

BIOGENIC FABRICATION AND CHARACTERIZATION OF SILVER NANOPARTICLES USING *BLEPHARIS CILIARIS* EXTRACT AND THEIR ANTIBACTERIAL APPLICATIONS

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(Received 24th Apr 2025; accepted 11th Jun 2025)

Abstract. Recently, multidrug-resistant bacteria have markedly reduced the effectiveness of antibiotics worldwide, a problem that is potentially solvable by the use of biogenic nanoparticles (NP). Here aqueous leaf extract of *Blepharis ciliaris* was used to synthesize biogenic silver nanoparticles (AgNPs) and to determine their antibacterial properties. UV-Visible spectroscopy was used to observe the formation of nanoparticles at various time intervals. The surface plasmon band changed color as the reaction time increased, with a maximum absorption recorded at 400 nm. Scanning electron microscopy (SEM) and transmission electron microscopy (TEM) were also used to analyze the size and morphology of the AgNPs. TEM revealed the spherical shape of the nanoparticles, which varied in size from 18 to 50 nm. The elemental composition of AgNPs was determined using energy dispersive spectroscopy (EDS), which showed strong signals at ~3 keV in the silver (Ag) zone. Finally, the antibacterial activity of AgNPs was assessed using the well diffusion method against some pathogenic bacteria. All of the bacteria tested proved susceptible, with *E. coli* and *B. subtilis* being inhibited more than *S. aureus* and *S. Typhimurium*.

Keywords: *Blepharis ciliaris*, plant, AgNPs, green synthesis, multidrug-resistant bacteria

Introduction

Nanotechnology is a field of research focused on the improvement of devices and materials at the molecular level. Current advancement in nanotechnology has introduced novel approaches which can be used to solve a number of problems which limit the progress of developing different countries, particularly with regard to environmental sustainability, food security, and health and sanitation (Khac et al., 2023). During the past few decades, there has been considerable novel global research, development, and industrial activity in this broad, multidisciplinary research area, including the biogenic synthesis of nanoparticles. The use of plant extracts, bacteria and fungi to produce various metal nanoparticles, including silver, gold, antimony, palladium, copper, platinum, and selenium has been previously described in literature (Ahmad et al., 2003; Senapati et al., 2005; Bhainsa et al., 2006; Shahverdi et al., 2007). The economic importance of silver metal (Ghotekar et al., 2019) means that silver nanoparticles (AgNPs) are of particular interest, as they can serve as substrates for surface-enhanced Raman scattering (SERS), can detect organic dyes (Chen, et al., 2021; Marimuthu, et al., 2020; Ahmed, et al., 2020) and transfer and deliver medications and biomolecules (Yin et al., 2020; Illanes Tormena et al., 2020). They also possess notable antibacterial and antiviral properties (Hue et al., 2021; Razek et al., 2019; He et al., 2017). The biogenic fabrication of NP using plant extracts, fungi, bacteria, and some proteins (including enzymes) is commonly used, largely because to its biocompatibility and low ecological

impact (Tagad et al., 2013; Husen and Siddiqi, 2014; Husen, 2017; Rana et al., 2024). Silver nanoparticles (AgNPs) are synthesized by a variety of chemicals available in plant extracts, such as alkaloids, phenols, glycosides, amines, quinines and terpenoids (Tagad et al., 2013; Siddiqi et al., 2018; Alsayed et al., 2024; Aljowaie and Aziz, 2025).

