Biosynthesis of Silver Nanoparticles using *Moringa oleifera* oil and their Antimicrobial Activity against *Escherichia coli*

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ABSTRACT

This work investigated the green synthesis of silver nanoparticles using *Moringa oleifera* oil as a capping agent and its antibacterial activity against *Escherichia coli*. The samples were characterized using Ultraviolet-visible spectroscopy (UV-vis), Fourier transform infrared spectroscopy (FTIR), Transmission electron microscopy (TEM), and Raman spectroscopy. UV-vis spectroscopy showed a surface plasmon resonance band at 420 nm related to the formation of silver nanoparticles. Fourier transform infrared analysis shows the functionalization of the silver nanoparticles with *M. oleifera* oil. The bioactive compounds in *M. oleifera* oil play a dual role by reducing the silver ions and stabilizing the resulting nanoparticles, forming both 30 nm and of 5 nm nanoparticles. Raman spectroscopy reveals the interaction of the nanoparticles with the *M. oleifera* oil by a Surface-Enhanced Raman Scattering (SERS) effect. The agar diffusion method was used to study the antibacterial efficacy of the nanoparticles with *M. oleifera*, revealing an effective antibacterial activity against *E. coli* combined with an interesting SERS effect that could be applied in the biomedical field.

Keywords: Green–synthesis; silver nanoparticles; antibacterial properties; *Moringa oleifera*; SERS.

Biosíntesis de Nanopartículas de Plata utilizando aceite de *Moringa oleifera* y su Actividad Antimicrobiana frente a *Escherichia coli*

RESUMEN

Este trabajo investiga la síntesis verde de nanopartículas de plata utilizando aceite de *Moringa oleifera* como agente de protección y su actividad antibacteriana contra *Escherichia coli*. Las muestras se caracterizaron mediante espectroscopia ultravioleta-visible (UV-vis), espectroscopia infrarroja por transformada de Fourier (FTIR), microscopía electrónica de transmisión (TEM) y espectroscopia Raman. La espectroscopia UV-vis mostró una banda de resonancia de plasmon superficial a 420 nm relacionada con la formación de nanopartículas de plata. El análisis infrarrojo por transformada de Fourier muestra la funcionalización de las nanopartículas de plata con aceite de *M. oleifera*. Los compuestos bioactivos del aceite de *M. oleifera* desempeñan una doble función al reducir los iones de plata y estabilizar las nanopartículas resultantes, formando nanopartículas de 30 nm y de 5 nm. La espectroscopia Raman revela la interacción de las nanopartículas con el aceite de *M. oleifera* mediante un efecto de dispersión Raman mejorada en la superficie (SERS). Se utilizó el método de difusión en agar para estudiar la eficacia antibacteriana de las nanopartículas con M. oleifera, revelando una actividad antibacteriana efectiva contra *E. coli* combinada con un interesante efecto SERS que podría aplicarse en el campo biomédico.

Palabras claves: Síntesis verde; nanopartículas de plata; propiedades antibacterianas; *Moringa oleifera*; SERS.

INTRODUCTION

Antibiotic resistance is a problem that continues to challenge the world's healthcare sector. To control that problem, designing new active compounds and antibiotics based on nanotechnology and biomaterials is imperative (1). Silver nanoparticles (AgNPs) have been used as antimicrobial agents against Gram-positive and Gram-negative bacteria, and they are potentially used as a substitute for traditional antibiotics due to their antimicrobial properties (2). The use of green synthesis to prepare silver nanoparticles offers a range of advantages, including environmental sustainability, reduced toxicity, energy efficiency, biocompatibility, cost-effectiveness, versatility, tunable properties, and alignment with sustainable development goals. The green synthesis methods, involve the use of microorganism, plants, seeds, and flowers. *M. oleifera* has been used in green synthesis as a natural reducing and stabilizing agent to produce silver nanoparticles. Its abundance of bioactive compounds, low-temperature process, biocompatibility, antimicrobial properties, and sustainability make them a valuable resource for creating nanoparticles with controlled properties for applications in various fields (3).

M. oleifera is a tree species indigenous to northwestern India, and it is considered an important crop in several other countries, such as the Philippines, Sudan, Ethiopia, and South Africa. It belongs to the genus *Moringa* and the family Moringaceae, and their seeds, flowers, and leaves are safe for human consumption (4). It has been reported that the extracts from the *M. oleifera* plant could present antibacterial activity against Gram-positive and Gram-negative bacteria (5).

