



# OPEN Endemic *Lilium ciliatum* as a novel biogenic platform for microwave-assisted green synthesis of antioxidant and antimicrobial silver nanoparticles

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Silver nanoparticles (AgNPs) are widely investigated as antimicrobial nanomaterials; however, their biological performance is strongly influenced by synthesis route, surface chemistry, and the nature of the stabilizing matrix. In this study, leaf and flower extracts of the endemic plant *Lilium ciliatum* P.H. Davis were used as distinct biogenic media for the microwave-assisted green synthesis of AgNPs. The extracts were obtained by ethanol-modified supercritical CO<sub>2</sub> extraction and chemically profiled by high-performance liquid chromatography coupled with diode-array detection (HPLC–DAD). Leaf extracts were mainly characterized by flavonoid-type compounds, particularly rutin and quercetin, whereas flower extracts contained a higher total quantified phenolic content and were dominated by gallic, protocatechuic, and chlorogenic acids. AgNP formation was optimized using 2% extract solution, 2 mL extract volume, 0.3 mM AgNO<sub>3</sub>, PEG-200, 90 W microwave power, and slightly acidic pH conditions. Ultraviolet–visible (UV–Vis) spectra showed characteristic surface plasmon resonance bands at approximately 400–420 nm, confirming AgNP formation. Transmission electron microscopy (TEM) analysis revealed mainly spherical nanoparticles with mean diameters of 12.138 ± 2.138 nm for leaf-extract-mediated AgNPs and 15.096 ± 2.922 nm for flower-extract-mediated AgNPs. X-ray diffraction (XRD) analysis confirmed the face-centered cubic crystalline structure of metallic silver, whereas Fourier-transform infrared (FT-IR) results supported the association of plant-derived functional groups with the nanoparticle surface, and Dynamic light scattering (DLS) and zeta potential findings indicated the contribution of these surface features to colloidal behavior. Flower-extract-mediated AgNPs exhibited stronger antioxidant activity and higher total phenolic content than leaf-derived AgNPs. They also showed broader antimicrobial efficacy, with the lowest minimum inhibitory concentration (MIC) values against *Escherichia coli* and *Candida albicans* at 0.78125 mg mL<sup>−1</sup>. Overall, both leaf and flower-derived *L. ciliatum* extracts provide promising biogenic routes for producing AgNP-based functional nanomaterials, with leaf extracts favoring colloidal stability and flower extracts enhancing biological performance.

**Keywords** *Lilium ciliatum*, AgNPs, Green synthesis, Microwave-assisted synthesis, Supercritical CO<sub>2</sub> extraction, Antimicrobial activity, Antioxidant activity

The increasing demand for safer antimicrobial materials has intensified research on metal-based nanostructures that can inhibit microbial growth while offering tunable physicochemical properties. This demand is particularly relevant for healthcare-related materials, food-contact surfaces, active packaging systems, water treatment technologies, wound-care products, and functional coatings, where microbial contamination may compromise

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product performance and user safety<sup>1,2,3</sup>. Among metal nanoparticles, silver nanoparticles (AgNPs) occupy a distinctive position because they combine nanoscale surface reactivity with broad-spectrum biological activity against bacteria and fungi<sup>4,2,3</sup>. Their antimicrobial effects are generally associated with multiple and complementary mechanisms, including interaction with microbial membranes, release of Ag<sup>+</sup> ions, disruption of cellular permeability, induction of oxidative stress, and interference with vital biomolecular processes<sup>2,3</sup>. In addition to their antimicrobial potential, AgNPs exhibit useful optical and catalytic features, making them attractive for multifunctional material systems<sup>2,3</sup>. Despite these advantages, the practical use of AgNPs depends strongly on how they are synthesized. Conventional chemical routes can provide good control over particle formation, but they often involve strong reducing agents, synthetic stabilizers, organic solvents, or reaction conditions that may limit environmental compatibility and increase concerns regarding residual chemicals<sup>5,6,7</sup>. For this reason, green synthesis strategies have become increasingly important in AgNP research<sup>1,2</sup>. In plant-mediated synthesis, naturally occurring metabolites such as phenolic acids, flavonoids, proteins, carbohydrates, organic acids, and reducing sugars may act as electron donors during the reduction of Ag<sup>+</sup> to Ag<sup>0</sup> and may also contribute to surface capping after particle formation<sup>4,8,1,2</sup>. This dual role makes plant extracts attractive alternatives to synthetic reducing and stabilizing agents. However, the biological origin of the extract also introduces variability, since the chemical composition of the plant material may differ depending on species, plant organ, extraction method, and processing conditions<sup>1,2</sup>. Recent studies on bio-derived noble metal nanoparticles have also demonstrated that plant-based extracts can provide an environmentally compatible route for producing functional nanoparticles with application-oriented properties<sup>9,10</sup>. A key challenge in plant-based AgNP synthesis is therefore not only to produce nanoparticles, but also to obtain reproducible particles with controlled size, surface chemistry, colloidal behavior, and biological performance<sup>1,2</sup>. The selection of extraction technique is important in this context. Supercritical CO<sub>2</sub> extraction modified with a small amount of ethanol offers a relatively mild and cleaner approach for obtaining phytochemical-rich fractions while reducing the dependence on large volumes of conventional organic solvents<sup>11</sup>. When such extracts are combined with microwave-assisted synthesis, the reduction process can be accelerated through rapid and more homogeneous heating<sup>9,11</sup>. Microwave irradiation may promote nucleation, shorten reaction time, and improve process efficiency compared with conventional heating<sup>9,3,11</sup>. Therefore, the combination of ethanol-modified supercritical CO<sub>2</sub> extraction and microwave-assisted synthesis represents a promising route for preparing plant-derived AgNPs under more sustainable conditions<sup>11</sup>.

The genus *Lilium* includes several species known for their ornamental, medicinal, and phytochemical value. Previous studies have reported that *Lilium* species contain various bioactive secondary metabolites, including phenolic compounds and flavonoids, which may contribute to antioxidant and biological activities<sup>12</sup>. Among these species, *Lilium ciliatum* P.H. Davis is an endemic plant of Türkiye and is naturally distributed in the Eastern Black Sea Region<sup>13</sup>. This endemic character makes *L. ciliatum* a valuable botanical resource and provides a strong rationale for investigating its potential in plant-mediated nanomaterial synthesis. From a green synthesis perspective, the selection of plant species and plant organ is highly important because the chemical composition of the extract may directly influence nanoparticle formation, surface capping, colloidal behavior, and biological activity<sup>1,2</sup>. Leaves and flowers may contain different groups and amounts of secondary metabolites. In the present study, this distinction was considered important because leaf and flower extracts were expected to provide different phytochemical environments for AgNP formation. Therefore, the use of both leaf and flower extracts allows the evaluation of plant-part-dependent effects on AgNP formation and biological performance.

Previous studies have shown that some *Lilium* species can serve as biogenic sources for AgNP synthesis. Al-Ansari,<sup>4</sup> synthesized AgNPs using *Lilium lancifolium* leaf extract and reported antibacterial and antibiofilm potential, while Du et al.,<sup>8</sup> prepared AgNPs using *Lilium casa blanca* petals and demonstrated antioxidant and surface-enhanced Raman scattering activities. However, AgNP synthesis using *L. ciliatum* leaf and flower extracts has not been sufficiently investigated. More importantly, limited information is available on how chemically distinct extracts obtained from different organs of an endemic *Lilium* species affect AgNP formation, colloidal properties, antioxidant activity, and antimicrobial efficacy. In this study, the endemic character of *L. ciliatum* was considered as a botanical and phytochemical rationale for selecting an underexplored plant resource rather than as a direct indicator of superior biological activity. The specific contribution of this work is the systematic comparison of leaf- and flower-extract-mediated AgNP synthesis in relation to phenolic profile, nanoparticle formation, colloidal behavior, antioxidant activity, and antimicrobial efficacy. Recent plant-mediated nanoparticle studies have also emphasized that bio-derived extracts can act not only as reducing agents but also as stabilizing and surface-modifying matrices that influence the functional properties of nanoparticles<sup>9,10</sup>. In this context, the present study introduces *L. ciliatum* as a novel endemic biogenic platform for the microwave-assisted green synthesis of AgNPs. By comparing leaf- and flower-extract-mediated AgNPs, this work provides a plant-specific and application-oriented contribution to the development of antioxidant and antimicrobial silver nanoparticles.

In this study, *Lilium ciliatum* leaf and flower extracts obtained by ethanol-modified supercritical CO<sub>2</sub> extraction were used for the microwave-assisted green synthesis of AgNPs. The study was designed to evaluate *L. ciliatum* not merely as a reducing source, but as a plant-derived platform capable of producing biologically active silver nanoparticles. For this purpose, the phenolic profiles of leaf and flower extracts were examined, and the synthesis process was optimized by considering extract concentration, extract volume, AgNO<sub>3</sub> concentration, microwave power, irradiation time, PEG-200 addition, and pH. The resulting AgNPs were characterized by UV-Vis spectroscopy, TEM, DLS, zeta potential analysis, XRD, and FT-IR spectroscopy to determine their optical behavior, morphology, size distribution, colloidal properties, crystalline structure, and surface functional groups. Their antioxidant capacity and antimicrobial activity against selected bacterial and fungal strains were also evaluated. Through the comparison of leaf- and flower-extract-mediated AgNP synthesis and their biological performance, this study provides a plant-specific contribution to green nanoparticle synthesis and supports

the evaluation of *L. ciliatum* as an underexplored endemic botanical resource for functional antimicrobial nanomaterials.

## Materials and methods

### Experimental design and botanical material

The experimental procedure was designed to obtain bioactive extracts from different organs of *Lilium ciliatum* P.H. Davis and to use these extracts as biogenic media for the microwave-assisted preparation of silver nanoparticles. Leaf and flower tissues were evaluated separately in order to determine whether plant-part-dependent differences affected AgNP formation and biological performance. Fresh *L. ciliatum* samples were collected from Haçkalı Baba Plateau in Düzköy, Trabzon, Türkiye, in June. The taxonomic identity of the plant material was confirmed by Prof. Dr. Salih Terzioğlu from the Department of Botany, Faculty of Forestry, Karadeniz Technical University. A voucher specimen was deposited in the Herbarium of the Faculty of Forestry, Karadeniz Technical University under the accession number KATO:19,481. Following collection, the leaf and flower parts were separated manually and washed several times with distilled water to remove dust, soil particles, and impurities. The washed plant materials were then dried under laboratory conditions at room temperature before extraction. No chemical sterilization procedure was applied in order to avoid possible alteration or loss of extractable phytochemical constituents.

### Preparation of leaf and flower extracts by ethanol-modified supercritical CO<sub>2</sub> extraction

Plant extracts were prepared using a supercritical fluid extraction system operated with carbon dioxide as the main extraction medium. Leaf and flower samples were processed independently to prevent cross-contamination between plant organs. For each extraction run, 30 g of fragmented plant material was placed into the extraction vessel. The system was operated at 150 bar and 40 °C, and 0.5 mL ethanol was used as a polarity modifier to improve the recovery of relatively polar phytochemicals<sup>11</sup>. The extract residue obtained after extraction was dissolved/dispersed in distilled water and used for AgNP synthesis. To determine the extraction duration to be used in the study, preliminary extraction trials were carried out for 2, 3, 4, and 6 h. Based on these preliminary trials, a 3 h extraction period was selected for the preparation of the leaf and flower extracts used in the subsequent HPLC–DAD analysis and AgNP synthesis experiments. This extraction duration provided a sufficient extract fraction for downstream analyses and a reproducible phytochemical profile suitable for nanoparticle formation. The extracts obtained from leaf and flower tissues were collected separately and stored under suitable conditions until use in phenolic analysis and AgNP synthesis.

