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Green Synthesis and Characterization of Silver Nanoparticles Using *Allium ampeloprasum* Leaf Aqueous Extract and Evaluation of Their Antioxidant Activity

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Abstract. The use of plants for the synthesis involve a route with lower toxicity compared to chemical methods, however variations between plant sources and/or processing conditions may affect reproducibility and level of statistical support assignable to published antioxidant activity. This study is not the first report of using this species but rather an assessment for AgNPs biosynthesis that was performed in specific laboratory-controlled environment (detailed below) with aqueous leaf extract from Iraqi-sourced *Allium ampeloprasum*. Extraction of fresh leaves (25 g) in 100 mL deionized water at 80 °C for the time period was conducted onto it. Added extract (5 mL) to 45 mL of 0.1 M silver nitrate (AgNO₃), pH adjusted with sodium carbonate to a milky colour and stir at 500 rpm for an hour under light restrictions [30]. It was characterized by ultraviolet–visible spectroscopy, Fourier-transform infrared spectroscopy (FTIR), scanning electron microscopy (SEM), X-ray diffraction (XRD) and atomic force microscope (AFM). When analysis by FTIR also showed the involvement of hydroxyl, carboxyl and amide groups in both reduction and capping, SEM images gave an idea that these aggregated structures contained approximately spherical domains dispersed throughout. XRD confirmed that silver gave face centred cubic with mean crystallite size of 45.0 nm. AgNPs had a concentration-dependent antioxidant activity (0.10–3.00 mg/mL) in the 2,2-diphenyl-1-picrylhydrazyl (DPPH)-assay with an IC₅₀ of apparent $-5 = 0.769$ mg/mL compared to ascorbic acid at 0.476mg/ml(n =3; ANOVA p <.001). The results demonstrate their antioxidant potential .

Keywords: Green synthesis; Silver nanoparticles; AgNO₃; *Allium ampeloprasum*; DPPH assay; Antioxidant activity.

1.1 Introduction

Oxidative stress is associated with the development and progression of several pathological conditions because excessive reactive oxygen species can damage cellular lipids, proteins, and nucleic acids. Antioxidant systems, including natural antioxidants and antioxidant-like nanomaterials, are therefore of continuing interest in pharmaceutical, biomedical, and materials research. In parallel, green nanochemistry has developed as an alternative to conventional chemical and physical nanoparticle synthesis because it can reduce the need for toxic reducing agents, high temperatures, and expensive processing conditions.

Silver nanoparticles (AgNPs) have attracted extensive attention because of their size-dependent optical, physicochemical, and biological properties. Plant-mediated synthesis is particularly attractive because plant extracts contain phenolics, flavonoids, terpenoids, proteins,

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sugars, and other phytochemicals that may reduce Ag^+ to Ag^0 and stabilize the resulting particles through surface capping interactions. Recent reviews emphasize that the final morphology, stability, and biological response of plant-derived AgNPs depend strongly on extract composition, AgNO_3 concentration, pH, temperature, reaction time, and purification procedure [1-3].

Species of the genus *Allium* contain organosulfur compounds, flavonoids, saponins, and other secondary metabolites that may participate in electron transfer and surface capping. Previous studies have already reported AgNP synthesis using *Allium cepa* and *Allium ampeloprasum* extracts [4–6]. Accordingly, the novelty of the present work is limited to the investigated Iraqi plant source, the defined aqueous protocol, and the combined FTIR, SEM, XRD, AFM, and statistically supported DPPH evaluation; it is not presented as the first AgNP synthesis from *A. ampeloprasum*.

A remaining practical gap is the limited integrated evidence for locally sourced Iraqi *A. ampeloprasum* under controlled extraction, pH, temperature, illumination, mixing, and purification conditions. Addressing this gap matters because variations in biomass composition and processing can alter nucleation, aggregation, surface chemistry, and biological response. The present study therefore reports the experimental conditions and measured DPPH and XRD data transparently and benchmarks the results against recent plant-mediated systems.

Table 1. Comparison of recent plant-mediated AgNP synthesis studies (2022–2025) with the present work.

