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A Comparative Analysis of the Antibacterial Effects of Green-Synthesized Silver Nanoparticles with Chemically Synthesized Silver Nanoparticles

Sama Bahraminezhad [a], **Mohammad Reza Mehrasbi** [a], **Pegah Homauoni** [b], **Zohre Farahmandkia** [a*]

a. Department of Environmental Health, School of Public Health, Zanjan University of Medical Sciences, Zanjan, Iran.
b. Department of Food Safety and Hygiene, School of Public Health, Zanjan University of Medical Sciences, Zanjan, Iran.

***Corresponding author:** Department of Environmental Health, School of Public Health, Zanjan University of Medical Sciences, Zanjan, Iran. Postal Code: 4515786349. E-mail: zfarahmand@zums.ac.ir

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ABSTRACT

Background: In recent years, the antimicrobial properties of nanoparticles have received significant attention due to increasing bacterial resistance to conventional antibiotics. In this study the antibacterial effects of green-synthesized silver nanoparticles with chemically synthesized silver nanoparticles have been comprised.
Methods: The physicochemical properties of the synthesized nanoparticles were evaluated through Ultraviolet-Visible (UV-Vis) spectrophotometry, Dynamic Light Scattering (DLS), Fourier Transform Infrared Spectroscopy (FT-IR), Field Emission Scanning Electron Microscopy (FESEM), and Energy Dispersive X-ray Analysis (EDAX) and antimicrobial activity by Minimum Inhibitory Concentration (MIC) and Minimum Bactericidal Concentration (MBC) values.
Results: DLS analysis revealed average sizes of 72.8 nm and 124.8 nm for green-synthesized and chemically synthesized AgNPs, respectively. FESEM images showed spherical shapes, while EDAX confirmed the presence of silver, carbon, and oxygen in the nanoparticles. Antimicrobial testing (MIC and MBC) showed that green-synthesized AgNPs exhibited stronger antibacterial activity than chemically synthesized ones. For *Escherichia coli*, the MIC and MBC for green-synthesized AgNPs were 0.028 mg/mL and 0.056 mg/mL, respectively, compared to 0.125 mg/mL and 0.25 mg/mL for chemically synthesized AgNPs. Similarly, for *Staphylococcus aureus*, the MIC and MBC values for green-synthesized nanoparticles were 0.226 mg/mL, while chemically synthesized nanoparticles had MIC and MBC values of 0.125 mg/mL and 0.25 mg/mL.
Conclusion: These results demonstrated a higher antimicrobial potential of plant-mediated silver nanoparticle synthesis over chemical methods, especially against Gram-negative bacteria.

1. Introduction

Nanotechnology has created new opportunities in various fields by manipulating the size of known antimicrobial compounds, thus altering their effects and expanding their applications (Lara et al., 2010). In recent years, the advancement of nanotechnology has led to the recognition of nanomaterials for their wide array of applications in both industry and biomedicine. This is attributed to their high specific surface area, extremely reactive surface sites, and the unique quantum effects they exhibit (Assadi et al., 2016). There has been growing interest in the synthesis and properties of noble metal nanoparticles such as gold, silver, and platinum in nanomedicine (Joseph et al., 2021). One of the notable properties of nanoparticles, particularly in medical and biological sciences, is their antibacterial activity. Given that many bacteria have developed resistance to traditional antibiotics, extensive research has been conducted on new methods and materials with antibacterial properties. Silver ions and silver-based compounds are regarded as more effective than other antimicrobial agents and antibiotics due to their distinct advantages. These

