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Green synthesis of silver nanoparticles using unicellular *Dunaliella salina* strain IPPAS D-294 extract

Ruslan Suleymanov[1*], Aynura Jalilova[2], Yashar Feyziyev[1]

[1]*Institute of Molecular Biology, Ministry of Science and Education of the Republic of Azerbaijan, 11 Izzat Nabiyev Str., AZ1073, Baku, Azerbaijan*
[2]*Baku State University, 23 Academician Zahid Khalilov Str., AZ1148, Baku, Azerbaijan*

[*]***For correspondence:*** *r.suleymanov@imbb.science.az*

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Green synthesis of silver nanoparticles (AgNPs) using microalgae represents an eco-friendly alternative to conventional chemical methods. In this study, biomass of *Dunaliella salina* strain IPPAS D-294, cultivated under 1.5 M NaCl conditions, was used for the biosynthesis of AgNPs. The extract obtained from *D. salina* was applied as a reducing and stabilizing agent for Ag^+ ions. UV-Vis spectroscopy revealed the formation of a broad absorption band in the range of 380-450 nm, associated with Surface Plasmon Resonance, indicating the partial formation of AgNPs. FTIR spectroscopy revealed the presence of hydroxyl, carbonyl, and polysaccharide-associated functional groups, indicating the involvement of biomolecules from *Dunaliella salina* extract in the reduction and stabilization of silver nanoparticles. This study highlights the properties of *D. salina* strain IPPAS D-294-mediated synthesis and provides insight into optimizing conditions for stable nanoparticle production.

Keywords: *Dunaliella salina*, *green synthesis, nanoparticle formation, UV-Vis spectra, FT-IR spectroscopy*

INTRODUCTION

Dunaliella salina is a single-celled green microalga that lives in very salty environments. It is widely regarded as an important biologically system for biotechnological use because it can naturally produce valuable biological active compounds (Barbosa et al. 2023; Pais et al., 2024). One of its most notable features is its strong ability to accumulate high amounts of carotenoids, especially β-carotene, when it is exposed to stressful conditions such as high salt concentration, strong light, or limited nutrients (Shariati et al., 2019).

D. salina has also been investigated for biomedical applications due to its high content of bioactive carotenoids such as β-carotene and zeaxanthin (Barbosa et al. 2023). Chitosan nanoparticles containing carotenoid-rich *D. salina* extracts exhibited significant wound-healing activity by enhancing tissue regeneration, reducing TNF-α expression, and increasing VEGF and collagen production, indicating strong antioxidant, anti-inflammatory, and antibacterial effects (El-Baz et al., 2023). *D. salina* is widely recognized for its high β-carotene accumulation capacity, although the regulatory mechanisms of carotenoid overproduction remain not fully understood (Lamers et al., 2008).

The growing use of metal nanoparticles in medicine has increased interest in eco-friendly synthesis methods. Biological systems such as bacteria and microalgae can reduce metal ions and form nanoparticles as part of detoxification processes. Some studies evaluated the ability of several *Lactobacillus* strains and algal species to synthesize silver nanoparticles under different silver nitrate concentrations (Mohseniazar et al., 2011).

The algal biomass contains various natural biomolecules, including proteins, polysaccharides,

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phenolic compounds, and pigments. These substances can function as natural reducing and stabilizing agents, which makes *D. salina* useful for environmentally friendly synthesis of nanoparticles without the need for toxic chemicals. Because of these properties, *D. salina* is increasingly recognized as an important organism in microalgal biotechnology, nanobiotechnology, and the development of sustainable bio-based products.

Green synthesis of metal nanoparticles using microalgae has gained significant attention. A photoinduced eco-friendly synthesis of gold nanoparticles (AuNPs) was carried out using an aqueous extract of the halotolerant microalga *D. salina* as both a reducing and stabilizing agent. The synthesized AuNPs exhibited a surface plasmon resonance peak at 560 nm and showed predominantly spherical morphology with an average particle size of 22.4 nm (Singh et al., 2019).