The plant used here, *Blepharis*, is the major genus in the family Acanthaceae, which includes some 126 species (Fig. 1). *Blepharis* occurs widely in hot, dry, and semi-arid areas of the old-world tropics and subtropics (McDade et al., 2005). *Blepharis ciliaris* is found throughout the world from the Arabian Peninsula to Pakistan and southern Iran (Vollesen, 2000). The flowering season for the perennial plant *Blepharis ciliaris* is from October to April. This species is rarely seen in sandy habitats; instead, it is typically found in the silt between rocks on plains and low places and also in gulleys, wadis and mountain slopes (Jongbloed et al., 2003; Feulner, 2016). Several species of *Blepharis*, including *B. maderaspatensis*, *B. ciliaris*, *B. linariifolia*, *B. edulis*, and *B. scindica* are commonly used throughout Africa and Asia to treat a variety of infectious and chronic disorders, inflammatory issues, bone problems, and parasite complications (Khare, 2001; Mohamed et al., 2015). Members of the genus *Blepharis* are also economically significant because of their use as natural dyes (El-Shanawany et al., 2013). The seeds also possess expectorant, diuretic, and aphrodisiac properties (Deshpande, 2006) and when crushed and roasted are used to treat inflammation and wounds. Additionally, the root's charcoal is used for treating eye inflammation and enhance vision (Boulos, 1981; Tackholm, 1974; Atanasov et al., 2015).



Figure 1. *Blepharis ciliaris* plant

In the present study, an aqueous leaf extract from *Blepharis ciliaris* was used for the biogenic fabrication of AgNPs, and characteristics of biosynthesized AgNPs were studied and tested as antibacterial agents against variety of pathogenic bacteria.

Materials and methods

Materials

Blepharis ciliaris plants were collected from Shabwah Governorate, south of Yemen (Fig. 1). Silver nitrate was used during synthesis process, nutrient broth and Muller-Hinton agar were used as bacterial growth media. The following bacteria were used; *Staphylococcus aureus*, *Bacillus subtilis*, *Escherichia coli* and *S. Typhimurium*.

Preparation of Blepharis ciliaris leaf extract

Plant spikes of plants were thoroughly washed with distilled water and then left to dry naturally at the room temperature, after which, the leaves were removed and ground into a fine powder using by grinder (Moulinex, France). Powder (10 g) was mixed with deionized water (100 ml) and heated for 15 min at 60°C. Finally, the mixture was filtered using Whatman No. 1 filter paper, and the resulting solution was kept at 4°C until the AgNPs synthesis was achieved.

Biogenic synthesis of silver nanoparticles

The leaf extract was added to a 90 mL aqueous solution of silver nitrate 0.01 mM. The solution's color changed overnight when the mixture was left at room temperature. The AgNPs were then separated from the solution by centrifugation at 15,000 rpm for 20 min. The nanoparticles were then dried in an oven at 40°C to produce AgNPs as dry particles.

Characterization of AgNPs

An Ultrospec 2100 Pro UV/visible spectrophotometer (Biochrom, UK) was used to perform UV–Vis spectroscopy at wavelengths between 200 and 800 nm. EDS analysis was done using by an Altima IV device (Regaku, Japan). To determine the morphology and size of the AgNPs, TEM pictures were obtained with an 80 keV accelerating voltage using a JEOL microscope (JEM 1011). The particles were vacuum-dried (Model 1:53 liter vacuum oven, Vinci Technologies, France) before imaging.

Antibacterial studies

The antibacterial activity of the AgNPs was investigated using the agar well diffusion method. Each bacterium was inoculated into petri plates with 20 milliliters of Mueller Hinton Agar media. Using a sterilized agar borer, wells 6 mm in diameter were made into the medium. The synthesized nanoparticles (100 µl) were then poured in to the well and the dishes were incubated for 24 h 37°C. A clear area surrounding the well indicated growth inhibition. Distilled water was used as the control.

Statistical analysis

The statistical analyses of antibacterial activity results were done with a SAS program. Three replicates were used in experiment. Data were expressed as the mean ± standard deviation. Using Duncan's multiple range test, the significance between sample means was determined.

Results and discussion

UV-vis spectra

The formation and stability of AgNPs was determined by surface plasmon resonance band (SPR) using UV–Vis spectroscopy at wavelengths from 200 to 800 nm. *Figure 2* shows the sample's graphic calorimetric pattern following the reaction between AgNPs and plant extract. The development of AgNPs was indicated by the colorless AgNO₃ solution becoming dark brown, and the appearance of an absorption peak circa, 400 nm

(Fig. 3). Typically, spherical AgNPs synthesized using plant extracts such as *Acanthospermum hispidum* leaves extract (Ghotekar et al., 2019), *Indigofera Oblongifolia* leaves extract (Salmen et al., 2019) and *Aloe vera*, *Portulaca oleracea* and *Cynodon dactylon* (Abalkhil et al., 2017) have previously been reported in the 400–475 nm visible range.

