

# Biosynthesis of AgNPs by extract from waste leaves of *Citrullus lanatus* sp. (watermelon); characterization, antibacterial and antifungal effects

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**Abstract.** Silver nanoparticles (AgNPs) are valuable materials with a large number of sectors used. Green synthesis is very important for biomedical applications as they show biocompatible properties. In this study, AgNPs were easily synthesized using the environmentally friendly green synthesis approach using agricultural waste parts of *Citrullus lanatus* sp. plant grown in the Diyarbakır region. Characterization of synthesized AgNPs was made. Fourier Transform Infrared Spectroscopy (FTIR) analysis was used to evaluate the phytochemicals responsible for effective reduction in the formation of AgNPs. UV-visible spectrophotometer (UV-Vis.) Spectra were also used to determine the presence of AgNPs. X-Ray Diffraction Diffractometer (XRD), Scanning Electron Microscope (SEM), Transmission Electron Microscope, Zeta potential analyzes were performed to define the crystal structures, dimensions and surface charges of AgNPs, respectively. In these data, it was determined that AgNPs showed maximum absorbance at 460 nm, spherical appearance, 21.27 crystal nano size and -30.05 mV zeta potential. Antimicrobial effects of AgNPs on gram-positive *Staphylococcus aureus* (*S. aureus*) and *Bacillus subtilis* (*B. subtilis*) bacteria, gram negative *Escherichia coli* (*E. coli*) and *Pseudomonas aeruginosa* (*P. aeruginosa*) bacteria, as well as on yeast *C. albicans* pathogenic microorganisms It was analyzed by specifying the Minimum Inhibition Concentration (MIC) by microdilution.

**Key words:** Antimicrobial, MIC, SEM, TEM, Zeta potential

## Introduction

Metallic nanoparticles are valuable materials. Metallic nanoparticles such as Ag (silver) (1), zinc (Zn) (2), Au (gold) (3), palladium (Pd) (4), copper (Cu) (5) are used in many fields. It has uses as anticancer and antimicrobial agents in medical applications (6, 7), bioremediation studies (8, 9), cosmetics industry (10), electronics (11). Different methods are used to synthesize nanoparticles (12). These methods are biological, physical and chemical methods. Biological methods are more advantageous in the synthesis process as they do not contain harmful toxic chemicals and the cost is low (13, 14). Biological sources such as

plants, fungi, bacteria, algae are used in the synthesis of nanoparticles (15, 16).

Synthesis of AgNPs by using plant sources, obtaining more in quantity, not requiring special conditions for synthesis, easy and simple application is advantageous in biological methods (17, 18). In addition, the stable structure of AgNPs synthesized using plant sources is the reason for the interest in this field. In the synthesis of AgNPs with plant sources, leaves (19), fruits (20), flowers (21), roots (22) of the plant are used. Phytochemicals found in the leaves, fruits, flowers and roots of the plant in the synthesis of AgNPs with plant sources are bioactive products. Bioactive compounds such as aromatic compounds, alcohols,

phenolic compounds, flavonoids are involved in the reduction of AgNP synthesis by reducing  $\text{Ag}^+$  ions in aqueous the environment and forming  $\text{Ag}^0$  (23, 24).

Watermelon is a plant that grows in the Diyarbakır region with its unique characteristics and creates a serious income source. In this study, *Citrullus lanatus* sp. (watermelon) is aimed to be environmentally friendly, easy synthesis and characterization of AgNPs and the antimicrobial effects of the obtained AgNPs on pathogenic strains with the extract created from the leaves of the plant that emerged as agricultural waste after harvesting the fruits of the plant.

## Materials and Methods

### *Citrullus lanatus* sp. Preparation of extract from plant leaves and 5 millimolar (mM) silver nitrate ( $\text{AgNO}_3$ ) solution

*Citrullus lanatus* sp. The leaves of the plant were collected after harvest. After washing with tap water, it was washed with distilled water and dried in room conditions. 100 g of dried plant and 400 ml of distilled water were mixed, and heat treatment was applied until it boiled. It was then cooled and filtered. It was stored at +4 °C to be used for synthesis.

