AMERICAN JOURNAL OF BIOLOGY AND NATURAL SCIENCES

https://biojournals.us/index.php/AJBNS

ISSN: 2997-7185

Article

Green Synthesis and Antimicrobial Effects of Silver Nanoparticles Using Seeds of *Plantago lanceolata*

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Citation: Al-Mothafer, S. M. A. Green Synthesis and Antimicrobial Effects of Silver Nanoparticles Using Seeds of Plantago lanceolata. American Journal of Biology and Natural Sciences 2026, 3(5), 121-132.

Received: 30th Mar 2026
Revised: 20th Apr 2026
Accepted: 05th May 2026
Published: 21st May 2026

Abstract: Nanotechnology has garnered the interest of researchers owing to the diminutive size and elevated surface-to-volume ratio of nanoparticles, resulting in alterations in chemical and physical properties. Green-synthesize silver nanoparticles (AgNPs) from the *Plantago lanceolata* plant and investigate their potential uses as antibacterial agents. Nanoparticles were constructed using P. c seeds extract by green synthesis method. The properties of nanoparticles were characterised using UV-vis, SEM, FTIR, and XRD then the antimicrobial activity and inhibition zone were measured using the diffusion method. The paper has managed to prepare silver nanoparticles (AgNPs) with the help of *Plantago lanceolata* seed extract, which has been proven by a color change to light yellow and a UK absorbance max at 432 nm. The SEM analysis showed the presence of nanoparticles of a spherical shape with 41.28 nm to 68.04 nm as the sizes, and XRD proved that they had a crystalline structure. Grameen AgNPs biosynthesized were also found to have high antibacterial activity against Gram-positive and Gram-negative bacteria. It is notable that the test with the greatest inhibition was obtained with Acinetobacter baumanii (22 mm) compared to the conventional antibiotic ampicillin which is considerably lower. These results show that AgNPs synthesized in green have a potential of being a powerful antimicrobial agent against multidrug-resistant microbes. The paper was able to show the green synthesis of silver nanoparticles (AgNPs) using *Plantago lanceolata* seed extract as a reducing agent which is both sustainable and environmentally friendly.

Keywords: AgNPs, *Plantago lanceolata,* Nanotechnology, Antibacterial Agents

Introduction

Nanotechnology is an emerging research field that has numerous uses in science and technology especially in the production of different nanomaterials and nanoparticles. Nanoparticles (NPs) are minute particles with a diameter between 1 nm and 100 nm in size that have been of significant interest to researchers due to their diverse use in different fields of science, such as biomedicine, catalysis, sensing, agriculture, pharmaceuticals, textiles, food technology, mechanics, electronics, and optics [1]. Silver nanoparticles are known to possess unique biological capabilities such as food preservation, drug delivery, wound healing, cosmetics, biomarking, sensing and water purification. It is also used in unusual applications in paints, electronics, textiles and catalysis. Medicinal herbs play a vital role because of the secondary metabolites that act as reducing and stabilizing agents of nanoparticles at nanoscale [2]. Various synthesis techniques are available to Ag nanoparticles in aqueous solutions, such as chemical, physical and biological. Both exhibit unique characteristics in terms of volume control, morphology, and scalability [3,4].

They are also employed as vectors to transport medicines and therapeutic substances to target locations to improve the efficacy of a treatment due to the size of the AgNPs that allow them to penetrate cells [5]. Moreover, due to their unique electrical and optical properties, they are used in detection systems and sensing [6]. In the measurement of low concentrations of target particles, such as heavy metals [7], biomarkers, and environmental contaminants [8]. They are flexible and therefore cannot be replaced in various product applications, such as water filtration systems [9], and antibacterial coatings [10]. According to recent studies, nanoparticles impact the entire spectrum of life, including bacteria and humans, and the metals that are used to manufacture them are detrimental to nature [11]. The use of natural products to solve the global health challenges is considerable. They have anti-inflammatory, antioxidant, antidepressant, anti-cancer, and antibacterial properties [12].

