J Nanostruct 13(2): 417-430, Spring 2023

RESEARCH PAPER

Biosynthesis and Antibacterial Activity of Gold Oxide Nanoparticles by Nocardia asteroids Isolated from Soil

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ARTICLE INFO

Article History:
Received 04 January 2023
Accepted 23 March 2023
Published 01 April 2023

Keywords:
Antibacterial activity
Biosynthesis gold oxide nanoparticles
Nanoparticle characterizations
Nocardia bacteria

ABSTRACT

Nanoparticle synthesis is an advanced approach in the field of modern nanotechnology. Recent studies aim to characterize gold oxide nanoparticles produced by bacteria. These studies are of great interest and signify a significant technological advancement, particularly in the context of microorganisms. In this context, *Nocardia* bacteria were isolated from soil and subjected to a detailed. *Nocardia* is isolated from the soil. Bacterial isolation was confirmed using advanced molecular techniques. Fragments of RNA 16S were amplified and sent for the following sequence following the isolation of cell-free *Nocardia asteroides*, they were employed in the biosynthesis of gold oxide nanoparticles (Au_2O_3) by reducing a gold salt solution ($HAuCl_4$) to form nanocrystals. The formation of Au_2O_3 was monitored by observing color change and the UV-VIS spectrum confirmed the decrease in gold ions. The resulting nanoparticles were characterized using Scanning Electron Microscopy (SEM), Fourier-transform infrared spectroscopy (FTIR), Transmission Electronic Microscopy (TEM), Atomic Force Microscopy – (AFM), and X-ray Diffraction (XRD). Gold oxide nanoparticles (Au_2O_3 NPs) were synthesized using *Nocardia asteroides*, and SEM analysis revealed predominantly spherical particles with an average size of 19 nm. TEM results indicated that the biologically prepared of gold oxide nanoparticles in this study exhibited sizes ranging from 12 to 23 nanometres in various shapes. XRD indicates that the produced material consists of gold oxide nanoparticles. The antibacterial activity of the gold oxide nanoparticles was assessed against pathogenic bacteria, but they exhibited no inhibitory effect on the tested bacterial isolates.

How to cite this article
Hassan A F., Hateet R R., Al- Shakban M. Biosynthesis and Antibacterial Activity of Gold Oxide Nanoparticles by Nocardia asteroids Isolated from Soil. J Nanostruct, 2023; 13(2):417-430. DOI: 10.22052/JNS.2023.02.012

INTRODUCTION

"Nano" is a word that comes from the Greek language and means "very small" or "dwarf". In science and technology, the unit of measurement is used "nanometers" (nm) to indicate this smallness. A nanometer is one billionth of a meter (10-9 meters). This scale is much smaller than the wavelength of visible light and 100,000 times less than the thickness of a human hair [1]. Nanoscience is defined as events that occur in materials at the nanoscale, involving nanostructures such as RNA, DNA, and subcellular organelles. Nanoscience has close ties to biology and biochemistry, and nanotechnology is interdisciplinary and encompasses a wide range of science and technology. Nanoparticles are synthesized through a variety of physical and chemical processes, regarding chemical

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and physical techniques, they have exhibited several adverse impacts like the requirement of high pressure or temperature, as well as the generation of toxic and hazardous byproducts. Conversely, the biological approach, also referred to as green manufacturing or biomanufacturing, has demonstrated environmentally sustainable, hygienic, and secure outcomes [2].

Gold nanoparticles and compounds are among the most important metal nanoparticles mentioned due to their extensive history in therapeutic applications. Gold nanoparticles and compounds are considered the most common in previous research [3]. Chemical and physical methods have shown many undesirable effects such as the need for pressure, high temperature, or the production of toxic and dangerous substances as secondary substances. On the other hand, the biological approach (also known as green manufacturing or biomanufacturing has been shown to produce environmentally friendly, clean, and safe results [4]. Gold oxide is a rare metal oxide that decomposes back into gold and oxygen over time or after heating due to its instability, gold oxide reacts with some chemicals to form other compounds, for example, it reacts with acids to form gold salt, and with reducing substances to convert it into metal gold. [5]. The most common composition of gold oxide is Au_2O_3, but it can also, in rare cases, be Au_2O or AuO, depending on the conditions and method of preparation. Gold oxide compounds are prepared using physical and chemical methods, the most important of which is the method of deposition precipitation, which is the intermediate compound ($AuOH_4$), which decomposes at high temperatures (300) degrees Celsius to form the compound of Au_2O_3 [6, 7].

Nocardia bacteria are an aerobic radial bacillus, catalyst-positive, and cram-positive dye with a branched filamentous shape first described in 1888 by Edmund Nocard [8]. *Nocardia* is found worldwide in a myriad of environments [9]. *Nocardia* is found in freshwater, salt water, dust, soil, decaying plants and decaying organic matter. Later, these bacteria were classified as important environmental bacteria due to their universal presence in soil, decaying plants, and water [10]. There are no documented biological processes in the scientific literature for synthesizing gold oxide nanoparticles by using plants. This research aimed to produce gold oxide nanoparticles using *Nocardia* bacteria obtained from soil samples. The bacteria were identified through chemical tests and scan technology. The nanoparticles were then subjected to physical tests to determine their properties, and the study investigated the potential antibacterial activity of these particles.

MATERIALS AND METHODS

Soil samples collection

Soil samples were collected from different locations in Maysan Province, Iraq, including sugar cane fields, rice fields and home gardens, Samples were taken from different depths ranging from (8 cm to 10 cm) and were collected in sterile plastic bags and sterile plastic bottles. After that, the sterile bags were transferred to the laboratory to complete the process of sterilizing and drying the soil, followed by the serial dilution process.

Bacteria Isolation

Bacteria were isolated based on phenotypic traits and chemical tests, then bacterial isolates were identified based on the diagnostic characteristics of Holt et al. (1994) and then confirmed by molecular diagnosis and sequencing technique. The soil samples were dried for about three hours in a hot air oven at 60 °C and then the soil samples were taken and transferred to sterile transfer tubes, 0.1 g of calcium carbonate was added to every 1 g of soil and incubated at room temperature for 7 days [11]. Then the soil samples were diluted in sterile test tubes containing 9 ml of sterile distilled water. The tubes were sequentially coded as 1, 2, 3, and 4. 1 gram of dried soil containing calcium carbonate was added to tube No 1, and thoroughly shaken, then 1 ml from tube No. 1 and added to tube No. 2 using a micropipette. This serial dilution was performed until tube No. 4, and then 1 ml was discarded from tube No.4, this process yielded concentrations of 10^{-1}, 10^{-2}, 10^{-3} and 10^{-4} g/ml [11]. We prepared the Petri dishes with the culture medium to isolating *Nocardia* bacteria, and each dish was labeled with the name and number of the corresponding tube from which the sample was obtained, 1 ml was taken from each tube and spread on a Petri dish by L Shape pattern, and then the dishes are incubated in the incubator for 14 days at 30 ° C [12].