A solution with a concentration of 5 mM was prepared from the  $\text{AgNO}_3$  salt for the Sigma Aldrich brand synthesis to obtain AgNPs in the Green synthesis.

### Synthesis of AgNPs

*Citrullus lanatus* sp. 200 ml of plant extract and 400 ml of 5 mM  $\text{AgNO}_3$  solution were mixed and placed on the floor under room conditions without any special conditions. The color change was observed. After the reaction, the synthesis liquid was centrifuged at 9000 rpm for 10 minutes. The particles settle in the bottom part were dried after washing with distilled water and made ready for characterization processes.

### Characterization of AgNPs

Characterization of the AgNPs Perkin Elmer One brand UV-vis. Spectrophotometer and FTIR device

were determined by Rigaku Miniflex 600 model XRD device and RadB-DMAX II computer controlled EDX device data. Also EVO 40 LEQ brand SEM, TEM, Jeol Jem. 1010 Transmission Electron Microscopy (TEM) devices and Malvern brand zeta potential device data were also used for characterization.

The wavelength scans were performed to determine the presence of AgNPs with UV-vis spectrophotometer. Crystal structures were determined with XRD data and crystal nano sizes were determined by the Debye-Scherrer equation (25).

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

In the equation: D = particle size, K = constant value,  $\lambda$  = X-ray wavelength value,  $\beta$  = half of the FWHM value of the maximum peak,  $\theta$  = Bragg angle of the high peak.

The functional groups responsible for reduction were evaluated with spectra of FTIR device. Morphology of AgNPs in SEM and TEM micrographs, element content in EDX profile and surface charge distributions with zeta potential values were determined.

### Determining the antimicrobial effects of AgNPs

The antimicrobial effects of AgNPs obtained by Green synthesis on pathogenic microorganisms were evaluated by determining MIC by microdilution. Pathogenic strains were procured from Inonu University Medical Faculty Hospital Microbiology Laboratory and Mardin Artuklu University Microbiology Research Laboratory. Gram-positive *Staphylococcus aureus* (*S. aureus*) ATCC 29213, *Bacillus subtilis* (*B. subtilis*) ATCC 11774 bacteria and gram-negative *Escherichia coli* (*E. coli*) ATCC25922, *Pseudomonas aeruginosa* (*P. aeruginosa*) ATCC27833 bacteria were used in the experiment. In addition, AgNPs were also applied on fungus *Candida albicans* (*C. albicans*).

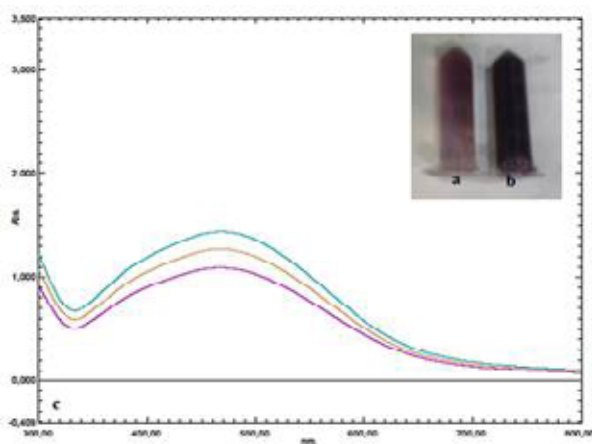
Bacteria in nutrient agar medium and yeast *C. albicans* in Sabora dextros agar medium were allowed to grow for one day at 37 °C. After the growth control the next day, suspensions were prepared from the cultured microorganisms according to the McFarland standard 0.5 (26) turbidity. Muller Hinton broth and Roswell Park Memorial Institute (RPMI) 1640 broths were prepared for microdilution application. A suitable medium prepared for microorganisms was added

to the microplate wells. One well was set for sterilization control and another well for growth control. The solutions containing appropriate concentrations of the AgNPs were pipetted into the first wells of the microplate. A series of micro dilutions were applied to the other wells that followed. The microorganism suspension was then added to the wells. Plates were left to incubate at 37 °C for a day at the end of the application. The next day, the growth control was performed and the concentration of the well before the concentration at which the growth started compared to the control was determined as MIC.