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properties make silver and its nanoparticle derivatives highly effective against infectious microbial agents. They have been recognized as antimicrobial agents with medical significance since 1000 BC and have been used as efficient additives in the Ayurvedic medicine of China and India (Golabi et al., 2019; Gupta & Silver, 1998). Silver nanoparticles are metallic nanoparticles ranging from 1 to 100 nanometers in size and containing 20 to 15,000 silver atoms (Chen & Schluesener, 2008). Due to their broad-spectrum antimicrobial activity, silver nanoparticles have become one of the most widely used sterilizing nanomaterials in consumer and medical products (Ahmadi, 2020), with applications in biomedical fields, water and air purification, food production, textiles, and various household products. To date, there are no definitive reports of bacterial resistance to silver nanoparticles (Zhang et al., 2023). Particles of different sizes can be obtained during nanoparticle synthesis using various techniques (physical, chemical, and biological processes). Physical and chemical methods have drawbacks, including high energy consumption, the release of toxic and harmful chemicals, and the use of complex equipment and specific synthesis conditions, which is why they are being replaced by green synthesis methods (Husain et al., 2023; Ying et al., 2022). Green chemistry methods mediated by plants have emerged as an active area of research in nanobiotechnology, as plant extract reactions are very fast and do not require specific conditions for nanoparticle synthesis (Krishnaraj et al., 2012). Moreover, plant extracts may be more advantageous for nanoparticle synthesis than microorganisms due to their simplicity in quality enhancement and reduced biological risks (Al Mashud et al., 2022). Secondary metabolites, enzymes, proteins, essential oils, carbohydrates, lipids, phenols, tannins, acids, vitamins, pigments, sulfur compounds, resins, and terpenoids play a crucial role in the synthesis of metal nanoparticles by plants (Dousti et al., 2019). *Agrimonia eupatoria L.*, known as agrimony, belongs to the *Rosaceae* family and is distributed across Europe, Asia, Africa, North America, and in various regions of Iran (Mazandaran, Gilan, Hamadan, Kermanshah, Markazi, Khorasan, and around Tehran) (Saffari et al., 2021). This plant is recognized as a raw material for extracting medicinal compounds or producing pharmaceuticals. It not only has antioxidant and antibacterial properties but also has anti-inflammatory, neuroprotective, antidiabetic, hepatoprotective, and anticancer effects (Kesić et al., 2023). In traditional medicine, nearly all parts of the agrimony plant have documented medicinal uses, including treatment of liver, kidney, and lung diseases, eye infections, as well as in diabetes treatment and cardiovascular disease prevention. Its anticancer, antimicrobial, and antiviral effects have been proven. Studies on the secondary metabolites of this plant include polyphenols, flavonoids such as procyanidins, quercetin, catechins, and kaempferol, tannins, and triterpenoids, which contribute to its reducing capabilities (Khazaei & Mirazi, 2018). Since *Agrimonia eupatoria L.* is a rich source of beneficial compounds with multiple therapeutic effects, this study aims to investigate the antibacterial effects of green-synthesized silver nanoparticles from this plant and compare them with chemically synthesized silver nanoparticles.

2. Materials and Methods

2.1 Materials

The *Agrimonia eupatoria L.* (AEL) was purchased from the Agricultural Research Center, Hamadan, Iran. After collection, various parts of the plant, such as the aerial parts (branches, leaves, and fruits), were washed with deionized water, and then dried under appropriate conditions in the shade and at room temperature. The plant was then ground into powder using an electric grinder for extract preparation. Additionally, the colloidal nano-silver solution was obtained from Pishgaman Nanomaterials Iranian Company, Mashhad, Iran.

2.2 The preparation of Agrimonia eupatoria L. leaf extract

5 gr of the plant material was combined with 500 mL of distilled water and heated in a water bath at 55°C for 30 min. Following this, the mixture was allowed to cool to room temperature and subsequently filtered. Finally, the resulting solution was stored at 4°C in a refrigerator for future use.

2.3 The synthesis of silver oxide nanoparticles

20 mL of the plant aqueous extract was added to 40 mL of a 0.6497 mg/mL silver nitrate solution and kept in the dark at room temperature (25 ± 1°C) for 24 h. When the color of the solution changed from pale yellow to dark brown, the silver nanoparticles synthesized by the plant extract were formed, and subsequent tests were conducted to confirm their presence and characterize their properties.

