

Original Article



Morus alba L. Leaves-Based Silver Nanoparticles for Aging-Related Infectious Diseases

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Abstract

Objectives: One of the most common types of aging-related diseases is infection for which ample medications are available, and most antibacterial agents are ineffective as drug resistance spreads globally.

Design: An original study.

Setting(s): Batman University, Batman, Turkiye.

Outcome measures: The presence of silver nanoparticles was analyzed with their maximum wavelength absorbance. Morphological appearances and distributions of synthesized nanoparticles were evaluated using transmissive electron microscope (TEM) and atomic power microscopy, respectively. The crystal patterns and sizes of nanoparticles were determined using an X-ray diffraction (XRD) instrument, and the crystal nanosizes were calculated by the DebyeScherrer equation. Minimum inhibition concentrations (MICs) were used for identifying an antibacterial effect on Gram-positive and Gram-negative strains of the bacteria *Staphylococcus aureus* ATCC 29213 and *Escherichia coli* ATCC 25922.

Results: The Malatya Orduzu region provided the leaves of the *Morus alba* L. plant used in this study. The resulting extract was used to create silver nanoparticles. The outcomes of several microscopic and spectroscopic analyses were used to evaluate the properties of silver nanoparticles.

Conclusions: *Morus alba* L. leaves were used to create silver nanoparticles (Ma-AgNPs), which had a size distribution below 100 nm and a maximum absorbance of 457.92 nm. Ma-AgNP's antibacterial activity was used for pathogen gram-positive and gram-negative bacteria.

Keywords: Infection diseases, Green syntheses, Antibacterial, Microorganism

Introduction

Nanoparticles (NPs), which are frequently studied, are used in numerous application areas such as water treatment, catalytic activity, chemical and biological sensors, and wireless electronics.¹⁻⁴ Metallic NPs, which are in the NP class, are becoming more popular with their wide usage areas, and the interest in them is increasing day by day. Silver nanoparticles (AgNPs), which are included in metallic NPs, are used quite frequently, especially in biomedical applications due to their antimicrobial effect.⁵ Synthesis studies using plant sources for these methods attracted attention with advantages such as being cheap, being easy, not requiring special conditions to obtain the source, and showing biocompatible properties.^{6,7}

Plant parts used as supplies for synthesis include leaves, flowers, fruits, seeds, and roots. Extracts from plant sources are a rich source of phytochemicals. These phytochemicals have functional groups such as alcohol/phenol, amine, and carboxylic acids which show bioactive properties.^{8,9} By converting the ionized Ag⁺ chargeable

state to the Ago state, bioactive components with certain functional groups actively contribute to the synthesis, stability, and capping of AgNPs.¹⁰⁻¹²

The Moraceae family includes the species *Morus alba* L., which is indigenous to China, Korea, and Japan. It is well known that this plant, which can be found growing all over the world, has had medicinal uses for thousands of years. This plant with a rich content of bioactive components has many effects such as antimicrobial, antidiabetic, and anticancer.¹³

This study aimed to analyze the eco-friendly and low-cost economic synthesis, characterization, and antibacterial effect of AgNPs using *M. alba* L. (Mulberry) tree leaves grown in Orduzu, Malatya region (Figure 1).

Methods

Morus alba L. Leaf Extract and Metal Solution Preparation

Mulberry leaves were harvested in the Orduzu Malatya region by the end of August and were repeatedly cleaned



