Original article

Fast and Low-Cost Biosynthesis of AgNPs with Almond Leaves: Medical Applications with Biocompatible Structures

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Abstract. In this study, silver nanoparticles (AgNPs) were obtained in a low cost, easy and simple way by using the leaf extract of *Prunus dulcis L.* (almond tree) growing in the Mardin region. Characterization of AgNPs obtained by biosynthesis: Fourier Transform Infrared Spectroscopy (FT-IR), X-Ray Diffractometer (XRD), UV-visible spectrophotometer (UV-Vis.), Scanning Electron Microscope (SEM), Transmission Electron Microscope (TEM), Zeta potential and Zeta size analysis was done through data. It was determined that AgNPs have maximum absorbance at 443 nm wavelength, they exhibit 14.67 crystal nano size, -19.9 mV zeta potential in spherical appearance. The Minimum Inhibition Concentration (MIC) of the obtained AgNPs was determined by using microdilution method on the growth of pathogen strains.

Key words: AgNPs, biocompatible, Antimicrobial effect, MIC, TEM, Zeta potential

Introduction

Nanoparticles are structures with superior properties. Thanks to its properties such as large surface area and high resistance to heat treatments,(1) biomedical applications (2, 3) food industry (4) bioremediation studies (5) cosmetics sector (6) catalysis studies (7) Ag (silver) (8), Au (gold) (9), zinc (Zn) (10), copper (Cu) (11), palladium (Pd) (12). are some of the famous metallic nanoparticles. Obtaining nanoparticles with different properties is done by bottom-up or top-down approaches (13, 14). Physical, chemical and biological methods are used to synthesize nanoparticles. In addition to the use of toxic chemicals in the synthesis stages, the high energy requirement is among the factors that make chemical and physical methods disadvantageous (15, 16). Silver nanoparticles (AgNPs) obtained through biosynthesis studies are environmentally friendly, their synthesis stages are easier, their advantages such as low cost and being economical and biocompatible increase the interest in these methods (17). Biological sources such as plant sources (18), bacteria (19), fungi (20), algae (21) are used to obtain AgNPs by biosynthesis.

The main reasons for the interest in this field are factors such as synthesis of AgNPs by using plant sources in large quantities, their stable structure, easy application, no risk of pathogenicity and no special conditions (8, 22). In synthesis with plant sources, the leaves of the plant (23), flowers (24), fruits (25), the plant itself (26) or roots (27) are used. Bioactive phytochemicals such as alcohols, phenolic compounds, flavonoids, aromatic compounds found in vegetable sources are biomolecules that form AgNPs by reducing Ag^+ ions in the aqueous environment and converting them to Ag form (28, 29). Medical applications of AgNPs as antimicrobial agents and anticancer agents have an important place 30, 31]. It is equally important to detect the toxic effects of these particles that may occur with their use (32, 33).

The almond plant, Prunus species (*Prunus dulcis* L.), belongs to the Prunoideae subfamily of the Rosaceae family. Almond tree leaves are a potential source of bioactive substances. Almond leaves are thrown away as agricultural waste (34). In the present study, it was aimed to characterize AgNPs obtained by the economic, environmentally friendly, easy synthesis of AgNPs with the extract obtained using almond tree leaves grown in the Mardin Şeyhan region and examination of biomedical applicability.

Materials and Methods

Preparation of leaf extract and silver nitrate solution

Almond tree leaves were collected in the Mardin Şeyhan region in August. It was subjected to washing with a series of taps and distilled water. It was then left to dry at room temperature. 100 grams of dried leaves were weighed and boiled with 400 ml of distilled water. The obtained extract was cooled in a sealed container and made ready for biosynthesis after filtration.

For the synthesis of AgNPs, the solid $AgNO_3$ (silver nitrate) salt Sigma Aldrich was used. At the time of biosynthesis, a solution of 5 mM (millimolar) concentration was prepared from the $AgNO_3$ salt.