2.4 Silver nanoparticles characterization

2.4.1 Green synthesized silver nanoparticles

The formation of silver nanoparticles (AgNPs) was monitored using UV-Vis spectroscopy in the wavelength range of 300-800 nm. The functional groups present on the AgNPs were identified and confirmed through Fourier-transform infrared (FTIR) spectroscopy in the range of 400-4000 cm^{-1}. The surface morphology and the structural properties of AgNPs were characterized by FESEM, elemental compositions were analyzed by EDX, particle size and distribution of nanoparticles were determined using DLS. An aliquot of nanoparticles was diluted with pure water and then sonicated for 10 min before the measurements.

2.4.2 Chemically synthesized silver nanoparticles

The size distribution of the nanoparticles made by the chemical process using the DLS method was measured simultaneously with the green synthesis nanoparticles, and the results provided by the selling company are also shown in next figures. Also, the images of these particles provided by the selling company are shown in next figurs.

2.5 Antibacterial effects of silver nanoparticles

Standard microbial strains, including *Escherichia coli* (ATCC 11303) and *Staphylococcus aureus* (ATCC 6538), were obtained from the Iranian Research Institute of Industrial Research (IROST). The minimum inhibitory concentration (MIC) and minimum bactericidal concentration (MBC) were determined by a microdilution method, Muller-Hinton broth, and an inoculum of 1.5×10^4 CFU/mL. The MIC value corresponded to the concentration that inhibited 99% of bacterial growth and the MBC value corresponded to the concentration where 100% of the bacterial growth was inhibited. Initially, 95 µL of Mueller-Hinton broth was added to each well. Then, 100 µL of silver nanoparticles, synthesized via the green method, was added to the first well of the first four rows. The contents of the first well were mixed thoroughly, and 100 µL was transferred to the second well. This twofold serial dilution process was repeated until the ninth well. Finally, the remaining 100 µL of the nanoparticle solution in the pipette was discarded. From row 3 onward, the same dilution process was performed, but using silver nanoparticles synthesized chemically. In rows 1 and 3, 5 µL of *Escherichia coli* suspension (1.5×10^4 CFU/mL), and in rows 2 and 4, 5 µL of *Staphylococcus aureus* suspension (1.5×10^4 CFU/mL) were added. For the positive control samples, 95 µL of Mueller-Hinton broth was added to each well, and 5 µL of *E. coli* suspension was added to the sixth row, while 5 µL of *S. aureus* suspension was added to the seventh row. For the negative control samples, 95 µL of broth and 5 µL of silver nanoparticles (green synthesis) were added to each well in the last row. At the end, the microplates were transferred to an incubator at 37 °C for 24 hours. After incubation, the results were read using a microplate reader at a wavelength range of 587-630 nm, and the obtained results were recorded. The MBC test was performed on plates containing Mueller-Hinton agar. From wells 1, 2, 3, 4, and 5 of each row, 10 µL samples were taken and inoculated onto the agar plates as nanoparticle droplets. The plates were then incubated at 37°C for 24 h.

3. Results and Discussion

3.1 Synthesis and Characterization

The synthesis of silver nanoparticles was carried out by adding 2 mL of plant extract to 4 mL of 0.6497 mg/mL silver nitrate solution and allowing the reaction to proceed for 24 h. The solution color changed from yellow to dark brown, indicating successful nanoparticle formation. The observations related to the formation of nanoparticles from plant extract and the results obtained are similar to the study conducted by Kavoosi and Yaghoubi (2017). UV-Vis spectrophotometry analysis, conducted in the 300-800 nm range, revealed the maximum absorption peak at approximately 450 nm, confirming the presence of silver nanoparticles (Figure 1). The optimal concentration for nanoparticle synthesis was found to be 0.6497 mg/mL, as it produced the highest absorption and stability, consistent with previous studies. Higher metal ion concentrations may result in incomplete nanoparticle synthesis or aggregation into larger particles. The reduction and stabilization of silver nanoparticles are attributed to secondary metabolites in the plant extract, such as polyphenols, flavonoids, tannins, and triterpenoids (Khazaei & Mirazi, 2018).

