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Eco-friendly Synthesis of Silver Nanoparticles from *Allium carolinianum* and Evaluation of their Antibacterial and Antioxidant properties

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Abstract
This work presents an inexpensive, environmentally safe approach to the synthesis of silver nanoparticles using the methanolic leaf extract of *Allium carolinianum*. In this method, the plant extract served as both a reducing and capping agent, facilitating particle formation and stabilization. A distinct UV-Vis absorption peak at 435nm confirms the successful formation of AgNPs. FTIR spectroscopy further established that several functional groups in the extract were directly involved in the reduction and stabilization of silver ions, while SEM and TEM images revealed that the nanoparticles were mainly spherical and cubic, with a size range of 30nm to 90nm. XRD analysis showed diffraction patterns consistent with a face-centered cubic crystalline structure (fcc), while EDX spectra verified silver as the major elemental component. The synthesized silver nanoparticles (AgNPs) displayed potent antibacterial performance, with *Escherichia coli* showing the largest inhibition zone (31.67±1.25mm at 40mg/ml. They also exhibited notable antioxidant potential, with DPPH radical scavenging activity of 62.33% at 100 µg/mL. Overall, AgNPs derived from *A. carolinianum* demonstrated meaningful antibacterial and antioxidant properties, highlighting their promise for biomedical and pharmaceutical applications.

Keywords: Ag NPs, *Allium carolinianum*, SEM, EDX, antimicrobial activity, DPPH assay

1. Introduction

Nanotechnology is a versatile science, engineering, and technology that combines and manipulates materials at the nanoscale (1-100nm) [1]. It is widely used across fields such as chemistry, catalysis, materials science, energy, food science, biomedical applications, and electronics [2]. The introduction of antibiotics was a significant breakthrough in contemporary medicine [3]. But their overuse and misuse have led to the development of antimicrobial resistance (AMR), which is now a severe global health hazard [4]. The selective pressure exerted on microbial populations by the persistence of antibiotic residues in the environment favors the horizontal dissemination of antibiotic resistance genes. It accelerates the spread of resistant strains [5].

Furthermore, biomedical waste is not easily degraded by natural processes and thus poses threats to environmental and human health, as well as contributing to the spread of antimicrobial resistance (AMR)[6]. The solution to multidrug-resistant bacteria lies in increased demand for sustainable antimicrobial agents that combine natural bioactive compounds with high-tech technologies [7, 8]. The most recent breakthroughs in nanotechnology have enabled the creation of Speciale nanoparticles (NPs) with improved antimicrobial, antifungal, and antiviral efficacy [9]. Nanoparticles are effective antimicrobial agents, but can also be used as delivery vehicles for herbal as well as pharmaceutical formulations [10]. Among the different methods of synthesizing nanoparticles (NPs), green synthesis has been established as a safer, cost-effective, green, and sustainable alternative to chemical and physical methods [11]. Green nanotechnology involves the use of plant extracts and microorganisms as reducing and stabilizing agents, providing an environmentally responsible pathway for producing nanomaterials [12]. Among the most extensively researched nanomaterials are silver nanoparticles, which possess remarkable electrical conductivity, thermal stability, catalytic activity, and potent antimicrobial properties [13, 14]. With the help of biological agents like bacteria, fungi, and algae, and plant extracts that possess a variety of biomolecules, the functionality, size, shape, and surface properties of nanoparticles can be altered [15, 16]. The effective new production of AgNPs with different plant-based extracts, including those from grape and orange waste [17], shikakai and reetha extracts [18], jatropha latex[19], and *Hagenia abyssinica* [17]. These extracts contain phytochemicals, including phenols, alkaloids, flavonoids, and terpenoids, that serve as natural reducing, stabilizing, and capping agents during nanoparticle (NP) synthesis [18].

Allium carolinianum, also known as the sprout alpine onion, is a high-altitude species and a member of the Amaryllidaceae family, which extends from western China to Pakistan[22]. Allium species contain various edible parts of the plant, including the flowers, leaves, roots, and bulbs. They are rich in bioactive compounds, including sulfur-containing compounds, alkaloids, flavonoids, and saponins, all of which play essential roles in

human health and immunity [19]. These plants also contain cysteine sulfoxides, secondary metabolites derived from cysteine, which contribute to their therapeutic properties[20]. The *Allium* species have been linked to several pharmacological effects such as prevention of diabetes, cancer, cardiovascular diseases, inflammation, and liver disorders[21]. The fact that *Allium carolinianum* (*A. carolinianum*) leaf extract has a variety of biologically active compounds like phenolic compounds, flavonoids, terpenoids, and alkaloids, which facilitate the reduction of silver ions into metallic silver nanoparticles without any use of harmful chemical reagents[22-24]. In the current study, silver nanoparticles (AgNPs) were synthesized using *Allium carolinianum* leaf extract through a green synthesis approach. This environmentally friendly and sustainable method provides an efficient pathway for nanoparticle production using natural materials as precursors.