Study and plant source	Reported reducing/capping species	Purification or recovery	Particle size or yield	Reported stability
Liaqat et al. (2022) [7] Eucalyptus camaldulensis aqueous leaf extract	Polyphenols, flavonoids and tannins; FTIR: O–H, N–H and C=O groups.	10,000 rpm, 20 min; pellet washed with distilled water and lyophilized.	DLS: 43 nm; numerical yield NR.	$\zeta = -26 \pm 4.61$ mV; UV–Vis profile unchanged after 30 days.
Ansari et al. (2023) [8] Azadirachta indica aqueous leaf extract	Phenol/alcohol, carbonyl/amide and methoxy groups identified by FTIR.	Repeated centrifugation and redispersion in distilled water; speed and duration NR.	22–30 nm; mass or percentage yield NR.	Described as stable after optimization; ζ and time-resolved stability NR.
Abdel-Rahman et al. (2024) [9] Portulaca oleracea aqueous leaf extract	FTIR-associated C–O/C=O, carbohydrate/polysaccharide and aliphatic C–H groups.	Final AgNP purification NR; the leaf-extract supernatant was pre-cleared at 10,000 rpm for 7 min.	TEM: 5–40 nm; mean 13.75 ± 8.70 nm; yield NR.	$\zeta = -36$ mV; strong electrostatic colloidal stability.
Akalin (2024) [10] Calluna vulgaris aqueous leaf extract	Hydroxyl/carboxylate groups associated with polyphenols/polysaccharides, plus amines.	5,000 rpm, 15 min, three cycles with distilled-water washing; vacuum freeze-dried and stored at 4 °C.	XRD: 44.77 ± 0.50 nm; AFM mean: 49.8 nm; numerical yield NR.	$\zeta = -23.1 \pm 0.6$ mV; UV–Vis signal stable for >2 h.
Wang et al. (2025) [11] Colocasia esculenta aqueous leaf extract	Polysaccharides, phenolics and flavonoids; O–H, N–H and C=O groups supported surface capping.	10,000 rpm, 10 min; pellet rinsed twice with distilled water, freeze-dried and stored at 4 °C.	DLS: 90.17 ± 12.65 nm; yield NR.	$\zeta = -31.6 \pm 1.26$ mV; stable aqueous dispersion.
Present study Allium ampeloprasum Iraqi aqueous leaf extract	FTIR-consistent hydroxyl, carboxyl and protein-related amide functionalities.	12,000 rpm, 20 min; three deionized-water washes and two absolute-ethanol washes; vacuum-dried at 60 °C for 12 h.	SEM: 42.5 ± 5.3 nm; XRD crystallite size: 45 nm; yield NR.	No ζ or time-resolved stability measurement; aggregation was evident in SEM/AFM.

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Accordingly, this study aimed to determine whether an aqueous extract of Iraqi-sourced *A. ampeloprasum* leaves could mediate AgNP formation under a controlled protocol and to characterize the resulting material and its DPPH radical-scavenging response. The research questions were: (1) Does reduction of Ag^+ to Ag^0 occur when the extract and AgNO_3 are stirred for 60 min at room temperature (35 °C), pH 8.5, under light-restricted conditions? (2) What optical, functional-group, morphological, and crystallographic features characterize the product? (3) How does AgNP DPPH inhibition vary across 0.10–3.00 mg/mL relative to ascorbic acid, and is the concentration effect statistically significant?

2. Materials and Methods

2.1. Chemicals and reagents

Analytical-grade silver nitrate (AgNO_3 ; 99.8% purity) was obtained from Cat.) 209139; Batch No. STBF8432V), 2,2-diphenyl-1-picrylhydrazyl (DPPH; Cat. It is purchased from Merck (Darmstadt, Germany; Cat. No. A92902) were purchased from Sigma-Aldrich (Germany). Analytical-grade methanol (99.9%; Cat. DPPH assay powder (No. 322415; Merck, Germany) was used for preparing the DPPH reagent and its corresponding assay solutions.

2.2. Plant collection, authentication, and extract preparation

Fresh, healthy *A. ampeloprasum* leaves were collected from a commercial agricultural market in Baghdad, Iraq, in March 2025. The leaves were washed under running tap water, rinsed three times with deionized water, and shade-dried at an ambient temperature of 25 °C. Finely chopped fresh leaves (25 g) were immersed in 100 mL of deionized water and heated at 80 °C for 45 min. After cooling, the mixture was filtered through Whatman No. 1 filter paper (11 µm nominal pore size). The clear, pale-yellow filtrate was stored at 4 °C and used within 24 h.