### HPLC–DAD analysis of phenolic constituents

The phenolic profiles of the leaf and flower extracts were determined by high-performance liquid chromatography coupled with diode-array detection (HPLC–DAD). HPLC–DAD analysis was carried out using an Agilent 1260 series HPLC system equipped with a 1260 DAD WR detector, 1260 quaternary pump, 1260 autosampler, and G7116A thermostatic column compartment (Agilent Technologies, USA). Chromatographic separation was performed on an ACE 5 C18 reversed-phase column (250 × 4.6 mm, 5 µm). The column temperature was maintained at 35 °C, the flow rate was set to 0.7 mL min<sup>−1</sup>, and the injection volume was 10 µL. Two mobile-phase systems were used to improve compound detection. In the first chromatographic program, acetonitrile was used as solvent A and water containing 1.5% acetic acid was used as solvent B. In the second program, methanol was used as solvent A and water containing 1.5% acetic acid was used as solvent B. Detection was performed at 250, 270, and 320 nm for the acetonitrile-based method and at 280, 290, 320, 370, and 535 nm for the methanol-based method, according to the procedure described by Aytar et al.,<sup>14</sup>. The resulting chromatographic data were used to compare the phenolic composition of the leaf and flower extracts before nanoparticle synthesis.

### Microwave-assisted preparation of AgNPs

Aqueous leaf and flower extract solutions were prepared at concentrations of 1% and 2% and evaluated separately as biogenic reaction media for AgNP formation. In preliminary experiments, both extract concentrations were screened for their ability to induce AgNP formation under microwave irradiation. For the synthesis experiments, extract volumes ranging from 0.1 to 6.0 mL were added to 100 mL of aqueous AgNO<sub>3</sub> solutions. AgNO<sub>3</sub> concentrations ranging from 0.01 to 10 mM were evaluated to identify the most suitable concentration range for nanoparticle formation.

The reaction mixtures were exposed to microwave irradiation using a domestic microwave oven at power levels of 90, 180, and 360 W. Irradiation times ranging from 1 to 60 min were tested under different pH conditions. AgNP formation was monitored visually by the color change from pale yellow to brown and spectroscopically by the appearance of the characteristic surface plasmon resonance (SPR) band in the UV–Vis spectra. The evaluated synthesis parameters included extract concentration, extract volume, AgNO<sub>3</sub> concentration, microwave power, irradiation time, pH, and PEG-200 addition. Based on the position, intensity, definition, and reproducibility of the SPR band, together with the observed dispersion behavior, 90 W was selected as the most suitable microwave power within the tested range. PEG-200 was included as an auxiliary stabilizer to support colloidal stabilization and reduce aggregation tendency.

Each optimization condition was evaluated in three replicate experiments conducted under identical conditions. The UV–Vis spectra presented in Figs. 2 and 3 are representative of these replicate experiments. Control experiments were performed under the same reaction conditions using AgNO<sub>3</sub> alone, leaf extract alone, flower extract alone, PEG-200 alone, and AgNO<sub>3</sub>/PEG-200 mixtures without plant extract. These controls were used to determine whether the characteristic SPR band of AgNPs could form in the absence of the complete plant-extract-mediated reaction system.

### Physicochemical characterization of AgNPs

The optical, morphological, colloidal, and structural properties of the synthesized AgNPs were examined using complementary characterization techniques. UV–Vis spectra were recorded using a Shimadzu UVP-1240 UV–Vis spectrophotometer (Shimadzu, Japan) in the wavelength range of 300–800 nm to monitor nanoparticle formation and surface plasmon resonance behavior. Microwave-assisted synthesis was performed using a Bosch domestic microwave oven (Bosch, Germany) operated at 90, 180, and 360 W. Hydrodynamic particle size, polydispersity index (PDI), and zeta potential were measured using a Zetasizer Nano ZSP analyzer (Malvern, United Kingdom) to evaluate dispersion quality and colloidal stability. Since plant-mediated nanoparticle systems contain both metallic cores and surface-associated biomolecules, DLS values were interpreted as hydrodynamic diameters rather than direct particle core sizes. The morphology and primary particle size of the AgNPs were examined using a Hitachi HT-7700 transmission electron microscope equipped with a LaB<sub>6</sub> electron source (Hitachi, Japan). Particle size distributions were calculated from TEM images to determine the average core size of the nanoparticles. The crystalline structure of the synthesized AgNPs was investigated using a PANalytical X'Pert<sup>3</sup> Powder X-ray diffractometer (PANalytical, Netherlands) with Cu K $\alpha$  radiation ( $\lambda = 1.54059 \text{ \AA}$ ) at 45 kV and 40 mA. Diffraction patterns were used to identify the metallic silver phase, and crystallite size was estimated using the Debye–Scherrer equation. FT-IR spectra were recorded using a PerkinElmer Frontier FT-IR spectrometer (PerkinElmer, USA) in the range of 4000–550 cm<sup>-1</sup> to identify surface-associated functional groups and evaluate the possible involvement of plant-derived biomolecules in AgNP reduction and stabilization<sup>15</sup>.

### Antioxidant activity and total phenolic content

The antioxidant activity of the extracts and synthesized AgNPs was evaluated using the 2,2-diphenyl-1-picrylhydrazyl (DPPH) radical scavenging activity radical scavenging assay<sup>16</sup>. Sample aliquots were mixed with DPPH solution and incubated in the dark. After incubation, absorbance was measured at 517 nm. The radical scavenging capacity was expressed as IC<sub>50</sub>, and Trolox was used as the reference antioxidant standard. Total phenolic content was determined using the Folin–Ciocalteu method<sup>17</sup>. Gallic acid was used to prepare the calibration curve, and absorbance was measured at 760 nm. The results were expressed as milligrams of gallic acid equivalents (GAE) per gram of dry sample weight.

### Antimicrobial activity assays

The antimicrobial activity of the synthesized AgNPs was tested against selected Gram-positive bacteria, Gram-negative bacteria, and yeast strains. The tested microorganisms included *Staphylococcus aureus*, *Enterococcus faecalis*, *Escherichia coli*, *Pseudomonas aeruginosa*, and *Candida albicans*. Minimum inhibitory concentration values were determined using the broth microdilution method in 96-well microplates in accordance with CLSI guidelines<sup>18</sup>. AgNP samples were prepared as two-fold serial dilutions, and microbial suspensions adjusted to 0.5 McFarland standard were added to the wells. After incubation under appropriate conditions, microbial growth was evaluated, and the MIC was defined as the lowest concentration that inhibited visible microbial growth. Disk diffusion assays were also performed on Mueller–Hinton agar. Microbial suspensions were spread uniformly onto agar plates, and sterile disks loaded with AgNP solutions were placed on the inoculated surfaces. After incubation, inhibition zone diameters were measured in millimeters. Imipenem and fluconazole were used as positive controls, while DMSO was used as the negative control.

### Statistical analysis

All quantitative experiments were performed in triplicate ( $n = 3$ ), where applicable, and the results are presented as mean  $\pm$  standard deviation (SD). Statistical comparisons between two independent groups were performed using Welch's t-test. Differences were considered statistically significant at  $p < 0.05$ . Multi-level UV–Vis optimization experiments and MIC results were evaluated descriptively.

## Results and discussion

### Phenolic composition of *Lilium ciliatum* extracts

HPLC–DAD analysis was performed to determine the phenolic constituents of *Lilium ciliatum* leaf and flower extracts used in AgNP synthesis. To the best of our knowledge, a comparative HPLC–DAD profile of phenolic and flavonoid constituents in *Lilium ciliatum* leaf and flower extracts specifically used for AgNP synthesis has not been previously reported in detail. Therefore, this comparison provides a phytochemical basis for evaluating plant-organ-dependent effects on AgNP formation and biological performance. The quantified HPLC–DAD results are summarized in Table 1, and representative chromatograms with peak labels and retention times are shown in Fig. 1. The results showed that the two extracts differed considerably in both total quantified phenolic content and dominant compound classes. The leaf extract contained a total quantified phenolic content of 34.96 mg L<sup>-1</sup> and was mainly characterized by flavonoid-type compounds. Rutin and quercetin were the most abundant constituents, with concentrations of 13.83 mg L<sup>-1</sup> and 9.79 mg L<sup>-1</sup>, respectively. Pyrogallol was also detected at 6.99 mg L<sup>-1</sup>, whereas baicalein, (+)-catechin, rosmarinic acid, and myricetin were present at lower levels. This profile indicates that the leaf extract provided a flavonoid-rich reaction medium for AgNP formation.

In contrast, the flower extract showed a higher total quantified phenolic content of 65.08 mg L<sup>-1</sup> (Table 1). The dominant compounds in this extract were phenolic acids, particularly gallic acid, protocatechuic acid, and chlorogenic acid, with concentrations of 19.88, 15.84, and 11.00 mg L<sup>-1</sup>, respectively. In addition, (-)-epicatechin and quercetin were detected at notable levels, while pyrogallol and trans-cafeic acid were present in smaller amounts. These findings demonstrate that the flower extract had a phenolic acid-rich composition compared with the leaf extract. The difference between the two extracts is important for AgNP synthesis because phenolic acids and flavonoids can contribute to both silver ion reduction and nanoparticle surface stabilization. The leaf and flower extracts exhibited distinct phenolic profiles, which may contribute to the differences observed

| No. | Compound                   | Compound class    | Leaf extract (mg L <sup>-1</sup> ) | Flower extract (mg L <sup>-1</sup> ) | Dominant source |
|-----|----------------------------|-------------------|------------------------------------|--------------------------------------|-----------------|
| 1   | Pyrogallol                 | Phenolic compound | 6.99                               | 2.00                                 | Leaf            |
| 2   | Rosmarinic acid            | Phenolic acid     | 0.82                               | n.d.                                 | Leaf            |
| 3   | Rutin                      | Flavonoid         | 13.83                              | n.d.                                 | Leaf            |
| 4   | Quercetin                  | Flavonoid         | 9.79                               | 5.84                                 | Leaf            |
| 5   | Baicalein                  | Flavonoid         | 2.18                               | n.d.                                 | Leaf            |
| 6   | (+)-Catechin               | Flavonoid         | 0.91                               | n.d.                                 | Leaf            |
| 7   | Myricetin                  | Flavonoid         | 0.44                               | n.d.                                 | Leaf            |
| 8   | Gallic acid                | Phenolic acid     | n.d.                               | 19.88                                | Flower          |
| 9   | Protocatechuic acid        | Phenolic acid     | n.d.                               | 15.84                                | Flower          |
| 10  | Chlorogenic acid           | Phenolic acid     | n.d.                               | 11.00                                | Flower          |
| 11  | Trans-cafeic acid          | Phenolic acid     | n.d.                               | 0.49                                 | Flower          |
| 12  | (-)-Epicatechin            | Flavonoid         | n.d.                               | 10.03                                | Flower          |
|     | Total quantified phenolics |                   | 34.96                              | 65.08                                | Flower          |

**Table 1.** HPLC–DAD-based comparative profile of quantified phenolic and flavonoid compounds in *Lilium ciliatum* leaf and flower extracts used as biogenic media for AgNP synthesis. n.d.: not detected or below the quantification limit under the applied HPLC–DAD conditions. Total quantified phenolic/flavonoid compounds indicate the sum of all quantified compounds detected in each extract. The dominant source was assigned according to the extract showing the higher quantified concentration for each compound.

between the corresponding AgNP systems. Therefore, the higher antioxidant and antimicrobial performance observed for flower-extract-mediated AgNPs can be partly related to the richer phenolic composition of the flower extract. Overall, the HPLC–DAD results indicate that *L. ciliatum* leaves and flowers provide chemically different phytochemical environments for AgNP synthesis. The leaf extract was mainly flavonoid-dominant, while the flower extract was richer in phenolic acids and had a higher total phenolic content. These compositional differences provide supportive context for interpreting the observed AgNP properties.