2.0. Materials and Methods

2.1 Chemicals and reagents

All chemicals used in this study were of analytical grade and were purchased from Sigma-Aldrich (USA) without further purification. The primary reagents included silver nitrate ($AgNO_3$), methanol, sulfuric acid (H_2SO_4\), potassium hydroxide (KOH), and chloroform ($CHCl_3$). Double-distilled water was used throughout all experimental procedures to ensure purity and accuracy of the reactions.

2.1. Collection and preparation of plant materials

Fresh *A. carolinianum* leaves (8kg) were collected during the flowering season from the Shangla district**,** Khyber Pakhtunkhwa, Pakistan. Botanical identification was conducted at the Department of Botany, University of Swat, and a voucher specimen was deposited for future reference. The collected leaves were thoroughly rinsed with tap water, then with distilled water, to remove dust, debris, and surface impurities. The cleaned leaves were shade-dried at room temperature to prevent the loss of thermolabile compounds and subsequently ground into a fine powder using an electric grinder [25].

2.2. Preparation of methanolic extract

The powder plant materials were macerated in methanol, an eco-friendly solvent. 1kg of powder leaves were soaked in methanol for 15 days with occasional shaking to enhance extraction efficiency[26]. The solution was passed through Watman No. 1 filter paper, and the resulting filtrate was concentrated under reduced pressure using a rotary evaporator operated at (80ºC, 85-95 rpm), yielding a thick gummy crude extract. The extract was stored at 4ºC until further use [27].

2.3. Fractionation of crude alkaloids

A portion of the methanolic extract was dissolved in 2.0 L of 0.5 N H_2SO_4 and agitated for one hour to obtain an acidic aqueous fraction with a (pH of 1-2). This fraction was subjected to three successive extractions with 2.0 L of chloroform to eliminate non-alkaloid components. The chloroform layer was concentrated using a rotary evaporator to yield a non-alkaloid acidic fraction, which tested negative for alkaloids using Dragendorff's reagent. The remaining aqueous phase was basified to pH 8-10 using a 10% KOH solution and continuously extracted with chloroform until the aqueous layer became clear. The combined organic layers were concentrated to obtain the basic alkaloid fraction, which tested positive with Dragendorff's reagent [25]. Finally, the residual aqueous phase was neutralized to pH 7 using 0.5 N H_2SO_4 and stored for subsequent use.

2.4. Biosynthesis AgNPs

Initially, a Silver Nitrate ($AgNO_3$) solution was prepared by dissolving 169.87 mg of silver nitrate in 100.0 mL of distilled water, then storing it at room temperature to form a 10.0 mM solution. A crude alkaloid solution was prepared by dissolving 250 mg of Allium carolinianum crude alkaloid extract in 500.0 mL of distilled water, followed by thorough mixing with an electric shaker. Silver nanoparticles (AgNPs) were synthesized according to a reported green synthesis protocol, with minor modifications to optimize reaction conditions [25]. Briefly, 5.0mL of $AgNO_3$ solution was transferred into a clean beaker. Subsequently, 5.0mL of the crude alkaloid extract was added, and the pH was adjusted to approximately 8 using a calibrated pH meter. The solution was heated and stirred simultaneously at 40ºC for 40 minutes; a color change was observed, indicating the successful synthesis of AgNPs. The reaction mixture was centrifuged at 15000 rpm for 15 minutes, and the resulting pellet was washed with distilled water to eliminate the unbound phytochemicals. The effect of pH on nanoparticle formation was also investigated by conducting parallel reactions under varying pH conditions.

2.5. Characterization of synthesized AgNPs

The synthesized AgNPs were characterized using multiple analytical techniques such as UV-Visible Spectroscopic Analysis where the optical characteristics were analyzed (300-500 nm) employing a Perkin Elmer UV-Vis spectrometer (USA) with the plant extract serving as the blank reference. Transmission electron microscopy (TEM) was used to obtain high-resolution images with a JEM-2100 microscope (JEOL Japan) operated at 200 kV. X-ray Diffraction (XRD) was applied to determine the crystallinity using Cu kα radiation at 40 KV. Additionally, Fourier Transform Infrared Spectroscopy was used to determine functional groups using a Cary 630 FTIR spectrometer (Agilent Technologies, USA).

Elemental composition was determined using Energy-dispersive X-ray Spectroscopy (EDX), which confirmed the presence of silver peaks corresponding to AgNPs. While morphological features of the synthesized NPs were examined through Scanning Electron Microscopy (SEM) using JSM-5910 SEM (JEOL Japan) operating at 30Kv.

2.6. Antibacterial Assay

Eight bacterial strains *Salmonella typhi, Klebsiella pneumonia, Bacillus subtilis, Escherichia coli*, *Pseudomonas aeruginosa*, *Acinetobacter, Bacillus anthracis and Clostridium difficile* were selected for antibacterial evaluation. The strains were cultured in modified growth medium at 37ºC. The composition of this medium (per liter) was as follows: sucrose (10g), glycerol (2g), soybean meal (15g), yeast extract (8g), and anhydrous sodium carbonate (1.8g). Prior to testing, all bacterial strains were subcultured onto fresh agar plates and incubated at 37ºC for 24 hours.

