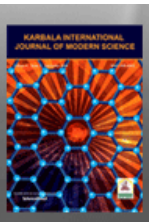
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Antioxidant and Antifungal Activity of Lactuca serriola L. Plant and Silver Nanoparticles Against Aspergillus Species.

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Medicinal plants remain a valuable source of antifungal agents, and recent developments in green nanotechnology have increased the prospects for *Lactuca serriola* L. as a useful bioresource. In the current study, leaves and roots were extracted by ultrasonic-assisted chloroform, ethanol, and aqueous extraction methods for the biological mediation of silver nanoparticles (Ag NPs). Screening of Phytochemicals for extracts revealed the present secondary metabolites. Ag NPs were verified through UV-visible spectroscopy, indicating a peak at (408-423 nm) corresponding to a surface plasmon resonance, FT-IR functional groups of O–H, C=O, and C–N binding. The morphological analysis revealed an irregular-to-quasi-spherical shape, with an average size of 27.4-54.41 nm, as observed by FE-SEM. Elemental analysis (EDX) reports the presence of silver atoms, and standard XRD patterns show characteristic planes of face-centered cubic structure corresponding to the (111), (200), and (220). The results of the DPPH radical scavenging activity assay (0.625-5 mg/mL) revealed a concentration-dependent antioxidant activity of crude extracts, with notable differences ($P \leq 0.05$) compared with ascorbic acid. The Ag NPs also showed scavenging activity lower than that of ascorbic acid. Crude extract shows a minimum inhibitory concentration (MIC) value between 12.5 and 50 mg/mL. Ag NPs exhibit a lower MIC value ranged from 1.56 to 6.25 mg/mL, with *Aspergillus* species. In general, the results showed that *Lactuca serriola* L. extracts and their biosynthesized Ag NPs possess antioxidant and antifungal activity, indicating that effective alternatives to chemicals used for antimicrobial purposes are available from an environmentally friendly perspective.

Keywords

AgNPs, Antioxidant, Anti-aspergillus, Green synthesis, and Lactuca serriola L.

Cover Page Footnote

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RESEARCH PAPER

Antioxidant and Antifungal Activity of *Lactuca serriola* L. Plant and Silver Nanoparticles Against *Aspergillus* Species

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Abstract

Medicinal plants remain a valuable source of antifungal agents, and recent developments in green nanotechnology have increased the prospects for *Lactuca serriola* L. as a useful bioresource. In the current study, leaves and roots were extracted by ultrasonic-assisted chloroform, ethanol, and aqueous extraction methods for the biological mediation of silver nanoparticles (Ag NPs). Screening of Phytochemicals for extracts revealed the present secondary metabolites. Ag NPs were verified through UV-visible spectroscopy, indicating a peak at (408−423 nm) corresponding to a surface plasmon resonance, FT-IR functional groups of O−H, C═O, and C−N binding. The morphological analysis revealed an irregular-to-quasi-spherical shape, with an average size of 27.4−54.41 nm, as observed by FE-SEM. Elemental analysis (EDX) reports the presence of silver atoms, and standard XRD patterns show characteristic planes of face-centered cubic structure corresponding to the (111), (200), and (220). The results of the DPPH radical scavenging activity assay (0.625−5 mg/mL) revealed a concentration-dependent antioxidant activity of crude extracts, with notable differences ($P \leq 0.05$) compared with ascorbic acid. The Ag NPs also showed scavenging activity lower than that of ascorbic acid. Crude extract shows a minimum inhibitory concentration (MIC) value between 12.5 and 50 mg/mL. Ag NPs exhibit a lower MIC value ranged from 1.56 to 6.25 mg/mL, with *Aspergillus* species. In general, the results showed that *Lactuca serriola* L. extracts and their biosynthesized Ag NPs possess antioxidant and antifungal activity, indicating that effective alternatives to chemicals used for antimicrobial purposes are available from an environmentally friendly perspective.

Keywords: AgNPs, Antioxidant, Anti-aspergillus, Green synthesis, *Lactuca serriola* L

1. Introduction

The Kurdistan Region is regarded as an abundant center for medicinal plant resources due to its distinctive topographic distribution and diverse climatic conditions, which support high biodiversity and provide a source of new bioactive compounds. For modern drug discovery and sustainable healthcare, the documentation and scientific validation of traditional Phytotherapeutic knowledge are indispensable for preserving cultural heritage [1]. The family Asteraceae is the largest family of dicotyledons in the flora of Iraq, comprising three subfamilies and 16 tribes. In all, 123 genera and 433 species have been noted, of which 14 taxa are cultivated [2]. *Lactuca serriola* L. (Asteraceae), a medicinal wild plant known as 'prickly lettuce'. The domesticated form is likely derived from wild populations spread around the Mediterranean and parts of Southeast Asia. Usually a biennial herb with smooth yellowish-green stems adorned with small black dots. The plant has deeply divided, toothed leaves and is characterized by having prickles on stems and leaves [3]. The species