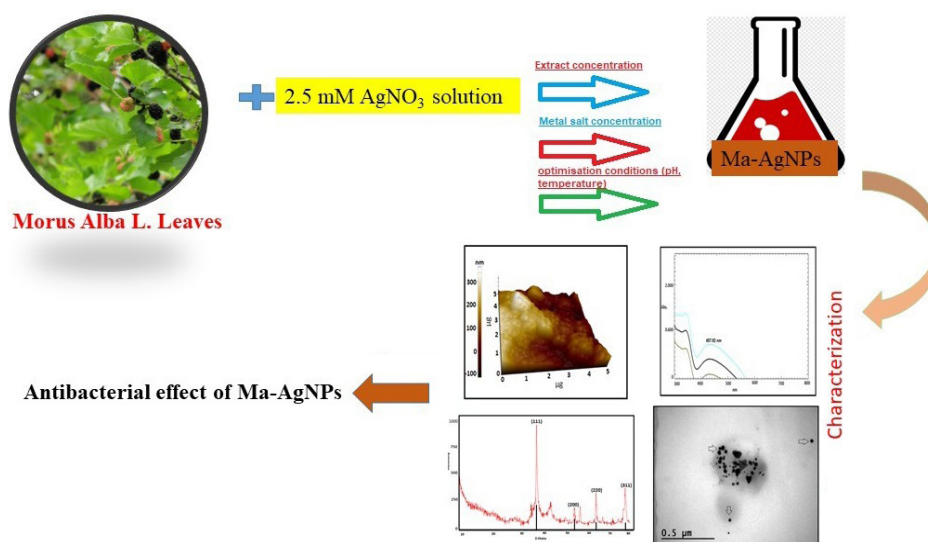


Figure 1. Schematic Illustration of Nanoparticle Synthesis Steps in Detail

with distilled and fountain water.¹⁻³ Then, 250 mL of purified water and 30 g of dried leaves were combined, and the mixture was brought to a boil.¹⁻³ The liquid extract was prepared for synthesis after cooling and filtering.⁴ Silver nitrate (AgNO₃), a solid compound salt from Sigma Aldrich, was dissolved in water at a concentration of 2.5 mM for use in green synthesis.⁴

Synthesis of Silver Nanoparticles with Extract Prepared from Mulberry Leaves

The leaf extract and 2.5 mM AgNO₃ solution were mixed in a suitable container as one unit of the extract and two units of metal solution and kept at room temperature at 25 °C. The color change was observed by observation against time.⁴ By taking samples from the synthesis medium at regular intervals, 300-800 nm wavelength scans were performed through a Perkin Elmer One, ultraviolet-visible spectrophotometer (UV-Vis).⁵ The presence of Ma-AgNPs was analyzed with their maximum wavelength absorbance.³

Characterization of Synthesized Silver Nanoparticles

Morphological appearances of synthesized Ma-AgNPs are presented in a Jeol Jem. It was evaluated using micrographs taken via the 1010 transmissive electron microscope (TEM).² The topographic structures and distributions of Ma-AgNPs were determined using a Park System XE-100 atomic force microscopy (AFM).³ The crystal patterns and sizes of Ma-AgNPs reflected on the plane between 20-80 at 2θ were determined using a Rigaku Miniflex 600 model X-ray diffraction (XRD) instrument. Using the full width at half maximum (FWHM) value of the highest peak obtained through XRD data, the crystal nano sizes were calculated by the Debye-Scherrer equation.¹⁰⁻¹⁴

Antibacterial Effects of Synthesized Silver Nanoparticles

Using the microdilution approach, the minimum inhibition concentrations (MICs) of created Ma-AgNPs have been determined on the development of pathogenic bacteria.^{15,16} The microorganisms utilized in the

experiment came from the microbiology lab of Mardin Artuklu University in Mardin, Turkey. Gram-positive and gram-negative strains of the bacteria *Staphylococcus aureus* ATCC 29213 and *Escherichia coli* ATCC 25922 were used to test the antibacterial action. In order to prepare the bacteria for antibacterial research, the bacteria were cultured in acceptable media and adjusted according to the McFarland standard 0.5¹⁷⁻¹⁹ turbidity requirements. Then, 96-well microplates were filled with the appropriate medium. The sterilization process and growth control procedures involved the use of some wells. Furthermore, transferring Ma-AgNPs made at various concentrations to the wells of the microplate served as the microdilution process. The prepared bacteria (McFarland 0.5) were then delivered into the wells, and they (McFarland 0.5) were then transferred into the wells after being prepared. To allow bacteria to interact with Ma-AgNPs, antibacterial microplates were placed in a hot oven at 37 °C for 24 hours. Following incubation, growth control was carried out, and MIC was calculated.

Results

Evaluation of the Properties of Synthesized Silver Nanoparticles

A color change from yellow to brown was observed 20 minutes after mixing the extract and AgNO₃ solution for synthesis. The rapid color change was a macroscopic finding indicating the formation of Ma-AgNPs.¹⁴⁻²⁰ In addition to this finding given in Figure 2, the maximum absorbance bands at 457.92 nm taken with UV-vis were characteristic spectrums showing the presence of Ma-AgNPs with the formation of the surface plasma resonance.²¹⁻²⁵

The determination of the morphological appearance and distribution of the synthesized AgNP was made with the images obtained from the TEM and AFM micrographs given in Figures 3 and 4. TEM pictures illustrated that Ma-AgNPs have a morphological appearance that was

predominantly spherical, and a small number of them seemed triangular. In AFM findings, Ma-AgNPs showed a distribution below 100 nm.²⁶⁻²⁹

The crystal structures and sizes of the particles synthesized using mulberry leaves were determined by the data received at 2θ via XRD. Bragg's expansions at 111o, 200o, 220o, and 311o showed that Ma-AgNPs mostly have a central faceted cubic pattern (fcc),³⁰⁻³³ and the values obtained in the data were measured as 38.68, 44.54, 64.76, and 77.38, respectively (Figure 5). The crystal nano dimensions of Ma-AgNPs were calculated using the FWHM value of Debye-Sherer's high peak, and the crystal size of Ma-AgNPs was 63.56 nm. In a green synthesis study in which different extraction processes were applied, it was calculated that the extracts have crystal sizes of 56 nm and 67 nm, respectively.³⁴

Findings of Antibacterial Effect of Synthesized Silver Nanoparticles

Ma-AgNPs synthesized from mulberry leaves were determined on pathogenic Gram-negative and Gram-positive bacteria by MIC microdilution method. MIC values of Ma-AgNPs suppressing the growth of the strains are presented in Table 1. As can be seen, 0.25-0.50 $\mu\text{g mL}^{-1}$ concentrations were found to have a suppressive effect on *E. coli* and *S. aureus*. These concentrations were lower than the commercial antibiotics used in practice (Table 1).

