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Green synthesis of Gold Nanoparticles and Their Effect on Pyocyanin Pigment Production from Local *Pseudomonas aeruginosa* Isolates

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Abstract

Pseudomonas aeruginosa is an opportunistic bacterium that lives in all wet and dry environments and animal tissues**.** It is also one of the main pathogens, especially in patients with the urinary tract, burns, cystic fibrosis, and bacteremia**.** *P. aeruginosa* produces several extracellular pigments that are important virulence factors associated with its pathology and virulence**,** among them is pyocyanin pigment**.** This study was designed to the green synthesis of gold nanoparticles and study their effect on the production of pyocyanin pigment in local *Pseudomonas aeruginosa* isolates. All isolates were identified as *P. aeruginosa* according to morphological, cultural, biochemical characteristics, VITEK-2 and *16S rRNA*. AuNPs were prepared using black tea leaf extract and characterized by UV-vis, XRD, SEM, FT-IR analysis. The results showed that the pyocyanin pigment production increased under the influence of gold nanoparticle with concentrations 20, 40, 80, 160, 320, and 100000 µg / ml.

Key words: Pigment production, Nanoparticles, Extraction of pyocyanin pigment, and purification of pyocyanin pigment.

1-Introduction

Pseudomonas aeruginosa is a significant opportunistic pathogen that significantly affects immunocompromised patients and burn victims and induces lung infections in patients with serious underlying diseases such as cystic fibrosis (CF), bronchectasis and chronic obstructive pulmonary disease (1), and possesses a natural resistance to many antibiotics and it causes widespread deaths in immunocompromised patients (2). *P. aeruginosa* is an occasional pathogen able to invade nearly all tissues. Furthermore, the *P. aeruginosa* is a germ widely distributed across the world (3). *P. aeruginosa* produces several extracellular pigments that are important virulence factors associated with its pathology and virulence (4), among them are pyocyanin and pyomelanin pigment (5). Pyocyanin pigment blue phenazine follows phenazines, which are redox-action pigments also, and it considered an antibiotic for several microorganisms (6, 7, and 8).

Several studies reported pigment production from *P. aeruginosa* isolated from urinary tract patients and chronic infection of cystic fibrosis patients (9). As a means of protection from light and free radicals for survival, it is also involved in the iron reduction and acquisition and extracellular electron transport (10). Gold NPs have been used for a long time due to their unique optical, photochemical, and electronic properties (11). The Chinese used colloidal red gold as medicine, and it was used in Indian medicine (12). At present, the gold NPs are used in cosmetics and hair tonics (13). Gold NPs emerged as an effective gene carrier due to their large surface area, low cell toxicity, and stability (14). Also, it can escape from the lysosome digestions, therefore it protects the DNA or the genes that carry from degradation (15). The gold NPs synthesized by bacteria did not exhibit a toxic effect on *P. aeruginosa*, but it increases the inhibition of

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pyocyanin pigment production (16). It also inhibits biofilm formation (17). Gold NPs, interfere with the bacterial cellular communication systems, which influence the expression of many virulence factors (18).

2-Materials and Methods

Isolation of *Pseudomonas aeruginosa*

The pigments-producing bacterium was isolated from 162 samples king A media agar plates for several days. Sixty-four isolates produced pyocyanin. They were picked out and identified as *P. aeruginosa*, based on their morphological and biochemical characteristics, VITEK-2 and *16SrRNA* the primer F(5-` GAGAGAGGGCAACTCGCTAC-3`) R(5`ACAACCTGCTGGACTATCGC-3`) (19) was supplied in a lyophilized form by the Macrogen company.

Screening of pigment production from local *P. aeruginosa* isolates:

Ninety-five isolates of *P. aeruginosa* were collected from several disease cases. Sixty-four isolates produced pyocyanin pigment. The isolates were able to produce the pyocyanin pigment on king A agar medium after 24-48 hr. at 37°C.

Extraction and purification of pyocyanin pigment

Separation of pyocyanin pigment from the culture medium (KingA broth) of *P. aeruginosa* isolates (P13) was performed according to (20)

Three ml of crude pyocyanin was added to the column, which was packaged by Sephadex G100, accurately and eluted with mobile phase chloroform in methanol solution (1:1). The flow rate was 30 ml/1hr. Pyocynin in the eluted fractions was detected using a UV spectrophotometer at a wavelength of 520 nm. After complete elution, results were plotted as optical density and fraction number.

Characterization of purified pyocyanin pigment

Purified pyocyanin was characterized using a UV-1800visible spectrophotometer (SHIMADZU), chloroform was used as the blank to record maximum absorption (21). Infrared spectra of pyocyanin separated from isolate (P13) culture was done and compared with the standard pyocyanin which was recorded by IR spectrometer, Shimadzu (Japan) from 4000- 400 cm^{-1} (22).

Preparation of gold nanoparticles (AuNPs):

The gold nanoparticles were prepared according to (23) as follows:

1.Preparation stock solution (12 mM) of HAuCl4

Dissolved 1gram of HAuCl4 (99% Germany) in 200 ml distilled water in a flask of 500 ml to obtain a solution of HAuCl4 (12 mM), and it was stored in a brown bottle.

