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Original article

ANTIBACTERIAL ACTIVITY OF GREEN SYNTHESIZED SILVER NANOPARTICLES USING AQUEOUS EXTRACT OF AELUROPUS LITTORALIS AND THEIR ANTICANCER AND ANTIOXIDANT PROPERTIES

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Abstract

Background. The biosynthesis of nanoparticles using plant extracts offers an eco-friendly and cost-effective method for producing stable nanoparticles with various applications in medicine, agriculture, and environmental science.

Purpose. To investigate the ability of *Aeluropus littoralis* aqueous extract to biosynthesize silver nanoparticles (AgNPs) and of antibacterial activity, anticancer and antioxidant properties.

Materials and methods. *A. littoralis* aerial parts were washed, dried, and ground. Extract was prepared by boiling 7 g in 100 mL water, filtered, and dried. Aelu-AgNPs were biosynthesized by mixing extract with $AgNO_3$, incubating until color change, then centrifuging and washing. Nanoparticles were characterized by UV-Vis, FTIR, XRD, SEM, EDX, and zeta potential. Antibacterial activity was tested against MDR bacteria using agar diffusion, MIC, and MBC assays. Cytotoxicity was evaluated with MTT on cancer and normal cells. Antioxidant activity was measured by DPPH assay. Data analysis used SPSS and GraphPad Prism.

Results. The aqueous extract of *A. littoralis* operated as a reducing agent for $AgNO_3$, resulting in the formation of AgNPs (Aelu-AgNPs), which was confirmed by spectroscopy at the greatest peak of 410 nm. Based on FESEM analysis, the Aelu-AgNPs were around 41.43 nm in size. The XRD study demonstrated a face-centered cubic structure, whereas zeta potential assessment suggested significant stability at -40.4 mV. *Pseudomonas aeruginosa*, *Citrobacter freundii*, *Proteus mirabilis*, *Enterobacter cloacae*, *Escherichia coli*, *Providencia rettgeri*, *Klebsiella pneumoniae*, *Staphylococcus aureus*, *S. haemolyticus*, *S. epidermidis*, *S. hominis*, and *Enterococcus faecium* were all proven to be susceptible to the Aelu-AgNPs. Among the bacteria tested, *P. mirabilis* showed the most sensitivity, with a 19.5 mm

inhibition zone. The MIC ranged from 62.5 to 7.8 μg/ml and the MBC from 62.5 to 15.6 μg/ml for all the bacterial isolates that were examined. The results showed that the Aelu-AgNPs had a potential antioxidant activity higher than ascorbic acid at a concentration of 12.5 μg/ml. The MTT assay validated superior efficacy in inhibiting the A375 cancer cell line with an IC_{50} of 71.04 μg/ml, in contrast to 148.6 μg/ml of the normal cell line (HdFn).

Conclusion: The biosynthesized silver nanoparticles obtained from *A. littoralis* extract exhibit potential as antibacterial, antioxidant, and anticancer agents.

Keywords: silver nanoparticles; anticancer activity; antioxidant activity; antibacterial activity; *Aeluropus littoralis*

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Introduction

Antibiotic resistance has become an important issue the past few years, primarily due to the widespread and incorrect use of antibiotics. Consequently, bacteria have evolved various resistance mechanisms to various antibiotics, rendering them inefficient against pathogenic bacteria. As a result, the prevention and treatment of diseases and injuries induced by these bacteria has become increasingly complex and difficult, the risk of infection and the degree of severity of wounds have grown greater, and in certain cases, therapy has become impossible, leading to a substantial rise in death rates attributable to these infections [1; 2].

This situation requires finding innovative therapeutic agents that can limit the spread of these resistant strains with high efficiency. Among these agents, silver nanoparticles (AgNPs) have become known as a powerful alternative since to their antibacterial activity and their ability to enhance the effectiveness of antibiotics when combined with these particles [3]. AgNPs have antibacterial activities via multiple mechanisms including leakage of cell contents by disrupting the bilayer of phospholipid in the plasma cell, it also damaging the cell wall and releasing reactive oxygen species. Furthermore, silver nanoparticles inhibit bacterial growth by forming complexes with DNA and modifying gene expression by interference with RNA transcription [4; 5].

The biological application of AgNPs extends more to their function as antibacterial agents since they additionally play an important role in cancer therapy

by specifically targeting cancer cells without damaging healthy cells. The ability to select a target arises from the distinctive surface features of the AgNPs, enabling them to associate with particular receptors on cells that are cancerous. Once these particles bind to tumor cells, they initiate multiple mechanisms that inhibit the spread of cells with cancer, and subsequently result in cell apoptosis [6; 7]. Biosynthesized AgNPs exhibit notable antioxidant activity which can be applied in biomedical and pharmaceutical fields to prevent various disorders related to oxidative stress [8].

Hazardous materials are used in the production of AgNPs in traditional synthesis methods such as chemical reduction, which results in environmentally harmful by-products that require careful management and disposal. There is a recent trend towards adopting green synthesis methods to produce silver nanoparticles, which are considered safe for health and the environment, it also provides cost-effectiveness and the ability to synthesize nanoparticles with precise size and shape [9; 10]. A wide range of biological sources can be utilized to synthesize silver nanoparticles using environmentally friendly methods including fungi, yeast, bacteria, algae, lichens and plant extracts. There are various techniques that use plant extracts to synthesize AgNPs, the most common of which is the reduction of Ag^+ using phytochemicals including terpenoids, phenols and flavonoids [11; 12]. The halophytic plant *Aeluropus littoralis*, a member of the Poaceae family, is very important to ecosystems due to its unique ways of surviving in salty conditions. The secondary metabolites produced by *A. littoralis* are known for their beneficial health properties and positive impact on the environment, and may have potential medicinal uses [13].