The suspension culture of *Arabidopsis thaliana* cells and *D. salina* microalgae was shown to reduce gold ions, leading to nanoparticle formation. The average size of gold nanoparticles synthesized using *D. salina* was smaller (≈8 nm) compared to those obtained with *A. thaliana* cells (≈25 nm). It was also demonstrated that only cell-conditioned culture media, after removal of biomass, exhibited gold-reducing activity, while fresh media showed no such effect (Chumakov et al., 2019). One of the cultivation methods for *D. salina* strain IPPAS D-294 described in the literature involves growth in Conway medium under controlled light-dark conditions (12 h light/12 h dark, 9000 lux) at 18°C for 2 weeks (Shantkriti et al., 2023).

FTIR analysis indicated that functional groups present in *D. salina* biomass, including hydroxyl (–OH), carbonyl (C=O), and amine (–NH) groups, participate in the reduction and stabilization of gold nanoparticles (GNPs). TEM and SEM analyses further revealed that the synthesized nanoparticles were predominantly spherical with an average size of approximately 50 nm (Basiratnia et al., 2021). Green synthesis of Fe_3O_4 nanoparticles using *D.salina* extract resulted in highly stable and biocompatible nanomaterials with enhanced biomedical potential compared to chemically synthesized nanoparticles (Jafari et al. 2024). Gold nanoparticles biosynthesized using *D. salina* extract were found to be small in size (approximately 12 nm) and exhibited selective cytotoxic activity against cancer cells (Chumakov et al., 2019). Biosynthesized silver nanoparticles produced using *Trichodesmium erythraeum* extract exhibited strong antioxidant, antibacterial, and anticancer activities, with crystalline cubical morphology and an average size of 26.5 nm (Sathishkumar et al., 2019).

Some studies have evaluated the use of silver nanoparticles synthesized from *Chlorella pyrenoidosa* and *Dunaliella salina* as feed additives in shrimp culture. The results indicated that AgNPs derived from microalgae positively influenced the growth and survival rate of vannamei shrimp, suggesting their potential to improve aquaculture performance and support more sustainable farming conditions (Mishbach et al. 2024).

The aim of this study was to investigate the feasibility of green synthesis of silver nanoparticles using an aqueous extract of *Dunaliella salina* strain IPPAS D-294 cultivated under laboratory conditions. Particular attention was given to the use of microalgal biomass as an environmentally friendly reducing and stabilizing agent for AgNP production. The present study demonstrates the potential of *D. salina* as a biological source for environmentally friendly synthesis of silver nanoparticles.

MATERIALS AND METHODS

Microalgae cultivation: The microalga *Dunaliella salina* strain IPPAS D-294 was cultivated according to the method of Abdullayev and Semenenko (1974) with minor modifications. The growth medium contained 10 mM Tris-HCl (pH 7.2), 0.5 M NaCl, 5 mM KNO_3, 1 mM KH_2PO_4, 5 mM $MgSO_4 \cdot 7H_2O$, 5 mM $NaHCO_3$, 0.03 mM $FeSO_4 \cdot 7H_2O$, and 1 mM EDTA. The medium was supplemented with a micronutrient solution at a rate of 2 mL L^{-1}. The stock micronutrient solution contained 48 mM H_3BO_3, 9.1 mM $MnCl_2 \cdot 4H_2O$, 0.8 mM $ZnSO_4 \cdot 7H_2O$, 1.4 mM $(NH_4)_2MoO_4 \cdot 4H_2O$ and 0.2 mM NH_4VO_3. Cultures were maintained at 27±1ºC under a 16:8 h light/dark photoperiod. Illumination was provided at an intensity of 20 W m^{-2} throughout the light phase.

