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Gold Nanoparticles: Green Synthesis, Characterization and Biological Activities

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Abstract

This study aims to introduce a green, eco-friendly, fast and cost-effective method to synthesis gold nano-sized particles (Au NPs) from actinomycete strain; Streptomyces sp. U30 (KP109810), isolated from metal containing rock. The biosynthesized gold nanoparticles were formed at lower conditions of pH, mass weight and incubation time (7 hours only). The bio-formed Au NPs were fully characterized with different techniques to study their nano-metrological measurements. The X-ray diffraction (XRD) technique and high-resolution transmission electron microscopy (HR-TEM) were used to determine the crystal structure, size and shape of Au NPs. Fourier transform infrared spectroscopy (FTIR) studies were done to investigate the distinct functional groups present on the surface. The zetasizer technique was used to define the stability of biosynthesized gold nanoparticles. The antimicrobial activities of biosynthesized gold nanoparticles were studied against gram positive and gram-negative bacteria at three different concentrations, 10, 30 and 50 µg/disc, using agar well diffusion method. The obtained results prove that the biosynthesized Au NPs gave different antimicrobial action which was strain dependent. All the tested bacterial strains were inhibited by the examined concentrations with variations in the inhibition zone diameters, except Candida albicans showed resistant to the biosynthesized Au NPs. The bio-formed gold nanoparticles can be used in different applications.

Keywords: Gold nanoparticles; Characteristics, ISO, Nano-metrological measurements, Antimicrobial activity.

1. Introduction

The rapid development of using nanomaterials in many sectors has raised a strong need for international guidelines to study their classifications, characterization and measurements. In 2005, the international organization for standardization (ISO) was established a technical committee (TC 229) in the field of nanotechnology. This committee published many standards and guides related to definitions of nano-objects, classification, characterization, and methods of measurements as ISO/TS 27687:2008, ISO/TR 11360:2010, ISO/TR 18196:2016 and ISO 21363:2020 respectively. Nanometrology, the field of measurements at the nano-scale level, includes measurements of length, size, shape, size distribution, chemical composition, nanoparticles concentration, and mass, electrical, force and other properties [1]. In the ISO/TS 80004 Nanotechnologies-vocabulary series, nanomaterial was defined as the material with any external dimension in the nano-scale (<100 nm). Biological and environmental interactions associated with these nanomaterials can be affected by size, aspect ratio, core chemistry, agglomeration state, physical state, surface properties and others. The extraordinary physical and chemical properties of nano-sized particles give them an immense interest in important applications in many fields, ranging from electronics, bio-medicine and agriculture to water treatment, energy and cosmetics. Nano-sized particles are considered successful candidates for bio-medical applications at nanometer level [2-4]. Metal nanoparticles are unique in their properties and received increasing interest as antimicrobials. These metal nanoparticles include gold, silver, zinc oxide, titanium oxide, copper oxide and magnesium oxide nanoparticles [5]. At present, gold nanoparticles are used in various fields of research and have potential applications in physics, chemistry, material science,

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biology, medicine and their different interdisciplinary fields [6]. Antibacterial activity of metal nanoparticles [7-11] is widely utilized for textile fabrics, water treatment, food packaging, drug and gene delivery, surgical devices and diagnostics. Gold nanoparticles (Au NPs) demonstrated high antibacterial activity [12]. For the synthesis of Au NPs, many physical and chemical methods have been well developed but because of using toxic and hazardous chemicals in the synthesis process, these methods are toxic and may hurt the human beings as well as the environment [13]. Synthesis of nanoparticles from biological methods is recommended as a possible alternative to chemical and physical methods.