## Results and Discussion

### UV-vis. Spectroscopy

After mixing watermelon plant extract and 5 mM AgNO<sub>3</sub> solution, the color change to dark brown was observed after 20 minutes. The color transformation occurred due to the formation of AgNPs as a result of the reduction of silver ions through functional groups found in phytochemicals (27, 28). Due to the excitation of free electrons in surface plasma resonance (SPR) bound UV-vis spectra, absorption bands at 460 nm are characteristic data regarding the presence and formation of AgNPs (29, 30) (Figure 1).



**Figure 1.** a. Watermelon plant extract b. The formation of AgNPs, the dark brown color change of the post-synthesis fluid, c. UV-vis. spectra showing the formation and presence of AgNPs

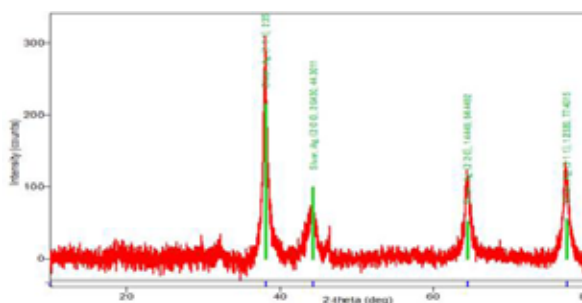
### Crystal structure and size analysis with XRD analysis data

To evaluate the crystal structures and sizes of the AgNPs obtained as a result of the synthesis, the data obtained with the XRD device at 2 $\theta$  were evaluated. The peaks (111), (200), (220) and (311) showing the crystal structure of AgNPs show that these particles exhibit a cubic crystal structure (31-33). The values of these peaks showing the values of the spectra of these peaks at 2 $\theta$  were read as 38.13, 44.30, 64.30 and 77.27, respectively (Figure 2). The highest peak value in (111) is 38.13. To determine the crystal nano sizes of AgNPs, calculations were made using the Debye-Scherrer equation from the data taken at 2 $\theta$ .

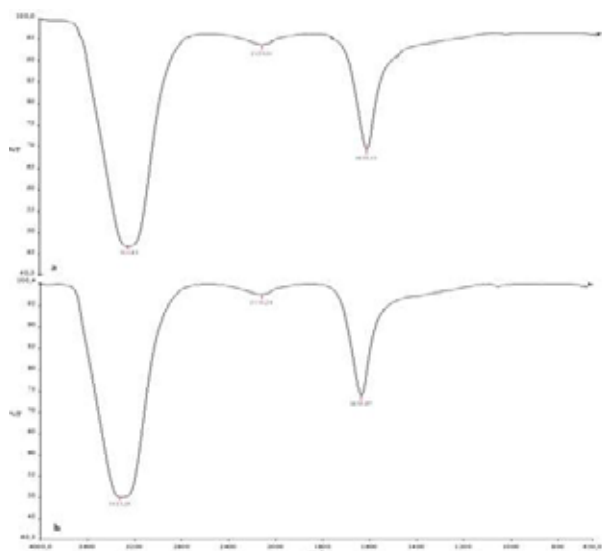
As a result of the calculation, it was determined that the crystal nano size of AgNPs was at 21.27 nm. The crystal nano sizes of the AgNPs obtained as a result of green synthesis studies were calculated as 21 nm (32) and 23.66 (34) nm.

### FTIR spectroscopy

Functional groups responsible for the reduction in synthesis were evaluated in reaction liquid both after extract and after synthesis by FTIR spectroscopy spectra. The frequency shift occurring in the spectra 3323.25-3311.83 cm<sup>-1</sup> is associated with the hydroxyl groups (-OH) (35), the shift alkyne groups (C≡C) (36) at 2116.24-2129.01 cm<sup>-1</sup>, and the frequency shift at 1635.07-1635.11 cm<sup>-1</sup> is the I amine groups (-NH<sub>2</sub>) (37) suggests that it can play a role in reduction (Figure 3).



**Figure 2.** XRD pattern of the crystal structures of AgNPs



**Figure 3.** FTIR spectra, a. the reaction liquid after synthesis, b. extract

### *SEM and TEM micrographs of AgNPs and EDX elemental compositions of the particles obtained*

In electron microscopy analyzes performed to determine the morphological structures of AgNPs, it

was determined that AgNPs were in spherical appearance (Figure 4a and b) (38, 39).

