Evaluation of Cytotoxicity and Antibacterial Activity of Green Synthesized Silver Nanoparticles using *Hedera helix* extract.

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ABSTRACT

Nowadays, silver nanoparticles (AgNPs) have drawn significant interest due to their unique properties, making them advantageous in biomedical applications, sensors, antimicrobial agents, catalysts, and optical fibers. Green synthesis is the safest and easiest method for producing silver nanoparticles (AgNPs). This study aimed to investigate the antibacterial and cytotoxic activities of silver nanoparticles synthesized using aqueous extracts of *Hedera helix* (AHE) against *S. aureus*, *P. aeruginosa*, and A549 lung cancer cell lines. Silver nanoparticles (Hh-AgNPs) were synthesized using the aqueous extracts of *Hedera helix* (AHE) as a reducing agent and polyvinylpyrrolidone (PVP) as a stabilizer and characterized by UV-visible spectrophotometry and particle size analysis via dynamic light scattering (DLS). The silver nanoparticles (Hh-AgNPs) were successfully synthesized, showing maximum absorption at 448 nm, with enhanced cytotoxic activity against A549 lung cancer cell lines (IC_{50} = 15.16 µg/ml) and antibacterial activity against *S. aureus* (MIC = 0.156 mg/ml) and *P. aeruginosa* (MIC = 0.3125 mg/ml), compared to AHE alone. Biological methods are cost-effective and eco-friendly and thus can serve as an economical and efficient alternative for the large-scale synthesis of silver nanoparticles.

Keywords: *Hedera helix*, Silver Nanoparticles, Green synthesis, *S. aureus*, *P. aeruginosa*, A549 lung cancer cell lines.

1. INTRODUCTION

Nanotechnology is a multidisciplinary scientific field that deals with designing, synthesizing, and manipulating particle structures with sizes ranging from about 1 to 100 nm. Applications for nanoparticles (NPs) are numerous and include many areas such as biomedical sciences, agric *Hedera helix* ulture, cosmetics, food technology, catalysis, drug delivery, drug-gene delivery, energy science, single electron transistors, chemical industries, light emitters, nonlinear optical devices, electronics, drug delivery, and photo-electrochemical applications [1,2].

Nowadays many researchers focus on silver nanoparticles (AgNPs) due to their unique characteristics (e.g., size and shape depending on optical, antimicrobial, and electrical properties). There have been reports of several preparation methods for the synthesis of silver nanoparticles: namely physical, chemical, and biological synthesis [3-5]. Nowadays biosynthetic methods, i.e., green chemistry, using natural reducing agents such as polysaccharides, biological micro-organisms such as bacteria and fungus or plant extracts have emerged as simple and viable alternatives to the chemical and physical

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synthetic procedures to obtain AgNPs to overcome their drawbacks including the use of toxic chemicals, high temperature, pressure, and yielding of hazardous by-products[6]. Recently AgNPs were synthesized by using different plant extracts as a potential reducing agent [7].

Hedera helix L. (Araliaceae Juss.), commonly known as Ivy, is an evergreen perennial plant native to the Northern Hemisphere zones [8,9]. Extract of *H. helix* is utilized for treating respiratory tract ailments characterized by excessive mucus production, respiratory tract infections, and persistent cough associated with the common cold. The aqueous extraction of Ivy leaves has been utilized in traditional medicine to treat respiratory diseases since the 19th century [10]. Several controlled clinical investigations have shown the effectiveness and safety of medications containing Ivy leaf extract [10-12]. Today, the use of Ivy leaf extract in medicines has been standardized by a Commission monograph of the "German regulatory authority" since 1988. These formulations include syrup, tablets, drops, and suppositories [10, 12], and can be used to treat the symptoms of chronic and acute inflammatory bronchial diseases, as well as the common cold with cough [13]. The primary bioactive compounds responsible for Ivy leaf extract therapeutic properties are triterpene, phenols and saponins. It possesses a range of beneficial effects, including spasmolytic/antispasmodic properties, anti-inflammatory actions, antimicrobial activity, pain relief, anthelmintic properties (against parasitic worms), antitrypanosomal and antileishmanial effects (against specific parasites), antitumor activity, antimutagenic properties, molluscicide activity (against mollusks), antioxidant effects, and antithrombin activity [14,15].

This study aimed to synthesize Hh-AgNps stabilized by PVP without using any chemical reducing agent and to evaluate their antimicrobial and cytotoxic. There are studies in the literature concerning the antibacterial activity of AgNps synthesized by using extracts of *Hedera helix* against *Bacillus subtilis* and *Klebsiella pneumoniae* [16]. Moreover, there is no published data in the literature concerning the evaluation of the antibacterial and cytotoxic activity of Hh-AgNPs stabilized by PVP against *S. aureus*, *P. aeruginosa* and A549 lung cancer cell lines.