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Lactuca serriola L. abound in phytochemicals and have a long history as an anti-asthmatic, in the treatment of digestive disorders, urinary infections, and inflammatory conditions. They are also promising candidates in the treatment of neurodegenerative diseases and exhibit significant antioxidant, anticancer, and antimicrobial activities [4]. Ultrasonic-accelerated extraction is widely recognized as an eco-friendly and efficient technique for extracting bioactive substances from plant materials due to the microbubble implosion effect of ultrasonic waves, which causes cell disruption, mass transfer, and solvent penetration, and is more environmentally friendly due to reducing extraction time, solvent use, and energy consumption compared with the conventional method [5,6]. Nanoparticles (NPs), commonly with particle sizes ranging from 1 to 100 nm, are characterized by their specific structure and properties which have extended their various applications in medical science [7]. The synthesis of nanomaterials using traditional methods is expensive and toxic, whereas green synthesis with plant extracts offers a safer, more environmentally friendly option [8]. Among the bunch of nanostructured Ag materials, the exceptional properties of Ag NPs and their wide range of applications have made them the most studied metal-based NPs. They are widely used in medicine, agriculture, environmental cleaning, and catalysis [9]. Nanoparticles are emphasized for their ability to significantly enhance thermal conductivity, mass transport, and flow controllability under external magnetic fields. When dispersed in biological fluids, Ag NP-based nanofluids can improve transport efficiency, cellular interactions, and localized thermal regulation [10,11]. Despite the growing interest in medicinal plants for green nano-technology, the application of *Lactuca serriola* L. plant in Kurdistan region as a biological origin utilized for a fabrication of silver nanoparticles by both plant parts, roots and leaves unstudied, previous studies have largely focused on single plant parts, conventional extraction methods, or a narrow range of solvents, with insufficient comparison of how extraction strategy and plant tissue influence nanoparticle's characteristics and biological activity. In particular, the combined effects of ultrasonic-assisted extraction, solvent polarity, and plant parts on the physicochemical properties and antimycotic efficacy of biosynthesized Ag NPs have not been systematically investigated. The purpose of this investigation was to assess the antifungal efficacies of Ag NPs green-synthesized and leaf and root extracts of *Lactuca serriola* L. against *Aspergillus* sp., as well as determine antioxidant activity and phytochemical compositions.

2. Materials and methods

2.1. Geographical study of the location area

The sample of *Lactuca serriola* L. plant, roots, and leaves were collected in the summer of 2024 in Khoran village, situated in the Safeen mountain, Erbil, Kurdistan region, Iraq (44.347180°E, 36.325380°N; 666 m above sea level), as shown in Fig. 1A and B. The plant material was kept in normal light (away from direct sunlight) at room temperature until used.

2.1.1. Plant preparation and extraction

Subsequently to collecting the plants, the roots and leaves were cleaned with tap water to remove extraneous dust and soil particles, then dried at 38–40 °C until they reached constant weight [12]. Then the roots were manually and carefully cut with a cutter tool, and the roots and leaves were pulverized using an electrical grinder (IKA M 20 Universal Mill, IKA Group, Wilmington, NC, Germany). The resulting powder was subsequently passed through an 80-mesh (80 μm) sieve to achieve a uniform particle size, and then preserved in a glass jar at 5–15 °C for further studies [13]. 10 g of each part's roots and leaves of *Lactuca serriola* L. were extracted sequentially by chloroform (99.5%) and ethanol (99.9%) through an ultrasonic-assisted extraction system (40 kHz, 30 °C, 30 min) and with shaking for 1 h. The crude extract was obtained by concentrating the filtrate under vacuum conditions using a rotary evaporator at 40 °C, then drying it in an oven for 24 h at 40 °C [14]. For synthesis, silver nanoparticles (Ag NPs), 5 g of powdered leaves and

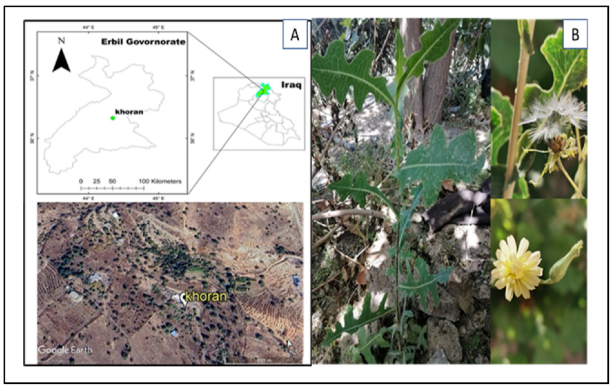

Fig. 1. (A) Geographical study of the location area of Erbil, Kurdistan Region, Iraq, (B) Lactuca serriola L. plant.

roots, were separately weighed and dispersed in 100 mL of deionized distilled water. The suspension was maintained at 40 °C for 1 h under constant agitation using a magnetic stirrer to facilitate the extraction of the bioactive compound. The resulting extract was passed through Whatman No. 1 filter paper to obtain a clear extract, which was then stored in a refrigerator at 4 °C until use [15,16].

2.1.2. Qualitative analysis of phytochemical components

Selected bioactive phytochemicals of the chloroform, ethanolic, and aqueous leaves and roots extracts of *Lactuca serriola* L. were screened according to the method described below [17–20].

a. **Terpenoids (Salkowski test):** To each 0.5 mL of plant extract, 2 mL of concentrated sulfuric acid followed by 2 mL of chloroform. Chloroform separates terpenoids from other phytochemicals. Then, concentrated H_2SO_4 reacts with the terpenoids to produce a reddish-brown interface, indicating the presence of terpenoid compounds
b. **Saponins (froth test):** For this assay, 2 mL of the plant extract was blended with 2 mL of distilled water and mixed vigorously for 15 min. Saponins are surface-active compounds containing polar glycosyl groups, non-polar steroid groups, and triterpenoids. When shaken with water, the polar groups face outward, and their non-polar counterparts face inside, causing foam formation of a stable froth that persisted for ≥1 cm.
c. **Phenols (ferric chloride ($FeCl_3$) test):** 1 mL of plant extract, 2 mL of distilled water, and subsequently a few drops of ferric chloride 10% were added. Phenolic compounds bind with ferric ions, producing an intense color, which may vary from blue-green, indicating the occurrence of phenolic compounds.
d. **Flavonoids (alkaline reagent test):** For the determination of flavonoids, the extract (2 mL) was added to 1 mL of 2 N NaOH and properly mixed. The formation of yellow coloration due to the reaction of flavonoid with the hydroxide ions confirms the presence of flavonoid compounds.
e. **Tannin (ferric chloride test):** 1 mL of ferric chloride 5% solution and 2 mL of extract was mixed. The appearance of a dark blue or blackish-green color indicated the presence of tannin due to complex formation between ferric ions (Fe^{3+}) and phenolic hydroxyl groups.
f. **Resins (turbidity test):** For the turbidity test, 10 mL of the plant extract was mixed with 20 mL of acidified distilled water (4% hydrochloric acid). Based on turbidity, when an alcoholic plant extract is diluted with water reflecting the hydrophobic nature of resinous compounds.