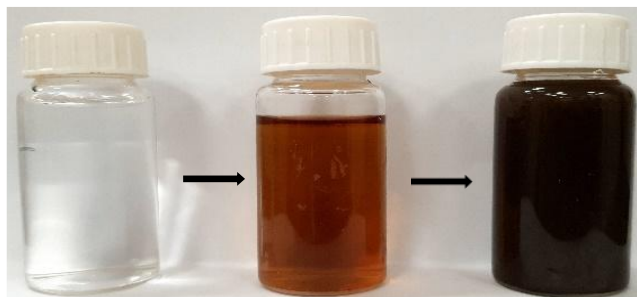


Figure 2. Color change after a mixture of *Blepharis ciliaris* extract with silver nitrate (AgNO_3) solution

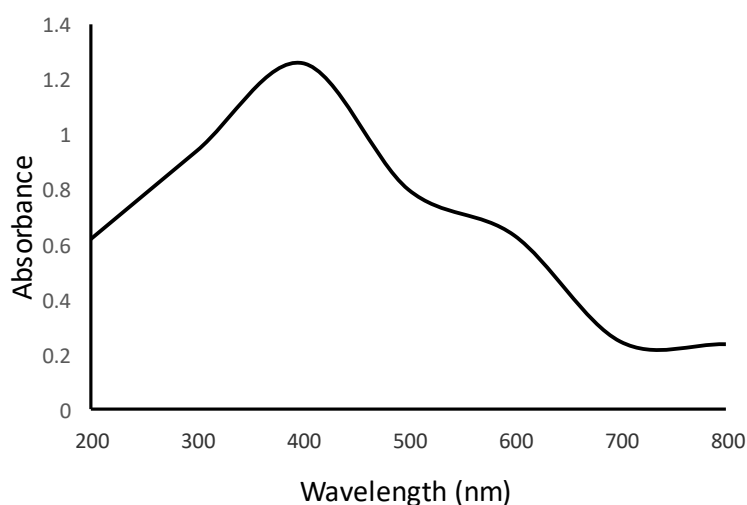


Figure 3. UV-vis absorption spectrum of the AgNPs biogenic synthesized using *Blepharis ciliaris* extract

EDS analysis

The elemental composition of AgNPs biosynthesized using *Blepharis ciliaris* extract was examined using EDX (Fig. 4). Strong signals were found at ~3 keV in silver (Ag) zone for AgNPs biogenic fabricated using *Blepharis ciliaris* extract. The signal's appearance at around 3 keV was considered confirmation that Ag elements were present in the AgNPs. The presence of a number of additional chemical components in the nanoparticles suggests that these components possibly originate from plant biomolecules attached to the AgNPs' surface. A pure silver metal ion is present when peaks appear before 5 keV (Kgatshe et al., 2019). Kgatshe et al. (2019) also reported that the pattern observes reflects peaks originating from the binding energies of carbon, chlorine, and oxygen, all of which can be related to contaminants occurring during the nanoparticle-drying process.