2. MATERIALS AND METHODS

2.1 Chemicals and Reagents

Different reagents and chemicals were employed in this study including : Silver nitrate 99.8 % purity (AgNO3), Mueller-Hinton agar and Mueller-Hinton broth(Oxoid Ltd., UK) and 3-(4,5 – dimethylthiazol-2-yl)- 2,5. diphenyltetrazolium bromide (MTT)(Promega kit, USA), Dulbecco's Modified Eagle Medium (DMEM), Clarithromycin (CLR-15 µg), Ciprofloxacin (CIP-5 µg), Clindamycin (DA-2 µg), Metronidazole (MET-5 µg), Doxycycline (DO-30 µg) , all antimicrobial disks were supplied by (Bioanalyse, Turkey), standard strain of *P. aeruginosa* ATCC 9027 and *S. aureus* ATCC 9027(Manassas,VA,USA), Folin-Ciocalteu reagent, Aluminum chloride ≥ 99% purity(AZ chem for chemicals, South Africa), 2,2-Diphenyl-1-picrylhydrazyl Radical (DPPH) free radial 95% purity (Sisco Research Laboratories, India), 2,2'-azinobis-(3-ethylbenzothiazoline-6-sulfonic acid) (ABTS) free radical ≥ %98 purity (Sigma Aldrich, USA), Dimethyl sulfoxide (DMSO) 98% purity, Gallic acid 98% purity, Sodium carbonate anhydrous 99.5-100.5 % purity, Sodium acetate trihydrate 99% purity, Polyvinylpyrrolidone (PVP) (Joswee medical, Jordan), Trypsin enzyme(Euroclone, Italy), Quercetine (Santa Cruz Biotechnology, USA), Absolute Ethanol and methanol , Potassium persulfate(Sigma Aldrich, USA), Phosphate buffered saline (Euroclone, Italy) and Deionized water.

2.2 Collection of plants

The *H. helix* leaves has been collected during spring period year 2022, from local plants nursery allocated in Jerash, Jordan. A specimen sample was authenticated and prepared by Dr. Hatem Tayfour at the Royal Botanical Garden, Jordan and kept at Al-Aliya Amman University

research laboratory. Fresh leaves were immediately cleaned and air dried to remove the moisture, grinded and kept until used. Followed by grinding the dried leaves into fine powder.

2.3 Preparation of Aqueous Leaf Extract (AHE)

Aqueous extract of *H. helix* (AHE) was prepared by placing a 25 g of dried *H. helix* plant (Collected and dried previously by our research group) inside a Soxhlet extractor with 400 mL of distilled water for 4 hrs. the resultant extract was then dried using Rotary evaporator for 24 hrs. and left inside fume hood for 48 hrs. to dry completely. The dried Aqueous extract is then stored at 4 ^{o}C for further tests.

2.4 Extraction yield calculation

The extraction yield is calculated to determine the relative amount of the extarct inside raw plant material. After the extract is dried and weighed, the weight of the extarct is divided on the total weight of raw plant material as per equation : Extraction yeild %= (weight of Extraction / weight of raw plant material) * 100%

All measurements were measured in triplicates. The average and standard deviations were used for further calculations.

2.5 Qualitative Phytochemical tests

2.5.1 Detection of saponins

Qualitative phytochemical analysis of the AHE was performed using the standard experimental procedures to detect the extract phytoconstituents. Among these phytoconstituents are saponins that have been analyzed by foam formation [17] and hemolysis tests [18]. The findings of these tests were indicated qualitatively as positive (+) or negative (-) indicating the presence or absence of saponins, respectively.

2.6 Quantitative Phytochemical Analysis and Antioxidant Properties

2.6.1Total Phenolic Content (TPC) Determination

The folin-Ciocalteu method was used to calculate the total phenolic content of AHE. All experiment setups were made in triplicate. The total phenolic content (TPC) of each extract was expressed as mg equivalent to gallic acid/ g of AHE (mg GAE/g AHE) [19]. All measurements were measured in triplicates. The average and standard deviations were used for further calculations.

2.6.2 Total Flavonoid Content (TFC) Determination

To determine the total flavonoid content, the Aluminum chloride test was utilized with some minor modifications according to the method described by Chang et al. [20]. The total flavonoid content (TFC) in the extract was expressed as mg equivalent to quercetin/ g of AHE (mg Qr/g AHE). All measurements were measured in triplicate]. The average and standard deviations were used for further calculations.

