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Research article

Characterization and antibacterial/cytotoxic activity of silver nanoparticles synthesized from *Dicranum scoparium* moss extracts growing in Armenia

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Abstract: This study focuses on a simple, non-toxic, and environmentally friendly method for the green synthesis of silver nanoparticles using *Dicranum scoparium* moss extract. It includes the characterization of the biosynthesized nanoparticles and an evaluation of their antibacterial, antifungal, and anticancer activities. Transmission electron microscopy (TEM) and dynamic light scattering (DLS) analyses confirmed that the biosynthesized silver nanoparticles were within the nanoscale range (50–100 nm) and exhibited an irregular morphology. The biogenic nanoparticles demonstrate antibacterial activity against bacterial strains *Staphylococcus aureus*, *Bacillus mesentericus*, *Escherichia coli,* and *Pseudomonas aeruginosa*. The results indicate a pronounced antibacterial activity against *E. coli* and *P. aeruginosa* compared to the tested Gram-positive bacteria, which is attributed to differences in the bacterial cell wall structure. Additionally, the green synthesized silver nanoparticles inhibited the growth of *Mucor plumber*, *Geotrichum candidum*, *Cladosporium herbarum,* and *Aspergillus flavus* mold fungi. Additionally, they expressed considerable cytotoxic properties against cancer cells.

Keywords: Ag-NPs; green synthesis; moss *Dicranium scoparium*; antibacterial activity; antifungal activity

1. Introduction

Metal nanoparticles have become integral to advancements in nanotechnology, driven by their unique physical, chemical, and biological properties [1]. Among these, silver nanoparticles (Ag-NPs) are particularly renowned for their potent antibacterial and antifungal activities, making them highly valuable in various applications [2]. Metals such as gold, silver, copper, and zinc are commonly used to synthesize nanoparticles. Silver, in particular, is favored due to its potent bactericidal properties. Certain flavonoids can chelate metal ions through their carbonyl groups or π-electrons, thus facilitating nanoparticle formation by participating in the nucleation and aggregation phases and bio-reduction. Additionally, flavonoids and flavonoid glycosides can actively induce the formation of metal nanoparticles (NPs) [3].

Traditional synthesis methods for metal NPs generally involve physical and chemical processes, which have drawbacks such as high energy consumption and the release of polluting chemicals into the environment. Biological synthesis methods for NPs offer a sustainable and viable alternative to conventional approaches, thus aligning with the goals for environmental and economic sustainability. Compared to traditional techniques, these methods offer advantages such as environmental friendliness, cost-effectiveness, and low energy consumption. The green synthesis of Ag-NPs by biological methods utilizes the reducing potential of compounds in living organisms, with plants and microorganisms serving as valuable resources due to their accessibility and safety. Furthermore, using biomass waste in the green synthesis process contributes to circular economy practices [1]. During "green" synthesis, bio-compounds coat the surfaces of NPs, thus enhancing their biological properties compared to those produced through chemical reduction. Additionally, compounds help prevent the contamination of NPs with toxic by-products, which may be hazardous compounds added during the physicochemical synthesis [4]. The biosynthesis of endogenous NPs is based on the ability of certain organisms to extract and hyperaccumulate metals from their environment. Research into the bioaccumulation mechanism has revealed that plants can retain metals in the form of nanoparticles. This synthesis occurs within the cell cytosol, which has a high reducing capacity, with cellular enzymes and other biomolecules playing a key role in the process [5]. Metal NPs are a category of nanomaterials that can exist in either amorphous or crystalline forms, and their surfaces can serve as carriers for liquid or gas droplets. Among NPs, those made from noble metals such as silver, gold, and platinum are particularly effective and widely used. Traditionally, silver has been used to control infections in the body and prevent food spoilage [6]. Additionally, it is employed as an agent for the treatment of ulcers [6]. Ag-NPs have garnered significant attention due to their unique properties, making them effective alternatives to traditional antibacterial compounds in antibiotic therapies [7,8]. These properties enhance the antimicrobial efficacy of silver. Over the years, the antibacterial effects of Ag-NPs have been demonstrated against more than 500 bacterial strains [8–11].

In recent years, there has been growing interest in harnessing the potential of lower plants, including bryophytes (mosses, liverworts, and hornworts), for NP synthesis. Bryophytes are primitive plants that lack a cuticle on their leaves and stems, which facilitates the easy absorption and transport of water. They possess the ability to efficiently retain water, which is supported by rapid absorption and low transpiration rates. Additionally, bryophytes can contribute to environmental humidity through evaporation. They are capable of quickly absorbing water from sources such as dew, fog, and mist—resources seldom utilized by other plants. These characteristics make bryophytes well-suited for the production of Ag-NPs using green synthesis methods. Moreover, the novelty of this work lies

in the synthesis of Ag-NPs for the first time using an extract from a moss species common in Armenia. Unlike angiosperms, bryophytes offer an advantage due to their simple and primitive structure, which may result in lower interferences from biochemical substances. Research on bryophytes remains relatively scarce compared to other plant groups [12].

