IJSM International Journal of Secondary Metabolite

International Journal of Secondary Metabolite
2025, Vol. 12, No. 2, 289–296

https://doi.org/10.21448/ijsm.1526393

journal homepage: https://dergipark.org.tr/en/pub/ijsm

Research Article

Biological activities of silver nanoparticles synthesized using *Olea europaea* L. leaves

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ARTICLE HISTORY

Received: Aug. 01, 2024
Accepted: Nov. 16, 2024

KEYWORDS

Antibiofilm,
Antimicrobial,
Antioxidant,
Olea europaea,
Silver nanoparticle.

Abstract: Recent advancements in nanotechnology have led to an increased utilization of silver nanoparticles (AgNPs) across various domains, including health, medicine, environmental chemistry, nanobiotechnology, and biosensors. The primary focus of this study is the green synthesis of AgNPs utilizing *Olea europaea* L. leaves. AgNP was characterized through UV-Vis Spectroscopy, SEM, EDS, and TEM. Furthermore, the study explored the antimicrobial, antibiofilm, and antioxidant activities, along with the growth kinetics of *Staphylococcus aureus* ATCC 25923, for the synthesized AgNPs. Characterization tests confirmed the synthesis of spherical nanoparticles with a size ranging from 51 to 56 nm. AgNPs demonstrated effectiveness, particularly against *Acinetobacter baumannii* ATCC 19606 and *Proteus vulgaris* ATCC 13315 bacteria, in terms of antimicrobial and antibiofilm activities. Moreover, the AgNPs exhibited noteworthy antioxidant activity. This study provides evidence that this environmentally friendly and cost-effective method can be applied for large-scale AgNP synthesis.

1. INTRODUCTION

Nanotechnology research has gained global prominence and is employed in numerous products, ranging from sunscreens, cosmetics, textiles, and sports equipment to applications in drug delivery, plant disease prevention, environmental pollutant remediation, biosensors, and various biomedical applications (Boisseau & Loubaton, 2011). The advancement in nanotechnology prompts consideration of eco-friendly technologies for selecting and producing nanoparticles tailored to specific purposes. Silver nanoparticles (AgNPs) hold a prominent position, exhibiting excellent antagonistic properties, sterilant capabilities, as well as pharmacological features such as biofilm removal, antioxidant effects, cell-level degradation, and anticancer effects. Plant-based sources prove to be efficient reducing agents in the synthesis of AgNPs (De Matteis *et al*., 2019). Plant nanoparticle synthesis is gaining importance as an environmentally friendly and sustainable method. As an alternative to traditional chemical synthesis techniques, nanoparticles derived from plants offer biocompatible and non-toxic compounds (Alsulami *et al*., 2023). Plants naturally contain a wealth of biochemical compounds, enabling high efficiency and activity in nanoparticle synthesis. Additionally,

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e-ISSN: 2148-6905

utilizing plant sources helps reduce the use of toxic chemicals, contributing to the protection of ecosystems (Piro *et al*., 2023). These nanoparticles have significant application potential in various fields, including agriculture, medicine, food safety, and environmental engineering, providing safer solutions for health and the environment while making important contributions to the advancement of scientific research and industrial applications. Therefore, plant nanoparticle synthesis holds substantial significance both scientifically and practically (Rahimzadeh *et al*., 2022). Nanoparticle synthesis through plants occurs based on natural biochemical processes and interactions of plant metabolites. This process typically begins with the combination of plant extracts, derived from various parts such as leaves, roots, or fruits, with metal salts. Plants possess the ability to reduce and stabilize metal ions due to their rich content of phenolic compounds, flavonoids, and other biologically active substances. This reduction process facilitates the transformation of metal ions into nanoparticles at the nanoscale. Furthermore, plant compounds can influence the surface properties of the resulting nanoparticles, enhancing their stability and reactivity. Consequently, this biological method provides an environmentally friendly and sustainable approach to nanoparticle production, contributing to the reduction of harmful waste associated with traditional chemical methods. This mechanism enables the use of plants' natural capabilities to produce high-quality nanoparticles with significant application potential across various fields (Barzinjy *et al*., 2020).