Silver nanoparticles are utilized to deliver medication, treat wounds, cancer, and inhibit the growth of microbial infections [13]. Silver nanoparticles (AgNPs), which are currently available in the market and present in various goods, offer numerous advantages, including antibacterial properties that eradicate bacteria or impede their proliferation [14]. Optimal mechanisms of AgNPs have been documented: silver ion release, cell membrane disruption, inhibition of DNA replication, and generation of reactive oxygen species (ROS). These mechanisms can surmount bacterial barriers, including forming robust polysaccharide walls that serve as physical impediments, diminished cellular metabolic activity, activation of efflux pumps, heterogeneous resistance within bacterial biofilms, and genetic adaptations [15,16]. *P. lanceolata* is used as an herbaceous plant in many countries due to its luxurious repertoire of active secondary metabolites. The plant can be used as a solution to a wide range of health problems, including dermatological disorders (abscesses and acne) [17].

Plants are also used in nanoparticle synthesis because of phytochemicals such as vitamins, proteins, polyphenols, sugars, organic acids and terpenoids that act as reducing agents and coating to composite nanoparticles according to particular shapes and sizes. Silver is given preference over other elements of nanoparticle biosynthesis due to its powerful angiogenesis, anti-inflammatory, antibacterial, and antifungal activities [18]. Multidrug-resistant bacteria represent a significant threat to global public health, as they are responsible for numerous life-threatening infectious illnesses. The prevalence of multidrug-resistant bacterial strains is steadily rising due to mutations, pollution, altered environmental conditions, and the overuse of pharmaceuticals. To address this issue, scientists are endeavoring to create novel pharmaceuticals for treating microbial diseases. Green chemical AgNPs have been beneficial in combating multidrug-resistant bacterial strains. This study aims to green-synthesize silver nanoparticles (AgNPs) from the *Plantago lanceolata* plant and investigate their potential uses as antibacterial agents.

Methodology

Plant collection

Plantago lanceolata was collected from a farm in the Safwan border region with Kuwait. The plant was presented to a professor of taxonomy at the College of Education for Pure Sciences, University of Basrah, who classified and confirmed its identity. The plant was washed with distilled water to remove dirt and then dried in the shade before being ground into a powder using a mechanical machine. The powder was stored in a refrigerator at 4°C until use.

Preparation of an aqueous extract of *Plantago lanceolata* seeds

1 gram of the plant powder (**Figure 1**) was weighed and dissolved in 100 ml of distilled water (10 mg/ml). The solution was placed in a water bath for 10 minutes, after which the solution was filtered by a filtering device to remove impurities. The aqueous extract was kept in the refrigerator at 4°C until its nanoparticles were prepared [19].

Figure 1. *Plantago lanceolata* seeds

Preparation of silver nanoparticles from *Plantago lanceolata* seeds

Ten millilitres of the aqueous extract were mixed with ninety millilitres of a two millimolar aqueous solution of silver nitrate ($AgNO_3$). This aqueous solution was formulated by dissolving 0.39 g of silver nitrate in one liter of deionized distilled water to create silver nanoparticles to reduce Ag^+ ions. The mixture was maintained slightly above room temperature 30 °C for 4 hours to facilitate complex formation. Ultimately, the transition to a pale-yellow hue commenced, signifying the synthesis of nanoparticles [20]. A centrifuge was used to precipitate the nano solution, then distilled water was added to the precipitate and the process was repeated three times to prepare for the tests of AgNps characterization. The plant extract, silver nitrate ($AgNO_3$) and McFarland solution were prepared using deionized distilled water.

AgNps characterization

UV–vis spectrum

The UV-visible spectrum was obtained via a UV-1800 model from Shimadzu Corporation, Japan. Continuous scanning within the wavelength range of 200–800 nm was employed for analysis, and the spectra of green silver nanoparticles have been compared with the control [21].