Bryophytes possess a predominantly gametophytic life cycle and lack a clearly defined vascular system for water and nutrient absorption. Additionally, they are referred to as resurrection plants because they directly absorb water through the surface of their thalli. Bryophytes have a long-life cycle and are easy to select, identify, and replant [13]. Their crude extracts are rich in secondary metabolites, including phenolic acids, flavonoids, alkaloids, and terpenoids. These compounds play a crucial role in reducing ionic forms into bulk metal nanoparticles. Both primary and secondary metabolites are actively involved in the oxidation-reduction reactions that drive the synthesis of environmentally friendly nanosized particles [14]. The green synthesis method enables a large-scale production of nanoparticles that are cost-effective, free from toxic chemicals, simpler to produce, convertible into products in a short time, and well-defined in size and morphology. [6] Moss species contain B vitamins, tocopherols, prostaglandin-like polyunsaturated fatty acids, and various phenolic compounds. Moreover, mosses produce enantiomeric mono-, sesqui-, and diterpenoids similar to those found in vascular plants, as well as bibenzyls, bisbenzyls, and polyketides. Many of these compounds exhibit antimicrobial, antiviral, and anti-inflammatory activities, as well as cytotoxicity against cancer cell lines, muscle relaxant effects, and antioxidant properties [15].

It has been shown that many biological systems, including plants and algae, can convert inorganic metal ions into metal NPs through a reduction process that involves enzymes and metabolites present in these organisms. Secondary metabolites of bryophytes, such as sugars, terpenoids, polyphenols, alkaloids, phenolic acids, and proteins, play a crucial role in reducing metal ions to NPs, thus ensuring their subsequent stability.

Flavonoids found in *D. scoparium* moss [16] can incorporate metal ions into chelate complexes and reduce them. These flavonoids contain various functional groups that promote the formation of NPs. It is hypothesized that tautomeric transformations of flavonoids from the enol form to the keto form can release a reactive hydrogen atom, which, in turn, reduces metal ions to form NPs.

Flavonoids present in the leaves of *D. scoparium* moss may participate in the bioreduction of silver ions ($Ag^+ + Ag^0$). This process could be attributed to the electron released during the reduction of NO_3^- NO_2^- and the flavonoids that stabilize the synthesized NPs [17].

This study is dedicated to synthesizing AG-NPs using the extract of mosses of the genus *Dicranum*, which is found across temperate and subarctic regions of the Northern Hemisphere, including Europe, Asia, North America, and Armenia. In our earlier studies, we have already shown a high content of flavonoids, sugars, and amino acids in the moss *Dicranum scopariu* [16]. Therefore, the aim of the present work is to synthesize silver nanoparticles using a green synthesis method that utilizes the crude water extract of *D. scoparium* moss, thus highlighting both the novelty and the potential sustainable benefits of using bryophytes in NP fabrication.

2. Materials and methods

2.1. Chemical and reagents

Peptone was sourced from Carl Roth GmbH (Germany). Tryptone, glucose, $AgNO_3$, dimethyl

sulfoxide (DMSO), phosphate-buffered saline (PBS), 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide (MTT), and other chemicals were obtained from Sigma-Aldrich GmbH (Taufkirchen, Germany). The following bacteria were obtained from were purchased from ATCC (Manassas, VA, USA): *P. aeruginosa* ATCC3032, *E. coli* BW25113, *S. aureus* ATCC65380, and HeLa ATCC-CCL-2 cancer cells.

2.2. Collection and extraction of D. scoparium plant material for the synthesis of AG-NPs

The moss *D. scoparium* was collected from the Floristic region of Lori, the Bazum Mountain Range, and the Gulakarak Arboretum, Armenia at an altitude of 1400–1500 m above the mean sea level. The plant materials were identified by Dr. A. Poghosyan from the Department of Botany and Mycology, Yerevan State University (YSU), Armenia. The confirmed specimens were deposited in the Takhtadjyan Herbarium of the Department of Botany and Mycology at YSU under Voucher No. 13453. The collected mosses were shade-dried and made into powder.

Upon collection, the mosses were shade-dried to preserve their bioactive compounds and subsequently ground into a fine powder. An aqueous extract of *D. scoparium* was prepared using the maceration technique at a 1/20 (w/v) ratio to serve as a reducing and stabilizing agent for the synthesis of Ag-NPs. Specifically, 5 grams of the dried moss powder were mixed with 100 mL of deionized water. This mixture was placed on a mechanical shaker at 150 rpm for one hour. The extraction was performed at a temperature of 60 ℃; after the extraction period, the mixture was allowed to cool to room temperature. Then, the resulting crude extract at a 50 mg DW/mL concentration was filtered through Whatman No. 1 filter paper to remove any undissolved particulate matter, thus yielding a clear filtrate. This filtrate, which is rich in reducing agents, was subsequently utilized in the synthesis of Ag-NPs.