Olea europaea L., a prominent member of the Oleaceae family comprising approximately 29 genera and 600 species, holds great importance (Özcan & Matthäus, 2017). The leaves and fruits of *O. europaea* are the most commonly utilized parts of this plant (Alesci et al., 2022). Among the bioactive compounds present in *O. europaea* are phenolic compounds, carotenoids, secoiridoids, and flavonoids. Oleuropein is the primary phenolic compound in olives, while other notable phenolic compounds include hydroxytyrosol, verbascoside, luteolin-7-glucoside, and apigenin-7-glucoside (Bonvino *et al*., 2018). *Olea europaea* is of considerable medical significance and has a historical association with various health benefits, including cardiovascular, antioxidant and anti-inflammatory properties, anticancer, anti-diabetic effects, antimicrobial, and positive effects on skin health (Salık & Çakmakçı, 2021). *O. europaea* has great potential for nanoparticle synthesis due to its high phenolic compound content and various biological activities. Consequently, there is a need to explore the use of waste materials with high phenolic compound content, such as olive leaf (OL), in these processes.

The objective of this study was twofold: to transform a significant waste product of the olive tree into a value-added product and to unveil the biological activities of the resultant nanotechnological product. To achieve this goal, AgNPs were synthesized using olive leaf extract (OLE) through a straightforward, cost-effective, and environmentally friendly green synthesis method. The nanoparticles were characterized, and its antimicrobial, antibiofilm, antioxidant and growth kinetic effects were determined.

2. MATERIAL and METHODS

2.1. Preparation of OLE and Synthesis of AgNPs

OLs collected from Çanakkale were washed and dried before being boiled in 100 mL of sterile distilled water for 7 min. The prepared OLE was subjected to a reaction with $AgNO_3$ at 25°C. The reaction proceeded until the anticipated development of a dark color, signifying the reduction of silver ions to a brown hue. After centrifugation (10.000 rpm, 5 min.), the liquid phase was removed, and the remaining solid phase was washed several times with pure water. The resulting AgNPs were then dried in an oven (at 65°C for 48 hr) (Bayğu, 2020).

2.2. Characterization of AgNPs

The morphology, size, and chemical analysis of the AgNPs were determined using a dual-beam spectrophotometer (UV-VIS, 200- 800 nm) Scanning Electron Microscope (SEM), Transmission Electron Microscopy (TEM) and Energy Dispersive X-ray Spectroscopy (EDS).

2.3. Antimicrobial and Antibiofilm Activities

The antimicrobial activities of OLE and AgNPs were determined using disk diffusion and microdilution methods against 8 test cultures (*Acinetobacter baumanii* ATCC 19606, *Escherichia coli NRRLB 3704*, *Pseudomonas aeruginosa* ATCC 27853, *Proteus vulgaris* ATCC 13315, *Bacillus subtilis* ATCC 6633, *Staphylococcus aureus* ATCC 25923, *Staphylococcus haemolyticus* ATCC 43252, *Candida albicans* ATCC 10231) (CLSI, 2006). By utilizing the findings of minimum inhibitory concentration (MIC) results, the minimum bactericidal concentrations (MBC) were also determined. The analyses were performed in triplicate.

The antibiofilm activities AgNP was conducted according to O'Toole (2011). AgNPs were utilized at MIC and MIC/2 concentrations. The percentage of biofilm inhibition is calculated using the formula:

% Inhibition=[1−(OD_{620} of cells treated with AgNPs/OD_{620} of negative control)]×100

2.4. Determining the Effect of AgNPs on The Growth Kinetics of *S. aureus*

S. aureus was incubated with AgNP at different concentrations (6.25 and 3.13 µg/mL), and absorbance measurements were taken at 540 nm at various time intervals (2-14 hours) to assess the effect on bacterial growth. (Erci, 2018). The analyses were performed in triplicate.