Molecular diagnosis of bacteria

The DNA of bacteria grown on nutrient agar was extracted using Presto™Minig DNA bacteria kit according to the manufacturer's

instructions. The genomic DNA was stored at -20 °C. All bacteria isolated by gene 16 S rDNA (1500 bp) gene by amplified by thermocycling device General prefixes Front starter ('5-AGAGTTTTTGATCCTGGCTCAG -3') and the reverse initiator (5'-TACCTTGTTACGACTT-'3) As for the special prefixes in *Nocardia* bacteria, it is the initiator of the front (ACCGACCACAAGGGGG 16 Bp) the reverse initiator (GGTTGTAACCTCTTCGA 17 Bp). Note that both public and private prefixes were designed by Al-Musaib Bridge Company in Iraq – Baghdad. The reaction mixture was carried out with a volume of 20 µL, where a DNA mold, primers and distilled water were added to each tube of Maxime™PCRPreMix Kit (i-Taq).

Each sample was classified and sent to the South Korean biotechnology company Macrogen for purification as a product and sequencing analysis for the general initiator Next, the sequence results were compared with the sequences of the front- and reverse prefixes by the same company. (Https://www.ncbi.nlm.nih.gov) It is then analyzed to detect the closest match to bacterial isolates.

Biosynthesis of gold oxide nanoparticles

Synthesis of gold oxide nanoparticles (Au_2O_3-NPs) from Nocardia bacteria

The bacteria were fermented on the nutrient broth medium for 3 days at a temperature of 30 ° C in the vibrating incubator at a rate of 120 rpm. A swab was then used to inoculate a 500 ml glass flask containing 100 ml of Nutrient broth medium with the growing bacterial culture. After the bacteria grew, the bacterial cultures were separated by a centrifuge at a speed of 10,000 cycles for 20 minutes. Subsequently, 5 ml of each bacteria filtrate was added individually to 50 ml of 2 mmolar gold chloride solution. The mixture was heated on the magnetic vibrating thermal plate at 50 ° C for 15 minutes and then incubated in the vibrating incubator (120 rpm) at 30 ° C for three days. The biosynthesis of gold oxide nanoparticles was monitored visually for changes in the color of the reaction mixture. The centrifuged gold oxide nanoparticles were deposited at 10,000 rpm for 30 minutes and washed three times with deionized water [13].

Physical Diagnosis of Biosynthetic Gold Oxide Nanoparticles

UV-vis spectroscopy

The detection of gold nanoparticles commences with a visible color change based on the principle of surface plasmon resonance. This color change happens as particle size increases, and for gold oxide, it shifts from deep red to purple. The visible color variations observed are a result of the phenomenon known as the surface localized resonance of electromagnetic plasmon, also referred to as SPR. This phenomenon is responsible for the color changes observed within the visible region of the electromagnetic spectrum. This means that a specific part of the wavelength in the visible region is absorbed while others are reflected, resulting in a distinctive color emission [14].

Fourier transform infrared spectroscopy (FTIR)

FTIR analysis was performed to determine the presence of functional groups in manufactured Au_2O_3 using infrared spectroscopy, samples were measured (in powder form) in the range of 400-4000 cm^{-1} [15].

X-Ray diffraction

The X-ray diffraction technique was used to investigate the crystalline structure of materials since the X-ray wavelengths between (0.2 and 10) nm are comparable to the interatomic spacing of crystalline solids. The diffraction of X-rays by a crystal is explained by the Bragg law, which is defined as the relationship between the wavelength of the X-rays. The interatomic spacing is given by the following equation [16].

Field Emission Scanning Electron Microscope)FE-SEM)

SEM is a surface imaging technique in which an incident electron beam scans over the specimen surface and interacts with it, generating signals that represent the specimen's atomic composition and topographic information. SEM generates many better-resolution images by using accelerated electron beams and electrostatic or electromagnetic lenses [17].

Transmission electron microscope (TEM)

TEM was used to analyze the shape, size, and distribution of NPs. To prepare the TEM grids, the suspensions of NPs were transferred onto copper grids coated with carbon. Before imaging, the grids were dried by air and then individual images were taken at 200 kV using TEM [18].

Anti-bacterial activity of prepared -Au_2O_3NPs

The determination of antibacterial activity was made by the method of disc propagation. A sterile tablet was taken, and the sterile tablet was impregnated with the nanomaterial (Au_2O_3NPs) for 24 hours, then the suspension of the tested bacteria was culture on a dish containing Muller hinton agar medium, and then the tablets saturated with nanomaterial were placed above the inoculated bacterial suspension, and tablets saturated with normal saline solution were placed, then the dishes were incubated in the incubator at 37 ° C for 24 hours [19].

RESULTS AND DISCUSSION

Isolation of soil bacteria

After transplantation on dishes containing humic acid VB medium. This medium is suitable for the growth and extraction of *Nocardia*, as it supports the growth of bacteria as well as spore growth, and this medium was developed as the only source of nitrogen and carbon, It was observed that this particular nutrient medium outperformed other media in terms of its ability to isolate a greater number of actinobacteria, including *Nocardia* bacteria [20]. Researchers in [21] suggested that colonies typically appear white and exhibit a powdery texture as shown in Figs. 1 and 2.

Biochemical Tests

The results of the biochemical examination show that the *Nocardia asteroides* isolate is consistent with what was mentioned in [9], which showed that the *Nocardia asteroids* bacteria was positive for the Starch, Catalase, and Urease tests and negative for the Casein, and Tyrosine tests (Fig. 3).

