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Biosynthesis, characterization, and antibiofilm activity of Ag nanoparticle prepared by *Verbascum scamandri* (Kazdağı Sığırkuyruğu)

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Abstract

The biosynthesis of silver nanoparticles (AgNPs) could play a significant role in the development of commercial antibiofilm agents. Herein, the biosynthesis of AgNPs was studied using the locally known as Kazdağı Sığır Kuyruğu (*Verbascum scamandri*). After that, the morphology was described via transmission and scanning electron microscopy (TEM - SEM), and spherical particles were found ranging from 72 to 97 nm. Zeta potential analysis showed that AgNP had negative surface charges, indicating stability. FTIR analysis revealed functional groups responsible for reduction, including -OH, -CN, and C=O bonds. EDX analysis indicated high silver content, with 90.32% Ag. The antibiofilm activity was found to be the highest against *Staphylococcus haemolyticus* bacteria, with a rate of 54.45%.

Keywords: Silver nanoparticles; *Verbascum scamandri*; Spectrophotometric; Biofilm; Biosynthesis

1. Introduction

Biofilm is a complex bacterial structure formed by microbial cultures and an extracellular polymer matrix (EPM), which consists of polysaccharides, proteins, glycopeptides, nucleic acids, and lipids. This slimy, three-dimensional structure is heterogeneous and contains channels for transport and water. Biofilm formation occurs in three stages: adhesion, colonization, and maturation. Once mature, microorganisms can detach and disperse into the environment. Overall, biofilms are intricate biological structures, resembling the complexity of multicellular organisms [1].

Biofilms protect microorganisms from harmful environmental factors such as temperature changes, desiccation, ultraviolet radiation, and immune responses, making them resilient and difficult to destroy. This protection leads to challenges in various areas, including water treatment [2], food production [3], implantology [4], and the treatment of chronic diseases [5].

Conventional antimicrobial treatments are often ineffective against biofilms, as antibiotics required to eliminate biofilms are much higher in concentration than those needed for planktonic bacteria [6-8]. This tolerance to antibiotics leads to chronic infections. While biofilm structure was once thought to block antibiotic penetration, studies show the extracellular matrix does not hinder diffusion. Instead, antibiotics may lose their effectiveness upon binding to biofilm components or fail to reach sufficient concentrations. Additionally, nutrient and oxygen gradients within biofilms slow bacterial metabolism, further reducing antibiotic efficacy [9].

The rise of biofilms formed by multidrug-resistant strains has worsened the challenge of treating biofilm-related infections in medicine and other industries. This has created an urgent need for new strategies to inhibit and eliminate

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biofilms. A promising solution is transitioning from traditional therapies to advanced, nanomaterial-based technologies [10-11].

Nanoparticles (NPs), particularly metal nanoparticles like AgNPs, are gaining attention for their ability to treat bacterial biofilms and overcome antibiotic resistance. Studies show that NPs often have superior biological properties compared to the parent metals, making them a promising area of research in the scientific community [12-13].

Verbascum scamandri Murb. (*VS*) is a biennial plant species, 50-80 cm high, with short and densely stellate-tomentose or glabrescent [14]. This species is distributed on mountain slopes in Northwest Anatolia, Türkiye-Kazdağı, known as "Kazdağı Mullein". The conservation status of this species is declared as endangered (EN, B1-B2a) according to IUCN criteria [15]. The *Verbascum* genus contains various secondary metabolites, such as flavonoids, alkaloids, essential oils, neolignan glycosides, terpenes, phenolics, iridoids, and phenylethanoids, which contribute to its biological properties [16-17].

In this study, the characterization of silver nanoparticles synthesized using *VS* plants collected from Bayramiç district (Çanakkale) at 625 m altitude in August 2021 was performed, along with their antibacterial and antifungal activities against some pathogenic bacteria, and most importantly, their antibiofilm activity.

2. Material and methods

2.1. Synthesis and Characterization of AgNPs

The plant sample collected from the 39°47'03.2" N; 26°53'57.5" E coordinates of Bayramiç (Çanakkale) in August 2021 was first washed in the laboratory and then left to dry at room temperature.