In our present study, we reported a novel synthesis of AgNPs utilizing extraction of *A. littoralis* aerial part with characterization of these particles as well as evaluated the biomedical applications such as antimicrobial activity against MDR-bacteria, cytotoxicity toward the cell lines HdFn and A375, and finally antioxidant activity.

Materials and methods

Collection plant parts and preparation for extraction

Aeluropus littoralis (Echrich, Iraq) fresh aerial parts were obtained from University of Southern Technical in Basra Governorate and rinsed extensively with tap water, thereafter washed multiple times with distilled water. The dried, cleaned component was ground into a fine powder after being left at room temperature in shade. Subsequently, 7 grams of the powder was added to 100 ml of deionized water (100°C) with shaking for 30 minutes at environment tem-

perature. The mixture was filtered using filter paper type Whatman No. 1 in an oven at 40 °C the filtered mixture evaporated. At 4°C, the dried extract was stored in an airtight bottle for subsequent examinations. For future testing, the dehydrated extract was sealed in a container and kept at 4°C.

Biosynthesis of Aelu-AgNPs

According to [14], Aelu-AgNPs were biosynthesized with certain modifications. In a satirized bottle, 7% aerial part aqueous extract was applied to 1 mM $AgNO_3$ solution (1:9 v/v) and stirred at 35 °C for 30 minute, the bottle incubated in darkness at room temperature until observing color change from light to dark color, the Aelu-AgNPs were collected by centrifuging the entire combination at 4000 rpm for 15 min, discarded the supernatant to remove unreacted compound, while the pellet was purified by washing with deionized water 3-5 times, the pellet was dried and then kept at 4°C until use in subsequent tests and examinations.

Characterization of Aelu-AgNPs

The synthesized Aelu-AgNPs were characterized by carrying out the UV-Vis spectrophotometer (Thermo, Biomate5) with the spectrum of 200–800 nm. To check the possible functional groups accountable for the reduction of Ag^+, the technique of fourier transmission infrared (FTIR) spectroscopy (IRAffinity-1, SHIMADZU) was performed at a spectrum frequency of 4000-400 cm^{-1} for each plant extract and AgNPs to compare between them. The X-ray diffraction (XRD) type Philips PW1730 was employed to examine the crystallization characteristics of the green produced Aelu-AgNPs in the range of 2 theta from 10°C to 80°C. The morphology of Aelu-AgNPs were analyzed using the FESEM (field-emission scanning electron microscopy) model TESCAN, MIRA3, the size of nanoparticles (NPs) was estimate via the software ImageJ. The type and proportion of elements present in the AgNPs were determined by EDX (energy dispersive X-rays). Zeta potential analyzer (HORIBA SZ-100) was examined the stability of the NPs.

Antibacterial activity of Aelu-AgNPs

We used [15], agar well diffusion method with some modifications to investigate AgNPs efficacy against MDR bacteria. Twelve pathogenic bacteria were collected from patients at the Al-Fayhaa Burn Centre at Al-Fayhaa teaching hospital in Basra, Iraq: *Pseudomonas aerugenosa*, *Staphylococcus aureus*, *Proteus mirabilis*, *Escherichia coli*, *Enterobacter cloacae*, *S. haemolyticus, Enterococcus faecium*, *S. epidermidis*, *Klebsiella pneumoniae*, *S. hominis*, *Citrobacter freundii*, and *Providencia rettgeri* (Table 1). Bacterial inoculum with 0.5 McFarland were applied on Mueller-Hinton agar (MHA) plates utilizing a ster-

ilized cotton swab. A 6-mm sterile cork borer drilled six wells into each plate after 30 minutes of room temperature incubation. A stock solution was prepared by dissolving 15 mg of Aelu-AgNPs powder in 15 ml of dimethyl sulfoxide (DMSO), then added 100 µl of two folded serial concentrations (1000 – 62.5 µg/ml) to the wells. Furthermore, a negative control treatment was applied by adding the same amount of DMSO to the well. Finally, the plates were incubated for 24 h at 37°C. The hollow zone formed around a well was denoted as inhibitory activity of AgNPs.

Table 1.

Antibiogram of selected pathogenic bacteria against antibiotics

Bacterial isolates	Antibiotics
P. aeruginosa	TC, TCC, PRL, TZP, CAZ, FEP, ATM, IMI, MRP, AK, CN, TOB, CIP
E. coli	TC, TCC, PRL, TZP, CAZ, FEP, ATM, IMI, MRP, CN, TOB, CIP, MN, SXT
K. pneumoniae	TC, TCC, PRL, TZP, CAZ, FEP, ATM, IMI, MRP, AK, CN, TOB, CIP, SXT
P. mirabilis	TC, CAZ, FEP, ATM, TOB, MN
P. rettgeri	TC, TCC, PRL, TZP, CAZ, FEP, ATM, IMI, MRP, TOB, CIP, MN, SXT
C. freundii	TC, TCC, PRL, TZP, CAZ, FEP, IMI, MRP, MN
E. cloacae	TC, TCC, PRL, TZP, CAZ, CAV, CTX, TZC, FEP, ATM, IMI, MRP, CN, TOB, CIP, SXT
S. aureus	CX, BNZ, OX, CN, TOB, LV, MXF, E, TEC, VA, TE, FOS, FC, SXT
S. epidermidis	CX, BNZ, OX, CN, TOB, LV, MXF, E, CD, VA, TE, FOS, FC, RD, SXT
S. hominis	CX, BNZ, OX, E, FC, SXT
S. haemolyticus	CX, BNZ, OX, CN, TOB, LV, MXF, E, CD, TE, FC, RD, SXT
E. faecium	P, E, LNZ, TE, FOS