M. oleifera seed has attracted a lot of attention as an excellent source of oil and various other products. This oil is rich in proteins, carbohydrates, phenols, vitamins, kaempferol, potassium, calcium, and amino acids (2). Moringa *oleifera* oil, being a natural product, may also provide stability and biocompatibility to the silver nanoparticles (6). Combining *M. oleifera* oil as a capping agent with silver nanoparticles could stabilize the nanoparticles in a biocompatible media, and they may offer a synergistic effect as an antibacterial material. Therefore, the present study investigated the green synthesis of silver nanoparticles using *M. oleifera* oil as a capping agent and the antibacterial activity against *Escherichia coli* bacteria.

MATERIALS AND METHODS

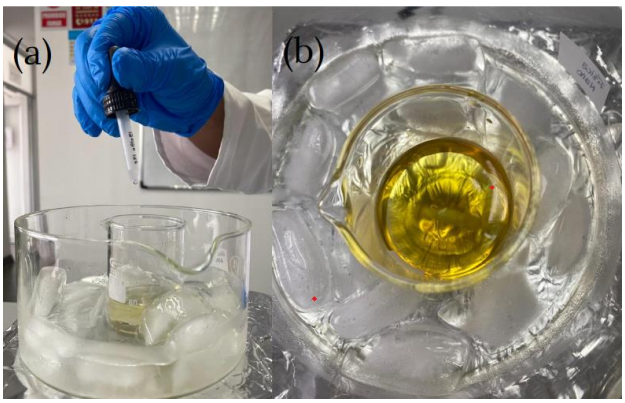
1.1. Chemicals

Silver nitrate and sodium borohydride were purchased from Sigma Aldrich. Moringa oil was obtained from a local store in Ecuador. All chemicals were of analytical grade and were used as received without further purification.

1.2 Synthesis of silver nanoparticles using *M. oleifera* oil

Silver nanoparticles were prepared using the protocol reported by L. Mulfinger (7) with 10 mL of 1.0 mM of silver nitrate added dropwise to 30 mL of 2.0 mM sodium borohydride solution in an ice bath (Figure 1 (a)). The reaction mixture was stirred, and the solution turned light yellow, as shown in Figure 1(b). Then, the colloidal silver solution was stored at room temperature for further characterization. Figure 1(c and d) shows the Tyndall effect of the colloidal solutions by the scattering of light due to the formation of the silver particles (8). The yellow color of the solutions and the Tyndall effect are the first evidence of the silver nanoparticle formation.

Silver nanoparticles capped with *M. Oleifera* oil (Ag + MO) were prepared with 1 mL *M. oleifera* oil as a capping agent (Figure 1 e and f) using the process previously described. The *M. oleifera* oil was added to the $AgNO_3$ solution, and the reduction process occurred. Then, the sodium borohydride was added, and the silver ions were reduced into free metallic atoms, forming the nanoparticles. The silver nanoparticles capped with *M. oleifera* oil turned brilliant yellow and maintained the color for months suggesting that *M. Oleifera* oil could prevent the oxidation and precipitation of the nanoparticles (Figure 1f).