The halophilic microalga *D. salina* strain IPPAS D-294 was cultivated under laboratory conditions in cultivation media containing 1.5 M NaCl. Cell growth was monitored spectrophotometrically by measuring optical density (OD) at 720 nm. The recorded OD values for the culture grown at 1.5 M NaCl were 0.3 on the initial day of cultivation, increasing to 1.6 on the following day and reaching 3.3 during further growth. The growth curve obtained in this study is presented in Fig. 1.

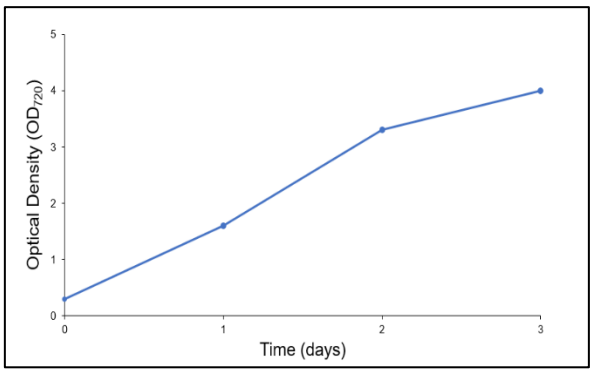

Fig. 1. Changes in optical density of *Dunaliella salina* culture during cultivation.

Biomass selection and preparation: The biomass was harvested by centrifugation and dried in the dark at 20°C. After drying, the obtained biomass was ground into powder and subsequently used for extract preparation.

Preparation of algal extract: The algal extract was prepared using distilled water as a solvent. Distilled water was preheated to 100°C. The dried and powdered biomass of *D. salina* was weighed and mixed with preheated distilled water at a ratio of 1 g per 50 mL (equivalent to 20 mg/mL). The mixture was subjected to continuous stirring at 500 rpm using a magnetic stirrer and maintained at 80-90°C for 45 minutes. After extraction, the mixture was allowed to cool to room temperature and subsequently filtered through filter paper to obtain a clear extract. The obtained extract exhibited a transparent green-yellow coloration, indicating the presence of water-soluble pigments and biologically active compounds extracted from the algal biomass. The extract was stored in the dark at low temperature until further use in nanoparticle synthesis.

Biosynthesis of silver nanoparticles: Silver nanoparticles were synthesized using the prepared algal extract and silver nitrate solution. The pH of the reaction mixture was adjusted to 11.0 using an appropriate alkaline solution. The extract was mixed with a 6 mM solution of silver nitrate ($AgNO_3$) in a 1:1 volume ratio. The reaction mixture was subsequently incubated at room temperature in the dark to minimize photoinduced reactions during nanoparticle synthesis and then incubated under continuous stirring at 60°C and 600 rpm for 4 hours. After incubation, the reaction mixture was kept under ambient temperature in the dark for an additional 3 days to allow further development of the reaction. The primary step involves the reduction of Ag^+ ions from $AgNO_3$ to metallic silver (Ag^0). In *D. salina* strain IPPAS D-294 extract, several classes of biomolecules, including polyphenols, proteins containing $–NH_2$ and –SH functional groups, reducing sugars, and pigments such as carotenoids and chlorophyll derivatives, may act as reducing agents during nanoparticle synthesis. A simplified reaction can be represented as:

$$Ag^+ + Biomolecule_{Red} \rightarrow Ag^0 + Biomolecule_{Ox}$$

Biomolecules present in the *D. salina* extract act as reducing agents, converting Ag^+ ions into metallic silver nanoparticles while being oxidized during the process.

From a chemical standpoint, the synthesis process represents a redox reaction in which Ag^+ ions act as electron acceptors, while biomolecules present in the algal extract function as electron donors. Hydroxyl (–OH) and carbonyl (–C=O) groups are especially important, as they can undergo oxidation while transferring electrons to Ag^+.

Once Ag^0 atoms are formed, the synthesis proceeds through the classical stages of nanoparticle formation, including supersaturation of Ag^0 atoms, nucleation through the formation of small atomic clusters, and subsequent particle growth via atom addition or coalescence. The initial light gray coloration corresponds to nucleation, while the transition to brown indicates growth and increased particle density. The formation of silver nanoparticles is clearly demonstrated in Table 1.