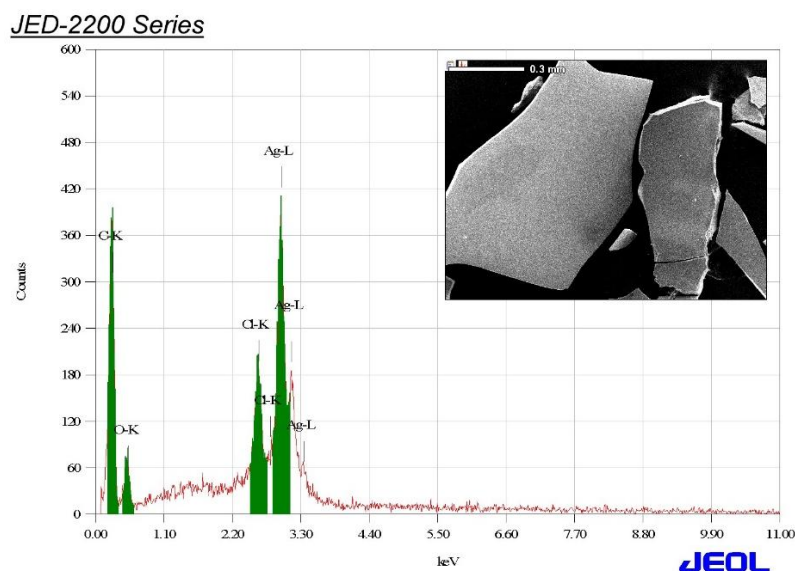


Figure 4. EDS analysis of AgNPs biogenic synthesized using *Blepharis ciliaris* extract

TEM analysis

TEM was used to determine the size and shape of the biosynthesized AgNPs using *Blepharis ciliaris* extract. Figure 5 shows TEM images of AgNPs, which are mostly spherical in shape and exhibit a wide range of NP size, from between 18 and 50 nm. Agglomeration of smaller particles that may have occurred during the powder sample preparation procedure caused the formation of bigger particles (Ibrahim, 2015; Salari et al., 2016). The presence of a translucent film of biomolecular coating around the nanoparticles serves as evidence of the capping activity of the phytochemical components present in the plant extracts, which contributes to the AgNPs stability (León-Silva et al., 2016). The silver nanoparticles synthesized using *Blepharis ciliaris* extract have a morphology that is essentially similar to that of the AgNPs biogenically fabricated using other extracts (Venugopal et al., 2017; Kgatshe et al., 2019).

Antibacterial activity

The antibacterial activity of AgNPs was determined against *S. aureus*, *B. subtilis*, *E. coli* and *S. Typhimurium*. Figure 6 shows the antibacterial activity of AgNPs at different concentrations. The susceptibility or resistance of the studied bacteria to AgNPs and the control was investigated by measuring the zones of inhibition around the test compounds. All bacteria showed growth susceptibility; *E. coli* and *B. subtilis* produced larger growth-inhibition zones than *S. aureus* and *S. Typhimurium*, showing that they were more sensitive to the inhibitory effects of the AgNPs. As expected, the control sample did not produce inhibition zones. There are several interpretations for the mechanism of the antibacterial effect of AgNPs. Bactericidal effects are due to silver ions released from AgNPs as a result of their interaction with bacteria; silver ions penetrate the cytoplasm, denature the ribosome, inhibit proteins and enzymes, and finally inhibit their metabolic activity, leading to death. Other interpretations for the mechanisms involved in the antibacterial activity of Ag NPs were reported by Siddiqi et al. (2018) including, interference during synthesis of bacterial cell wall, suppression during protein biosynthesis, disruption of transcription process and disruption of primary metabolic pathways.