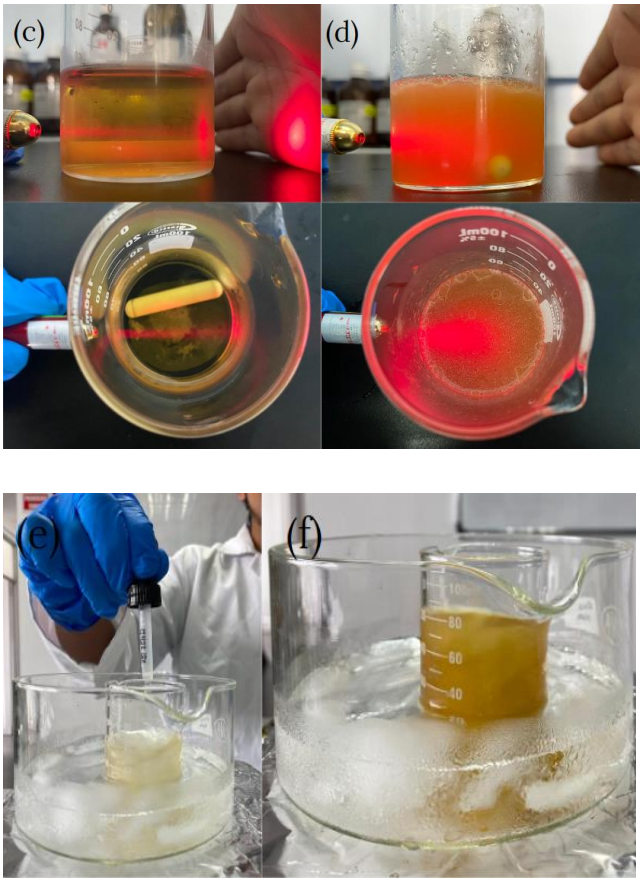


Figure 1. Synthesis of silver nanoparticles. (a) 10 mL of 1.0 mM of silver nitrate added dropwise to 30 mL of 2.0 mM sodium borohydride solution in an ice bath. (b) Light yellow colloidal silver solution. Tyndall effect of (c) AgNPs and (d) AgNPs with *M. oleifera oil.* Synthesis of silver nanoparticles with *M. oleifera* oil. (e) 10 mL of 1.0 mM of silver nitrate added dropwise to 30 mL of 2.0 mM sodium borohydride solution in an ice bath. (f) Silver nanoparticles with *M. oleifera* oil (Ag+MO).

1.3. Agar Diffusion Test

To conduct the Agar Diffusion Test, we prepared LB agar medium by combining 1% tryptone, 1% NaCl, 0.5% yeast extract, and 1.5% agar. A strain of *E. coli* ATCC®25922™ was grown overnight in liquid LB medium. The cell density was measured at 600 nm using a Nanodrop apparatus (Thermo Fisher Scientific). 10^6 cells were spread on an LB agar plate. We used 100 µg of ampicillin from a 100 mg/mL stock at the center of the agar plate to serve as a positive control and introduced droplets of various types, each measuring 5.0 µL, onto the agar plate to assess their effect on bacterial growth. Specifically, the droplets used in the experiments included the staples of silver nanoparticles (Ag NPs), *M. oleifera* (MO), and the silver nanoparticles capped with *M. oleifera* (Ag+MO). Finally, the agar plate was incubated at 37 °C for 24 hours.

1.4. Characterization

The optical properties were measured using a Genova Nano Jenway UV-Vis spectrophotometer. Infrared analysis was performed using an Agilent Technologies spectrometer Cary 360 with a diamond attenuated total reflectance (ATR) accessory. Transmission Electron micrographs were obtained at 80 kV using an FEI-Tecnai G20 Spirit Twin microscope equipped with an Eagle 4k HR camera. Raman measurements were acquired using a HORIBA LabRAM HR Evolution spectrometer.

RESULTS AND DISCUSSION

Ultraviolet-Visible spectroscopy (UV-Vis)

The formation of the silver nanoparticles was confirmed by UV-Vis spectroscopy. Figure 2a show the characteristic absorption peak at 420 nm, indicating the presence of silver nanoparticles due to the surface plasmon resonance phenomenon, which occurs due to the excitation of the surface plasmon on the outer surface of the silver nanoparticles, which gets excited to the applied electromagnetic field (9).

M. oleifera oil showed a main absorption peak in the range between 200 and 300 nm (Figure 2b), due to the presence of conjugated double bonds related to organic molecules, such as carotenoids, chlorophyll, tocopherols, phenolic compounds, and other chromophores (10). The absorption spectrum of the silver nanoparticles capped with *M. oleifera* oil showed a surface plasmon absorption band with a maximum of 420 nm (Figure 2c), related with the silver nanoparticles formation.

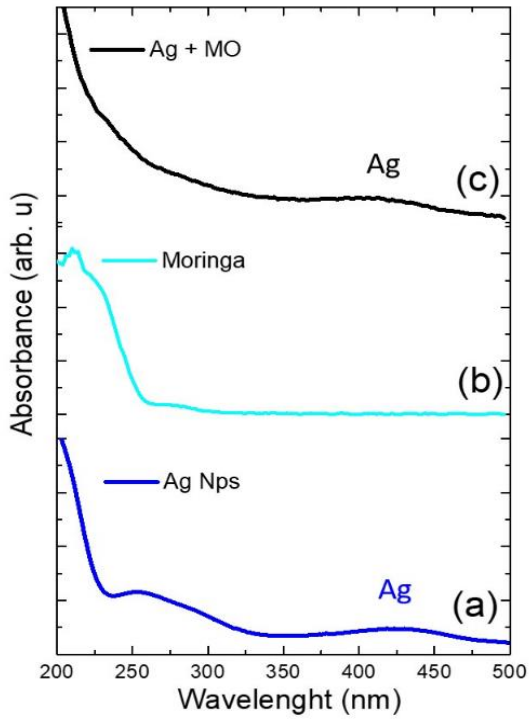


Figure 2. UV-vis absorption spectrum of (a) silver nanoparticles (Ag NPs), (b) *M. oleifera*, and the (c) silver nanoparticles capped with *M. oleifera* (Ag + MO).