2.Preparation of black tea leaves the solution

Using 0.34 gram from black tea leaves in 100 ml of distilled water on the magnetic hot plate for 15 min at 70-80 °C, then filtered with Whatman filter paper No.1.

-Synthetic AuNPs were synthesized by reducing HAuCl4 to Au**.** 100 ml of HAuCl4 solution (12 mM) were mixed with black tea leaves extract (100 ml) on a magnetic stirrer hotplate at 80°C, the color of the mixture turned purple within 10-15 minutes, indicating the production of gold nanoparticles, then sterilize the

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solution using a filter syringe 0.22µm. The AuNPs were then harvested by centrifugation at 12000 rpm for 20 min, washed with sterile distilled water three times, and dried at 40°C for 48hr.

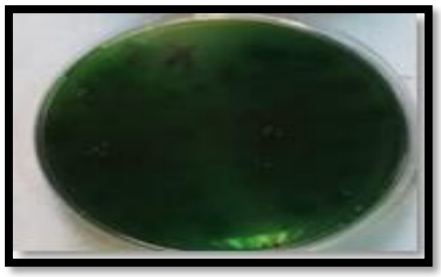
Characterization of AuNPs:

The nanoparticles have been characterized by several investigations to know some of their chemical and physical properties, such as UV-visible, SEM, FTIR, XRD (24). The analysis was performed to identify the absorbance of the visible and UV light spectrum at wavelengths from 400-600 nm to monitor the interaction between gold ions and tea extract (23). The gold NPs solution was diluted with sterile distilled water in a ratio of 1:9 ml using DW as a blank. Gold nanoparticles have been subject to FTIR analysis to know functional groups of the nanoparticles after being deposited on a glass slide to dry and then mixing them with potassium bromide (KBr) granules at a ratio of 1:100 until they are in the form of a disk, and then the model was measured by (FTIR-4100; Shimadzu-Japan) spectrophotometer (22). The structural characterization of the manufactured gold nanoparticles was analyzed as the sample was placed in powder form on a glass slide (25). X-ray diffraction device operating at a wavelength of 1.54060 with a Step Size of [°2Th] 0.0500. The aim of the Scanning Electron Microscopy (SEM) characterization is to determine the morphological structure of the surface layer (22). The examination is based on scanning the sample with a focused electron beam accurately to obtain microscopic images of the surface of the gold nanoparticles that have been prepared (26).

3-Results and Discussion

Screening of pigment production from local *P. aeruginosa* isolates:

Ninety-five isolates of *P. aeruginosa* were collected from several disease cases. Sixty-four isolates produced pyocyanin pigment. The isolates were able to produce the pyocyanin pigment on king A agar medium after 24-48 hr. at 37°C figure (1). The percentage of isolates producing pyocyanin pigment was 21.87% from ear infections, 39.05% from UTI, 20.31% from burns, 12.5% from RTI, 3.12% from both wounds, and CSF. The isolates of *Pseudomonas aeruginosa* that grew on the cetrimide agar (0.3%) media subjected microscopic, cultural biochemical analysis, VITEK-2 and *16SrRNA* these tests were compared with those reported by Kumar (27) figure (2) showed one band of 188 bp (product size) in the produced pigment isolates and the control as a result on agarose gel compare with DNA marker (100-1500 bp).



Figure(1): Pyocyanin pigment produced by *P.aeruginosa* on KingA agar medium

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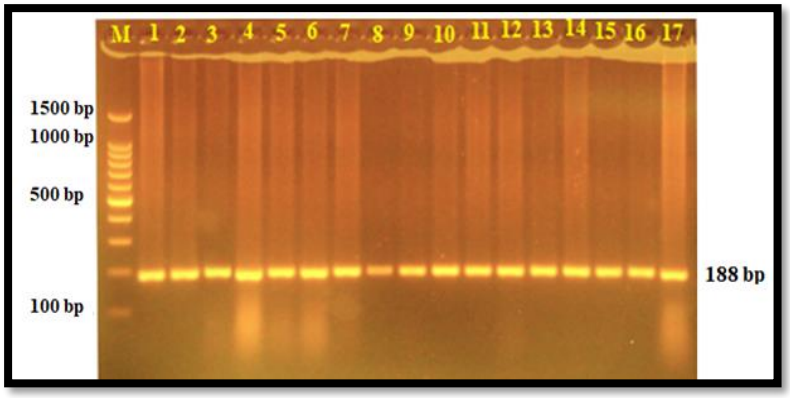

Figure (2): Agarose gel electrophoresis of PCR product for *16S rRNA* gene (188bp) of *Pseudomonas aeruginosa*.