Ma-AgNPs were ionized and exhibited considerable reactivity in liquid media. This reactivity was created by the engagement of electrostatic attraction force interactions with the microorganisms present in the same environment.³⁵⁻³⁹ By causing damage to microorganisms' membrane structures, they enhanced the production of reactive oxygen species and altered the biomolecules'

Table 1. Concentrations of Ma-AgNPs, Antibiotics, and Silver Nitrate Solution Where They Exert an Antimicrobial Effect on the Growth of Microorganisms

Organism	AgNPs $\mu\text{g mL}^{-1}$	Silver Nitrate $\mu\text{g mL}^{-1}$	Antibiotic $\mu\text{g mL}^{-1}$
<i>Staphylococcus aureus</i>	0.50	4.00	2.00
<i>Escherichia coli</i>	0.25	2.00	2.00

Note. Ma-AgNPs: Synthesized silver nanoparticles.

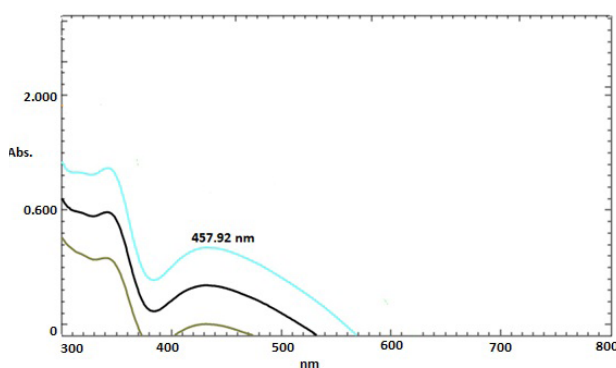


Figure 2. UV-vis Spectra Showing the Maximum Wavelength Absorbance of Ma-AgNPs Synthesized through Mulberry Leaves. Note. UV-vis: Ultraviolet-visible; Ma-AgNPs: Synthesized silver nanoparticles

structures, which caused a decline in their functions and the extinction of microorganisms.^{18,40} In this study, Ma-AgNPs were synthesized easily and quickly with the extract obtained by collecting the leaves of the *M. alba* L. plant in Orduzu, Malatya region. The outcomes of several microscopic and spectroscopic analyses were used to evaluate the characteristics of Ma-AgNPs. Ma-AgNPs can be produced as a powerful substitute, particularly in the struggle against microorganisms with multiple drug resistance.

Discussion

Rapid color change was a macroscopic finding indicating the formation of Ma-AgNPs.²⁰⁻²⁴ In addition to this finding given in Figure 1, the maximum absorbance bands at 457.92 nm taken with UV-vis were characteristic spectrums showing the presence of Ma-AgNPs with the formation of on the plasma surface resonans (SPR).²¹⁻²⁵ It was observed that Ma-AgNPs were mostly spherical in morphological appearance in TEM images, and they also showed triangular appearance, although a small

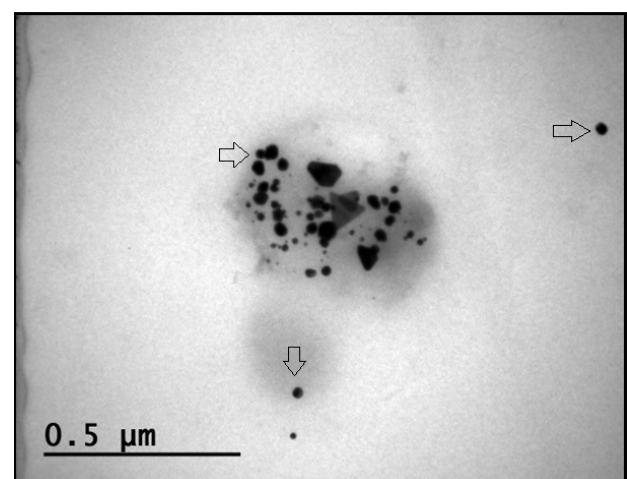


Figure 3. TEM Picture Displaying the Ma-AgNPs' Structural Appearances. Note. TEM: Transmissive electron microscope; Ma-AgNPs: Synthesized silver nanoparticles