Biosynthesis of AgNPs

The almond leaf extract and 5 mM $AgNO_3$ solution were mixed at a ratio of 1:2 and kept under room conditions on a fixed surface, and color change was observed. At the end of the synthesis, the dark colored reaction liquid was centrifuged at 8000 rpm for 20 minutes. Particles in the bottom sediment were washed with distilled water. It was then dried and used for characterization.

Characterization of AgNPs

To determine the presence of AgNPs, the maximum absorbance data were examined by performing wavelength scans in the range of 200-800 nm with samples taken against time with color change with a Perkin Elmer One brand UV-Vis. device (35).

To specify the functional groups of phytochemicals responsible for bioreduction, the spectra of both leaf extract and liquids obtained after biosynthesis were evaluated with the Perkin Elmer One brand FT-IR device.

Nano dimensions were calculated by determining the crystal structures of AgNPs with the data read between 20-80 at 2θ of the Rigaku Miniflex 600 model XRD device. The nano sizes of AgNPs were calculated with the Debye-Scherrer equation given below (17).

$D = K\lambda/(\beta \cos\theta)$ (1)

In the equation: D = particle size, K = constant value, λ = X-ray wavelength value, β = half of the FWHM value of the maximum peak, θ = Bragg angle of the high peak.

The morphological structures of AgNPs were determined by EVO 40 LEQ brand SEM, FE-SEM, Jeol Jem 1010 Transmission Electron Microscopy (TEM) micrographs. The elemental compositions of the particles synthesized with RadB-DMAX II computer-controlled EDX device data were evaluated.

Determining the anti-microbial effects of AgNPs

The antimicrobial effects of AgNPs on pathogenic strains were examined by the microdilution method with minimum inhibition concentration. Gram-positive, gram-negative bacteria and yeast *C.albicans* pathogen strains were used in the application. Gram positive *Staphylococcus aureus* (*S.aureus*) *ATCC 29213* and *Bacillus subtilis* (*B.subtilis*) *ATCC 11774*, gram negative *Escherichia coli* (*E.coli*) *ATCC25922* and *Pseudomonas aeruginosa* (*P. aeruginosa*) *ATCC27833* bacteria were used. *S. aureus*, *E. coli* and *C. albicans* strains were obtained from Microbiology Laboratory of İnönü University Medical Faculty Hospital. *P. aeruginosa* and *B. subtilis* strains were procured from Mardin Artuklu University Microbiology Research Laboratory.

Bacteria in nutrient agar medium and yeast C. albicans in Sabora dextrose agar medium were grown by incubating overnight at 37 °C for one night. Solutions were prepared according to the turbidity value of McFarland standard 0.5 (36) with microorganisms grown from the medium plates. Then, for microdilution, Muller Hinton broth was prepared for bacteria and Roswell Park Memorial Institute (RPMI) 1640

broths for C. albicans yeast. An appropriate medium prepared for each strain was added to the wells in 96 microplates. Some wells were identified for sterilization and growth control.

The solution containing AgNPs with a concentration of 16 μg/mL^{-1} was pipetted into the first wells. A series of micro-dilutions were applied to the other wells along with the first well. Then, the solutions prepared for each microorganism were added to the microplate wells. Plates were incubated overnight at 37 °C after micro-dilution. The MIC value was calculated by detecting the wheregrowtstarted.

Results and Discussion

UV-vis Spectrophotometer Data

Color change from yellow to dark brown occurred 30 minutes after the almond leaf extract and 5 mM $AgNO_3$ solution were mixed. This color change due to the formation of AgNPs by the bioreduction of Ag^+ ions (13, 38) is caused by the Surface Plasmon Resonance vibration (SPR). Bands at 443 nm confirme the synthesis of AgNPs due to the excitation of free electrons in the UV-vis absorption spectra (15, 39) (*Figure.1*).

