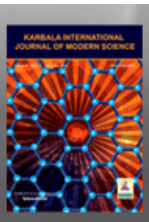

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Synthesis of Gold Nanoparticles Using Hordeum vulgare Leaf Extract and their Antibacterial Activity

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Abstract

Plant extracts and gold nanoparticles are promising alternatives for combating antibiotic resistance in light of the increasing bacterial resistance. Leaf extract of barley was used to synthesize gold nanoparticles (AuNPs). Barley gold nanoparticles (BL-AuNPs) were produced by adjusting some reaction parameters. These BL-AuNPs were characterized through employing the UV-visible spectroscopy technique, the scanning electron microscope (SEM), Fourier transform infrared spectroscopy (FTIR), and energy dispersive X-ray spectroscopy (EDX). BL-AuNPs were tested for antibacterial efficacy against two strains of Gram-negative bacteria, clinically isolated and considered as multidrug-resistant pathogens, *Acinetobacter baumannii* and *Salmonella typhi*. The antimicrobial efficiency of the compounds was evaluated via diffusion and microdilution assays. UV spectra were used to characterize the extract, providing a peak at 530 nm. The biomolecules associated with the AuNPs conjugated with plant extracts were identified to describe C-H, carbonyl (C-O), carbonyl OH, and C≡C groups based on the FTIR technique. The SEM analysis indicated that barley agglomerates of formed gold nanoparticles ranged in size from 263.2 nm to 214.7 nm. The AFM analysis exhibited that their size was 36.29, and the surface roughness and root mean square were 3.34 and 4.18, respectively. The results of the antibacterial activity of BL-AuNPs showed that *A. baumannii* and *S. typhi* strains were not affected at 1 mM of AuNPs, while the free gold nanoparticles showed moderate antibacterial action. The BL-AuNPs exhibited higher antibacterial activity, with inhibition zones ranging from 14 to17 mm for *A. baumannii* and 10 to18 for *S. typhi*, compared to AuNPs and barley leaf extract individually, which showed lower antibacterial activity. In conclusion, gold nanoparticles synthesized from the extract of *Hordeum vulgare* leaves offer a rapid, cost-effective, and eco-friendly method. This green approach yields high quantities without releasing toxic substances. The stability and antibacterial activity of synthesized gold nanoparticles revealed considerable promise for development in biomedical applications. Future studies should include Gram-positive and fungal strains to better evaluate the full antibacterial spectrum and investigate further for outstanding and improved biological uses.

Keywords

Gold nanoparticles, Barley leaves, Salmonella typhi, A. baumannii, antibacterial activity

RESEARCH PAPER

Synthesis of Gold Nanoparticles Using *Hordeum vulgare* Leaf Extract and Their Antibacterial Activity

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Abstract

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Keywords: Gold nanoparticles, Barley leaves, *Salmonella typhi*, *A. baumannii*, Antibacterial activity

1. Introduction

Nanomaterials are the core element of nanotechnology and are defined as metallic materials with sizes ranging from 1 to 100 nm. Due to their unique properties in physical and biological fields, these materials made a revolution in many applications, particularly in drug delivery, due to their large surface-to-volume ratio [1]. The incidence of infectious diseases and drug consumption has considerably grown globally as a result of unintentional drug usage and the rising of antibiotic resistance in pathogenic microorganisms [2]. Researchers have reported that metallic nanoparticles can serve as an alternative to conventionally used antimicrobial agents [3−5]. Among different types of nanomaterials, noble metal nanoparticles gained considerable attention due to their special catalytic, electronic, and optical properties [6]. Gold (Au) is known for its excellent chemical stability,

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biocompatibility, and the ease with which it can be synthesized using green methods. Furthermore, gold nanoparticles (AuNPs) have unique optical and electrical properties, making them ideal for a variety of biological and catalytic applications [7,8]. Novel methods for the synthesis of gold nanoparticles (AuNPs) occupied a great interest in green production to show high biocompatibility and non-toxicity, which brings attention compared to other conventional methods [9]. The use of natural resources to reduce environmental problems is one of the most important basic goals of creating nanomaterials from sustainable green sources [10].