Scanning Electron Microscope (SEM)

We employed scanning electron microscopy to enhance the analysis and examination of the morphological characteristics of the synthesized nanoparticles. To achieve high-quality photos, the material was thoroughly desiccated. The drying procedure involved; dissolving 0.01 g of green nanoparticles in acetone, subjecting the sample to an ultrasonic bath, and maintaining it in an oven at 40 °C for 8 hours. A thin layer of gold was added to the nanoparticle powder to improve surface conductivity, and scanning electron microscopy was used to examine the structure. The silver nanoparticle powder had to be placed on an aluminium conductor [22].

X-ray diffraction (XRD)

The crystallite size of silver nanoparticles was ascertained using X-ray diffraction at angles of 78.2°, 64.4°, 44.3°, and 38.1° (2θ), which correspond to the peaks (111), (200), (220), and (311). The full width at half maximum intensity (FWHM) was determined using the subsequent method Basalius et al., [23]:

$$D = (K\lambda) / (\beta \cos \theta).$$

D represents the crystallite size, β denotes the peak width at half maximum intensity, θ symbolizes the Bragg angle of the peak, λ indicates the X-ray wavelength, and K is a constant value of 0.9.

Fourier transform infra-red (FTIR)

The purified AgNPs and plant extract were analyzed for several phytochemicals using a Fourier transform infrared (FT-IR) Shimadzu (IR Prestige-21) spectrometer from Japan.

Antibacterial activity

A diffusion assay against microorganisms evaluated the antibacterial efficacy of silver nanoparticles. Bacterial colonies were streaked onto Mueller-Hinton agar plates, andthen 6 mm holes were made and filled with plant extract and silver nanoparticles by placing 50 μl of the solutions and incubated at 37 °C for 18 hours. Various degrees of inhibitory zones were quantified [24]. The antibacterial efficacy of the optimized stable colloidal solution of AS-AgNPs was evaluated using the agar disc diffusion method. Notably, Ampicillin (0.1 μg/mL) was used as the positive control, and the aqueous AS-extract functioned as the negative control, demonstrating the scientific method employed in the study. The investigation employed four bacterial strains, two Gram-negative and two Gram-positive bacteria, to evaluate the antibacterial effectiveness of the biosynthesized AS-AgNPs.

Statistical Analysis

The Statistical Package for the Social Sciences (SPSS) application (2019) was used to identify the impact of various factors on study parameters. In the present study, the least significant difference (LSD) was employed to compare means and standard deviations significantly (ANOVA/One-way: probability 0.05).

Results and Discussion

Synthesis of AgNPs

This binding is contingent upon the nanoparticles' composition, shape, and absorbency. Nanoparticles are utilized in environmental applications in three distinct manners: primarily, producing sustainable products via green synthesis to mitigate pollution [25], second, the bioremediation of pollutants in the environment [26], and third, serving as sensors to detect alterations in ambient conditions [27,28]. This study demonstrates that adding plant extract to the aqueous solution of silver ion complex resulted in a color transition from translucent to light yellow, indicative of silver ion reduction and the creation of AgNPs, as illustrated in the (**Figure 2. A & B**).

Figure 2. A. Silver nanoparticles of *Plantago lanceolata* seeds extract after 1 hour, B. Silver nanoparticles of *Plantago lanceolata* seeds extract after 4 hours

A change in the color of the solution that is visible is usually a sign of the formation of silver nanoparticles. Although this needs confirmation with further testing, the change in color is one of the first signs of the presence of silver nanoparticles, and the results of the study are in line with the outcomes of the study Sukweenadhi et al., [29]. The results of the study are consistent with a prior study Shah et al., on the changes in color during the production of silver nanoparticles using the *Plantago lanceolata* extract [30]. This research finding is consistent with that of the study Ahmed et al., which shows that the color of the solution changed to dark yellow with the addition of different amounts of the plant extract indicating the formation of silver nanoparticles [31].