The biosynthesis of gold nanoparticles is becoming dominant because of the easy reduction of their salts at room temperature, non-toxicity, cost effectiveness and getting different geometrical shapes of nanoparticles [14]. Synthesis of Au NPs from chloroauric acid biologically was reported [15-18]. Microorganisms have been reported as a good factory for Au NPs [19-25]. Amongst bacteria, Lactobacillus sp., Rhodopseudomonas capsulate and Pseudomonas spp., were used to biosynthesis gold nanoparticles [26-29]. These kinds of bacteria were considered as micro-factories and it can mediate the intracellular or extracellular synthesis of nanoparticles. Genera of the actinobacteria, particularly *Streptomyces*, declared the richest source of bioactive compounds including antimicrobial and antitumor compounds [30-32], antibiotics [33], it also active in bioremediation [34, 35], wastewater treatment [36] and bio-mining [37]. In this study, Au NPs biosynthesized form actinomycete strain; *Streptomyces sp*. U30 (KP109810) that was isolated from metal containing rock and their nano-metrological measurements were done with different techniques. Their antimicrobial activities were tested against seven pathogen strains. The biosynthesized gold nanoparticles can be used in different applications like cosmetics, diagnostics, drug and gene delivery, cancer therapy and water treatment.

2. Experimental

In this experiment, multi-metal resistant Streptomyces sp. U30 (KP109810) was isolated previously from sandstone at Um Bogma village in west-central Sinai, Egypt to be used in the biosynthesis process [33]. Streptomyces sp. U30 (KP109810) spores were inoculated into starch casein broth and incubated at 30 ˚C under continuous agitation at 100 rpm for 3 days. The bio-mass was harvested by centrifugation and then rinsed twice with sterile distilled water. The washed pellets were resuspended in sterile tap water and the dry weight was measured. For synthesis of Au NPs, 100 mg dry weight equivalent, after rewashing with sterile distilled water, was added to 100 ml of HAuCl4 (the final concentration 100 mg/l) of pH 4, the mixture was agitated at 100 rpm. The biosynthesis of gold nano-sized particles was observed through a visual change in the color of the mixture to purple. The flasks were left static to settle the bio-mass and the suspended Au NPs were separated to study their characteristics and biological activities. Many international standards were introduced by the experts of technical committee (TC 229) to facilitate characterizing of the nanomaterials such as sample preparation, ISO/TR 20489:2018 (Nanotechnologies – Sample preparation for the characterization of metal and metal-oxide nano-objects in water samples), measuring the concentration as ISO/TS 19590:2017 (Nanotechnologies - Size distribution and concentration of inorganic nanoparticles in aqueous media via single particle inductively coupled plasma mass spectroscopy), surface characterization as ISO/TS 14101:2012 (Surface characterization of gold nanoparticles for nanomaterial specific toxicity screening: FT-IR method) and morphology as ISO 21363:2020 (Nanotechnologies – Measurements of particle size and shape distributions by transmission electron microscope). In this study, the biosynthesized gold nanoparticles (Au NPs) were characterized for absorption by UV-vis spectrophotometer (ShimaDZU, UV3101PC) at room temperature. The structure analysis was carried out by X-ray diffraction (XRD, D8 Advance, BRUKER) with Cu Ka (λ= 1.54056 Å) to confirm the biosynthesis of gold nano-sized particles. The functional groups on the surface of Au NPs were investigated by Fourier transform infrared spectrophotometer (JASCO, FT/IR-4100typeA). The stability of nanoparticles was defined by Zetasizer (Nano ZS90, Malvern Instrument). The size and shape of nanoparticles were investigated by the nanometrological technique, high resolution transmission electron microscope (HR-TEM, Jeol JEM 2100). The concentration of the solution was measured by inductively coupled plasma spectrophotometer (ICP Spectrophotometer, Perkin Elmer, Optima 7300DV).

Biological and physical sciences share a common interest in nanoparticles, especially in the fight against economically and socially significant bacterial species [39]. In this study, the antimicrobial activity of the biosynthesized Au NPs was tested against seven pathogen strains. The type cultures were gram negative {Escherichia coli (ATCC25922),

Pseudomonas aeruginosa (ATCC27853) and Salmonella typhimurium (ATCC14028)}, gram positive {Bacillus subtilis (ATCC6633) and Staphylococcus aureus (ATCC25923)}, yeast Candida albicans (ATCC10231), and MRSA, a clinical culture. Agar well diffusion method was used to study the antimicrobial activity of the biosynthesized Au NPs [40]. Nutrient agar plates were inoculated by 107 CFU of each bacterial strain by spreading over the entire agar surface. A hole of 8 mm diameter was perforated aseptically with a sterile cork borer, and a volume that contains a desired concentration of the biosynthesized Au NPs was introduced into the well. After wells imbibition and dryness, the plates were incubated at 37 ˚C for 24 h. Four wells in each plate were perforated, three loaded with the biosynthesized Au NPs (10, 30 and 50 µg/well) and a blank well was loaded with distilled water used as a negative control.