VS leaves were crushed and extracted with sterile distilled water at 65–70°C for 60 minutes. For AgNP production, an aqueous solution of $AgNO_3$ (1 mM) and *VS* extract was mixed at a 1:9 ratio and reacted at room temperature. The resulting dark solution was centrifuged, and the pellet (AgNP) was dried at 50°C for 48 hours [18]. The size and surface charge of the AgNPs were determined using dynamic light scattering and a Zetasizer device. UV-Vis absorption spectroscopy, FTIR analysis, and electron microscopy (SEM and TEM) were used to characterize the AgNPs, with elemental composition assessed by EDX. All analyses were performed at Atatürk University's DAYTAM center.

2.2. Antibacterial/Antifungal and Antibiofilm Activities of AgNP

The antibacterial/antifungal activities of synthesized AgNP were evaluated using the microdilution method, determining the Minimum Inhibition Concentration (MIC) and Minimum Bactericidal/Fungicidal Concentration (MBC/MFC) against various bacterial strains (*Staphylococcus haemolyticus*, *Acinetobacter baumanii*, *Staphylococcus aureus*, *Escherichia coli*, *Bacillus subtilis*, *Proteus vulgaris*, *Pseudomonas aeruginosa*) and *Candida albicans* yeast [19]. The inhibition of biofilm formation by *VS*-AgNP plant extracts was assessed using the micro plate biofilm method [20]. The antibiotic effect was measured by calculating the percentage reduction of absorbance using the formula: % Inhibition = $[(A_{control} - A_{sample}) / A_{control}] \times 100$, where $A_{control}$ is the absorbance of the control and A_{sample} is the absorbance of the test compounds.

3. Results and discussion

This study presents the investigation into the synthesis of AgNP from *VS* and its antibiofilm activity, a plant species endemic to the local region.

3.1. Characterization results of AgNP

The UV-Vis absorption spectrum was used to determine the optical properties of the synthesized silver nanoparticles. A prominent absorption peak (0.103 A) is observed at approximately 432 nm (Figure 1). This peak corresponds to the surface plasmon resonance (SPR) band, which can vary depending on the size, shape, and synthesis conditions of the silver nanoparticles. The absorption peak observed at 432 nm supports the presence of nanoparticles with a spherical or slightly elliptical morphology.

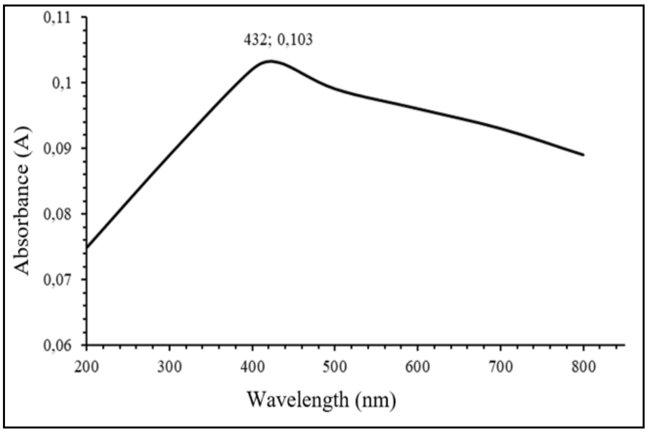

Figure 1 UV-Vis spectra of *VS-AgNP*

Zeta potential analysis was performed to assess the surface charge and colloidal stability of the particles. The results indicate that electrostatic stability is low, suggesting that the nanoparticles may tend to agglomerate (bind). The nanoparticle particle size was determined to be 800.4 ± 13.33 nm, the polydispersity index was 0.774, and the zeta potential charge was -12.3 ± 0.643 mV (Figure 2).