2.2. Antioxidant assay (DPPH method)

For antioxidant evaluation, using the DDPH (2,2-diphenyl-1-picrylhydrazyl) assay, both chloroform and ethanol extracts from roots and leaves, as well as silver nanoparticles (Ag NPs), were analyzed. Serial concentrations of 5, 2.5, 1.25, and 0.625 mg/mL of samples and control were used [21,22]. DPPH is a stable nitrogen-centered radical generating violet color in ethanolic solution, which fades on reduction to diphenyl picryl hydrazine when an antioxidant donates a hydrogen atom. Each test sample (1:1, v/v) was combined with 0.04% DPPH solution in 99.5% ethanol. Following 30-min incubation at room temperature under light-protective conditions, the absorbance was read at 517 nm [23].

The free radical inhibition percentage was calculated by the equation presented below.

$$Inhibition\ \% = \frac{Ac - As}{Ac} * 100 \quad (1)$$

Ac = the absorbance value of the control (ethanol used in place of the sample).

As = the absorbance value of the sample solution or positive control (ascorbic acid).

2.2.1. Calculation of IC50

The IC_{50} value, which was defined as the concentration necessary to achieve 50% radical inhibition, was determined using the interpolation method. The inhibition percentages were initially plotted against the corresponding sample concentrations, and the regression relationship was assessed. Since the inhibition curve did not show a pure linear relationship, the IC50 was determined using four experimental points that flank 50% inhibition. A line was produced between them, and the equation of linear regression is shown in Equation (2).

$$Y = ax + b \quad (2)$$

where Y is the inhibition ratio, and X is the sample concentration. The concentration (X) at 50% inhibition was determined by substituting Y = 50 into the equation and recorded as the IC_{50} [24].

2.3. Green-synthesis of silver nanoparticles (Ag NPs)

For green-synthesis of silver nanoparticles using 10 mM of Silver nitrate, which was prepared by dissolving 0.169 g of AgNO3 in 100 mL of deionized water [25]. The plant extract was prepared separately from each plant and its parts, and the extract was gradually added to the AgNO3 solution in a reaction flask at 60 °C under a magnetic stirrer until the color changed to brown at pH 8. After that, the reaction mixture was incubated undisturbed overnight at room temperature to ensure complete reduction of Ag^{+} ions and further nanoparticle stabilization. The resulting suspension was centrifuged at 6000 rpm (HERMLE, Germany) for 30 min. The supernatant was decanted, and the pellet containing Ag NPs was washed at least three times with deionized distilled water. Each wash involved resuspending the pellet in fresh water, followed by centrifugation under the same conditions. The purified silver nanoparticles (Ag NPs) were subsequently dried at 90 °C for 48 h to yield dry nanoparticle powder suitable for downstream characterization and applications [21].

2.3.1. Assessment of silver nanoparticles (Ag NPs) characterization

The synthesized nanoparticles were characterized by using a UV–Visible spectrophotometer (UV-1900i, Shimadzu, Japan) and Fourier Transform Infrared spectroscopy (FTIR, Shimadzu 8400 S), carried out at the Department of Chemistry, College of Science. Their surface morphology and elemental composition were measured using Field Emission Scanning Electron Microscopy (FE-SEM; Tescan, MIRA3, Czechia-Iran) equipped with Energy Dispersive X-ray Spectroscopy (EDX). An X-ray diffraction (XRD) technique was used to analyze the crystalline structure at the Research and Application of Science Center, Soran University.

2.4. Fungal sample

Four species of Aspergillus were isolated from aspergillosis lung infection chicks (Ross-308) in the biology department of Salahaddin University, in the laboratory. The *Aspergillus* species are *A. niger, A. fumigatus, A. flavus,* and *A. terreus.*

2.4.1. Preparation of fungal spore suspension

Spore suspensions of the species were derived from 7-day pure cultures on Sabouraud Dextrose Agar (SDA). The spores were harvested by administering 2 mL of Phosphate-Buffered Saline (PBS, 0.85%) to each Petri plate. The suspensions were resuspended, vortexed well, and quantified. The grade of the spores was adjusted to 1.0×10^5 by appropriate dilution using 0.85% saline solution, and an accurate count of viable spores was obtained using a hemocytometer [26].

2.4.2. Microdilution method

The antifungal properties of the extracts and silver nanoparticles (Ag NPs) were determined by the microdilution assay in accordance with previously reported methods [27]. The sterile 96-well microplates were used for this assay. The fungal inoculum was standardized spectrophotometrically to 0.08–0.10 wavelength at 625 nm (1 cm light path). Then, 100 μL of Sabouraud Dextrose Broth (SDB) was introduced to each well of the microplate. The first well received 100 μl of the sample, prepared at a concentration of 100 mg/mL. Subsequently, 100 μL from the first well was transferred to the next well in the same row and mixed thoroughly. This serial transfer procedure was repeated along the row to obtain a series of two-fold dilutions 1:2, 1:4, 1:8, 1:16, etc., (50, 25, 12.5, 6.25, 3.125, and 1.56 mg/mL) [28]. In this case, the sample concentration will decrease, while the broth with fungi is positive and the broth-only media is negative. Each well was inoculated with microbial suspension prepared from the working solution. Absorbance was read at 630 nm without incubation by an ELISA microplate reader (Epson, Biotech, UK). The microplate was kept at 25 °C for 48 h. The next day, after incubation, the absorbance was measured again and then compared to the earlier reading. The MIC was determined as the concentration at the breakpoint by comparing the absorbance values before and after incubation. All sample dilutions were sub-cultured onto fresh solid media to confirm the results. The experiment was performed in triplicate for each fungal growth.

