STUDYING OF THE SILVER NANOPARTICLES BIOACTIVITY AGAINST MANY HARMFUL BACTERIA

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Abstract
Metal nanoparticles with a diameter of less than 100 nm have had a significant influence on a variety of biological applications throughout the past several decades. In this article, the eco-friend method of preparing silver nanoparticles (Ag-NPs) utilizing Lactuca sativa leaves extract as a stabilizer and AgSO4 salt as a precursor is described. The Ag-NPs are then used as antibacterial agents. The product's characteristics were assessed using UV spectroscopy, FE-SEM, TEM, and XRD. Ag-NPs' size was determined by UV-vis analysis to be 13.7 nm. An analysis using X-ray diffraction (XRD) revealed that the silver nanoparticles had a size of 34 nm. Different sizes, shapes of silver nanoparticles were revealed by FE-SEM images. Additionally, utilizing the agar-well diffusion method, the antibacterial properties of Ag NPs nanoparticles at 100 and 200 µg/mL concentrations were evaluated against a variety of harmful bacteria, including Bacillus licheniformis, Acinetobacter baumani, Escherichia coli, and Staphylococcus aureus.

Keyword: Ag nanoparticles; Lactuca sativa leaves extract; antibacterial.

Introduction

Nanomaterials and nanotechnology have grown in importance in modern research. The term "nanoparticles" describes particles in at least one of the three possible dimensions that are in the size range of 1 nm to 100 nm. Individual atoms, molecules, and the corresponding bulk materials have properties that are very different from the physical, chemical, and biological properties of nanoparticles in this size range. Nanoparticles may be made from a wide variety of chemical materials, but the most widely utilized ones include metals, metal oxides, silicates, non-oxide ceramics, polymers, organics, carbon, and biomolecules.[1-3] Nanoparticles come in a variety of shapes, such as tubes, spheres, cylinders, and platelets.[4] Water disinfectants, electronics, household appliances, cosmetics, odor-resistant clothes, food supplements, and packaging materials. One of the materials utilized in nano formulation is nano silver. Due to its antibacterial properties, silver has also been utilized in drinking water and swimming pool filters.[5] Metallic silver has been reduced to ultrafine particles using a number of processes, including electrochemical reduction, spark discharge, solution irradiation, and chemical synthesis, to synthesis nano silver. Twenty to fifteen thousand silver atoms make up the bulk of silver nanoparticles, which are smaller than 100 nm.[6] Adding silver nanoparticles to objects is a typical use since silver is a soft white exotic metal. However, the development of nano silver compounds is primarily driven by their highly effective antibacterial characteristics.[7] Silver nanoparticles are among the most promising products in the nanotechnology industry.[8] Modern nanotechnology research is primarily concerned with creating dependable processes for the synthesis of silver nanoparticles.[9] In this regard, efficient in vitro techniques have been established recently for the bio-reduction of metal ions from their nanoparticles using plant extracts. Extracts from a range of plant parts, such as leaves, flowers, seeds, bark, and roots, have been utilized to create Ag-NPs.[10,11] Plant extracts may serve as capping and reducing agents in the synthesis of Ag-NPs. Aslam et al. reported that Ag-NPs were made with a plant aqueous extract of S. procumbens.[12] A range of techniques, such as UV-Visible, FT-IR, SEM, XRD, and EDX, were successfully used to characterize the produced Ag-NPs.[13] Since a wide range of tiny organisms have been shown to be poisonous to silver (Ag), silver-based combinations have been extensively used for their antibacterial properties. [14] As bactericidal agents, a few salt forms of Ag and their subclasses are commercially useful. Silver particles have a very noteworthy bactericidal effect on microorganisms; in any event, the bactericidal mechanism is still not fully understood. Research findings indicate that Ag+ ions form a strong bond with the thiol (-SH) linkages of essential molecules, thereby disabling them. [15] Experiments have demonstrated that when Ag-based particles are administered to microorganisms, the DNA's ability to replicate itself is lost. Various assessments have confirmed fundamental changes in the cell layer and the distribution of small electron-thick granules formed by sulfur and silver.[16] When compared to other salts, Ag NPs have superior bactericidal qualities because of their larger surface area, which promotes better microbe interaction. It has been noted that Ag NPs frame a low sub-atomic weight within the bacterial cell when they penetrate the cell membrane. [17] As a result, the bacteria group together to protect the DNA from the Ag NPs. As a result, Ag NPs target the respiratory system in their optimum fashion, causing longer-lasting cellular division and subsequent cell death.[18] The goal of the current work was to create silver nanoparticles using extracts from the medicinal plant Lactuca sativa, describe the finished product, and assess the antibacterial properties of the particles.

