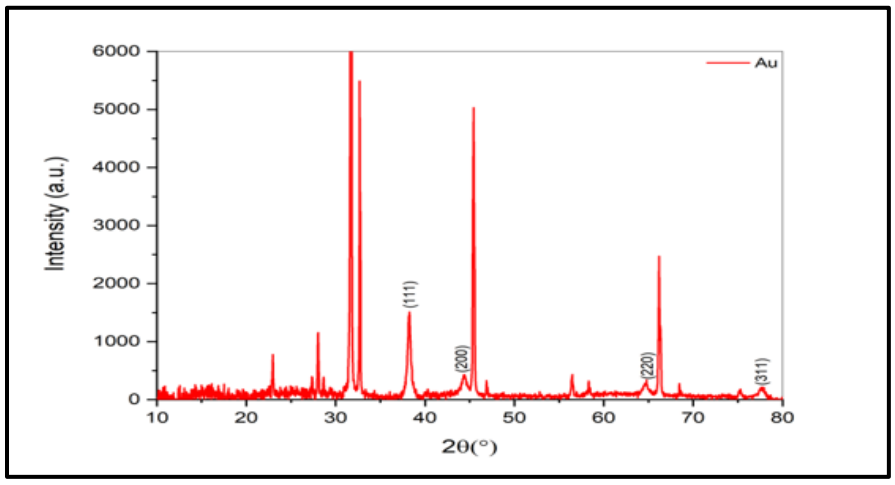
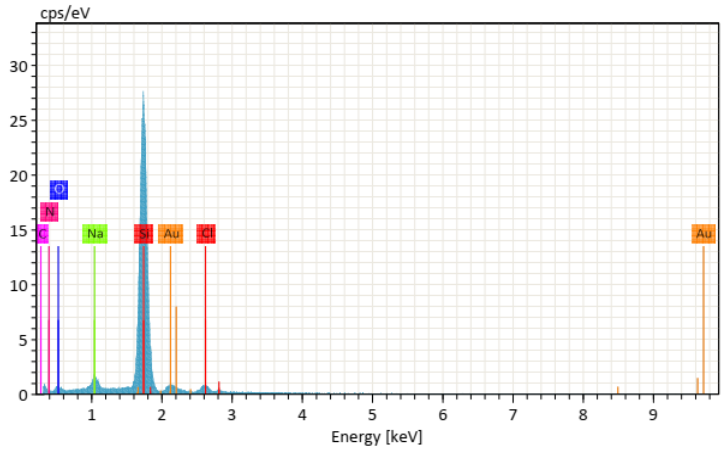


Fig (7) XRD analysis of AuNPs produced by *A. baumannii*

Table (3) Debye-Schwarr equation of AuNPs

2 theta	theta (deg)	thata (rad)	Cos theta	FWHM (β) (deg)	FWHM ((rad)	D
38.236	19.11801	0.33350306	0.9449014	0.25584	0.004462987	32.86633
44.357	22.1785	0.38689161	0.92608636	0.374804	0.006538248	22.89024
64.676	32.338175	0.5641215	0.84505864	0.51168	0.008925973	18.37472
77.549	38.774375	0.67639743	0.77983294	0.614016	0.010711168	16.593
D-average						22.6811

The presence of an elemental gold was confirmed during AuNPs by EDS analysis . The peaks in Fig (8) can be attributed to the chemicals found in the bacterial extracts, and the signals (oxygen, carbon, silicon, chloride, etc.) may have originated from organic biomolecules or phenolic compounds on the nanoparticle surface (Hatipoğlu,2021). Fig (9) displays AuNPs' EDX-Mapping images, which display a map of the atom distribution for each NP element.