3. Results and Discussion

Biosynthesis of gold nanoparticles

Materials at nano-scale show different physical and chemical characteristics than their bulk state [38]. Au NPs exhibit unique optical properties in the visible region due to its interaction with light. Because of their surface Plasmon oscillation of free electrons [38], the color of gold nano-sized particles ranged from deep red to purple [15]. The synthesis of gold nano-sized particles was preliminary characterized through the visible observation in the color change of the solution from pale yellow to purple, the characteristic color of gold nano-sized particles [41], as shown in figure 1. However, the color change depends on the size and shape of gold nanoparticles. Different size and shape of gold nanoparticles possess different color change.

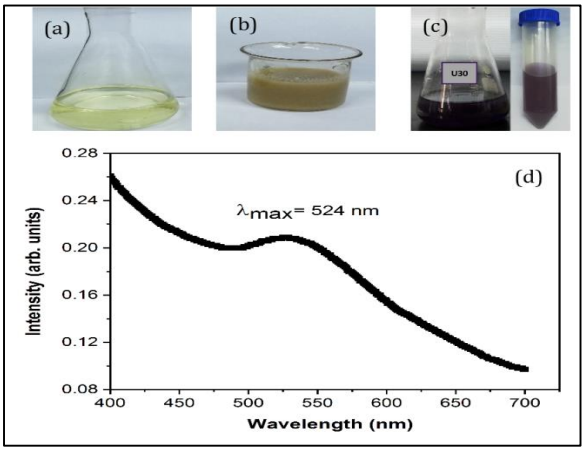

Fig. 1. Biosynthesis of Au NPs and color change of gold solution into purple. (a) gold solution, (b) *Streptomyces* sp. U30 biomass, (c) biosynthesized Au NPs, and (d) UV-visible absorption spectra

The optical properties of nanoparticles are particle size dependent [42,43]. The absorption spectrum is considered an important characteristic of the formation of nanoparticles. In the current study, UV-visible characterization of gold nano-sized particles was done by recording the absorbance measurements over the wavelength range of 400 -700 nm using quartz cuvette with 1 cm path length. The synthesis of gold nano-sized particles was confirmed by a well-defined absorption peak appeared at 524 nm as shown in figure 1(d). This peak appeared after 7 hours and this time is less than the optimum biosynthesis time that was stated by Naimi-Shamel et al. (48 hours) [19], Waghmare et al. (72 hours) [44] and Balagurunathan et al. (24 hours) [45]. The obtained peak corresponds to the surface plasmon resonance (SPR) wavelength of Au NPs [46-48]. The absorption band of SPR appeared because of free electrons variation of metal nanoparticles combined with the light wave in resonance [49].

X-ray diffraction analysis

XRD analysis of gold nano-sized particles was done over the spectrum of 2ɵ values from 20˚ to 80˚ to provide information about the crystalline nature of biosynthesized nanoparticles. The sample was prepared by depositing the solution on a glass substrate. In figure 2, six Bragg diffractions patterns were observed at 27.05º, 30.99º, 38.18º, 44.64º, 64.74º and 77.31º. Four of them were typical XRD patterns of Au NPs corresponding to (111), (200), (220) and (311) facets of the face-centered cubic crystal structure (fcc). The unknown peaks marked with x could be appeared due to crystalline bio-organic compounds from the extract. These results are consistent with those reported for gold nanocrystals [41, 50-51]. The results confirmed the presence of Au NPs formed by actinomycete strain; *Streptomyces* sp. U30 (KP109810), isolated from metal containing rock.