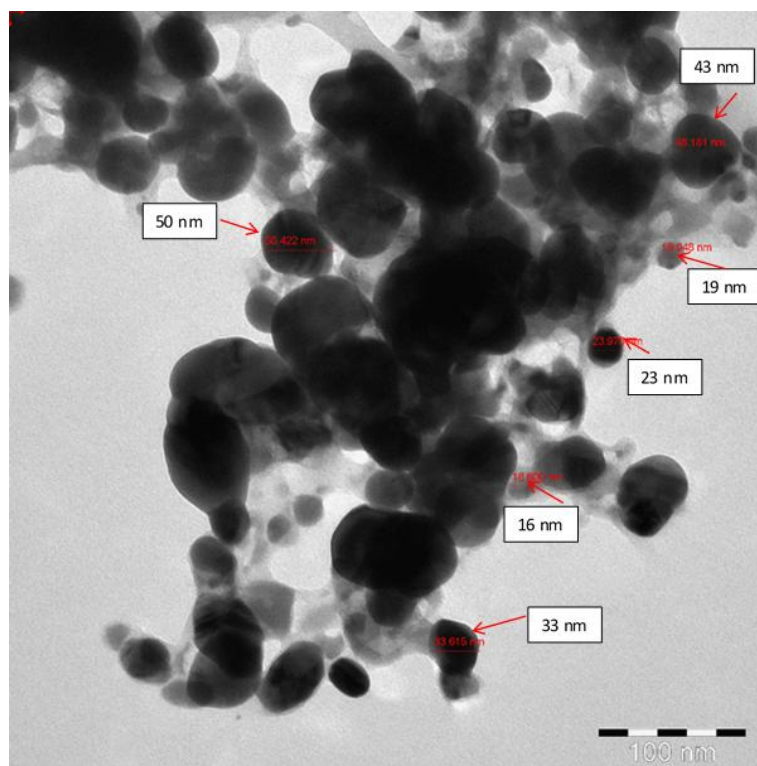


Figure 5. TEM image of AgNPs biogenic synthesized by *Blepharis ciliaris* extract

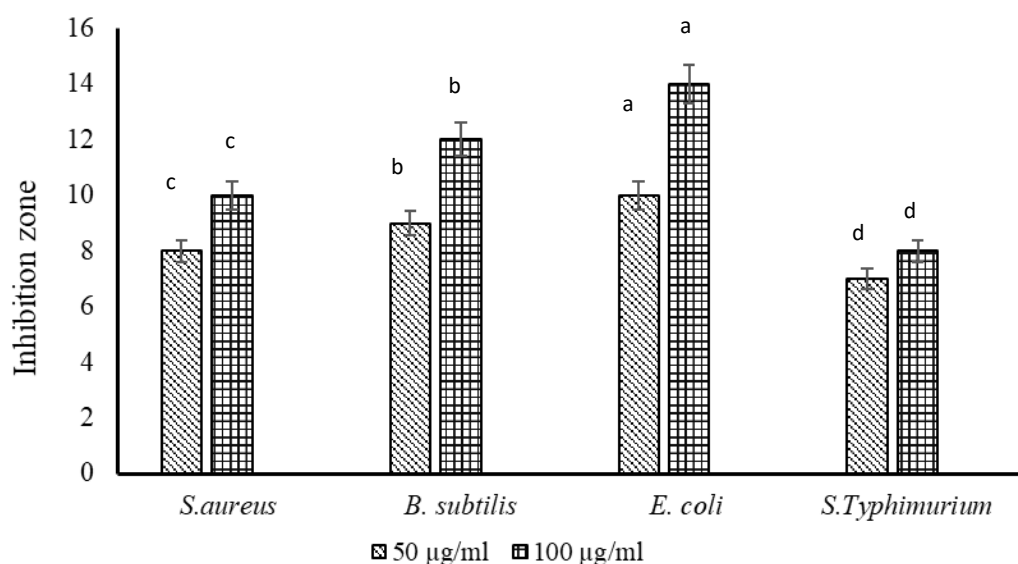


Figure 6. Antibacterial activity of AgNPs synthesized by *Blepharis ciliaris* extract. Means with different letters on each column are significantly different ($P < 0.05$)

Conclusion

In this study, the biogenic fabrication of silver nanoparticles mediated by *Blepharis ciliaris* plant was achieved. Extracts of this plant was considered to serve as a reducing agent in the rapid and environmentally safe synthesis of silver nanoparticles. AgNPs-properties were determined using a number of different techniques including, UV-

Visible spectroscopy, EDS, SEM and TEM. Fabrication of AgNPs was confirmed by UV-Visible spectroscopy at 400 nm with sizes ranging from 18 to 50 nm. EDS analysis was showed strong signals at ~3 keV in silver zone. AgNPs also showed a stronger antibacterial activity. Finally, the plant extract's ability to act as a reducing, stabilizing and capping agent in AgNPs, factors which relate to their possible applications in biological and pharmacological technology.

Acknowledgements. This project was supported by Researchers Supporting Project number (RSP2025R385), King Saud University, Riyadh, Saudi Arabia.

Conflict of interests. The author declares that no conflict of interests.