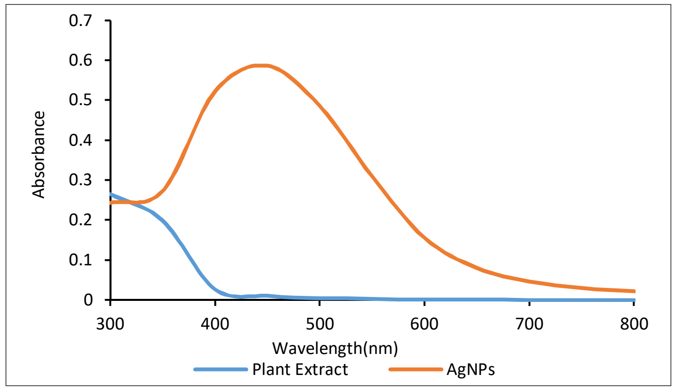

Figure 1. UV-vis absorption spectra of Ag nanoparticles and plant extract

The characterization of the synthesized silver nanoparticles was conducted using various techniques, including DLS, FT-IR, FESEM, and EDAX. DLS analysis provided the size distribution of the nanoparticles, showing that larger particles exhibited higher peak intensity. Dynamic Light Scattering was employed to determine the hydrodynamic diameter of both plant extract-synthesized and chemically synthesized silver nanoparticles (Figure 2). The results indicated that the hydrodynamic diameter of the green-synthesized nanoparticles was approximately 72.8 nm, while the chemically synthesized nanoparticles had a hydrodynamic diameter of about 124.8 nm. The results of the DLS analysis presented by the seller company showed the average size of the chemically synthesized nanoparticles as 117 nm. FT-IR analysis revealed absorption peaks that indicated the presence of reducing agents on the nanoparticles. As shown in Figure 3, the FT-IR spectra of the plant extract and the green-synthesized silver nanoparticles display absorption peaks corresponding to functional groups in the plant extract. These peaks suggest the involvement of reducing agents in the synthesis of the nanoparticles. FESEM analysis of the chemical and green-synthesized silver nanoparticles, shown in Figure 4, revealed spherical-shaped particles with an average size of 184.49 nm. The uniformity and shape of the nanoparticles suggest successful synthesis and stability. The FESEM images confirmed that the average size of the nanoparticles was 184.49 nm and nearly spherical, which aligns with the findings of a study by Bijari (2023). EDAX analysis of the dry plant (Figure 5) revealed trace amounts of silver, along with significant peaks for carbon (C), oxygen (O), and nitrogen (N), indicating the presence of phytochemicals. In contrast, the EDAX spectrum of the green-synthesized silver nanoparticles showed higher concentrations of carbon and oxygen, followed by silver (Ag) and nitrogen. This suggests that plant-derived biomolecules play a key role in stabilizing and reducing silver ions during nanoparticle synthesis. The EDAX analysis exhibited strong signals for carbon and oxygen, with weaker signals for silver and nitrogen, confirming the presence of silver nanoparticles. These results are consistent with those reported by Al Mashud et al. (2022), Zhang et al. (2023), Ahmadi (2020), and Lakkim et al. (2020).