2.5. Antioxidant Activity

2.5.1. *DPPH radical scavenging activity*

The determination of DPPH was carried out according to the method described by Blois (1958). The 2,2-diphenyl-1-picrylhydrazyl (DPPH) radical was utilized, and the absorbance value was measured at 517 nm. The study standard was butylated hydroxytoluene (BHT). The absorbance values (A) were evaluated relative to the control. The percentage of inhibition is calculated using the formula: %Inhibition=($A_{control}$−A_{sample})/$A_{control}$)×100

2.5.2. *ABTS$^{•+}$ radical cation scavenging activity*

The 2,2'-azino-bis (3-ethylbenzothiazoline-6-sulfonic acid (ABTS$^{•+}$) radical cation scavenging activity assay was determined according to the Blois (1958). The inhibition was calculated for the obtained values and these results were evaluated by comparing them with the positive control (BHT).

ABTS$^{•+}$ Radical Cation Scavenging Activity Inhibition (%): [(A0-A1)/A0]x100

A0: ABTS$^{•+}$ of control, A1: ABTS$^{•+}$ of sample.

3. RESULTS and DISCUSSIONS

The synthesis of AgNPs from OL occurred at room temperature and was detected through a color change from yellow to brown within 2 hours (Figure 1). SEM and TEM studies confirmed the development of NPs with almost the same spherical in 4 shapes and dimensions particle size ranged between 51 to 56 nm (Figure 2A-B).

In EDS analysis, the peak at 3 keV is attributed to silver 1 atoms (Figure 2C), while other peaks are identified as chlorine (Cl) and oxygen. These peaks are reported to originate from phenolic and flavonoid components found in the oil (Veisi *et al*., 2019). The UV-Vis spectra of OL and AgNPs reveal distinct peaks at 372 and 449 nm, respectively (Figure 2D). The observed surface plasmon resonance (SPR) bands closely resemble data found in the literature for AgNPs, supporting the evidence of AgNP synthesis (Atalar *et al*., 2022). Our study's SEM and EDS analysis findings align with the literature information (Sellami *et al*., 2021; Atalar *et al*., 2022). The differences in AgNP sizes are thought to vary depending on the synthesis steps, extract concentration, and the phytochemical composition of the collected olive leaves. SEM and EDS analyses indicate that the nanoparticles obtained have both a spherical form and a size suitable for entering cells, highlighting their potential use in biological and medical studies (Erdoğan *et* *al*., 2019). The morphology of the AgNPs obtained in our study is suitable for potential applications in this regard.

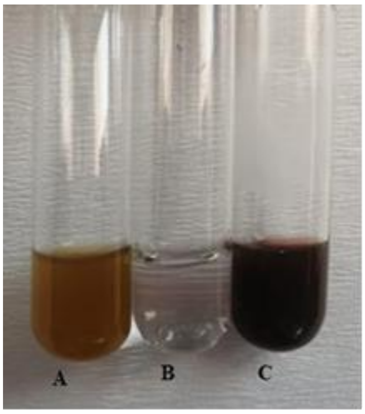

Figure 1. A) OLE, B) $AgNO_3$, C) Synthesised AgNPs.