TC Ticarcillin, PRL Piperacillin, TCC CAZ Ceftazidime, Ticarcillin/Clavulanic Acid, TZP Piperacillin/Tazobactam, CAV Ceftazidime/Avibactam, CTX Cefotaxime, TZC Ceftolozane/Tazobactam, ATM Aztreonam, FEP Cefepime, MRP IMI Imipenem, Meropenem, CIP Ciprofloxacin, AK Amikacin, TOB Tobramycin, CN Gentamicin, MN Minocycline, SXT Trimethoprim/Sulfamethoxazole, CX

Cefoxitin, P Pencillin, BNZ Benzylpenicillin, OX Oxacillin, LV Levofloxacin, E Erythromycin, MXF Moxifloxacin, CD Clindamycin, TEC Teicoplanin, TE Tetracycline, VA Vancomycin, FOS Fosfomycin, FC Fusidic Acid, RD Rifampicin.

MIC and MBC procedure

The MIC (minimum inhibitory concentrations) of *Al*-silver nanoparticles was estimated utilizing the micro-dilution in 96-well microtiter plates. The bacterial suspension of each tested isolate was prepared according to (CLSI, 2024) protocol [16]. To prepare a stock solution with 1000 µg/ml AgNPs, dissolving 10 mg of powder in DMSO, mixing 17.5 ml of Mueller-Hinton broth (MHB) with 2.5 ml of stock solution to obtain a 125 µg/ml concentration, serial dilutions of Aelu-AgNPs were prepared from 125 to 3.9 µg/ml. 100 µl of each concentration was applied to the wells in the rows from A to F. The wells in the rows from A to G, 20 µl of each bacterial suspension was added to it (every single column was represented a distinct bacterial species). 100 µl of MHB added to rows of G and H, which represented the positive and negative controls, respectively (Fig. 1). After incubation for 24 h at 37°C, 20 µl of 0.016% resazurin dye solution (color blue) was applied to the entire wells and incubated at the same conditions for two hours to observe the color change, color blue indicating no bacterial growth, while color pink or purple referring to bacterial growth. The last well in a column with no color change was scored as the MIC value. Calculating MBC (Minimum bactericidal concentrations) involved direct culturing the contents of the MIC well and the higher concentration well on MHA plates. MBC/MIC was discovered.

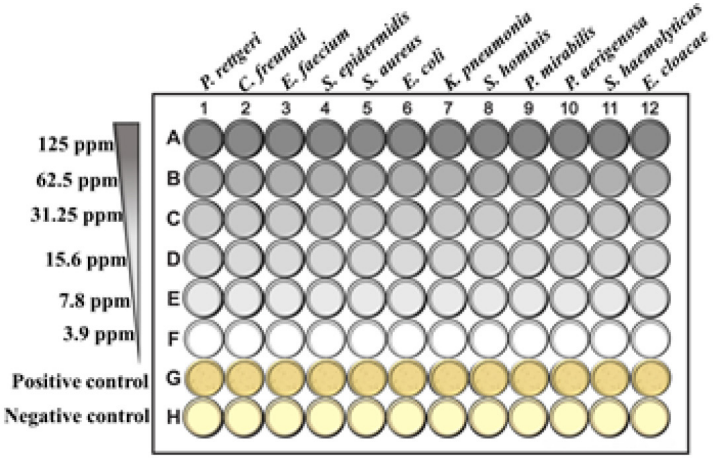

Fig. 1. A MIC procedure

Anticancer activity of Aelu-AgNPs

The cytotoxicity effect of Aelu-AgNPs synthesized by aqueous extract of *A. littoralis* were estimated according to [17] by a 3-(4, 5-dimethylthiazol-2-yl)-2, 5-diphenyl-tetrazolium bromide (MTT) assay using A375 (Human malignant melanoma) as cancer cell line compared with HdFn (Human dermal fibroblasts, neonatal) as a normal cell line. Concentrations 400, 200, 100, 50, and 25 µg/mL were applied for 48 hours to both A375 and HdFn cell lines, the viability percentage and the IC_{50} were calculated after 4 hours of adding the MTT solution.

Antioxidant activity of Aelu-AgNPs

A stable DPPH 2, 2-diphenyl-1-picrylhydrazyl radical scavenging experiment was utilized to test antioxidant activity according to [18]. To modify concentrations, 0.3 mL of DPPH solution (0.1 mg/mL) and 3 mL of methanol-DMSO (9:1 v/v) were added to separate reaction tubes with 0.5 mL of AgNPs (200, 100, 50, 25, and 12.5 µg/mL). After a quick shaking, the tubes incubated at room temperature in the dark for one hour. An ELIZA reader assessed absorbance at 517 nm after incubation. DPPH and methanol-DMSO were the negative controls, while ascorbic acid was the reference standard.

Statistical analysis

All data in this study was expressed as mean plus or minus standard deviation. The data of antibacterial activity analyzed using one-way analysis with SPSS version 22 software. Statistical significance was considered when *p*-value equal or greater than 0.05. To analyze the antioxidant and anticancer effects, we used Graph Pad Prism 10.4.0, and to display the FTIR and XRD findings, we used Origin Pro 10.15.

Results and discussion

Biosynthesis of Aelu-AgNPs

As shown in Fig. 2, reducing silver ions into AgNPs using the aqueous extract of *A. littoralis* was observed by the change in the solution color after 7 days of incubation in the dark at room temperature from yellowish brown to deep brown due to stimulation of surface plasmon resonance (SPR) in Aelu-AgNPs. It typically takes several hours to complete the bioreduction process, which might explain why the color shift is happening so slowly at room temperature, when AgNPs develop and proliferate slowly [19]. The combination color changed because several secondary metabolites in the plant extract reduced Ag^{+} ions to Ag^{0}. Many prior studies, like [20] and [21], confirmed AgNPs by color change.