XRD Analysis Data

Crystal structures and sizes of AgNPs were evaluated in the data taken in XRD 2 θ (111), (200), (220) and (311) are peaks showing the crystal structure of silver. The values of these peaks showing that it has a cubic crystal structure were read as 38.11, 44.30, 64.45 and 77.40, respectively (*Figure. 2*) (39, 40). The Debye-Scherrer equation given below was used to determine the crystal nano sizes of AgNPs (41)

$D=K\lambda/(\beta \cos\theta$ (2)

In equality; D = particle size, K constant value (0.90), λ = X-ray wavelength value (1.5418 Å), β = FWHM value of high peak and cosθ = Braggθ angle of high peak.

In the calculation made with the Debye-Scherrer equation, the crystal nano dimensions of AgNPs were determined as 14.67 nm. By using this equation in some green synthesis studies, crystal nano sizes of AgNPs were calculated as 14.58 nm (26) and 16 nm (42)

FT-IR spectroscopy data

To evaluate the functional groups involved in the reduction in the biosynthesis of AgNPs, FT-IR spectra of both the extract and the reaction liquid after synthesis were evaluated. The frequency shifts in the spectra

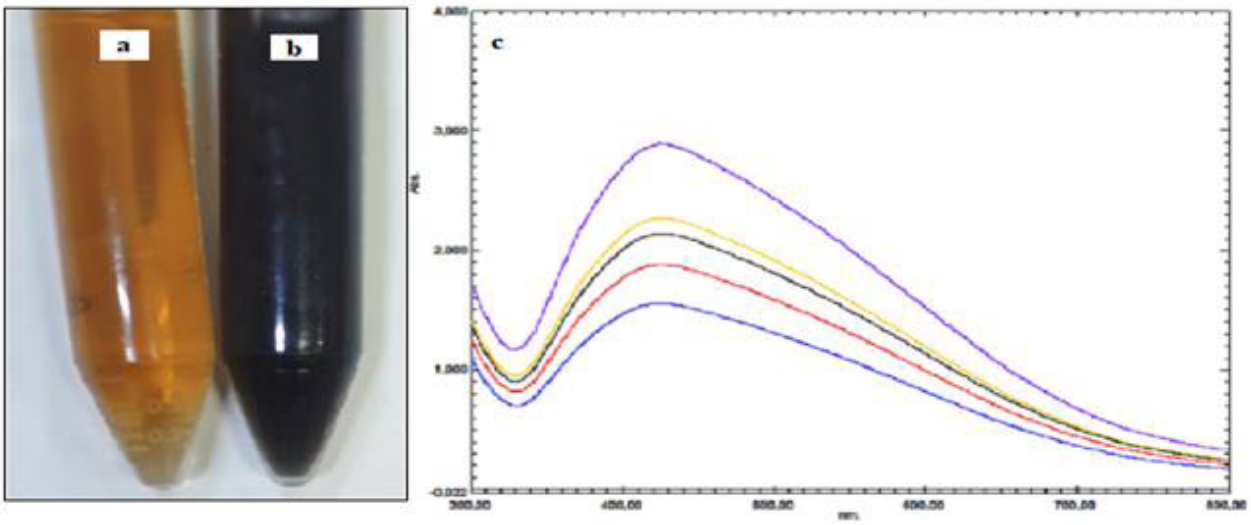

Figure 1. a. Leaf extract b. Dark brown color change due to the Formation of AgNPs, c. UV-vis. indicating the presence of AgNPs synthesized from Prunus dulcis L. leaf extract spectra.

at 3336.83 –3332.80 cm^{-1}, 2107.27–2107.15 cm^{-1} and 1635.02–1635.16 cm^{-1} suggest that hydroxyl groups (43), methylene groups (26) and carboxyl groups (44) are effective in reduction (*Figure.*3).