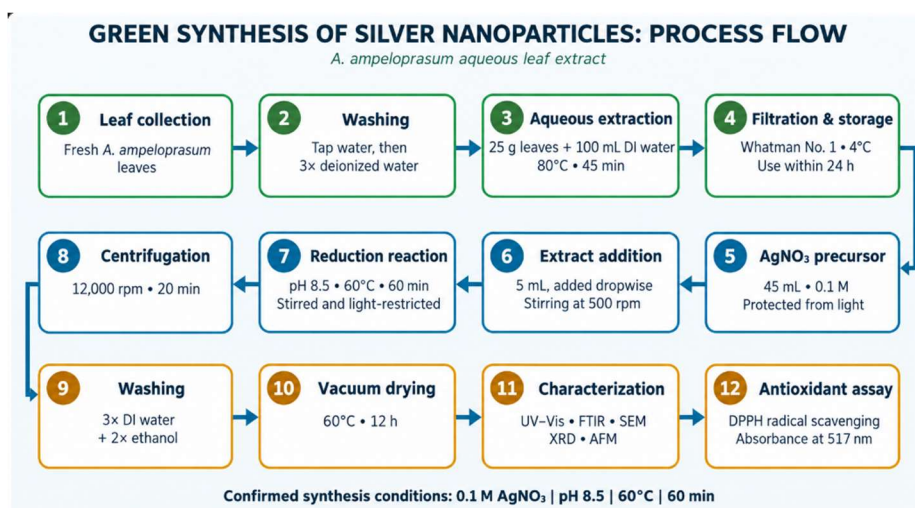


Fig. 1. Fresh *Allium ampeloprasum* leaves used for aqueous extract preparation.

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2.3. Biosynthesis of silver nanoparticles

An aqueous 0.1 M AgNO₃ precursor was prepared in an amber volumetric flask. Fresh extract (5 mL) was added dropwise to 45 mL of AgNO₃ under magnetic stirring at 500 rpm. The reaction was maintained for 60 min at room temperature (35 °C) and pH 8.5, adjusted with 0.1 M sodium hydroxide, in a light-restricted environment. Thus, reduction was not treated as an uncontrolled spontaneous process: pH adjustment, mixing, defined temperature, reaction time, and restricted illumination were external process controls. The colour changed from pale yellow to honey/dark brown. The suspension was centrifuged at 12,000 rpm for 20 min, washed three times with deionized water and twice with absolute ethanol, and vacuum-dried at 60 °C for 12 h.



Scheme 1 Schematic representation of the green synthesis, purification, characterization, and antioxidant evaluation of silver nanoparticles using aqueous *Allium ampeloprasum* leaf extract.

2.4. Characterization of AgNPs

Ultraviolet-visible spectroscopy was conducted using a Shimadzu UV-1900i double-beam spectrophotometer (1 nm resolution, wavelength range 300–700 nm) against deionized water baseline blank. FTIR spectra were collected on a Bruker Alpha II spectrometer in the range of 4000–400 cm⁻¹ (32 scans, resolution: 4 cm⁻¹) for identifying functional groups involved with reduction and capping. Particle morphology and aggregation was characterized by SEM using an FEI Nova NanoSEM 450 at 15 kV. Crystalline phase and crystallite size were examined using XRD, and surface topography and grain-size distribution of prepared samples were investigated using AFM.

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For every indexed XRD peak, crystallite size was calculated applying the Debye–Scherrer equation ($D = K\lambda/(\beta \cos \theta)$), where D is half of the total wavelength [$D=K(1/2)(\text{diameter})$], $K = 0.9$ shape factor; $\lambda = \text{Cu } \alpha \text{ wavelength} = 0.15406 \text{ nm}$, β background-corrected FWHM in radians and θ is Bragg angle)22–24.