Table 1. Visual color changes during silver nanoparticle synthesis using *Dunaliella salina* strain IPPAS D-294 extract.

Time (min, day)	Solution color	Visual observation	λmax (nm)	Absorbance (a.u.)	Process interpretation
0 min	Green	Clear green extract	—	—	Presence of pigments (chlorophylls, carotenoids); no AgNPs
1 hour	Light gray / slightly turbid	Initial turbidity	~410–420	Low	Onset of nucleation ($Ag^+ \rightarrow Ag^0$)
4 hours	Gray	Increased turbidity	~420	Increasing	Formation of nanoparticles
1 day	Grayish-brown	Visible color development	~420–430	Moderate	Growth phase of nanoparticles
3 days	Brown	Intense coloration	~430	High	Surface plasmon resonance of AgNPs
4 days	Dark brown	Strong color intensity	~430–440	Very high	Increased concentration of AgNPs
Other days	Dark brown / nearly black	Possible slight opalescence	~440+	Plateau / stabilization	Particle growth and possible aggregation

A gradual color change from green to dark brown was observed upon the addition of $AgNO_3$ to the *D. salina* strain IPPAS D-294 extract, indicating the formation of silver nanoparticles. This visual transformation is associated with the excitation of surface plasmon resonance, typically observed in the range of 420-440 nm. The increase in absorbance intensity over time suggests the progressive formation and growth of AgNPs.

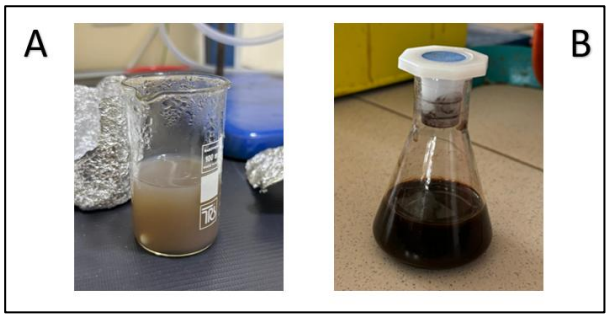

Fig. 2. Visual appearance of the reaction mixture during the synthesis of silver nanoparticles using *D. salina* strain IPPAS D-294 extract (A - immediately after synthesis (day 0); (B) after 4 days of incubation).

The broadening of the absorption peak further suggests a certain degree of size distribution and possible polydispersity of the synthesized nanoparticles. The biosynthesis of silver nanoparticles using *D. salina* strain IPPAS D-294 extract can be explained through a coupled reduction–stabilization mechanism mediated by biomolecules present in the algal matrix.

UV-Vis spectroscopy: The spectral properties of the synthesized nanoparticles were analyzed using a Shimadzu 1280 spectrophotometer in the wavelength range of 200-800 nm. The formation of nanoparticles was evaluated based on absorption features associated with the Surface Plasmon Resonance frequency range.

Fourier Transform Infrared Analysis: Fourier transform infrared (FTIR) spectroscopy was performed to identify the functional groups involved in the reduction and stabilization of silver nanoparticles synthesized using algal extract. The FTIR spectra of the samples were recorded using a Thermo Scientific Nicolet iS10 FT-IR spectrometer (USA) in the wavelength range of 4000-400 cm^{-1}. The samples were dried prior to analysis and measured at room temperature. The obtained spectra were analyzed to determine the presence of biomolecules such as proteins, polysaccharides, phenolic compounds, and other metabolites responsible for the bioreduction and capping of AgNPs. Characteristic absorption bands corresponding to different functional groups were identified and compared with previously reported studies (Dogmaz and Cavas, 2023).