Extraction and purification of pyocyanin pigment

The pyocyanin pigment was extracted from KingA broth in three isolates successfully using chloroform, 0.1N HCl, which are considered more efficient in extracting the pigment. The extraction process was done by treating the pigment broth with chloroform with king A broth in a ratio of 2:1, then treating the blue layer with hydrochloric acid modifying the pH to7 using 0.4M NaOH, this process was repeated 3-4 times until the pigment was completely extracted. Pyocyanin pigment can exist in either oxidized or reduced form, the latter being an unstable form of pyocyanin that reacts rapidly with molecular oxygen pyocyanin using this protocol is easy and inexpensive (29). Extraction of. Purification of the crude pyocyanin pigment of isolates was done by using gel filtration using Sephadex G100 due to its suspected low molecular weight. The pyocyanin assay is based on the absorbance of pyocyanin at 520 nm (29) and the result was in agreement with many previous studies (30, 29).

Characterization of purified pyocyanin pigment

1. UV-VIS spectrophotometer

The absorbance spectrum of purified pyocyanin pigment was measured at a wavelength of 200-800 nm. The pigment showed maximum absorbance peaks at 310, 379, 660, and 696 nm figure (5). This result complied with results of the UV spectrum of standard pyocyanin pigment at wavelength 310, 529, 699, and 254.5 nm by (31). These results came in the same direction as who studied the UV spectrum for pyocyanin pigment and found absorption peaks at 316, 367, and 700 nm (32).

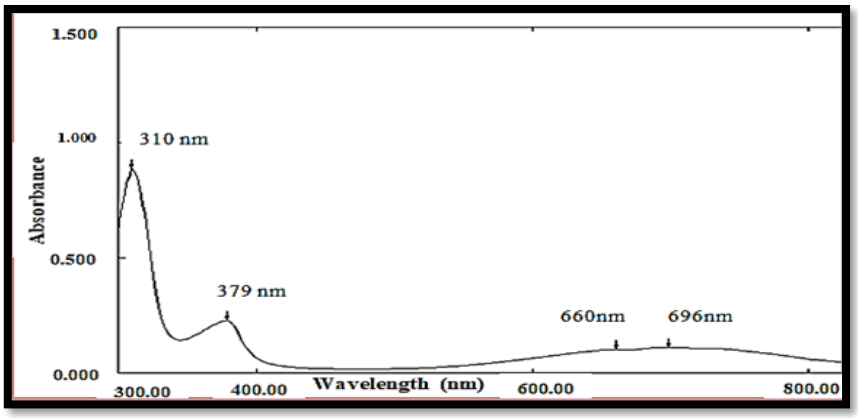

Figure (5): UV-vis for purified pyocyanin pigment.

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2.IR-spectra analysis for pyocyanin pigment:

The purified pyocyanin pigment was subjected to an infrared absorption analysis to characterize the pigment using maximum absorbance spectra of the functional groups included in the chemical composition of the pyocyanin pigment figure (6). The results of the IR-spectra demonstrated the absorbance peaks between 3400-3500cm^{-1} region, 3438 cm^{-1} indicating the presence of the –OH group and absorbance peak at 3120 cm^{-1} representing -C-H stretch for group belonging to the aromatic compound and peak at 2925cm^{-1}, 2856 cm^{-1} representing aldehyde group (CHO). As for the area of 1650-1780 cm^{-1}exhibited a peak at 1739.67, which is referring to the present carbonyl group (C=O) (33), while absorption peaks appeared in the region of 600-1400cm^{-1}, including the absorption peaks at 1267.14 and 1315.36 were indicative of C-N bonds in aromatic stretching and C-O, respectively (33). The region at1590-1690 cm^{-1}presented a peak at 1645.17cm^{-1}indicative unsaturated bonds of C-N (34), a peak at 1035.70 cm^{-1} indicating the alkali C-O (34). These results showed similarities with FTIR for standard pyocyanin pigment (35).

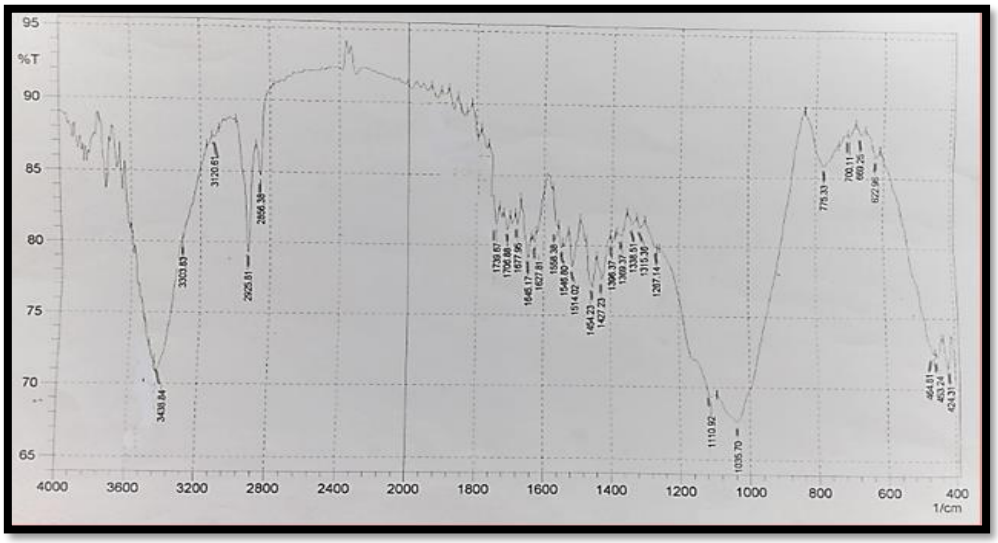

Figure (6): FTIR for purified pyocyanin pigment from local isolates.