2.6.3 Quantification of Radical Scavenging activity (DPPH)

To determine the half inhibitory concentration (IC_{50}) of AHE, it is needed to scavenge 50% of the 2,2-Diphenyl-1-picrylhydrazyl Radical (DPPH) DPPH• free radical in a solution, as described by Matusiewicz et al. [21] with some modifications. DPPH solution (0.2Mm) in absolute methanol was mixed vigorously with AHE (1:1, v/v). After 30 minutes of incubation, the mixture was centrifuged (15,000 x g, 10 minutes) and the absorbance of the supernatant was recorded at 517nm, using UV -Vis spectrophotometer (Shimadzu, Japan). The IC_{50} of AHE was compared to the IC_{50} of Vitamin C, which was used as a standard antioxidant. All measurements were measured in triplicates. The average and standard deviations were used for further calculations.

2.6.4 Scavenging Activity of 2.2′-Azino-bis (3-ethylbenzthiazoline-6-sulfonic Acid) Radical Cation (ABTS•+)

The total antioxidant capacity was determined using the 2.2′-Azino-bis (3-ethylbenzthiazoline-6-sulfonic Acid) Radical Cation (ABTS) scavenging activity as described by Re et al., (1999) [22] with some modifications.7mM stock solution of ABTS with 2.45mM potassium persulfate solution was prepared and diluted with methanol until it

reached an absorbance value of 0.7 at 734nm. 1 ml samples of AHE and Hh-AgNPs within a concentration range of (64 to 4.06 µg/ml) were prepared. These samples were added to 2ml of ABTS•+solution. After exactly 1hr, the final absorbance was measured at 734nm. Ascorbic acid (Vitamin C) was used as a reference compound for comparison (Re et al., 1999) [22].

2.7 Preparation of Silver Nanoparticles (Hh-AgNPs)

Silver nanoparticles (Hh-AgNPs) were synthesized using different concentrations of Polyvinylpyrrolidone (PVP) as a stabilizer, and compared by means of particle size, surface charge, and polydispersity index (PDI) as described by Zein et al., [23], with slight modifications.

A stock solution of (PVP) was prepared in de-ionized water at concentrations of (0,10, 15, 20 mg/mL). A stock solution of $AgNO_3$ in de-ionized water was prepared at concentration of (3.5 mg/mL). 20 mL of each solution was mixed and stirred for 15 minutes at room temperature using a magnetic stirrer. A 4 mL of *H. helix* extract (30 mg/mL) was then added to the mixture and was further stirred for 24 hours. After the time elapsed, the color of the resultant nanoparticle suspension turned brownish yellow. The probe sonicator (Sonopulus ultrasonic homogenizer model HD 4000, Bandelin,Germany) was used at 80% amplitude for 2 hours to uniform the size of the NPs, followed by centrifugation for 5 minutes at 10,000 RPM and 25 ºC, the supernatant was discarded. The remaining powder was then deep frozen for 24 hours and lyophilized using freeze dryer (Zirbus, Germany) for another 24 hours. The resultant powder was then stored at 4 ºC for further examination.

2.8 Characterization of the prepared Hh-AgNPs

2.8.1 Ultraviolet -Visible Absorbance Spectroscopy

According to the method described by Zein et al., [23] the bioproduction of silver ion (Ag^+) into silver NPs (Ag^0) was monitored in aqueous solution prepared by dissolving 7 mg sample of the prepared Hh-AgNPs in 10 mL de-ionized water. Then, 1ml of the solution was scanned using Shimadzu UV-Vis spectrophotometer at regular intervals within wavelength ranges (200 to 800 nm). The formation of peak spectra confirms the formation of the expected nanoparticles. This assay was performed in triplicates.

2.8.2 Dynamic Light Scattering (DLS)

According to the method described by Zein et al.,[23], the particle size, surface charge (Zeta potential), and polydispersity index (PDI) of the prepared Hh-AgNPs was measured for 11 times over a duration of 2 weeks (excluding the nonworking days) using Malvern Zetasizer Nano-Z® device (Malvern, Model ZEN-3600).

A 7 mg sample of the prepared Hh-AgNPs was weighed and dissolved in 10 mL de-ionized water. A 10 µL of the prepared suspension was then diluted with 990 µL De-ionized water in an Eppendorf tube and vortexed for one minute to ensure homogeneous distribution of Hh-AgNPs in the vehicle. The sample was then transferred into a Zeta sizer Quartz cuvette to measure the above-mentioned parameters (Size, Charge, and PDI).

All measurements were measured in triplicates. The average and standard deviations were used for further calculations.