2.6.1. Measurement of minimum inhibitory concentration (MIC)

The minimum inhibitory concentration (MIC) of the synthesized silver nanoparticles (AgNPs) was determined using the broth dilution method. Bacterial cultures were adjusted to a final concentration of 5×10^6 CFU/mL. A four-fold serial dilution of AgNPs ranging from 40 to 10 µg/mL was prepared using DMSO as the solvent. Each well of a 96-well microplate contained 50 µL of bacterial suspension and 50 µL of the corresponding AgNP dilution**,** giving a final volume of 100 µL per well.

A positive control containing bacterial suspension without AgNPs and a negative control containing only broth and AgNPs were included. A solvent control (DMSO without AgNPs) was also used. Chloramphenicol was employed as a reference standard antibiotic. The plates were incubated at 37°C for 24hr, and the MIC was defined as the lowest concentration of AgNPs that completely inhibited visible bacterial growth[28].

2.6.2. The DPPH radical scavenging Assay

The antioxidant potential of the synthesized AgNPs was evaluated using the 1,1-diphenyl-2-picrylhydrazyl (DPPH) radical scavenging method [29]. A 1.0mM DPPH solution was prepared in absolute ethanol and mixed with varying concentrations of AgNPs (10, 15, 25, 50, and 100 µg/mL). Ascorbic acid was employed as a reference control. The mixture was vortexed thoroughly and incubated in the dark for 30 minutes to allow interaction between the DPPH radicals and the samples. All experiments were performed in triplicate to ensure accuracy and reproducibility. Absorbance (RSA) was measured at 517nm using UV- UV-Vis spectrometer. The percentage of radical inhibition was evaluated using the subsequent equation:

$$RSA\ (\%) = \frac{Abs\ of\ contol - Abs\ of\ sample}{Abs\ of\ conrol} \times 100$$

3.0. Results and Discussion

Proper characterization is essential for nanoparticles because these properties directly influence a nanoparticle's stability, reactivity, and performance in scientific, industrial, and biomedical applications. The characterization of biosynthesized Ag nanoparticles was conducted to measure and analyze their key physical and chemical properties at the nanoscale. It typically involves determining parameters such as size, morphology, surface charge, composition, and crystallinity using techniques like Scan electron microscopy, spectroscopy, and dynamic light scattering.

3.1. UV-Vis spectroscopic analysis

The synthesis of AgNPs was monitored using UV-visible spectroscopy to optimize the reaction conditions. The Spectra was recorded after 40 minutes of reaction between silver ions (Ag^+) and the crude leaf extract of *Allium carolinianum* within a wavelength range of 300-700nm. A distinct surface plasmon resonance (SPR) absorption peak was observed at 434nm (Fig. 1), consistent with previous reports [30, 31].

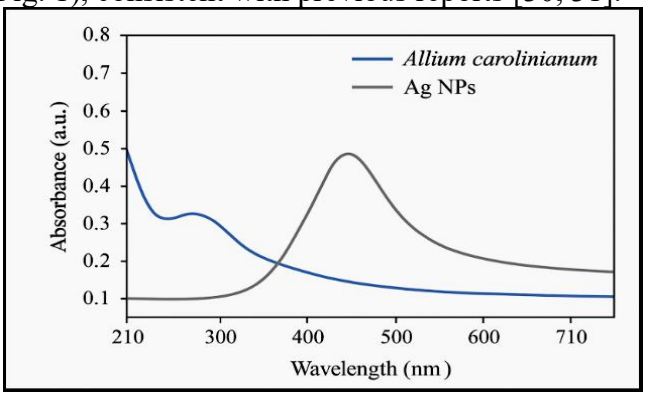

Figure (1): UV-Visible spectra of silver nanoparticles using Allium carolinianum leaf extract.

3.2. Stability Studies

The stability of the synthesized silver nanoparticles (AgNPs) was assessed under varying pH and temperature conditions. The results showed that the AgNPs exhibited maximum stability within the temperature range 25ºC to 45ºC, maintaining consistent color intensity and absorbance profile. At neutral (7) pH, nanoparticle formation was minimal; however, synthesis efficiency increased progressively with increasing alkalinity, reaching an optimum at pH 10. The enhanced synthesis and stability observed under alkaline conditions can be attributed to the increased availability of hydroxyl ions, which facilitate the reduction of Ag^{+} to Ag^{o} and improve nanoparticle dispersion. The findings confirmed the successful and stable formation of AgNPs under moderate thermal and alkaline conditions a shown in (Fig.2a & 2b).