Diagnosis using polymerase chain reaction technology (PCR)

The BLAST program was used to analyze and match the DNA sequences and results of the isolates with their reference strains in GenBank. Samples were screened to determine the partial sequence of the 16S rRNA in *Nocardia*. The NCBI BLASTn search engine showed a high 99% sequence similarity between the sequenced samples and the reference target sequences of *Nocardia asteroids*. By comparing the specific DNA sequences of these examined samples with those of *Nocardia asteroids* (GenBank acc. MT355849.1), the exact locations and other details of the recovered PCR fragments were

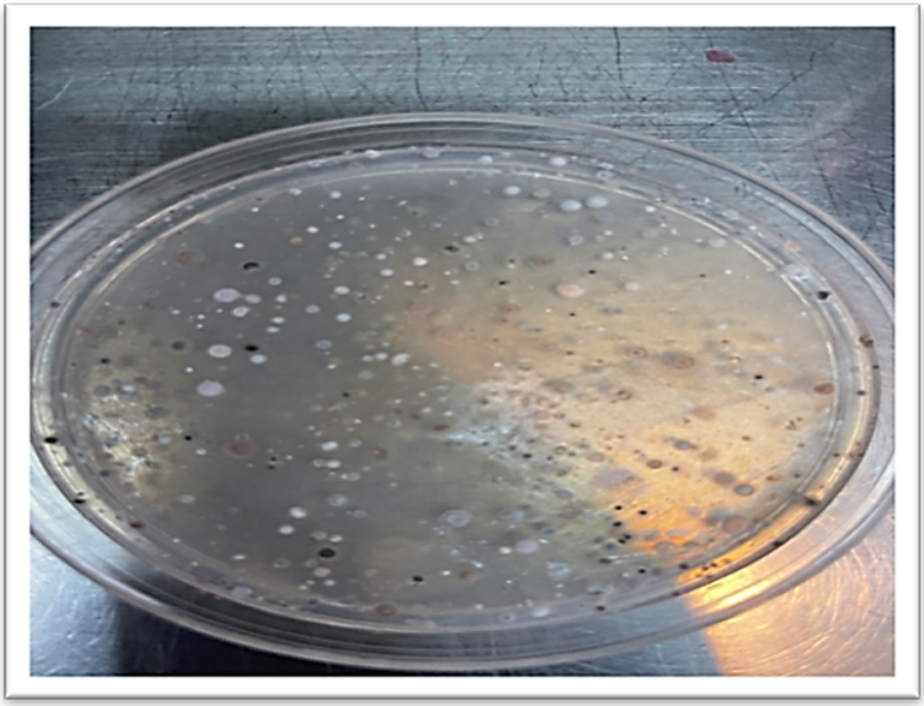

Fig. 1. The shape of the isolated colony on the medium Humic acid VB agar.

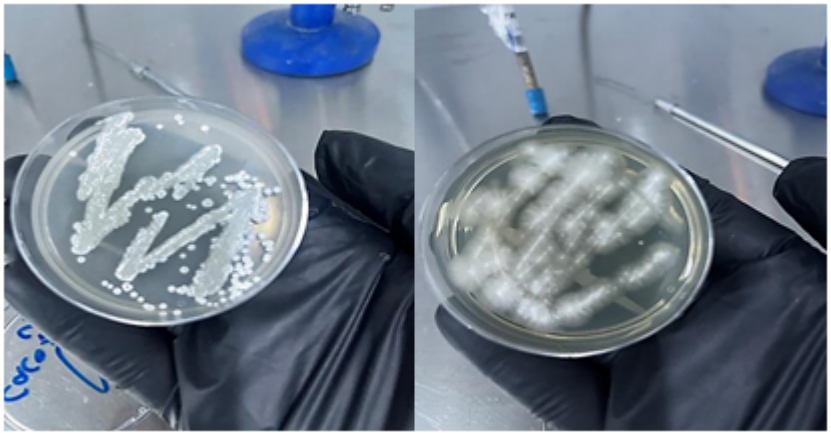

Fig. 2. Color and texture of the colony of *Nocardia* bacteria cultured on medium Humic acid.

determined. The total length of the target site was analyzed using the NCBI server, and the locations of the target site within the most matching bacterial target gene were confirmed. It became apparent that the *Nocardia* asteroid isolate has been identified as a new isolate and was recorded in NCBI under accession number OR625083 and the isolate was named Sameh-S2.

Synthesis and characterization of nanoparticle

After mixing the cell-free bacterial extract with a gold chloride solution, biosynthesis results showed that the color change occurred 45 minutes after adding the bacterial extract to the gold chloride dissolved in deionized water, as the color changed from colorless white to light yellow as shown in Fig. 4. This confirms a reaction

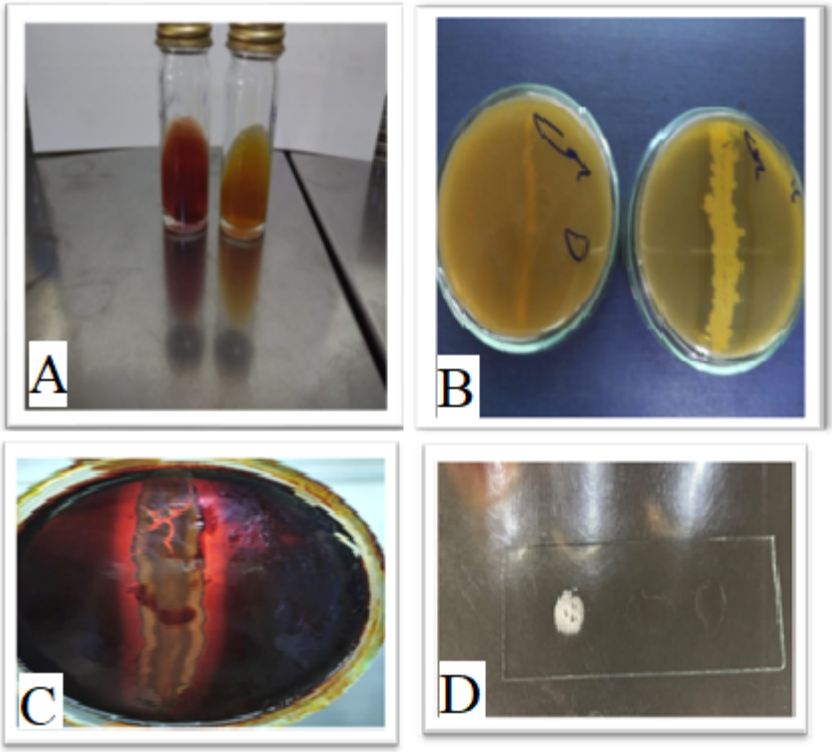

Fig. 3. The biochemical tests, where (A) represents the urea test, (B) the casein test, (C) the starch test and (D) the catalase test.

between the active secondary compounds in the bacterial extract with the gold chloride salt, causing the process of reduction and particle formation to occur after 48 hours, the color change was observed from light yellow to orange as it appears in Figs. 4 and 5. The color change is the initial evidence of the formation of gold oxide nanoparticles and the positivity of green biosynthesis. The color changes that occurred are due to the phenomenon of Plasmon Surface Resonance, and this property occurs in parts of metals such as gold, silver and other metals [22].