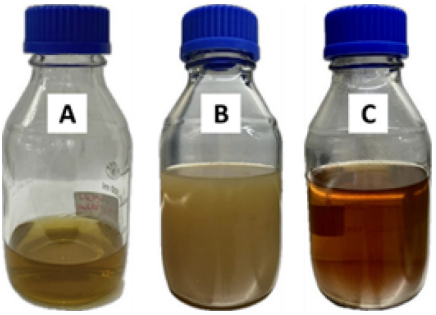

Fig. 2. *A. littoralis* aqueous extract (A), immediately after the addition of $AgNO_3$ (B), color shifting stability after 7 days of adding $AgNO_3$ (C)

Characterization of Aelu-AgNPs

The UV-Vis spectrophotometer analysis employs a simple, efficient and highly sensitive technique. Fig. 3 shows Aelu-AgNPs' UV-Vis absorption spectra, which mainly exhibit a singular narrow absorption peak at 410 nm. The SPR band originates from the closeness of the conduction and valence bands of silver nanoparticles, allowing collective electron oscillation in resonance when exposed to a light wave, thereby generating the SPR absorption band. Size and morphology affect nanoparticle absorption in dielectric medium [22]. According to [23], spherical AgNPs are generally indicated by the distinctive band at 400-420 nm. The present study concurs with several investigations that identified an absorption peak between 400-450 nm, including [24] study, which confirmed the SPR peak at 410 nm.

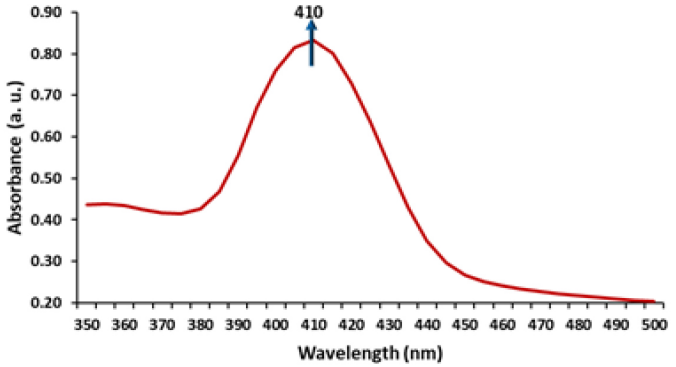

Fig. 3. Uv-Vis spectrum of the green synthesized Aelu-AgNPs utilizing *A. littoralis* aqueous extract

The FTIR spectroscopy was utilized to identify the biomolecules that may have reduced Ag^{+} to Ag^{0} nanoparticles. The spectra of the *A. littoralis* aerial parts extract and Aelu-AgNPs showed similarities, with minor shifts and alterations as seen in Fig. 4. The Aelu-AgNPs exhibited visible bands at 3324, 2316, 2110, 2024, 1957, 1637, 960, 597, and 514 cm^{-1}. The O-H stretching vibrations of the phenolic functional groups were responsible for the broad peak at 3324 cm^{-1}. The bands detected at 2110 cm^{-1} suggested the binding of Aelu-AgNPs with the carbon-carbon triple bonds stretching vibrations of alkyne functional groups. 2024 and 1957 cm^{-1} bands were suggested the binding of Aelu-AgNPs with the C-H bands of aromatic compound functional groups. The peak at 1637 cm^{-1} was a result of the alkene functional groups' C=C stretching. Findings from earlier research pointed the formation of AgNPs, capped with a variety of biomolecules, when observed bands in the spectra between 1700 and 1600 cm^{-1} [20; 25]. Thus, the extracted compound plays a role in biosynthesis, including its reducing, stabilizing, and capping functions.

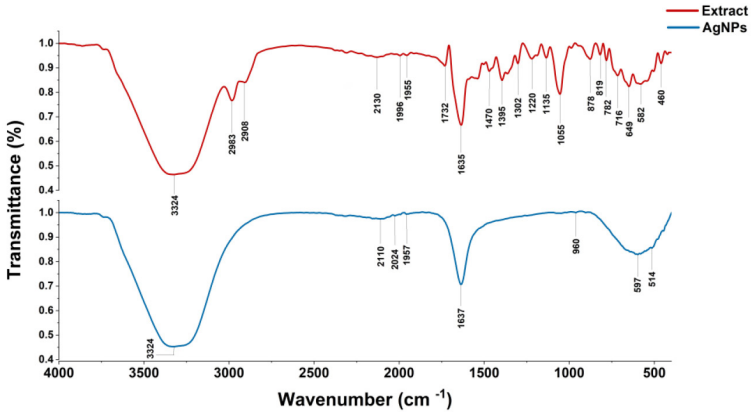

Fig. 4. The spectrum of FTIR for Aelu-AgNPs and *A. littoralis* aqueous extract

The synthesized Aelu-AgNPs were examined via XRD analysis to investigate their crystalline structure. The spectrum pattern in Fig. 5 shows that the main diffraction peaks at 2θ values of 28.14°, 32.52°, 46.64°, 55.22°, 57.82°, 67.79°, 74.95°, and 77.23° reflect 111, 200, 220, 311, 222, 400, 331, and 410 lattice planes of silver. These planes showed that the Aelu-AgNPs were crystalline in nature and had a face-centered cubic lattice (JCPDS file no 84-0713 and 04-0783). The current XRD findings consistent with those of earlier research [26; 27].