2.3. Green synthesis of DS-Ag-NPs

High-purity silver nitrate ($AgNO_3$, 99.0% purity, Sigma-Aldrich, USA) was used to synthesize biogenic Ag-NPs using *D. scoparium* extract (DS-Ag-NPs). An aqueous solution of 1 mM $AgNO_3$ was used as the precursor. Specifically, 45 mL of the 1 mM $AgNO_3$ solution was added to 5 mL of the moss extract solution at a volume ratio of 9: 1 [18]. The concentration of the extract was 50 mg DW/mL. The mixture was shaken at room temperature for 50–60 minutes under illumination. The color of the reaction mixture changed from yellowish to dark brown, thus indicating the formation of Ag-NPs. Then, the reaction mixture was centrifuged at 14,000 rpm for 5 minutes, after which the precipitate was collected and dissolved in deionized water for further use.

2.4. Characterization of synthesized silver nanoparticles

The reduction of biosynthesized Ag-NPs was subjected to (perkin-Elmer Lamda 35) UV-visible spectroscopy in the range of 200–800 nm. Fourier Transform Infrared Spectroscopy (FTIR) spectra were obtained using a spectrophotometer (Spectrum RX 1, Perkin Elmer) in the spectral region of 4000–400 cm^{-1}. The morphology, size, and elemental analysis of the prepared Ag-NPS were examined using Field emission scanning electron microscopy (FESEM) with EDX (Carl Zeiss, SUPRA 55 model). The distribution pattern of the DS-Ag-Nps particle size was evaluated by a Dynamic light

scattering analysis (DLS) and the stability was determined by the Zeta potential analysis by zeta sizer (Malvern instrument Ltd, UK).

2.5. Determination of the antibacterial activity of Ag-NPs and MOS extract

The antibacterial activity of the moss extract and synthesized biogenic DS-Ag-NPs was determined against Gram-negative *P. aeruginosa* ATCC3032, *E. coli* BW25113, and Gram-positive *B. mesentericus* wild type (WT) (isolated from soil), *S. aureus* ATCC65380 bacteria. The strains used were obtained from the culture repository of the Department of Biochemistry, Microbiology, and Biotechnology at YSU. The bacterial strains were cultured in a nutrient medium containing peptone and tryptone, with glucose as the carbon source (pH 7.5, at a temperature of 37 °C) [19]. 150 µl of an aqueous extract of *D. scoparium* moss (50mg DW/m), 50, 100, and 150 µl of DS-Ag-NPs (1 mg/mL) were added to 10 ml bacterial growth medium containing cultured bacteria. The final concentrations of the extract and the bacterial growth medium were 0.75 mg DW/mL and 5, 10, and 15 µg/mL respectively. Bacteria cultivated without Ag-NPs and moss extract served as the negative control. The growth rate of bacteria in the presence of either the NPs or moss extract was determined using a densitometer (DEN-1B McFarland, Biosan, Latvia) during 6 h of growth. The specific growth rate of bacteria was calculated according to growth rate = $(\ln OD_t - \ln OD_0)/t$, where OD_0 is the initial value of optical density (OD); OD_t is the value of OD after *t* h [19].

Additionally, the antibacterial activity of the synthesized biogenic Ag-NPs was assessed by the determination of colony forming units (CFU) after the exposure of NPs using the Petri plate smear method. Bacteria at a concentration of 10^8 CFU/mL were incubated for 1 h in the presence of Ag-NPs (AgNPs 5µg/mL)) and the DS extract (4.54 mg DW/mL) at 37 °C. Then, 100 µL of each sample was applied to nutrient agar plates, and the growth of the bacterial colonies was counted after incubation for 24 hours at 37 °C. The number of CFU was determined by counting the viable bacterial colonies in each sample as described by Gabrielyan et al. [19].

2.6. MTT cytotoxicity assay

The MTT assay was performed to evaluate the cytotoxic effects on HeLa cells following the exposure to various concentrations of *D. scoparium* (DS) extract and DS green-synthesized Ag-NPs (DS-Ag-NPs) over periods of 4, 24, and 72 hours, as described earlier [20]. Each experiment included three independent replicates with three technical replicates each. The cytotoxicity was determined as a percentage of growth inhibition compared to the control cells treated with the solvent alone, set as 100% growth. The IC50 values for DS-Ag-NPs were calculated using a nonlinear regression with a variable slope.

2.7. Statistical analysis

All experiments were conducted in triplicate (n = 3), with the data reported as mean ± SD or ± SEM. A statistical analysis was performed using either a Student's t-test or an ordinary one-way analysis of variance (ANOVA) with a multiple comparisons test to evaluate significant differences between experimental series, with a p-value of ≤ 0.05 considered statistically significant.