2.9 Determination of Antibacterial Activity of Biosynthesized Hh-AgNPs

2.9.1 Agar well diffusion method

The biosynthesized Hh-AgNPs were tested for their antibacterial potential under sterile conditions, and according to guidelines of the Clinical and Laboratory Standards Institute [24] using *Staphylococcus aureus* (Gram-positive bacteria) and *Pseudomonas aeruginosa* (Gram-negative bacteria). For testing, the desired bacteria were inoculated in Mueller–Hinton broth and incubated 2-6h in the incubator shaker at 37 °C, and the turbidity of the bacterial culture was standardized at OD_{600}=0.1 (contrast to 0.5 McFarland) to get $1.5x10^8$ CFU/mL before swabbing the agar plates.

A sterile borer was used to make wells of 8mm diameter into the Mueller–Hinton agar plates (Oxoid Ltd., UK). A cotton swab was immersed in the previously standardized broth culture of the bacteria and swabbed

uniformly on the agar plates. An aliquot of 100 µL of AHE, AgNO3, and Hh-AgNPs at concertation of 10 mg/ml was transferred into each well to measure the antibacterial activity, while the negative control well had distilled water [24].

2.9.2 Minimal inhibitory concentration (MIC)

The MIC was performed to determine the lowest concentration needed that prevent visible bacterial growth. Serial descending dilutions were prepared from Hh-AgNPs, AHE, and AgNO3 (range of 5 - 0.078 mg/ml). A 100µL of each dilution was placed in a well and plates were streaked with the desired previously standardized bacterial inoculum of *P. aeruginosa* and *S. aureus*. Plates were incubated at 37 °C overnight, and the inhibition zones were recorded in millimeters (mm)[25].

2.9.3 Antibiotics Susceptibility Test

To determine the potency of Hh-AgNPs and AHE, the antimicrobial activity was compared to the antibacterial activity of selective standard antibiotics against the tested bacteria. According to the Handbook of Performance Standards for antimicrobial susceptibility testing, (M100S, 26th edition) [26], sterile plates of Mueller–Hinton agar were streaked with a bacterial culture using a sterile cotton applicator dipped into the standardized bacterial inoculum (0.5M McFarland equivalence). The agar plates were left to dry for a few minutes before distributing the discs of selected antibiotics on the surface. The plates were incubated at 37 °C for 24hrs., then inhibition zones were measured millimeter (mm). For *P. aeruginosa*, Doxycycline (DO-30 µg), Metronidazole (MET-5 µg), and Ciprofloxacin (CIP-5 µg) were used as a positive control. Similarly, for *S. aureus*, Clarithromycin (CLR-15 µg), Clindamycin (DA-2 µg), and Ciprofloxacin (CIP-5 µg), were used.

2.10 Determination of Anticancer activity of the prepared Hh-AgNPs against Lung cancer cell line (A549)

2.10.1 Splitting of A549 lung cancer cell line

According to the method described by Mosmann et al., [27]. Splitting of the A549 cells was carried out by aspirating and removing old Dulbecco's Modified Eagle Medium (DMEM) media from the T-75 flask, and the cells were washed with sterile phosphate-buffered saline (PBS). Next, a trypsin solution was applied to the flask to release the cells from the surface, and the flask was then placed in a humid environment with 5% CO2 at a temperature of 37°C for 3-5 minutes. The cells were gently agitated to facilitate detachment and then neutralized with fresh a DMEM culture medium. The resulting cell suspension was moved to a sterile centrifuge tube and centrifuged.

Then, the supernatant was drawn off, leaving the cells to be resuspended in a new medium. After counting the cells, they were placed into a new tissue culture flask and incubated until they reached a coverage level of approximately 70-80%. This process was repeated to continue the expansion of the cells. A sterile environment was maintained throughout the procedure and were checked for possible cells contamination regularly.

2.10.2 Counting of A549 lung cancer cell line

According to the method described by Maurya et al. in 2010,[26] the cells were obtained as a cell suspension through trypsinization. This suspension was then diluted in a buffer to reach a concentration of 1-2 x 10^6 cell/ml. A trypan blue solution was created by dissolving 0.4 g of trypan blue powder in 100 mL of phosphate-buffered saline. The cell suspension was combined with the trypan blue solution and left to incubate for 3-5 minutes to allow the dye to enter the cells. The trypan blue-stained cell suspension was then placed onto the Hemo-cytometer Automated Cell Counter following the manufacturer's instructions for counting the cells.

The cell concentration was determined by dividing the number of viable cells by the volume of the cell suspension loaded onto the Hemo-cytometer. To ensure accuracy, the counting procedure was repeated several times, and the average cell concentration was calculated. Throughout the process, aseptic conditions were maintained to prevent contamination of the cell suspension, and suitable

protective equipment was used to avoid exposure to hazardous materials.