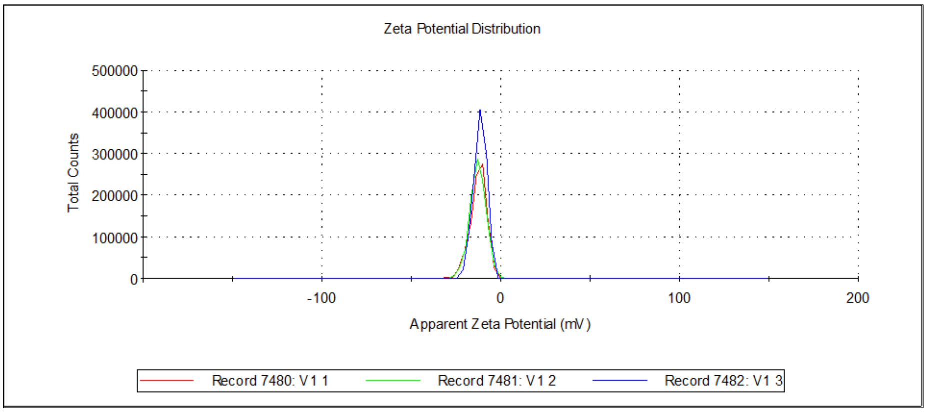

Figure 2 Surface charge of the colloidal system of *VS-AgNP*

FTIR analysis indicates the presence of functional groups derived from plant extracts on the surface of the nanoparticles. The spectrum shows fluctuations between 3200-3800 cm^{-1}, corresponding to O-H groups, peaks in the range of 1650-1750 cm^{-1}, representing C=O groups, and peaks between 1000-1300 cm^{-1}, which correspond to C-N or N-H groups (Figure 3). These functional groups suggest that the nanoparticles are stabilized by plant compounds and are coated with biochemical components. Additionally, the fluctuations between 1200 cm^{-1} and 1600 cm^{-1} are believed to originate from the characteristic features of flavonoids/phenolic groups.

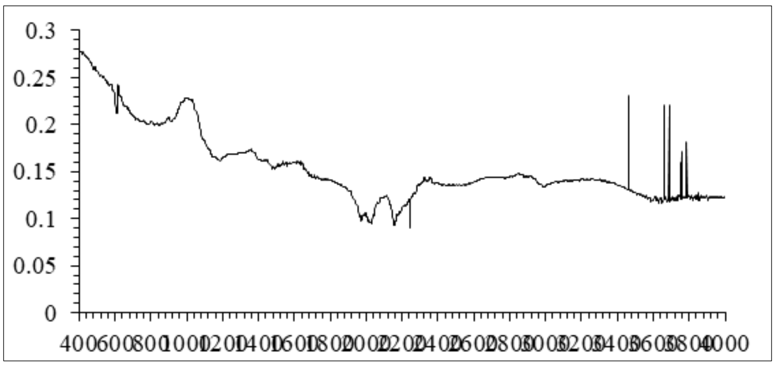

Figure 3 FTIR analysis of *VS-AgNP*

The SEM image shows that the particles exhibit a heterogeneous distribution and tend to agglomerate in certain regions. The measured particle sizes were found to range from 72.88 nm to 97.03 nm. The images reveal irregular and dense structures, suggesting that the particles partially agglomerate. The TEM image indicates that the particles generally have a spherical or slightly elliptical morphology and are well dispersed in the solution. However, agglomeration is observed in some areas. This is consistent with the low zeta potential, indicating particle aggregation due to weak electrostatic repulsion (Figure 4).