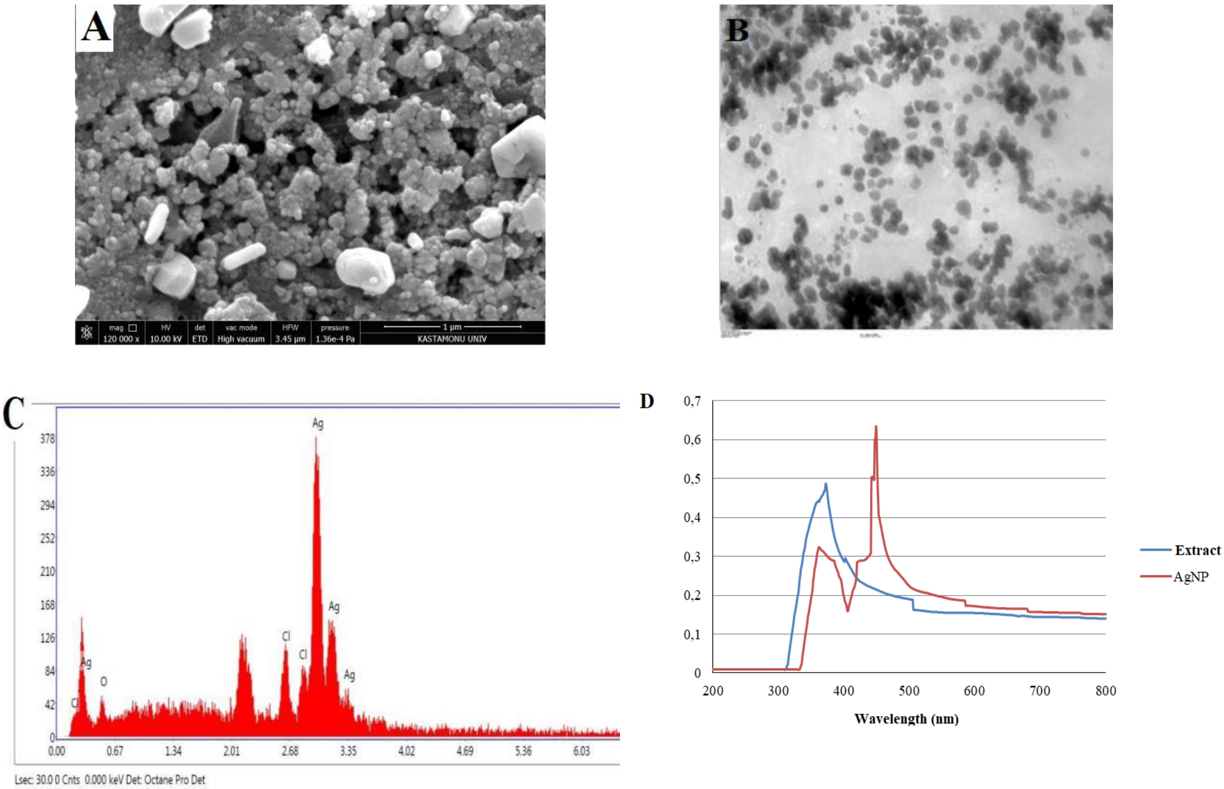

Figure 2. Characterization of AgNPs; A) SEM image, B) TEM image, C) EDS analysis, D) UV-Vis spectrum.

It was observed that OLE exhibited a higher antagonistic effect against *P. aeruginosa* and *S. haemolyticus* compared to P10. AgNP demonstrated a higher antibacterial effect than the reference antibiotic against *P. aeruginosa*, *P. vulgaris*, and *A. baumannii*. The MIC of AgNP also revealed a bacteriostatic effect higher than the S10 against *P. vulgaris* (0.625 µg/mL) and *A. baumannii* (2.5 µg/mL), consistent with the results of the disk diffusion. These findings were further supported by MBC results (Table 1).

Sellami *et al*. (2021) reported that AgNPs caused logarithmic decrease against *B. subtilis*, and some gram (-) bacteria at levels equivalent to commercial antibiotics. The study also suggested that higher MIC values against gram (-) bacteria might be due to the easier penetration of particles into the cell wall composition of gram (-) bacteria. Atalar *et al*. (2022) observed inhibitory activity against all pathogens in varying proportions ranging from 0.03 to 1.0 mg/mL of AgNPs. It is believed that the positive charge carried by Ag ions is a significant factor in the antagonistic effect observed in interaction with microorganisms (Klueh *et al*., 2000). Therefore, the combination and synthesis of AgNPs with plant agents such as olive leaves, which possess high antimicrobial effects, may present a new strategy against microbial resistance to existing antibiotics due to the potential for high synergistic effects, as demonstrated once again in this study.

Table 1. The antimicrobial activities of OLE and AgNPs.

Test Cultures	Antimicrobial Test Methods									
	*Disc Diffusion[a]		Control (mm)		MIC (µg/mL)			MBC		
	OLE	AgNP	P10	NY100	OLE	AgNP	S10	NY100	OLE	AgNP
E. coli	9.0+0.01	8.0+0.03	16.0	NT	10.0	10.0	4.0	NT	10.0	10.0
P. aeruginosa	14.0+0.01	12.0+0.02	8.0	NT	5.0	5.0	1.0	NT	5.0	5.0
P. vulgaris	8.0+0.01	14.0+0.02	13.0	NT	1.25	0.625	4.0	NT	1.25	0.625
B. subtilis	8.0+0.01	9.0+0.01	14.0	NT	20.0	20.0	2.0	NT	20.0	20.0
S. haemolyticus	17.0+0.05	10.0+0.01	14.0	NT	1.25	10.0	4.0	NT	1.25	10.0
A. baumannii	11.0+0.01	17.0+0.01	12.0	NT	10.0	2.5	4.0	NT	10.0	2.5
S. aureus	12.0+0.012	12.0+0.05	15.0	NT	10.0	10.0	5.0	NT	10.0	10.0
C. albicans	16.0+0.03	8.0+0.04	NT	16.0	0.625	10.0	NT	2.5	1.25	10.0