### UV–Vis-guided optimization of microwave-assisted AgNP formation

UV–Vis spectroscopy was used to monitor AgNP formation and identify the most suitable synthesis conditions within the tested experimental ranges. The appearance of a characteristic SPR band at approximately 400–420 nm, together with the color change of the reaction mixtures from pale yellow to brown or dark brown after microwave treatment, supported the formation of AgNPs. The first optimization step examined extract concentration. The 1% aqueous extracts did not produce sufficiently clear and reproducible SPR bands under the tested conditions, whereas the 2% leaf and flower extracts showed distinct and repeatable spectral responses. Therefore, 2% extract solutions were used in subsequent experiments. Extract volume also influenced the UV–Vis profiles. Among the tested volumes, 2 mL produced the most distinct and reproducible SPR bands for both leaf- and flower-mediated systems and was therefore selected for further synthesis experiments (Figs. 2a–c and 3a–c). AgNO<sub>3</sub> concentration affected the intensity and definition of the SPR bands. The most suitable spectral response was obtained at 0.3 mM AgNO<sub>3</sub> for both extract-mediated systems. Higher precursor concentrations produced broader and less regular absorption profiles; therefore, 0.3 mM was selected for subsequent experiments (Figs. 2d–f and 3d–f). PEG-200 addition improved SPR band definition compared with PEG-free samples and was consequently included in the selected formulation (Figs. 2g and 3g). Microwave power also influenced SPR development. Higher power levels produced broader and less regular spectra, whereas 90 W yielded the most distinct and reproducible SPR profiles. Accordingly, 90 W was selected for subsequent synthesis (Figs. 2h–j and 3h–j). Under these conditions, slightly acidic media produced the most favorable spectral responses, with pH values of approximately 5.9 for the leaf-mediated system and 5.2 for the flower-mediated system (Figs. 2k and 3k). These pH values should be regarded as system-specific conditions within the investigated experimental range rather than as general optimum values for plant-mediated AgNP synthesis. In the control experiments, samples containing AgNO<sub>3</sub> only, leaf or flower extract only, PEG-200 only, or AgNO<sub>3</sub>/PEG-200 without plant extract did not exhibit the characteristic SPR band at approximately 400–420 nm. This observation supported the conclusion that AgNP formation occurred only in the complete reaction systems containing AgNO<sub>3</sub> and *Lilium ciliatum* extract under microwave irradiation. Overall, the most suitable synthesis conditions were identified as 2% extract solution, 2 mL extract volume, 0.3 mM AgNO<sub>3</sub>, PEG-200, 90 W microwave power, and slightly acidic pH. These conditions were selected based on the reproducibility, position, intensity, and definition of the SPR band, together with spectral broadening and observed dispersion behavior, rather than absorbance intensity alone. The SPR bands observed at approximately 400–420 nm are consistent with the characteristic absorption range previously reported for plant-mediated AgNPs<sup>4,8</sup>. Previous studies using *Lilium lancifolium* leaf extract and *Lilium casa blanca* petals similarly demonstrated the ability of *Lilium*-derived extracts to mediate AgNP formation<sup>4,8</sup>. Related studies on bio-derived noble-metal nanoparticles have also highlighted the importance of extract-derived constituents and synthesis conditions in nanoparticle formation and colloidal behavior<sup>9,10</sup>. Nevertheless, the proposed effects of pH, microwave power, extract composition, and PEG-200 should be regarded as possible explanations for the observed UV–Vis and colloidal behavior rather than as experimentally established mechanisms. PEG-200 functioned primarily as an auxiliary stabilizer rather than as an independent

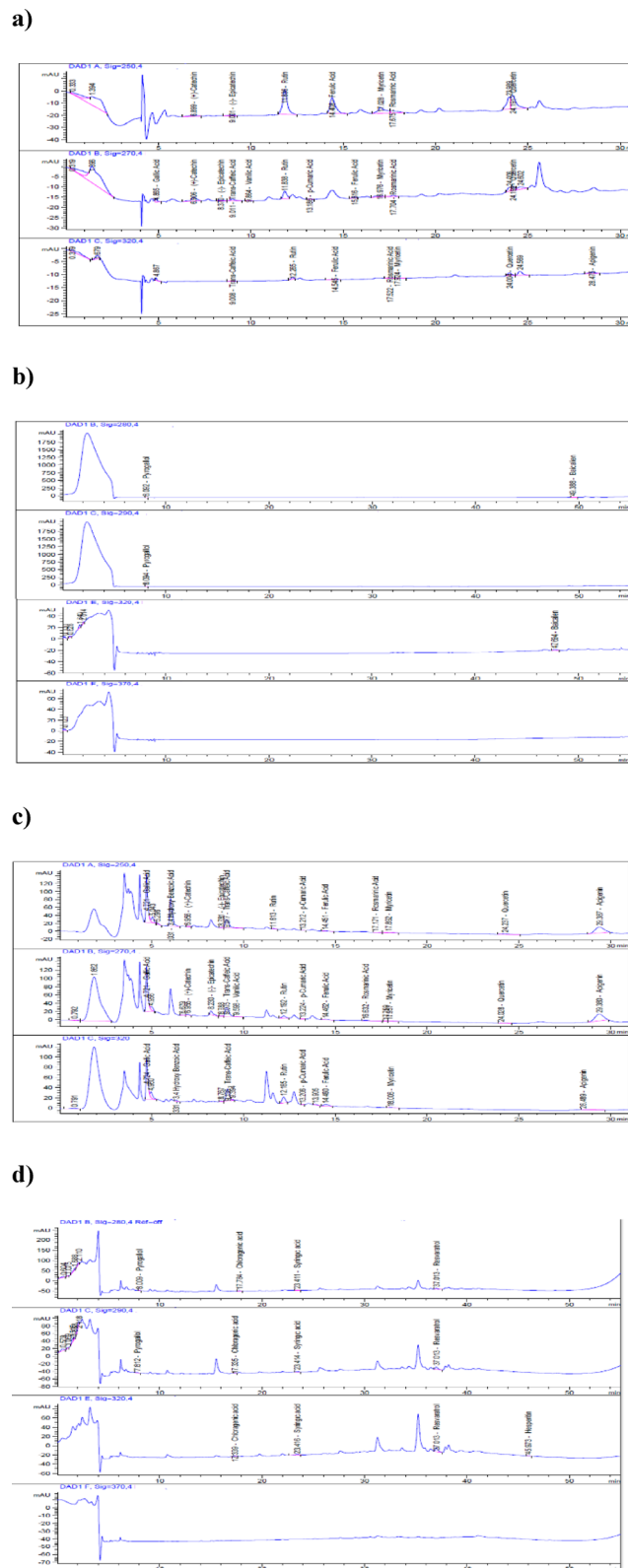


**Fig. 1.** Representative HPLC–DAD chromatograms of *Lilium ciliatum* extracts. **(a)** Leaf extract analyzed under chromatographic method 1, **(b)** leaf extract analyzed under chromatographic method 2, **(c)** flower extract analyzed under chromatographic method 1, and **(d)** flower extract analyzed under chromatographic method 2. Peak labels and retention times are shown on the chromatograms.

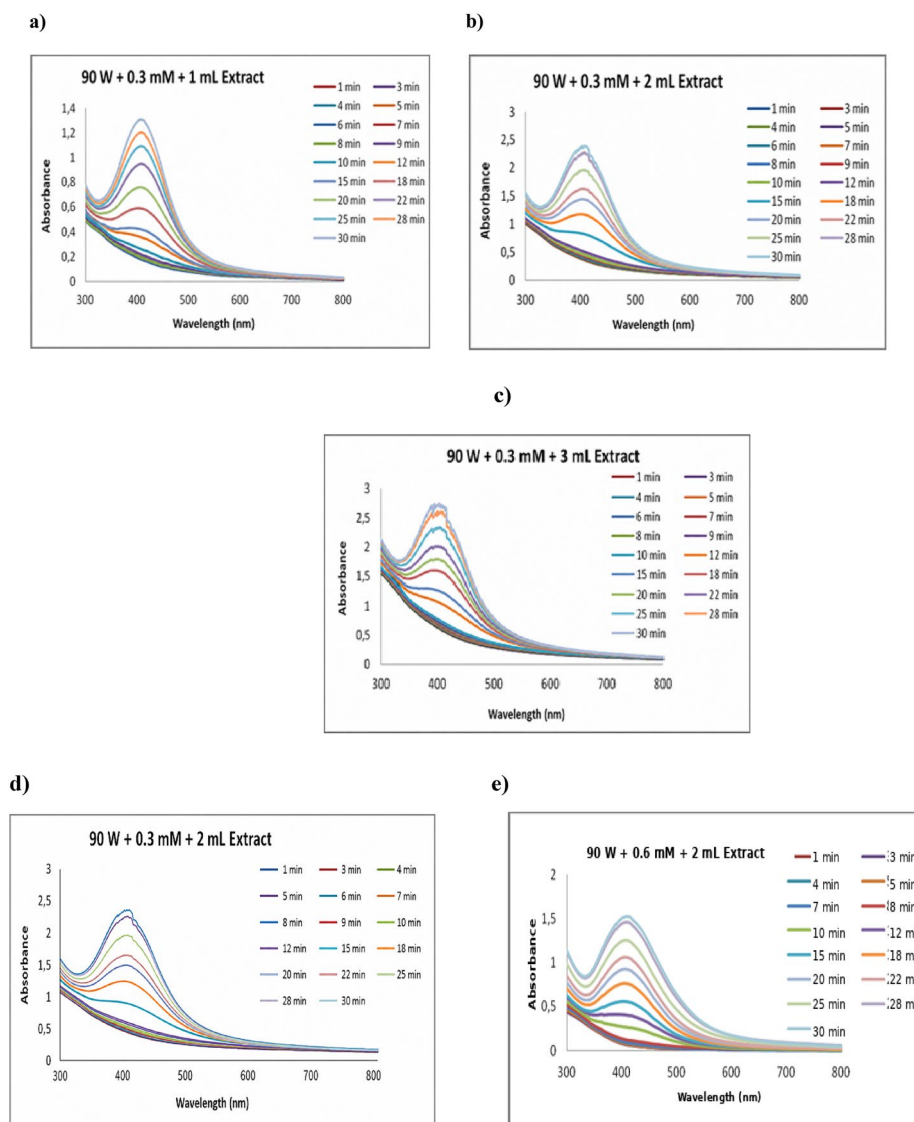


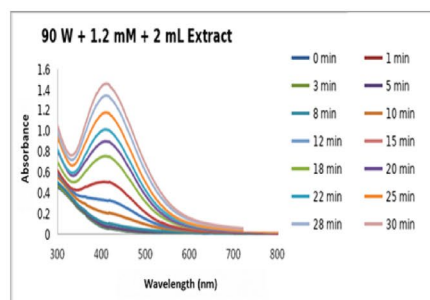
**Fig. 2.** UV-Vis-based optimization of AgNP synthesis using *Lilium ciliatum* leaf extract. Effects of (a–c) extract volume (1, 2, and 3 mL), (d–f) AgNO<sub>3</sub> concentration (0.3, 0.6, and 1.2 mM), (g) PEG-200 addition, (h–j) microwave power (90, 180, and 360 W), and (k) pH on AgNP formation are shown.

reducing agent or a plant-derived capping agent. However, because PEG-free AgNPs were not characterized by TEM, its specific influence on primary particle size and morphology could not be directly evaluated.