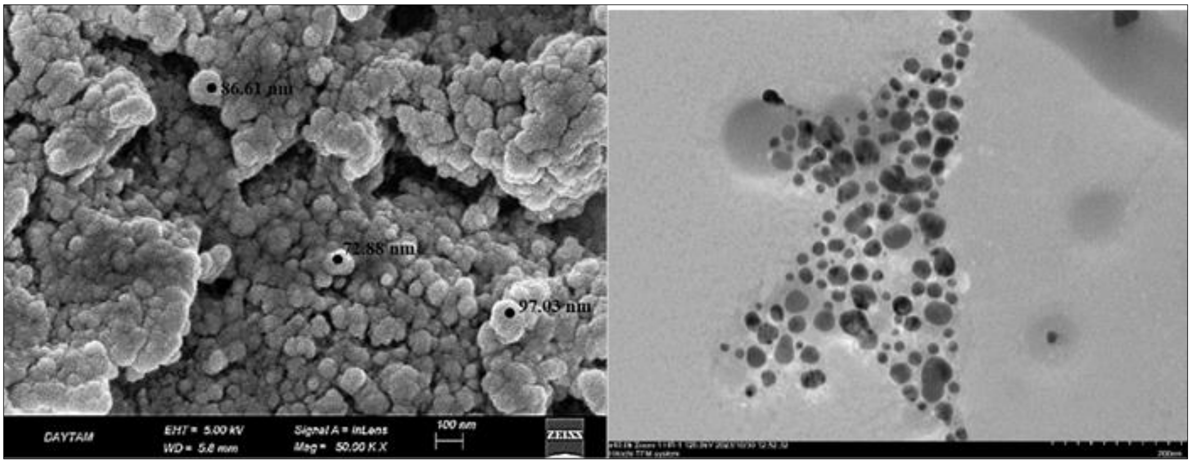

Figure 4 SEM and TEM images of *VS-AgNP*

EDX analysis was performed to determine the elemental composition of the nanoparticles. Characteristic peaks for the Ag element were observed around 3.0 keV in the spectrum. The silver signal confirms that the synthesized nanoparticles are of high purity. Additionally, signals for carbon and oxygen elements were also detected, suggesting the presence of organic compounds derived from the plant extract on the nanoparticle surface (Figure 5).

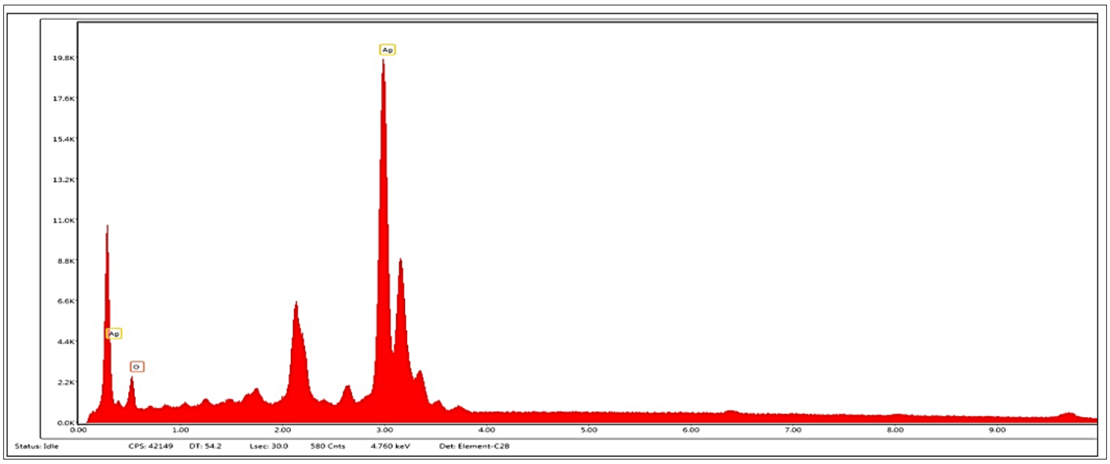

Figure 5 EDX analysis of *VS-AgNP*

Based on the analysis results, it is evident that the physicochemical properties of silver nanoparticles biologically synthesized from the *Verbascum* plant have been successfully demonstrated. UV-Vis spectroscopy confirmed the optical properties of the nanoparticles, and the surface plasmon resonance band was observed at 432 nm. The zeta potential values indicate that the particles have a low surface charge, and that stability may be limited. SEM and TEM analyses revealed that the nanoparticles have a spherical morphology but may show agglomeration in specific regions. EDX analysis confirmed that the nanoparticles are of high purity. These results suggest that *VS*-AgNPs could be a potential candidate for antimicrobial and environmental applications.