Biosynthesis of gold nanoparticles by green chemistry method:

An eco-friendly method was used to prepare gold nanoparticles, where dry black tea leaves were used that contain polyphenols, which is a good reducing agent for the formation of NPs (36), and phytochemicals which include water-soluble catechins that act as a reducing agent and envelops the surface of the gold particles to prevent their clumping. Water has also been used as an environmentally harmless solvent (21). The preparation was done by using a stock solution of gold chloride with a stock solution of tea leaves mixed at 60^{o}C for 15 min, where the solution turned into deep purple color, indicating the formation of gold nanoparticles (AuNps) figure (7), the color change is a sign of interaction between tea extract and gold chloride tetrahydrate ($HAucl_4$), this color change is due to the excitation of the Plasmon surface as a result of the collective movement of free electrons present in the nanoparticles in the visible light area (37).

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Figure (7): Biosynthesis of gold nanoparticles using black tea extract as a reducing agent.

Characterization of gold nanoparticles (AuNps) :

1.UV-visible analysis :

The visible and ultraviolet spectrum analysis was used to studying the properties of gold nanoparticles, the maximum absorption of AuNPs was at 546 nm figure (8), as the nanoparticles possess free electron, and when subjected to a UV-visible source, the surface of the Plasmon is irritated and the electrons vibrate each other, which leads to an increase in the absorbance of the Plasmon (38). This result was in agreement with Acta *et al* who recorded an absorbance peak at 546 nm for AuNPs prepared using an extract of beluntas leaf *Pluchea indica* as a reducing agent (25).

2.Scanning Electron Microscopy (SEM) for the detection of gold nanoparticles:

The gold nanoparticles deposited on the slide were examined using a scanning electron microscope (SEM), which was accurately prepared for the surface topography of the NPs. Scanning electron microscope (SEM) showed the shape and the size of the AuNPs, which were spherical or semi-spherical in shape and size was19.11 nm figure (9). As for Nun et al, they indicated that the size of the AuNPs was 15-45 nm and spherical shape after its preparation by tea extract as a reducing agent (39).

3.FT-IR analysis of gold nanoparticles (AuNps):

The infrared spectrum analysis was used to identify the functional groups responsible for the formation and stability of AuNPs. The results of the analysis showed in figure (10), which showed the presence of a strong peak at 3479 cm^{-1} represent a bond in the O-H group of tea extract that is involved in the biosynthesis of AuNPs (40). Also, the presence of a strong absorbance band at 2970 cm^{-1} indicated the C-H bond in alkane groups, absorbance peak at 1947 cm^{-1} represented (C=C), band of absorbance at 1710 cm^{-1} indicated C=O stretching in carbonyl group (16). The band of absorbance at 1610 cm^{-1} for C=N stretching of the amide group in the proteins. The peak located at 2541cm^{-1} was attributed to the N–H stretching vibrations (41). The absorbance peaks at 1070 cm^{-1} were for C-OH aliphatic amines, and the weak band at1375 cm^{-1} for C-N functional group in aromatic amines. The band at 1218 cm^{-1} for C-O stretching is related to polyphenols (42). The presence of an absorbance peak at 3479cm^{-1}showed the involvement of the -OH group of polyphenols as catechins in the green synthesis of AuNPs, band at1392 cm^{-1}illustrate the presence of caffeine (43). The absorbance peaks at 1070,1610 and 1218 cm^{-1} demonstrate that AuNPs may bound to the protein through the carboxylate group (42). These results are in accordance with previous reports (17, 16) and demonstrated the presence of black tea polyphenols as reducing agents in the biosynthesis of AuNPs, which gave stability to the NPs (44)

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4. Detection of gold NPs by X-ray diffraction analysis (XRD):

X-ray diffraction analysis (XRD) was used to find out the crystal structure of AuNPs fixed on a glass slide by using an X-ray beam incident on it. The crystal structure of the AuNPs appeared with three peaks figure (11), corresponding to the standard Prague diffraction or reflections (111, 200, and 220). The strong peak at 38.37 corresponding to (111), where peaks at 44.53˚and 64.72˚correspond to (200) and (220), respectively. These results were in agreement with that reported by Mishra et al, who indicated the results of XRD gold NPs with the appearance of three peaks at 38.1, 44.56, and 64.74 (45).

