

Antibacterial Effects of Silver Nanoparticle-doped “Black Physician: Tar”

Erdi BİLGİÇ*, Sinem GÜRKAN**, Fevzi AK***, Arzu ÖZGEN****

Abstract

Aim: This study evaluated the antibacterial activity of silver nanoparticles (AgNPs) synthesized using *Juglans regia* (walnut) leaf extract and coated with *Cedrus libani* tar against Gram-positive (*Staphylococcus aureus*) and Gram-negative (*Escherichia coli*) bacteria.

Method: AgNPs were biosynthesized using walnut leaf extract as the reducing agent and subsequently coated with cedar tar. The antibacterial activities of the individual components (walnut extract and tar), uncoated AgNPs, and tar-coated AgNPs were assessed via standard disc diffusion assays and compared with conventional antibiotics.

Results: Both walnut extract and tar exhibited inherent antibacterial properties, though they were less effective than standard antibiotics. AgNPs synthesized with walnut extract showed enhanced antibacterial activity; incorporation of tar further improved performance, yielding inhibition zones comparable to those produced by antibiotics against both bacterial strains.

Conclusion: Tar coating synergistically enhanced the antibacterial efficacy of biosynthesized AgNPs without requiring higher nanoparticle concentrations. Tar-coated AgNPs synthesized using walnut leaf extract may therefore serve as promising alternative antibacterial agents.

Keywords: Silver nanoparticles, walnut leaf extract, cedar tar, antibacterial activity, biosynthesis.

Gümüş Nanopartikül Katkılı “Siyah Hekim: Katran”ın Antibakteriyel Etkileri

Öz

Amaç: Bu çalışmada, *Juglans regia* (ceviz) yaprağı ekstresi kullanılarak sentezlenen ve *Cedrus libani* katranı ile kaplanan gümüş nanopartiküllerin (AgNP'ler) Gram-pozitif (*Staphylococcus aureus*) ve Gram-negatif (*Escherichia coli*) bakterilere karşı antibakteriyel aktiviteleri değerlendirildi.

Yöntem: Gümüş nanopartiküller, indirgeme ajanı olarak ceviz yaprağı ekstresi kullanılarak biyosentezlendi ve ardından sedir katranı ile kaplandı. Bireysel bileşenlerin (ceviz ekstresi ve katran), kaplanmamış

Özgün Araştırma Makalesi (Original Research Article)

Geliş / Received: 03.05.2025 **Kabul / Accepted:** 11.03.2026

DOI: <https://doi.org/10.38079/igusabder.1687362>

* Asst. Prof., Antalya Bilim University, Department of Medical Services and Techniques, Vocational School of Health Services, Antalya, Türkiye. E-mail: erdi.bilgic@antalya.edu.tr [ORCID](https://orcid.org/0000-0002-2892-274X) <https://orcid.org/0000-0002-2892-274X>

** Asst. Prof., Istanbul Gelisim University, Faculty of Applied Sciences, Aircraft Maintenance and Repair, Istanbul, Türkiye. E-mail: sgurkan@gelisim.edu.tr [ORCID](https://orcid.org/0000-0002-5146-8936) <https://orcid.org/0000-0002-5146-8936>

*** Lecturer, Alanya Alaadin Keykubat University, Department of Social Service and Consultancy, Department of Social Services, Alanya Chamber of Commerce and Industry Vocational School, Antalya, Türkiye.

E-mail: fevzi.ak@alanya.edu.tr [ORCID](https://orcid.org/0009-0008-6522-3914) <https://orcid.org/0009-0008-6522-3914>

**** Asst. Prof., Istanbul Gelisim University, Vocational School of Health Services, Medical Laboratory Techniques, Istanbul, Türkiye. E-mail: aozgen@gelisim.edu.tr [ORCID](https://orcid.org/0000-0003-2104-6019) <https://orcid.org/0000-0003-2104-6019>

AgNP'lerin ve katran kaplı AgNP'lerin antibakteriyel aktiviteleri standart disk difüzyon yöntemleriyle değerlendirildi ve konvansiyonel antibiyotiklerle karşılaştırıldı.