RESULTS AND DISCUSSION

Visual observation of nanoparticle formation: The formation of silver nanoparticles was initially evaluated by visual observation. As shown in Fig. 2, the reaction mixture exhibited a light brown coloration immediately after synthesis

using *D. salina* extract. This color change is commonly associated with the formation of silver nanoparticles due to Surface Plasmon Resonance. After 4 days of incubation, a noticeable change in the appearance of the solution was observed (Fig. 2B), indicating further evolution of the reaction system.

At later stages (e.g., 45-60 min): Slight darkening and red shift may indicate: Ostwald ripening or aggregation due to insufficient capping. High ionic strength (especially relevant for *D. salina*) may also influence: double-layer compression and reduced colloidal stability.

Overall, the synthesis of AgNPs using *D. salina* extract is governed by a synergistic mechanism in which algal biomolecules simultaneously function as reducing and stabilizing agents. The process follows classical nucleation and growth kinetics, while spectroscopic evidence (SPR band at ~420-440 nm) confirms the formation and evolution of metallic nanoparticles. The system represents a typical example of green nanochemistry, where complex biochemical matrices replace conventional chemical reductants and surfactants. The synthesis conditions (pH 11.0 and 60°C) were selected based on previous studies on algal-mediated synthesis of silver nanoparticles as well as our earlier experiments with marine macroalgae. These conditions were chosen as suitable for facilitating the reduction of Ag^+ ions and the formation of silver nanoparticles using biological extracts. The successful synthesis of AgNPs under these conditions was confirmed by visual observation, UV-Vis spectroscopy, and FTIR analysis.

UV-Vis spectroscopic analysis: The formation of silver nanoparticles was further confirmed by UV-Vis spectroscopy in the wavelength range of 200-800 nm (Fig. 3).

The recorded spectra showed a broad absorption band in the region of approximately 400-430 nm, which is characteristic of silver nanoparticles and corresponds to Surface Plasmon Resonance.

The appearance of a broad absorption band in the UV-Vis spectrum indicates the formation of silver nanoparticles exhibiting surface plasmon resonance. The broad nature of the band suggests a distribution of particle sizes rather than a monodisperse nanoparticle population. However, the observed absorption peak was relatively broad and this might indicate that the synthesized nanoparticles are polydisperse, meaning that they vary in size and possibly in shape. In contrast to monodisperse systems, where nanoparticles exhibit a narrow and symmetric surface plasmon resonance band, this suggests the presence of particles with different dimensions, leading to overlapping optical responses. This behavior may be attributed to the rapid reduction of silver ions under alkaline conditions and at relatively high precursor concentration, which can promote uncontrolled nucleation and growth processes. Additionally, biomolecules present in the extract of *D. salina* may not provide sufficient stabilization, resulting in a heterogeneous nanoparticle population. Therefore, the spectral characteristics reflect a non-uniform distribution of nanoparticle sizes, which significantly influences the optical properties of the system.

Time-dependent formation of nano-particles: The kinetics of nanoparticle formation was evaluated by monitoring the absorbance at 420 nm over time.

An increase in absorbance with time was observed, indicating the gradual formation of silver nanoparticles in the reaction mixture. This trend suggests a continuous reduction of silver ions during the incubation period (Fig. 4).

The synthesis was carried out under alkaline conditions (pH 11.0) and relatively high silver ion concentration (6 mM $AgNO_3$). These parameters significantly influence the formation process of nanoparticles.

The obtained spectral features indicate that the reaction conditions play a crucial role in determining the optical properties of the synthesized nanoparticles.

Fourier-transform infrared spectroscopy analysis: FTIR analysis was performed to identify the functional groups present in the synthesized silver nanoparticles and to evaluate the possible biomolecules involved in the reduction and stabilization processes. The FTIR spectrum of AgNPs synthesized using *D. salina* extract is presented in Fig. 5.