Natural plants basically contain leaves, roots, and fruits and various compounds of antioxidants, such as the presence of water-soluble polyphenols that can reduce gold trivalent ions (Au^3) to gold nanoparticles with a high affinity to binding on the surface of gold nanoparticles, achieving stability and eco-friendly features [11,12]. Barley (*Hordeum vulgare*) is one of the most important cereal crops globally, valued not only for its nutritional and economic significance but also for its medicinal properties. Economically, barley is widely cultivated for animal feed, malting for the production of beer, making it an essential component in the fields of agriculture and industry [13]. In terms of medicine, barley grains are high in nutritional fiber, polyphenols, beta-glucans, and antioxidants, which contribute to their cholesterol-lowering, and anti-diabetic effects, and anti-inflammatory [14]. The experiment's barley leaves were seedlings, which are 20–30 cm long and contain various antioxidant compounds that help avoid aging and cell deterioration, including vitamins C, carotene, chlorophyll, and flavonoids [15]. A naturally occurring flavonoid compound with potent antioxidant properties is 2-O-glycosylisovitex (2-O-GIV) which was extracted from barley leaves. Additionally, three novel antioxidants were isolated from barley leaves:4-glucosyl-6-sinapoylsaponarin, 6-sinapoylsaponarin, and 6-feruloysaponarin [16]. The barley leaves can be used in the environmentally friendly synthesis of gold nanoparticles as a result of their high reducing capacity [17]. The production of biocompatible nanoparticles using the barley-mediated technique is not only economical and not polluting for the environment, but it also exhibits exceptional antibacterial efficacy against harmful microorganisms [18]. It was possible to generate stable gold nanoparticles based on barley leaf extracts as efficient templates, reducing agents, and stabilizers. In the face of rising demand for eco-friendly NPs with improved stability and efficacy, our study approached the challenge of sustainable synthesis by exploiting the plentiful and easily accessible barley species. This study utilized many techniques to study the generated gold nanoparticles to ascertain their properties. The particle sizes of the AuNPs were measured by EDX, FTIR, UV-VIS, AFM, and SEM. Additionally, the antibacterial effects of AuNPs against *Salmonella typhi* and *A. baumannii*, two Gram-negative (G-^{ve}) bacteria, were studied.

2. Materials and methods

2.1. Materials

Dimethyl sulfoxide (DMSO) and 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide (MTT) were bought from Sigma (Aldrich). Chloroauric acid ($HAuCl_4$), sodium hydroxide (NaOH), and other chemical substances were ordered from Shanghai Chemicals Reagents (China). Fresh barley leaves were obtained locally, and the experiment was conducted using ddH_2O double-distilled water (18.2 MΩ Millipore system). The bacterial strains of *Acinetobacter baumannii* and *Salmonella typhi* were taken from the University of Mosul-Faculty of Life Sciences strain bank. The standard bacteria of *S. typhi* (ATCC 6539) and *A. baumannii* (ATCC 19606) were considered as a reference strain and brought from Medya Diagnostic Center (MDC) in Erbil.

2.2. The barley leaf extract preparation

Fresh barley (*Hordeum vulgare*) leaves were collected from agricultural fields located in Tal Afar District, Nineveh Governorate, Iraq. Authentication of the plant was done by the herbarium of the Biology Department, College of Education, Salahaddin University-Erbil. The barley (*Hordeum vulgare*) extract was prepared using an aqueous extraction method. The barley leaves were cleaned, dried, and pounded to powder in a mortar. Twenty grams of powdered leaf suspended in 400 mL of deionized water and soaked for 30 min, then vacuum-filtered using two Whatman filter paper layers (size No 5). The generated solution was filtered by a disposable 200 nm-diameter needle filter to remove any leftover fibers and then kept at 4 °C [19].

2.3. BL-Au NP synthesis

After dissolving one gram of salt powder in 100 mL of ddH_2O, a solution of golden chloroacetic acid ($HAuCl_4$) 0.03 mol/L was prepared [20]. The AuNPs were developed by adding 1 mL of $HAuCl_4$ solution (24.89 mmol/L).

To 5 mL of barley leaf extract with continuous magnetic stirring under reflux, followed by slowly

adding 0.05 mL of 1 mol/L of NaOH solution at the boiling point. After stirring the mixture for 5 h at room temperature, the color of the solution gradually changed from pale yellow to ruby red. The nanoparticles were thoroughly washed by repeated centrifugation and resuspension in distilled water to remove unbound phytochemicals. It was filtered by using a 0.45 mm syringe filter before transferring to a new glass bottle. The resulting material was filtered for 8 h to obtain a pure solution by discarding the excess amount of NaOH and the remaining suspension. In the final step, the concentration of the nanoparticle formation was determined to be one-tenth the volume in the oven at 30 °C and stored in a refrigerator at 4 °C [21], for further use as shown in Fig. 1.

2.4. Characterization procedures

BL-AuNPs' structure and crystals have been studied by employing a JEOL JEM-2100 SEM (scanning electron microscope) set to 200 kV. AuNPs size designation and zeta potential value were defined utilizing a Malvern Zetasizer Nano ZS90 particle size potency meter. Furthermore, the UV–visible spectral features of AuNPs were examined by operating a Varian Cary-Eclipse 500 spectrophotometer. The specimen FTIR Fourier transform infrared spectrum was specified using a Nicolet 380 spectrometer, and the Nano system was imaged by a Brilliance 64-slice CT (Philips Healthcare)." (Philips Healthcare, Andover, MA).