Fourier Transform Infrared spectroscopy (FTIR)

FTIR spectra in Figure 3 and Table 1 show the main vibration peaks related to unsaturated fatty acids in the *M. oleifera* oil at 2922 cm^{-1} correspond to the asymmetric and symmetric stretching of the C–H in CH_2 group. The carbonyl C=O stretching vibration of the ester functional group in triglycerides appears as a strong band in the FTIR spectrum at 1744 cm^{-1} (11). These absorption peaks is a characteristic features of vegetable oils and are related to the presence of fatty acid esters in the *M. oleifera* oil used. The peak at 1587 cm-1 is attributed to the stretching of the C–N deformation of the N–H linking proteins. The absorption bands in the fingerprint region at 600-1500 cm^{-1}, due to the bending and stretching vibrations of C-H bonds in the aliphatic chains. Our results revealed that the silver nanoparticles were successfully capped with *M. oleifera* oil (Figure 3b).

Table 1: FTIR main peaks of silver nanoparticles (Ag NPs), *M. oleifera* oil (MO), and the silver nanoparticles capped with *M. oleifera* oil (Ag+MO).

Ag NPs (cm^{-1})	MO (cm^{-1})	Ag+MO (cm^{-1})	Assignment
-	2922	2922	C-H
-	1744	1744	C=O
	1587	1600	N-H

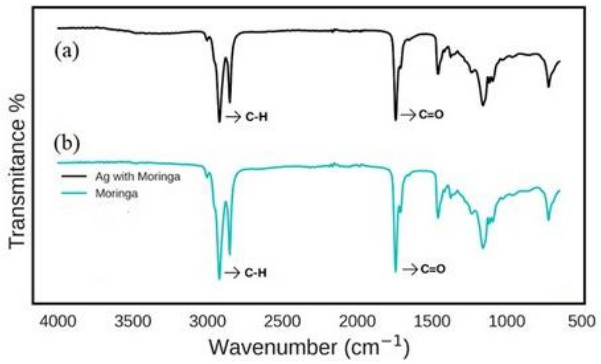


Figure 3. FTIR spectra of (a) silver nanoparticles capped with *M. Oleifera* oil (Ag + MO) and (b) *M. Oleifera* (MO).

Transmission electron microscopy (TEM)

Transmission electron micrographs in Figure 4a shows the formation of silver nanoparticles with an average particle size of 30 nm and a semispherical shape. Meanwhile Figure 4b shows the formation of nanoparticles of nanoparticles of 5 nm around the 30 nm nanoparticles. The formation of 5 nm nanoparticles around larger ones is explained by the seed-mediated growth during the synthesis of silver nanoparticles using *M. oleifera* oil as a reducing and stabilizing agent. This process involves two stages: the initial formation of larger silver nanoparticles and the subsequent growth of smaller nanoparticles around them. *M. oleifera* oil, contains bioactive compounds, such as polyphenols, flavonoids, and reducing agents. These compounds act as reducing agents in the synthesis of silver nanoparticles. Initially, silver ions from silver nitrate are reduced by the active compounds in *M. oleifera* oil. This reduction process results in the formation of the seed's silver nanoparticles. The seed nanoparticles serve as nucleation sites for further silver ions reduction. Larger

silver nanoparticles can grow by depositing additional silver atoms onto these seed nanoparticles. *M. oleifera* oil also plays a crucial role in stabilizing the newly formed nanoparticles. They can attach to the nanoparticle surfaces, preventing agglomeration and ensuring the nanoparticles remain dispersed in the solution. As the reaction progresses, the larger silver nanoparticles continue to grow, while the seed nanoparticles act as templates for depositing more silver atoms. This leads to the formation of tiny nanoparticles around the larger ones, as we observed in Figure 4b.