3. Results

A UV-visible spectroscopic analysis was performed to confirm the NPs synthesis. The formation of silver NPs in the reaction mixture was measured using the absorption method, which shows an SPR band. The UV-visible spectrum revealed a specific SPR peak at 441 nm, which is consistent with the data for Ag-NPs (Figure 1 A). Silver nitrate dissolved in deionized water forms free silver ions. The extracellular synthesis of Ag-NPs was confirmed by the color change of the solution (Figure 1 B, C, and D). According to the literature, the SPR range for Ag-NPs produced via "green" synthesis is typically between 400–450 nm [17]. When the plant extract is added to silver nitrate, these free silver ions receive electrons and are converted into elemental silver. The appearance of a reddish-brown color is due to surface plasmon resonance (SPR) (Figure 1 D).

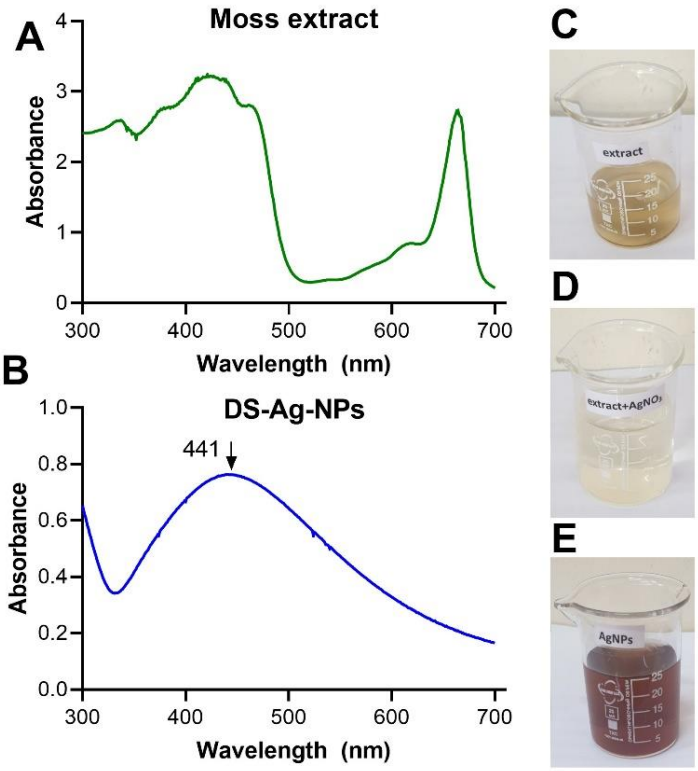

Figure 1. A-UV/Vis spectrum of reaction mixture containing biogenic Ag-NPs. B-UV/Vis spectrum of *D. scoparium* crude extracts. Visual observation of biosynthesized Ag-NPs by color change. C, D, and E represent the color change during syntheses of DS.Ag-NPs.

3.1. Transmission electron microscopy (TEM) and dynamic light scattering (DLS) analysis

For the biotechnological and medical use of synthesized NPs, data on their phytochemical properties are essential. The hydrodynamic radii distribution of green synthesized Ag-NPs has been analyzed by applying DLS (Figure. Before DLS measurements, an initially centrifuged and resuspended NP suspension was filtered through a 0.22 μm membrane filter. Data collection was

performed using the Mobius DLS instrument (Wyatt Technology, USA; laser wavelength: 532 nm; scattering detector angle: 168.7°). The measurements were conducted in triplicates, and 40 DLS acquisitions were recorded for each sample. Data analysis was carried out by applying the DYNAMICS software and Origin Pro.