Element	At. No.	Mass [%]	Atom [%]
Carbon	6	0.00	0.00
Nitrogen	7	8.14	21.38
Oxygen	8	4.26	9.78
Sodium	11	2.11	3.38
Silicon	14	47.55	62.27
Chlorine	17	2.16	2.24
Gold	79	5.02	0.94
		69.24	**100.00**

Fig (8) EDX analysis of AuNPs produced by *A. baumannii*

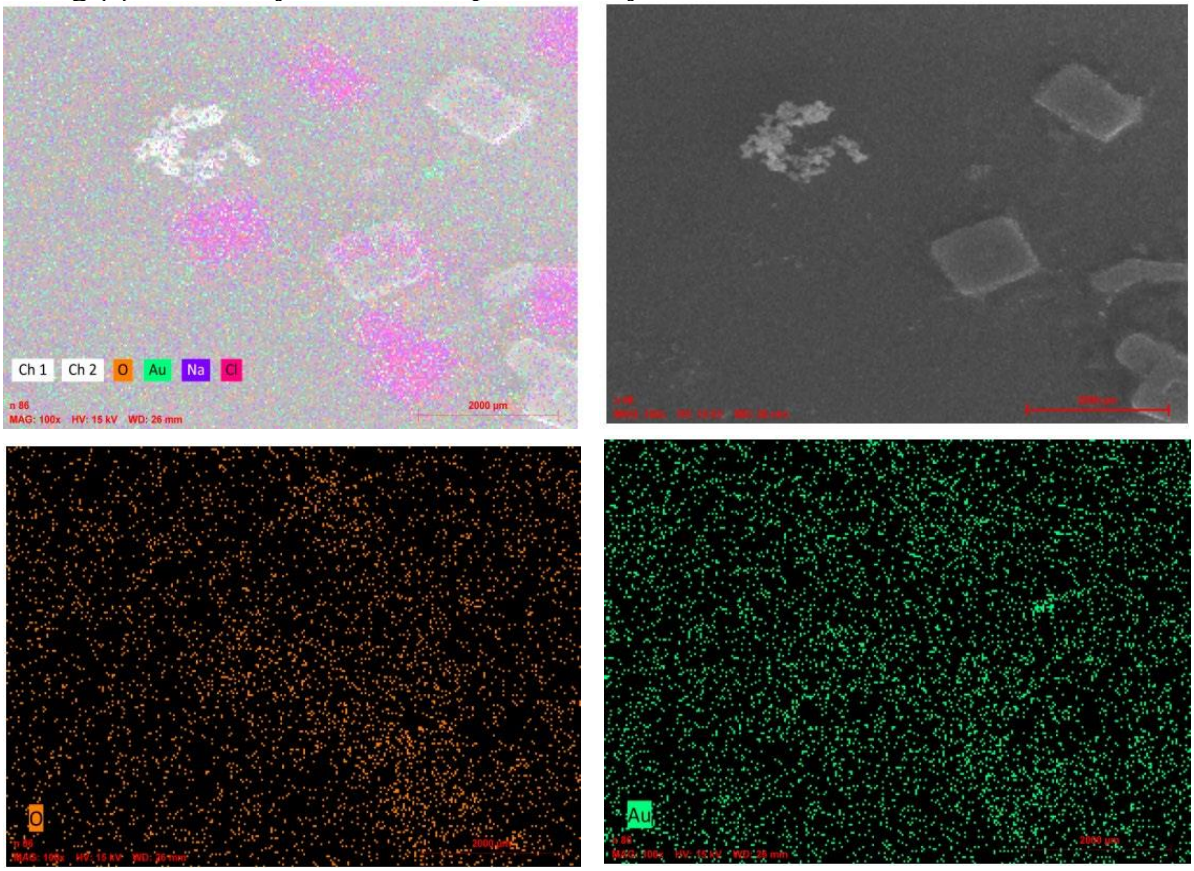


Fig (9) EDX-Mapping image of AuNPs produced by *A. baumannii*

4-Testing anti-bacterial activity of bio-synthesized AuNPs:

The antibacterial activity of AuNPs synthesized from pathogenic bacteria filtrates *A. baumannii* was tested on Gram-positive *S. aureus* and Gram-negative *E. coli* bacteria using agar disc diffusion, and the antibiotic gentamicin was used as a positive control. The inhibition zone of AuNPs on bacteria *E. coli* was 22 mm Fig (10) whereas the inhibition zone on bacteria *S. aureus* was 24 mm Fig (11). These results demonstrate the considerable activity of AuNPs against both positive and negative bacterial isolates.i.e.a significant result compared to the antibiotic gentamicin (20mm).