Characterization of AgNPs

UV–vis spectrum

Surface plasmon resonance (SPR) is a phenomenon of metals that can be measured by a spectrophotometer of UV Vi. The solution of Pl-Ag NPs showed the highest absorbance at 432 nm in the case of *P. lanceolata* extract, as shown in (**Figure 3**), the search result Shah et al., is the same [30]. The surface plasmon resonance (SPR) absorbance peak of silver nanoparticles is at 400450 nm [32,33]. Additional investigations suggest that the peak near 420 nm aligns with the surface plasmon resonance of silver nanoparticles [34,35]. SPR shifting may occur due to an augmentation in particle size and plant extract. The silver nanoparticles obtained from *Passiflora caerulea* extract exhibit a peak at approximately 379 mm [36].

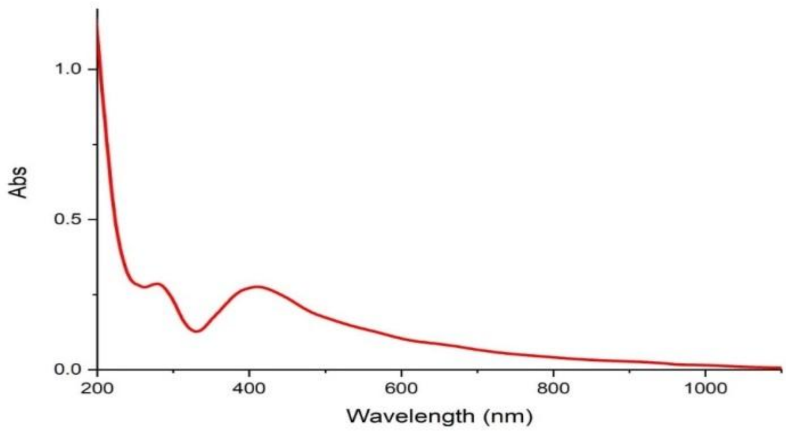

Figure 3. UV-vis of Pl-Ag NPs

Scanning Electron Microscope (SEM)

Scanning Electron Microscopy (SEM) was employed to characterize the morphology of the synthesized silver nanoparticles (Ag NPs). This study utilized SEM microscopy on a dried solid CP-AgNPs sample. The SEM pictures of PL-AgNPs (**Figure 4**) reveal that the synthesized MNPs are spherical, with sizes ranging from 41.28 nm to 68.04 nm.