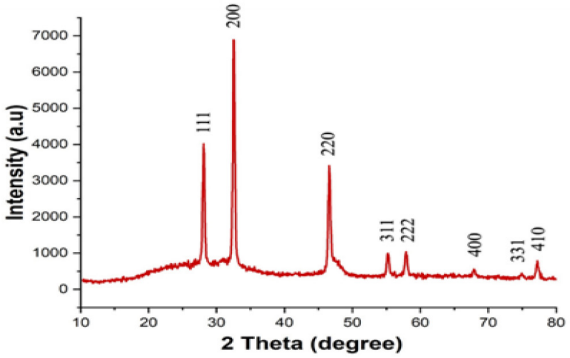

Fig. 5. The spectrum pattern of X-ray diffraction analysis of Aelu-AgNPs

The surface topography and morphology of synthesis Aelu-AgNPs were investigated by FESEM, which depicts that the shape of NPs was mostly spherical and semispherical. The sizes distribution of the synthesized Aelu-AgNPs were estimated under FESEM visualization using ImageJ software, the particle diameters ranging from 20 to 78 nm, averaging 41.43 ± 10.16 nm (Fig. 6). The biosynthesis silver nanoparticles' elemental composition was studied using EDX analysis (Fig. 7), which revealed the existence of silver element at 3 kiloelectron volt (KeV) due to SPR, with high weight percentage (76.51%), indicating excellent purity. while the appearance of other element such as chlorine and oxygen at low weight percentage of 22.05% and 1.31%, respectively may be due to organic biomolecule that capped on silver nanoparticles surface [28]. The shape, size, and chemical composition of the Aelu-AgNPs correspond with the range described in previous investigations [29; 30].

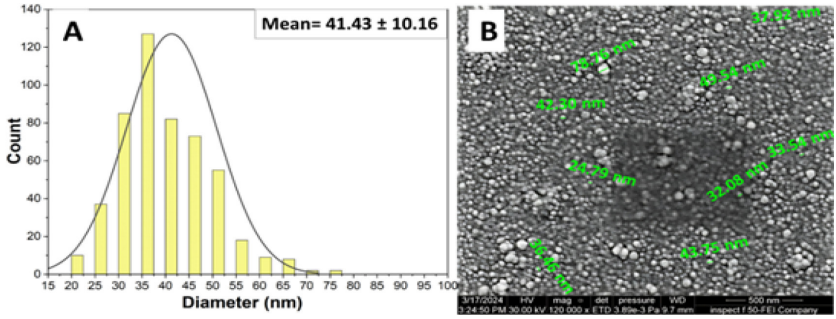

Fig. 6. The Aelu-AgNPs size distribution (A); FESEM image (B)

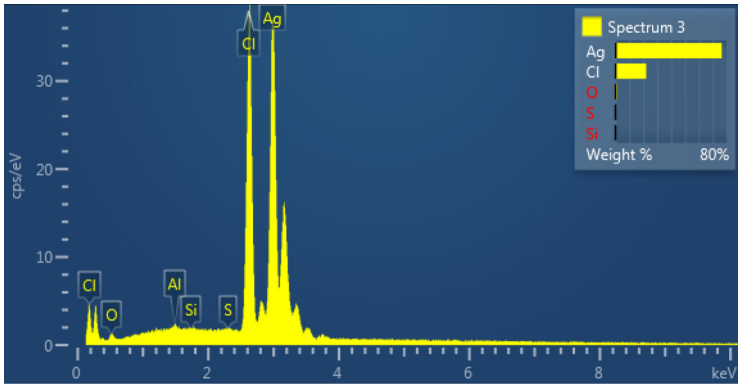

Fig. 7. The spectroscopy of EDX for Aelu-AgNPs

The zeta potential of the green synthesized Aelu-AgNPs was observed as a sharp peak at -40.4 mV (Fig. 8). The nanoparticles' surface seems to be negatively charged and distributed in the colloidal solution. Their strong stability is demonstrated by the negative value, which further supports the repulsion between the particles. Several studies have shown that silver nanoparticles are quite stable around -40 mV or below [31].