Experimental part

Biosynthesis of Ag NPs: In 50 milliliters of deionized water, $AgNO_3$ (0.00588 mol) was dissolved to create the salt solution. After three days, the extract solution was progressively added to salt solution at room temperature. Silver nanoparticles, or Ag-NPs, were then obtained by filtering the mixture, collecting the precipitate, and washing it with ethanol and deionized water (see figure 1).

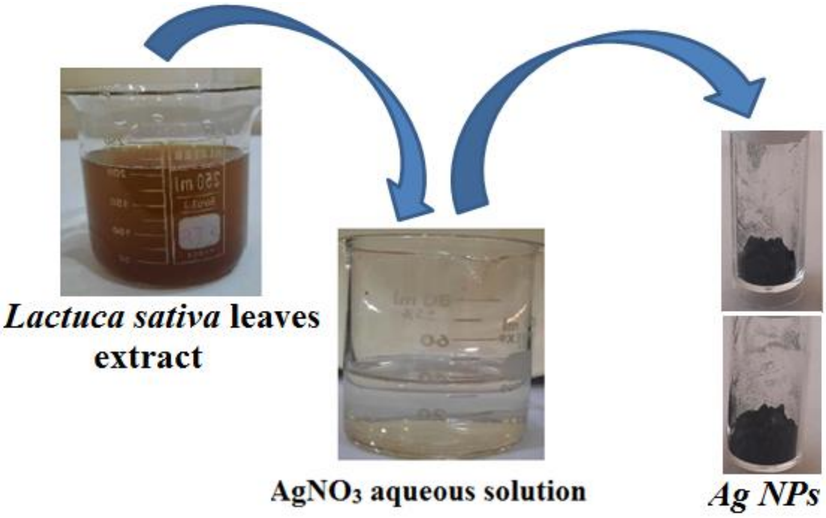

Fig.1. Using an extract from Lactuca sativa leaves, silver nanoparticles (Ag NPs) is produced.

Preparation the Culture Media

Muller Hinton agar was made by dissolving 38 grams in 1.0 liter of distilled water, autoclaving it at 121 degrees Celsius and 15 pounds of pressure for 15 minutes, and then pouring the sterile mixture onto sterile plates and storing it in the refrigerator until it was needed.(see figure 2)

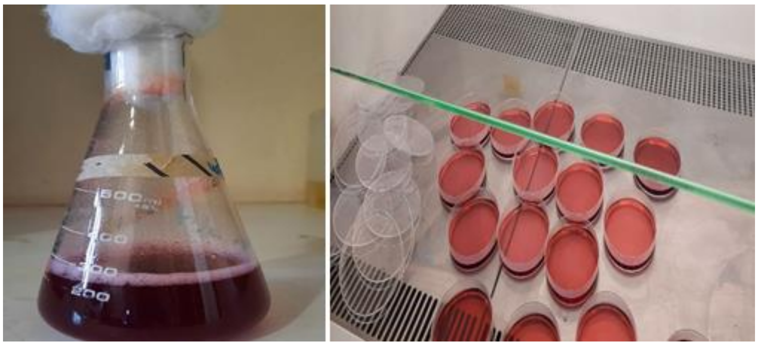

Fig.2. Muller Hinton agar

Assessing Antimicrobial Activity using the Agar Well Diffusion Method

To prepare the suspended bacteria and place them in tubes containing brain heart infusion broth to activate the bacteria, many bacterial colonies have been transferred by loop. The tubes were incubated at 37 °C for 18–24 hours. The suspended bacteria were compared to the usual MacFarland solution (1.5×108) cells/mL. Subsequently, the bacteria were distributed using a sterile swab and placed on Muller-Hinton agar plates. The plates were then allowed to air-dry for some time. Using a sterilized cork borer, a 5 mm-diameter hole was cut in the culture medium. 100 µL of the substance (concentration 100, 200 mg/mL) was introduced to each hole separately using a micropipette. Subsequently, incubate the dishes at 370 °C. The concentrations of piperacillin (100 µg), tetracycline (100 µg), ciprofloxacin (100 µg), and Staphlococcus aureus (100 µg) were arranged in the center of the petri dishes containing Acinetobacter baumani, Escherichia coli, Staphlococcus aureus, and Bacillus licheniformis. These concentrations were chosen in accordance with the Clinical and Laboratory Standards

Institute. The diameter of the inhibition zone surrounding each hole was measured in order to assess the efficacy of each concentration and antibiotic.