2.5. Antioxidant activity by DPPH assay

Antioxidant activity was evaluated employing DPPH radical-scavenging assay. Suspensions of AgNP and ascorbic acid were tested at the concentrations 0.10, 0.25, 0.50, 0.75, 1.00, 1.50, 2.00 and 3 mg/mL with three measurements per concentration. An equal volume of 0.1 mM DPPH solution was added to each aliquot, vortexed and incubated in the dark at room temperature (35 °C) for 30 min. Readings were taken at 517 nm for the absorbance. Methanol was used as the spectrophotometric blank, DPPH without sample served to give a negative control and ascorbic acid was chosen for positive reference.

Absorbance corrected for blank absorption was calculated from $A_{\text{corrected}} = A_{\text{reading}} - A_{\text{blank}}$. Radical-scavenging activity was subsequently calculated as: DPPH % inhibition = $[(A_{\text{control,corrected}} - A_{\text{sample,correct}})/A_{\text{control,correct}}] \times 100$

2.6. Statistical analysis

DPPH results are reported as mean $\pm$ standard deviation (SD), with $n = 3$ per concentration. For each material, differences among the eight concentrations were assessed by one-way analysis of variance (ANOVA). Statistical significance was set at $p < 0.05$. Apparent IC_{50} values were estimated by linear interpolation between adjacent concentrations bracketing 50% inhibition on the rising response branch.

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3. Results:



Fig. 2. Visual stages of AgNP synthesis: (a) aqueous leaf extract after filtration; (b) silver nitrate solution before reaction with plant extract; and (c) reaction mixture after the color change suggesting AgNP formation.

3.1. UV-visible spectroscopy

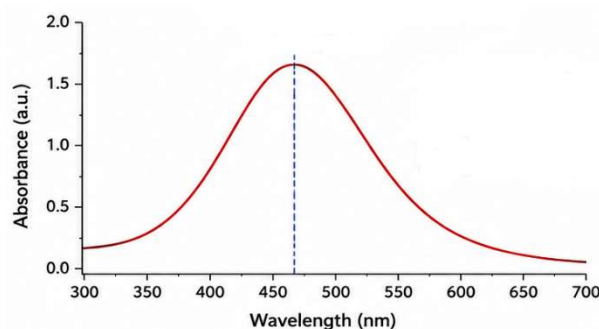


Fig. 3. Ultraviolet-visible absorption spectrum of the synthesized AgNPs showing the SPR maximum at 435 nm.

The reaction mixture changed from pale yellow to dark brown within 15 min and stabilised visually by 60 min (Fig. 2). The ultraviolet-visible spectrum showed a single, relatively symmetric absorption band centred at 435 nm (Fig. 3). This band is consistent with the surface plasmon resonance (SPR) commonly reported for plant-mediated AgNPs and supports the

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reduction of Ag^+ to metallic Ag^0 [1–3]. The optical result alone does not establish particle size or colloidal stability and was therefore interpreted together with SEM, AFM, and XRD.

3.2. FTIR analysis

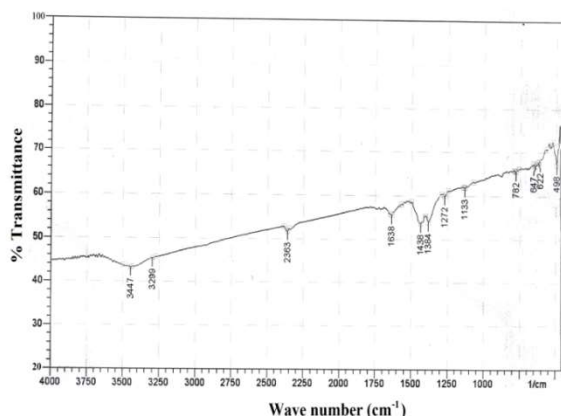


Fig. 4. FTIR spectrum of synthesized AgNPs over 4000–400 cm^{-1} .

Functional groups related to the plant matrix and AgNP surface were identified by Fourier-transform infrared spectroscopy (FTIR) (Fig. 4; Table 2). Bands at 3412, 2360, 1612, 1384, 1245 and 586 cm^{-1} were assigned to hydroxyl/amine, aliphatic C–H, amide/carbonyl (90 [43]), amine (82 [38]), ether/organosulfur (63) and metal–oxygen-related vibrations [34]. Alcoholic extracts of these assignments were in accordance to previous reports on phytochemical and proteinaceous components from plant-mediated AgNP systems [1–6].