2.10.3 Treatment of A549 lung cancer cell line

To examine the effects of AHE, Hh-AgNPs, and $AgNO_3$ on A549 lung cancer cell lines, a 96-well microtiter plate was used. The cells were seeded into the wells at a density of 2000 cells per well and allowed to attach overnight. On the next day, the old medium was replaced with fresh medium containing varying concentrations of the drug, with the concentration range being from 1000 µg/ml to 15.62 µg/ml. The plate was then placed in a humid environment with 5% CO_2 and incubated at 37°C for 24, 48, and 72 hours. Control wells containing only the solvent (DMSO) without the drug were also included as described by Mosmann in 1983 [27].

After the incubation period, MTT assay was used to assess cell viability. The absorbance measurements at 570 nm were obtained using an ELISA microplate reader, and the data was analyzed to determine the half-maximal inhibitory concentration (IC_{50}) of each treatment.

3. RESULTS AND DISCUSSION

The phytochemical analysis of AHE reveals the presence of saponins, phenolic compounds and flavonoids. The presence of these compounds not only reduce Ag+ ions to AgNPs but also acted as capping/stabilizing agents which prevent aggregation of NPs[27] .In this regard, phenolic compounds were confirmed by measuring their content in AHE, it was found that the total phenolic content (TPC) was (43.98 ± 1mg/ g equivalent to gallic acid), and the total flavonoid content was (28.81 ± 0.076 mg /g equivalent to quercetin). The results of a recent study on the antioxidant potential of *H.helix* provides support for a theory regarding the role that antioxidant molecules from aqueous leaf extract may play in the biogenic synthesis of silver nanoparticles. Previous studies have shown that plants contain phenolic compounds and flavonoids, which have strong antioxidant properties and so promote the biosynthesis of nanoparticles [28-31].

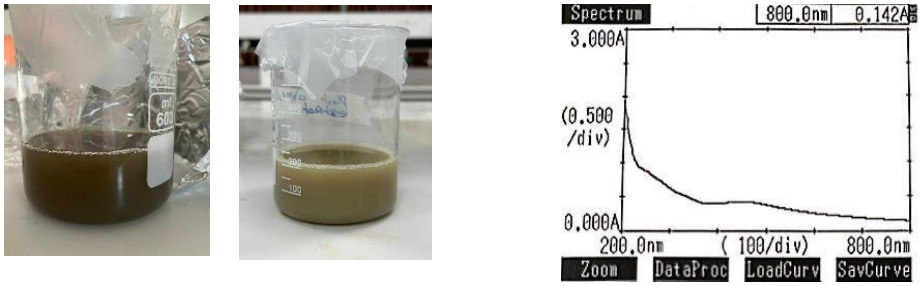
Hh-AgNP showed an enhanced antioxidant activity compared with AHE, the antioxidant activity of synthesized Hh-AgNPs and AHE was evaluated by DPPH radical scavenging assay. Ascorbic acid was used as a positive control. The IC_{50} of AHE and Hh-AgNPs were 39.03 ± 0.557 µg/ml and 13.19±0.13 µg/mL respectively. Also, their antioxidant activity was evaluated by ABTS assay, it was found that IC_{50} of AHE and Hh-AgNPs were 24.81± 0.74 and 8.66± 0.64 respectively. According to previous literature obtained by Mishra et al., in 2012 [32], there are differences between the antioxidant activity of a compound when tested using DPPH or ABTS free radical's assays. Differences were because of variations in the chemical properties, reaction conditions, and the principle of the DPPH and ABTS assays, resulting in variations in the IC_{50} values obtained for the tested extract [32].

Kharat and Mendahulkar [33] studied the antioxidant activity of synthesized NPs using DPPH assay and observed their antioxidant potential. They suggested that these photosynthesized NPs can be used as a potential free radical scavenger. Divya, K. S et al in 2019 studied *In vitro* antioxidant activity of methanol and silver nanoparticles extract from *Trigonella foenum-graecum* and found significant free radical scavenging potential [34]. The results strongly recommended the application of AgNPs as useful natural antioxidants against different oxidative stress.

In a current study, NPs of *H. helix* crude extract was synthesized and evaluated for their cytotoxic effect and antimicrobial activity. Synthesis of Hh-AgNPs through bio-green method was identified when the color was changed to brownish upon adding AHE in $AgNO_3$ solutions (Fig 1. A). The intensity of the color was increased with increasing the incubation time. Collective oscillation of free electrons of reduced Hh-AgNPs were responsible for change in color of the reaction mixture [35]. Further, UV-visible spectrometric analysis of nanoparticles showed the maximum absorption of nanoparticles at a wavelength of at λ= 448 nm (Fig 1.B).