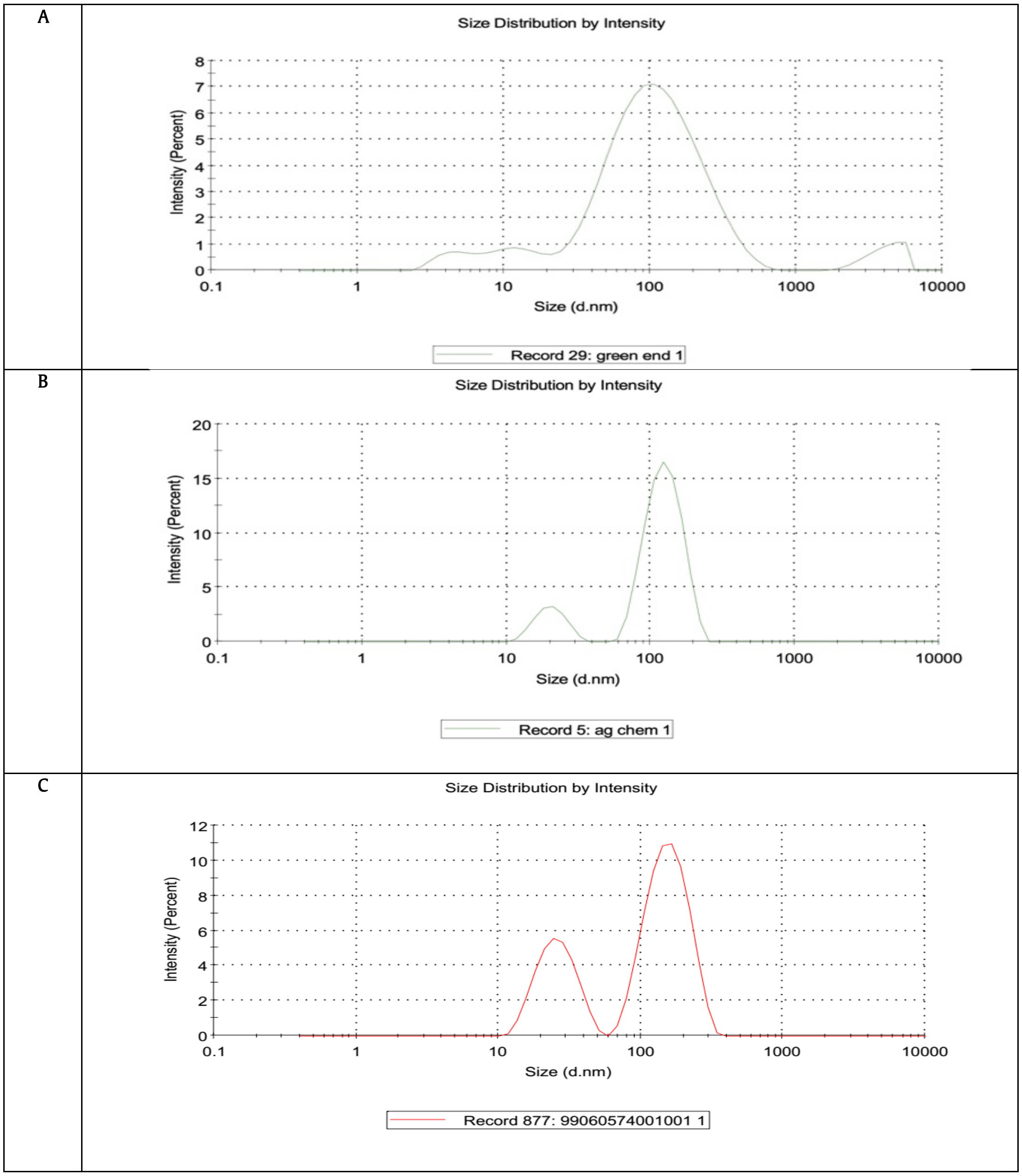

Figure 2. (A): DLS of green synthesized silver nanoparticles, (B): DLS of chemically synthesized silver nanoparticles, (C): DLS of chemically synthesized silver nanoparticles presented by the seller company

3.2 Antibacterial Ability of Nano Silver

The antibacterial activity of green-synthesized and chemical silver nanoparticles was evaluated using the microdilution method in 96-well microplates against *Escherichia coli* (ATCC 35218) and *Staphylococcus aureus* (ATCC 25923). The MIC and MBC were determined for both types of nanoparticles, as shown in Table 1. For *Escherichia coli*, the MIC of the green-synthesized silver nanoparticles was 0.028 mg/mL, while the MBC was 0.056 mg/mL. In contrast, the chemical silver nanoparticles exhibited a higher MIC of 0.125 mg/mL and an MBC of 0.25 mg/mL. For *Staphylococcus aureus*, both the MIC and MBC of the green-synthesized silver nanoparticles were 0.226 mg/mL. The chemical silver nanoparticles showed similar MIC and MBC values of 0.125 mg/mL and 0.25 mg/mL, respectively, which were identical to those observed for *Escherichia coli*.