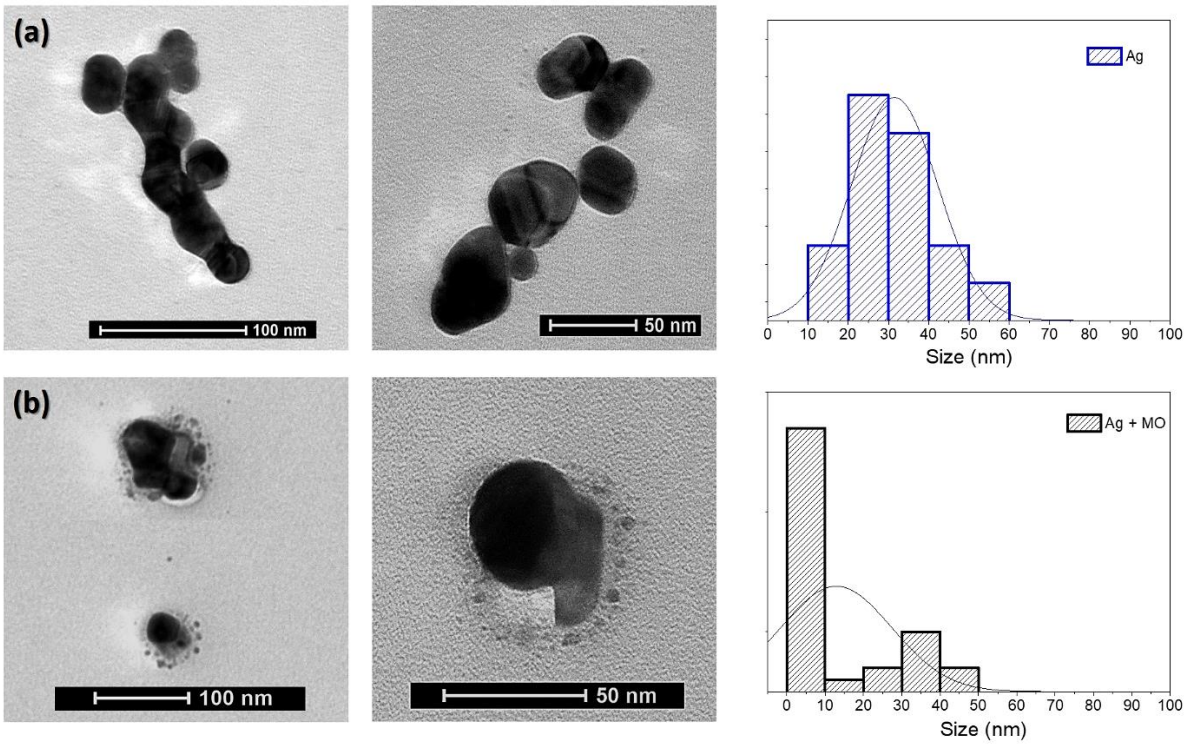


Figure 4. Transmission electron micrographs and average particle size distribution of (a) silver nanoparticles and (b) silver nanoparticles with *M. oleifera* oil.

Raman spectroscopy

Raman spectra of silver nanoparticles are shown in Figure 5. In these spectra, we observe the Raman signal of *M. oleifera* oil at 1003 cm^{-1} associated with the presence of characteristic carbon-carbon double bonds C=C of the unsaturated fatty acids from the oil. This vibrational mode is known as the cis C=C stretching mode, and it is commonly observed in vegetable oils that contain unsaturated fatty acids, such as oleic acid and linoleic acid (12). Raman spectra of silver nanoparticles consists of vibrational modes at 66, 1378, and 1574 cm^{-1} (13). Raman signal of the silver nanoparticles capped with *M. oleifera* oil at 2558 cm^{-1} corresponds to the vibrational mode associated with the carbon-hydrogen (C-H) stretching vibrations in the aliphatic hydrocarbon chains on the silver nanoparticles' surface.

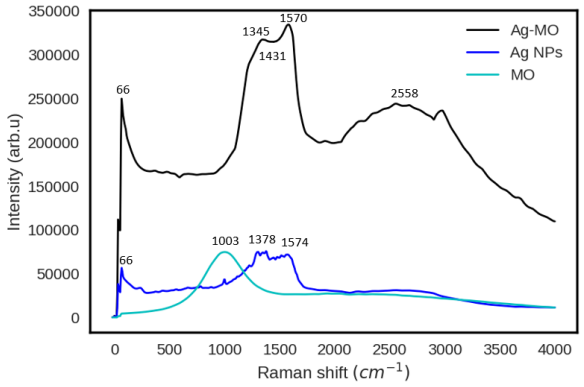


Figure 5. Raman spectra of silver nanoparticles (Ag NPs), *M. oleifera* (MO), and the silver nanoparticles capped with *M. oleifera* (Ag+MO).