REFERENCES

- [1] Abalkhil, T. A., Sulaiman, A. A., Salmen, S. H., Wainwright. M. (2017): Bactericidal activity of biosynthesized silver nanoparticles against human pathogenic bacteria *Biotechnol. – Biotechnol. Equip.* 31: 411-417.
- [2] Ahmad, A., Mukherjee, P., Senapati, S., Mandal, D., Khan, M. I., Kumar, R., Sastry, M. (2003): Extracellular biosynthesis of silver nanoparticles using the fungus *Fusarium oxysporum*. – *Colloids Surf B* 28: 313-318 13.
- [3] Ahmed, T., Noman, M., Shahid, M., Niazi, M. B. K., Hussain, S., Manzoor, N., Wang, X., Li, B. (2020): Green synthesis of silver nanoparticles transformed synthetic textile dye into less toxic intermediate molecules through LC-MS analysis and treated the actual wastewater. – *Environ. Res.* 191. <https://doi.org/10.1016/j.envres.2020.110142>.
- [4] Aljowaie, R. M., Aziz, I. M. (2025): Anticancer and antimicrobial effects of greensynthesized silver nanoparticles using *Teucrium polium* leaves extract. – *Green Processing and Synthesis* 14: 20240227. <https://doi.org/10.1515/gps-2024-0227>.
- [5] Alsayed, M. F., Alodaini, H. A., Aziz, I. M., Alshalan, R. M., Rizwana, H., Alkhelaiwi, F., ALSaigh, S. M., Alkubaisi, N. A. (2024): Silver nanoparticles synthesized using aerial part of *Achillea fragrantissima* and evaluation of their bioactivities. – *Scientific Reports* 14:24703. <https://doi.org/10.1038/s41598-024-75558-z>.
- [6] Atanasov, A. G., Waltenberger, B., Pferschy-Wenzig, E. M., Linder, T., Wawrosch, C., Uhrin, P., Temml, V., Wang, L., Schwaiger, S., Heiss, E. H., Rollinger, J. M., Schuster, D., Breuss, J. M., Bochkov, V., Mihovilovic, M. D., Kopp, B., Bauer, R., Dirsch, V. M., Stuppner, H. (2015): Discovery and resupply of pharmacologically active plant-derived natural products: a review. – *Biotechnol. Adv.* <https://doi.org/10.1016/j.biotechadv.2015.08.001>.
- [7] Bhainsa, K. C., D'Souza, S. F. (2006): Extracellular biosynthesis of silver nanoparticles using the fungus *Aspergillus fumigates*. – *Colloids Surf B* 47: 160-164 12.
- [8] Boulos, L. (1981): Medicinal plants of North Africa. – Reference Publications, Inc., Alonac.
- [9] Chen, J., Liu, Y., Xiong, Y., Wei, D., Peng, J., Mahmud, S., Liu, H. (2021): Konjac glucomannan reduced-stabilized silver nanoparticles for mono-azo and di-azo contained wastewater treatment, *Inorg. – Chim. Acta* 515. <https://doi.org/10.1016/j.ica.2020.120058>.
- [10] Deshpande, D. J. (2006): A Handbook of Medicinal Herbs. – Agrobios, Jodhpur, pp 342-343.
- [11] El-Shanawany, M. A., Sayed, H. M., Ibrahim, S. R. M., Fayed, M. A. A., Radwan, M. M., Ross, S. A. (2013): A new isoflavone from *Blepharis ciliaris* of an Egyptian origin. – *Med. Chem. Res.* 22: 2346-2350. <https://doi.org/10.1007/s00044-012-0228-2>.