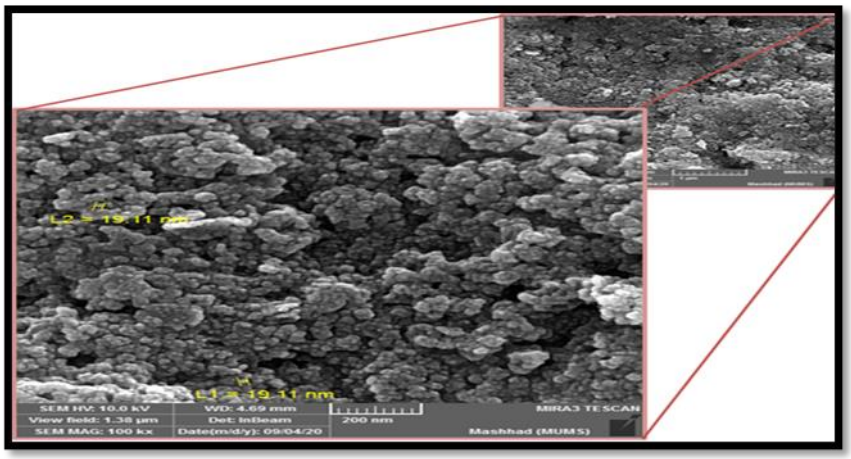

Figure (8): UV-visible for AuNPs green synthesis by black tea extract.

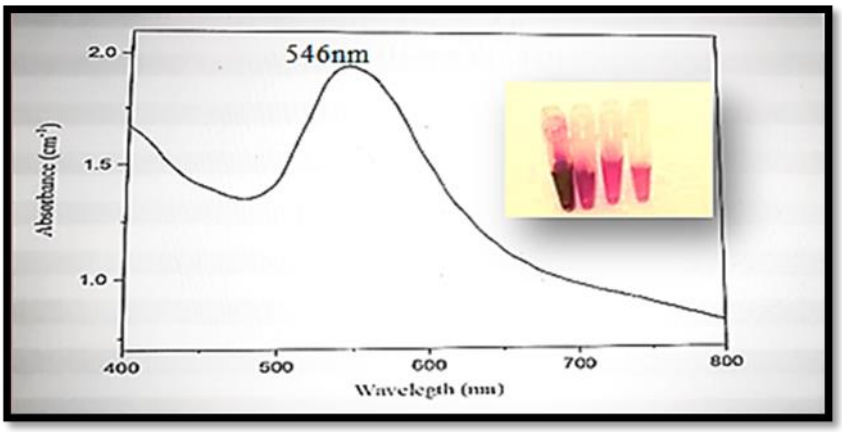

Figure (9): Scanning Electron Microscopy (SEM) for green synthesized AuNPs

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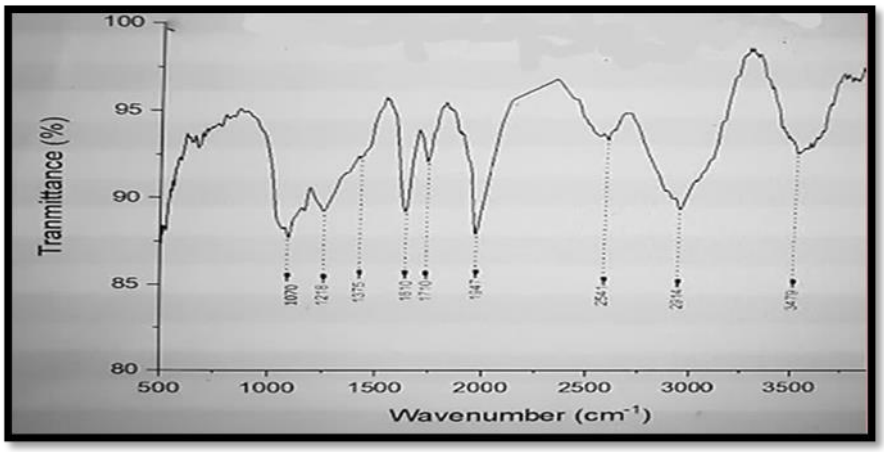

Figure (10): IR-spectra analysis for green synthesized AuNPs.

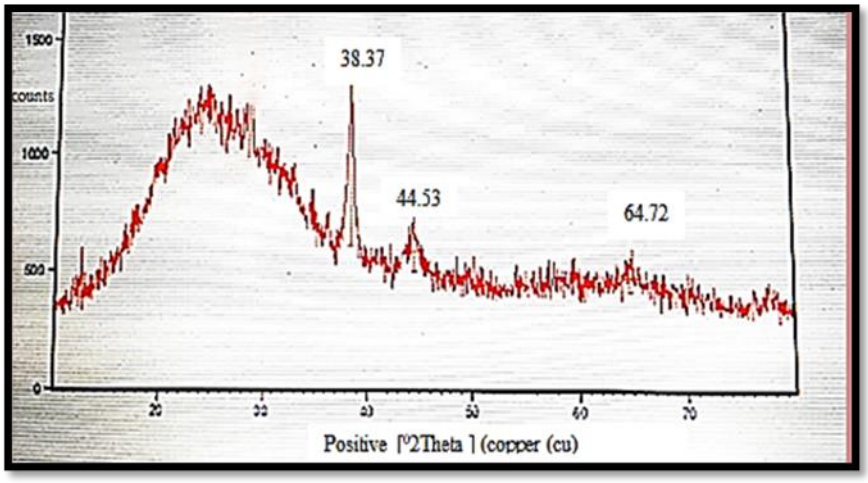

Figure (11): X-ray Diffraction of gold NPs surface.