Table 2: Raman shift of silver nanoparticles (Ag NPs), *M. oleifera* (MO), and silver nanoparticles capped with *M. oleifera* (Ag + MO).

AgNPs (cm^{-1})	MO (cm^{-1})	Ag + MO (cm^{-1})	Assignment
66	-	66	Ag-O
-	1003	1345	C=C
-	-	1431	C=C
1574	-	1570	N-H
-	-	2558	C-H

When *M. oleifera* compounds capped the surface of silver nanoparticles, they might affect the plasmonic resonance of the silver nanoparticles, leading to shifts in the absorbance and scattering spectra of the nanoparticles. Figure 5 shows a Surface-Enhanced Raman Scattering (SERS) effect when silver nanoparticles are capped with *M. oleifera* oil. The plasmonic enhancement of the silver nanoparticles could explain this behavior. Silver nanoparticles have a high affinity for enhancing the Raman signals of nearby molecules through the plasmonic effect. When illuminated with a laser at 633 nm, the silver nanoparticles generate localized surface plasmon resonances. These plasmon resonances lead to strong electromagnetic fields around the silver nanoparticles (14). When *M. oleifera* oil is adsorbed on or near the silver nanoparticles' surface, their Raman signals experience a significant enhancement due to the intensified electromagnetic field within the vicinity of the silver nanoparticles.

M. oleifera oil contains various functional groups corresponding to lipids, phenolics, and flavonoids (Figure 3). When these molecules adsorb onto the surface of the silver nanoparticles, they can undergo chemical interactions, leading to additional Raman signal enhancement via the chemical enhancement mechanism (15). The interactions between the *M. oleifera* oil and the silver nanoparticles alter the molecular polarizability and electron distribution, thereby further amplifying the Raman signals (16). The combination of the plasmonic enhancement of the silver nanoparticles and the chemical enhancement with the *M. oleifera* oil results in a significant overall increase in Raman signal intensity, allowing for the detection and identification of molecules from *M. oleifera* oil even at very low concentrations.

Antibacterial Activity Assessment

This work investigated the antibacterial activity of Ag NPs cappped with *M. oleifera* against pathogenic bacteria *E. coli,* C. Figure 6 shows the inhibition zone of the positive control ampicillin (100 µg) at the center of the agar plate and introduced droplets of various Ag NPs types (5.0 µL) onto the agar plate to assess their effect on bacterial growth. The droplets used in the experiments include the staples of silver nanoparticles (Ag NPs), *M. oleifera* (MO), and the silver nanoparticles capped with *M. oleifera* (Ag+MO). After the agar plate incubation at 37 °C for 24 h, we observed the inhibition zone of the silver nanoparticles capped with *M. oleifera* oil, maybe due to a synergistic effect.

The antibacterial activity of the silver nanoparticles with *M. oleifera* oil could be explained by the intrinsic physicochemical properties of the silver nanoparticles and the binding to the organic compounds or proteins of the *M. oleifera* oil, resulting in the disruption of the bacterial cell wall and respiratory chain disorder, leading to the generation of reactive oxygen species (17). The cell membrane disruption could also result in the dissemination of the nanoparticles into the cells, generating high concentrations of reactive oxygen species by the silver nanoparticles' surface reactions leading to oxidative stress and cell death. In addition, the penetration of the 5 nm nanoparticles into the cells could inhibit DNA replication which ultimately distresses the protein synthesis and further cellular metabolism, resulting in bacterial cell death (18). These nanoparticles' detailed mode of action on pathogens must still be clearly elucidated. The nonspecific mode of action of Ag NPs with the *Moringa* oil against *E. coli* bacteria provides a sustainable route for developing antimicrobial agents. From this study, it can be suggested that the silver nanoparticles capped with *M. oleifera* oil might have worked in synergy and resulted in enhanced antibacterial activity against *E. coli*. However, the silver nanoparticles were not effective against the bacteria, probably due to the low concentration of nanoparticles obtained during the synthesis and the large average particles size, as shown in Figures 2 and 4.