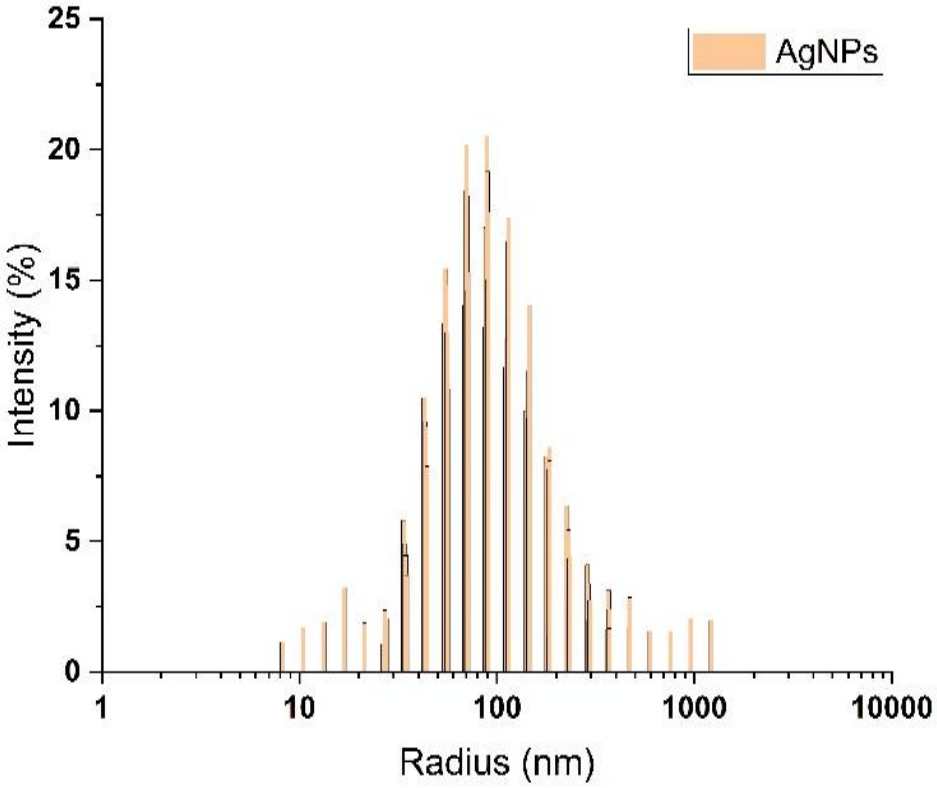

Figure 2. DLS measurements reveal the hydrodynamic radius of biosynthesized Ag-NPs.

As estimated from the DLS measurements, the cumulative hydrodynamic radius size of green synthesized Ag-NPs was 67.72 ± 3.3 nm. However, the polydispersity of ~ 32.6 ± 2.4% corresponds to the cumulative radius.

3.2. Antibacterial activity of Ag-NPs synthesized using D. scoparium moss extracts

The effect of Ag-NPs synthesized using *D. scoparium* moss extract on the specific growth rate of following bacteria *E. coli*, *P. aeruginosa*, *S. aureus*, and *B. mesentericus* was studied (Figure 3). The impact of the moss extract on the colony formation of these microorganisms was also assessed (Figure 4). The moss extract reduced the growth rate of the tested bacteria, with a more pronounced effect observed on *P. aeruginosa* (Figure 3D). At a concentration of 5 µg/ml, growth inhibition was observed as follows: *P. aeruginosa* ≈ 77%, *E. coli* ≈ 65%, *S. aureus* ≈ 59%, and *B. mesentericus* ≈ 40%. Gram-positive bacteria demonstrated less susceptibility to Ag-NPs as compared to Gram-positive *ones*.

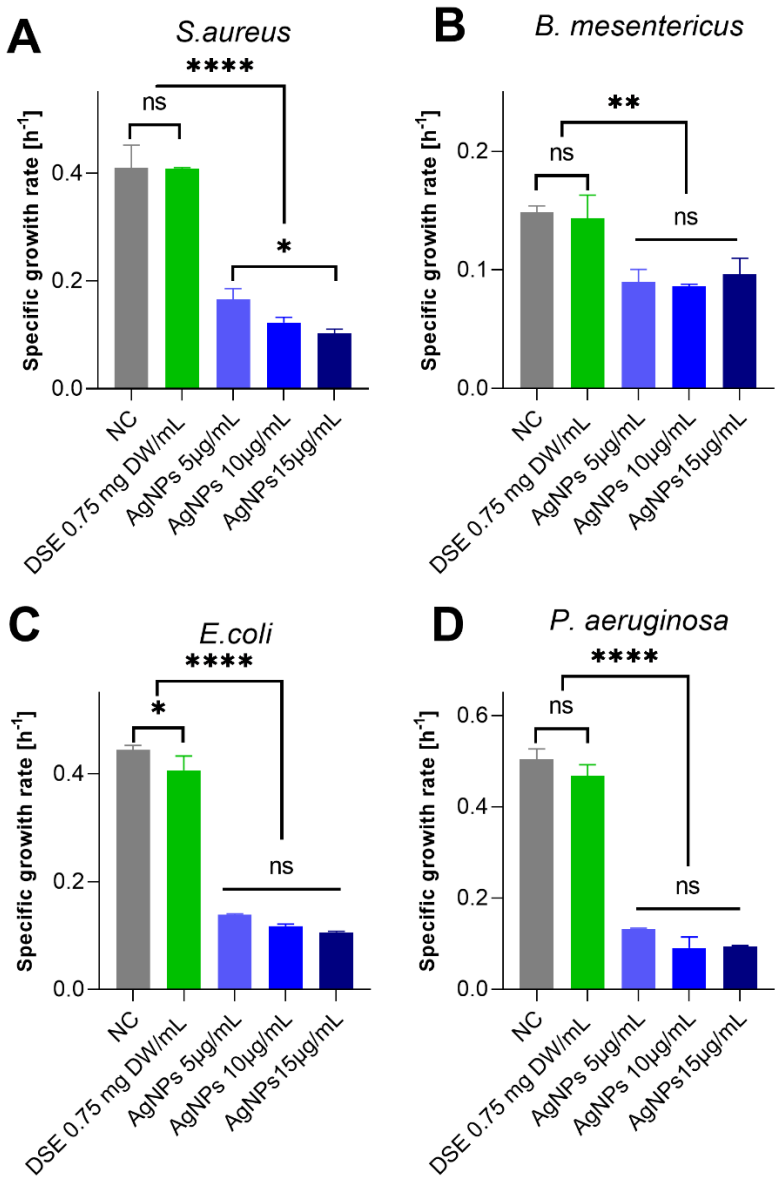

Figure 3. Growth rate changes of Gram-positive: *S. aures* (A) and *B. mesentericus* (B) and Gram-negative *E. coli* (C) and *P. aeruginosa* (D) bacteria after treatment with *D. scoparium* moss extract and DS-Ag-NPs. NS–none significant, *$p \leq 0.05$, **$p \leq 0.01$ ***$p \leq 0.001$, ****$p \leq 0.0001$.