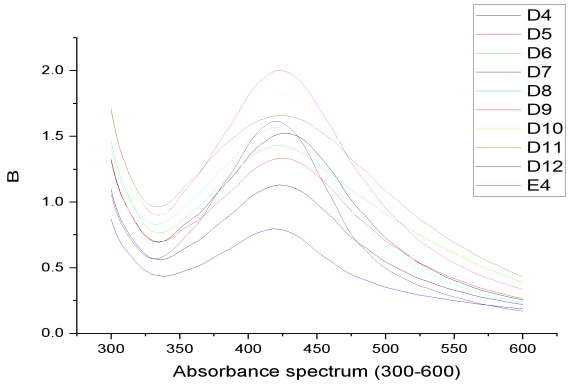

Figure (2a): Effect of and temperature on the formation and stability of biosynthesized AgNPs

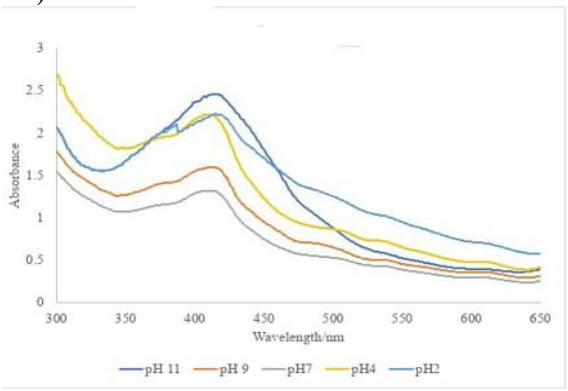

Figure (2b): Effect of PH on the formation and stability of biosynthesized AgNPs

3.3. FTIR Spectral Analysis

The FTIR spectrum (Fig. 3) provided valuable insight into the functional groups that play key roles in the reduction and stabilization of silver nanoparticles synthesized from an *Allium carolinianum* leaf extract.

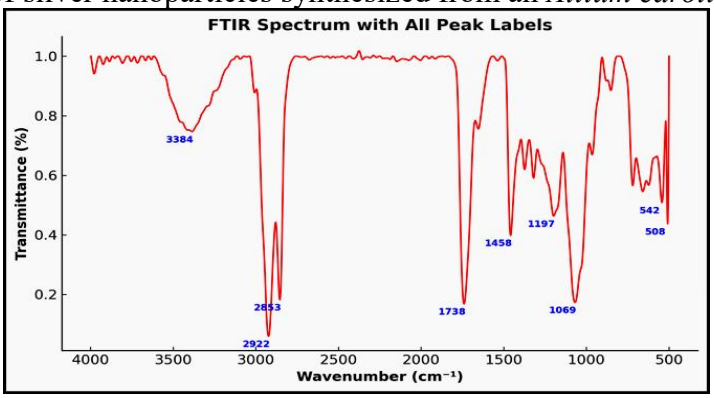

Figure (3): FTIR spectrum of green-synthesized AgNPs showing major functional groups involved in reduction and stabilization.

Table (1) showed a visible broad absorption band observed at 3384 cm^{-1}, corresponding to O-H stretching vibrations of hydroxyl groups present in alcohols and phenolic compounds, indicating the involvement of these biomolecules in the reduction of silver (Ag^{+}) ions [32]. The peaks at 2922 cm^{-1} and 2853 cm^{-1} are associated with C-H stretching vibrations, characteristic of aliphatic hydrocarbons, particularly methyl and methylene groups. A strong peak at 1738 cm^{-1} is assigned to the C=O stretching of carbonyl-containing functional groups such as esters, aldehydes, and carboxylic acids, suggesting the participation of these groups in nanoparticle formation. The absorption band at 1458cm^{-1} is indicative of C-H bending, whereas the peaks at 1197cm^{-1} is assigned to C-O stretching of ester, ether, or carboxyl groups. An additional absorption band at 1069cm^{-1} indicates C-O stretching in polysaccharides and proteins. Minor peaks at 542 cm^{-1} and 508 cm^{-1} are attributed to C-X (halogen) stretching vibrations (Fig. 3). Collectively, these bands indicate the presence of diverse functional groups that act as reducing and capping agents, facilitating the green synthesis and stabilization of AgNPs.

Table 1: Peak counts, Wavenumber, Intensity, and corresponding Functional groups.

Wavenumber (cm^{-1})	Vibrational Mode	Functional group/Bond type	Possible Source or compound class
3384	O-H stretching	Hydroxyl groups	Alcohol, phenols (reducing agents)
2922	C-H asymmetric stretching	Alkyl groups	Aliphatic hydrocarbons
2853	C-H asymmetric stretching	Methylene and methyl groups	Aliphatic compounds from biomolecules
1738	C=O stretching	Carbonyl groups	Esters, aldehydes, carboxylic acids
1458	C-H bending	Alkane groups	Hydrocarbons, protein side chain
1197	C-Os stretching	Ester/Ether/Carboxyl group	Polysaccharides, flavonoids
1069	C-O stretching	Alcohol or polysaccharide groups	Phenolic or carbohydrate residues
542	C-X stretching	Halogenated compounds	Phytochemical derivative
508	C-X stretching	Halogenated compounds	Bioactive metabolites