FE-SEM, SEM and TEM micrographs of AgNPs

Morphological structures of synthesized AgNPs were determined by electron microscopy images. Micrographs were seen to be spherical in appearance and monodisperse (*Figure. 4*) (30, 45, 46)

EDX Analysis Data

Strong peaks of silver in the EDX profile of the particles synthesized in *Figure. 5* show the presence of AgNPs in the element content (39, 42). Strong silver peaks are the result of silver nanoparticles absorbing a large amount of energy due to SPR (47), while weak peaks such as C, Cl and O are due to the phytochemicals contained in the extract (48, 49)

Zeta potential and zeta sizer distribution data of AgNPs

The average zeta size distribution of AgNPs synthesized by biosynthesis was determined to be 181 nm (*Figure. 5a*). In one of the environmentally friendly synthesis studies of AgNPs, the zeta size distribution was analyzed as 78.2-160.6 nm (50).

The AgNPs showed a zeta potential distribution of -19.9 mV in the zeta potential analysis data made to determine the surface charge. This result shows that the surface charge distribution of AgNPs is completely

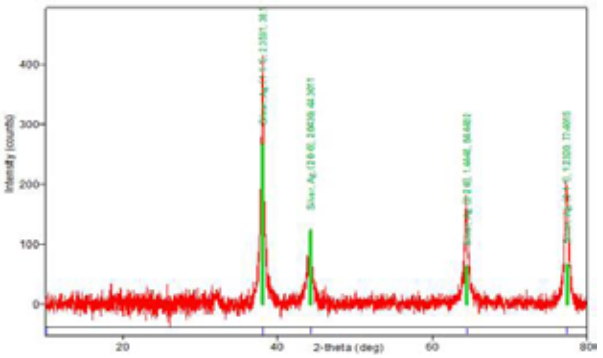

Figure 2. XRD pattern of the crystal structures of AgNPs synthesized from Prunus dulcis L. leaf extract

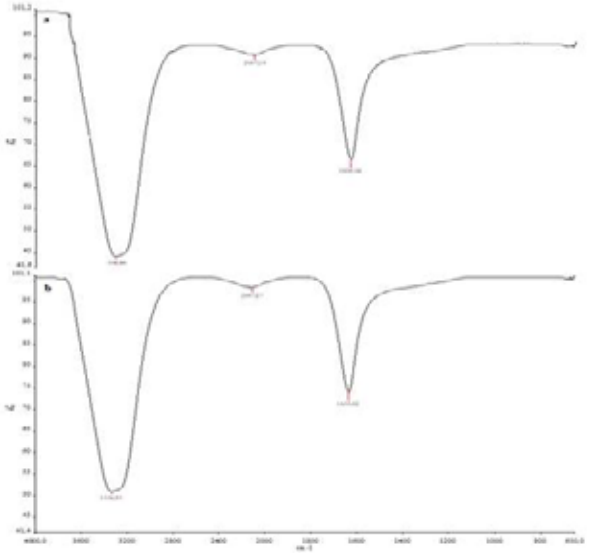

Figure 3. Spectra of functional groups that play a role in bioreduction in the formation of AgNPs synthesized from Prunus dulcis L. leaf extract a. The liquid formed after the synthesis of AgNPs, b. extract

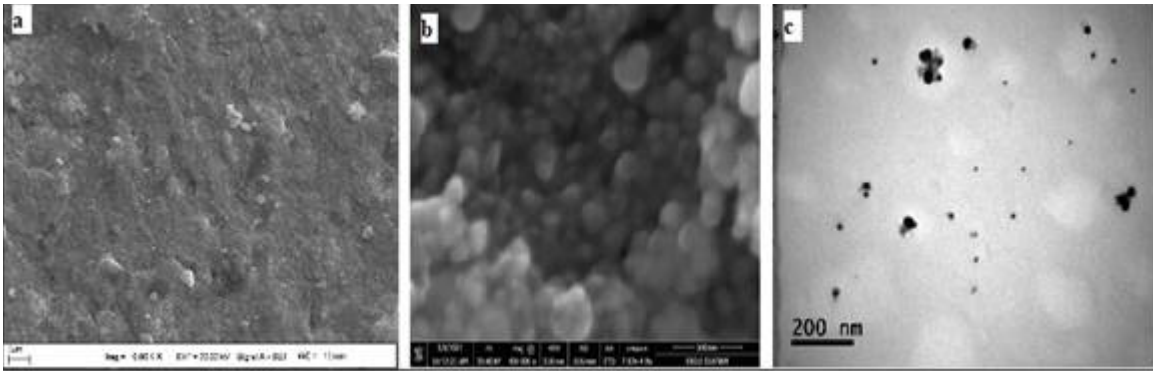

Figure 4. Micrographs of AgNPs synthesized from Prunus dulcis L. leaf extract a. SEM, b. FESEM, c. TEM images