UV-Visible

In the context of gold oxide extraction using bacteria, the goal is to convert the gold metal in the raw materials into gold oxide using biological processes. In this study, *Nocardia* bacteria were used for this purpose. There was a shift in the absorption peak towards shorter wavelengths, in the ultraviolet region, at the wavelengths of 265 nm. That indicates an increase in a light

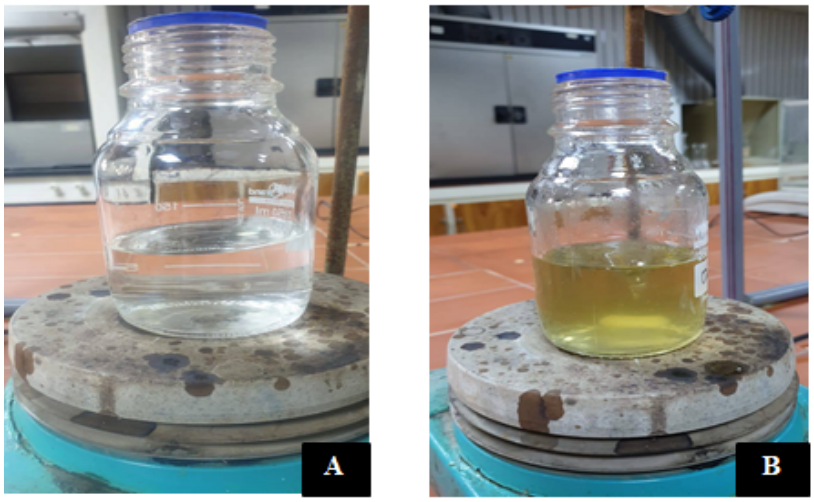

Fig. 4. The color change from transparent white to light yellow for the filtrate of the *Nocardia asteroid* bacteria culture after 45 minutes of reaction time

Fig. 5. The color changes from transparent white to the orange of the *Nocardia asteroid* bacteria filtrate after 48 hours.

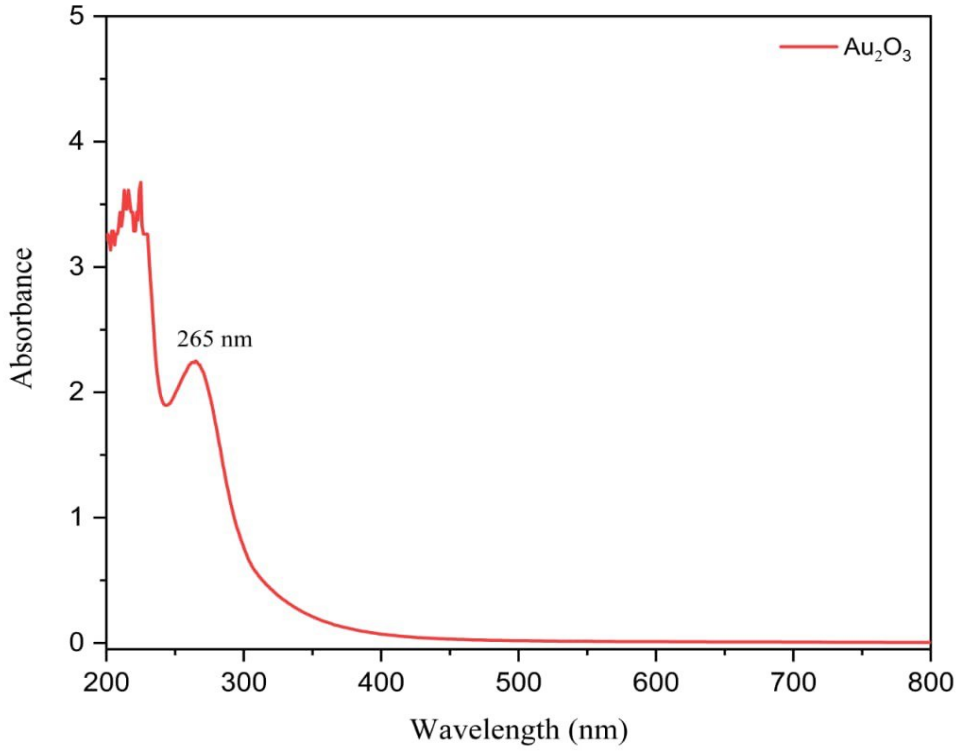

Fig. 6. Ultraviolet spectrum of the bacterial filtrate *Nocardia asteroid.*

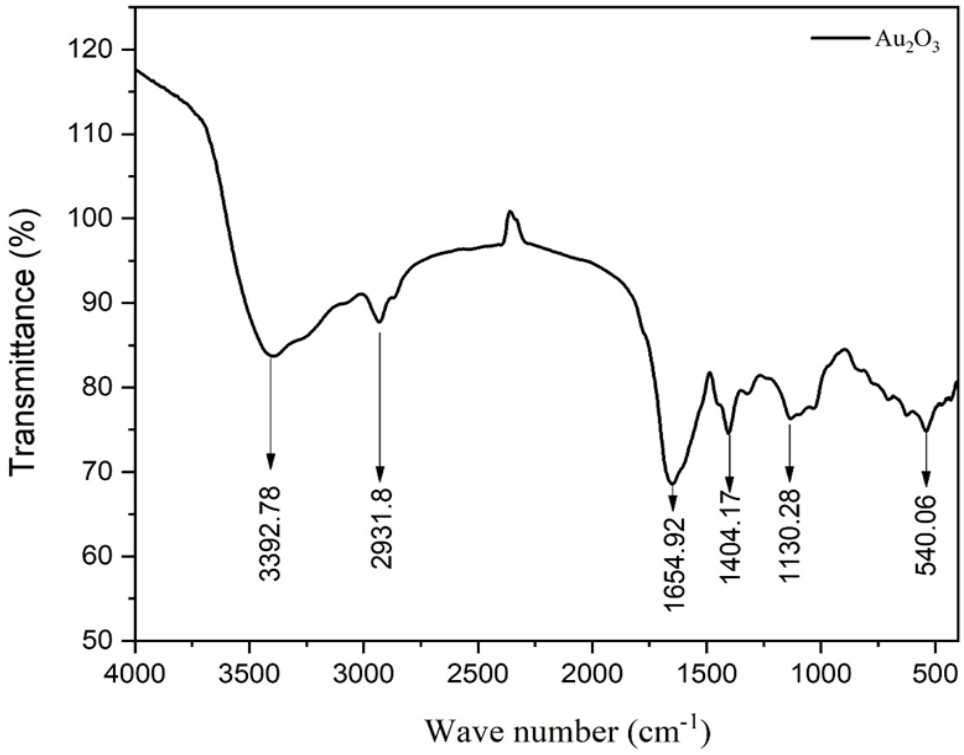

Fig. 7. Infrared spectrum of gold oxide nanoparticles manufactured from *Nocardia asteroid* bacteria.