2.5. Statistical evaluation

Data were evaluated using GraphPad Prism (version 10). Results are presented as mean intergroup comparisons, and controls were evaluated by one-way ANOVA. Dunnett's post hoc multiple comparison test. A P-value <0.05 was considered statistically significant.

3. Results and discussions

3.1. Qualitative phytochemical determination in different plant parts of Lactuca serriola L.

Table 1 shows the qualitative estimation of phytochemicals in *Lactuca serriola* extracts, with a significant variation depending on the solvent and plant part employed. The absence of bioactive compounds was scored (−), and the presence was scored (+), slight (++), moderate, and (+++) highly. In chloroform leaf extracts, terpenoids, phenolics, flavonoids, and tannins were found, whereas in the root, mainly terpenoids and saponins were detected. For the leaves, ethanol was the best solvent, with the highest phenolic (+++), flavonoid (++), and tannin (++) contents, and moderate amounts of resin were also present. However, the aqueous extracts showed a high concentration of tannins (+++) and phenolic compounds (++), mainly in leaves, and moderate levels of saponins and flavonoids in both leaves and roots. These differences were attributed to solvent polarity affecting the solubility of phytochemicals. Polar solvents, for example, ethanol and water, showed greater extracting ability of phenolic, flavonoid, and tannin compounds, whereas non-polar in nature chloroform concentrated on terpenoids and resins. These phytochemical compounds were the major contributors to the plant's bioactivity and antioxidant properties, as well as its medicinal properties. The finding showed that the presence of terpenoids in the plant may contribute to its antimicrobial activity [29]. Phenolic compounds are considerably antimicrobial, antifungal, and sources of antioxidant properties [30]. The bioactive molecules (phenolic acids) were found at higher concentrations in the phenolic extracts in vitro, which was digested before, consistent with the strong antibacterial activity exhibited by these extracts [31]. Several studies have demonstrated that chlorogenic acid, a prominent phenolic compound, exhibits antimicrobial activity against various fungi at concentrations ranging from 0.04 to 10 mg/mL [32]. Astragalin and iso quercitrin isolated from chamchwi (*Aster scaber* Thunb.) have an antioxidant activity [33]. Flavonoids and phenolic compounds participate in antiviral, anti-inflammatory, antioxidant, and antifungal activities [34]. Reference [35] The resin highly exhibits antifungal activity against all tested dermatophytes in agar diffusion assays. Tannins are polyphenolic compounds and have been shown to exhibit antifungal, antibacterial, and antiviral activities. Antimicrobial action is attributed to inhibition of various enzymes and suppression of nucleic acid synthesis [36]. Phenolic, flavonoid, and tannin, which are present in aqueous extract of leaf and root of *Lactuca serriola* L., which shown in Table 1, are considered effective reducing and capping agents in the green synthesis of silver nanoparticles (Ag NPs) [15].

3.2. Characterization of silver nanoparticles

3.2.1. UV-visible spectrophotometric evaluation

Fig. 2 shows the absorption spectrum of *Lactuca serriola* L. leaf and root extracts and their Ag NPs. The *Lactuca serriola* L. leaf and root extracts exhibited absorption peaks at approximately 271 and 225 nm, respectively, which shifted to 408 and 423 nm, respectively, after the addition of silver nitrate. The detection of red shift in the absorption spectrum validates the reduction of Ag^{+} ions to Ag^{0} nanoparticles. UV−Vis spectroscopy is commonly used as an indirect yet accurate technique for nanoparticle formation, as confirmed by the appearance of a unique, sharp surface plasmon resonance (SPR) band characteristic of silver nanoparticles (Ag NPs). As described in prior research [37], the typical SPR peak is observed between 400 and 500 nm, suggesting that silver nanoparticle fabrication was successful. Reference [38] showed in their study that the SPR peak at 419 nm indicated the production of Ag NPs.

Table 1. Qualitative test for phytochemical compound content of Lactuca serriola L. leaf and root chloroform, ethanol, and aqueous extract.

Solvents	Compounds						
	Plant parts	Terpenoid	Saponin	Phenol	Flavonoid	Tannin	Resins
Chloroform	**Leaf**	+	-	++	+	++	-
	Root	+	+	-	+	-	-
Ethanol	**Leaf**	-	-	+++	++	++	+
	Root	-	-	+	++	+	++
Water	**Leaf**	+	+	++	+	+++	-
	Root	-	+	+	+	+	-

- Absent, + slightly present, ++ moderately present, +++ highly present.

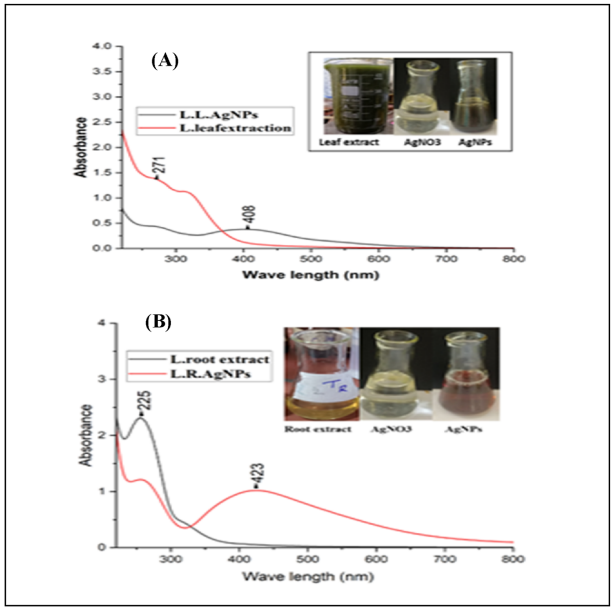

Fig. 2. (A) UV-Vi spectrum of aqueous leaf extract of Lactuca serriola L. and their silver nanoparticles (Ag NPs), (B) UV-Vi spectrum of aqueous root Lactuca serriola L. extraction and their silver nanoparticles (Ag NPs). L = Lactuca serriola L., L = Leaf, R = Root.