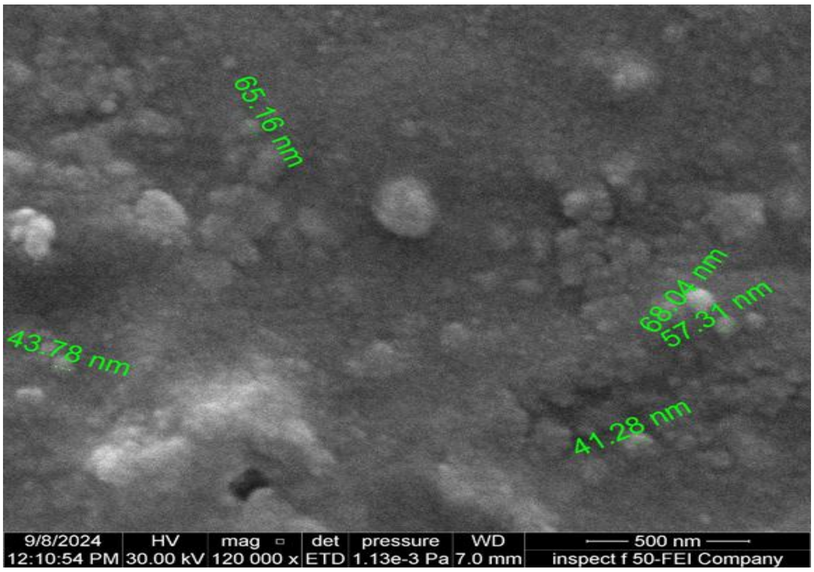

Figure 4. SEM of PL AgNPs

The results concur with the work referenced in Shah *et al*., [30], SEM analysis revealed a spherical morphology with an average size of 30 ± 4 nm, but some particles exhibited either larger or smaller dimensions. The results agree with Sukweenadhi *et al*., study which concluded that the Ag Nps using *Plantago major* leaf extract have a spherical shape and (size 10-20 Spherical) have an effect toward the pathogens *S. aureus, E. coli*, *P. aeruginosa* [29]. The bottom-up approach typically generates particles [37,38]. Another study supports this conclusion, indicating that surface plasma resonance (SPR) in the 400–450 nm range implies that the particles exhibited a spherical morphology [39]. Lohrasbi *et al*., research details the synthesis of (IONPs) utilizing an aqueous leaf extract of *Plantago* major, demonstrating a straightforward and environmentally acceptable process [40]. Synthesized nanoparticles exhibited a spherical morphology with sizes ranging from 4.6 to 30.6 nm, consistent with current studies on nanoparticle size.

X-ray diffraction (XRD)

The XRD pattern exhibits a pronounced peak across the spectrum of 2θ values from 20° to 40°, confirming the presence of silver constituents in the synthesized nanoparticles (**Figure 5**). This is consistent with the findings of a study Sukweenadhi *et al*., which investigated silver nanoparticles utilizing an ethanol extract of *Plantago* central L. leaf extract [29]. A supplementary report corroborates this finding. The study by Shah *et al*., focused on silver nanoparticles synthesized with *Plantago lanceolata* extract, concluding that the pronounced intensity of peaks indicated the high crystallinity of the AgNPs [30]. Importantly, the primary component of the Ag. NPs were confirmed to be Ag metal. The findings of Ahmed *et al*., do not align with the results of the study [31]. The XRD study of AgNPs reveals four unique peaks, confirming their crystalline structure. Comparable findings were also documented by Sher *et al*. [41], and Bar *et al*., [42]. The four distinctive peaks are characteristic of the face-centered cubic configuration of silver [43].

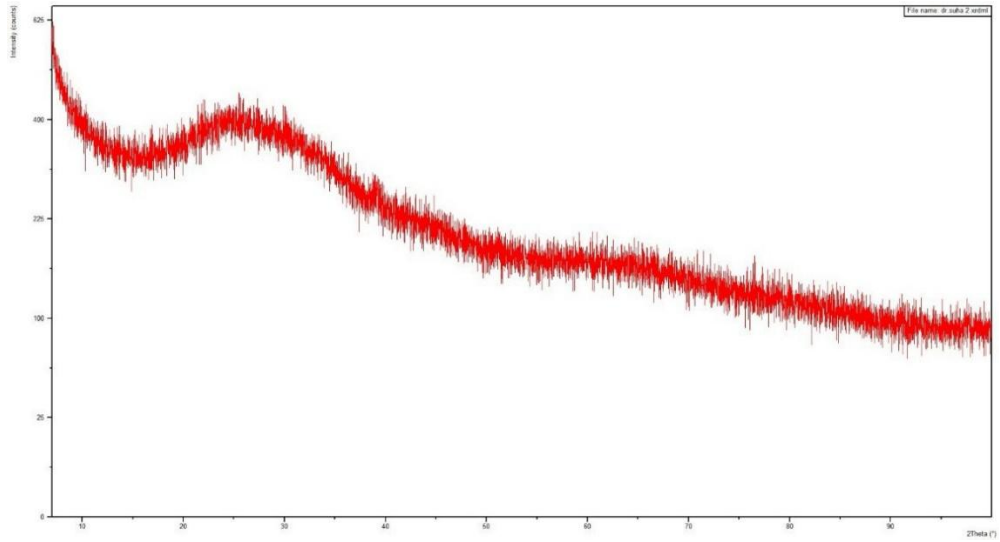

Figure 5. XRD of Pl-Ag NPs

Fourier transform infra-red (FTIR)

FTIR analysis was employed to identify the functional groups in the *P. lanceolata* seed extract and to quantify the quantity of silver nanoparticles (**Figure 6**).