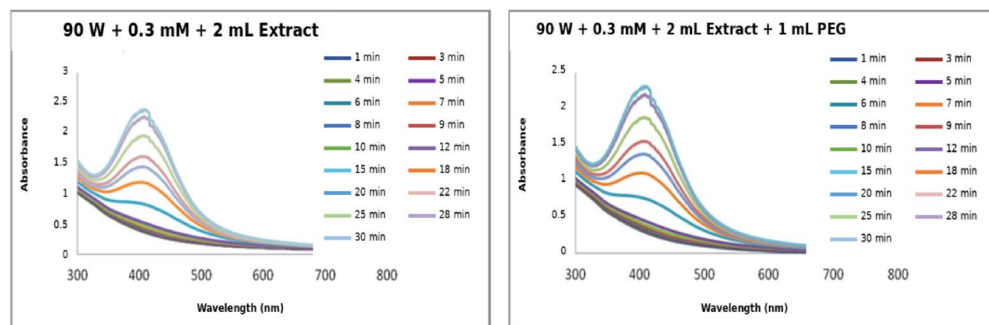
### Morphological features of AgNPs observed by TEM

TEM analysis was used to examine the morphology and primary particle size of the AgNPs synthesized under the selected microwave-assisted conditions. The micrographs showed that both leaf- and flower-extract-mediated systems produced nanoscale particles with predominantly spherical and slightly irregular spherical morphologies. No large bulk-like silver structures were observed in the examined TEM fields. The mean particle diameter was  $12.14 \pm 2.14$  nm for leaf-extract-mediated AgNPs and  $15.10 \pm 2.92$  nm for flower-extract-mediated AgNPs (Figs. 4 and 5). In both samples, most particles were distributed within the 10–20 nm range. The observed difference in mean particle size may be associated with variations in extract composition and synthesis conditions. These findings are consistent with previous studies on AgNP synthesis using other *Lilium* species. Du<sup>8</sup> reported predominantly spherical AgNPs with an average size of  $5.60 \pm 1.50$  nm using *Lilium casa blanca* petals, whereas Al-Ansari et al.<sup>4</sup> obtained *Lilium lancifolium*-mediated AgNPs with a broader size range and more heterogeneous morphology. Compared with these reports, the AgNPs synthesized in the present study exhibited a relatively narrow size distribution and were smaller than those reported for *L. lancifolium*-mediated AgNPs. Overall, the TEM results demonstrate that both *L. ciliatum* leaf and flower extracts can mediate the formation of small, predominantly spherical AgNPs. The TEM-derived particle sizes were larger than the XRD-derived crystallite sizes, which is expected because TEM measures the overall physical particle dimensions, whereas XRD estimates the size of coherent crystalline domains. In plant-mediated nanoparticle systems, such

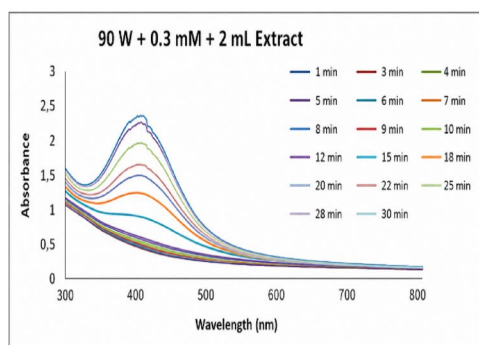
f)



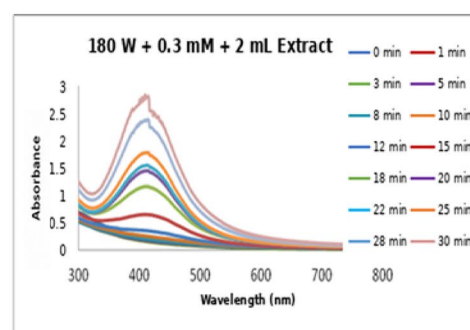
g)



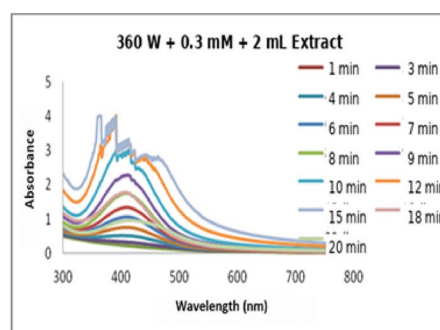
h)



i)



j)



k)

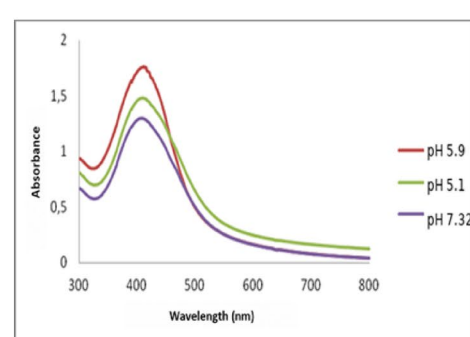


Fig. 2. (continued)

differences may also be associated with polycrystalline structures, structural defects, instrumental broadening, and surface-associated organic components<sup>9,10,1, 19,20,21</sup>.

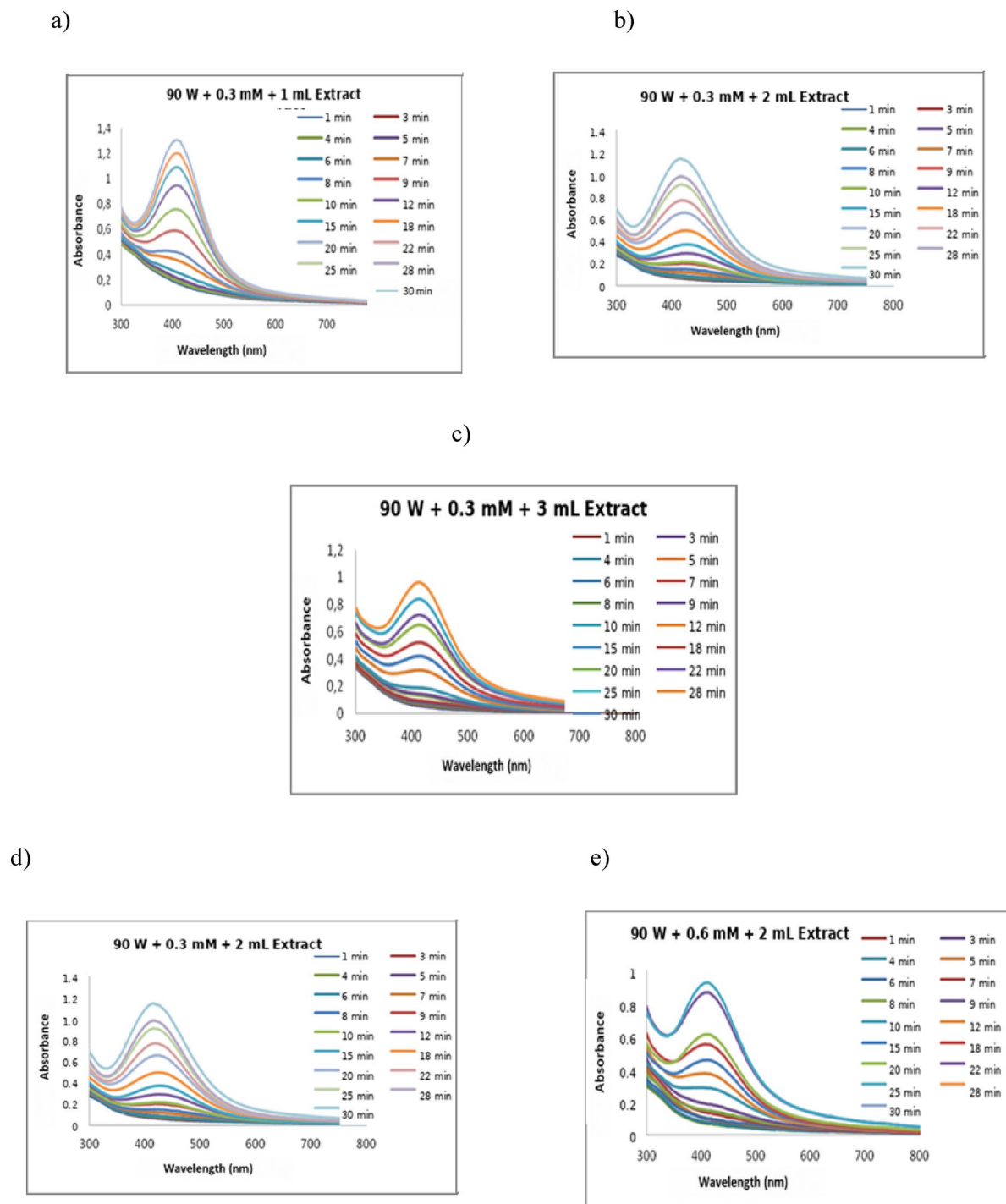
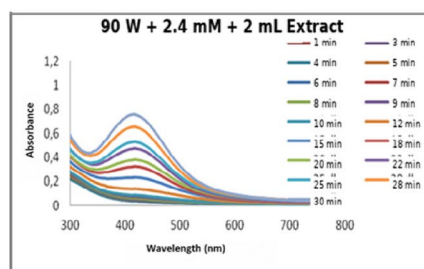


**Fig. 3.** UV-Vis-based optimization of AgNP synthesis using *Lilium ciliatum* flower extract. Effects of (a–c) extract volume (1, 2, and 3 mL), (d–f)  $\text{AgNO}_3$  concentration (0.3, 0.6, and 2.4 mM), (g) PEG-200 addition, (h–j) microwave power (90, 180, and 360 W), and (k) pH on AgNP formation are shown.