2.5. The study of BL-Au NPs stability

The strength of BL-Au NPs was assessed by using the UV–visible method. The colloidal BL-Au NPs were dissolved in ddH_2O, serum, phosphate-buffered saline (PBS), and Dulbecco's Modified Eagle's Medium (DMEM) for at least 7 day at room temperature. BL-Au NPs (200 μg/mL) were dissolved in ddH_2O at room temperature to gain high stability. The solutions were maintained at various temperatures (4, 25, 37, and 50 °C) for 30 min to be ready for measurements. The BL-Au NPs solution (200 μg/mL) at different pH values (pH = 5, 6, 7, and 8) was stored for 30 min at room temperature to verify the pH constancy [19].

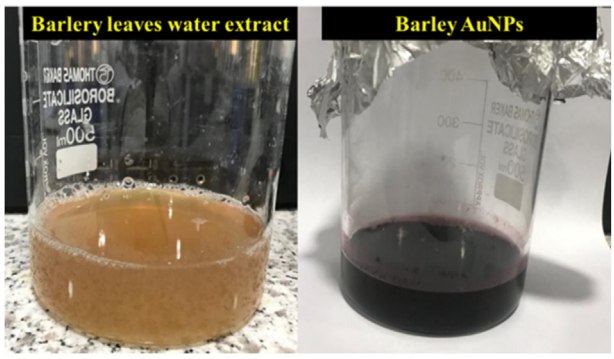

Fig. 1. Barley leaves mediate the production of Au nanoparticles.

2.6. X-ray attenuation

The X-ray properties of BL-Au NPs were evaluated by mixing the BL-Au NPs solution and Ultravist in a 1.5 mL Eppendorf container tube with different active element concentrations [Au] = 0, 1.125, 2.25, 4.5, 9,18 mmol/L and [I] = 0, 1, 2, 5, 10, 20, 40 mmol/L). The received CT photographs were analyzed by a GSI Viewer, which revealed the disparity progress in Hounsfield units [19,22].

2.7. Measurement of antibacterial agents activity

The antibacterial efficacy of gold ions and AuNPs was evaluated during a radial diffusion assay. To create bacterial suspensions, one pure colony was grown in Luria-Bertani broth for an entire night with a clearness of 0.5 McFarland standards. The bacterial solution and 0.1 mg of the gold nanoparticles were dissolved in 1 mL of distilled water (D.W.) and then added to Mueller-Hinton agar plates. Wells with a diameter of 6 mm were created by using a conventional punch. Independently, 50 μL of each barley extract conjugated with AuNPs, gold nanoparticles, and barley extract were added to each well and incubated for 24 h at 37 °C. The gap diameter was subtracted from the total area of inhibition to estimate the zone of inhibition (ZOI). All these experiments were conducted using duplicates [23,24].

2.8. Measurement of both minimum inhibitory concentrations (MICs)and minimum bactericidal concentrations (MBCs)

Employing the microtiter plate (MTP) method on a 96-well microtiter plate, the MICs and MBCs of AuNPs were ascertained [25]. Barley extract was utilized as a negative control at a concentration of 0.5 mmol/L, with the dilution set up to produce 5 mL of Luria-Bertani broth containing 10–100 μg/mL of gold nanoparticles. The MIC was calculated by inoculating 50 μl of the medium broth with a single strain of bacteria that had been suspended from Luria-Bertani agar plates. Once the suspensions had been incubated for 24 h, they were diluted in Milli-Q Ultrapure water to produce final samples

containing 5×10^5–5×10^6 colony-forming units (CFU)/ml. Luria-Bertani broth was used to create two-fold serial dilutions of the AuNP solution on 98-well plates, starting with a 10^{-2} M base solution. Each bacterial inoculum was added in equal volume to an MTP that held 0.05 mL of the serial compound dilution. MIC was measured using a POLAR star OPTIMA microplate reader following a 24 h incubation period at 35 °C (BMG LABTECH GmbH, Germany). The absorbance contrasted with the negative control wells, which comprised broth composed of AuNPs, no inoculum, and the chemical at the descending concentrations. Three independent replicates' mean values are used to express the results [26,27].

2.9. Statistical analysis

To measure the particle size distribution of AuNPs, the image processing application developed in Java was implemented. Experiments were performed in triplicate, and bacterial growth was reported as mean ± standard deviation (n = 3) via analysis to calculate the MIC according to three separate samples.