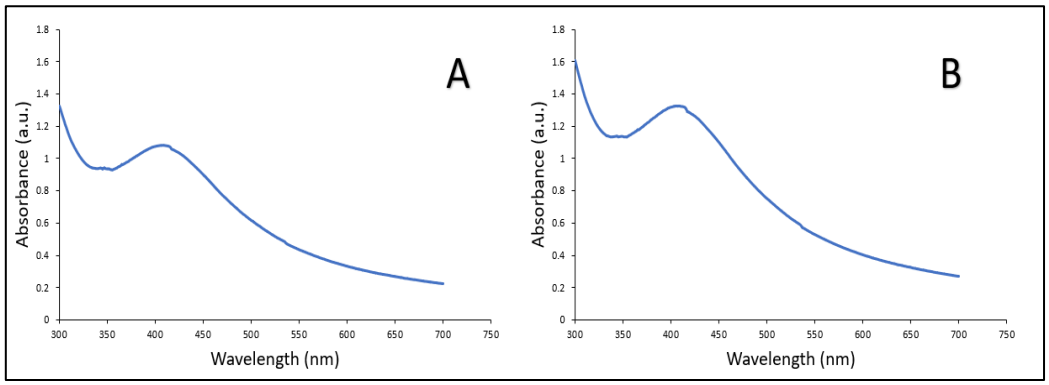

Fig. 3. UV-Vis spectra of *Dunaliella salina* silver nanoparticles. A – at 72 hours of synthesis B – at 96 hours of synthesis.

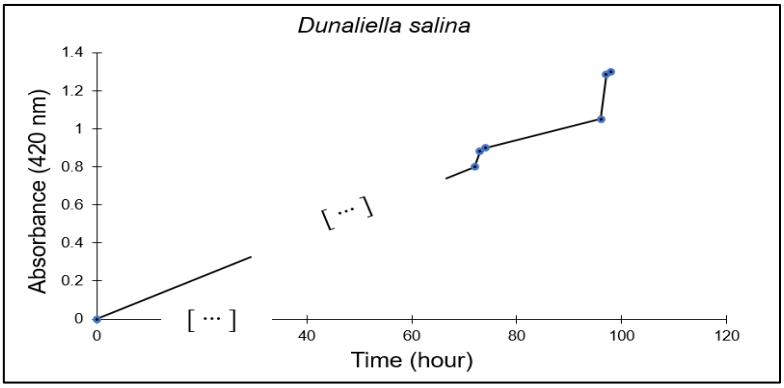

Fig. 4. Time-dependent formation of silver nanoparticles using *D. salina* extract.

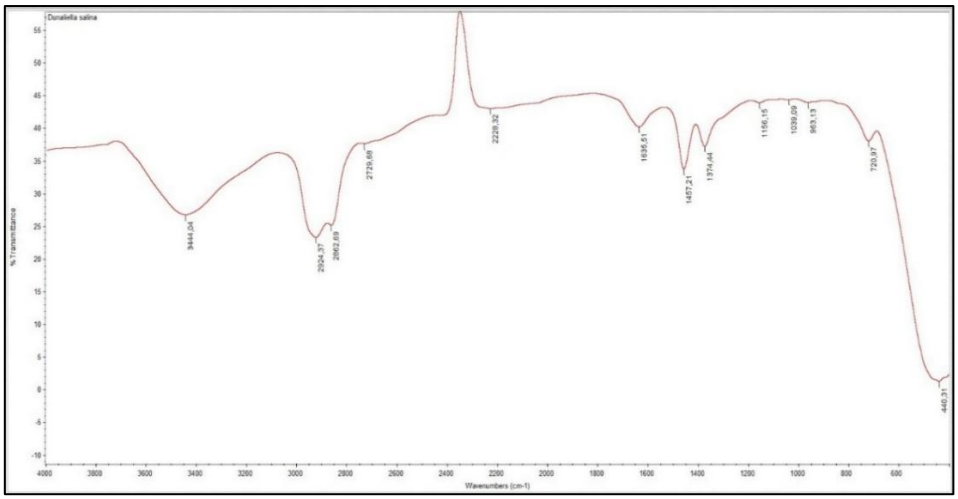

Fig. 5. FTIR spectrum of biosynthesized silver nanoparticles obtained using *Dunaliella salina* extract.