3.2.2. FT-IR spectrophotometer

The Fourier-Transform Infrared (FTIR) spectra of *L. serriola* leaf extract, root extract, and their Ag NPs are shown in Fig. 3. The FTIR spectrum of the leaf extract showed significant bands at (3295, 2133, 1628, 1281, 1126, and 645–478) cm^{-1} attributed to O–H/N–H stretching; C≡C or C≡N stretching; C=O or aromatic C=C vibrations models, and C–O/C–N functional groups, respectively. After the synthesis of L. L. Ag NPs, shown in Fig. 3A spectral shift and intensity changes were observed, such as shifting of broad O–H/N–H band from 3295 cm^{-1} to 3329 cm^{-1}, remaining but altered 1628 cm^{-1} peak, and appearance of new peaks at 2008 cm^{-1} and 1437 cm^{-1} indicates strong interaction between bioactive constituents and silver ions. However, Fig. 3B shows the FTIR spectrum of root extract showed a broad pick at 3300 cm^{-1} (O–H/N–H stretching), C–H stretching at 2970–2915 cm^{-1}, strong band at 1633 cm^{-1} (amide I/C=O or aromatic C=C), characteristic features at 1211 cm^{-1} (C–O/C–N) and 560–690 cm^{-1}. After Ag NPs formation (L.R. Ag NPs), O–H/N–H envelope peaks changed to 3323 cm^{-1} with less intensity and a new peak appeared at 1697 cm^{-1}, and enhanced sensitivity between 1435 and 1400 cm^{-1} and the modification in the region at 1000–1200 cm^{-1} was observed. These spectral changes, such as bathochromic frequency shift (red shift) and band broadening, together validate the involvement of phenolic, amine, and carbonyl functionalities of the extract in the reduction and capping of silver nanoparticles, in agreement with previous reports claim that plant-derived biomolecules serve as both reducing and capping agents during green synthesis [39].

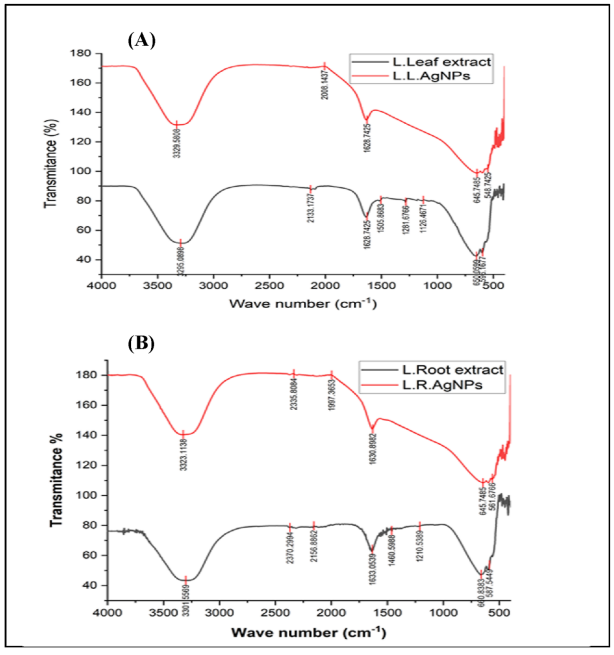

Fig. 3. FT-IR of (A) Leaf Lactuca serriola L. aqueous extract and Leaf-Ag NPs, (B) Root Lactuca serriola L. aqueous extract and root-Ag NPs, L = Lactuca serriola plant, L = Leaf, R=Root, Ag NPs = silver nanoparticles.

3.2.3. Assessment of size, shape, and distribution of silver nanoparticles

The surface morphology and structure characteristic of nanoparticles was detected by Field Emission Scanning Electron Microscopy (FE-SEM), as shown in Figs. 4A and 5A. The FE-SEM images showed that the particles were mainly irregular to quasi-spherical in shape. Aggregation preferentially occurred as a consequence of the large surface energy and inter-particle attraction. The particle sizes (Mean ± S.D.) of leaf-Ag NPs and Root-Ag NPs, measured from ImageJ analysis over hundreds of individual particles, result in (27.4 ± 8.8 nm) and as large as (54.4 ± 42.9 nm), respectively. The histograms reveal that Fig. 4B has a narrow size distribution, whereas Fig. 5B has a broader dispersion. The nanoscale dimensions of both Leaf-Ag NPs and Root-Ag NPs indicate successful synthesis of particles within the optimal range for applications in biomedicine and drug delivery (less than 100 nm), thereby improving cellular uptake and bioavailability [40,41]. These morphological features render

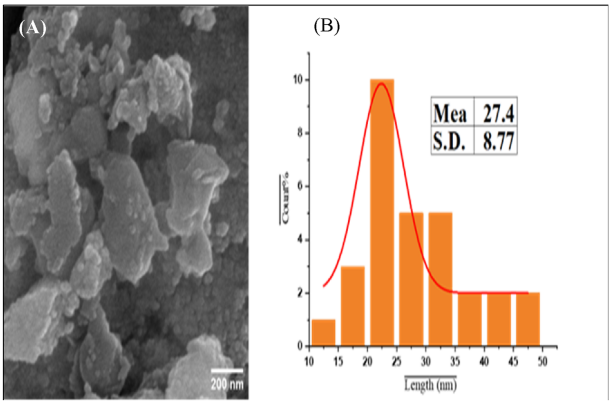

Fig. 4. (A) FE-SEM of silver nanoparticles (Ag NPs) synthesized by aqueous leaf Lactuca serriola L. extract, (B) Histogram showing the distribution size of Ag NPs.