Previous study reported that AgNPs give absorption peak at 420- 450nm because of its plasma resonance (SPR) character [31,36]

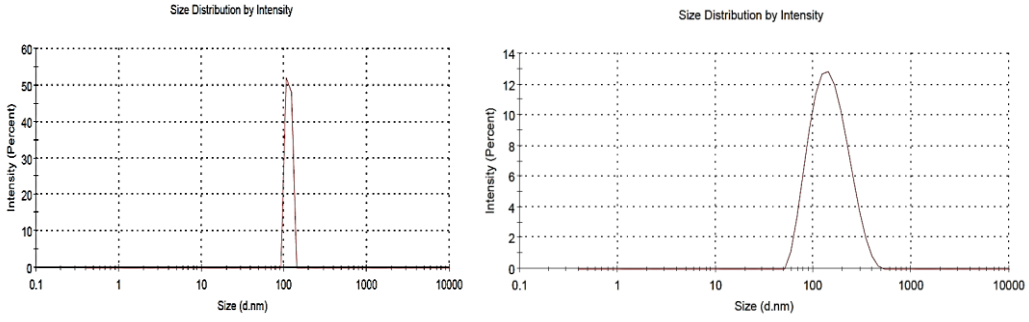
Dynamic Light Scattering (DLS) demonstrated the size and surface charge distribution of biosynthesized Hh-AgNPs. It has been found that the average size and charge of Hh-AgNPs were125.1nm and -10 mv respectively (Fig 2. A). The size was 90.1nm with charge -16.0mv when 15%mg/ml pf PVP was used (Fig 2. B).



A **B**

Fig 1. A) Color changes from yellow to brown upon synthesis of AgNPs. B) UV/visible spectra of Hh-AgNPs.

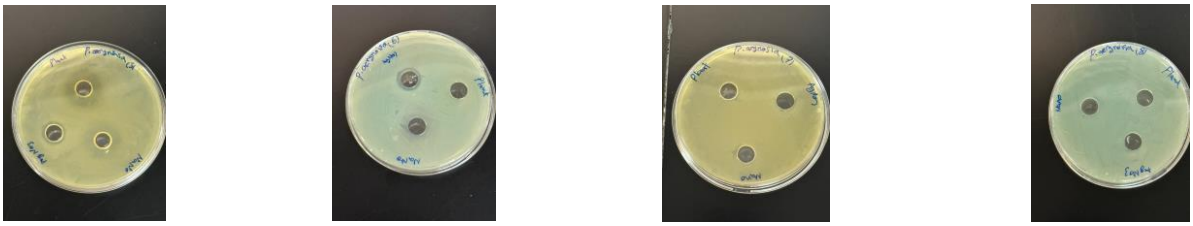
AgNPs synthesized by plant extract most often are negatively charged [37,38]. This highly negative zeta potential value describes the presence of the bioactive compounds with a negative charge on the surface of AgNPs as a capping agent [39]. As we know, the zeta potential of AgNPs is one of the essential factors in preventing additional NP aggregation and stabilizing NPs by generating electronic repulsion [40]. On the other ha the negative zeta potential leads to increased cellular uptake and subsequent cytotoxicity observed from the positive-charge AgNPs [41].



A **B**

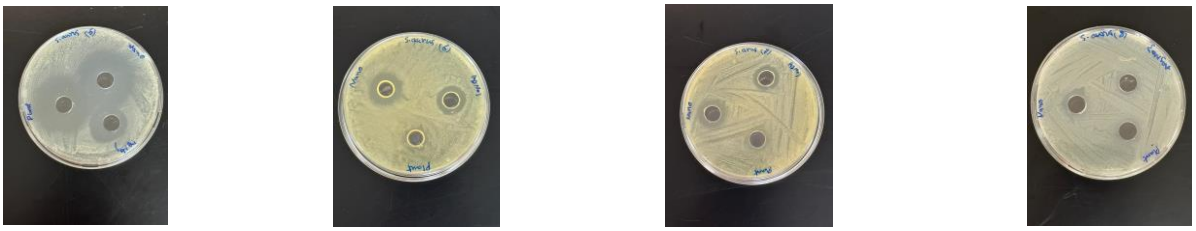
Fig 2. DLS spectra A) Hydrodynamic size distribution of Hh-AgNPs without PVP B) Hydrodynamic size distribution of Hh-AgNPs with 15% mg/ml PVP

Broad spectrum antibacterial activity of AHE has been reported [42, 43]. The antibacterial activity of Hh-AgNPs against *S. aureus* and *P. aeruginosa* were determined by measuring the zone of growth inhibition (mm). The diameter of inhibition zone for both genus using Hh-AgNPs is slightly higher than that for AHE at all tested concentrations (Figure 3, and 4).