3.2. Antagonistic activity results of *VS*-AgNP

The MIC values of *VS*-AgNPs range between 2.5 - 5.0 µg/mL. Higher MIC values were obtained against *S. haemolyticus* and *C. albicans*, compared to the control antibiotic. The MBC/MFC values *VS*-AgNPs 5 µg/mL for all test cultures. The highest biofilm percentage was obtained against *S. haemolyticus* with 54.45±1.1%. Furthermore, significant antibiofilm activity has been observed against other test cultures except *C. albicans* (Table 1).

Table 1 Antimicrobial and antibiofilm activities of *VS*-AgNP

Test Cultures	**Antimicrobial**			**Antibiofilm**	
	MIC (µg/mL)		**MBC or MFC (µg/mL)**	**Concentration (µg/mL)**	
	AgNP	**Control S10/NY100**	**AgNP**	**MIC**	**MIC/2**
A. baumanii ATCC 19606	5.0	2.0/ NT	5.0	30.45±1.14	-
E. coli NRRL B-3704	5.0	4.0/ NT	5.0	31.01±0.1	-
P. vulgaris ATCC 13315	5.0	4.0/ NT	5.0	32.01±0.2	-
P. aeruginosa ATCC 27853	5.0	1.0/ NT	5.0	33.12±1.14	-
S. haemolyticus ATCC 43252	2.5	5.0/ NT	5.0	54.45±1.1	34.25±1.1
S. aureus ATCC 6538P	5.0	4.0/ NT	5.0	33.12±0.10	-
B. subtilis ATCC 6633	5.0	4.0/ NT	5.0	30.78±1.20	-
C. albicans ATCC 10231	5.0	NT/2.5	5.0	11.05±0.2	3.65±0.25

S10: Streptomycin (10 µg/disc); NY100: Nystatin (100 µg/disc); NT: No Tested

Numerous studies have reported the antimicrobial activity of *Verbascum* species [21-24]. These studies have demonstrated that *Verbascum* L. species exhibit antimicrobial effects against Gram-positive bacteria and yeasts, but no activity was observed against Gram-negative bacteria using the disc-diffusion method [22, 25].

Microbial biofilms are considered significant virulence factors due to their increased resistance to both chemotherapeutics and the host immune system [26]. Bacteria within biofilms exhibit greater antibiotic resistance than planktonic cells because they are protected by the EPM. This highlights the need for exploring new and natural antibiotic sources, such as medicinal plants, to combat biofilms. Biofilm formation is regulated by quorum sensing, a bacterial communication system [27].

However, findings regarding the antimicrobial and antibiofilm activity of silver nanoparticle synthesis from *Verbascum* species are quite limited [28-29].

Ercan et al. [28] were synthesized of zinc oxide nanoparticles (VcZnO) and selenium nanoparticles (VcSe) from hybrid *Verbascum calcicolum* Hub.-Mor. and evaluate their antioxidant and antimicrobial properties. The results showed that VcZnO was effective against both Gram-positive and Gram-negative bacteria, with varying MIC, while VcSe had better antioxidant properties. Zinc nanoparticles exhibited stronger antibacterial effects compared to selenium nanoparticles. The study concludes that different plant-derived nanoparticles can be more effective for various applications, and that *Verbascum calcicolum* is a promising source for nanoparticle synthesis.

In a study by Cambaz et al. [29], compared AgNPs synthesized via *VS* collected from 675 and 800 meters with those produced through *in vitro* grown plant. They identified strong antimicrobial and antibiofilm activity of *VS* against *A. baumanii* bacteria *in vitro* grown plant.

4. Conclusion

The strong antimicrobial and antibiofilm activity of *V. scamandri* against *S. haemolyticus* indicates the potential of this plant as a pharmaceutical raw material. Antimicrobial activity could provide effective alternative treatment options, particularly against pathogens that have developed resistance to antibiotics. Additionally, its antibiofilm properties offer a significant advantage in cases where microbes form biofilms, enhancing their resistance to antibiotics. These characteristics suggest that *V. scamandri* could contribute to the treatment of various infections. However, further research is needed, especially in terms of safety, bioavailability, efficacy, and production processes, to fully evaluate its potential as a pharmaceutical raw material.

Compliance with ethical standards

Disclosure of conflict of interest

On behalf of all authors, the corresponding author states that there is no conflict of interest.

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