- [12] Feulner, G. R. (2016): The flora of Wadi Wurayah National Park, Fujairah: an annotated checklist and selected observations on the flora of an extensive ultrabasic bedrock environment in the northern Hajar Mountains. – *Tribulus* 24: 4-84.
- [13] Ghotekar, S., Pansambal, S., Pawar, S., Pagar, T., Oza, R., Bangale, S. (2019): Biological activities of biogenically synthesized fluorescent silver nanoparticles using *Acanthospermum hispidum* leaves extract. – *SN Applied Sciences* 1: 1342. <https://doi.org/10.1007/s42452-019-1389-0>.
- [14] He, R. X., Liang, R., Peng, P., Norman Zhou, Y. (2017): Effect of the size of silver nanoparticles on SERS signal enhancement. – *J. Nanoparticle Res.* 19: 8.267. <https://doi.org/10.1007/s11051-017-3953-0>.
- [15] Hue, D. T., Thao, N. T. P., Khoi, T. K., Ha, C. V. (2021): Multi-shaped silver meso-particles with tunable morphology for surface enhanced Raman scattering. – *Opt Commun.* 497. <https://doi.org/10.1016/j.optcom.2021.127200>.
- [16] Husen, A. (2017): Gold Nanoparticles from Plant System: Synthesis, Characterization and Their Application. – In: Ghorbanpourn M., Manika K., Varma A. (eds.) *Nanoscience and Plant–Soil Systems*. Vol. 48. Springer, Cham, pp. p. 455-479.
- [17] Husen, A., Siddiqi, K. S. (2014): Phytosynthesis of nanoparticles: concept, controversy and application. – *Nano Res Lett.*,9:229.
- [18] Ibrahim, H. M. M. (2015): Green synthesis and characterization of silver nanoparticles using banana peel extract and their antimicrobial activity against representative microorganisms. – *Journal of Radiation Research and Applied Sciences* 8: 265-275.
- [19] Illanes Tormena, R. P., Rosa, E. V., Mota, B. O., Chaker, J. A., Fagg, C. W., Freire, D. O., Martins, P. M., Silva, I. C. R., Sousa, M. H. (2020): Evaluation of the antimicrobial activity of silver nanoparticles obtained by microwave-assisted green synthesis using *Handroanthus impetiginosus* (Mart. ex DC.) Mattos underbark extract. – *RSC Adv.* 10 20676-20681. <https://doi.org/10.1039/d0ra03240a>.
- [20] Jongbloed, M. V. D., Feulner, G. R., Boer, B., Western, A. R. (2003): *The Comprehensive Guide to the Wild Flowers of the United Arab Emirates*. – Environmental Research and Wildlife Development Agency, Abu Dhabi.
- [21] Kgatshe, M., Aremu, O. S., Katata-Seru, L., Gopane, R. (2019): Characterization and antibacterial activity of biosynthesized silver nanoparticles using the ethanolic extract of *Pelargonium sidoides* DC. *J. of nanomaterials.* 10: 3501234. – <https://doi.org/10.1155/2019/3501234>.
- [22] Khac, K. T., Phu, H. H., Thi, H. T., Thuy, V. D., Thi, H. D. (2023): Biosynthesis of silver nanoparticles using tea leaf extract (*Camellia sinensis*) for photocatalyst and antibacterial effect. – *Heliyon* 9: e20707.
- [23] León-Silva, S., Fernández-Luqueño, F., López-Valdez, F. (2016): Silver nanoparticles (AgNP) in the environment: a review of potential risks on human and environmental health. – *Water, Air, Soil Pollution* 227: 9: 306.
- [24] Marimuthu, S., Antonisamy, A. J., Malayandi, S., Rajendran, K., Tsai, P., Pugazhendhi, A., Ponnusamy, V. K. (2020): Silver nanoparticles in dye effluent treatment: a review on synthesis, treatment methods, mechanisms, photocatalytic degradation, toxic effects and mitigation of toxicity. – *J. Photochem. Photobiol. B* 205. <https://doi.org/10.1016/j.jphotobiol.2020.111823>.
- [25] McDade, L., Daniel, T., Kiel, C., Vollesen, K. (2005): Phylogenetic relationships among Acantheae (Acanthaceae): major lineages present contrasting patterns of molecular evolution and morphological differentiation. – *Syst. Bot.* 30: 834-862.
- [26] Mohamed, G. A., Ibrahim, S. R. M., Elkhayat, E. S., Ross, S. A., Sayed, H. M., El-Moghazy, S. A. M., El-Shanawany, M. A. (2015): Blepharisides A and B, new flavonol glycosides from *Blepharis ciliaris* growing in Saudi Arabia. – *Phytochem. Lett.* 11: 177-182. <https://doi.org/10.1016/j.phytol.2014.12.018>.