Effect of biosynthesized AuNPs on pigments production

By mixing 0.1 gram of AuNPs with 10 ml of distilled water (10 mg/ml), the stock solution of gold nanoparticles was prepared in concentrations (10, 20, 40, 80, 160, 320, and 100000)µg /ml by mixing the known amount of the stock solution with sterile distilled water. The bacteria suspension was prepared and compared with McFarland No.0.5, then 100 µl of bacterial suspension were transferred to Luria broth and tyrosin broth inoculated with 100µl of AuNPs concentrations (0, 10, 20, 40, 80, 160, 320 and 100000µg / mL) figure (12) then incubation of tubes at 37 for 18-24 hrs. (23, 46).

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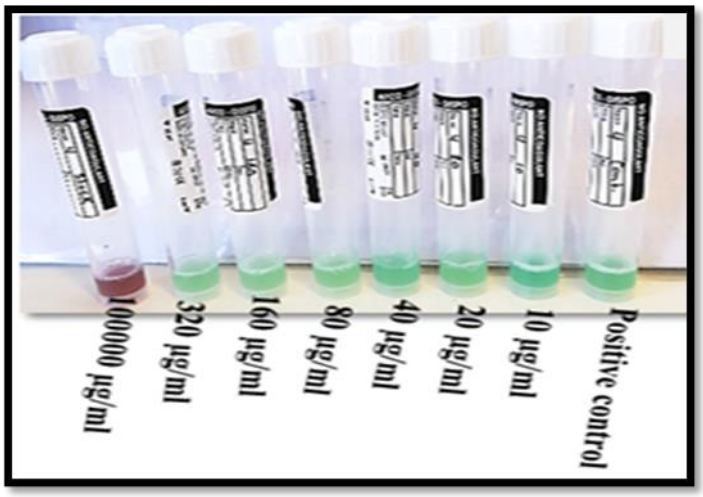

Figure(12): *P. aeruginosa* isolates treated with different concentrations of AuNps in Luria broth medium (LB).

The treatment with gold NPs leads to increase pyomelanin pigment production at a concentration of 80 µg / ml 160 µg/ml concentration, 320 and 100000 µg / ml concentration, respectively in comparison to the control.

The results indicated an increase in pyocyanin pigment under the effect of AuNPs compared to untreated isolates or control. Pigment production increased at a concentration of 20, 40, 80, 160, 320, and 100000 µg / ml in comparison to the control sample. Ellis et al reported that pyocyanin pigment could protect *P. aeruginosa* from the influence of silver NPs and prevent their death. It was also reported that exposure *P. aeruginosa* to silver NPs increases the expression of the pyocyanin, pyoverdin, and pyochelin (47). While Lee et al used Zinc oxide NPs to study their effect on pyocyanin, pyoverdin, and pyochelin pigments in *P. aeruginosa* and indicated that ZnO NPs led to the suppression production of pyocyanin and pyochelin pigments and induced production of pyoverdine pigment (48). Gold NPs can penetrate the cell membrane where accumulate in the cytoplasm and can interact with DNA, causing various biological and biochemical effects, which leads to mutations and genetic instability. Gold nanoparticles caused variations in the expression of many genes, Tian *et al* reported that these NPs upregulated 374 genes and reduced the expression of 135 genes responsible for many cellular activities of cells (49). The results of the statistical analysis showed a significant effect on the increased pigment's production, thus increasing the production of pigments as a means of resistance, and these gold NPs did not have a toxic effect on the *P. aeruginosa* bacteria.

References

1. Hardalo C, Edberg SC. *Pseudomonas aeruginosa*: assessment of risk from drinking water. Crit Rev Microbiol. 1997;23(1):47–75.
2. Bodey GP, Bolivar R, Fainstein V, Jadeja L. Infections caused by *Pseudomonas aeruginosa*. Rev Infect Dis. 1983;5(2):279–313.
3. Shaebth LJ. Molecular identification and sequencing of *Pseudomonas aeruginosa* virulence genes among different isolates in al-diwaneyah hospital. Iraqi J Vet Sci. 2018;32(2):183–8.
4. Prof A, Shamaa SDA-, Shababa L. Production of Extracellular Pigments as a Virulence Factor of *Pseudomonas aeruginosa*. 2011;11(2):689–97.
5. Lau GW, Hassett DJ, Ran H, Kong F. The role of pyocyanin in *Pseudomonas aeruginosa* infection. Trends Mol Med. 2004;10(12):599–606.