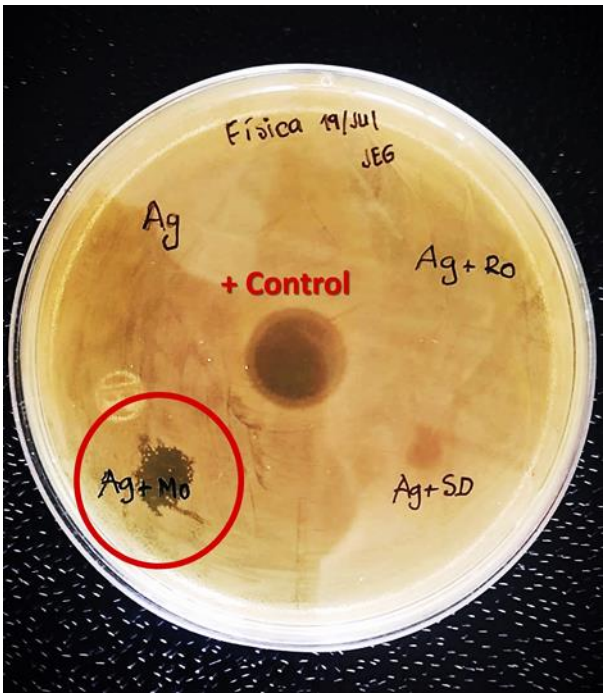


Figure 6. Antibacterial agar diffusion test of silver nanoparticles (Ag NPs) and silver nanoparticles capped with *M. oleifera* oil (Ag + Mo). The samples: silver nanoparticles capped with rosemary (Ag + Ro) and silver nanoparticles capped with Dragon's blood (Ag + SD) are not considered in this work.

CONCLUSIONS

The results suggested that the silver nanoparticles prepared by a green approach using *M. Oleifera* oil could be an alternative antibacterial agent against *E. coli.* The use of *M. Oleifera* oil in synthesizing silver nanoparticles involves forming seed nanoparticles followed by their controlled growth through the reduction of silver ions. The bioactive compounds in *M. Oleifera* oil play a dual role by reducing the silver ions and stabilizing the resulting nanoparticles, forming both larger and smaller nanoparticles, which can have unique properties for various applications. Combining the SERS effect measured with Raman with *M. Oleifera* oil-capped silver nanoparticles might have potential applications in the biomedical field. For instance, it could be used for targeted drug delivery, where the *M. Oleifera* oil components act as carriers for therapeutic molecules. At the same time, the SERS effect allows for real-time tracking and monitoring of drug release at specific sites.

Authors' Contributions

The authors contributed equally to writing the manuscript.

Conflict of interest

The authors declare that they have no conflict of interest.

REFERENCES

[1] Peng Tan, Huiyang Fu, Xi Ma, (2021), Design, optimization, and nanotechnology of antimicrobial peptides: From exploration to applications, Nano Today, 39,101229, https://doi.org/10.1016/j.nantod.2021.101229

[2] Hafiza Mahreen, Mehwish Muhammad Shahid Riaz Rajoka Yongai, Xiong Huiming Cai Rana Muhammad Aadil Qaisar Mahmood Zhendan He Qinchang Zhu, (2021). Green synthesis of a silver nanoparticle using Moringa oleifera seed and its applications for antimicrobial and sun-light mediated photocatalytic water detoxification. Journal of Environmental Chemical Engineering, 9, Issue 4, 105290. https://doi.org/10.1016/j.jece.2021.105290

[3] Gufran Mahmood Mohammed, Sumaiya Naeema Hawar, (2022), Green Biosynthesis of Silver Nanoparticles from Moringa oleifera Leaves and Its Antimicrobial and Cytotoxicity Activities, International Journal of Biomaterials, 4136641, 10, https://doi.org/10.1155/2022/4136641

[4] G. Tovar, S. Briceño, J. Suarez, S. Flores, G. González. (2020), Biogenic synthesis of iron oxide nanoparticles using Moringa oleifera and chitosan and its evaluation on corn germination. Environmental Nanotechnology, Monitoring & Management. 14, 100350.https://doi.org/10.1016/j.enmm.2020.100350

[5] Jerushka S Moodley, Suresh Babu Naidu Krishna, Karen Pillay, Sershen and Patrick Govender, (2018), Green synthesis of silver nanoparticles from *Moringa Oleifera* leaf extracts and its antimicrobial potential, *Adv. Nat. Sci: Nanosci. Nanotechnol.* 9 015011. DOI 10.1088/2043-6254/aaabb2

[6] Javed, R., Zia, M., Naz, S. et al, (2020), Role of capping agents in the application of nanoparticles in biomedicine and environmental remediation: recent trends

and future prospects. J Nanobiotechnol 18, 172 https://doi.org/10.1186/s12951-020-00704-4