A broad absorption band observed around 3444 cm^{-1} corresponds to O–H stretching vibrations associated with hydroxyl groups of alcohols and phenolic compounds. The peaks detected near 2924 cm^{-1} and 2862 cm^{-1} are attributed to C–H stretching vibrations of aliphatic compounds. The absorption band at approximately 1635 cm^{-1} may be related to C=O

stretching or amide groups originating from proteins and other biomolecules present in the algal extract. Peaks observed in the region of 1457–1374 cm^{-1} are associated with bending vibrations of C–H and O–H functional groups.

Additional peaks around 1156 cm^{-1} and 1039 cm^{-1} indicate the possible presence of C–O and C–O–C groups characteristic of polysaccharides and carbohydrates. These biomolecules may play an important role in both the reduction of Ag^{+} ions and stabilization of the synthesized nanoparticles. The obtained FTIR results confirm the involvement of biologically active compounds from *D. salina* extract in the biosynthesis of silver nanoparticles.

Similar FTIR profiles for algae-mediated silver nanoparticles have previously been reported, particularly regarding the involvement of hydroxyl, carbonyl, and polysaccharide-associated functional groups in nanoparticle synthesis and stabilization (Navarro et al., 2019). FTIR results are consistent with previous reports on *D. salina*-mediated silver nanoparticles, where hydroxyl, amide, and carbonyl groups were identified as key functional groups involved in the reduction and stabilization of AgNPs (Singh et al., 2017).

CONCLUSION

The obtained results indicate that a sufficient amount of *D. salina* biomass is required to ensure effective synthesis of silver nanoparticles, since the availability of bioactive metabolites plays a key role in the reduction and stabilization of Ag^{+} ions.

The study demonstrates that, under properly controlled extraction and reaction conditions, including temperature, pH, and biomass concentration, unicellular *D. salina* can successfully be used as a biological platform for nanoparticle synthesis. The obtained results demonstrate the potential of *Dunaliella salina* biomass as a sustainable biological resource for silver nanoparticle synthesis. The synthesized AgNPs may serve as promising materials for future catalytic studies, including applications related to biohydrogen production.

Furthermore, the findings suggest that optimization of cultivation parameters is essential for improving nanoparticle yield and stability. In particular, future studies should focus on large-scale cultivation of *D. salina* under different salinity conditions (e.g., 2-3 M and other gradients), as salinity is expected to significantly influence the metabolic profile and, consequently, the efficiency of nanoparticle biosynthesis.

In addition, further research is needed to explore different aspects of the synthesis process, including the role of specific biomolecules, extraction conditions, and reaction kinetics, in order to better understand and control the formation of stable and uniformly dispersed nanoparticles.

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CONFLICT OF INTEREST

The author declares no conflict of interest related to this study.

AUTHOR CONTRIBUTIONS

Ruslan Suleymanov conceived and designed the study, conducted the experimental work, analyzed the spectroscopic data, and prepared the initial manuscript draft. Aynura Jalilova contributed to data interpretation, laboratory analyses, and critical revision of the manuscript. Yashar Feyziyev supervised the research, contributed to the study design and scientific interpretation of the results, and reviewed and approved the final version of the manuscript. All authors read and approved the final manuscript and agree to be accountable for all aspects of the work.

AI STATEMENT

The authors declare that no artificial intelligence (AI) tools were used to generate, analyze, interpret, or validate the experimental data and scientific findings presented in this study. Any AI-assisted technologies, if used, were limited to language editing, grammar correction, or formatting support. The authors take full responsibility for the accuracy, originality, and integrity of the manuscript.

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ORCIDs:
Ruslan Suleymanov: https://orcid.org/0009-0005-1708-8592
Aynura Jalilova: https://orcid.org/0000-0003-4924-4316
Yashar Feyziev: https://orcid.org/0009-0003-3528-9043