- [27] Rana, J., Sharma, A., Rana, J., Sagar, A. (2024): Phytochemical analysis and biogenic synthesis of silver nanoparticles from *Phlomis bracteosa* Royle ex Benth. and screening of their antimicrobial and antioxidant potential. – Micro and Nano Systems Letters 12: 29. <https://doi.org/10.1186/s40486-024-00218-w>.
- [28] Razek, S. A., Ayoub, A. B., Swillam, M. A. (2019): One step fabrication of highly absorptive and surface enhanced Raman scattering (SERS) silver nano-trees on silicon substrate. – Sci. Rep. 9:13588 <https://doi.org/10.1038/s41598-019-49896-2>.
- [29] Salari, Z., Danafar, F., Dabaghi, S., Ataei, S. A. (2016): Sustainable synthesis of silver nanoparticles using macroalgae *Spirogyra varians* and analysis of their antibacterial activity. – J. Saudi Chem. Soci. 20: 459-464.
- [30] Salmen, S. H., Alwhibi, M. S. –Alharbi, S. A. (2019): Characterization and antibacterial activity of silver nanoparticles biosynthesized using (*Indigofera oblongifolia*) leaves extract. – Appl. Ecol. Environ. Res. 17(6): 12869-12876.
- [31] Senapati, S., Ahmad, A., Khan, M. I., Sastry, M., Kumar, R. (2005): Extracellular biosynthesis of bimetallic Au–Ag alloy nanoparticles. – Small 1: 517-520.
- [32] Shahverdi, R., Minaeian, S., Shahverdi, H. R., Jamalifar, H., Nohi, A. A. (2007): Rapid synthesis of silver nanoparticles using culture supernatants of enterobacteria: a novel biological approach. – Proc Biochem 42: 919-923 14.
- [33] Siddiqi, K. S., Husen, A., Rao, R. A. K. (2018a): A review on biosynthesis of silver nanoparticles and their biocidal properties. – J Nanobiotechnol. 16: 14.
- [34] Siddiqi, K., S., Rashid, M., Rahman, A., Tajuddin, Husen, A., Rehman, S. (2018b): Biogenic fabrication and characterization of silver nanoparticles using aqueous ethanolic extract of lichen (*Usnea longissima*) and their antimicrobial activity. – Biomater. Res. 22(23). <https://doi.org/10.1186/s40824-018-0135-9>
- [35] Tackholm, V. (1974): Student's Flora of Egypt. 2nd Ed. – Cairo University, Cairo, pp. 501-502.
- [36] Tagad, C. K., Dugasani, S. R., Aiyer, R., Park, S., Kulkarni, A., Sabharwal, S. (2013): Green synthesis of silver nanoparticles and their application for the development of optical fiber-based hydrogen peroxide sensor. – Sensors Actuators B Chem. 183:144-149.
- [37] Venugopal, K., Ahmad, H., Manikandan, E., Thanigai Arul, K., Kavitha, K., Moodley, M. K., Rajagopal, K., Balabhaskar, R., Bhaskar, M. (2017): The impact of anticancer activity upon Beta vulgaris extract mediated biosynthesized silver nanoparticles (ag-NPs) against human breast (MCF-7), lung (A549) and pharynx (Hep-2) cancer cell lines. – Journal of Photochemistry and Photobiology B: Biology 173: 99-107.
- [38] Vollesen, K. (2000): Blepharis (Acanthaceae). A Taxonomic Revision. – Royal Botanic Gardens, Kew.
- [39] Yin, I. X., Zhang, J., Zhao, I. S., Mei, M. L., Li, Q., Chu, C. H. (2020): The antibacterial mechanism of silver nanoparticles and its application in dentistry. – Int. J. Nanomed. 15. 2555-2562. <https://doi.org/10.2147/IJN.S246764>.