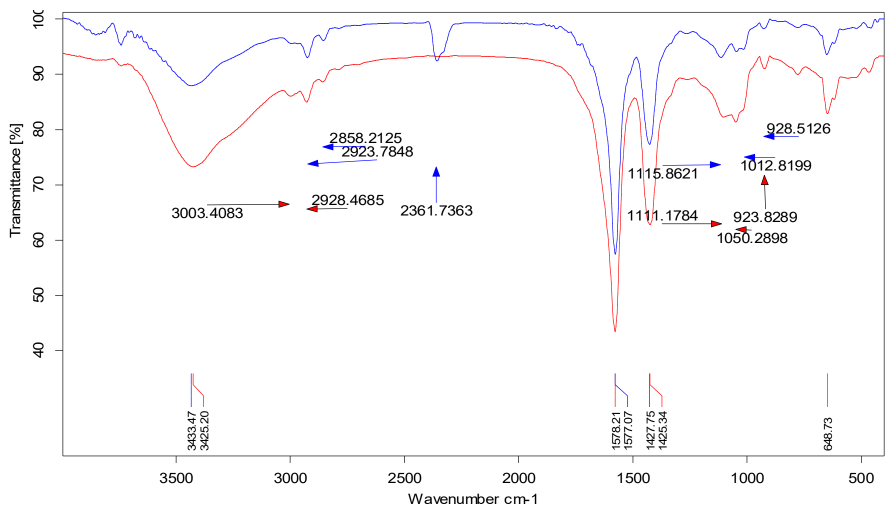

Figure 3. FTIR spectra of plant extract and AgNPs

These results indicate that the green-synthesized silver nanoparticles demonstrate significantly better antibacterial activity against *Escherichia coli*, whereas the chemical silver nanoparticles exhibited similar antibacterial efficacy against both *Escherichia coli* and *Staphylococcus aureus*. These findings support the hypothesis that green-synthesized silver nanoparticles possess stronger antimicrobial properties, particularly against Gram-negative bacteria. The smaller size of the green-synthesized nanoparticles likely contributes to their higher antimicrobial activity, as smaller nanoparticles can more easily penetrate bacterial cells, especially in Gram-negative bacteria, which have thinner peptidoglycan layers (Dousti et al., 2019; Behravan et al., 2019; Sohrabi et al., 2023; Srikhao et al., 2021; Tolouietabar & Hatamnia, 2017).

Table 1. Results of the MIC and MBC of bacteria induced by green-synthesized and chemically synthesized silver nanoparticles

Bacterial names	MIC (mg\mL Ag)		MBC (mg\mL Ag)	
	Green-Synthesized	Chemical-Synthesized	Green-Synthesized	Chemical-Synthesized
E.coli	0.028	0.125	0.056	0.25
S.aureus	0.226	0.125	0.226	0.25