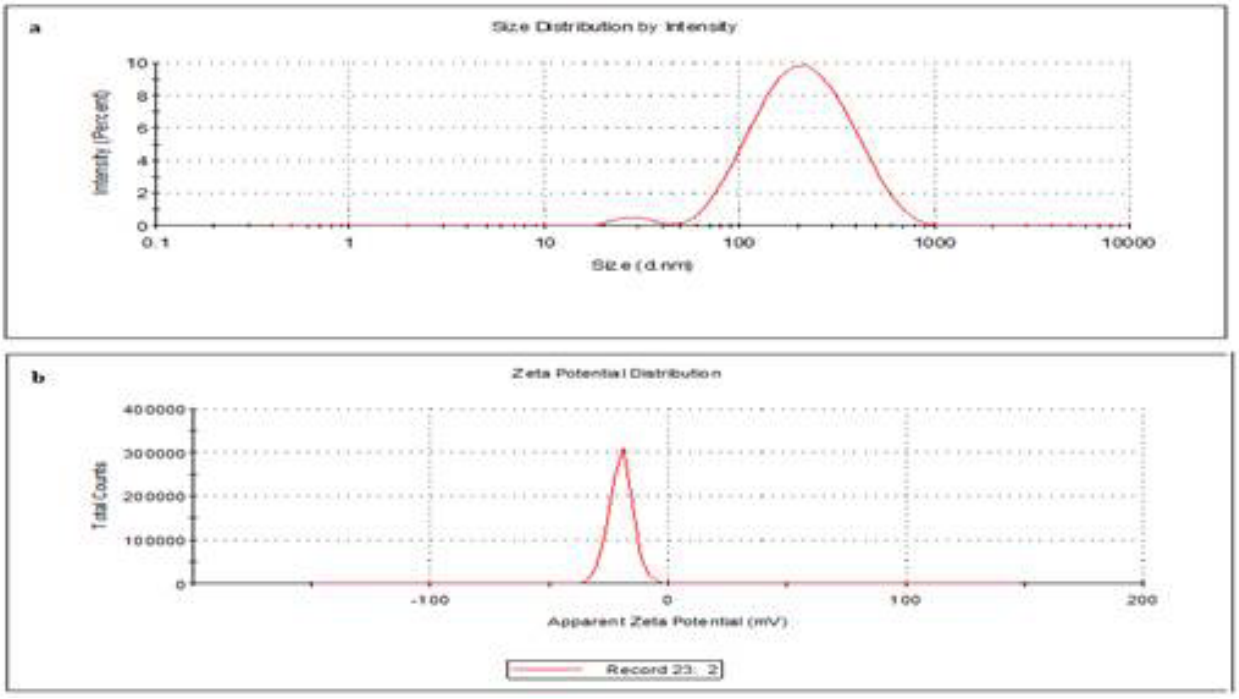

Figure 5. Analysis data of AgNPs synthesized from Prunus dulcis L. leaf extract a. Zetasizer, b. zeta potential distributions,

negatively charged (*Figure. 5b*). The difference in the surface load of AgNPs causes results such as agglomeration and accumulation (32) and negatively affects their stable structure (51). In a study conducted, zeta potential distributions of AgNPs were found as -19 mV (52) in a study.