Figure 4 shows the effect of biogenic Ag-NPs on the CFU of the bacteria studied. The data indicate that, in the presence of silver nanoparticles, the CFU-forming ability of *E. coli* and *S. aureus* decreased by ≈ 86% and ≈ 84%, respectively, while the CFU of *P. aeruginosa* and *B. mesentericus* decreased by ≈ 51% and ≈ 50%, respectively. Thus, biogenic Ag-NPs demonstrated significant antibacterial activities against the tested bacteria.

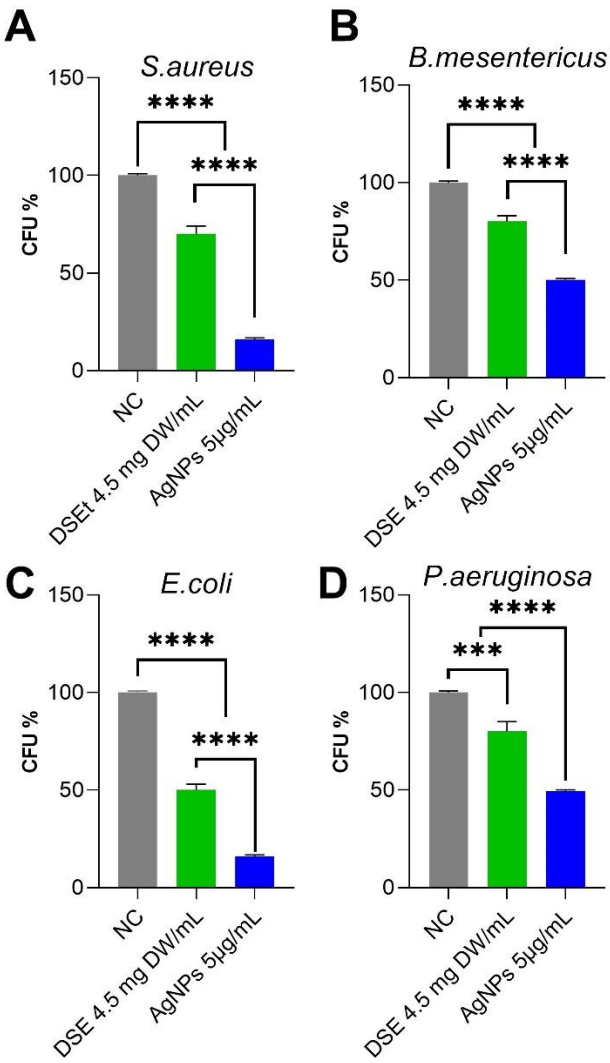

Figure 4. The Effect of DS-Ag-NPs on the Colony-Forming Ability of S. *aures* (A) and *B. mesentericus* (B), *E. coli* (C), and *P. aeruginosa* (D) bacteria after treatment with *D. scoparium* moss extract and DS-Ag-NPs. ***$p \leq 0.001$, ****$p \leq 0.0001$.

3.3. Growth-inhibiting properties of D. scoparium extract and green synthesized DS-Ag-NPs tested by MTT assay

The study assessed the growth-inhibitory effects of the *D. scoparium* (DS) extract and DS green-synthesized Ag-NPs (DS-Ag-NPs) on HeLa cancer cells. The effectiveness of the DS extract and DS-Ag-NPs was tested at various concentrations and exposure durations (4, 24, and 72 hours), with the results shown in Figures 5A, and B.

The DS extract alone did not inhibit the HeLa cell growth, even at the highest tested concentration (7.2 mg/mL) and the longest exposure time (72 hours). In contrast, the DS-Ag-NPs displayed considerable, concentration-dependent cytotoxic effects on the HeLa cells. At 24 and 72 hours, DS-Ag-NPs induced substantial growth inhibition at higher concentrations starting from 16.5 µg/mL, while lower concentrations did not highly impact the growth rate of the HeLa cells (Figure 5B).

The DS-Ag-NPs showed a time-dependent cytotoxic effect, although the response was not strictly

linear with an increasing exposure duration. Both the 24-hour and 72-hour exposures led to comparable levels of inhibition, whereas the 4-hour exposure resulted in a significantly lower inhibition. While the DS-Ag-NPs displayed some degree of concentration-dependent cytotoxicity, the observed inhibition remained below 50% across all the tested concentrations. Consequently, it was not possible to calculate the IC50 values within this concentration range, as the threshold for 50% inhibition of the HeLa cells was not reached.