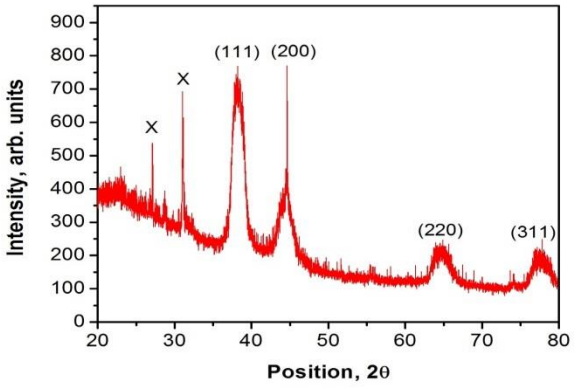

Fig.2. X-ray diffraction patterns of biosynthesized gold nanoparticles showing peak indices.

The average crystal size of biosynthesized gold nanoparticles was calculated by the Debye-Sherrer equation after fitting as a Gaussian distribution. It was found to be 7.6 nm. For more accurate measurement of particle size and shape, analysis of HR-TEM images were done.

FTIR spectroscopy studies

FTIR measurement of biosynthesized Au NPs was carried out in the range of 400-4000 cm^{-1} using JASCO spectrophotometer to define the distinct functional groups existent on the surface. Figure 3 displayed the FTIR spectroscopy analysis of biosynthesized Au NPs. The broad peak at 3451.96 cm^{-1} was interpreted as O–H stretching, peak located at 1640.16 cm^{-1} was assigned to C=O bonds in reduction of gold (III) ions to gold atoms [34], peak at 1463.71 cm^{-1} was characteristic for amine and amino-methyl stretching groups and peak at 1155.15 cm^{-1} marked to C–O bonds. Biomolecules linked to nanoparticles surface through free amino groups in the proteins [52, 53]. The existence of these proteins over the metal nanoparticles worked as capping agents [54, 55] and they were supported the formation and stabilization of gold nanoparticles.

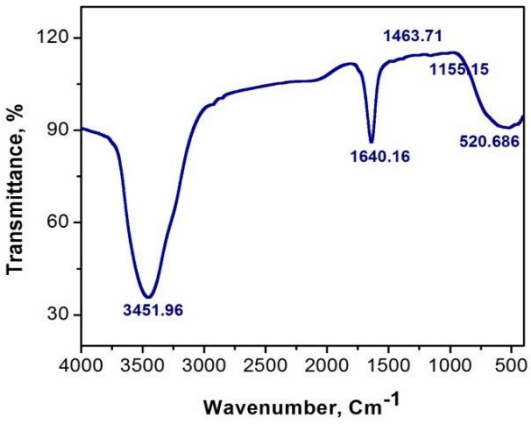

Fig.3. FTIR spectra of biosynthesized gold nanoparticles.

Zeta potential analysis

Zeta potential was measured to define the degree of stability of biosynthesized gold nanoparticles by using the Zetasizer technique, Nano ZS 90. A diluted solution was used to avoid the aggregation of Au NPs. The measured value of zeta potential of biosynthesized Au NPs was -27.8 ± 6.86 mV. This result indicated the stability and monodispersity of biosynthesized Au NPs due to the electrostatic repulsion between charged nano-sized particles in the solution. In literature, nanomaterials with surface charge value between ±10 to ± 30 mv are considered stable products [56, 57].

TEM analysis

Recently a new standard ISO 21363:2020 (Nanotechnologies – Measurements of particle size and shape distributions by transmission electron microscope) has published by the technical committee (TC 229) to study the morphology of nanoparticles. The biosynthesized gold nanoparticles were investigated using high resolution transmission electron microscope (HR-TEM) operated at 200 keV. For TEM investigation, the sample was prepared by putting a drop of the solution on a grid of carbon-coated copper which was dried at room temperature. The shape, size and particle size distribution of Au NPs were obtained from TEM measurements. Statistical analysis of the size distribution from the TEM images reveals that the nanoparticles were well dispersed with diameter size ranging from 12 to 35 nm. The mean diameter was estimated to be 23.93 ± 6.4 nm. The morphological analysis displayed that the shape of the nanoparticles was spherical and hexagonal as shown in (figure 4a) and a little aggregation of Au NPs appeared in TEM image (figure 4b) due to their extremely small dimensions with high surface energy. The histogram of particle size distribution is shown in (figure 4c).