Antimicrobial effects of AgNPs

The antimicrobial effects of AgNPs on pathogenic microorganisms were determined by microdilution. MIC values causing antimicrobial effects were compared with antibiotics and 5 mM silver nitrate solution. It was determined that 0.13-2.00 µg/mL concentrations were suppressive MIC affecting their growth on microorganisms. It was determined that P. aeruginosa was effective against antibiotics at a lower concentration, but at a higher concentration than silver nitrate solution. In all other pathogen strains, AgNPs were found to exhibit much lower antimicrobial effects against antibiotics and silver nitrate solution (*Table 1, Figure. 6*).

AgNPs that are ionized in an aqueous structure show very high reactivity. Electrostatic attraction force interacts with microorganisms after they are ionized (21,33, 53). Reactive oxygen species (ROS) increase as a result. Some biomolecules have high affinity for ROS such as DNA and RNA. In addition, thiol groups of important enzymes can establish strong bonds with these species. As a result of this situation, these enzymes become incapable of functioning (35). Cell death occurs due to the disruption in the structures of biomolecules such as cell membrane and nucleus with the increase of ROS (34).

In a green synthesis study, it was stated that AgNPs were effective on gram-positive *S. aureus* at a concentration of 250 µg / mL, while concentrations of 15 µg / mL and 30 µg / mL were effective on gram-negative *E. coli* and *P. aeruginosa* bacteria (28). In another synthesis study, AgNPs were effective at 50 µg / mL concentration on E. coli, 25 µg / mL concentration on *B. subtilis* and *C. albicans* strains in antimicrobial activity (37). In a study, it was stated that AgNPs synthesized with *Gundelia tournefortii* extract were effective at a concentration of 0.367 µg/mL on *C. albicans* (54).

Table 1. MIC values where AgNPs synthesized from Prunus dulcis L. leaf extract, silver nitrate and antibiotics have antimicrobial activity on microorganisms (antibiotics used in experimental studies: vancomycin, frucosanol and colistin antibiotics were used)

ORGANISM	AgNPs µg/mL	Silver Nitrate µg/mL	Antibiotic µg/mL
S. aureus ATCC 29213	0.13	2.65	2
B.subtilis ATCC 11774	0.13	1.32	1
E. coli ATCC25922	1.00	0.66	2
P. aeruginosa ATCC27833	2.00	1.32	4
C. albicans	0.25	0.66	2

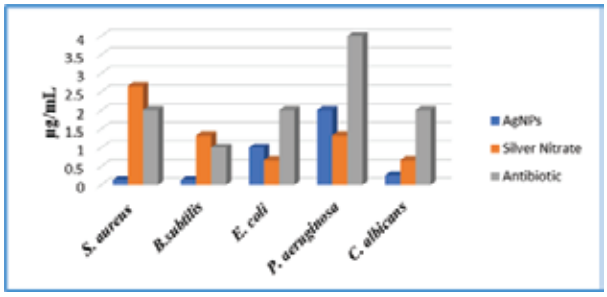

Figure 6. Comparison of the antimicrobial effects of AgNPs synthesized from Prunus dulcis L. leaf extract, AgNO3 solution and antibiotics on microorganisms

Some properties have the quality that determines the effectiveness of nanoparticles in toxic activity. These features of AgNPs include surface charge, concentrations, interaction time, shapes, degree of deposition, and dimensions (37), (55).

Conclusion

Almond tree leaves create a great deal of agricultural waste. Obtaining useful products by recycling wastes has a very important place in today's world. The biosynthesis of AgNPs with the extract obtained from the leaves of almond trees growing in the Mardin region was carried out in an easy, cost-effective, environmentally friendly and rapid manner. AgNPs formed by biosynthesis were characterized by FT-IR, TEM, SEM, FE-SEM, XRD, EDX, UV-Vis., Zeta sizer and Zeta potential analyzes. It was observed that AgNPs were effective in antimicrobial activity at concentrations lower than antibiotics at concentrations of 0.13-2.00 µg/mL. By working on the parameters that affect the properties of AgNPs, it can be contributed to medical applications as antimicrobial agents.

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