3.4. XRD Analysis

The XRD profile of biosynthesized silver nanoparticles (AgNPs) is presented in Fig. (4), the diffraction peaks observed at 2θ values of 38.1°, 44.3º, 64.4º, and 77.3º corresponding to (111), (200), (220), and (311) lattice planes, respectively of the face centered cubic (fcc) structure of metallic silver (JCPDS No. 04-0783). The intense reflection at the (111) plane indicates a preferred orientation and a high degree of crystallinity of the nanoparticles. The sharp, well-defined peaks indicate the formation of highly crystalline AgNPs with minimal structural defects. The absence of additional impurity peaks confirms that the synthesized nanoparticles are composed solely of elemental silver, free of secondary phases or oxides. These results are consistent with previously reported diffraction patterns of green-synthesized AgNPs [35, 36], further validating the successful bio-reduction of Ag^{+} to Ag° by *Allium carolinianum* leaf extract.

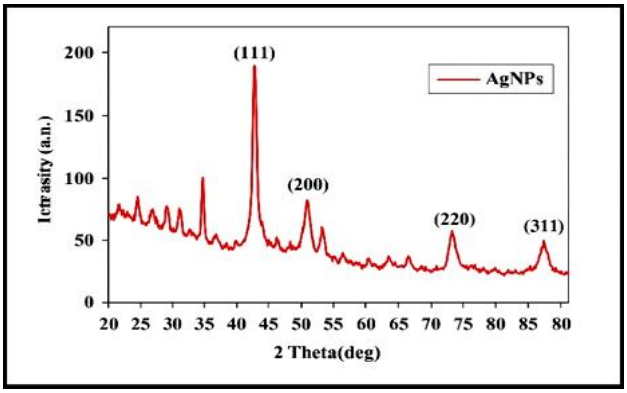

Figure (4): XRD spectrum of synthesized AgNPs

3.5. Energy Dispersive X-ray Spectroscopy (EDX)

The elemental composition of the biosynthesized silver nanoparticles (AgNPs) was analyzed using energy dispersive X-ray (EDX) spectroscopy (Fig.5). The EDX spectrum confirmed the successful formation of AgNPs, with a prominent signal from metallic silver (Ag) as the dominant element. In addition to silver, other trace number of oxygens (O), carbon (C), nitrogen (N), chlorine (Cl), calcium (Ca), potassium (K), magnesium (Mg), silicon (Si), and sulfur (S) were detected.

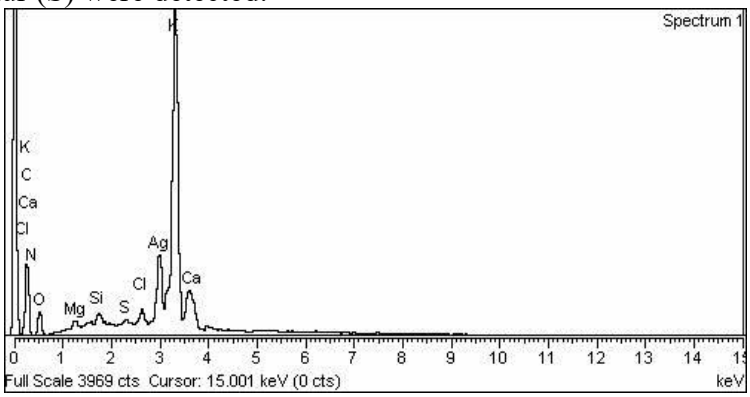

Figure (5): EDX spectrum of biosynthesized AgNPs confirming elemental composition.

Figure (5) and the corresponding EDX elemental composition data (Table 2) confirm the biosynthesis and purity of silver Nanoparticles (AgNPs).The presence of these elements is attributed to the phytochemicals in the Allium carolinianum leaf extract, which act as reducing and stabilizing agents during synthesis. The

absence of any extraneous peaks further indicates the purity of the synthesized AgNPs. These findings validate the role of plant-derived biomolecules in the bio-reduction and capping of Ag^{+} ions, ensuring nanoparticle synthesis, stability, and surface passivation.

Table 2: Elemental composition of synthesized AgNPs

Element	Weight (%)	Atomic (%)	Probable Source or Role
C (K)	18.73	34.55	Phytochemicals (organic compounds)
N (K)	9.53	15.07	Alkaloids and proteins
O (K)	18.09	25.06	Oxygenated Biomolecules
Mg (K)	0.75	0.68	Minerals residues (Enzyme cofactors)
Si (K)	0.82	0.65	Trace Phyto silicates
S (K)	0.37	0.25	Sulfur compounds
Cl (K)	1.10	0.69	Chlorinated organic compounds
K (K)	31.91	18.08	Plant-derived mineral content
Ca (K)	3.24	1.79	Cell wall and ionic residues
Ag (L)	15.47	3.18	Silver nanoparticles (core element)
Total	100	-	-