3. Results and discussion

3.1. Gold nanoparticle (AuNP) biosynthesis

In this study, barley leaf extract was used as both a reducing and stabilizing agent in the presence of $HAuCl_4$ (0.03 mol/L) and trisodium citrate to synthesize the gold precursor. The reduction of $HAuCl_4$ was visibly detected by the color change of barley leaf extract from pale yellow to ruby red, confirming the formation of BL-AuNPs (less than 30 nm) with a red color resulting from surface plasmon resonance [28]. A simple and more environmentally friendly method was employed to synthesize gold nanoparticles: the analysis operating image revealed that 90% of the particles were spherical and dispersed, highlighting the green synthesis features compared to using harsh and expensive chemicals. Green synthesis methods are gradually replacing physical and chemical methods because of their high energy requirements, the emission of harmful and dangerous compounds, the usage of complicated equipment, and synthesis conditions [29]. A phytochemical-driven reaction involves complicated reducing compounds such as antioxidants, enzymes, and phenolic moieties in the plant extract to convert gold ions into AuNPs, according to the recognized putative mechanism for the synthesis of NPs in this manner [30]. The presence of phytochemicals (barley leaves) drives the hypothetical lowering of $HAuCL_4$ to form zero-valent gold, which in turn triggers an accumulation of gold molecules in nanosized particles. The general mechanism behind the synthesis of metal nanoparticles using plant extract is the reduction of metal ions by phytochemicals to reduced metal ions; then, the reduced metal ions begin to aggregate into small clusters (nucleation), and finally, stabilization by phytochemicals prevents the nanoparticles from clumping together [31]. The role of phytochemicals (flavonoids, phenolic acids, and terpenoids) is to act as natural reducing and capping agents. These compounds influence the reduction kinetics of Au^{3+} to Au^0, which affects the nucleation and development of nanoparticles, resulting in smaller particle sizes and increased stability compared to chemically synthesized counterparts [21]. Furthermore, a biological coating produced from plant metabolites can synergistically increase the antioxidant and antibacterial activities of AuNPs, expanding their potential for medicinal and environmental applications [32]. Green synthesis's eco-friendliness and scalability make it particularly useful in sustainable nanotechnology [21]. BL-Au NPs were produced in this study utilizing a simple one-pot green synthesis methodology that reduced the Au^{3+} in $HAuCl_4$ in an alkaline setting using barley leaf extract.

Barley leaves helped make gold nanoparticles more stable, easier to dissolve, and safer for use in biological applications. In addition, barley leaves were utilized to stabilize gold nanoparticles, which increased their stability and usability in biological applications [19].

Phenolic antioxidants were considered among other active chemicals readily available in barley leaves, which may cause a decrease in MNPs [33]. For the environmentally agreeable production of MNPs, barley leaf aqueous extract may be used as a bioactive source.

The leaves were refluxed in distilled water for 2 h and dried in the oven, as shown in Fig. 1. After the filtration process, a brown solution was produced. Some reaction parameters, such as the metallic ion concentration, temperature, and duration, may optimize the synthesis of stable MNPs. UV–Vis measurements produced an indicator that the nanomaterials could be formed as barley AuNPs by changing the colloid solutions from yellow to purple.

3.2. SEM analysis

It is one of the most commonly employed instruments for studying and analyzing solid objects-micro- and nanoparticles. Many reasons make SEM

a useful device, including high resolution, which can be obtained when bulk objects are examined. SEM is the most ideal technique for particle size analysis with a resolution of up to 10 nm. It can be combined with techniques such as EDX/EDS to analyze the structure and crystal properties [34]. Fig. 2 provides scanning electron micrographs showing the gold nanoparticle agglomerates of barley prepared using the green technique. The agglomerates ranged in size from 263.2 nm to 214.7 nm when compared to a previous study carried out in 2023 [20]. The current data revealed finer AuNPs using an SEM to display spherical particles with an average grain size of 27.08 nm and few aggregations. A few microcracks are also visible in the micrographs, which may be the result of the preparation process.

3.3. AFM analysis

Atomic force microscopy (AFM) photography shows images close to atomic resolution for assessing the topography of surfaces. This device is capable of measuring samples' surface roughness to less than the angstrom level. Fig. 3 shows a 3-dimensional image by atomic force microscope for barley AuNPs prepared by plant extracts. The result shows the sample has an average size of 36.29, which is slightly higher than SEM analysis. The surface roughness and root mean square were 3.34 and 4.18, respectively. The histogram of the sample shown in Fig. 3 represents the distribution of the nanoparticles of the sample, where most particle sizes distribute between 10 and 16 nm.