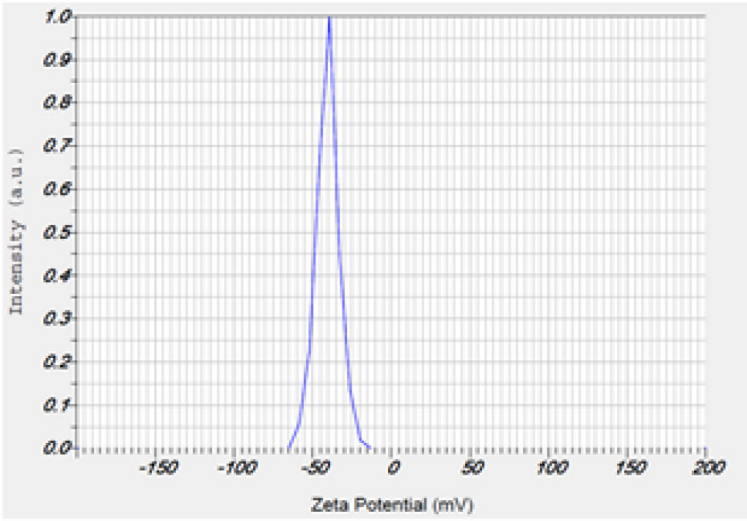

Fig. 8. The zeta potential of biosynthesized Aelu-AgNPs

Antibacterial activity of Aelu-AgNPs

The green synthesized Aelu-AgNPs were diluted in series through a 2-fold manner from 1000 to 62.5 µg/ml and investigated their effectiveness as antibacterial agents against twelve MDR pathogenic bacterial species, including *P. aerugenosa*, *S. aureus*, *P. mirabilis*, *E. coli*, *E. cloacae*, *S. haemolyticus, E. faecium*, *S. epidermidis*, *K. pneumoniae*, *S. hominis*, *C. freundii*, and *P. rettgeri*, utilizing the agar well diffusion method. *P. mirabilis* was most susceptible to the synthesized Aelu-AgNPs, with an inhibitory zone of 19.5 mm, followed by *S. hominis* at 18.9 mm, as illustrated in Table 2 and Fig. 9. In contrast, the least susceptible bacteria were *E. coli* and *E. cloacae*, with inhibitory zones of 15 mm and 15.2mm, respectively. The concentration of 250 µg/ml exhibited the most effective, with the 125 µg/ml concentration coming as a close second. Green synthesized Aelu-AgNPs showed remarkable antibacterial efficacy at various concentrations against all studied bacterial species [32] and [33] reported the efficient antibacterial properties of green synthesized AgNPs derived from the shoot extract of *Carthamus oxycantha* and the leaf extract of *Moringa oleifera*, respectively.

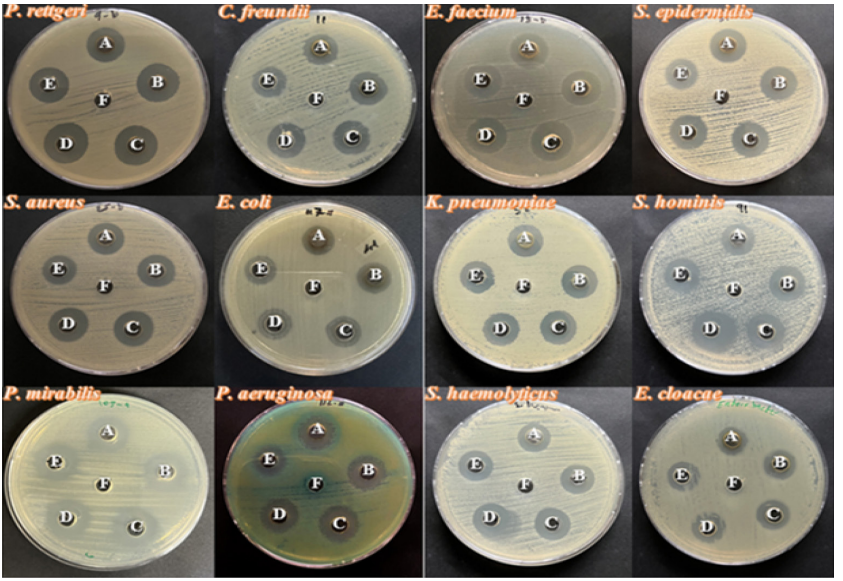

Fig. 9. The antibacterial effects of Aelu-AgNPs obtained from *A. littoralis* aqueous extract. The characters A, B, C, D, E, and F were represented by1000 µg/ml, 500 µg/ml, 250 µg/ml, 125 µg/ml, 62.5 µg/ml, and DMSO, respectively

Several bacterial strains were significantly inhibited by eco-friendly synthesized AgNPs. The unique effect of these nanoparticles may be due to their surface area is much larger than their size, which allows them to interact with bacterial cells more effectively, thus modifying plasma membrane permeability, leading to cell wall rupture, release of cellular contents, and subsequent cell death [34]. AgNPs with very tiny size and high kinetic energy facilitate their penetration into bacterial cells, causing internal alterations leading to the generation and accumulation of free radicals, consequently inducing intracellular oxidative stress and finally resulting in apoptosis. Furthermore, it restricts ATP generation and the functioning of respiratory chain enzymes by binding to their active sites, in addition to preventing mRNA transcription and DNA replication. The synthesis of protein molecules in cells can be impeded by their interaction with ribosomes [35; 36]. In contrast to the efficacy of antibiotics that target a single site within pathogenic bacteria, silver nanoparticles perform their effects on multiple sites at the same time, thereby preventing bacteria from developing resistance mechanisms to these nanoparticles.

Table 2.

Inhibition zone (mm) resulting from the antibacterial properties of Aelu-AgNPs synthesized by *A. littoralis* aqueous extract

Bacterial isolates	Concentrations of Aelu-AgNPs (µg/ml)					Average ($p \leq 0.05$)
P. aerigenosa	16.5 ± 0.98	16.9 ± 0.96	17.6 ± 0.35	17.3 ± 0.35	14.5 ± 0.40	$16.6 \pm 1.25^{a*}$
E. coli	14.9 ± 0.36	15.5 ± 1.12	15.2 ± 0.31	15.0 ± 0.71	14.7 ± 0.62	15.0 ± 0.29^{b}
K. pneumonia	17.4 ± 0.87	17.8 ± 0.32	17.8 ± 1.45	17.0 ± 0.79	15.3 ± 0.25	17.0 ± 1.04^{ac}
P. mirabilis	18.5 ± 0.70	20.4 ± 0.70	19.7 ± 0.89	19.9 ± 1.69	19.0 ± 0.70	19.5 ± 0.75^{d}
P. rettgeri	17.4± 0.96	18.7 ± 0.57	19.1 ± 0.36	19.8 ± 0.42	17.5 ± 0.97	18.5 ± 1.06^{e}
C. freundii	16.3 ± 0.91	17.2 ± 0.47	16.9 ± 0.42	18.0 ± 0.76	15.4 ± 1.07	16.8 ± 1.00^{acf}
E. cloacae	14.0 ± 0.65	14.8 ± 1.15	15.3 ± 0.60	15.5 ± 0.96	16.2 ± 0.80	15.2 ± 0.84^{b}
S. aureus	17.2 ± 1.01	17.6 ± 0.35	18.5 ± 0.75	17.7± 1.29	15.4 ± 0.78	17.3 ± 1.14^{acfg}
S. epidermidis	18.6 ± 0.36	19.2 ± 1.05	19.7 ± 0.99	20.0 ± 0.21	15.9 ± 0.76	18.7 ± 1.62^{eh}
S. hominis	18.7 ± 0.30	19.3 ± 0.91	20.1 ± 0.26	20.4 ± 0.85	15.8± 0.57	18.9 ± 1.78^{dehi}
S. haemolyticus	15.8 ± 0.65	16.5 ± 0.65	17.3 ± 0.61	17.3 ± 0.06	16.1 ± 0.15	16.7 ± 0.87^{acfg}
E. faecium	17.4 ± 0.67	17.8 ± 0.25	20.6 ± 0.91	19.0 ± 0.81	17.8 ± 0.72	18.5 ± 1.30^{chi}
Average ($p \leq 0.05$)	$16.9 \pm 1.47^{a*}$	17.6 ± 1.62^{ab}	18.2 ± 1.79^{bc}	18.1 ± 1.77^{bc}	16.1 ± 1.33	