It contributes the surface adsorption and electron donation from constituents of extract as indicated by bands related with hydroxyl, carbonyl, amide and ether. In classic chemical synthesis, Ag^+ reduction generally uses an exclusive reducing agent and a traditional stabiliser. Multiple extract components involved in $\text{Ag}^+ \rightarrow \text{Ag}^0$ reduction and capping may be co-contributing at the same time. However, FTIR does not provide evidence that any one fraction controlled the reaction process because individual phenolic and organosulfur compounds were not quantified.

Table 2. FTIR spectral peak assignments for *A. ampeloprasum* extract and synthesized AgNPs.

Synthesized AgNPs (cm^{-1})	Functional Group Assignment	Phytochemical Family Origin
3412	O–H Stretching / N–H stretching	Phenolics, Flavonoids, Proteins
2360	C–H stretching vibrations	Aliphatic matrix components
1612	C=O Stretching (Amide I band)	Proteins / Secondary metabolites
1384	C–N stretching / N–H bending	Proteins / Amide links
1245	C–O stretching / C–S bonds	Organosulfur compounds / Esters
585	Ag–O / metallic-core interaction	Metal–oxygen / metal–biomass binding

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3.3. SEM :

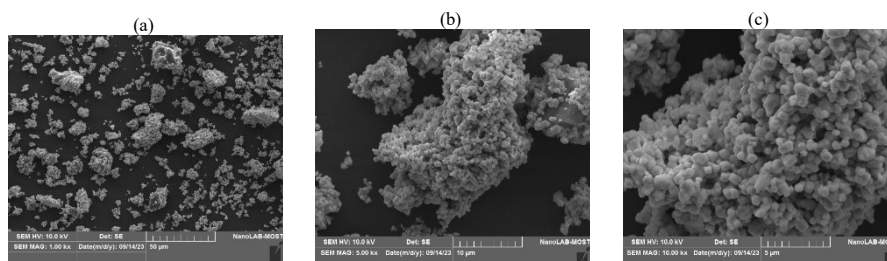


Fig. 5. (a) Overview of clusters at low-magnification; (b) Network of clusters aggregated together and (c) domains that are nearly spherical viewed under higher magnifications in the SEM images from AgNPs. This will retain scale information that was embedded in the original micrographs.

SEM showed approximately spherical primary domains within a strongly aggregated cluster network (Fig. 5a–c). ImageJ analysis of 100 domains gave a mean diameter of 42.5 ± 5.3 nm. Drying and interactions among surface-bound biomolecules may have contributed to aggregation, but the observed clustering must also be considered a functional limitation: aggregation can reduce dispersion, accessible surface area, and the number of exposed sites available for radical interaction. This interpretation is consistent with the decline in AgNP DPPH inhibition at 3.00 mg/mL, although direct colloidal measurements were not performed.

3.4. XRD analysis

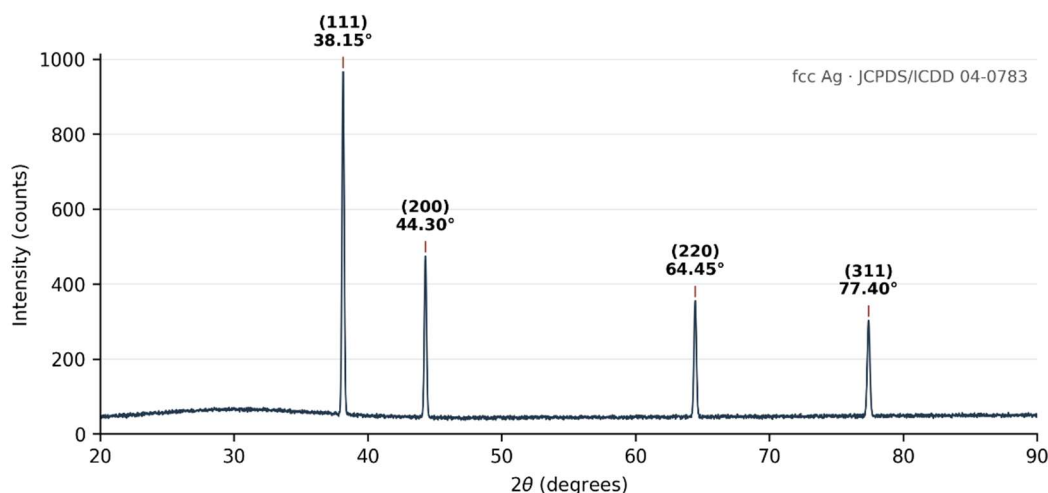


Fig. 6. Indexed XRD pattern of synthesized AgNPs showing the (111), (200), (220), and (311) reflections of fcc silver (JCPDS/ICDD 04-0783).