Bulgular: Hem ceviz ekstresi hem de katran, antibakteriyel özellikler gösterdi, ancak standart antibiyotiklere göre daha az etkiliydi. Ceviz ekstresi ile sentezlenen AgNP'ler artırılmış antibakteriyel aktivite sergiledi; ancak, katranın dahil edilmesi, her iki bakteri suşuna karşı antibiyotiklere benzer inhibisyon zonları ile antimikrobiyal performansı önemli ölçüde artırdı.

Sonuç: Katran kaplamasının AgNP'ler üzerindeki sinerjik etkisi, daha yüksek nanoparçacık konsantrasyonlarına gerek kalmadan antibakteriyel etkinliği artırdı ve ceviz yaprağı ekstresi kullanılarak sentezlenen katran kaplı AgNP'lerin, alternatif antibakteriyel ajanlar olarak umut verici olabileceğini göstermektedir.

Anahtar Sözcükler: Gümüş nanopartiküller, ceviz yaprağı ekstresi, sedir katranı, antibakteriyel aktivite, biyosentez.

Introduction

Humans have long relied on plants as sources of food, materials, dyes, fuels, and medicines. Over time, specific plants have been distinguished for their distinctive properties and used to treat diseases in both humans and animals^{1,2}. The therapeutic use of tar obtained from cedar wood has persisted for centuries. In folk medicine, tar has been used for treating wounds, asthma and upper respiratory diseases³, ear pain⁴, acne⁵, hemorrhoids⁴, and ulcers³.

With the development of nanotechnology, interest in plant-derived materials has increased further. Beyond textiles, chemistry, and industrial applications, plants are now widely used in nanoparticle synthesis. In green synthesis, plant extracts act as reducing and stabilizing agents. Metal nanoparticles produced by this method have demonstrated antibacterial⁶⁻⁸, antifungal⁹, and antiviral¹⁰ activity. Numerous studies have reported antibacterial effects of nanoparticles (NPs)⁶, particularly against bacteria such as *Escherichia coli*⁷ and *Staphylococcus aureus*⁸, and antiviral and antifungal effects have also been described; for example, Lamsal et al. (2011) reported antifungal activity of AgNPs⁹.

AgNPs synthesized by green methods have been frequently explored for biomedical applications. In one study, wound dressings incorporating AgNPs were shown to promote wound healing. In parallel, tar has long been used in folk medicine for wound care^{11,12}. While the use of tar for injuries has been associated with deterring insects, worms, and other parasites, the contribution of AgNPs likely derives from their antibacterial activity.

Material and Methods

Synthesis of AgNPs

AgNPs were synthesized via green synthesis using walnut leaf extract as the reducing agent and AgNO₃ as the silver source. Formation kinetics were monitored by time-dependent UV–Vis spectroscopy, and particle morphology was characterized by transmission electron microscopy (TEM).

Preparation of cedar tar

Firewood from the resin-rich portions of cedar wood was placed in a metal container with a small hole at the base. External heating caused pyrolysis; the tar volatilized from the wood and condensed into a collecting vessel placed below.

Preparation of walnut extract

Walnut leaves (1 g) were added to 100 mL of distilled water and boiled under magnetic stirring (JKI-JK-PMS-HS-130109) for 1 hour. The mixture was filtered and centrifuged (General Purpose-17022810) at 10,000 rpm for 15 minutes to obtain a homogeneous extract.

Preparation of Walnut-AgNO₃ solutions

Equal volumes (1:1, v/v) of 2 mM AgNO₃ and walnut leaf extract were mixed to yield a final reaction mixture with 1 mM AgNO₃.