absorption in the short wavelength region because of electronic transitions in the gold oxide atoms. The appearance of the absorption peak at the wavelength of 265 nm indicates the presence of gold oxide atoms affected by quantum size effects, which affects their optical behavior and leads them to absorb light in this region (Fig. 6). The quantum size effect can have a significant impact on the optical and electronic properties of nanomaterials, making them interesting in areas such as nanotechnology, advanced materials, and medical fields [23, 24].

Fourier-transform infrared spectroscopy (FTIR)

FTIR spectroscopy was performed here to identify the functional peaks and bands, which serve as a unique identifier of gold oxide nanoatoms. The broad energy absorption bands in the region of 3392.78 cm^{-1} indicate the presence of stretched frequencies of oxygen-hydrogen (O-

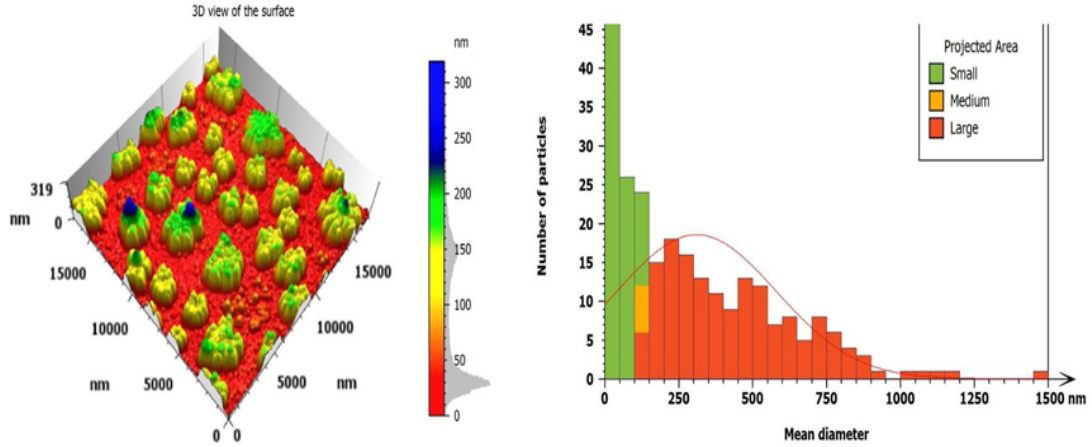

Fig. 8. Three-dimensional atomic force microscope images and a diagram of the average height diameter of gold oxide atoms.

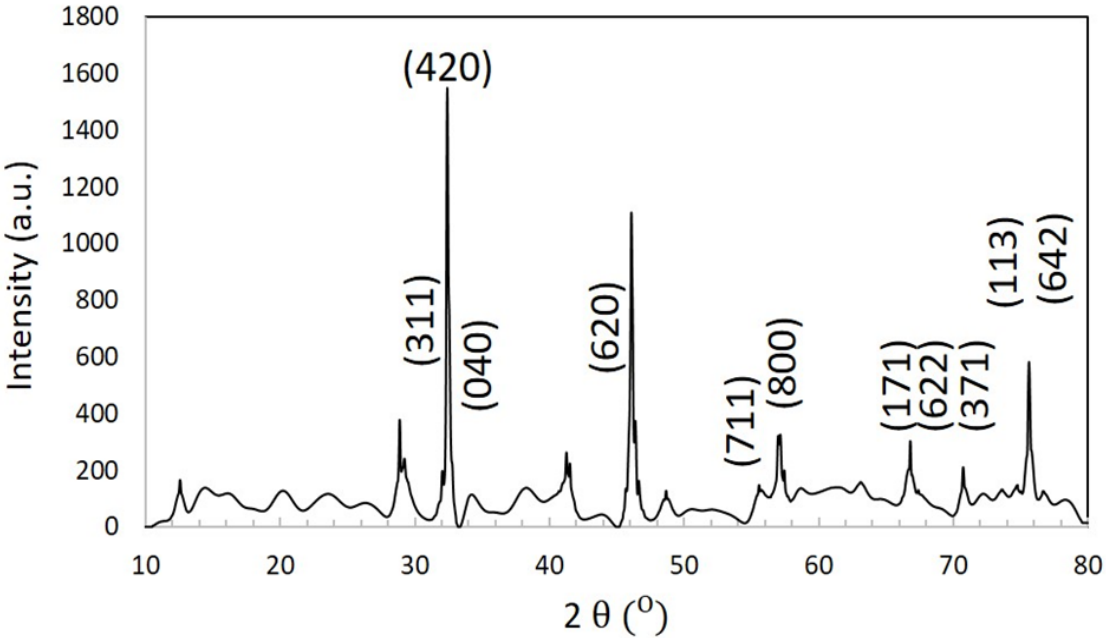

Fig. 9. X-ray diffraction pattern of gold oxide nanoparticles synthesized from *Nocardia asteroid.*

H) and the bands in the region of 2931.8 cm^{-1} correspond to the stretching vibration of the bond. C-H. The peak in the region 1654.92 cm^{-1} is attributed to the presence of the amide group from the carbonyl expansion (C=O). In addition, the bands in the region of 1404.17 cm^{-1} and 1130.28 cm^{-1} are attributed to the C-O-H spherical vibration of carboxylic acids and C-O fracture frequencies sequentially as appears in Fig. 7. The band located at 540 cm^{-1} shows the study of the infrared spectrum of gold oxide particles synthesized using *Nocardia* bacteria surrounded by some proteins and secondary compounds, such as alkaloids that contain functional groups such as hydroxyl amines group, alcohols group, phenols group and carboxylic acids group and other groups [25, 26].