Result and Discussion

Characterization of synthesized silver nanoparticles: XRD, FE-SEM, EDX, and UV spectra of the resonance band at about 300–450 nm were used to examine the synthesis of silver nanoparticles, which were made using a method utilizing NaOH solution as a precipitation agent. One of the distinctive characteristics of metal nanoparticles is their surface plasmon resonance, which has been used in optical spectroscopy to calculate the size of these nanoparticles using the Haiss equation.[19]

$$d= \ln (\lambda SPR - \lambda°)/L_1/L_2$$

Where d represents the Ag-NPs' particle diameters, λSPR and λ° denote the wavelengths of maximum and minimum absorption, respectively, and L1 and L2 are parameters equal to 6.53 and 0.0216. The UV-Vis spectra reveal characteristic absorption peak of the Ag-NPs at shown in figure 3.

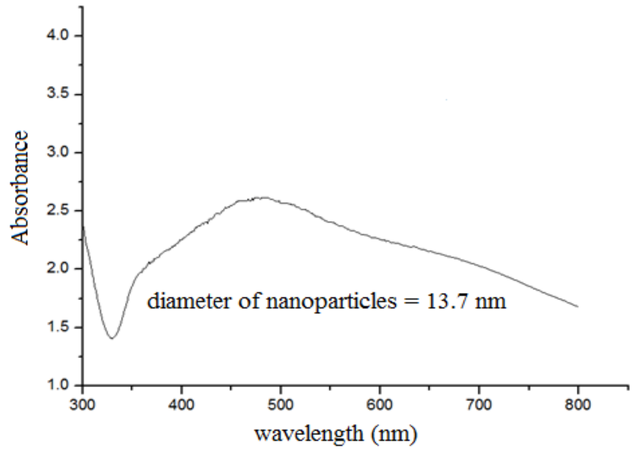

Fig. 3. Absorbance spectra of synthesized Ag- NPs using Lactuca sativa leaves extract plant.

Figure 4 displays the XRD of Ag-NPs that was prepared using a Lactuca sativa extract leaves. Ag-NPs have been seen to have a cubic structure, with an average crystalline size of 34.27 nm. The large peaks that are seen signify the creation of the silver nanocrystalline phase.

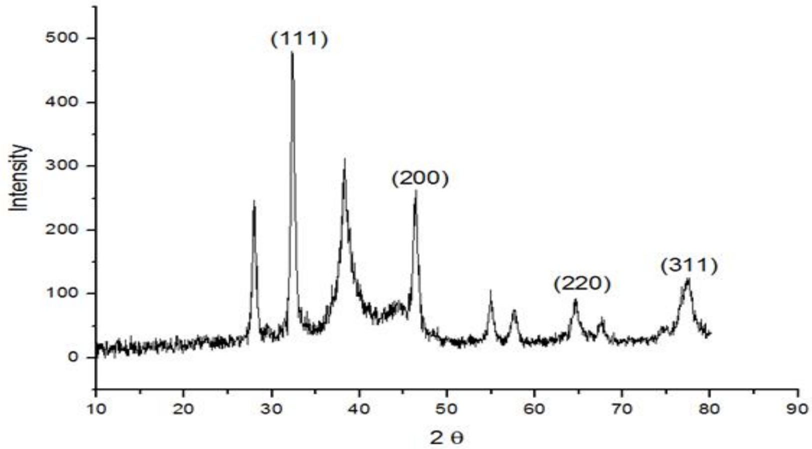

Fig.4. XRD pattern of Ag-NPs synthesized by Lactuca sativa leaves extract

Silver nanoparticles morphology was investigated by FE-SEM and TEM techniques which has used to visualize the size and shape of the nanoparticles. FE-SEM micrographs of silver nanoparticles have given in figure 5. Figure 5 shows the FE-SEM image of the synthesized silver nanoparticles (Ag NPs) with spherical in shape and average size about 93.15 nm.