6. Baron SS, Rowe JJ. Antibiotic action of pyocyanin. Antimicrob Agents Chemother. 1981;20(6):814–20.
7. Abdul-Hussein, Atia. Antimicrobial Effect of Pyocyanin Extracted from *Pseudomonas aeruginosa* Keywords : Vol. 6, iMedPub Journals. 2016. p. 4–
8. Turick CE, Knox AS, Becnel JM, Ekechukwu AA, Milliken CE. Properties and function of pyomelanin. Biopolymers. 2010;449:72.
9. Hunter RC, Newman DK. A putative ABC transporter, hatABCDE, is among molecular determinants of pyomelanin production in *Pseudomonas aeruginosa.* J Bacteriol. 2010;192(22):5962–71.
10. Wang Z, Lin B, Mostaghim A, Rubin RA, Glaser ER, Mittraparp-arthorn P, et al. Vibrio campbellii hmgA-mediated pyomelanization impairs quorum sensing, virulence, and cellular fitness. Front Microbiol. 2013;4:379.
11. Higby GJ. Gold in medicine. Gold Bull. 1982;15(4):130–40.
12. Richards DG, McMillin DL, Mein EA, Nelson CD. Gold and its relationship to neurological/glandular conditions. Int J Neurosci. 2002;112(1):31–53.
13. Skrabalak SE, Chen J, Au L, Lu X, Li X, Xia Y. Gold nanocages for biomedical applications. Adv Mater. 2007;19(20):3177–84.
14. Thakur S, Saini R V., Singh P, Raizada P, Thakur VK, Saini AK. Nanoparticles as an emerging tool to alter the gene expression: Preparation and conjugation methods. Mater Today Chem [Internet]. 2020;17:100295.
15. Panyam J, Zhou W, Prabha S, Sahoo SK, Labhasetwar V. Rapid endolysosomal escape of poly (dl-lactide-coglycolide) nanoparticles: implications for drug and gene delivery. FASEB J. 2002;16(10):1217–26.
16. Diego KDGS, Alindayu JIA, Baculi RQ. Biosynthesis of gold nanoparticles by bacteria from hyperalkaline evaluation of their inhibitory activity against pyocyanin production. 2018.
17. Yu Q, Li J, Zhang Y, Wang Y, Liu L, Li M. Inhibition of gold nanoparticles (AuNPs) on pathogenic biofilm formation and invasion to host cells. 2016;6(May):26667.
18. García-Lara B, Saucedo-Mora MÁ, Roldán-Sánchez JA, Pérez-Eretza B, Ramasamy M, Lee J, et al. Inhibition of quorum sensing dependent virulence factors and biofilm formation of clinical and environmental *Pseudomonas aeruginosa* strains by ZnO nanoparticles. Lett Appl Microbiol. 2015;61(3):299–305.
19. Suleiman A, Turkey A yuosif A. Molecular Variation Study of Clinical Isolates of Pseudomonas aeruginosa for *16SrRNA,pvdE, toxA*, and *phzM* Genes Related with Virulence and Characterization Features. 2015;
20. Alqahtani FS. Pyocyanin Pigment Extracted from *Pseudomonas aeruginosa* Isolate as Antimicrobial Agent and Textile Colorant. Orig Res Pap. 2016;5(9):467–70.
21. Sudhakar T, Karpagam S, Shiyama S. Analysis of pyocyanin compound and its antagonistic activity against phytopathogens. Int J ChemTech Res. 2013;5:1101–6.
22. Alhamadany AH, Rajaa MA, Jassim EA, Zaid NA. Synthesis and characteristics of gold nanoparticles/Epoxy polymer Nano composite material. J Phys Conf Ser. 2019;1279(1):6–12.
23. Sharma RK, Gulati S, Mehta S. Preparation of gold nanoparticles using tea: A green chemistry experiment. J Chem Educ. 2012;89(10):1316–8.
24. Nur Y. Gold Nanoparticles : Synthesis, Characterisation and their Effect on *Pseudomonas Fluorescent* By Yusuf Nur. 2013;(April).
25. Samanta S, Singh BR, Adholeya A. Intracellular Synthesis of Gold Nanoparticles Using an

Ectomycorrhizal Strain EM-1083 of Laccaria fraterna and Its Nanoanti-quorum Sensing Potential Against *Pseudomonas aeruginosa*. Indian J Microbiol. 2017;57(4):448–60.