Atomic Force Microscopy – (AFM)

This type of quantitative analysis is useful for studying and understanding surfaces and their applications in areas such as nanotechnology, biomaterials, electronics, energy, etc. [24]. Fig. 8 shows us three-dimensional images of the surface of gold oxide atoms. The surface of the atoms contains heights ranging between (56.1-319 nm) and areas whose diameter ranges between (24.7-1473 nm) and the average diameter of the area of the peaks is 309 nm. The surface of the material is considered "rough" based on atomic force microscopy (AFM) images because it contains peaks of different dimensions. The presence of surface roughness of gold oxide atoms increases the effectiveness of killing bacteria and cancer cells [27,28].

X-ray diffraction

An X-ray diffraction (XRD) device was used to study the crystal structure of gold oxide nanoparticles that were synthesized from *Nocardia* bacteria. The diffraction angles, which represent the angles of the gold oxide nano atoms, were monitored with values of 32.12, 32.41, 34.47, 45.7, 56.26, 57.24, 67.24, 67.96, 75.4, and 75.71, and these values correspond to the Meller coefficient: (311), (420), (620). , (711), (800), (171), (67.96), (622), (371), (113), and (642) respectively, which is consistent with reference file 1039-43JCPDS. The X-ray diffraction (XRD) pattern shows that the gold oxide particles synthesized using the green method have a cubic orthorhombic crystal structure.

Field emission Scanning Electronic Microscope and EDX

Images were taken at different magnifications,

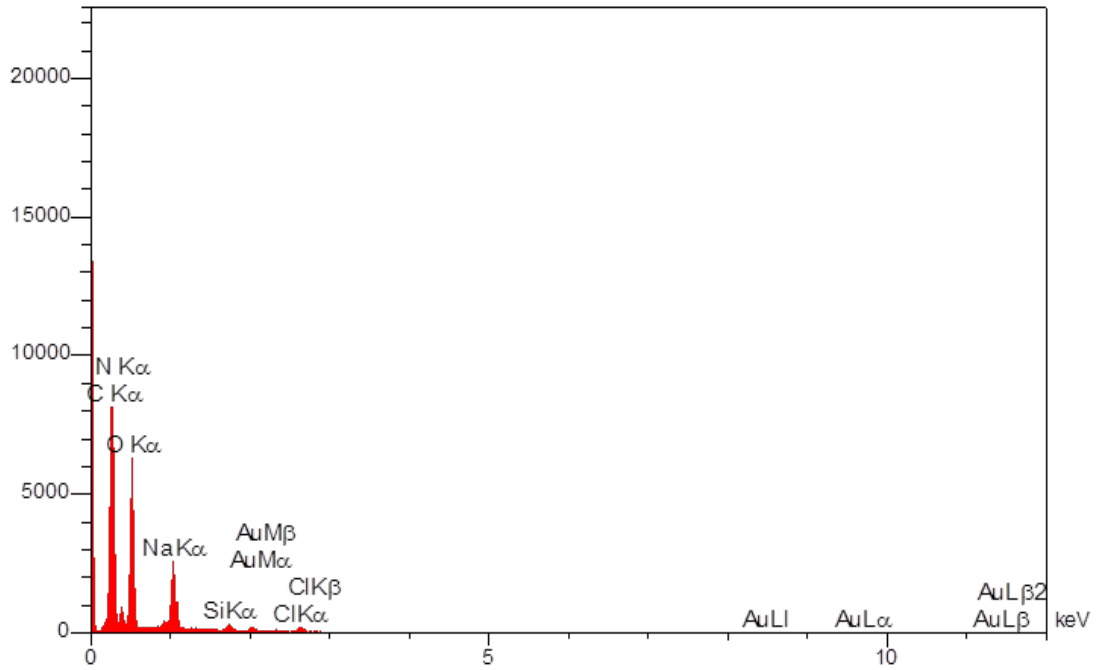

Fig. 10. EDX diagram of gold nanoparticles synthesized from *Nocardia asteroides* filtrate.

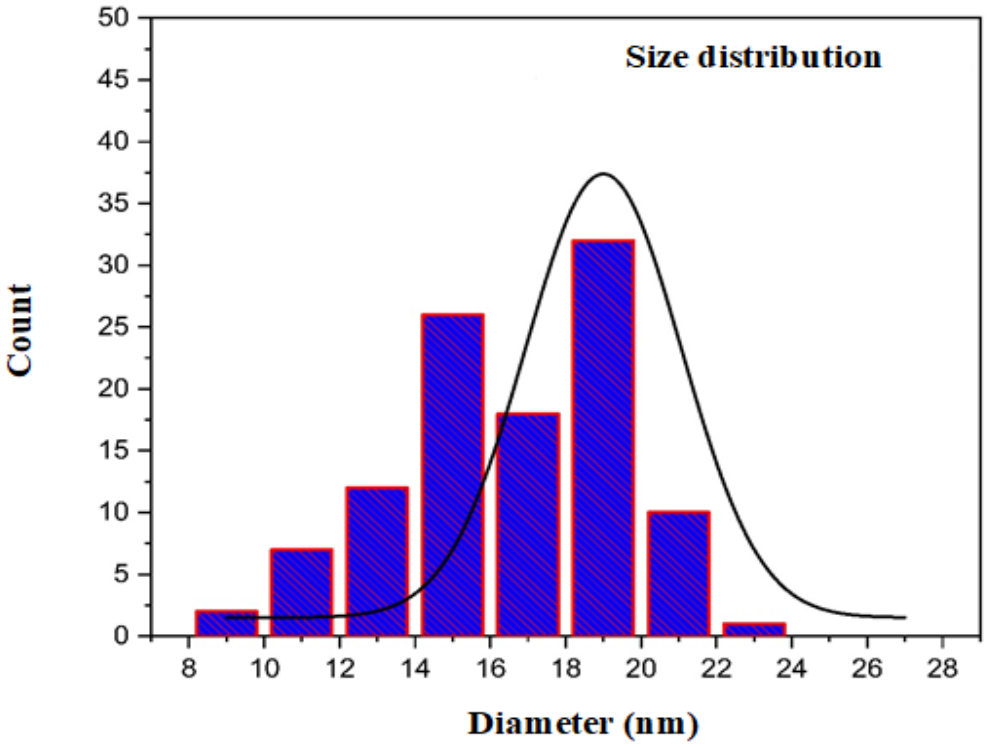

Fig. 11. Histogram distribution of the diameter of gold oxide nanoparticles.