Incorporation of AgNPs into tar

After 24 hours of reaction, water from the colloidal AgNP dispersions was evaporated. The resulting dry nanoparticles were added to tar and dispersed by sonication in an ultrasonic bath for 30 minutes to minimize aggregation.

UV–Vis spectroscopy (surface plasmon resonance)

Surface plasmon resonance (SPR) of the walnut–AgNO₃ reaction mixtures was analyzed using an Eon Microplate Spectrophotometer over 200–700 nm. Samples were collected after 24 hours of exposure to sunlight. Absorbance spectra were recorded and interpreted.

Image analysis of AgNPs

For TEM, aliquots of walnut–AgNO₃ reaction mixtures collected 24 hours post-mixing were drop-cast onto carbon-coated copper grids and air-dried in a clean environment. Nanoparticles were imaged using a LEO 906E TEM.

The antibacterial activity

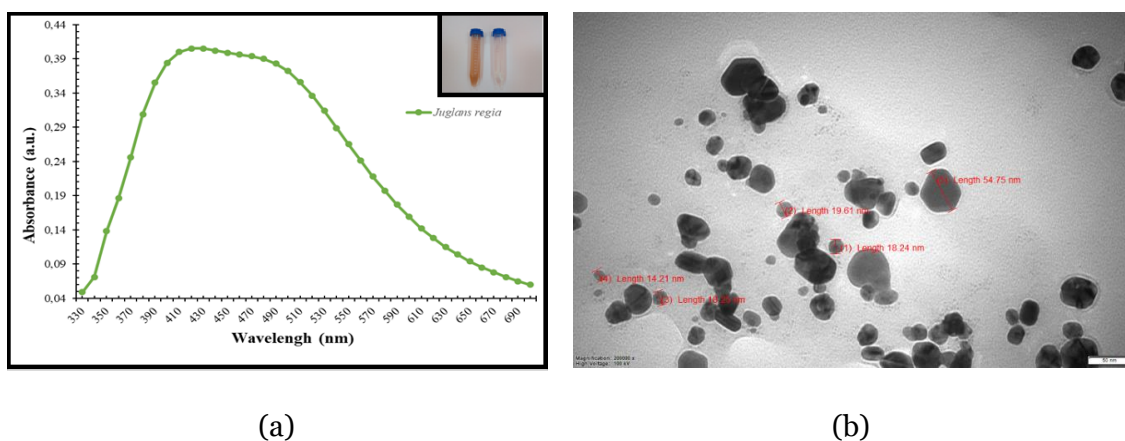
The antibacterial activities of AgNPs, walnut leaf extract, tar, and the combined tar+AgNPs formulation were evaluated against Gram-positive *S. aureus* ATCC 29213 and Gram-negative *E. coli* ATCC 25922 using the standard disc diffusion method on Mueller–Hinton agar (MHA), following established protocols^{13,14}.

Bacterial cultures were incubated for 12 hours at 37 °C in MHA medium. Suspensions were adjusted to 0.5 McFarland standard (approximately 1.5×10^8 CFU/mL) by 1:100 dilution in Nutrient Broth¹⁵. Subsequently, 100 µL of each suspension was spread onto MHA plates. Sterile 6-mm Whatman filter paper discs were placed on the agar and loaded with 50 µL of test substances: AgNPs (containing 8.5 µg Ag/disc), walnut leaf extract, tar, and tar+AgNPs. The walnut leaf extract and tar served as the negative control, and doxycycline (30 µg/disc), a broad-spectrum antibiotic, served as the positive control. Plates were incubated at 37 °C for 24 hours, and inhibition zone diameters were measured in millimeters. Each experiment was performed in duplicate.