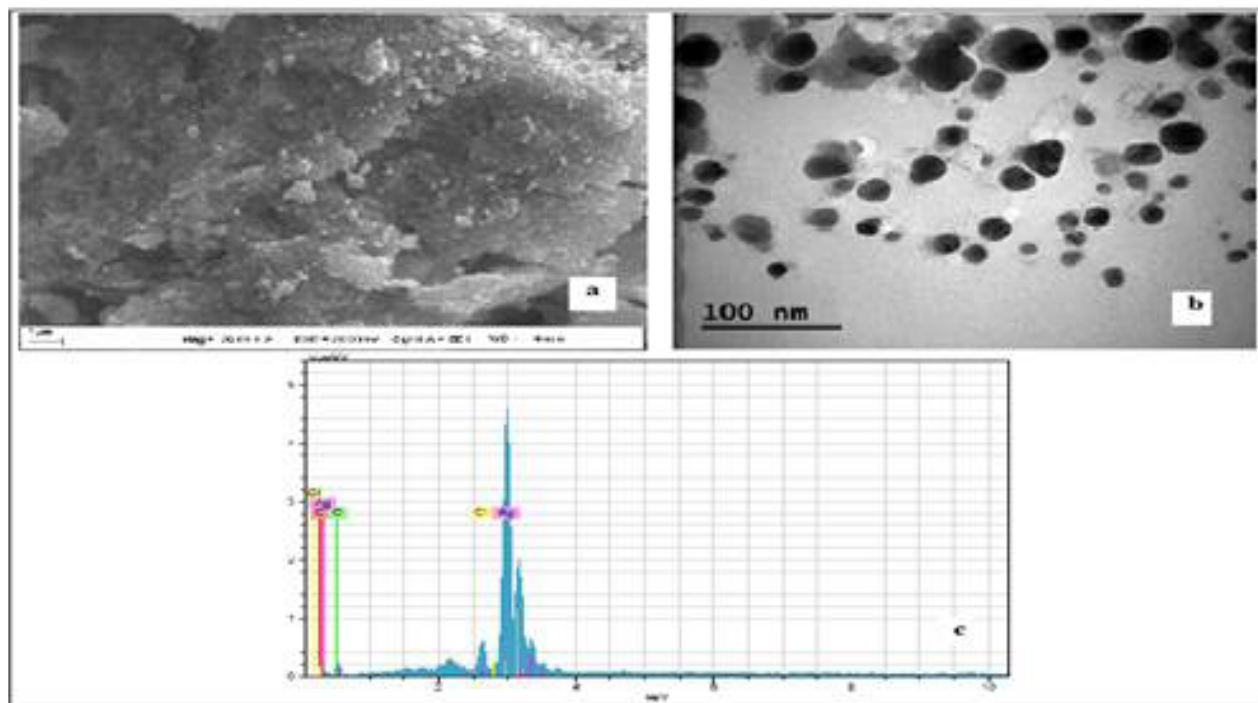
The EDX profile of the particles obtained in Figure 4c was evaluated. Strong peaks of silver show the presence of AgNPs in the element content (40). In addition, weak peaks such as C and O are also due to residues of the extracted content (36, 41).

### *Zeta potential distributions of AgNPs*

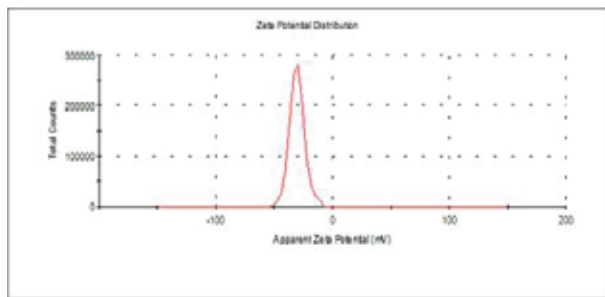
With the zeta potential analysis, it was determined that the surface loads of AgNPs showed a distribution of -30.5 mV (Figure 5). This result shows that AgNPs do not show aggregation and are stable (42, 43). In a study, it was stated that the zeta potential distributions of AgNPs were -30.4 mV in one study (44).