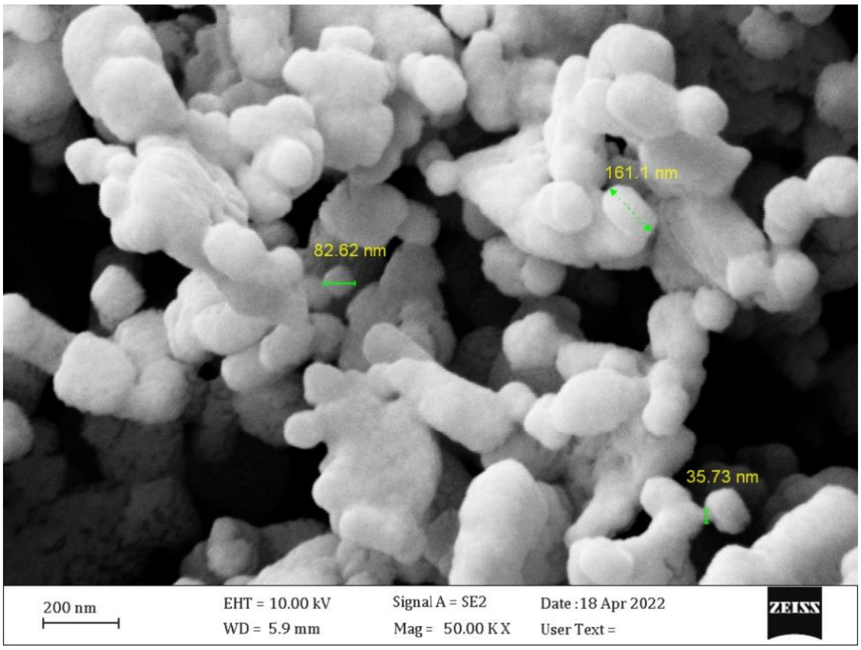

Fig.5**.** FE-SEM image of silver nanoparticles synthesized using Lactuca sativa leaves extract

The transmission electron microscope (TEM) picture of the Ag nanoparticles is shown in Figure 6. Silver nanoparticles were broadly spherical and tube-shaped forms and were between 10 and 14 nm in size.

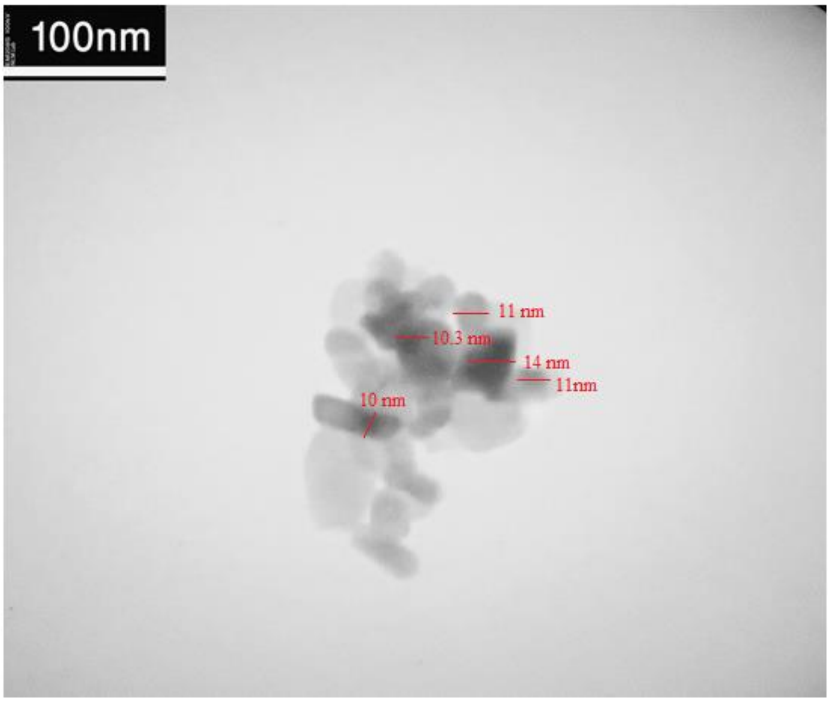

Fig.6. TEM image of silver nanoparticles synthesized using Lactuca sativa leaves extract

The Antibacterial Activities

Using the disk diffusion method, the biological activity of artificially created silver nanoparticles derived from plant extract was investigated against four pathogenic bacterial isolates found in the Baqubah Teaching Hospital. These isolates included two gram-positive bacteria (Staphlococcus aureus and Bacillus licheniformis) and two gram-negative bacteria (Acinetobacter baumani and Escherichia coli).