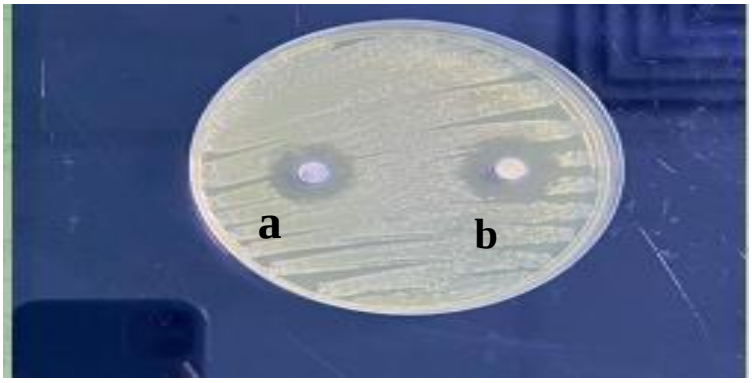


Fig (10) Inhibition zone on *E.coli* a) gentamycin b) AuNPs

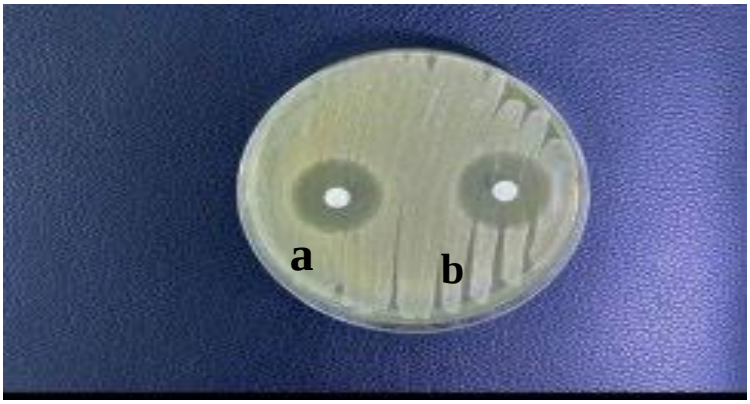


Fig (11) Inhibition zone on *S. aureus* a) gentamycin b) AuNPs

The results indicate that the biosynthesized AuNPs were more effective (more sensitive) on *S. aureus* bacteria compared to *E. coli* bacteria and this antibiotic is due to the fact that the NPs are able to adhere to the bacterial cell wall *S. aureus* and then penetrate its wall, which leading to cell death (Mohanlall&Biyela,2022). The size of NPs has a significant impact on their antibacterial action; smaller particles have demonstrated stronger antibacterial activity because of their increased capacity to penetrate bacteria (Yousaf *et al*.,2020).

Conclusion:

Using pathogenic bacteria found and identified by DNA extraction, the current study provides a positive outcome on the usage of AuNPs as efficient alternative antibacterials. These findings showed that, in the right circumstances, certain harmful bacteria can biosynthesize AuNPs. Subsequently, the synthesized AuNPs' biological activity was evaluated on two kinds of harmful bacteria to determine their inhibitory capacity, and they did, in fact, indicate a high likelihood of inhibition.

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Conflicts of Interest Statement……

Manuscript title:

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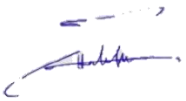
Hawraa Khalaf Abbood		2025-1-19
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