3.6. Scanning Electron Microscopy (SEM)

The surface morphology and structural features of biosynthesized silver nanoparticles were examined using scanning electron microscopy (SEM), as shown in Fig. (6). The micrographs reveal a dense population of uniformly distributed nanoparticles, confirming efficient reduction and stabilization by the Allium carolinianum leaf extract. At lower magnification, the nanoparticles appear well dispersed, with no significant agglomeration, indicating good colloidal stability. Higher-magnification images (Fig. 6a) display predominantly cubic and spherical particles with smooth, well-defined surfaces. Occasional star-like structures observed in (Fig. 6c) suggest antistrophic growth during nucleation, while the slightly wrinkled surfaces in Fig. 6d. may result from mild aggregation overlapping during the drying process. The overall particle size fall within the nanometer range, consistent with results from UV-Vis and XRD analysis. These morphological observations confirm the successful green synthesis of well-defined, stable silver nanostructures mediated by *A. carolinianum.*

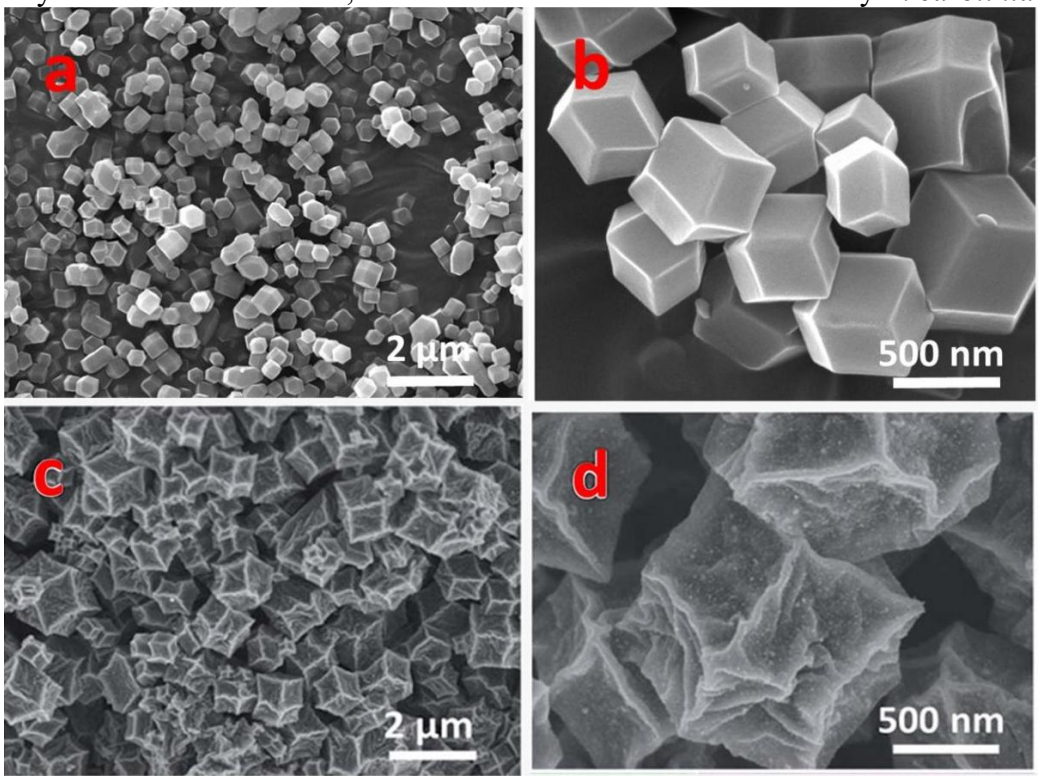

Figure (6): SEM micrographs of AgNPs

3.7. Antibacterial Assay

The antibacterial potential of biosynthesized silver nanoparticles was evaluated against several clinically important bacterial strains, *Salmonella typhi, klebsiella pneumoniae, Bacillus subtilis, Escherichia coli, pseudomonas aeruginosa, Acinetobacter SP*, *Bacillus anthracis, and Clostridium difficile* using the agar well

diffusion method at concentrations ranging from 10 to 40mg/ml by comparison with the standard antibiotic chloramphenicol as shown in (Table 3).

Table 3: Antibacterial Activity of Green Synthesized AgNPs from Allium carolinianum