P10 = Penicillin (10 µg/disc); NY100: Nystatin (100 µg/disc); S10: Streptomycin (10 µg/disc); NT: Not Tested

Both in AgNP and OLE, the highest antibiofilm percentage was observed against *A. baumannii* (Table 2). In recent years, numerous studies have been conducted on NP synthesis from plants to develop new antibiofilm strategies (Erci, 2018; Karakaş *et al*., 2023a; Karakaş *et al*., 2023b). The results obtained in these studies have shown that nanoparticles obtained through phytosynthesis exhibit high antibiofilm effects through synergistic interactions with phytochemicals present in the composition of plants. Literature information indicates that the antibiofilm activities of AgNPs synthesized from OLE were first identified in our study. Our findings provide evidence that phytosynthesized AgNPs could serve as a potential new source for developing novel antibiofilm drugs, particularly against *A. baumannii*.

Table 2. Antibiofilm activities of OLE and AgNPs.

Test Cultures	OLE		AgNP	
	MIC	MIC/2	MIC	MIC /2
E. coli	22.75 ±0.22	28.45±0.2	32.75 ±0.22	28.45±0.20
P. aeruginosa	47.51±2.10	27.51±2.10	55.72± 0.12	-
P. vulgaris	50.40±1.60	-	57.12±0.25	-
B. subtilis	30.78±1.20	-	35.12± 0.10	-
S. haemolyticus	46.12±0.11	-	60.19±0.06	-
A. baumannii	81.04±0.01	54.12±0.12	92.04±0.01	54.12±0.12
S. aureus	32.57±1.10	-	58.41±1.12	-
C. albicans	30.78±1.20	-	35.67±0.56	-

AgNPs, especially at a concentration of 6.25 µg/mL, slowed down the growth of *S. aureus* over time (Figure 3). Studies on the growth kinetics of *S. aureus* with AgNPs are quite limited in the literature (Erci, 2018).

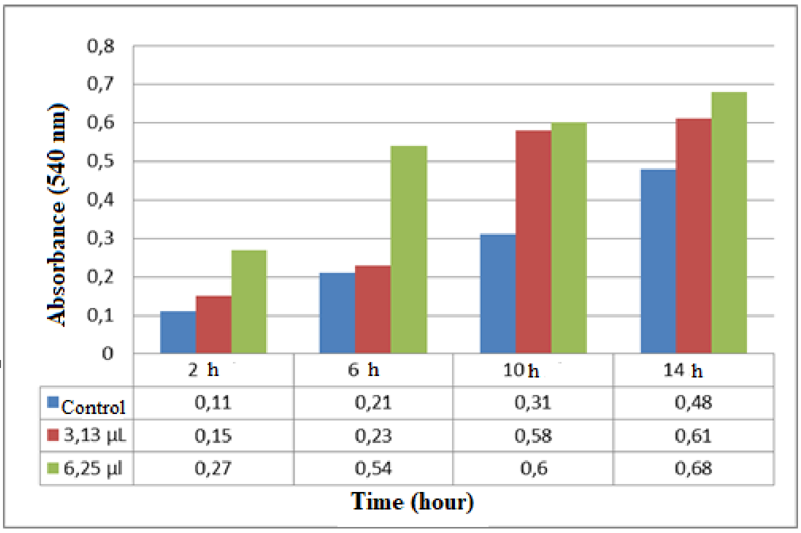

Figure 3. The time-dependent kinetics of AgNP at two different concentrations against *S. aureus.*

Although lower compared to OLE findings, an increase in antioxidant activity dependent on concentration has been achieved in the AgNP product (Figure 4A) In the CUPRAC method, although the control group is lower compared to ascorbic acid, significant antioxidant activity has been identified in both OLE and the AgNPs (Figure 4B).