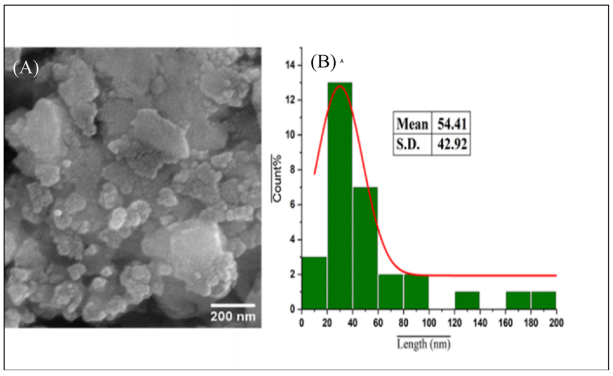

Fig. 5. (A) FE-SEM of silver nanoparticles (Ag NPs) synthesized by aqueous root Lactuca serriola L. extract, (B) Histogram showing the distribution size of Ag NPs.

these nanoparticles ideal candidates for targeted drug-delivery systems with high surface functionality and stability requirements [42]. According to geometric potential theory, the high surface curvature of nanosized particles generates curvature-dependent boundary energy, which enhances nanoparticle-cell membrane interactions and facilitates cellular penetration. In the case of Ag NPs, this increased geometric potential promotes improved cellular uptake and bioavailability [43,44]. The smaller nanoparticles (<50 nm) are reported to be more effective at penetrating biological membranes and may exhibit lower controlled-release behavior due to their greater surface reactivity [45]. The element profile of biosynthesized silver nanoparticles was identified through Energy-Dispersive X-ray Spectroscopy (EDS). Strong and sharp signals at around 3 keV are attributed to Ag Lα emission, indicating the existence of elemental silver as the main composition in both Fig. 6A&B. For Ag NPs shown in Fig. 6A, the percentage of silver weight in

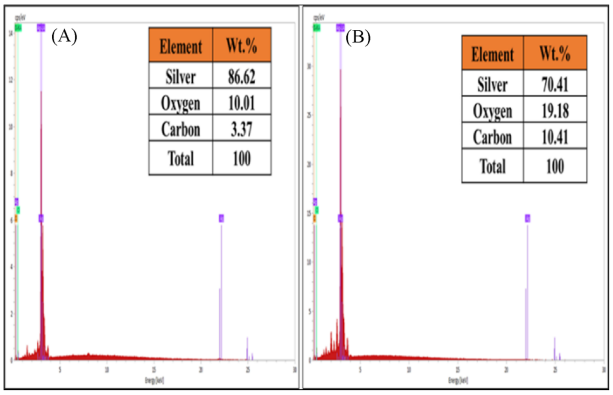

Fig. 6. EDX of Ag NPs mediated by (A) Leaf, (B) Root Lactuca serriola L. aquas extract.

leaf-Ag NPs is (86.62%), and these values are less (70.41%) for root-Ag NPs as seen from Fig. 6B, along with a higher portion of oxygen (10.1% and 19.18%) and carbon (3.37% and 10.41%), respectively. The oxygen and carbon peak presence due to surface adsorption of organic biomolecules, more likely arising from silver oxidation during synthesis or storage. These substances are generally attributed to the phytochemical components of extracts, which function as natural reducing and stabilizing agents during green synthesis [46].

3.2.4. X-ray diffraction (XRD)

The XRD analysis was used to identify the crystallographic properties of the green-synthesized Ag NPs, as illustrated in Fig. 7A and B. The diffraction pattern showed three sharp diffraction peaks of leaf-Ag NPs and root-Ag NPs at 2θ (37.46°, 43.63° and 63.85°) and four sharp diffraction peaks (37.35°, 43.73° and 63.81°) respectively the diffraction peaks within the measured range agree with those observed for the (111), (200), and (220) crystalline planes of face centric cubic (fcc) silver that are consistent with standard reference pattern (JCPDS file card No. 04-0783/ICDD PDF No. 98-006–4996), confirming formation of crystalline metallic silver. Sharp and strong peaks confirmed the high crystallinity of the nanoparticles. Of these, the (111) plane of leaf-NPs and root-NPs is the most intense, implying a preferential growth along this plane, a phenomenon previously reported for green-synthesized Ag NPs [47]. Unassigned diffraction peaks may be attributed to crystallization of the bio-organic residue on the nanoparticles' surface, and were therefore excluded from the crystallite size calculation [28]. The average crystalline size was determined by the Dybe-Scherrer formula shown in Equation (3).

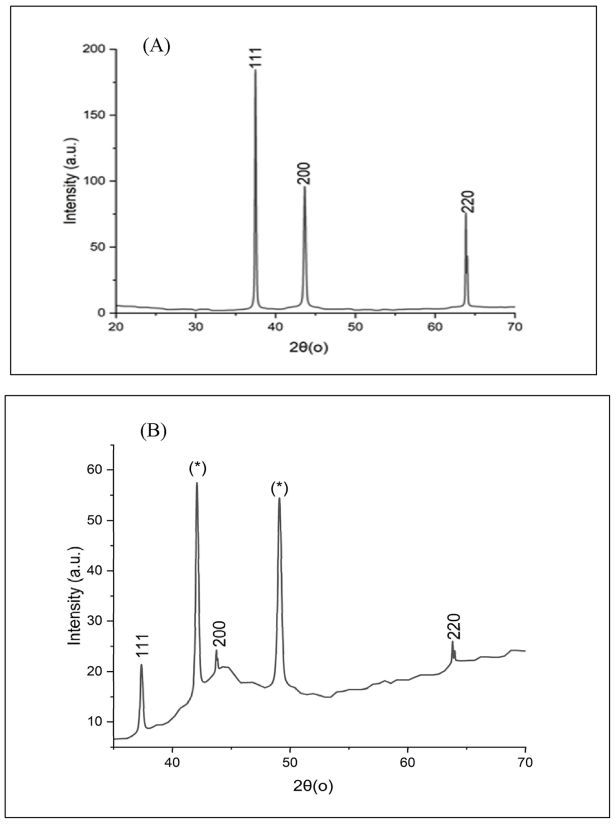

Fig. 7. XRD mediated (A) Leaf (B) Root aqueous Lactuca serriola L. plant extract. (*) unsigned peak.

$$D = \frac{0.94\lambda}{\beta \cos \theta} \quad (3)$$

When the crystalline dimension is D, the X-ray wavelength is λ (equal to 1.5406 Å), and the peak full width is β. As shown in Table 2, the average size (56.8 nm and 78.20 nm) of leaf-Ag NPs and root-Ag NPs, respectively, confirms nanocrystalline silver formation in both samples. The result was agreement with [48].