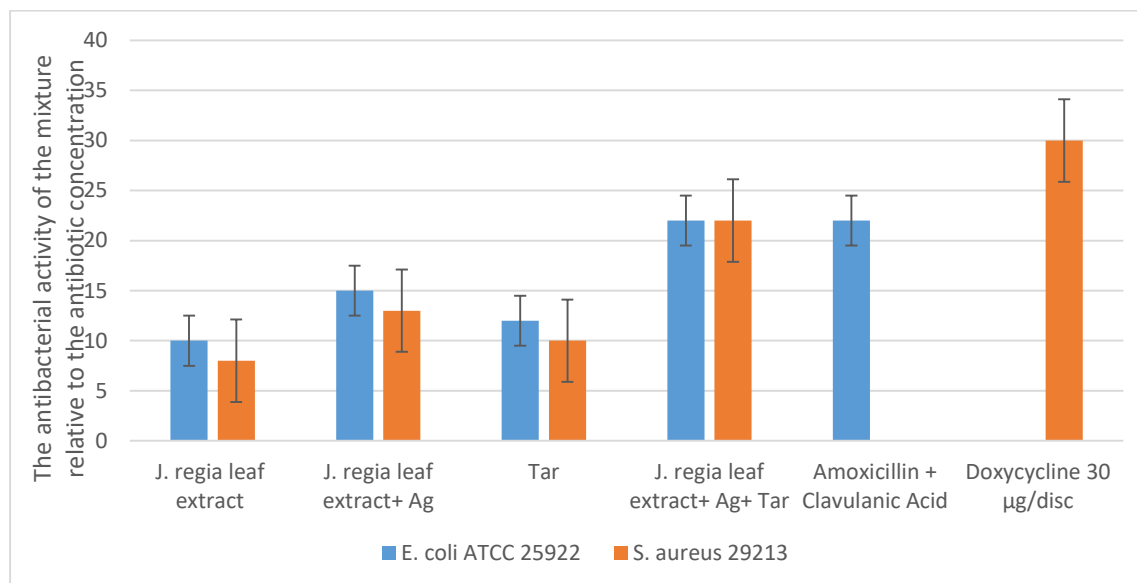
Results

In this study, the surface plasmon resonance (SPR) peaks of the synthesized AgNPs (Figure 1) were measured in the range of 200-700 nm using The Eon™ Microplate Spectrophotometer, and it was found that the SPR peaks had a broad Full Width at Half Maximum (FWHM) and peaked at 420 nm. The broad FWHM values of the SPR peaks indicate the heterogeneity in the size distribution of the nanoparticles.

Figure 1.a. SPR peaks of mixtures, **b.** TEM Images of AgNPs (Magnification: 200000x, High Voltage: 100kV)



According to transmission electron microscopy (TEM) data, the sizes of the nanoparticles were determined to be in the range of 8.25-54.75 nm and to have a circular shape (Figure 1.b.).

Figure 2. Antibacterial activity of synthesized AgNPs using the walnut leaf extract and tar.

The antibacterial activities of tar-coated silver nanoparticles (AgNPs) synthesized using walnut leaf extract were tested on Gram-positive (*S. aureus* ATCC 29213) and Gram-negative (*E. coli* ATCC 25922) bacteria. The findings indicate that the applications using walnut and tar alone exhibited antibacterial effects, but these effects were lower compared to antibiotics. However, it was determined that AgNPs synthesized using walnut extract had higher antibacterial effects compared to walnut extracts alone but were still less effective than antibiotics (Figure 2).

Discussion

The broad SPR peaks observed in UV–Vis spectra are consistent with a wide nanoparticle size distribution, as also confirmed by TEM analysis (8.25–54.75 nm, spherical morphology). Such broad absorption features are typically attributed to surface plasmon resonance damping caused by particle size heterogeneity and aggregation phenomena, both of which are common in green-synthesized nanoparticles due to the presence of multiple phytochemical reducing and capping agents. These findings are in good agreement with previous reports. For instance, Mohamad Kasim et al. (2020) reported plant-extract-mediated AgNPs with sizes of 49.26 ± 13.76 nm and predominantly spherical morphology¹⁶. Similarly, Azwatul et al. (2023) synthesized AgNPs through plant-mediated reduction with particle sizes ranging from 5 to 35 nm, showing a circular shape and homogeneous dispersion¹⁷. Such size variations are expected since biological extracts contain a variety of biomolecules (flavonoids, phenolics, terpenoids, and proteins) that simultaneously act as reducing and stabilizing agents, influencing the growth kinetics and final morphology of the nanoparticles.