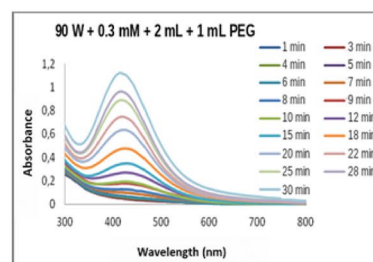
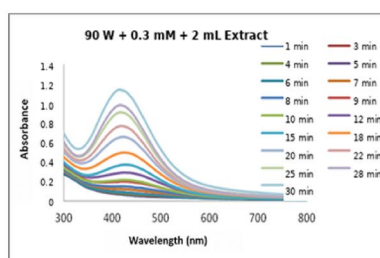
### Colloidal stability of AgNP dispersions

DLS and zeta potential measurements were used to evaluate how extract type, PEG-200 addition, and storage affected the colloidal properties of the synthesized AgNPs (Fig. 6). Since plant-mediated nanoparticle systems contain both metallic cores and surface-associated biomolecules, DLS values were interpreted as hydrodynamic diameters rather than direct particle core sizes. For leaf-extract-mediated AgNPs, the PEG-free sample showed a zeta potential of  $-11.7 \pm 0.056$  mV, a hydrodynamic diameter of  $99.44 \pm 3.16$  nm, and a PDI of  $0.568 \pm 0.0327$  (Fig. 6a, b). After PEG-200 addition, the hydrodynamic diameter decreased to  $29.19 \pm 0.690$  nm, while the zeta potential was  $-13.4 \pm 3.500$  mV and the PDI was  $0.627 \pm 0.008$  (Fig. 6c, d). After 2 months of storage, the PEG-200-assisted leaf AgNPs showed a hydrodynamic diameter of  $105.6 \pm 0.404$  nm, a zeta potential of  $-32.8 \pm 0.600$

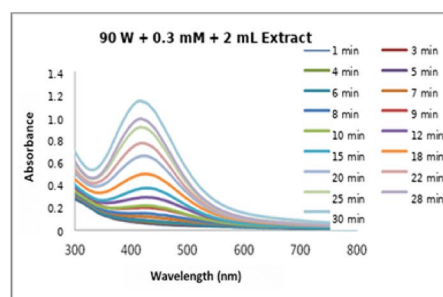
f)



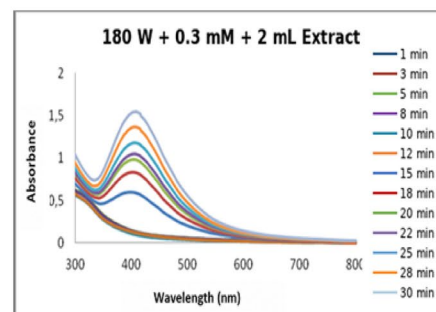
g)



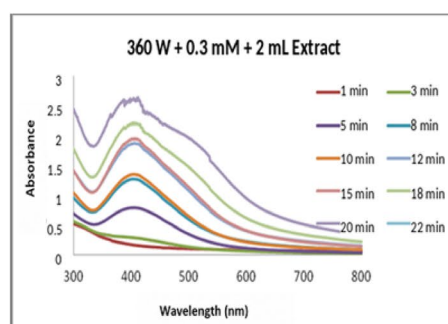
h)



i)



j)



k)

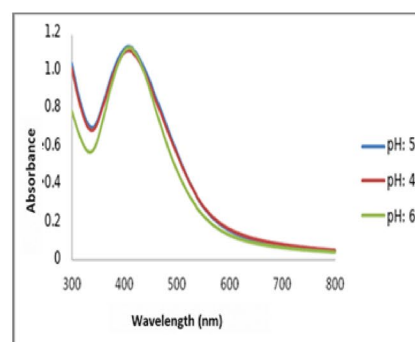


Fig. 3. (continued)

mV, and a PDI of  $0.268 \pm 0.009$  (Fig. 6e, f). For flower-extract-mediated AgNPs, the PEG-free sample exhibited a zeta potential of  $-16.6 \pm 1.779$  mV, a hydrodynamic diameter of  $62.23 \pm 1.56$  nm, and a PDI of  $0.483 \pm 0.009$  (Fig. 6g, h). The freshly prepared PEG-200-assisted flower AgNPs showed a hydrodynamic diameter of  $86.97 \pm 0.230$  nm, a zeta potential of  $-22.7 \pm 0.231$  mV, and a PDI of  $0.380 \pm 0.042$  (Fig. 6i, j). After 2 months of

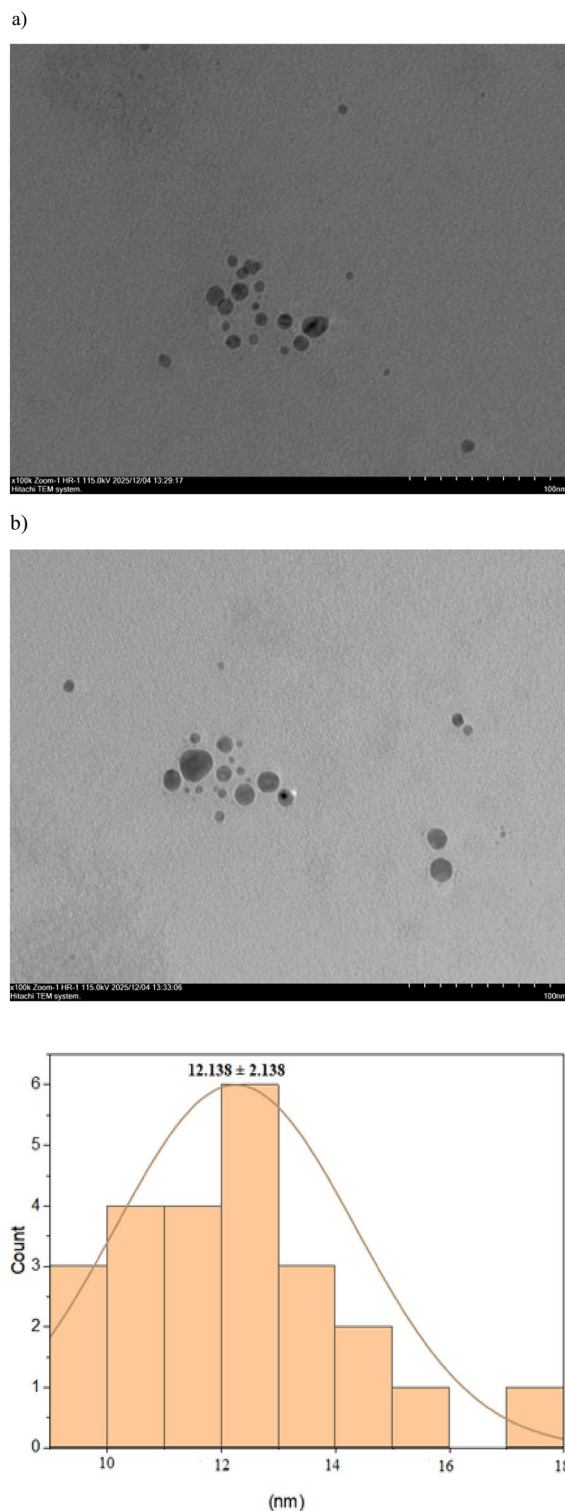


**Fig. 4.** TEM analysis of AgNPs synthesized using *Lilium ciliatum* leaf extract. (a) TEM image and (b) particle size distribution histogram of AgNPs prepared with 0.3 mM AgNO<sub>3</sub> and 2 mL leaf extract under microwave-assisted synthesis conditions.

storage, the hydrodynamic diameter was  $67.24 \pm 1.502$  nm, the zeta potential was  $-17.3 \pm 0.600$  mV, and the PDI was  $0.471 \pm 0.013$  (Fig. 6k, l). These results show that AgNP formation occurred even in the absence of PEG-200, confirming that PEG-200 was not the primary reducing component in the synthesis process. Instead, PEG-200 acted mainly as an auxiliary stabilizing agent and affected the colloidal behavior of the AgNPs in an extract-dependent manner. In the leaf-derived system, PEG-200 markedly reduced the initial hydrodynamic diameter,

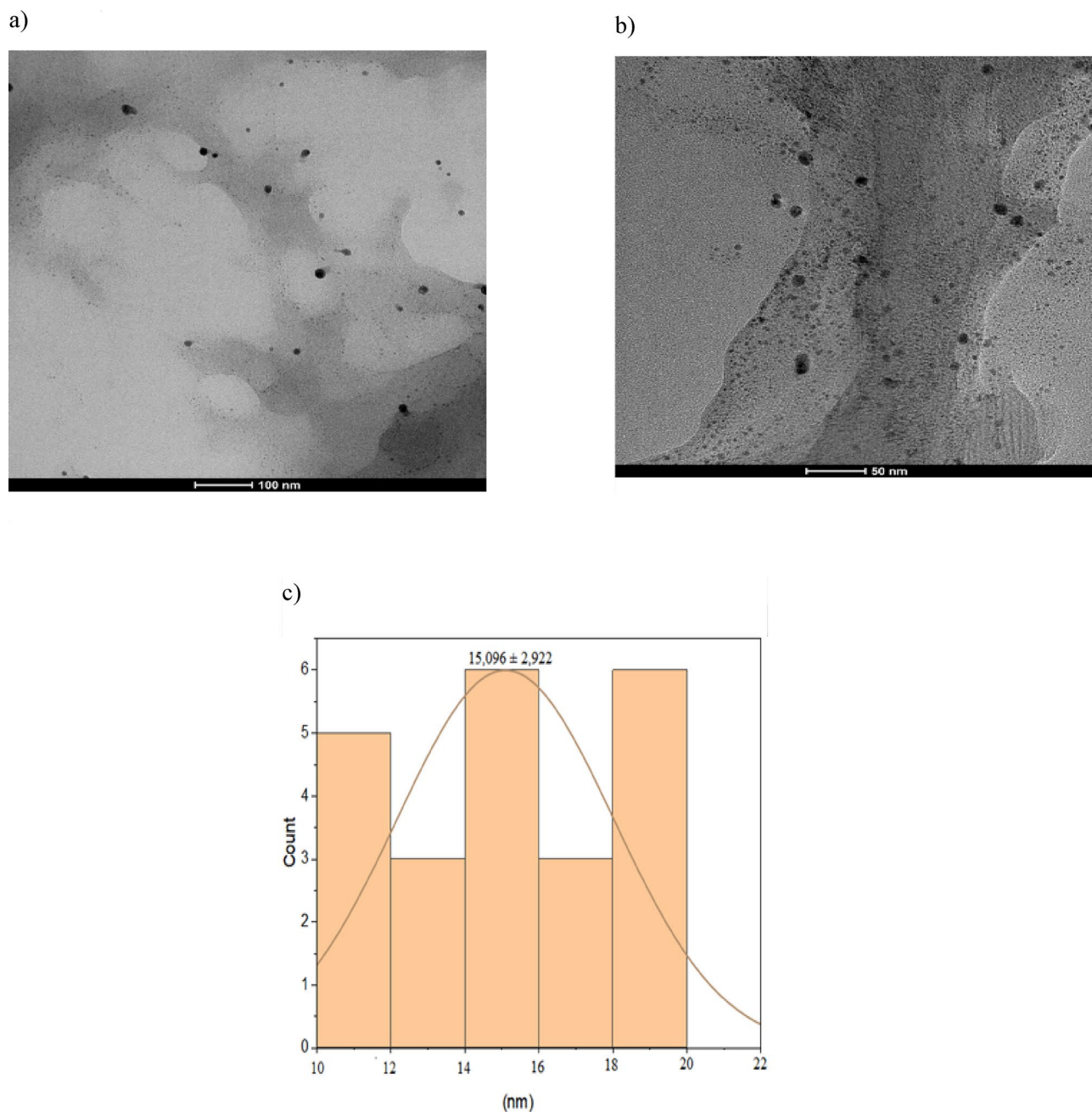


**Fig. 5.** TEM analysis of AgNPs synthesized using *Lilium ciliatum* flower extract. (a) TEM image and (b) particle size distribution histogram of AgNPs prepared with 0.3 mM AgNO<sub>3</sub> and 2 mL flower extract under microwave-assisted synthesis conditions.

whereas after storage the particles showed a larger hydrodynamic size but a more negative zeta potential and lower PDI. In the flower-derived system, PEG-200 improved the initial zeta potential and PDI, and the dispersion retained moderate colloidal characteristics after 2 months. Overall, the DLS and zeta potential results demonstrate that extract type, PEG-200 addition, and storage influenced the dispersion behavior and colloidal stability of the synthesized AgNPs.