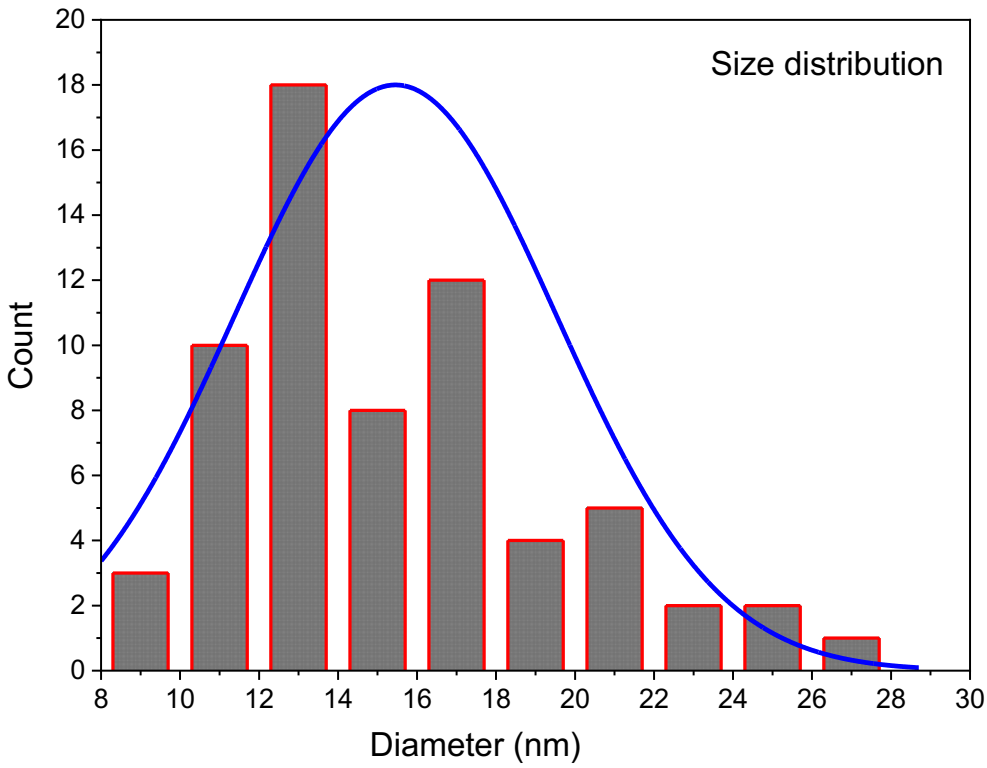

Fig. 12. Size distribution of gold nanoparticles from the bacterial filtrate *Nocardia asteroids*.

and all images showed the formation of spherical gold oxide nanoparticles. Image J software was used to analyze the size of these nanoparticles by plotting the histogram distribution of the particles, as shown in Fig. 9. The average diameter of the nanoparticles was calculated, and the final value of this diameter was 19 nanometers. This small nanoparticle diameter shows an explanation consistent with the results of the UV-visible optical absorption analysis, where light absorption was monitored in the ultraviolet region. This absorption is consistent with the small size of gold oxide nanoparticles and indicates their interaction with UV radiation [29].

Energy dispersive X-ray EDX analysis also revealed sharp and intense optical absorption peaks that reveal confirmation of the presence of the oxygen element in the gold oxide particles, by showing the strongest optical signal in addition to the presence of other metals such as nitrogen, carbon, and others. The metallic gold oxide nanocrystals show a strong optical absorption peak, which is A typical absorption pattern for gold nanocrystals and their compounds as shown in Fig.10 [30].

Transmission Electronic Microscope (TEM)

Through transmission electron microscope images, the presence of small, dark-colored particles indicating the formation of gold oxide nanoparticles was observed.

Most particles appear spherical, and some appear close to spherical. In figure (13), it is observed that there are gold oxide nanoparticles of different sizes ranging from 50-200 nanometers. According to the results of the ultraviolet (UV) spectrum, the wavelength value corresponds to the size of the nanoparticles [31]. It was noted that the smaller 50 nm particles have roughly spherical shapes, while the larger 200 nm particles display a range of geometric shapes such as triangles, truncated triangles, pentagons, and hexagons. These geometries are typical for gold nanoparticles and gold composites [32]. The average particle diameter was about 15.45 nanometers, as shown in Figs 11-13.

Gold oxide nanoparticles' antibacterial activity

The examination was conducted using the disk diffusion method, and the gold oxide nanoparticles

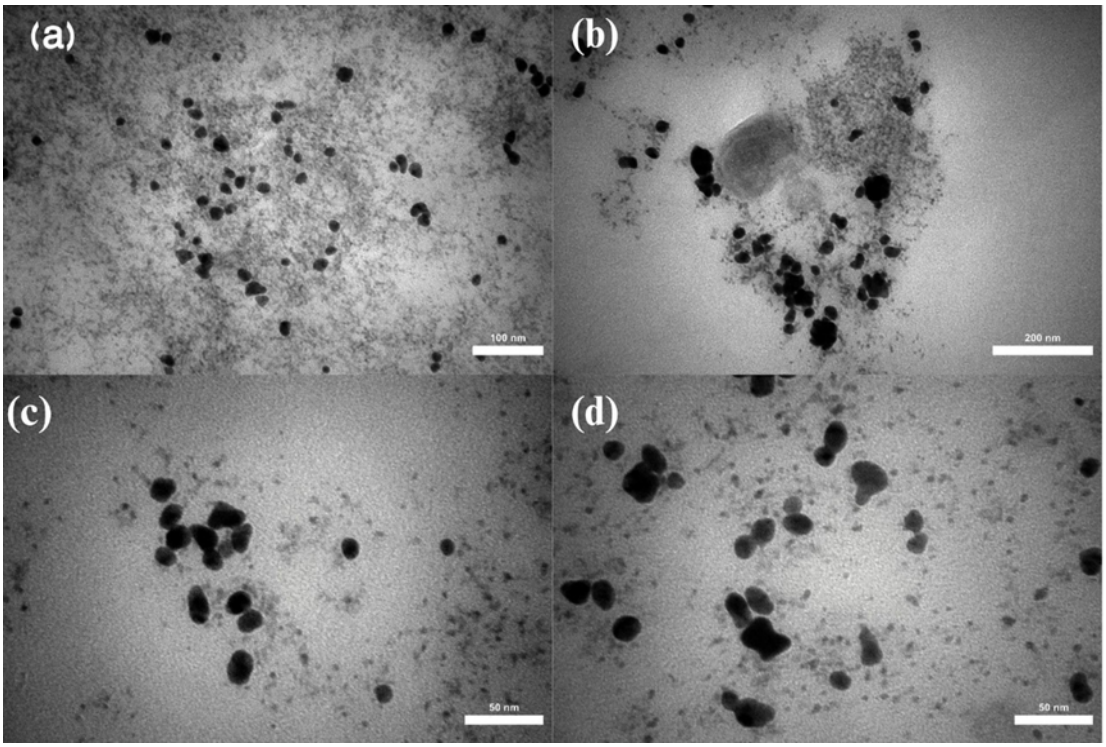

Fig. 13. Transmission electron microscope images at different magnification levels of gold oxide nanoparticles of the bacterial filtrate *Nocardia asteroids*