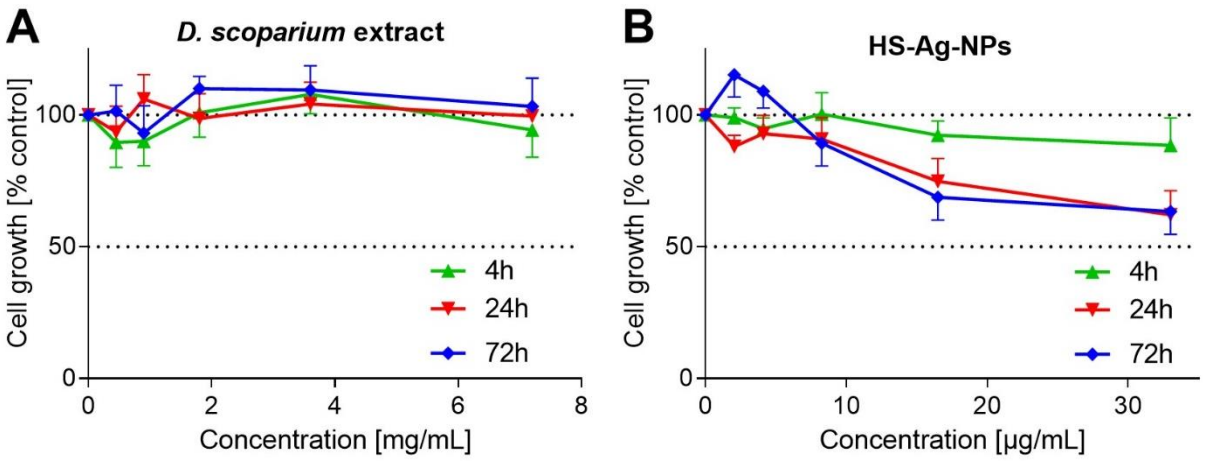

Figure 5. Proliferation of HeLa cells treated with *D. scoparium* extracts (A) and green-synthesized silver nanoparticles from *D. scoparium* (B) over 4, 24, and 72 hours. Data are presented as mean ± SD from three independent experiments, with SD ≤ 15%.

4. Discussion

Among all noble metal NPs, Ag-NPs occupy a special place, which is interesting due to their unique properties, such as chemical stability, good conductivity, catalytic, and, most importantly, antimicrobial and anti-inflammatory activity.

In this study, we successfully synthesized Ag-NPs using the aqueous extract of a *D. scoparium* moss extract through a green synthesis method. The formation of Ag-NPs was confirmed by the change in color of the reaction mixture and the characteristic SPR peak observed at 441 nm in the UV-visible spectrum. The DLS analysis revealed that the synthesized NPs had a hydrodynamic radius of approximately 67.72 nm, which falls within the nanoscale range. The relatively high polydispersity index suggests a moderate distribution in the particle size, which is common in green synthesis methods due to the natural variability of biological reducing agents.

The synthesized DS-Ag-NPs demonstrated significant antibacterial activities against both Gram-negative and Gram-positive bacteria. Notably, the NPs exhibited a more pronounced inhibitory effect on Gram-negative bacteria (*E. coli* and *P. aeruginosa*) compared to Gram-positive strains (*S. aureus* and *B. mesentericus*). This differential activity can be attributed to the structural differences in the bacterial cell walls. Gram-negative bacteria possess an outer membrane rich in lipopolysaccharides, which may facilitate the interaction and penetration of Ag-NPs, thus leading to an increased antibacterial efficacy. In contrast, the thicker peptidoglycan layer in Gram-positive bacteria could act as a barrier, thus reducing NPs uptake and subsequent antibacterial action.

The reduction in CFU further supports the potent antibacterial effect of DS-Ag-NPs. The significant decrease in CFU for *E. coli* and *S. aureus* indicates that the NPs not only inhibit bacterial growth, but also affect the cell viability. The mechanism of antibacterial action is likely multifaceted, involving the disruption of cell membranes, the generation of reactive oxygen species (ROS), and an interference with essential cellular processes such as DNA replication and protein synthesis. [21]. Extensive research has investigated the antibacterial properties of Ag-NPs against a wide variety of pathogenic bacteria. These NPs are highly effective at killing bacteria because they release silver ions, which can disrupt the bacterial cell membranes, inhibit enzymatic activities, and interfere with DNA replication. The mechanism of action involves Ag-NPs directly interacting with bacterial cell walls, thus leading to structural damage and subsequent cell death. Multiple studies have demonstrated that AgNPs can effectively eliminate both Gram-positive and Gram-negative bacteria, including drug-resistant strains [21].