The antibacterial efficacy of AgNPs has been shown to depend strongly on both particle size distribution and mass concentration. Smaller AgNPs generally display greater antibacterial activity due to their larger surface area-to-volume ratio, which enhances their interaction with bacterial membranes and facilitates silver ion release. Khan et al. (2023) demonstrated that antibacterial potency increases with higher AgNP concentrations; however, elevated concentrations are often associated with higher cytotoxicity to mammalian cells¹⁸. Therefore, identifying formulations that maintain antibacterial strength at reduced nanoparticle doses is crucial for biomedical applicability.

In this context, tar was incorporated into walnut leaf-mediated AgNPs with the aim of amplifying antibacterial effects while mitigating potential cytotoxicity. The results reveal a synergistic effect between tar and AgNPs, yielding antibacterial outcomes comparable to conventional antibiotics such as amoxicillin/clavulanic acid and doxycycline at equivalent doses. This synergy likely arises from complementary mechanisms.

These observations align with growing evidence that plant-extract-synthesized AgNPs can exhibit superior antibacterial efficacy compared to chemically synthesized counterparts¹⁹. The presence of phytochemicals on the nanoparticle surface not only enhances colloidal stability but also contributes to biological activity by providing additional functional groups capable of interacting with microbial cell walls. Moreover, combining AgNPs with other biocompatible or bioactive materials has been shown to further augment antimicrobial performance. For example, hybrid composites of AgNPs with chitosan, lignin, or phenolic-rich plant extracts have demonstrated enhanced bactericidal activity at lower silver doses, underscoring the value of synergistic formulations²⁰.

Nevertheless, while AgNPs possess remarkable antimicrobial potential, their dose-dependent cytotoxicity remains a major concern for clinical and environmental applications. Excessive release of Ag⁺ ions or accumulation of nanoparticles in tissues can trigger oxidative stress, mitochondrial dysfunction, and genotoxicity in mammalian systems²¹. Hence, optimizing concentration and exposure duration is essential to achieve an effective therapeutic window. In this study, the incorporation of tar -rich in phenolic and polyaromatic compounds- was explored as a strategy to boost antibacterial efficacy within safer dosing limits. The phenolics present in tar likely interact with AgNPs through surface adsorption, increasing their stability and enhancing ROS-mediated antibacterial mechanisms.

The present findings, demonstrating significant antibacterial activity of the AgNPs-tar composite against both Gram-positive (*S. aureus*) and Gram-negative (*E. coli*) bacteria, are consistent with previous studies reporting strong antibacterial effects of plant-synthesized AgNPs⁸. For instance, AgNPs synthesized from *Hypericum perforatum*⁸ and *Syzygium polyanthum*¹⁹ extracts have shown potent inhibitory zones against *E. coli* and *S. aureus*. Such broad-spectrum activity is attributed to multiple concurrent actions, including disruption of cell wall integrity, leakage of intracellular contents, enzyme inactivation, and induction of oxidative stress.

Mechanistically, AgNPs interact with bacterial cell membranes primarily through electrostatic attraction, resulting in structural disruption and increased membrane permeability²². The subsequent release of Ag⁺ ions allows them to bind with thiol (-SH) groups in membrane and cytoplasmic proteins, altering their tertiary structure and impairing essential enzymatic and metabolic processes²³. Moreover, silver-induced reactive oxygen species (ROS) generation contributes to lipid peroxidation, protein oxidation, and DNA damage, thereby intensifying bactericidal activity²⁴.