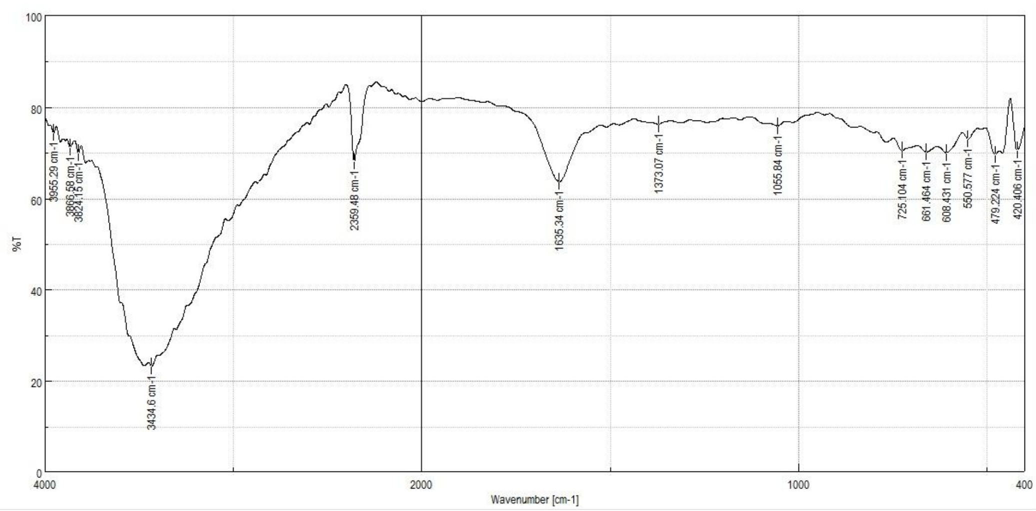

Figure 6. FTIR analysis of *P. lanceolata* seed extract

FTIR analysis was employed to identify the functional groups in the *P. lanceolata* seed extract and to assess the yield of silver nanoparticles (**Figure 2, b**). The vibration band at 3434.6 cm^{-1} is attributed to the O–H stretch, showing an enhancement of hydrogen bonding [44]. The band at 1635.34 cm^{-1} corresponds to C=O stretching, widely observed in proteins [45]. Another specific bond 1373.07 cm^{-1} corresponds to the phenol group vibrations are C–OH deformation vibration. The band is 2359.48 cm-1 due to Nitriles so it contains the C-N group. The peak at 1055.84 cm^{-1} is ascribed to the vibrations of C-O, C-O-C glycosidic, and C-O-H bands. The peak 550.577 showed the exitance of C-H deformation. This implies that these groups significantly reduce Ag^{+} and possess a considerable affinity for binding with AgNPs. The findings of this study align with those of the study Sukweenadhi *et al*., which similarly indicates the potential involvement of phenol, proteins [29], and alcohol in the green synthesis of silver nanoparticles (AgNPs) [46]. This corresponds with the previous report of the detection of Pm-Ag NPs peak during FTIR analysis, with the expected compound. These plant metabolites serve as stabilizing and capping agents for synthesized silver nanoparticles [34,47].

The plant *P. lanceolata* comprises tannins, phenolic acid, anthraquinone, terpenes, glycosides, sugar reducers, saponins, emodin, flavonoids, and Phlorotannin, all of which are crucial to producing AgNPs [48], corroborating the findings of the study with Shah *et al.,* [30]. Phytocomponents play a significant role in stabilizing nanoparticles in plant-mediated production of nanoparticles, which is essential for their functional qualities. These findings also align with the reports of Ahmed *et al*., [31].