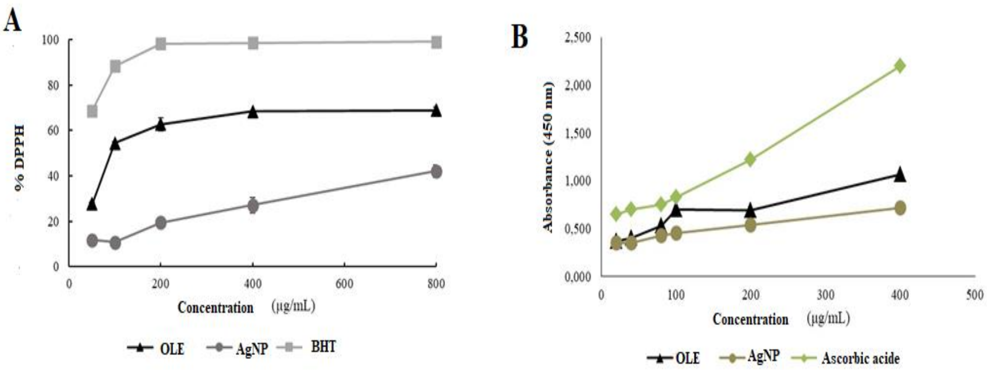

Figure 4. Antioxidant activites of extract and AgNPs. A: DPPH free radical scavenging activity, B: ABTS radical cation scavenging activity.

In the literature, while different biological activity studies on OLE-AgNP components are reported, antioxidant findings appear to be quite limited. Sellami *et al*. (2021) detected high antioxidant activity of OLE-AgNP compared to the ascorbic acid standard and OLE. They suggested that this could be attributed to metal chelation arising from electron-rich secondary metabolites (phenolics and flavonoids) derived from the plant (Khan et al., 2021; Sellami *et al*., 2021).

This study aims to highlight the potential utilization of olive leaves, a crucial component of the economically significant olive tree, as a green synthesis material in the production of AgNPs. The UV-Vis, SEM, EDS, and TEM analyses employed for characterizing the obtained AgNPs demonstrate the successful synthesis of silver nanoparticles. In addition to the morphological characteristics of the synthesized AgNPs, the antimicrobial activity was also investigated in this study, revealing particularly high activity against gram (-) bacteria (*P. vulgaris* and *A. baumannii*). Similarly, tests demonstrated a significant reduction in the biofilm formation capacity of *A. baumannii* bacteria, which is particularly important as it represents the first antibiofilm findings obtained in phytosynthesis studies with olive leaves. It was also shown that AgNPs at a concentration of 6.25 µg/mL slowed down the growth of *S. aureus*. Additionally, the obtained antioxidant activity findings of AgNPs were noteworthy. These results collectively suggest that the synthesized AgNPs can be a potentially versatile agent for various medical purposes.

In this study, AgNP synthesis was performed using *Olea europaea* plant leaves with an eco-friendly method. These AgNPs were characterized by UV-VİS, SEM, TEM and EDS techniques and their antimicrobial, antibiofilm and antioxidant activities were investigated. Biosynthesized AgNPs showed high antimicrobial, antibiofilm and antioxidant activities compared to plant extracts and standard controls. Therefore, AgNPs synthesized from *Olea europaea* and similar plants can be combined with medicinal phytochemicals, leading to the discovery of industrial raw materials with higher activity. Our results reveal that biologically synthesized AgNPs exhibited multifunctional properties and could be used as antimicrobial and antioxidant agents.

Acknowledgments

This study is part of Özge CEYLAN’s Master's thesis research.

Declaration of Conflicting Interests and Ethics

The authors declare no conflict of interest. This research study complies with research and publishing ethics. The scientific and legal responsibility for manuscripts published in IJSM belongs to the authors.

Authorship Contribution Statement

Özge Ceylan: methodology, validation, review and editing, Nurcihan Hacıoğlu Doğru: Supervision, investigation, methodology, resources, visualization, writing—review and editing.

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