3.3. Antioxidant activity

Table 3 shows the reducing power of *Lactuca serriola* L. (leaf and root) extracts, as well as chloroform and ethanol. Ag NPs mediated by *Lactuca serriola* L. aqueous extract of leaf and root were measured at 0.625, 1.125, 2.5, and 5 mg/mL concentration by DPPH free-radical scavenging assay, using ascorbic acid as the positive control. Fig. 8 shows that the %DPPH radical-scavenging activity increased with increasing concentration in all samples. The result shows that leaf and root extract by chloroform and ethanolic, as well as leaf and root Ag NPs, are significantly ($p \leq 0.0001$) lower than ascorbic acid in all concentrations. Among the leaf extracts, ethanol showed maximum scavenging activity (74.24% at 5 mg/mL), followed by chloroform (59.82%) and Ag NPs (14.77%). Ethanol extract showed activity comparable to that of ascorbic acid (79–85%). In the root extracts, the chloroform fraction showed the highest activity (81.10% at 5 mg/mL), nearly as ascorbic acid (85.23%). While root ethanol extracts demonstrated moderate activity, Ag NPs exhibited reduction-scavenging activity, ranging from 30.65% to 33.27%. Collectively, both plant parts were shown to exhibit dose-dependent antioxidant activity, with the leaf ethanol and root chloroform extracts exhibiting activity similar to that of ascorbic acid at maximum concentrations. In contrast, Ag NPs showed a weaker radical scavenging effect. Fig. 9 showed that the lower IC 50 value indicated a more potent antioxidant. There is a significant difference ($P \leq 0.05$) between (L. L. Ag NPs and ascorbic acid) with other treatments; however, no significant difference ($P \geq 0.05$) of (leaf and root chloroform and ethanol extract) and between (leaf ethanol extract and Root-Ag NPs). Among the samples tested, the ethanol extract of *Lactuca serriola* L. exhibited the most acceptable radical scavenger activity with a value of 2.59 mg/mL. In contrast, its chloroform extract followed with 3.155 mg/mL. While the *L. serriola* leaf Ag NPs exhibited the weakest antioxidant activity, with an IC 50 of 18.18 mg/mL. For root *L. serriola*, the chloroform extract presented the most potent antioxidant activity of 2.1708 mg/mL, which is even lower than the values found in its root Ethanol extract and root Ag NPs. From the assay, ascorbic acid, the standard, revealed more significant antioxidant activity, with a value of IC 50 (0.0441 mg/mL), confirming that the assay was successful. The variations in extract antioxidant activity might be related to solvent polarity and relative phytochemical content. Ethanol and chloroform can extract a wider range of phenolic acids, which are the main compounds contributing to antioxidant activity. These might supply hydrogen atoms or electrons to stabilize the radicals, thereby protecting against oxidative stress. The lower activity observed in the aqueous and Ag NPs forms

Table 2. Estimation of the crystallite size (Ag NPs).

NPs	NO.of peaks	planes	Pos. (°2Th.)	FWHM (°2Th.)	Size (nm)
Leaf-Ag NPs	1	111	37.4603	0.1378	60.8
	2	200	43.6283	0.2362	35.2
	3	220	63.8485	0.1181	74.3
			Average size		**56.8**
Root-Ag NPs	1	111	37.3524	0.2362	35.50
	2	200	43.7360	0.0900	95.11
	3	220	63.8111	0.0900	103.98
			Average size		**78.20**

Table 3. DDPH scavenging activity % of Lactuca serriola L. plant and Ag NPs.

Plant parts	solvents	DPPH scavenging activity %			
		Concentration (mg/mL)			
		0.625	1.25	2.5	5
Leaf	Ethanol	10.29 ****	24.89****	67.48 ****	74.24 ****
	Chloroform	26.72 ****	31.25****	52.90 ****	59.82 ****
	Ag NPs	6.91 ****	7.86****	10.04 ****	14.77 ****
Root	Ethanol	11.04 ****	16.85****	33.98****	50.10****
	Chloroform	30.28 ****	39.73 ****	73.41****	81.10 ****
	Ag NPs	1.903 ****	7.21****	24.74 ****	33.27 ****
Ascorbic acid		34.40	78.22	79.22	85.23

Values are means, Symbols *: indicate significant difference between treatment and control data (ascorbic acid). All data were significant at $P < 0.05$ (*), $P < 0.01$ (**), $P < 0.001$ (***), and $P < 0.0001$(****).

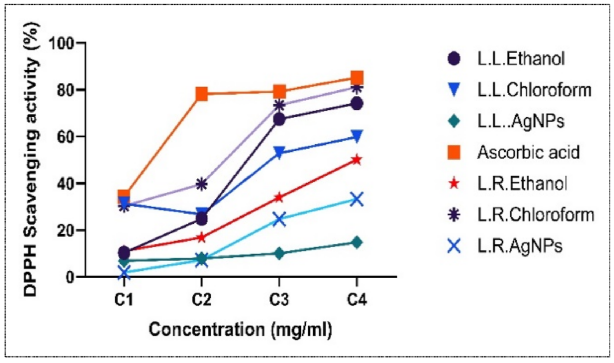

Fig. 8. DPPH scavenging activity % increased with increasing concentration. L= Lactuca serriola L., L=Leaf, R=Root.

could be due to lower yields of non-polar secondary metabolites during extraction and to chemical variation in phenolics during nanoparticle formation [49]. However, the antioxidant properties of *Lactuca serriola* L. plant extract alone exhibited much higher levels of scavenging activity compared to that of silver nanoparticles. A similar result was shown when using grapefruit pomace extract-Ag NPs and *Thymus vulgaris* or *Thymus citriodorus* [50].