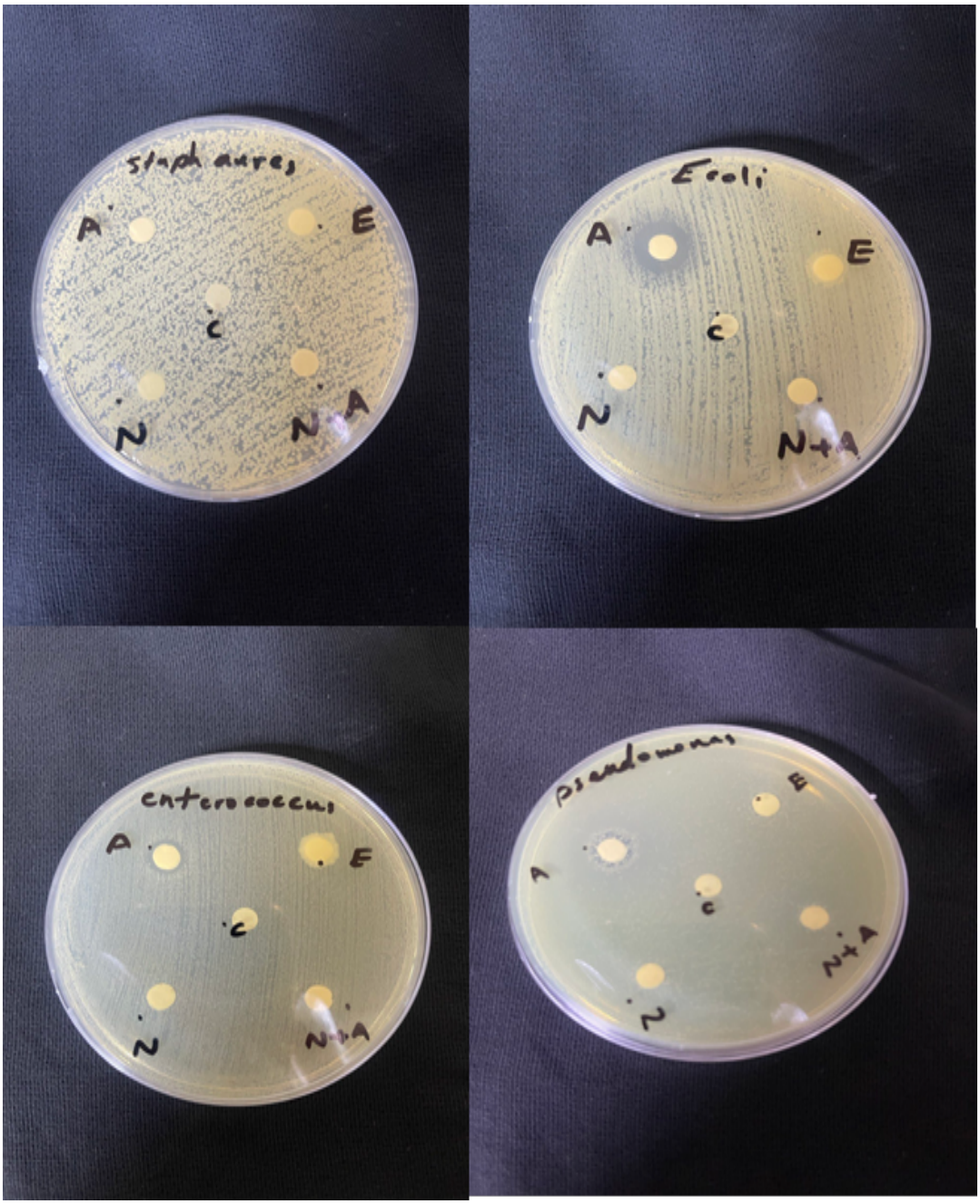

Fig. 14. Antibiotic activity of gold oxide nanoparticles (disc diffusion method) against *Stphylococcus aureus, E.coli, Pseudomonas, Enterococcus* bacteria N: nano, A: antibiotic, c: control

in the study did not show any inhibitory activity against the tested bacterial isolates, which are (*Staphylococcus aureus*, *E.coli*, Pseudomonas, *Enterococcus*) as shown in Fig. 14.

CONCLUSION

The primary aim of this investigation was to develop a novel method for producing gold oxide nanoparticles utilizing the Nocardia strain of bacteria. To begin, the selected *Nocardia* bacteria underwent rigorous identification procedures, including a series of chemical tests complemented by the extraction of DNA for confirmatory analysis. Following successful identification, the

research progressed to the synthesis phase, where gold oxide nanoparticles were derived from the bacteria. The resulting Au_2O_3 nanoparticles (Au_2O_3NPs) were then subjected to an extensive structural examination employing a range of advanced analytical techniques, such as UV-visible spectroscopy, field emission scanning electron microscopy (FE-SEM), atomic force microscopy (AFM), transmission electron microscopy (TEM), and X-ray diffraction (XRD). Post-synthesis, the biological efficacy of these nanoparticles was evaluated through their interaction with various pathogenic bacterial species to assess any potential inhibitory effects. Contrary to expectations, the synthesized Au_2O_3NPs demonstrated no significant antibacterial activity against the tested pathogens. This lack of inhibition might be attributed to possible resistance mechanisms present within the bacteria or to specific characteristics of the nanoparticle composition that may have impeded their antimicrobial action. Given these findings, it is advisable for future research to pivot towards exploring a broader spectrum of biological activities that Au_2O_3NPs may influence, or to consider adjusting the synthesis parameters to modify the properties of the nanoparticles. Such explorations could potentially unveil new applications for these nanoparticles or shed light on the nuances of their interaction with biological entities.

CONFLICT OF INTEREST

The authors declare that there is no conflict of interests regarding the publication of this manuscript.

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