Some synthetic compounds' biological activity as antibacterial agents were tested against a variety of identified and isolated bacteria, including two gram-positive isolates (Gr +ve) of Staphlococcus aureus and Bacillus licheniformis and two gram-negative isolates (G –ve) of Acinetobacter baumani and Escherichia coli, which were chosen due to their significance in the development of antibiotics and medical drugs. The study of the synthesized silver nanoparticles' antibacterial activity at two concentrations (100 and 200 mg/mL) was presented in Table 1; the results indicated moderate to good activity when compared to standard antibiotics (ciprofloxacin, Tetracycline, and piperacillin). The biological activity can be explained as follows.

Table 1**.** Result for synthesized Ag nanoparticles against Gr+ve **and** Gr-ve bacteria

Comp.	Bacterial inhibition diameter measured in millimeters							
	Staphlococcus aureus		Bacillus licheniformis		Acinetobacter baumani		Escherichia coli	
	100%	200%	100%	200%	100%	200%	100%	200%
Ag NPs	41	53	36	48	29	33	21	30
DMSO	N.A		N.A		N.A		N.A	
ciprofloxacin	19		28		12		9	
Tetracycline	N.A		N.A		N.A		N.A	
piperacillin	N.A		N.A		N.A		N.A	

At concentrations of 100 and 200 gm/mL, the activity of silver nanoparticles derived from plant extract Lactuca sativa leaves is evaluated against Staphlococcus aureus, Bacillus licheniformis, Acinetobacter baumani, and Escherichia coli bacteria. Table 1 presents the findings of the comparison of moderate to good activities with drug usage.

Conclusion

In this work, the aqueous extract of Lactuca sativa leaves was effectively used as a reducing and stabilizing agent in the green production of silver nanoparticles. Green technologies, which are less harmful and more cost-effective, were successfully used to synthesis pure silver nanoparticles, or Ag-NPs. Ag nanoparticles' crystalline size was estimated by UV spectroscopy to be around 13.7. Ag-NPs were synthesized in the cubic phase, as validated by XRD measurement, with an average crystalline size of around 34.27 nm. The morphology of silver nanoparticles was examined using FE-SEM and TEM methods, yielding sizes of 93.15 nm and 10–14 nm with spherical and tube-shaped morphologies, respectively. After being exposed to antibacterial activities, the prepared silver nanoparticles, or Ag-NPs, had a moderate to good influence on the chosen bacteria.