#### XRD-based structural confirmation of AgNPs

XRD analysis was performed to verify the crystalline phase of the AgNPs synthesized using *L. ciliatum* leaf and flower extracts. The diffraction patterns of both samples displayed characteristic reflections of metallic silver. The main peaks were observed at approximately  $2\theta = 38.5^\circ, 44.7^\circ, 64.8^\circ, 77.7^\circ$  and  $81.8^\circ$ , corresponding to the (111), (200), (220), (311) and (222) crystallographic planes, respectively (Fig. 7). For leaf-extract-mediated AgNPs, the diffraction peaks appeared at  $38.52^\circ, 44.69^\circ, 64.76^\circ, 77.67^\circ$  and  $81.79^\circ$  (Fig. 7a). For flower-extract-mediated AgNPs, the corresponding peaks were located at  $38.56^\circ, 44.74^\circ, 64.83^\circ, 77.76^\circ$  and  $81.89^\circ$  (Fig. 7b). These peak

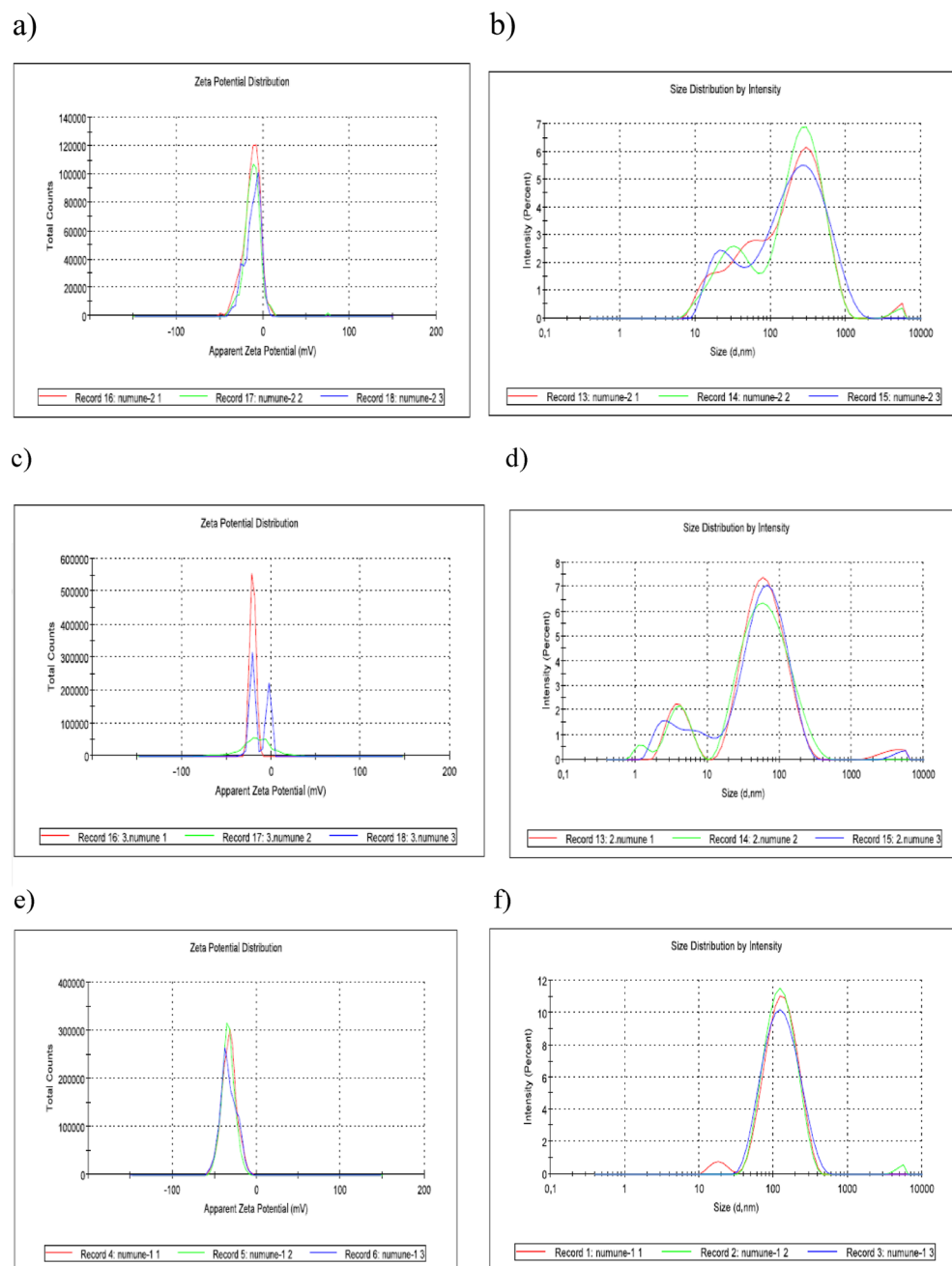


**Fig. 6.** Zeta potential and DLS size distribution profiles of *Lilium ciliatum*-mediated AgNPs under PEG-free, PEG-200-assisted, and 2-month storage conditions. (**a,b**) Leaf-extract-mediated AgNPs without PEG-200; (**c,d**) freshly prepared PEG-200-assisted leaf-extract-mediated AgNPs; (**e,f**) PEG-200-assisted leaf-extract-mediated AgNPs after 2 months of storage; (**g,h**) flower-extract-mediated AgNPs without PEG-200; (**i,j**) freshly prepared PEG-200-assisted flower-extract-mediated AgNPs; and (**k,l**) PEG-200-assisted flower-extract-mediated AgNPs after 2 months of storage.

positions confirm the formation of crystalline metallic silver with a face-centered cubic structure in accordance with JCPDS No. 04-0783. The crystallite size calculated by the Debye–Scherrer equation was 8.31 nm for the leaf-extract-mediated AgNPs and 10.70 nm for the flower-extract-mediated AgNPs. These crystallite sizes were smaller than the TEM-based particle diameters, which is expected because a single particle may contain crystalline domains together with surface-bound organic components. The slightly larger crystallite size of the flower-derived sample is consistent with its larger TEM-based mean particle size. These findings demonstrate that both *L. ciliatum* extracts were able to mediate not only AgNP formation but also the development of a well-defined crystalline silver phase under microwave-assisted conditions (Fig. 7).

These XRD findings agree well with previous *Lilium*-mediated AgNP studies. Al-Ansari,<sup>4</sup> reported AgNP diffraction peaks at 38.13°, 44.31°, 64.44° and 77.37°, while Du et al.,<sup>8</sup> reported peaks at 38.0°, 42.5°, 64.5° and

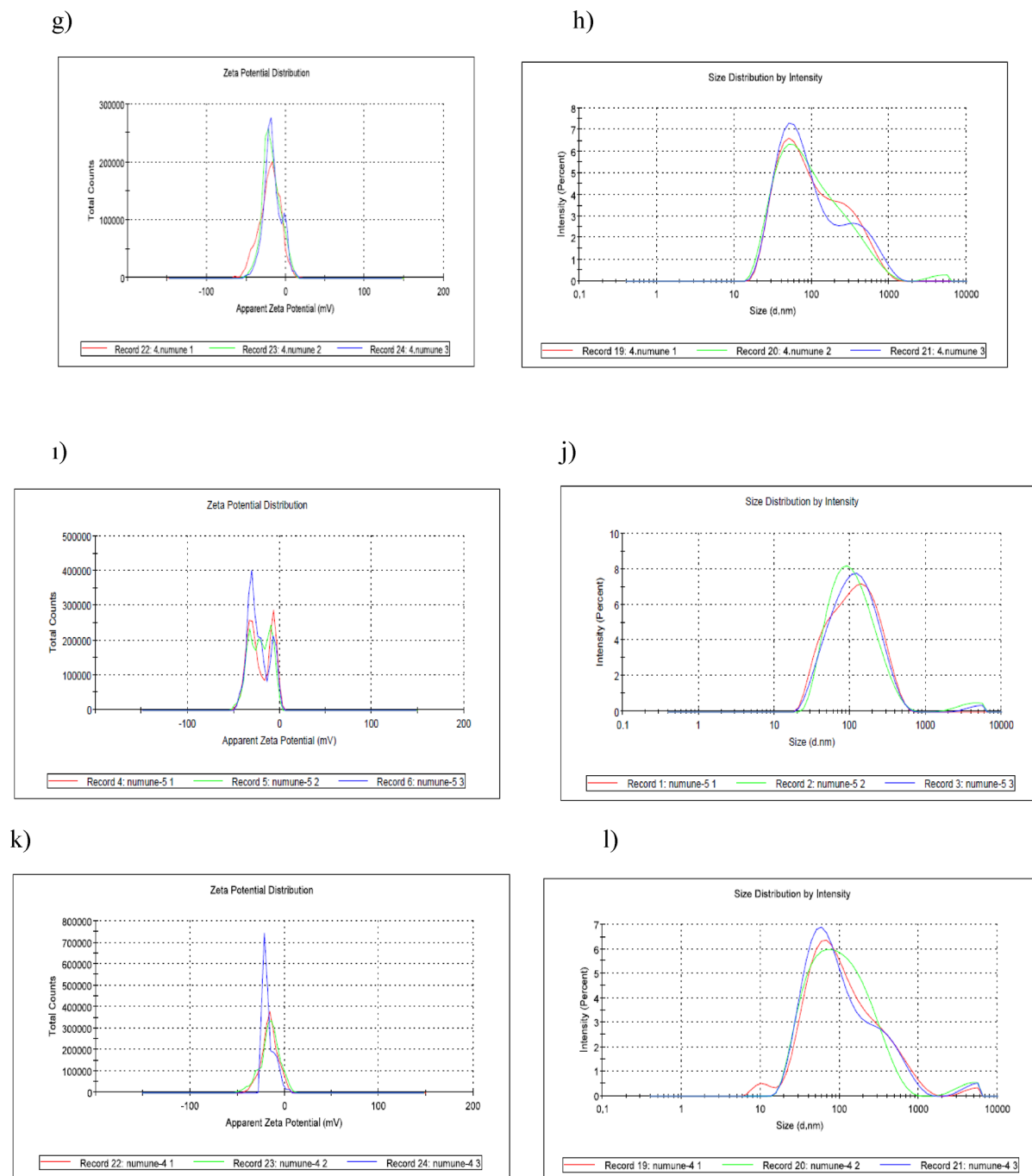


Fig. 6. (continued)

77.0°. The peak positions observed in the present study for leaf- and flower-derived AgNPs were close to these reported values and corresponded to the characteristic fcc planes of metallic silver. Therefore, the XRD results confirm that both *L. ciliatum* leaf and flower extracts successfully mediated the formation of crystalline AgNPs under microwave-assisted synthesis conditions.