Bacterial Strains	Zone of inhibition (mm)					
	Positive control	AgNPs 10 mg/ml	AgNPs 20 mg/ml	AgNPs 30 mg/ml	AgNPs 40 mg/ml	Chloramphenicol 20 mg/ml
Salmonella typhi	13.67±1.25	15.0±1.63	15.67±1.7	16.67±1.7	17.67±1.7	19.31±1.41
Klebsiella pneumonia	22.0±1.63	11..33±1.25	15.00±0.82	20.0±1.65	21.02±1.63	24.23±0.35
Bacillus subtilis	23.0±1.63	13.0±0.82	13.33±1.25	16.33±1.2	17.33±1.25	19.17±1.12
Escherichia Coli	19.33±1.25	22.33±1.25	24.33±1.25	27.33±1.2	31.67±1.25	20.61±1.15
Pseudomonas aeruginosa	13.33±1.25	13.33±2.05	17.00±1.63	21.0±0.82	26.03±0.82	23.31±1.23
Acinetobacter	11.67±1.7	16.03±0.82	19.67±1.25	23.33±2.0	26.02±0.82	25.9±0.25
Bacillus anthracis	12.56±1.11	19.32±1.41	20.31±1.20	20.61±1.2	21.35±1.21	18.33±1.21
Clostridium difficile	9.26±1.54	15.33±1.24	17.23±1.21	18.31±0.8	19.51±1.30	20.25±0.67

An apparent dose-dependent increase in the zone of inhibition was observed for all tested strains, indicating enhanced bacterial activity with increasing nanoparticle concentration (Table 4, Fig..7). Among the tested bacteria, *Escherichia coli* exhibited the highest sensitivity, showing a maximum inhibition zone of 31.67±1.25mm at 40mg/ml, compared to chloramphenicol (20.61±1.15 mm)[33], followed by *Acinetobacter sp.* (26.00±0.82mm). A comparable or slightly higher inhibition zone was also observed against *p. aeruginosa* (26.00±0.82mm vs 23.31±1.23mm), and *Bacillus anthracis* (21.35±1.21mm vs 18.33±1.21). In contrast, chloramphenicol showed greater inhibitory zones against *S. typhi, K. pneumonia*, *B. Subtilis,* and *Clostridium difficile*, whereas silver nanoparticles showed moderate but lower activity. The pronounced antibacterial efficacy against Gram-negative bacteria, especially *E. coli*, can be attributed to their thinner cell walls and higher membrane permeability, which facilitate nanoparticle penetration and the generation of reactive oxygen species (ROS). This finding confirms that *A. carolinianum*-mediated AgNPs possess potent, broad-spectrum antibacterial activity, making them promising candidates for biomedical and antimicrobial formulations.

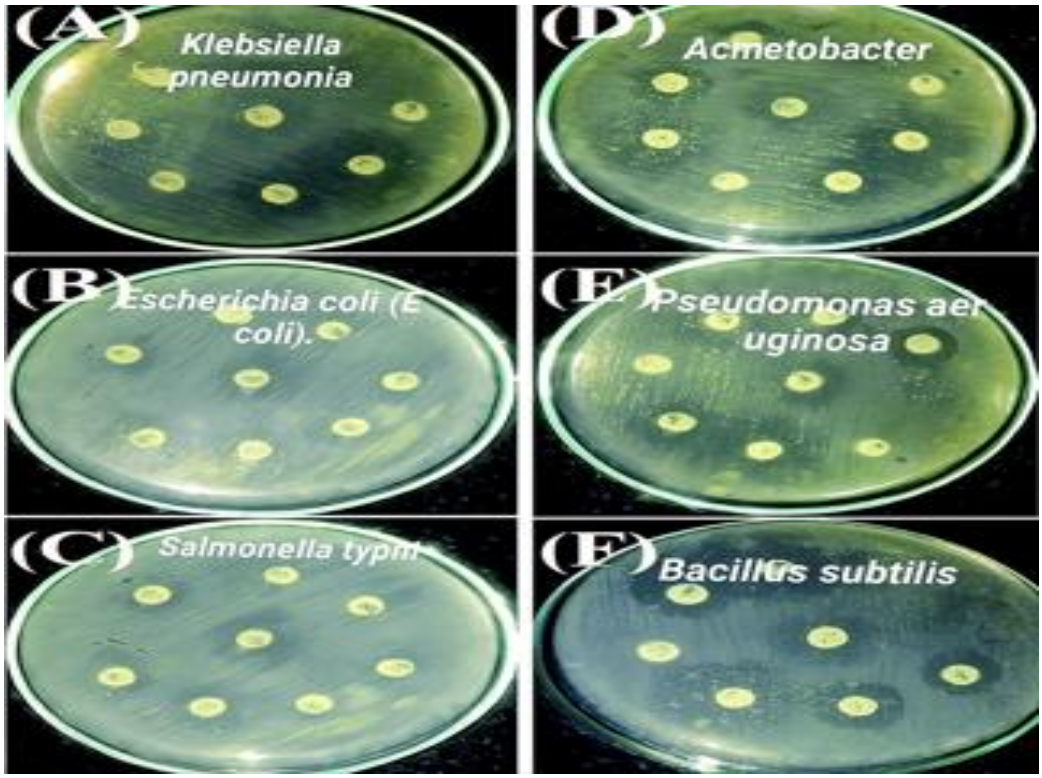

Figure (7): The Antibacterial Assay representation.