References

1. Nadeem Baig, Irshad Kammakakam, and Wail Falath abe. Nanomaterials: a review of synthesis methods, properties, recent progress, and challenges. Mater. Adv., 2021, 2, 1821-1871.
2. Nadeem Joudeh and Dirk Linke. Nanoparticle classification, physicochemical properties, characterization, and applications: a comprehensive review for biologists. Journal of Nanobiotechnology ; 2022, 20:262.
3. Paulina Szczyglewska, Agnieszka Feliczak-Guzik, and Izabela Nowak. Nanotechnology–General Aspects: A Chemical Reduction Approach to the Synthesis of Nanoparticles. Molecules. 2023, 28(13): 4932.
4. Vancha Harish, Mustafiz Ansari, Manish Gaur, Awadh Bihari Yadav, María-Luisa García-Betancourt, Fatehy M. Abdel-Haleem, Mikhael Bechelany, and Ahmed Barhoum.Nanoparticle and Nanostructure Synthesis and Controlled Growth Methods. Nanomaterials; 2022, 12(18), 3226.
5. AlexandruRus,Vasile-Dănuț Leordean and Petru Berce. Silver Nanoparticles (AgNP) impregnated filters in drinking water disinfection. MATEC Web of Conferences 137, 07007 (2017).
6. Hayelom Dargo Beyene, Adhena Ayaliew Werkneh, Hailemariam Kassa Bezabh, and Tekilt Gebregergs Ambayec. Synthesis paradigm and applications of silver nanoparticles (AgNPs), a review. Sustainable Materials and Technologies. 2017 Sep; 13: 18–23.
7. Sina Kaabipour and Shohreh Hemmati. A review on the green and sustainable synthesis of silver nanoparticles and one-dimensional silver nanostructures. Beilstein J Nanotechnol. 2021; 12: 102–136.
8. Xi-Feng Zhang, Zhi-Guo Liu, Wei Shen, and Sangiliyandi Gurunathan. Silver Nanoparticles: Synthesis, Characterization, Properties, Applications, and Therapeutic Approaches. Int J Mol Sci. 2016 Sep; 17(9): 1534.
9. Ngoc Phuong Uyen Nguyen, Ngoc Tung Dang, Linh Doan, Hoai Thi Thu Nguyen. Synthesis of Silver Nanoparticles: From Conventional to 'Modern' Methods—A Review. Processes 2023, 11, 2617 2 of 17.
10. Tijjani Mustapha, Norashiqin Misni, Nur Raihana Ithnin, Abdullahi Muhammad Daskum, and Ngah Zasmy Unyah. A Review on Plants and Microorganisms Mediated Synthesis of Silver Nanoparticles, Role of Plants Metabolites and Applications. Int J Environ Res Public Health. 2022 Jan; 19(2): 674.
11. Jumoke A. Aboyewa, Nicole R. S. Sibuyi, Mervin Meyer, and Oluwafemi O. Oguntibeju. Green Synthesis of Metallic Nanoparticles Using Some Selected Medicinal Plants from Southern Africa and Their Biological Applications. Plants (Basel). 2021, 10(9): 1929.
12. Madeeha Aslam, Fozia Fozia, Anadil Gul,3 Ijaz Ahmad, Riaz Ullah, Ahmed Bari, Ramzi A. Mothana, and Hidayat Hussain. Phyto-Extract-Mediated Synthesis of Silver Nanoparticles Using Aqueous Extract of Sanvitalia procumbens, and Characterization, Optimization and Photocatalytic Degradation of Azo Dyes Orange G and Direct Blue-15. Molecules. 2021, 26(20): 6144.

13. Hanadi Sawalha, Rambod Abiri, Ruzana Sanusi, Siti Aqlima Ahmad. Toward a Better Understanding of Metal Nanoparticles, a Novel Strategy from Eucalyptus Plants. Plants 2021, 10, 929.
14. Tamara Bruna, Francisca Maldonado-Bravo, Paul Jara, and Nelson Caro .Silver Nanoparticles and Their Antibacterial Applications. Int J Mol Sci. 2021, 22(13): 7202.
15. Doaa Safwat Mohamed, Rehab Mahmoud Abd El-Baky, Tim Sandle, Sahar A. Mandour, and Eman Farouk Ahmed. Antimicrobial Activity of Silver-Treated Bacteria against Other Multi-Drug Resistant Pathogens in Their Environment. Antibiotics (Basel). 2020, 9(4): 181.
16. Yael N. Slavin, Jason Asnis, Urs O. Häfeli, and Horacio Bach. Metal nanoparticles: understanding the mechanisms behind antibacterial activity. J Nanobiotechnology. 2017; 15: 65.
17. Jasminka Talapko,Tatjana Matijević, Martina Juzbašić, Arlen Antolović-Požgain, and Ivana Škrlec. Antibacterial Activity of Silver and Its Application in Dentistry, Cardiology and Dermatology. Microorganisms 2020, 8(9), 1400.
18. Pragati Rajendra More, Santosh Pandit, Anna De Filippis, Gianluigi Franci, Ivan Mijakovic, and Massimiliano Galdiero. Silver Nanoparticles: Bactericidal and Mechanistic Approach against Drug Resistant Pathogens. Microorganisms. 2023,11(2): 369.
19. Wolfgang Haiss, Nguyen TK Thanh, Nguyen TK Thanh, Jenny Aveyard, David G Fernig. Determination of Size and Concentration of Gold Nanoparticles from UV−Vis Spectra. Analytical Chemistry journal, 2007, 79(11):4215-21.