#### FT-IR evidence of biomolecular surface association

FT-IR spectroscopy was used to evaluate changes in the major functional groups of *Lilium ciliatum* extracts following AgNP formation. The spectra of the leaf and flower extracts and their corresponding AgNPs are presented in Fig. 8. In the leaf system, the broad band observed at 3257.25  $\text{cm}^{-1}$  in the raw extract shifted to 3286.20  $\text{cm}^{-1}$  after AgNP formation, while the band at 1569.04  $\text{cm}^{-1}$  shifted to 1595.31  $\text{cm}^{-1}$ . In addition, noticeable spectral changes were observed in the C–H and fingerprint regions. These differences are consistent with changes in the chemical environment of extract-derived biomolecules during nanoparticle formation. A similar trend was observed in the flower system. The broad band in the raw flower extract shifted from 3308.48

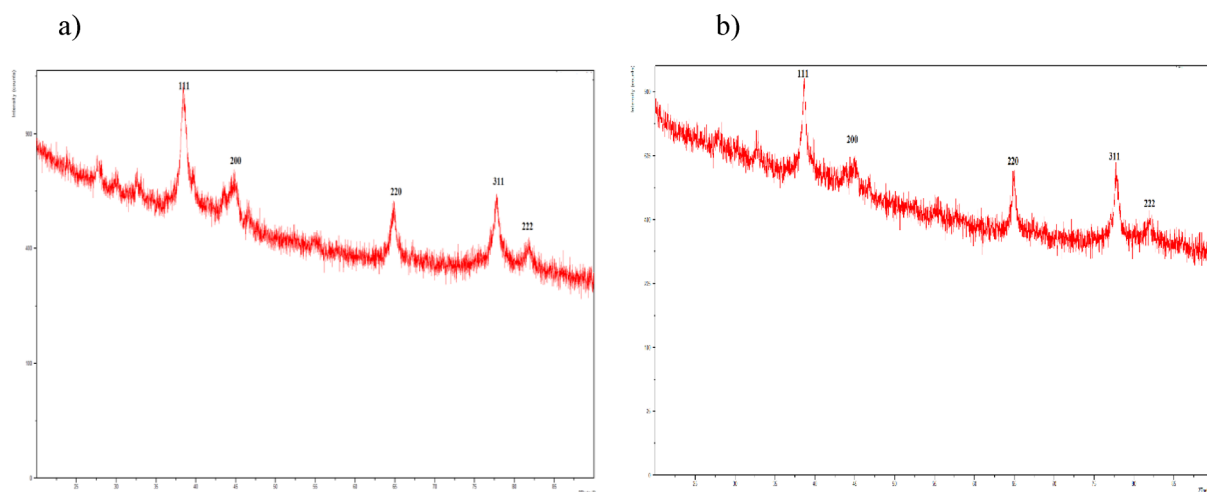


**Fig. 7.** XRD patterns confirming the crystalline structure of AgNPs synthesized using *Lilium ciliatum* extracts. AgNPs were prepared with 0.3 mM AgNO<sub>3</sub> and 2 mL extract under microwave-assisted synthesis conditions: (a) leaf-derived AgNPs and (b) flower-derived AgNPs.

to 3296.96 cm<sup>-1</sup>, while the band at 1635.40 cm<sup>-1</sup> shifted to 1652.99 cm<sup>-1</sup>. In addition, new bands and noticeable changes in band distribution were observed in the C–H, carbonyl, and fingerprint regions. These spectral differences indicate changes in the chemical environment of extract-derived organic constituents during AgNP formation and support their possible association with the nanoparticle surface. Overall, the FT-IR findings provide supportive evidence that biomolecules present in the leaf and flower extracts may contribute to AgNP formation and surface association. Nanoparticle stabilization was therefore interpreted based on the combined evidence obtained from FT-IR, DLS, zeta potential measurements, control experiments, and PEG-200-assisted dispersion behavior. The findings of the present study are broadly consistent with previously reported *Lilium*-based AgNP studies. Al-Ansari,<sup>4</sup> reported FT-IR bands in similar spectral regions for *Lilium lancifolium*-mediated AgNP synthesis, while Du et al.,<sup>8</sup> suggested that plant-derived constituents such as reducing sugars, proteins, and flavonoids may contribute to nanoparticle formation. These studies support the possible role of plant-derived biomolecules in AgNP synthesis and stabilization.

### Bioactive surface characteristics and antioxidant behavior of AgNPs

The antioxidant activity of *Lilium ciliatum*-mediated AgNPs and their corresponding crude extracts was evaluated using the DPPH radical scavenging assay, and the results were interpreted based on IC<sub>50</sub> values and concentration-dependent scavenging activity (Table 2). Since lower IC<sub>50</sub> values indicate stronger DPPH radical scavenging activity, flower-extract-mediated AgNPs exhibited a more favorable antioxidant profile than leaf-extract-mediated AgNPs. The IC<sub>50</sub> value of flower-extract-mediated AgNPs was significantly lower than that of leaf-extract-mediated AgNPs ( $p=0.040$ ). At the highest tested concentrations, flower-extract-mediated AgNPs reached 75.58 ± 1.47% scavenging activity, whereas leaf-extract-mediated AgNPs showed 47.57 ± 1.51% activity. Based on the IC<sub>50</sub> values, DPPH radical scavenging activity followed the order: crude flower extract > crude leaf extract > flower-extract-mediated AgNPs > leaf-extract-mediated AgNPs. The IC<sub>50</sub> values of the crude flower and leaf extracts were 0.385 ± 0.342 and 0.543 ± 0.021 mg mL<sup>-1</sup>, respectively; however, the difference between these values was not statistically significant ( $p=0.508$ ). Both crude extracts showed lower IC<sub>50</sub> values than their corresponding AgNP samples. This finding indicates that AgNP formation did not enhance the apparent DPPH radical scavenging potency compared with the free extract systems. The lower scavenging activity of the AgNP samples may be associated with changes in the accessibility of extract-derived antioxidant compounds during synthesis. The total phenolic content results were consistent with the observed differences in antioxidant behavior. The crude flower extract had a higher total phenolic content than the crude leaf extract, with values of 17.16 ± 1.87 and 4.79 ± 3.83 mg GAE g<sup>-1</sup>, respectively. After AgNP formation, flower-extract-mediated AgNPs also showed a higher measured phenolic content than leaf-extract-mediated AgNPs, with values of 10.07 ± 3.98 and 4.75 ± 2.91 mg GAE g<sup>-1</sup>, respectively. These findings provide compositional context for interpreting the stronger antioxidant response of the flower-derived AgNPs.

Overall, the antioxidant behavior of *L. ciliatum*-mediated AgNPs was associated with the phytochemical characteristics of the plant part used in synthesis. Although the crude extracts exhibited stronger direct DPPH radical scavenging activity than their corresponding AgNP samples, flower-extract-mediated AgNPs showed a higher phenolic content and significantly stronger DPPH radical scavenging activity than leaf-extract-mediated AgNPs.

### Antimicrobial activity of AgNPs

The antimicrobial activity of the synthesized AgNPs was evaluated using disk diffusion and minimum inhibitory concentration (MIC) assays against selected Gram-positive bacteria, Gram-negative bacteria, and yeast. The tested microorganisms included *Enterococcus faecalis*, *Escherichia coli*, *Staphylococcus aureus*, *Pseudomonas*

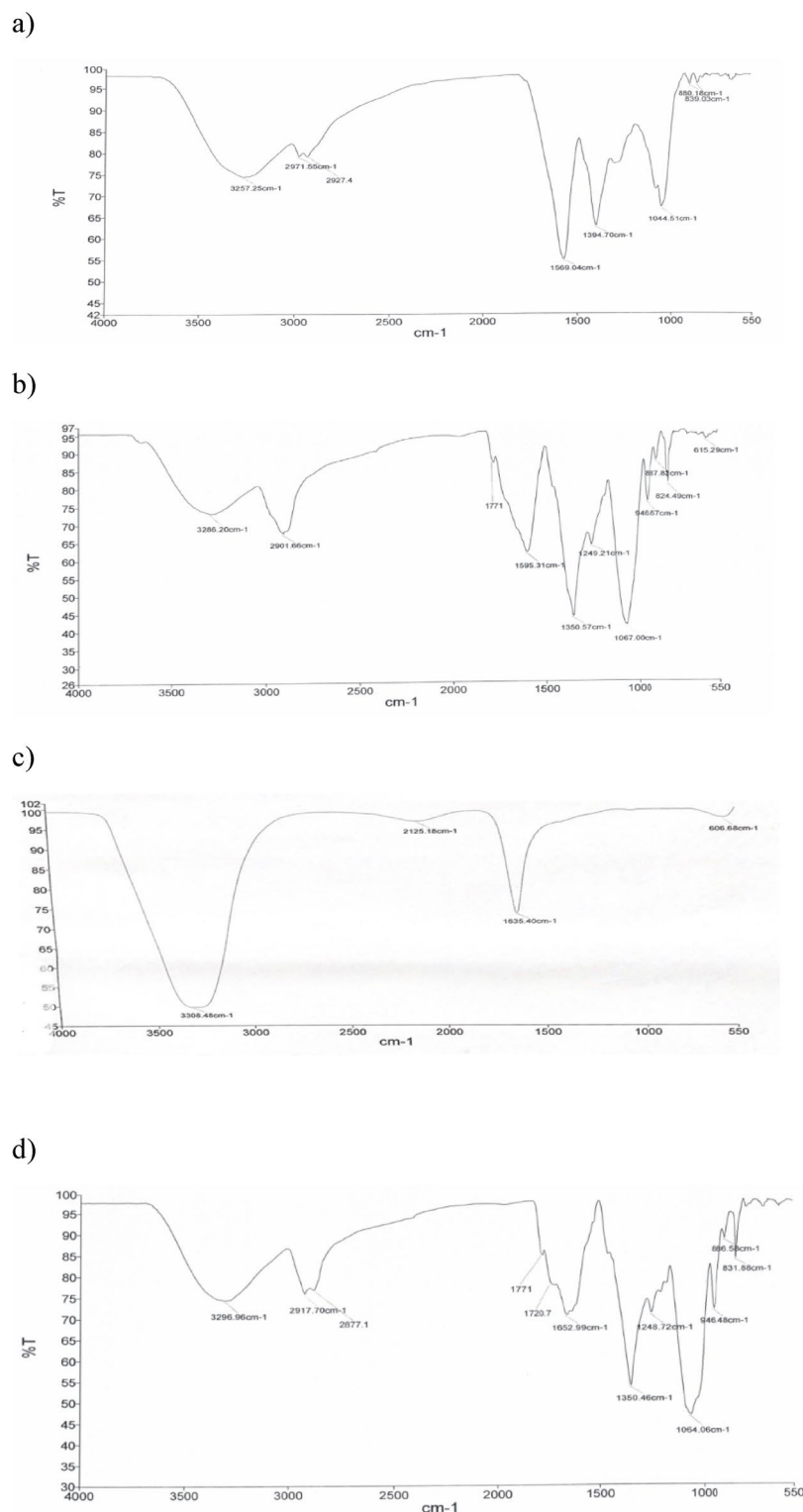


**Fig. 8.** FT-IR spectra of raw *Lilium ciliatum* extracts and AgNPs synthesized from the corresponding extracts. (a) Leaf extract; (b) AgNPs synthesized using the leaf extract; (c) flower extract; and (d) AgNPs synthesized using the flower extract. AgNPs were prepared using 0.3 mM  $\text{AgNO}_3$  and 2 mL extract under microwave-assisted synthesis conditions.