### *Antimicrobial effects of AgNPs*

AgNPs obtained as a result of the synthesis were effective on microorganisms in the concentration range 0.13-1.00  $\mu\text{g}/\text{mL}$ . AgNPs showed lower concentrations than silver nitrate solution and antibiotics on all strains. It was determined that AgNPs were



**Figure 4.** Micrographs of AgNPs a. SEM, b. TEM images, c. EDX profiles



**Figure 5.** Zeta potential analysis data of surface charges of AgNPs

effective on the growth of *B. subtilis* and *C. albicans* strains with the lowest concentration of 0.13  $\mu\text{g/mL}$ . The highest concentration was detected on gram negative *P. aeruginosa* with a concentration of 1.00  $\mu\text{g/mL}$  (Table 1 and Figure 7).

AgNPs are ionized in the liquid in which they show very high reactivity and contact microorganisms with electrostatic attraction force (21,33,45). After this interaction, it causes an increase in Reactive Oxygen Species (ROS). Some biomolecules have affinity for these species such as DNA, RNA and life enzymes. As a result, these biomolecules become incapable of functioning and cell death occurs (34, 35).

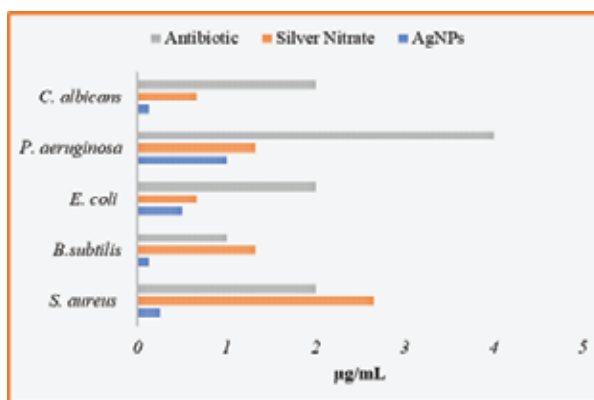
AgNPs obtained in a green synthesis study were reported as 10  $\mu\text{g/mL}$  MIC on gram negative *E. coli* growth (46). In another study, a concentration of 250  $\mu\text{g/mL}$  was effective on gram positive *S. aureus*, and a concentration of 30  $\mu\text{g/mL}$  was effective on gram negative *P. aeruginosa* bacteria (47). It was also stated in one of the studies that AgNPs were effective on *B. subtilis* and *C. albicans* strains at a concentration of 25  $\mu\text{g/mL}$  (48). In another environmentally friendly study, a concentration of 0.33  $\mu\text{g/mL}$  showed an inhibition effect on *E. coli* (49).

## Conclusion

AgNPs were successfully synthesized in an environmentally friendly manner with the extract prepared from above-ground plant parts in the form of the agricultural waste remaining in the soil after the watermelon harvest. Various analyzes were made to determine their characteristics. AgNPs were

**Table 1.** MIC values where AgNPs, antibiotics and silver nitrate solution are effective on antimicrobial activity on pathogenic microorganisms (The used antibiotics (Fluconazole, Vancomycin, and Colistin) were obtained commercially).

ORGANISM	AgNPs $\mu\text{g/mL}$	Silver Nitrat $\mu\text{g/mL}$	Standarts Antibiotic $\mu\text{g/mL}$
<i>S. aureus</i> ATCC 29213	0.25	2.65	2.00
<i>B. subtilis</i> ATCC 11774	0.13	1.32	1.00
<i>E. coli</i> ATCC25922	0.50	0.66	2.00
<i>P. aeruginosa</i> ATCC27833	1.00	1.32	4.00
<i>C. albicans</i>	0.13	0.66	2.00



**Figure 6.** Comparison of MIC values effective on microorganisms

characterized by using XRD, FTIR, UV-vis., Zeta potential, TEM, SEM, and EDX analysis data. The antimicrobial effects of the synthesized AgNPs were examined, and it was found that they showed lower concentrations of antimicrobial effects against antibiotics and silver nitrate solution. By developing the application steps, they can be used as an antimicrobial agent against the problem of antibiotic resistance.

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