26. Danied MC, Astruc D. Gold nanoparticles: assembly, supram-olecular chemistry, quantum-size-related properties, and applications toward biology, catalysis, and nanotechnology. Chem Rev. 2004;104:293–346.
27. Kumar S. Kumar, S. (2012). Textbook of Microbiology. Jaypee Brothers, Medical Publishers Pvt. Limited. https://books.google.iq/books?id=ikl-ds56O4ECextbook of Microbiology
28. Parsons JF, Greenhagen BT, Shi K, Calabrese K, Robinson H, Ladner JE. Structinconal and functional analysis of the pyocyanin biosynthetic protein PhzM from *Pseudomonas aeruginosa*. Biochemistry. 2007;46(7):1821–8.
29. Essar DW, Eberly L, Hadero A, Crawford IP. Identification and characterization of genes for a second anthranilate synthase in Pseudomonas aeruginosa: Interchangeability of the two anthranilate synthase and evolutionary implications. J Bacteriol. 1990;172(2):884–900.
30. Kurachi M. Studies on the biosynthesis of pyocyanine. (II): Isolation and determination of pyocyanine. 1958;
31. Priyaja P. Pyocyanin (5-Methyl–1-hydroxyphenazine) produced by *Pseudomonas aeruginosa* as antagonist to Vibrios in aquaculture: Over expression, downstream process and toxicity. 2012;159.
32. El-Fouly MZ, Sharaf AM, Shahin AAM, El-Bialy HA, Omara AMA. Biosynthesis of pyocyanin pigment by *Pseudomonas aeruginosa*. 2015;8(1):36–48.
33. El-Zawawy NA, Ali SS. Pyocyanin as anti-tyrosinase and anti tinea corporis: A novel treatment study. Microb Pathog [Internet]. 2016;100:213–20.
34. Bavithra H, Sathiyamurthy K. Research Article SCREENING , characterization,antimicrobial and cytotoxic potential of pyocyanin producing *Pseudomonas* spp . Isolation from the sponge. 2019;11(1):1471–6.
35. DeBritto S, Gajbar TD, Satapute P, Sundaram L, Lakshmikantha RY, Jogaiah S, et al. Isolation and characterization of nutrient dependent pyocyanin from *Pseudomonas aeruginosa* and its dye and agrochemical properties. Sci Rep. 2020;10(1):1–12.
36. KSV G. Green Synthesis of Iron Nanoparticles Using Green Tea leaves Extract. J Nanomedicine Biotherapeutic Discov. 2017;07(01):1–4.
37. Noginov MA, Zhu G, Bahoura M, Adegoke J, Small C, Ritzo BA, et al. The effect of gain and absorption on surface plasmons in metal nanoparticles. Appl Phys B. 2007;86(3):455–60.
38. Dorjnamjin D, Ariunaa M, Shim YK. Synthesis of silver nanoparticles using hydroxyl functionalized ionic liquids and their antimicrobial activity. Int J Mol Sci. 2008;9(5):807–20.
39. Nune SK, Chanda N, Shukla R, Katti K, Kulkarni RR, Thilakavathy S, et al. Green nanotechnology from tea: Phytochemicals in tea as building blocks for production of biocompatible gold nanoparticles. J Mater Chem. 2009;19(19):2912–20.
40. Lee YJ, Ahn E-Y, Park Y. Shape-dependent cytotoxicity and cellular uptake of gold nanoparticles synthesized using green tea extract. Nanoscale Res Lett. 2019;14(1):1–14.
41. Bankar A, Joshi B, Kumar AR, Zinjarde S. Banana peel extract mediated synthesis of gold nanoparticles. Colloids Surfaces B Biointerfaces. 2010;80(1):45–50.
42. Aswathy Aromal S, Philip D. Green synthesis of gold nanoparticles using *Trigonella foenum-graecum* and its size-dependent catalytic activity. Spectrochim Acta - Part A Mol Biomol Spectrosc [Internet]. 2012;97:1–5.
43. Rolim WR, Pelegrino MT, de Araújo Lima B, Ferraz LS, Costa FN, Bernardes JS, et al. Green tea

extract mediated biogenic synthesis of silver nanoparticles: characterization, cytotoxicity evaluation, and antibacterial activity. Appl Surf Sci. 2019;463:66–74.

44. Smitha SL, Philip D, Gopchandran KG. Green synthesis of gold nanoparticles using *Cinnamomum zeylanicum* leaf broth. Spectrochim Acta Part A Mol Biomol Spectrosc. 2009;74(3):735–9.
45. Mishra A, Harper S, Yun S Il. Interaction of biosynthesized gold nanoparticles with genomic DNA isolated from *E. coli* and *S. aureus*. Proc IEEE Conf Nanotechnol. 2011;1745–50.
46. San Diego KDG, Alindayu JIA, Baculi RQ. Biosynthesis of gold nanoparticles by bacteria from hyperalkaline spring and evaluation of their inhibitory activity against pyocyanin production. J Microbiol Biotechnol Food Sci. 2020;9(4):781–7.
47. Ellis DH, Maurer-Gardner EI, Sulentic CEW, Hussain SM. Silver nanoparticle antibacterial efficacy and resistance development in key bacterial species. Biomed Phys Eng Express. 2018;5(1):15013.
48. Lee JH, Kim YG, Cho MH, Lee J. ZnO nanoparticles inhibit *Pseudomonas aeruginosa* biofilm formation and virulence factor production. Microbiol Res [Internet]. 2014;169(12):888–96.
49. Tian B, Li J, Pang R, Dai S, Li T, Weng Y, et al. Gold nanoparticles biosynthesized and functionalized using a hydroxylated tetraterpenoid trigger gene expression changes and apoptosis in cancer cells. ACS Appl Mater Interfaces. 2018;10(43):37353–63.