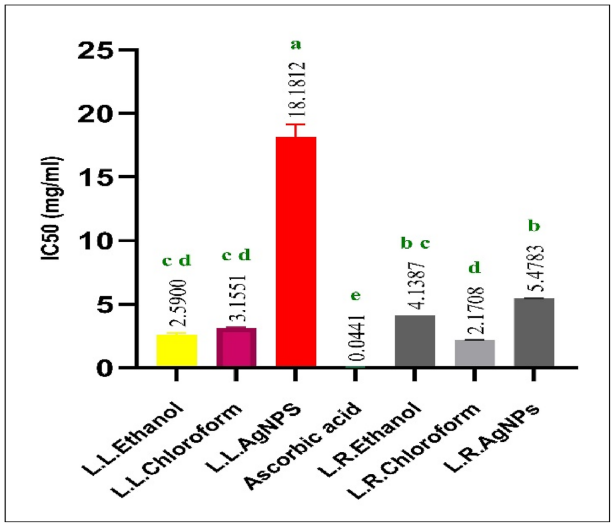

Fig. 9. Comparison of IC50 values for chloroform and ethanol extracts of Lactuca serriola L. roots and leaves, their corresponding Ag NPs, and ascorbic acid. Values are means with three replications; means followed by the same letter are not significantly different at P<0.05. L = Lactuca serriola L., L = Leaf, R = Root.

3.4. Minimum inhibition concentration (MIC) of Lactuca serriola L. leaf and root extract and Ag NPs on Aspergillus sp.

The MIC of Ag NPs and *Lactuca serriola* L. extract on *Aspergillus* sp. is observed in Table 4. The most antifungal activity of crude extracts of *Lactuca serriola* L. leaves, chloroform, and roots-ethanolic extract with *Aspergillus* sp., is moderate and most often within 25–50 mg/mL of MIC. The most active crude extract was leaf-ethanol 12.5 mg/mL agents *A. terreus*. The change in plant part and solvent slightly shifts the activity, indicating that the antifungal metabolites are solvent- and tissue-dependent. The power of Ag NPs after green synthesis increased dramatically to 6.25 mg/mL for all leaf-Ag NP species, and reduced root-Ag NPs to 1.56 mg/mL for *A. niger* and *A. flavus*, making them more effective than crude extracts. 'This activity is consistent with previous studies, which report that plant-mediated Ag NPs act via membrane damage and ROS generation [14]. The mechanism of Ag NPs, which affects antifungal activity, mainly relies on the induction of reactive oxygen species (ROS) and other free radicals, thereby increasing intracellular oxidative damage. Increased levels of ROS damage key cellular constituents such as lipids, proteins, and nucleic acids, causing structural and functional alterations that lead to eventual cell death. Although the involvement of ROS is well established, the precise molecular mechanisms underlying ROS-mediated antifungal activity remain poorly understood [51]. The toxic response observed with the accumulation of Ag^{+} ions may be due to their

Table 4. Minimum inhibition concentration (MIC) of Lactuca serriola L. extract and Ag NPs on Aspergillus sp.

plant	Plant parts	solvents	MIC (mg/mL)			
			Aspergillus sp.			
Lactuca serriola L.			*A. Niger*	*A. flavus*	*A. fumigatus*	*A. terreus*
	Leaf	Ethanol	50	25	50	12.5
		Chloroform	25	25	50	25
	Root	Ethanol	25	25	50	50
		Chloroform	50	50	25	25
	Leaf-Ag NPs		6.25	6.25	6.25	6.25
	Root -Ag NPs		1.56	1.56	3.125	25

binding to the microbial membrane, thereby inhibiting cellular signaling and mitochondrial respiration. Overproduction of free radicals leads to direct damage to the mitochondrial membrane, eventually resulting in necrotic cell death. The heads of these cells are also hyperoxidized across lipids, proteins, and DNA due to higher-than-normal ROS levels in those cells [52]. High levels of ROS production as a consequence of mitochondrial stress, ER stress, and inactivation of antioxidant enzymes amplify genotoxic effects. Genotoxicity induced by nanoparticles can lead to chromosomal anomalies, including DNA strand disruption, oxidative damage to DNA bases, and mutations [53]. And their nanoscale size is illustrated in Figs. 4 and 5. The reference [29] investigated various *Lactuca* sp. plants against different pathogenic fungi. Reference [54] reported that Ag NPs at 75 ppm completely suppressed mycelial development and spore germination of four kiwifruit rot pathogens. At the same time, SEM and TEM analyses revealed increased membrane permeability, intracellular leakage, hyphal deformation, and vacuolation, indicating severe cellular and organelle damage.

4. Conclusion

Lactuca serriola L. is one of the most important medicinal plants, containing many bioactive compounds used in various therapeutic applications. This study concluded that *Lactuca serriola* L. exhibits anti-aspergillus activity and antioxidant activity comparable to that of the chemical antioxidant ascorbic acid. The modern ultrasonic-assisted extraction method provides an effective approach to the extraction of phenol, flavonoid, saponin, resin, and tannin from plant leaves and roots, as confirmed by the ethanolic and chloroform extracts. As a result, plant-mediated synthesis of silver nanoparticles was successfully achieved using aqueous extracts of *Lactuca serriola* L. leaf and root, which are eco-friendly, non-toxic, and low-cost. Furthermore, Ag NPs exhibited good antioxidant and antifungal activity against four Aspergillus species. Crude extracts and Ag NPs can be used in the development of new antifungal agents and antioxidants. Further studies may be needed to confirm these findings and explore other potential treatment options for different diseases. Future in vivo studies are necessary to validate the safety and efficacy of these extracts and Ag NPs, thereby enabling the translation of in vitro results into real-world clinical and agricultural applications.

Ethical clearance

Local ethical committee approval was obtained from Salahaddin University/College of Science.

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Conflict of interest

The authors declared that no competing interests exist.

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