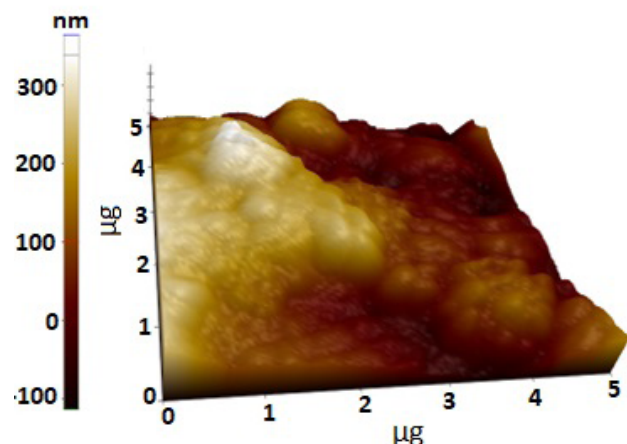


Figure 4. AFM Micrograph Showing Topographic Distributions of Ma-AgNPs. Note. AFM: Atomic Force Microscopy; Ma-AgNPs: Synthesized silver nanoparticles

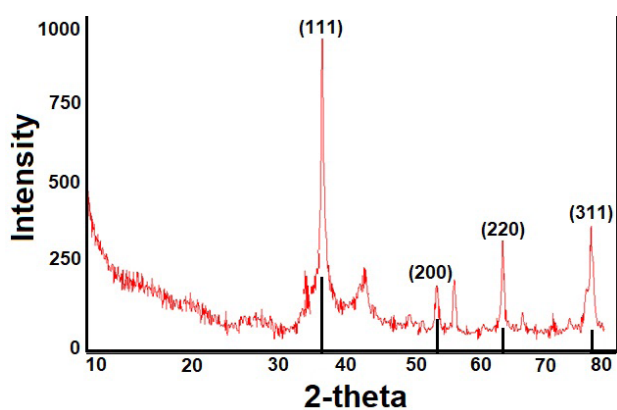


Figure 5. XRD Data Showing the Crystal Structures of Ma-AgNPs Synthesized by the Leaf Extract. Note. XRD: X-ray diffraction; Ma-AgNPs: Synthesized silver nanoparticles

number of them. In AFM findings, it was observed that they showed a distribution below 100 nm.^{28,29} Bragg's expansions at 111o, 200o, 220o and 311o showed that Ma-AgNPs mostly have a central faceted cubic pattern (fcc).³⁰⁻³³ The values obtained in the data were measured as 38.68, 44.54, 64.76, and 77.38, respectively (Figure 4). The crystal nano dimensions of Ma-AgNPs were calculated using the FWHM value of Debye-Sherer's high peak, and the crystal size of Ma-AgNPs was 63.56 nm. In a green synthesis study in which different extraction processes were applied, it was calculated that the extracts had crystal sizes of 56 nm and 67 nm, respectively.³⁴ As seen in the findings, 0.25-0.50 µg mL⁻¹ concentrations were found to have a suppressive effect on *E. coli* and *S. aureus*. These concentrations were lower than the commercial antibiotics used in practice (Table 1). Ma-AgNPs, In liquid medium are ionized and show high reactivity. They provide this reactivity by interacting with the microorganisms in the same environment with the electrostatic attraction force.³⁵⁻³⁹ They cause an increase in reactive oxygen species (ROS) by disrupting the membrane structure of microorganisms. They disrupt the structure and functions of RNA, DNA, and enzymes with high affinity for these species. The structures of biomolecules damaged by these species and as a result their functions deteriorate, resulting in the death of microorganisms.^{8,18,40}

Conclusions

Green synthesis methods are a very popular area that aims to design safer and more sustainable processes without the use of toxic chemicals. The Ma-AgNPs had size distributions below 100 nm, an ultimate absorbance of 457.92 nm, and a mainly spherical shape. By defining the MIC, the antibacterial properties of pathogen gram-negative and positive bacteria have been identified by microdilution. Furthermore, Ma-AgNPs were discovered to be more successful than generic antibiotics, according to the data.

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Formal analysis: Mehmet Firat Baran, Ahmet Meşe.

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Methodology: Mehmet Firat Baran, Ahmet Meşe.

Project administration: Mehmet Firat Baran.

Resources: Mehmet Firat Baran, Ahmet Meşe, and Aziz Eftekhari.

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Supervision: Mehmet Firat Baran.

Validation: Mehmet Firat Baran, Ahmet Meşe.

Visualization: Mehmet Firat Baran, Ahmet Meşe.

Writing—original draft: Mehmet Firat Baran, Ahmet Meşe.

Writing—review & editing: Aziz Eftekhari.

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Data availability statement

Not applicable.

Ethical approval

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Consent for publication

Not applicable.

Conflict of interests

The authors declare that there is no conflict of interests.

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