Antibacterial effects

The antibacterial efficacy of silver nanoparticles produced from the natural extract of *P. lanceolata* against five pathogenic pathogens was assessed using the disc diffusion method on Mueller-Hinton agar plates. The diameter of the inhibitory zones (in mm) surrounding each hole filled with the plant extract and nano solution was quantified, as illustrated in (Table 1).

Table 1. The standard antibiotic ampicillin (0.1 μg/mL) was a positive control, while the aqueous AS extract acted as a negative control

Type of Bacteria	Mean ±SD		
	Bacteria source	**PL-AgNPs (mm)**	**Ampicillin (0.1 μg/mL) (mm)**
Klebsiella pneumonia	Wound	17 ±0.58 bc	8 ±0.35 c
Acinetobacter baumanii	Sputum	22 ±1.06 a	9 ±0.42 bc
Pseudomonas aeruginosa	Sputum	19 ±0.74 b	11 ±0.57 a
Escherichia coli	Urine	17 ±0.58 bc	10 ±0.54 ab
Staphylococcus aureus	Urine	15 ±0.42 c	9 ±0.42 bc
LSD value	--	2.726 *	1.461 *

* Means having with the different letters in same column differed significantly. (P≤0.05).

The mechanism behind the antibacterial action of silver nanoparticles (AgNPs) remains ambiguous to this day. Silver nanoparticles (AgNPs) can generate superoxide radicals or reactive oxygen species (ROS) and hydroxyl ions, leading to rapid microbial mortality. The action involves breaking the cell membrane, generating reactive oxygen species (ROS), penetration of the membrane, and subsequent binding to DNA and proteins. The antibacterial action of AgNPs likely arises from their surface charge, which adheres to negatively charged bacteria [49]. Our investigation indicates that Gram (-) bacteria exhibit more susceptibility to PL-AgNPs than Gram (+) bacteria (Table 1), corroborating the findings with the study of Sukweenadhi *et al*., which investigated the antibacterial effects of nanoparticles derived from the leaves of *Plantago* major L [29]. This results from the structural variations in the cell membrane and cell wall of Gram-positive and Gram-negative bacteria [50]. The results from Ahmed *et al*., found that AgNPs and plant extract exhibited antibacterial activity at various concentrations (20, 40, 80, and 100 μg/ml), corroborating recent findings [31].

The AgNPs synthesized with *Plantago lanceolata* extract showed substantial efficacy against all four selected bacterial strains: *Proteus vulgaris, Agrobacterium tumefaciens, Escherichia coli, and Staphylococcus aureus,* corroborating recent findings. The findings indicated that the silver nanoparticles produced by PL-AgNPs had the most significant antibacterial efficacy against *A. baumanii*. The minimal antibacterial efficacy of the silver nanoparticles produced by PL-AgNPs was seen against *K. pneumoniae*. Plant extract functioned as the negative control, whereas ciprofloxacin (1 g/ml) acted as the positive control. The study of Sharifi-Rad *et al*., indicates that silver nanoparticles are highly efficient against *Escherichia coli*, *Shigella flexneri, and Staphylococcus aureus, Bacillus cereus,* corroborating the findings of the present investigation [51].

Conclusions

This study successfully demonstrated the green synthesis of silver nanoparticles (AgNPs) using *Plantago lanceolata* seed extract as a reducing agent that is sustainable and environmentally friendly. This paper demonstrate the effectiveness of plant-mediated synthesis as a safe and cost-efficient substitute to traditional physical and chemical procedures. Cytotoxicity studies should be done thoroughly to ascertain the safety of these nanoparticles in human clinical uses. The synergistic effects of these AgNPs and traditional antibiotics should be investigated further in order to overcome multiple drug-resistant (MDR) bacterial strains. Also, the outcomes are the basis of the creation of highly developed antimicrobial coatings and water purification systems.

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