* Identical letters indicate no significant differences

MIC and MBC

Resazurin-based microtiter dilution assays were performed to determine the MIC and MBC. which depend on the colorimetric shift of resazurin dye

(blue) to resorufin (pink or purple), referring to the existence of cells with metabolic activity within the sample (Table 3 and Fig. 10). The findings indicated that the MIC for all examined bacterial isolates varied from 62.5 to 7.8 μg/ml. *E. faecium*, *S. aureus*, and *S. haemolyticus* exhibited the highest MIC value at 62.5 μg/ml, whereas *P. rettgeri* and *P. mirabilis* demonstrated the lowest MIC at 7.8 μg/ml. Thereafter, we studied the MBC, which is defined as the least concentration that entirely prevents the bacterial growth on agar upon subculturing after each MIC test. The highest MBC was recoeded toward *K. pneumoniae*, *S. epidermidis*, *S. haemolyticus*, *S. aureus*, *E. faecium*, and *E. coli* at 62.5 μg/ml. In contrast, 15.6 μg/ml exhibited the least MBC against *P. rettgeri* and *P. mirabilis*. The MBC/MIC ratio for Aelu-AgNPs ranged from 1 to 2. An AgNPs are deemed as bactericidal whenever the MBC to MIC ratio is below 4, and as bacteriostatic long as it is above 4 [37]. Previous investigations have indicated differing MIC values for green-synthesized AgNPs against different pathogenic bacteria [38] revealed the MIC of biosynthesized AgNPs from *Areca catechu* extract against *P. aeruginosa* and *E. faecalis* was 5.6 and 11.25 μg/ml, respectively, while the MBC was 11.25 μg/ml against each one. In a separate investigation, the MIC of green formed AgNPs reduced by *Moringa oleifera* was 250 μg/ml, with a MBC of 500 μg/ml against *E. coli*. On the other hand, the MIC and MBC against *S. aureus* were 250 and 2000 μg/ml, respectively [39].

Table 3.

The values of MIC and MBC with the MIC/MBC ratio of biosynthesized Aelu-AgNPs from *A. littoralis* against tested pathogenic bacteria

	MIC (μg/ml)	MBC (μg/ml)	MBC/MIC
K. pneumoniae	31.25	62.5	2
C. freundii	15.6	31.25	2
S. epidermidis	31.25	62.5	2
E. faecium	62.5	62.5	1
E. cloacae	15.6	31.25	2
P. rettgeri	7.8	15.6	2
E. coli	31.25	62.5	2
S. hominis	15.6	31.25	2
P. mirabilis	7.8	15.6	2
P. aeruginosa	15.6	31.25	2
S. haemolyticus	62.5	62.5	1
S. aureus	62.5	62.5	1

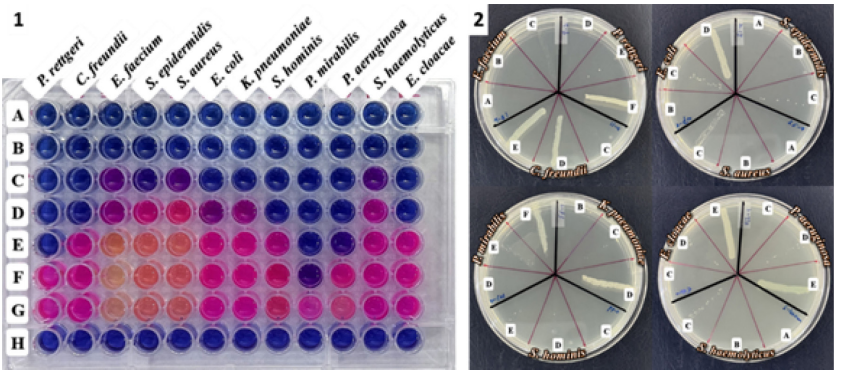

Fig. 10. The micro-titer plate of MIC test after incubation for 24 hours (1), The plates of MBC test after 24 hours of incubation (2), where A: (125 µg/ml), B: (62.5 µg/ml), C: (31.25 µg/ml), D: (15.6 µg/ml), E: (7.8 µg/ml), F: (3.9 µg/ml), G: (positive control), and H: (negative control)