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Table 3. Indexed XRD peaks, FWHM values, and Debye–Scherrer crystallite-size calculations ($K = 0.9$; $\text{Cu K}\alpha \lambda = 0.15406 \text{ nm}$; mean $D = 45.0 \text{ nm}$).

2θ ($^\circ$)	Plane (hkl)	FWHM ($^\circ$)	β (rad)	D (nm)	Phase assignment
38.15	(111)	0.1868	0.003260	45.0	fcc Ag; JCPDS/ICDD 04-0783
44.30	(200)	0.1906	0.003327	45.0	fcc Ag; JCPDS/ICDD 04-0783
64.45	(220)	0.2087	0.003642	45.0	fcc Ag; JCPDS/ICDD 04-0783
77.40	(311)	0.2262	0.003948	45.0	fcc Ag; JCPDS/ICDD 04-0783

The indexed XRD pattern (Fig. 6) contained peaks at $2\theta = 38.15^\circ$, 44.30° , 64.45° , and 77.40° , assigned to the (111), (200), (220), and (311) planes of face-centred cubic metallic silver (JCPDS/ICDD 04-0783). No distinct Ag_2O or AgO peaks were detected. Peak-specific FWHM values ranged from 0.1868° to 0.2262° and produced crystallite sizes of $45.0\text{--}45.0 \text{ nm}$ (Table 3). The mean Debye–Scherrer size was 45.0 nm , consistent with the workbook calculation.

3.5. AFM analysis

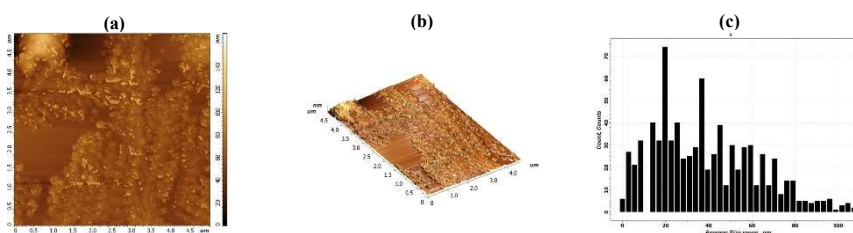


Fig. 7. AFM analysis of synthesized AgNPs: (a) two-dimensional topography; (b) three-dimensional surface topography; and (c) grain-size distribution.

AFM images (Fig. 7a–c) supported the clustered morphology observed by SEM. Across the $5 \mu\text{m} \times 5 \mu\text{m}$ scan area, the average roughness was 14.2 nm and the root-mean-square roughness was 18.6 nm . The grain-size distribution was consistent with heterogeneous surface topography and aggregated, capped particle domains; it does not by itself demonstrate colloidal stability in suspension.

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3.6. DPPH radical scavenging activity

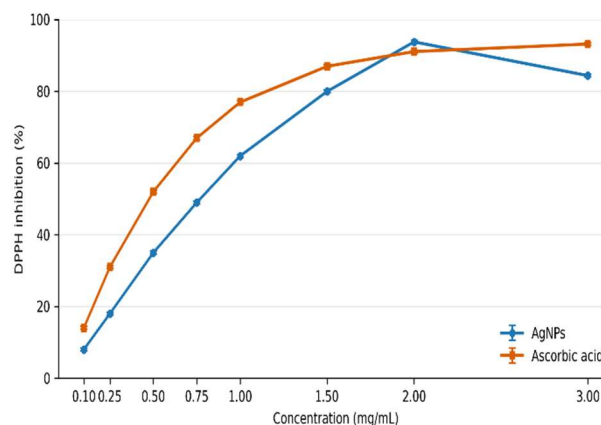


Fig. 8. DPPH inhibition by synthesized AgNPs and ascorbic acid over 0.10–3.00 mg/mL. Values are mean $\pm$ SD (n = 3).