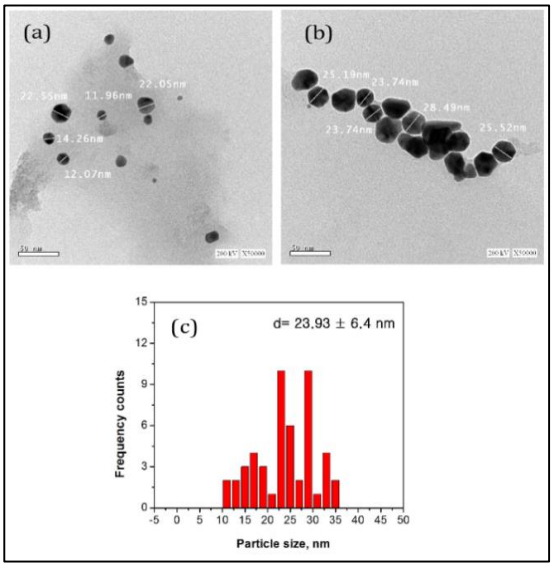

Fig. 4. TEM images of gold nanoparticles recorded from different regions of the sample at a magnification of 50000x. (a) TEM image of the spherical and hexagonal shaped Au NPs, (b) TEM image of a little aggregation of Au NPs and (c) particle size distribution histogram determined from TEM images, d is the mean diameter of particle size.

Antimicrobial activity of gold nanoparticles

In this experiment, the biosynthesized Au NPs was tested against seven pathogen strains using agar well diffusion method to study their antimicrobial activity. After incubation, the inhibition zones diameters were measured in the range of millimetres. As shown in table (1), all the tested bacterial strains were inhibited by the examined concentrations (10, 30, 50 **µg**/well) with variations in the inhibition zone diameters, while *Candida albicans* showed resistant to gold nanoparticles. As reported previously the existence of contaminants with Au NPs affects their antifungal and antibacterial activities, either by decreasing or increasing the activity [58]. Both *E. coli* and *P. aeruginosa* showed the same inhibition zones diameter (14 mm) at concentration 10 µg, while by increasing the concentration, *P. aeruginosa* was more sensitive (table 1 and figure 5). Zawrah and Abd El-Moez [59] obtained similar inhibition zone against *P. aeruginosa* (17 mm). In the current investigation the inhibition zones of *S. typhimurium* and *B. subtilis* were the same at the three tested concentrations. The inhibition zones for both were 16, 22 and 24 mm formed by 10, 30 and 50 µg concentrations, respectively. The type culture strain *St. aureus* was the least affected strains while, the clinical *St. aureus* (MRSA) was the most affected strain. These results are in line with that obtained by Shamaila *et al*. [60], who observed that E. coli was more sensitive to Au NPs than S. aureus. Table 2 shows the results of antibacterial activity of Au NPs reported by others [58-60]. Characteristically different Au NPs gave different antifungal and antibacterial activities. Previous reports concluded that the antibacterial action against gram negative is more than gram positive [58-61]. These studies used *E. coli* as gram negative and *St. aureus* as gram positive, while according to the obtained results, the antimicrobial action of Au NPs is strain dependent action. Many studies reported that *Streptomyces* is a good candidate for the synthesis of gold nanoparticles, but most of them used the metabolites which are mixture of diverse compounds and medium remnants. This leads to synthesis of heterogeneous nanoparticles due to the contribution of diverse agents in the synthesis process [62-64]. Using cell free extract [65] or direct contact [66] leads to synthesis of homogenous nanoparticles. In this study, strain of *Streptomyces* sp. U30 (KP109810) was used to synthesis homogenous gold nanoparticles at lower pH, incubation time and mass weight comparing to the study of Khadivi *et al*., [66].