(A) (B) (C) (D)

Figure 3 Diameter of the inhibition zone after treatment of *P. aeruginosa* with various concentrations of Hh-AgNPs, AHE. and silver nitrate. (A: 0.625mg/ml, B: 0.3125mg/ml, C: 0.156mg/ml, D: 0.078mg/ml)



(A) (B) (C) (D)

Figure 4 Diameter of the inhibition zone after treatment of *S. aureus* with various concentrations of Hh-AgNPs, AHE. and silver nitrate. (A: 0.625mg/ml, B: 0.3125mg/ml, C: 0.156mg/ml, D: 0.078mg/ml)

The MIC of Hh-AgNPs against *S. aureus* was determined to be 0.156mg/ml, while the MIC of Hh-AgNPs, and AHE against *P. aeruginosa* was determined to be 0.3125 and 0625mg/ml, respectively. In the present study Hh-AgNPs were found to have a higher antibacterial activity compared to AHE (table 1). Reddy et al. reported that the enhanced antibacterial activity of biosynthesized NPs using *Piper longum* compared to the extract supports our results [44]. Inhibition of DNA replication and hindrance of protein synthesis are the mechanisms behind the antibacterial activity of metallic NPs [45].

Table 1: Antibacterial activity measured as zones of inhibition (mm) of Hh-AgNPs, AgNO3 and AHE against *S. aureus* and *P. aeruginosa.*

Concentration mg/mL	Diameter of inhibition zone (mm) *S. aureus*			Diameter of inhibition zone (mm) *P. aeruginosa*		
	Hh-AgNPs	AHE	$AgNO_3$	Hh-AgNPs	AHE	$AgNO_3$
10	44±2.51	38±2.30	20±4.05	39±3.05	36±5.60	17±1.20
5	42±4.00	37±6.50	19±3.51	35±2.64	30±2.08	16±1.10
2.5	40±2.49	34±4.01	18±3.42	27±1.52	29±2.00	15±0.57
1.25	39±5.01	34±0.57	16±1.15	24±5.20	22±0.57	14±0.58
0.625	36±4.20	32±4.35	16±4.10	18±1.12	20±1.15	12±0.57
0.3125	21±4.05	13±3.51	14±2.20	10±0.57	R	R
0.156	19±2.51	12±2.51	12±1.00	R	R	R
0.078	R	R	R	R	R	R

Hh-AgNPs and AHE were more effective than some standard antibiotics used for treatment of *P. aeruginosa* and *S. aureus* (table 2). Bacterial resistance to drugs is one the major global health issue, so there is a need to anticipate and develop potent antibiotic agents against multidrug resistance microbes. The use of AgNPs as antibacterial agent can be traced from ancient Greece [46].

Table 2: Inhibition zone diameter (mm) of several standard antibiotics' controls against *P. aeruginosa* and *S. aureus*

Antibiotic	Zone of inhibition (mm)	
	P. aeruginosa	***S. aureus***
Doxycycline (30µg)	R	R
Metronidazole (5µg)	R	R
Ciprofloxacin (5µg)	35± 0.23	35± 0.55
Clarithromycin (15µg)	R	40± 1.02
Clindamycin (2µg)	R	36± 0.88

R, resistant

The biomedical applications of AgNPs are promising with their tremendous effects in the fields of medicine, drug delivery and anti-angiogenic property of cancer [47-49]. In the present study the cytotoxic effect of AHE, Hh-AgNPs and AgNO3 was tested against A549 cell lines by using MTT assay. The viability of the A549 cell lines was observed after 24hr, 48hr and 72hr of treatment with AHE, Hh-AgNPs and AgNO3. Table 3 shows the IC_{50} value against A549 cell line.

The IC_{50} values after 24hr were determined to be 73.57, 15.16 and 395.5 µg/ml for AHE, Hh-AgNPs and AgNO3 respectively. The Hh-AgNPs showed excellent anticancer activity against A549 cell compared with AHE. This study strongly revealed the significant antiproliferative activity of biosynthesized silver nanoparticles. However, such activity may be due to the synergistic effect of both nanosized silver and bioactive phytocompounds attached on the surfaces of the NPs. These days much attention is being given to metallic NPs and their anticancer activity.