3.4. FTIR analysis

By employing technology (IRAffinity-1-SHMADZU), AuNPs FTIR spectra are a beneficial tool for determining the atomic structure of chemical substances. The functional groups in the region of 200–4000 cm^{-1} were identified using FTIR techniques [20]. Fig. 4 illustrates the FTIR spectrum of gold nanoparticles extracted from barley. The most prominent peak was broad at 3435.85 cm^{-1}, which is due to the vibration of the extended O-H group [39]. The presence of CO2 molecules in the ambient air causes bands around 2076.10 cm^{-1}. The third indicated peak assigned to C-H stretching bound located at 2924.25 cm-1. The strong band of the O-H bending vibration mode is assigned at 1636.83 cm^{-1} [40]. The peak of C-H bending located at 1464.92 cm^{-1}, while the last peak indicates to C=C bending located at 714.92 cm^{-1}. All bonds and functional groups are tabulated in Table 1.

3.5. Antibacterial activities of barley extract, Au-NPs and BL- Au-NPs

Barley extract and Au-NPs were tested for their antibacterial properties using the well diffusion

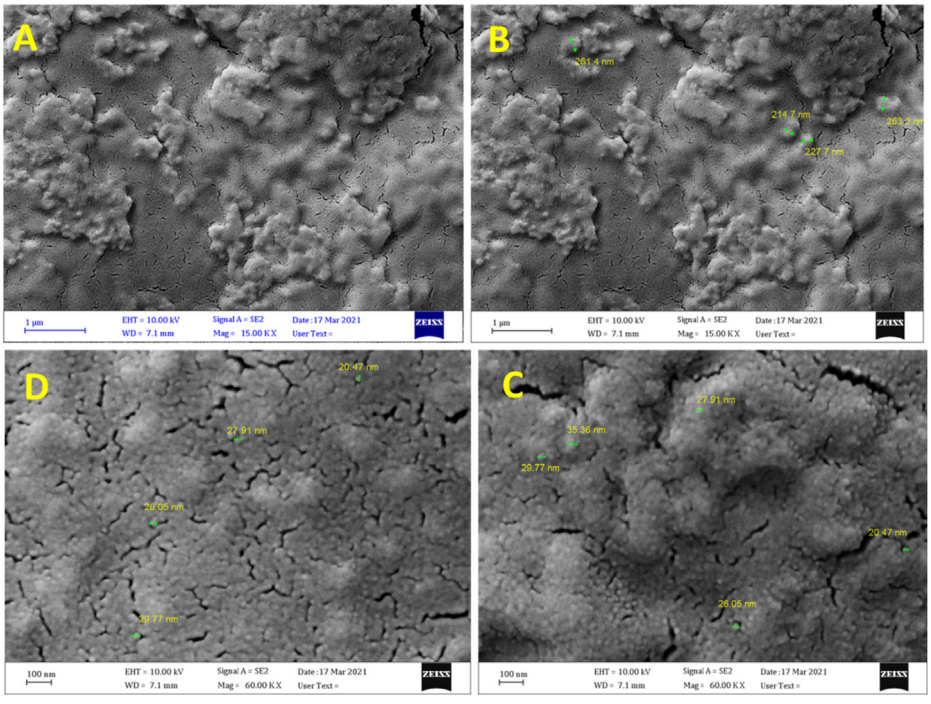

Fig. 2. Scanning electron microscopy SEM micrograph of Barley AuNPs prepared by plant extracts.

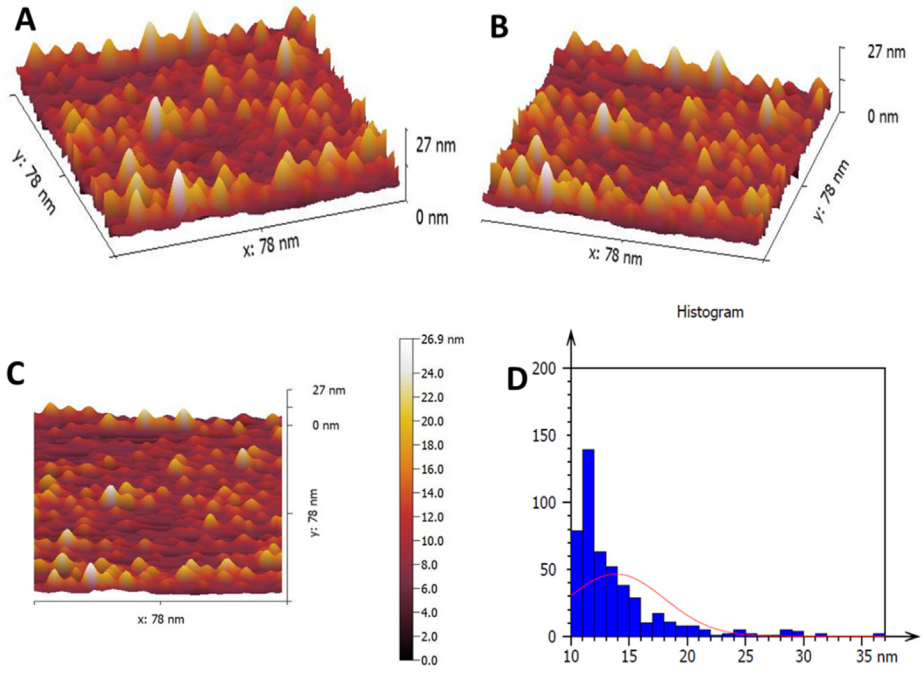

Fig. 3. AFM analysis of 3D-images for Barley AuNPs prepared by plant extract.