[7] Lorraine Mulfinger, Sally D. Solomon, Mozghan Bahadory, Aravindan V. Jeyarajasingam, Susan A. Rutkowsky, and Charles Boritz, (2007), Synthesis and Study of Silver Nanoparticles. Journal of Chemical Education 84 (2), 322. DOI: 10.1021/ed084p322

[8] Adrian Gołębiowski, Bogusław Buszewski, (2023), Characterization of colloidal particles of a biological and metallic nature, Microchemical Journal, 191, 108864, https://doi.org/10.1016/j.microc.2023.108864

[9] Benalcázar J, Lasso E, Ibarra-Barreno C, Arcos Pareja J, Santiago Vispo N, Chacón-Torres J, Briceño S, (2022), Photochemical Optimization of a Silver Nanoprism / Graphene Oxide Nanocomposite's Antibacterial Properties. ACS Omega. 7, 50, 46745 46755. https://doi.org/10.1021/acsomega.2c05793

[10] Hassan Barakat, Galal A. Ghazal, (2016), Physicochemical Properties of Moringa oleifera Seeds and Their Edible Oil Cultivated at Different Regions in Egypt, Food and Nutrition Sciences, 7 No.6, DOI: 10.4236/fns.2016.76049

[11] Xiaona Fu, Jiling Su, Li Hou, Ping Zhu, Ying Hou, Kai Zhang, Huowang Li, Xiangyi Liu, Chengxinzhuo Jia & Juan Xu, (2021), Physicochemical and thermal characteristics of Moringa oleifera seed oil. Adv Compos Hybrid Mater 4, 685–695 https://doi.org/10.1007/s42114-021-00302-4

[12] Moumita Gourai, Ashok K. Nayak, Partha S. Nial, Bijaylaxmi Satpathy, Rajashree Bhuyan, Saroj K. Singha and Umakanta Subudhi, (2023), Thermal plasma processing of Moringa oleifera biochars: adsorbents for fluoride removal from water, RSC Adv.,13, 4340-4350. https://doi.org/10.1039/D2RA07514H

[13] Meheretu, Getnet Melese, Dana Cialla and Juergen Popp, (2014), Surface Enhanced Raman Spectroscopy on Silver Nanoparticles. International Journal of Biochemistry and Biophysics, 2(4), 63-67, DOI:10.13189/ijbb.2014.020403

[14] S. Mohapatra; Y. K. Mishra; D. K. Avasthi; D. Kabiraj; J. Ghatak; S. Varma, (2008), Synthesis of Gold-silicon Core-shell Nanoparticles with Tunable Localized Surface Plasmon Resonance. Applied Physics Letters, 92, 103105, https://doi.org/10.1063/1.2894187

[15] Nanang Adrianto, Andi Marwanti Panre, Nurul Imani Istiqomah, Muhammad Riswan, Fajar Apriliani, Edi Suharyadi, (2022), Localized surface plasmon resonance properties of green synthesized silver nanoparticles, Nano-Structures & Nano-Objects, 31, 100895, https://doi.org/10.1016/j.nanoso.2022.100895

[16] Langer, J., Jimenez de Aberasturi, D., Aizpurua, J., Alvarez-Puebla, R. A., Auguié, B., Baumberg, J. J., Bazan, G. C., Bell, S. E. J., Boisen, A., Brolo, A. G., Choo, J., Cialla-May, D., Deckert, V., Fabris, L., Faulds, K., García de Abajo, F. J., Goodacre, R., Graham, D., Haes, A. J., Haynes, C. L., … Liz-Marzán, L. M, (2020), Present and Future of Surface-Enhanced Raman Scattering. ACS nano, 14(1), 28–117. https://doi.org/10.1021/acsnano.9b04224

[17] Joshi, Neha; Jain, Neha; Pathak, Abhishek; Singh, Jai; Prasad, Ram; Upadhyaya, Chandrama Prakash, (2018), Biosynthesis of silver nanoparticles using Carissa carandas berries and its potential antibacterial activities. Journal of Sol-Gel Science and Technology, 86 (3), 682–689. doi:10.1007/s10971-018-4666-2

[18] Syed Anees Ahmad, Sabya Sachi Das, Ayesha Khatoon, Mohammed Tahir Ansari, Mohd. Afzal, Md Saquib Hasnain, Amit Kumar Nayak, (2020), Bactericidal activity of silver nanoparticles: A mechanistic review, Materials Science for Energy Technologies, 3, 756-769, https://doi.org/10.1016/j.mset.2020.09.002