Additionally, the DS-Ag-NPs exhibited cytotoxic effects on HeLa cancer cells in a concentration-dependent manner. While the aqueous extract of *D. scoparium* alone did not show significant cytotoxicity, the incorporation of silver ions enhanced the anticancer properties. The NPs induced substantial growth inhibition at tested higher concentrations, suggesting their potential as anticancer agents. The cytotoxicity is possibly due to the ability of Ag-NPs to induce oxidative stress, thus leading to apoptosis through mitochondrial damage and the activation of apoptotic pathways. The anticancer potential of green-synthesized Ag-NPs was also shown in other studies [2].

Among all noble metal NPs, Ag-NPs occupy a special place due to their unique properties, such as chemical stability, good conductivity, catalytic potential, and, most importantly, remarkable antimicrobial, anticancer, and anti-inflammatory activities. Hence, our findings align with previous reports which highlighted the dual antimicrobial and anticancer properties of green-synthesized Ag-NPs [2,21,22].

In addition to these bioactivities, it is crucial to consider the growing difficulty of obtaining large amounts of plant materials for the green synthesis of Ag-NPs from nature in light of ongoing ecosystem degradation. For this reason, the ease of cultivating bryophytes *in vitro* emerges as a promising strategy for producing substantial biomass and extracting numerous bioactive compounds with interesting biological functions. Additionally, this approach is advantageous for studies in development, cell biology, molecular, biochemical, and ecophysiological research. *In vitro* bryophyte cultures can address the challenges posed by environmental stress, as they enable the mass production of biomass in controlled bioreactors. By optimizing the production conditions, the yield of terrestrial metabolites can be significantly enhanced, thus potentially offering new avenues to combat drug resistance. Continued research is required to fully understand the therapeutic scope of these moss-derived metabolites and their potential in the green synthesis of Ag-NPs [14].

A broader comparison with other established Ag-NPs synthesis methods underscores the advantages of the moss-based approach. Conventional chemical reduction methods often rely on strong reducing agents such as sodium borohydride or citrate, which allow for a fine control over particle size and shape but involve toxic chemicals and generate hazardous by-products. Microbial-mediated synthesis, using bacteria or fungi, is more environmentally friendly than chemical methods; however, it can be time-consuming, prone to contamination, and more challenging to scale up. In contrast, *D. scoparium* moss-based synthesis presents a more balanced and sustainable alternative. It leverages natural compounds for reduction and stabilization, thereby minimizing the environmental impact and toxic by-products, while also offering a relatively straightforward protocol that does not require highly

stringent conditions. Nonetheless, as with other green synthesis methods, natural variability in moss metabolites can lead to polydisperse NP populations, and consistent scaling-up requires optimized *in vitro* mass cultivation protocols.

Overall, by looking into the sustainable production of bryophytes and their inherent capacity for generating bioactive substances, this moss-based synthesis method offers an eco-friendly, cost-effective, and potentially scalable route to synthesizing Ag-NPs. Future work may focus on improving the nanoparticle uniformity, fine-tuning the growth conditions for higher metabolite yields, and investigating the full range of biological effects of moss-derived Ag-NPs, including the possibility of overcoming antibiotic-resistant pathogens and enhancing cancer therapies.

5. Conclusions

In conclusion, this study demonstrated the successful green synthesis of DS-Ag-NPs using the aqueous extract of *Dicranum scoparium* moss. The synthesized DS-Ag-NPs exhibited significant antibacterial activities against both Gram-negative and Gram-positive bacteria, with a more pronounced effect on the Gram-negative strains. Additionally, the NPs show considerable cytotoxic effects on HeLa cancer cells, thus highlighting their potential as antimicrobial and anticancer agents. The use of *D. scoparium* not only provides an eco-friendly and cost-effective method for NP synthesis, but also enhances the biological activities of the NPs due to the presence of bioactive phytochemicals. These findings suggest that DS-Ag-NPs could be promising candidates to develop new antimicrobial and anticancer therapeutics. While the study showcases the eco-friendly and cost-effective potential of using *D. scoparium* in NP synthesis, certain limitations must be acknowledged. The study did not evaluate the *in vivo* efficacy and safety of DS-Ag-NPs, thus leaving their therapeutic applicability largely unexplored. Future research should allow for deeper investigations into the molecular mechanisms underlying the bioactivity of DS-Ag-NPs. Additionally, future studies will include clinically relevant pathogens and antibiotic-resistant strains to broaden the scope and impact of the findings.

Data availability statement

The datasets and materials used and/or analyzed during the current study are available from the author upon reasonable request.

Use of generative-AI tools declaration

The authors declare they have not used artificial intelligence (AI) tools in the creation of this article.

Acknowledgments

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Conflict of interest

The authors declare no conflict of interest.

Author contributions

The study's conception and design were the results of collective contributions from all authors. The investigations and analysis of results were carried out by GS, IS, and MG. GS and MG wrote the manuscript. NA directed the project, and corrected, and edited the manuscript. All authors participated in the revision and approval of the final version of the manuscript.

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