In parallel, tar-derived phenolic compounds may function as redox-active agents, amplifying oxidative stress and exacerbating bacterial membrane perturbation. The observed synergistic antibacterial effect suggests that tar not only complements the intrinsic antimicrobial activity of AgNPs but may also act as a mild stabilizing matrix, reducing nanoparticle aggregation and promoting prolonged contact with bacterial surfaces.

Overall, the incorporation of tar into plant-mediated AgNP systems represents a novel and eco-friendly strategy for developing next-generation antimicrobial agents. This composite approach capitalizes on green synthesis principles, reduces the dependence on high silver concentrations, and harnesses the multifunctional properties of natural compounds to enhance antibacterial efficacy. Future investigations should focus on elucidating the detailed mechanistic pathways underlying this synergism through ROS quantification, membrane integrity assays, and cytotoxicity studies on mammalian cells to validate the biosafety and therapeutic applicability of the AgNP–tar composite system.

Conclusion

In this study, the antibacterial activities of tar-doped silver nanoparticles (AgNPs) synthesized through walnut leaf extract were successfully evaluated. In tests performed against both gram-positive (*S. aureus*) and gram-negative (*E. coli*) bacteria, it was observed that walnuts and tar alone had antibacterial effects, but they showed lower effects compared to antibiotics. It was determined that AgNPs synthesized with walnut extract exhibited stronger antibacterial effects compared to walnut extracts, but were still less effective than antibiotics. Current studies in the literature report that the antibacterial activity of AgNPs depends on their mass concentration and size, but high concentrations increase the risk of cytotoxicity. For this reason, we aimed to increase the antibacterial effects of low-concentration AgNPs by adding tar, which is rich in phenolic compounds, which we think will be biocompatible in increasing the antibacterial effects of low-concentration AgNPs in terms of controlling cytotoxicity. Thus, with the addition of tar, the antibacterial activity of AgNPs was increased and results comparable to antibiotics were obtained against both bacteria. These findings indicate that walnut leaf extract and tar-doped AgNPs can be evaluated as potential antibacterial agents in the future and results should be considered preliminary, as the study was conducted exclusively under in vitro conditions, and further in vivo studies are required to confirm their practical applicability.

REFERENCES

1. Altay V, Karahan F. Tayfur Sökmen Kampüsü (Antakya-Hatay) ve çevresinde bulunan bitkiler üzerine etnobotanik bir araştırma. *Karadeniz Fen Bilimleri Dergisi*. 2012;3(2):13-28.
2. Deniz L, Serteser A, Kargioğlu M. Uşak Üniversitesi ve yakın çevresindeki bazı bitkilerin mahalli adları ve etnobotanik özellikleri. *Afyon Kocatepe Üniversitesi Fen ve Mühendislik Bilimleri Dergisi*. 2010;10(1):57-72.
3. Kurt Y, Kaçar MS, Isik K. Traditional tar production from *Cedrus libani* A. Rich on the Taurus Mountains in southern Turkey. *Economic Botany*. 2008;62(4):615.
4. Ülger Z. Aydın (Merkez) ve Çevresinde Halk Hekimliği [master's thesis]. Türkiye: Aydın, Adnan Menderes Üniversitesi, Sosyal Bilimler Enstitüsü; 2012.
5. Gönenç A. Antalya İli Korkuteli İlçesinde Halk İnanışları ve Halk Hekimliği [doctoral thesis]. Türkiye: Konya, Selçuk Üniversitesi Sosyal Bilimler Enstitüsü; 2011.
6. Morones JR, Elechiguerra JL, Camacho A, et al. The bactericidal effect of silver nanoparticles. *Nanotechnology*. 2005;16(10):2346.
7. Bilgic E, Aydin SG, Ozgen A, Nizamlioglu M. Green synthesized silver nanoparticles: Morphology and antibacterial contact effects. *Medicine*. 2019;8(3):541-544.
8. Ozgen A, Bilgic E, Aydin, SG, Nizamlioglu M. Characterization of biosynthesized silver nanoparticles using *Hypericum perforatum* leaf and determination of their antibacterial activity. *Medicine Science*. 2019;8(3):503-507. doi: 10.5455/medscience.2018.07.8985.
9. Lamsal K, Kim S, Jung JH, Kim YS, Kim KS, Lee YS. Inhibition effects of silver nanoparticles against powdery mildews on cucumber and pumpkin. *The Korean Society of Mycology*. 2011;39:26-32. doi: 10.4489/MYCO.2011.39.1.026.
10. Carvalho-Silva JM, Ferreira I, Uehara L M, et al. Antiviral effect of silver nanoparticles against Influenza A: A systematic review of in vitro and in vivo studies. *Future Virology*. 2025;20(1-2):19-26.
11. Ak M. Yörüklerde halk baytarlığı. *ASOS J*. 2017;5(50):51-60.
12. Ak M. Yörüklerde halk hekimliği. *The Journal of Academic Social Science Studies*. 2017;57:395-405. doi: 10.9761/JASSS7124.