Table 3: Cytotoxic activity evaluation against A549 cell line

Sample	Anticancer activity against A549 lung cancer cell line (IC_{50}values)		
	24hr	**48hr**	**72hr**
AHE	73.57 µg/ml	87.96 µg/ml	103.7 µg/ml
AgNO3	395.5 µg/ml	341.3 µg/ml	542.5 µg/ml
Hh-AgNPs	15.16 µg/ml	18.61 µg/ml	25.55 µg/ml

4. CONCLUSION

In this study, stable bioactive silver nanoparticles were synthesized successfully using **H. helix** extract by bio-green method as a cost-effective manner. The nanoparticles possessed the added advantage of active phytoconstituents incorporated in them. Further, these nanoparticles were evaluated for their activities and showed higher antioxidant, antibacterial and cytotoxic activities compared with extract. The outcomes of this study illustrate a broad range of applications of bioactive silver nanoparticles synthesized by bio-green method.

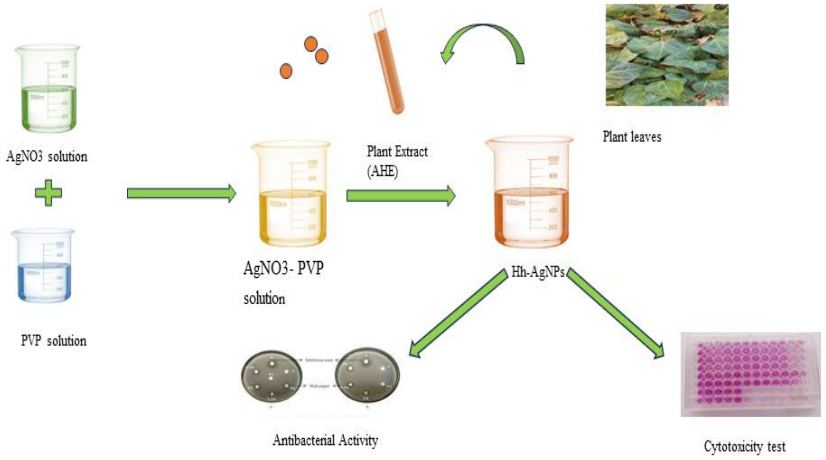
Conflicts of interest

There are no conflicts to declare.

Author Contributions

Reem Issa and Sina M. Matalqah conceptualization, methodology, analyse and discuss the results, drafting and editing the final manuscript. Ahmad Alfalahi prepared silver nanoparticles and characterised them and participated in performance of antimicrobial and cytotoxicity tests. Hala .AL-Dagestani designed and conducted the antimicrobial activity test while Anas abed designed and conducted the cytotoxicity activity test.

Graphic Abstract:



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تقييم السمية الخلوية والنشاط المضاد للبكتيريا لجسيمات الفضة النانونية المصنعة بالتكنولوجيا الخضراء باستخدام مستخلص نبات اللبلاب

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ملخص

في الوقت الحاضر جذبت جسيمات الفضة النانوية اهتمامًا كبيرًا نظرًا لخصائصها الفريدة التي تجعلها مفيدة في الطب الحيوي وأجهزة الاستشعار ومضادات الميكروبات، والمحفزات، وتطبيقات الألياف الضوئية. يعتبر التوليف الأخضر هو الطريقة الأكثر أمانًا وأسهل لإنتاج جسيمات الفضة النانوية. هدفت الدراسة إلى التحقق من النشاط المضاد للبكتيريا والسمية الخلوية لجسيمات الفضة النانوية المحضرة باستخدام المستخلص المائي لنبات اللبلاب ضد أنواع بكتيريا وخلايا سرطان الرئة. تم تصنيع جزيئات الفضة النانوية باستخدام المستخلص المائي لنبات اللبلاب كعامل اختزال والبولي فينايل بيروليدين كمثبت. ومن ثم تشخيص جزئيات الفضة النانوية المصنعة بواسطة مقياس الطيف الضوئي للأشعة فوق البنفسجية ومحلل حجم الجسيمات بطريقة تشتت الضوء الديناميكي. تم تصنيع هذه الجزئيات بنجاح وأظهرت أقصى امتصاص عند 448 نانوميتر مع تحسن في نشاطها السام لخلايا الرئة السرطانية بقيمة 15.16 ميكروغرام /مل كأدنى تركيز مثبط. وتحسن في نشاط هذه الجزئيات النانونية كمضاد للبكتيريا (المكورات العنقودية الذهبية) مقارنه مع المستخلص المائي. تعتبر الطرق البيولوجية فعالة من حيث التكلفة وصديقة للبيئة، وبالتالي يمكن أن تكون بديلاً اقتصاديًا وفعالاً لتخليق جسيمات الفضة النانوية على نطاق واسع.

الكلمات الدالة: اللبلاب (Hedera helix)، الجسيمات النانوية الفضية، التخليق الأخضر، المكورات العنقودية الذهبية، الزائفة الزنجاري، خلايا سرطان الرئة .A549.

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