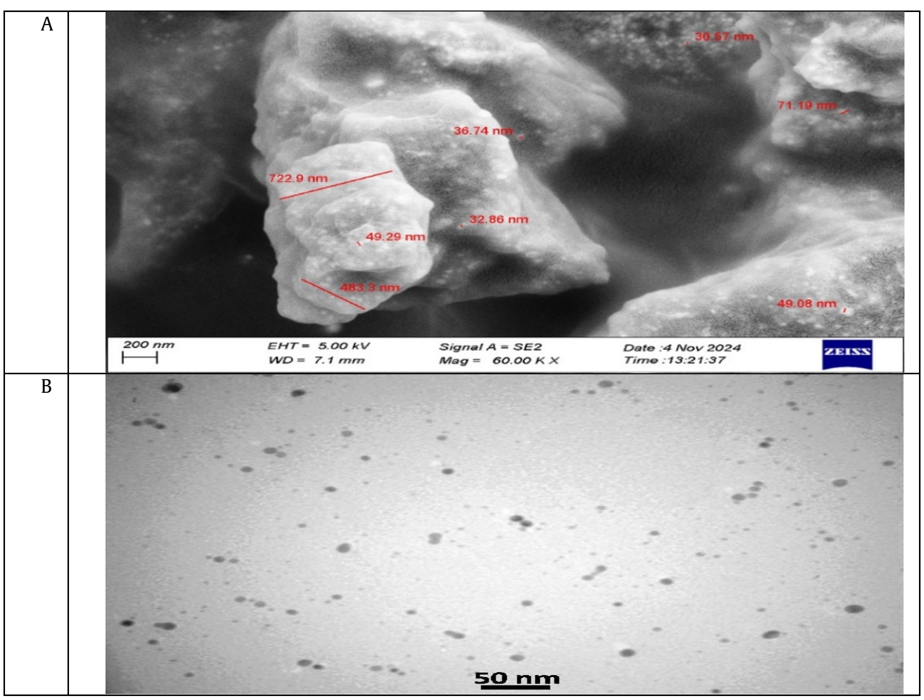

Figure 4. FESEM image of green synthesized AgNPs (A) and TEM image of chemical synthesizes AgNPs presented by seller company(B)

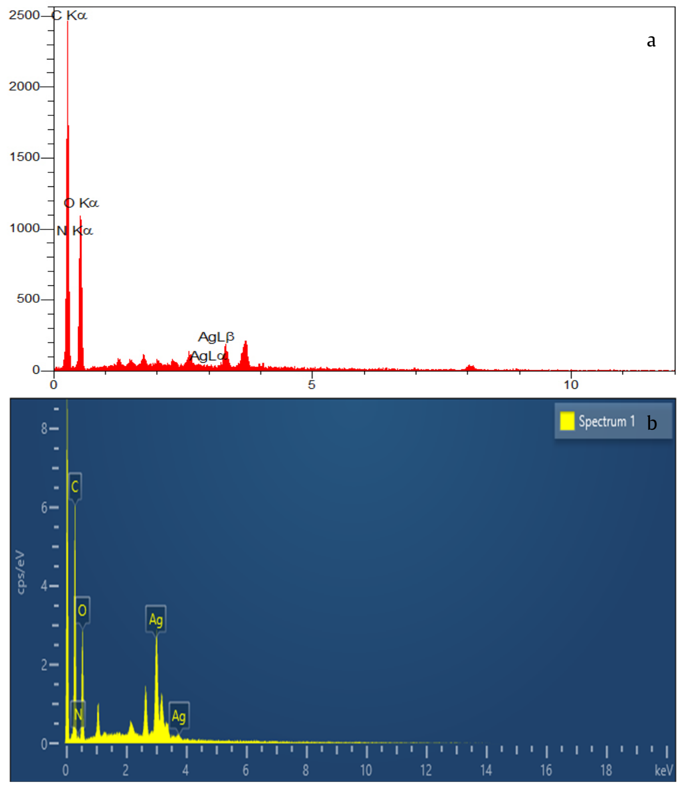

Figure 5. (a) EDAX of dry plant. (b) EDAX of AgNPs

4. Conclusion

Antibacterial activity of the green-synthesized silver nanoparticles and chemically synthesized silver nanoparticles was confirmed against *Escherichia coli* and *Staphylococcus aureus*. The green-synthesized silver nanoparticles demonstrated stronger antimicrobial activity, particularly against *Escherichia coli*, with MIC and MBC values of 0.028 and 0.056 mg/mL, respectively, compared to the chemical silver nanoparticles, which showed MIC and MBC values of 0.125 and 0.25 mg/mL. Similarly, against *Staphylococcus aureus*, the green-synthesized nanoparticles exhibited better efficacy, with MIC and MBC values of 0.226 mg/mL, while the chemical nanoparticles showed MIC and MBC values of 0.125 and 0.25 mg/mL.

Authors' Contributions

Sama Bahraminezhad, Pegah Homauoni: Designing the project; laboratory data collection. **Mohammad Reza Mehrasbi:** Contributing to the methodology section. **Zohre Farahmandkia:** Contributing to the data analysis.

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Conflicts of Interest

The authors declare no conflict of interest.

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Ethical considerations

This study was approved by the Ethics Committee of the University of Zanjan University of Medical Sciences (Code: IR.ZUMS.BLC.1402.049).

Using artificial intelligence

There was no use of artificial intelligence in this research.

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