13. Russell AD, Furr JR. The antibacterial activity of a new chloroxyleneol preparation containing ethylenediamine tetraacetic acid. *J Appl Bacteriol.* 1977;43(2):253-260.
14. Irobi ON, Moo-Young M, Anderson WA, et al. Antimicrobial activity of bark extracts of *Bridelia ferruginea* (Euphorbiaceae). *J Ethnopharmacol.* 1994;43:185-90.
15. Clinical and Laboratory Standards Institute. *Performance standards for antimicrobial disk susceptibility tests.* 7th ed. Wayne (PA): Clinical and Laboratory Standards Institute; 2012.
16. Mohamad Kasim AS, Ariff AB, Mohamad R, Wong FWF. Interrelations of synthesis method, polyethylene glycol coating, physico-chemical characteristics, and antimicrobial activity of silver nanoparticles. *Nanomaterials.* 2020;10(12):2475.
17. Azwatul HM, Uda MNA, Gopinath SC, et al. Plant-based green synthesis of silver nanoparticle via chemical bonding analysis. *Materials Today: Proceedings.* 2023. doi: 10.1016/j.matpr.2023.01.005.
18. Khan S, Rukayadi Y, Jaafar A H, Ahmad NH. Antibacterial potential of silver nanoparticles (SP-AgNPs) synthesized from *Syzygium polyanthum* (Wight) Walp. against selected foodborne pathogens. *Heliyon.* 2023;9(12). doi: 10.1016/j.heliyon.2023.e22771.
19. Shahzadi S, Fatima S, Shafiq Z, et al. A review on green synthesis of silver nanoparticles (SNPs) using plant extracts: A multifaceted approach in photocatalysis, environmental remediation, and biomedicine. *RSC Advances.* 2025;15(5):3858-3903.
20. Mert Sivri F. Introduction to nanoparticles, their synthesis, fabrication and applications. In: *Nanoparticles Integrated Functional Textiles.* Cham: Springer Nature Switzerland; 2025:1–30.
21. Akhtar M F, Irshad M, et al. Understanding the silver nanotoxicity: Mechanisms, risks, and mitigation strategies. *Journal of Nanoparticle Research.* 2025;27(4):1-18.
22. Rai M, Yadav A, Gade A. Silver nanoparticles as a new generation of antimicrobials. *Biotechnology Advances.* 2009;27(1),76-83.
23. Durán N, Durán M, et al. Silver nanoparticles: A new view on mechanistic aspects on antimicrobial activity. *Nanomedicine: Nanotechnology, Biology and Medicine.* 2016;12(3):789-799. doi: 10.1016/j.nano.2015.11.016.

24. Gurunathan S, Han JW, Kwon DN, Kim JH. Enhanced antibacterial and anti-biofilm activities of silver nanoparticles against gram-negative and gram-positive bacteria. *Nanoscale Res Lett.* 2014;9(1):373.