Table 4. DPPH inhibition by AgNPs and ascorbic acid. Values are mean $\pm$ SD (n = 3).

Concentration (mg/mL)	AgNPs inhibition (%)	Ascorbic acid inhibition (%)
0.10	8.00 $\pm$ 0.39	14.00 $\pm$ 0.40
0.25	18.00 $\pm$ 0.39	31.00 $\pm$ 0.40
0.50	35.00 $\pm$ 0.40	52.00 $\pm$ 0.40
0.75	49.00 $\pm$ 0.39	67.00 $\pm$ 0.40
1.00	62.00 $\pm$ 0.40	77.00 $\pm$ 0.40
1.50	80.00 $\pm$ 0.40	87.00 $\pm$ 0.40
2.00	93.80 $\pm$ 0.40	91.10 $\pm$ 0.40
3.00	84.40 $\pm$ 0.40	93.20 $\pm$ 0.40

The DPPH assay showed a concentration-related increase in AgNP inhibition from $8.00 \pm 0.39\%$ at 0.10 mg/mL to $93.80 \pm 0.40\%$ at 2.00 mg/mL (Table 4; Fig. 8), followed by a decline to $84.40 \pm 0.40\%$ at 3.00 mg/mL. Ascorbic acid increased from $14.00 \pm 0.40\%$ to $93.20 \pm 0.40\%$ over the same range. Concentration effects were significant for AgNPs, $F(7,16) = 19152.01$, $p < 0.001$, and ascorbic acid, $F(7,16) = 16366.04$, $p < 0.001$. Apparent IC_{50} values were 0.769 mg/mL for AgNPs and 0.476 mg/mL for ascorbic acid. The higher AgNP IC_{50} indicates lower potency than ascorbic acid at the 50% inhibition level.

Ascorbic acid showed higher mean inhibition than AgNPs from 0.10 to 1.50 mg/mL, whereas AgNPs were slightly higher at 2.00 mg/mL. These pointwise differences are descriptive because the available ANOVA tested the concentration effect within each material rather than direct between-material contrasts at individual concentrations. The AgNP decline at 3.00 mg/mL is compatible with reduced accessible surface area caused by high-concentration

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aggregation, but this mechanism remains tentative without dynamic light scattering or zeta-potential measurements.

4. Discussion

The data indicates that *A. ampeloprasum* extract from Iraqi grown source mediated Ag^+ reduction using 35 °C, pH 8.5 stirred light-reduced method protocol described here. In the current context line Pairs of the spectral optical, FTIR data and microstructural SEM, XRD as well as AFM results together support the growth process for capped after agglomeration crystalline fccAgNPs. While AgNPs had to be utilized in greater concentrations than ascorbic acid (50% DPPH inhibition) at 0.30 mg/mL, they reached 93.80% at the minimal concentration of ions used (2.00 mg/mL). The later drop at 3.00 mg/mL is more directly related to the morphological limitation than a vague statement of antioxidant potential on assay performance. As no profiling of phytochemicals was done, the study did not prove that this Iraqi cultivar has above average concentrations of folate or "rare" phenolics. Stability in suspension cannot be proclaimed either without time-resolved optical, dynamic light scattering or zeta-potential measurements.

5. Conclusion

The findings answer the three research questions. First, *A. ampeloprasum* extract mediated $\text{Ag}^+ \rightarrow \text{Ag}^0$ reduction when combined with 0.1 M AgNO_3 at pH 8.5 and stirred for 60 min at room temperature (35 °C) under light-restricted conditions. Second, the product showed a 435 nm SPR band, FTIR-associated capping groups, aggregated approximately spherical domains, and an indexed fcc Ag phase with a mean Debye–Scherrer crystallite size of 45.0 nm. Third, AgNPs displayed a significant concentration-dependent DPPH response from 0.10 to 2.00 mg/mL (ANOVA $p < 0.001$; apparent $\text{IC}_{50} = 0.769$ mg/mL), followed by reduced inhibition at 3.00 mg/mL. Accordingly, the material demonstrates antioxidant activity under the assay conditions, but aggregation and unmeasured colloidal stability limit broader biomedical interpretation.

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