Anticancer activity of Aelu-AgNPs

The MTT assay was used to evaluate the cytotoxic effect of silver nanoparticles at a various concentration (400, 200, 100, 50, 25 µg/ml) on the A375 as cancer cell line compared with HdFn as a normal cell line. After 24 hours of exposure to Aelu-AgNPs, a reduction in the viability of both cancer and normal cell lines was observed as the concentration of Aelu-AgNPs increased, with more cytotoxic effect against cancer cells compared to normal cells at all tested concentrations as shown in Fig. 11. The current study reported the IC_{50} of Aelu-AgNPs against A375 at 71.04 µg/ml, while in the normal cell line the IC_{50} was equal to 148.6 µg/ml, this variation in the IC_{50} value confirm the potential of anticancer activity of Aelu-AgNPs, it was observed that when the concentration was decreased, the most effective concentration of Aelu-AgNPs with lowest toxicity against normal cell was 50 µg/ml and 25 µg/ml with 6.8% and 5.2% inhibition respectively, while for cancer cell the inhibition for these concentration was 27.2% and 15.6% respectively.

The study found that IC_{50} value of AgNPs against A375 cell line was less than [40] result that recorded an IC_{50} of 121.7 µg/ml, while it was close to [41] study with 77.03 µg/ml. This variation between studies may be due to several factors, including the type of reduction agent, as well as nanoparticles characteristic including the stability, shape, and size, in addition to cell line used. The effect of AgNPs against A375 cell lines was reported by previous studies, including [42] that confirmed a decrease in cell membrane permeability and mi-

tochondrial membrane potential, which leads to an increase in cytochrome C in the cytosol, and thus activation of caspases which leads to cell death.

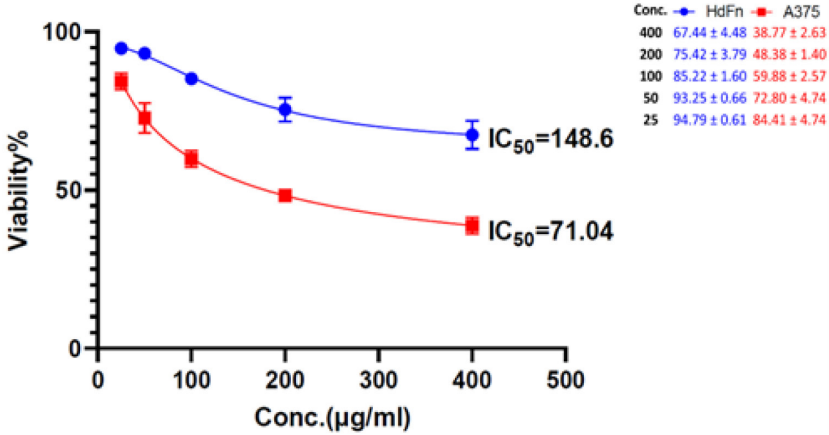

Fig. 11. The cytotoxicity of biosynthesized Aelu-AgNPs utilizing the MTT assay for HdFn and A375 cell lines with their IC_{50}

Antioxidant activity of Aelu-AgNPs

The activity of free radical scavenging of the green synthesized Aelu-AgNPs was evaluated by using DPPH assay, various concentration of Aelu-AgNPs (200, 100, 50, 25, 12.5 µg/ml) with ascorbic acid as a standard exhibited their ability for reduce a stable DPPH radical to DPPH-H as presented in Fig. 12.

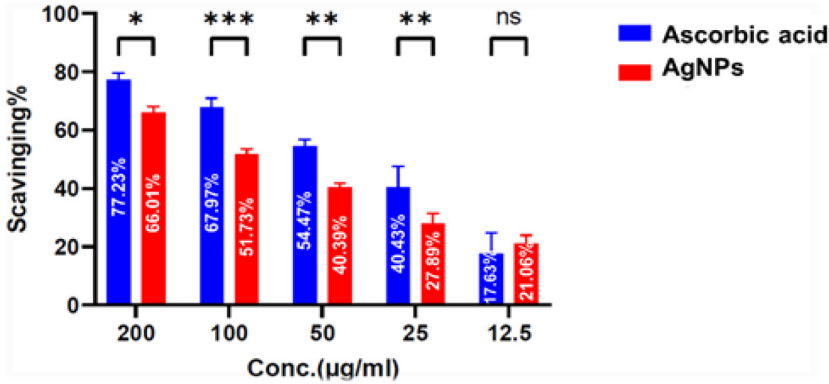

Fig. 12. The antioxidant activity of biosynthesized Aelu-AgNPs obtained from *A. littoralis* aqueous extract utilizing DPPH radical scavenging assay

The antioxidant activity of Aelu-AgNPs observed less than ascorbic acid in all concentration except for 12.5 μg/ml which was higher than a standard with no significant differences, the current investigation recorded the higher antioxidant activity of AgNPs as 66% at 200 μg/ml, at the same concentration this finding was similar to [43] study, which report the scavenging activity as 65.9%, and higher than [44] result with 50% inhibition. Scavenging activity of Aelu-AgNPs seems to be associated with the presence of biologically active compound such as phenols, which was suggested to be as capping and stabilizing agents by FTIR spectra in the current result.

Conclusion

This study found that *A. littoralis* aqueous extract has a considerable ability to produce AgNPs with spherical and semispherical shapes. The Aelu-AgNPs at various concentrations demonstrate high antibacterial activity against all examined pathogenic bacteria mentioned in this study. In comparison to normal cell lines, the green produced AgNPs exhibited a higher level of cytotoxicity against cancer cell lines. The antioxidant activity was found more effective at the lower concentration of biosynthesized Aelu-AgNPs compared to ascorbic acid. Consequently, the potential green synthesized AgNPs utilizing the *A. littoralis* aqueous extract are advantageous for biological applications, including antibacterial, anticancer, and antioxidant activity.

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