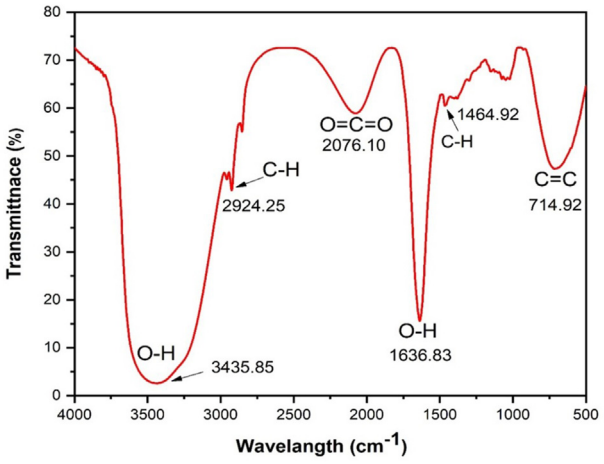

Fig. 4. Shows the FTIR spectra of barley gold nanoparticles chemically extracted by the green synthesis technique.

technique to evaluate their antimicrobial activity against multi-drug- resistant *Salmonella typhi* and *A. baumannii* isolates. The samples were thoroughly described before antibiotic testing to exclude any confounding factors that might have affected the activity. The presence of impurities in the sample may influence the antibacterial efficacy of gold nanoparticles [35]. Positive ions may inhibit or enhance the antibacterial activity, for instance (e.g., calcium) (e.g.m zinc) respectively [36,37]. The AuNPs have been shown to produce antimicrobial capabilities [35]. Size, shape, and surface charge are

Table 1. Shows the bond and functional group of AuNPs.

Peaks (cm^{-1})	Bond and functional group	Compound Class	Reference
3435.85	O-H stretching	Alcohol	[39]
2076.10	O=C=O stretching	Ambient	[40]
2924.25	C-H stretching	Alkane	
1636.83	C=C stretching	Alkene	
1464.92	C-H bending	Alkane	
714.92	C=C bending	Alkene	

important aspects of NPs that affect how they interact with bacterial cells, making their production techniques and properties essential in assessing their antibacterial efficiency [38]. The current study utilized the zone of inhibition assay to evaluate the antibacterial activity of the AuNPs produced. *A. baumannii* and *S. typhi* strains were not affected by gold nanoparticles at a concentration of 1 mM, while free gold ions exhibited antibacterial action, as seen in Fig. 5(A and B) and Table 2. The antibacterial performance of BL-AuNPs was determined via zone inhibition assay toward six strains of each *A. baumannii* and *S. typhi* corresponding to leaf extract of barley and Au NPs. Fig. 5(A and B) shows that BL-Au nanoparticles revealed higher antibacterial activity, with inhibition zones ranging from 14 to 17 mm for *A. baumannii* and 10–18 mm for *S. typhi*, compared to AuNPs and barley leaf extract individually, which showed relatively lower activity. It is important to note that the leaf extract exhibited no