*aeruginosa*, and *Candida albicans*. The disk diffusion and MIC results of leaf- and flower-extract-mediated AgNPs are summarized in Table 3, and representative disk diffusion plate images are presented in Fig. 9. In the disk diffusion assay, flower-extract-mediated AgNPs exhibited the broadest inhibition profile, producing measurable inhibition zones against all tested microorganisms, with mean zone diameters ranging from approximately 8.0 to

| Sample                        | Concentration (mg mL <sup>-1</sup> ) | DPPH scavenging activity (%) | IC <sub>50</sub> value (mg mL <sup>-1</sup> ) |
|-------------------------------|--------------------------------------|------------------------------|-----------------------------------------------|
| Leaf-extract-mediated AgNPs   | 0.249                                | 7.267 ± 2.087                | 4.786 ± 0.214                                 |
|                               | 0.498                                | 11.91 ± 0.544                |                                               |
|                               | 0.997                                | 16.34 ± 2.814                |                                               |
|                               | 1.995                                | 28.32 ± 2.556                |                                               |
|                               | 3.990                                | 47.57 ± 1.513                |                                               |
| Flower-extract-mediated AgNPs | 0.214                                | 7.477 ± 2.034                | 2.306 ± 0.946                                 |
|                               | 0.429                                | 14.95 ± 0.592                |                                               |
|                               | 0.858                                | 31.02 ± 0.706                |                                               |
|                               | 1.717                                | 50.41 ± 1.611                |                                               |
|                               | 3.435                                | 75.58 ± 1.465                |                                               |
| Raw flower extract            | 0.031                                | 0.486 ± 1.180                | 0.385 ± 0.342                                 |
|                               | 0.062                                | 0.199 ± 4.366                |                                               |
|                               | 0.250                                | 10.73 ± 0.096                |                                               |
|                               | 0.500                                | 18.62 ± 1.372                |                                               |
| Raw leaf extract              | 0.092                                | 11.83 ± 1.806                | 0.543 ± 0.021                                 |
|                               | 0.184                                | 25.89 ± 2.643                |                                               |
|                               | 0.368                                | 44.73 ± 2.500                |                                               |
|                               | 1.474                                | 84.34 ± 0.314                |                                               |
| Trolox standard               | 0.0015                               | 15.03 ± 5.451                | 0.006 ± 0.001                                 |
|                               | 0.0031                               | 42.46 ± 10.23                |                                               |
|                               | 0.0062                               | 51.94 ± 9.104                |                                               |
|                               | 0.0125                               | 91.19 ± 2.646                |                                               |

**Table 2.** Comparative DPPH radical scavenging activity of raw *Lilium ciliatum* leaf and flower extracts, corresponding extract-mediated AgNPs, and Trolox standard at the tested sample concentrations. Values are presented as mean ± standard deviation.

| A. Disk diffusion test results |                                               |                                           |                                             |                                                 |                                               |
|--------------------------------|-----------------------------------------------|-------------------------------------------|---------------------------------------------|-------------------------------------------------|-----------------------------------------------|
| Sample                         | <i>E. faecalis</i> DDT (mm)                   | <i>E. coli</i> DDT (mm)                   | <i>S. aureus</i> DDT (mm)                   | <i>P. aeruginosa</i> DDT (mm)                   | <i>C. albicans</i> DDT (mm)                   |
| ZÇAgNPs                        | 8.6, 8.3, 8.4                                 | 9.6, 9.6, 9.4                             | 9.4, 9.3, 9.1                               | 7.9, 8.1, 8.0                                   | 9.1, 9.3, 9.2                                 |
| ZYAgNPs                        | –                                             | 7.7, 7.9, 7.9                             | –                                           | –                                               | 7.4, 7.2, 7.5                                 |
| DMSO                           | –                                             | –                                         | –                                           | –                                               | –                                             |
| Chloramphenicol                | 14.1, 14.6, 14.5                              | 20.4, 20.9, 20.7                          | 18.7, 18.5, 18.4                            | 21.1, 20.9, 21.3                                | 23.1, 23.3, 23.3                              |
| B. MIC results                 |                                               |                                           |                                             |                                                 |                                               |
| Sample                         | <i>E. faecalis</i> MIC (mg mL <sup>-1</sup> ) | <i>E. coli</i> MIC (mg mL <sup>-1</sup> ) | <i>S. aureus</i> MIC (mg mL <sup>-1</sup> ) | <i>P. aeruginosa</i> MIC (mg mL <sup>-1</sup> ) | <i>C. albicans</i> MIC (mg mL <sup>-1</sup> ) |
| ZÇAgNPs                        | 1.5625                                        | 0.78125                                   | 3.125                                       | 1.5625                                          | 0.78125                                       |
| ZYAgNPs                        | 6.25                                          | 6.25                                      | 6.25                                        | 3.125                                           | 3.125                                         |
| Chloramphenicol                | 0.064                                         | 0.008                                     | 0.032                                       | 0.016                                           | 0.008                                         |

**Table 3.** Disk diffusion test (DDT) and MIC results of *Lilium ciliatum* leaf- and flower-extract-mediated AgNPs against tested microorganisms. DDT values are given as replicate inhibition zone diameters in millimeters. “–” indicates no detectable inhibition zone under the tested conditions. ZÇAgNPs: flower-extract-mediated AgNPs; ZYAgNPs: leaf-extract-mediated AgNPs.

9.6 mm. In contrast, leaf-extract-mediated AgNPs produced detectable inhibition zones only against *E. coli* and *C. albicans*. The representative plate images supported these findings, with visible inhibition zones around the disks labeled 2, corresponding to the respective AgNP samples (Fig. 9). Under the applied test conditions, flower-extract-mediated AgNPs therefore exhibited a broader antimicrobial spectrum than leaf-extract-mediated AgNPs. Statistical comparisons were performed only for microorganisms showing measurable inhibition zones in both AgNP groups. Flower-extract-mediated AgNPs produced significantly larger inhibition zones than leaf-extract-mediated AgNPs against *E. coli* and *C. albicans* ( $p < 0.001$ ).

The MIC assays further differentiated the antimicrobial responses of the two AgNP systems. Flower-extract-mediated AgNPs showed the lowest MIC values against *E. coli* and *C. albicans*, both at 0.78125 mg mL<sup>-1</sup>. MIC values of 1.5625 mg mL<sup>-1</sup> were obtained against *E. faecalis* and *P. aeruginosa*, whereas the MIC value against *S. aureus* was 3.125 mg mL<sup>-1</sup>. Leaf-extract-mediated AgNPs showed higher MIC values overall, with values of 6.25 mg mL<sup>-1</sup> against *E. faecalis*, *E. coli*, and *S. aureus* and 3.125 mg mL<sup>-1</sup> against *P. aeruginosa* and *C. albicans*. MIC values were interpreted descriptively because they represent endpoints obtained from two-fold serial dilution



**Fig. 9.** Representative disk diffusion assay images showing the antimicrobial activity of *Lilium ciliatum* flower- and leaf-extract-mediated AgNPs against selected microorganisms. The disks numbered 2 represent the corresponding AgNP samples.

assays. Among the tested microorganisms, *E. coli* and *C. albicans* showed the greatest susceptibility to flower-extract-mediated AgNPs, whereas *S. aureus* required a higher AgNP concentration for inhibition. The stronger antimicrobial response of flower-extract-mediated AgNPs may be associated with differences in phenolic content, particle properties, and surface-associated constituents. The antimicrobial findings are consistent with the previous *Lilium*-mediated AgNP study by Al-Ansari et al.<sup>4</sup>, who reported antibacterial activity of *Lilium lancifolium*-derived AgNPs against both Gram-positive and Gram-negative bacteria, including *E. coli* and *P. aeruginosa*. Similarly, in the present study, *L. ciliatum*-mediated AgNPs exhibited antimicrobial activity against the tested microorganisms. However, the present study extends the available literature by including MIC assessment and antifungal evaluation against *C. albicans*. It also provides a direct comparison between leaf- and flower-extract-mediated AgNPs, showing that flower-derived AgNPs exhibited a broader and stronger antimicrobial response under the tested conditions. Overall, flower-extract-mediated AgNPs exhibited stronger antimicrobial activity than leaf-extract-mediated AgNPs. This conclusion was supported by their broader disk diffusion response, significantly larger inhibition zones against *E. coli* and *C. albicans*, and generally lower MIC values.

## Conclusion

This study demonstrated the feasibility of using *Lilium ciliatum* leaf and flower extracts as distinct biogenic media for the microwave-assisted green synthesis of silver nanoparticles. Ethanol-modified supercritical CO<sub>2</sub> extraction yielded plant-derived fractions with different phytochemical profiles. Flavonoid-type compounds were more prominent in the leaf extract, whereas phenolic acids were present at higher levels in the flower extract. Reproducible AgNP formation was achieved using a 2% extract solution, 2 mL extract volume, 0.3 mM AgNO<sub>3</sub>, PEG-200, 90 W microwave power, and slightly acidic pH conditions. The formation and physicochemical properties of the AgNPs were characterized using complementary analytical techniques. UV-Vis spectra exhibited characteristic surface plasmon resonance bands at approximately 400–420 nm. TEM analysis revealed predominantly spherical particles with mean diameters of  $12.14 \pm 2.14$  nm for leaf-extract-mediated AgNPs and  $15.10 \pm 2.92$  nm for flower-extract-mediated AgNPs. XRD analysis confirmed the crystalline metallic silver structure, whereas FT-IR analysis indicated the association of plant-derived functional groups with the nanoparticle surface. DLS and zeta potential measurements further characterized the colloidal behavior of the two AgNP systems. The biological activity results revealed clear differences between the leaf- and flower-extract-mediated AgNPs. Although leaf-extract-mediated AgNPs exhibited more favorable colloidal behavior during storage, flower-extract-mediated AgNPs showed a higher measured total phenolic content, significantly stronger DPPH radical scavenging activity, and broader antimicrobial activity. Flower-extract-mediated AgNPs produced measurable inhibition against all tested microorganisms and generally exhibited lower MIC values. The greatest susceptibility was observed for *Escherichia coli* and *Candida albicans*. The association between the phenolic acid-rich flower extract and the stronger antioxidant and antimicrobial responses provides useful compositional context for interpreting the findings. The main contribution of this study lies in the systematic comparison of *L. ciliatum* leaf and flower extracts as separate biogenic systems for AgNP synthesis. The observed differences in phytochemical composition, particle properties, colloidal behavior, antioxidant activity, and antimicrobial

efficacy indicate that the plant organ selected for synthesis is an important factor in determining the properties of plant-mediated AgNPs. Under the tested conditions, flower-extract-mediated synthesis appeared particularly promising for producing AgNPs with stronger antioxidant and antimicrobial activity. Future studies may focus on evaluating the cytotoxicity and long-term stability of the synthesized AgNPs.

## Data availability

Included in the paper or Supplementary Information “The data supporting the findings of this study are included within the article. Additional raw data are available from the corresponding author upon reasonable request.”

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## Author contributions

Gönül Serdar and Melek Koç Keşir were responsible for conceptualization, methodology, experimental work, formal analysis, visualization, and manuscript writing. Fatma Tunali contributed to the experimental studies. Salih Terzioğlu identified the plant material. Abidin Gümrükçüoğlu performed the HPLC analysis. Tuğba Mazlum Şen conducted the antioxidant assays, and Deniz Canbolat Gültekin carried out the antimicrobial assays. All authors reviewed and approved the manuscript.

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## Declarations

### Competing interests

The authors declare no competing interests.

### Additional information

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