3.8. Antioxidant activity (DPPH Radical Scavenging Assay)

The silver nanoparticles (AgNPs) using the *Allium Carolinianum* leaf extract exhibited a strong antioxidant potential, as antioxidant ability was assessed by the DPPH free radical scavenging assay as shown in Figure (8) with the methanolic extract-mediated AgNPs is characterized to have a maximum activity of 62.33% pillar at

100µg/ml), which was close to the activity of the known antioxidant ascorbic acid (72.00%)[34]. To the contrary, the scavenging potential of liquid methanolic plant extract was relatively lower (43.21% at 100µg/ml). The increased radical scavenging activity of SNPs can be attributed to the presence of a variety of surface-bound phytochemicals, as suggested by the FTIR analysis, which indicated the presence of hydroxyl, carbonyl, and amine functional groups that could donate electrons and hydrogen atoms to overcome the scavenging of DPPH radicals. The functional groups not only facilitated the reduction of Ag+ ions to Ag^{o} during synthesis but also stabilized the resulting nanoparticles and increased their redox activity. Hydrogen-atom transfer by hydroxyl groups and the formation of stable phenoxy intermediates are the combined efforts of the abundant phytochemical profile of Allium carolinianum, which includes alkaloids, phenols, and proteins, contributing to its antioxidant performance [35]. All these results suggest that *A. carolinianum*-mediated AgNPs have the potential to be used as natural antioxidants for the mitigation of oxidative-stress-related diseases and for the generation of antioxidant nanotherapeutics [36].

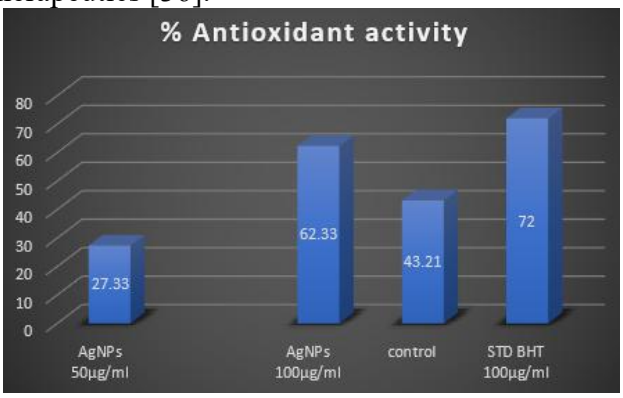

Figure (8): dose dependent antioxidant activity of AgNPs compared with standard

3.9. Phytochemical studies

The crude *A. carolinianum* leaf extract was qualitatively analyzed to identify its major phytochemical constituents using standard screening assays. The analysis confirmed the presence of alkaloids and flavonoids in appreciable quantities, whereas terpenoids and saponins were detected at lower levels. Alkaloids and flavonoids are well recognized for their ability to act as effective reducing and stabilizing agents during the conversion of Ag^{+} precursor ions in solution, thereby facilitating nanoparticle formation [37].

Table 4: Presence of phytochemicals in crude extract of *Allium carolinianum.*

#	Class	Test	Reagent	Observation	Result
1	Alkaloids	Hager's Reagent	A saturation solution of Picric acid	Yellow precipitation	positive
2	Flavonoids	Alkaline Reagent	2% NaOH + drop of HCL	Disappearance of yellow color with addition of HCl	Positive
3	Tannins	Braymer's Reagent	5% $FeCl_3$	Dark green color	Positive
4	Saponins	Foam Test	Distilled water	Formation of foam	Positive
5	Waxes	Saponification	Methanolic NaOH	No saponification	Negative

4. Conclusion

The current research demonstrated the sustainability, economic viability, and cost-effectiveness of green synthesis of silver nanoparticles using *Allium carolinianum* methanolic leaf extract. The bioactive phytoconstituents in the plant extract were effective reducing and stabilizing agents, resulting in stable crystalline AgNPs, as revealed by UV-Vis, FTIR, TEM, and XRD analysis. A UV-Vis spectrum showed a surface plasmon resonance band for AgNPs. In contrast, the FTIR revealed the presence of functional groups, including hydroxyl, carbonyl, and amine, during nanoparticle formation. XRD was used to confirm the presence of crystalline nanoparticles with a face-centered cubic structure. A biological assay has shown that the produced AgNPs have significant antibacterial and antioxidant properties, with the potential to be used as alternative therapeutic agents. It was shown that *A. carolinianum*-derived AgNPs have a promising biomedical outlook and can contribute to the further development of medicinal plants as sustainable sources of nanoparticle production. Research to be done in the Future must center on the detailed mechanism of action, cytotoxicity analysis, and the possible incorporation of these nanoparticles into pharmaceutical and biomedical systems.

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Conflict of Interest: The authors declare that no conflict of interest exists.

Author's contribution

Muhammad Younas, Mujeeb Ul Haq (wrote the original manuscript), Mian Gul Sayeed, Waqar Khan (supervised the work and provided FTIR facility), Irfan Ullah (conducted antibacterial and antioxidant activity at the Center for Biotechnology and Microbiology, CB&M, University of Swat), Rahmat Ali (conducted characterization facilities done at the central resources laboratory in Peshawar (CRL).

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