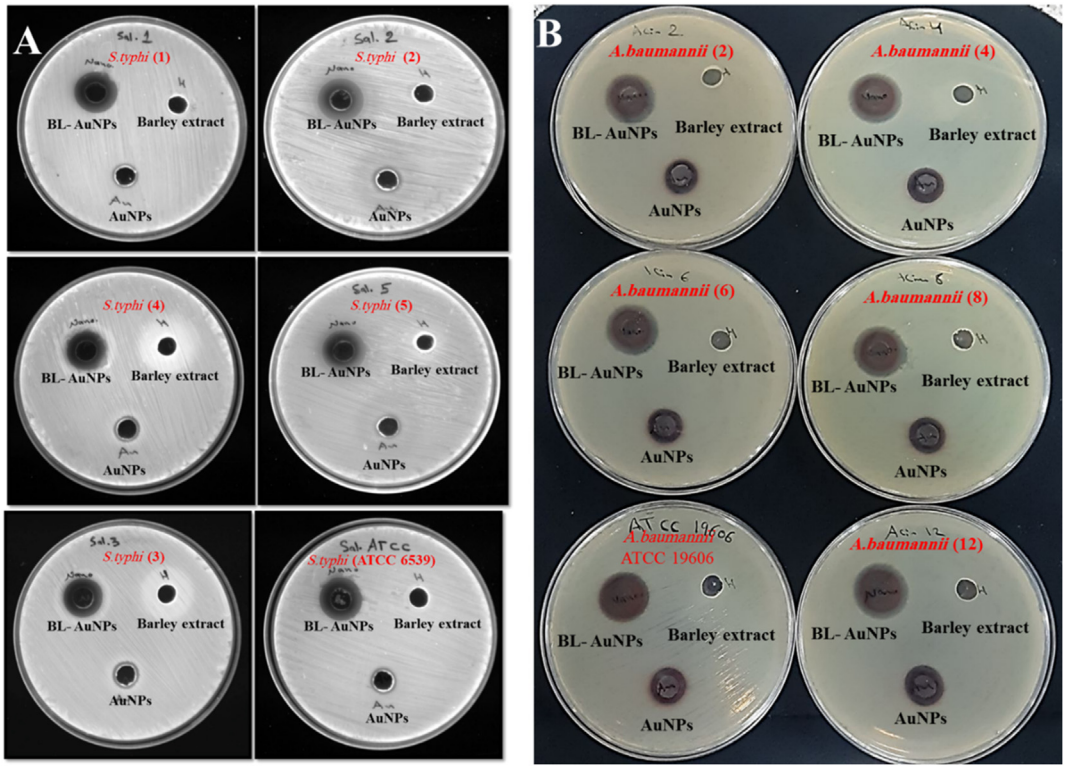

Fig. 5. Visible clear zone produced by leaf barley extract-mediated AuNPs against six strains of Salmonella typhi *(1, 2, 3, 4, 5 and ATCC 6539) (A), and six strains of* Acinetobacter baumannii *(2, 4, 6, 8, 12 and ATCC 19606) (B).*

Table 2. Mean diameter (mm) of growth inhibition zone of S. typhi *and* A. baumannii *strains.*

No. of the strain	Barely extract	AuNPs	BL-AuNPs
S. typhi ATCC (6539)	0	9	16
S. typhi 1	0	9	16
S. typhi 2	0	9	17
S. typhi 3	0	9	15
S. typhi 4	0	9	14
S. typhi 5	0	9	16
A. baumannii ATCC (19606)	0	11	16
A. baumannii 1	0	12	18
A. baumannii 2	0	12	18
A. baumannii 3	0	12	18
A. baumannii 4	0	13	18
A. baumannii 5	0	11	18

effect for any antimicrobial behavior toward the studied strains. The prepared AuNPs were biocompatible with the tested microorganisms and had a lower level of environmental toxicity, reflecting the absence of effective antibacterial plant compounds in the leaf extract. Subsequently, it is currently shown that the size, shape, synthesis process, and composition of the capping agent all significantly affect the antibacterial activity of MNPs [41,42]. The physical properties of the nanoparticles and the adsorption of biologically active Phytomolecules from barley leaf extract on their surface may work in concert to provide BL-AuNPs their higher antibacterial activity [43]. Additionally, the results indicated that the synthesized BL-Au NPs appeared to be more effective against *A. baumannii* strains than *S. typhi*. This may be due to the fact that *S. typhi* strains were more resistant toward prepared BL-Au NPs. According to a few reports, inorganic nanoparticles have the ability to interact with microorganisms and may therefore have antifungal and antibacterial properties [44,45].

Their potent cytotoxicity to a wide range of microorganisms may be the reason for their widespread antimicrobial activity; bacteria may be destroyed and rendered inactive as a result of their interaction with various surface-exposed functional groups on their cell surfaces. This activity is attributed to the surface coating of the gold nanoparticles or residual contaminants from the making process rather than to the core of the gold nanoparticles themselves [17]. Moreover, two published papers argue that AuNPs are safe regardless of their sizes (3.5, 4, 10, 12, or 18 nm) or capping agents such as sugars, citrate, cysteine, or biotin [46,47]. However, other publications report that the toxicity of 2 nm cationic AuNPs may appear due to the amount of dosage used. While the identical nanoparticles with

a negative surface charge were shown to be harmless in the same quantities [46,48]. On one hand, A. Folorunso, S. Akintelu, A. K. Oyebamiji, S. Ajayi, B. Abiola, I. Abdusalam, and A. Morakinyo showed that *Clostridium sporogenes* is highly sensitive to AuNPs at concentrations of 2 and 4 mg/L with zones of inhibition rates of 50% and 54%, respectively; on the other hand, two bacterial strains (*Staphylococcus aureus* and *Enterococcus faecalis*) produced low sensitivity with inhibition rates of 40% at the same concentration [49].

3.6. Microdilution assay

The lowest inhibitory concentration (MIC) of barley leaf extracts conjugated with AuNPs was obtained by employing the micro-dilution procedure. The concentration of conjugated AuNPs with plant extract varied from 0.8 to 0.0063 mg/mL. As seen in Table 3, barley extracts demonstrated a strong antibacterial action through coupling with AuNPs. It was observed that AuNPs conjugated with water extract against *S. typhi* and *A. baumannii* produce the least MIC value of 0.0063 mg/mL. The effectiveness of gold nanoparticles against bacteria proportionally rises with increasing concentration levels.