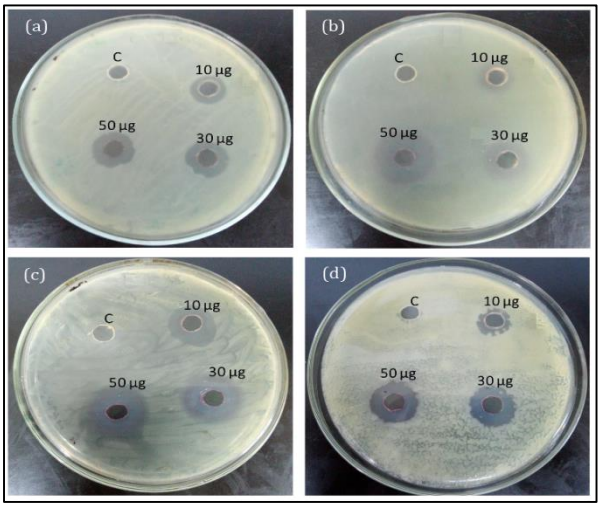

Fig. 5. Antimicrobial activity of the biosynthesized gold nanoparticles at different concentrations (10, 30 and 50 µg) against *E. coli* (a), *P. aeruginosa* (b), *S.* MRSA (c) and *S. aureus* indicated by inhibition zones, C: uninoculated well.

Table 1

Diameter of inhibition zones due to the biosynthesized gold nanoparticles at different concentrations (10, 30 and 50 µg) against type and clinical cultures

Type Culture Strains	Inhibition Zone Diameter (**mm**)	Concentration **µg** per well		
		10	*30*	50
Gram Positive Bacteria				
Escherichia coli (ATCC25922)		14	17	20
Pseudomonas aeruginosa (ATCC27853)		14	19	23
Salmonella typhimurium (ATCC14028)		16	22	24
Gram Positive Bacteria				
Bacillus subtilis (ATCC6633)		16	22	24
Staphylococcus aureus (ATCC25923)		13	18	20
Yeast				
Candida albicans(ATCC10231)		-	-	-
Clinical culture				
Staphylococcus aureus (MRSA)		19	23	25

Table 2

Diameter of inhibition zones due to gold nanoparticles at different concentrations against type cultures and wild type bacteria reported by others

Tested Organisms	Concentration	Inhibition Zone Diameter (mm)	Reference
E. coli (ATCC number 25922)	5 mg/ml	0.3	Ref. No. 58
E. coli O157	50 µl	12	Ref. No. 59
S. aureus	50 µl	13	Ref. No. 59
B. cereus	50 µl	14	Ref. No. 59
P. aeruginosa	50 µl	17	Ref. No. 59
E. coli	40 µl	35	Ref. No. 60
S. aureus	40 µl	25	Ref. No. 60

Economical study of biosynthesized Au NPs

The cost of chemicals used to biosynthesis gold nanoparticles was less than $250. 500 g of starch casein medium cost about $140 and 250 mg of HAuCl4 cost around $100. These quantities of purchased chemicals are sufficient to prepare big amount of Au NPs. In this study, only 1/25 of both medium and HAuCl4 were used to biosynthesis gold nanoparticles. For characterization and studying the antimicrobial activity of formed Au NPs, the laboratories at National Institute of Standards and Suez Canal University are fully equipped.

4. Conclusions

To conclude, the present study reported fast and non-toxic biosynthesis method of stable gold nanoparticles from actinomycete strain; Streptomyces sp. U30 (KP109810) which was isolated from metal containing rock. The biosynthesized gold nanoparticles were formed after only 7 hours of incubation time which is less than the optimum biosynthesis time stated by others. Au NPs fully characterized and tested against gram positive and gram negative bacteria. These nanoparticles showed significant antimicrobial activity which may include in the fight against pathogenic bacterial species especially resistant species like MRSA. According to the obtained results, the antimicrobial action of Au NPs is strain dependent action and the biosynthesized nanoparticles can be used in bio-medical applications such as drug and gene delivery, surgical devices and diagnostics.

5. Conflicts of interest

There are no conflicts to declare.

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