Previous studies have demonstrated that synthesized metal nanoparticles from plants are effective for drug delivery with strong antibacterial features that make them beneficial against a wide variety of microorganisms [11]. The constructed AuNPs were found to be extremely influential against bacteria and may be used to treat illnesses caused by bacteria. The bactericidal activities of BL-AuNPs were confirmed by the MIC/MBC test, where the MIC was identified as the minimum concentration, which reveals no significant growth *of S. typhi* and *A. baumannii,* as demonstrated in Table 3.

Table 3. Shows the MIC and MBC values of BL-AuNPs against twelve bacterial strains (S. typhi and A. baumannii), expressed in microliters.

Bacteria strain	MIC(mM)	MBC(mM)
S.typhi ATCC	0.46875	0.9375
S.typhi 1	0.23475	0.4695
S.typhi 2	0.234750	0.4695
S.typhi 3	0.234750	0.4695
S.typhi 4	0.46875	0.9375
S.typhi 5	0.234750	0.4695
A.baumannii ATCC (19606)	0.46875	0.9375
A. baumannii 1	0.46875	0.9375
A. baumannii 2	0.46875	0.9375
A. baumannii 3	0.46875	0.9375
A. baumannii 4	0.46875	0.9375
A. baumannii 5	0.46875	0.9375

The MICs of BL-AuNPs were found to be 0.46875 mM for all strains of *A. baumannii* and 0.23475 mM for four strains of *S. typhi* and 0.46875 mM for the other two *S. typhi.*

Nonetheless, it has been proposed that AuNPs engage in interactions with microorganisms via their cell walls. The Gram-negative bacteria have a thinner cell wall and may thus be more sensitive to nanoparticles. In contrast, the thick peptidoglycan coating of Gram-positive bacteria hinders the particles' ability to pass through their cell walls [33]. The minimum inhibition concentration of barley water extracts with bio-based AuNPs was determined using a micro-lag test, with the user concentration being between 0.3 and 0.0063 mg/mL, although some plant extracts showed no significant efficacy [50]. All plant extracts appear to be significantly more effective when combined with AuNPs, although it is challenging to document the bactericidal effect of using the extracts alone. It is difficult for plant extracts to exhibit full bactericidal activity [51].

The authors A. Murei, K. and Pillay, A. Samie reported in their study that the AuNPs produced a low biological bactericidal activity because the conjugates showed no MBC level; however, only a small number of chemically manufactured AuNPs conjugated to acetone extracts could destroy *E. coli* 25922. Therefore, chemically prepared gold nanoparticles are more effective as bactericides than biologically prepared ones [52]. Barley leaf extracts exhibited potent antibacterial activity in this investigation to produce a low MIC of 0.23475 mM. One of the major goals of creating complexes like nanoparticles conjugated with medicinal plants or even some medicines is to raise the activity of chemicals against bacteria. Utilizing the micro-dilution test, the synergistic influences of AuNPs and plant extracts were scrutinized against a few harmful microorganisms. Similarly, many researchers have discovered that conjugated nanoparticles exhibit greater antibacterial activity, showing that combinations were helpful against infectious illnesses. For instance, as in Ref. [53], the synergistic consequences of AuNPs coupled with norfloxacin against *P. aeruginosa*, *E. coli* ATCC 25922, *Streptococcus mutans*, and *Staphylococcus aureus* were reported. The conjugated synergistic effects of reducing bacterial strain survival at low pharmaceutical doses may be feasible.

4. Conclusions

In this work, the ecologically friendly synthesis of AuNPs without costly antioxidants by using a rapid and predictable technique was achieved. Leaf plant

extracts directly reduce gold ions to AuNPs by some chemical processes. "Medium monodisperse" nanoparticles were made by simply incubating a barley extract with aqueous gold ions at room temperature, indicating that the plant extract functioned as an effective reducing substance. This simple strategy offers some interesting benefits, including affordability, interoperability, and ease of fabricating growth. The BL-AuNPs exhibited higher antibacterial activity against the two gram-negative bacteria compared to AuNPs and barley leaf extract individually. Future studies should include Gram-positive and fungal strains to better evaluate the full antibacterial spectrum. As well, future studies could explore the mechanistic aspects of nanoparticle-biomolecule interactions and further evaluate the in vivo efficacy and safety of these green-synthesized nanoparticles in medical applications.

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Ethical statement

This study does not involve any human participants or animal experiments requiring ethical approval.

Conflicts of interest

The authors declare no conflict of interest.

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