INVESTIGATING ANTIMICROBIAL ACTIVITY OF SILVER NANOPARTICLES PRODUCED THROUGH GREEN SYNTHESIS USING LEAF EXTRACT OF COMMON GRAPE (*VITIS VINIFERA*)

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Abstract. In this study, a direct approach to fabricating silver nanoparticles (AgNPs) via the leaf extract of common grape (*Vitis vinifera*) has been demonstrated. The produced particles were found with a maximum wavelength of 452.47 nm, spherical shape and the crystal size of 18.53 nm through UV-Visible spectrophotometry, XRD (X-ray diffraction) and SEM (Scanning electron microscopy) characterization methods. Furthermore, the functional groups involved in the reduction were specified with FTIR (Fourier transform infrared spectroscopy), the elemental compounds were identified with EDX (Energy dispersive X-Ray spectroscopy) and the degradation points were determined with TGA-DTA (Thermal gravimetric analysis) methods. AgNPs were found to be effective against hospital pathogens, namely Gram-negative *Escherichia coli* ATCC 25922, Gram-positive *Staphylococcus aureus* ATCC 29213 and *Candida albicans* fungus at the concentrations of 0.314, 0.078 and 0.334 μg mL^{-1}, respectively.
Keywords: *green synthesis, metal nanoparticles, grape leaf extract, characterizations*

Introduction

Nowadays, nanoparticles are widely used. These particles have found their use in different areas such as electronics, photography, material science, dye removal, cosmetics, biomedicine, bioremediation etc. (Saha et al., 2017; Swamy et al., 2015; Chaudhry et al., 2018). Green synthesis, which includes biological methods, is highly advantageous compared to physical and chemical methods due to it being environmentally friendly and not using toxic chemicals during any step (Sinsinwar et al., 2018; Ramkumar et al., 2017; Pantidos and Horsfall, 2014). Particles between the sizes of 1-100 nm are defined as nanoparticles. These nanoparticles can be obtained through biological, physical and chemical techniques (Ahmed et al., 2017). Since it is inexpensive and easy to obtain, the use of plants for the synthesis of nanoparticles is becoming increasingly common. Due to the strong antimicrobial activity of silver (Ag) against fungi, viruses, and bacteria, AgNPs have been a subject of study by researchers from many different fields in recent years, and it has been stated that AgNPs have anti-inflammatory properties in addition to their antimicrobial properties (Shah et al., 2017). Silver nanoparticle synthesis and phytochemicals in plant leaf extracts create AgNPs by

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reducing Ag^+ ions present in the medium to Ag^0 and also provide stability (Geethalakshmi and Sarada, 2010; Prakash et al., 2013; Ahmed et al., 2018). Green synthesis is an important phenomenon that must be taken into consideration, especially since it supports the use of non-toxic chemicals, and environmentally friendly, renewable materials (Kaushal et al., 2016). Plant-based green synthesis generally uses different parts of plants (leaves, roots, seeds and fruits) (Majeed et al., 2018).

In this research, the green synthesis, which is an environmentally friendly, easy and simple method, was used to synthesize AgNPs from the leaf extract of the common grape (*Vitis vinifera*) and its antimicrobial effect on microorganisms was investigated.

Materials and methods

Preparation of Vitis vinifera extract and silver nitrate ($AgNO_3$) solution

Collected grape leaves from Mardin region in Turkey were initially washed with tap water, then washed with distilled water and finally dried under room conditions. After the drying process, the dried leaves were grounded, then boiled with distilled water and filtered at room temperature using coarse filter paper followed by Whatman 1 filter paper and the extract was obtained for the synthesis of AgNPs. Afterwards, 1 mM solution was prepared using solid $AgNO_3$ (purity 99.8%, Sigma-Aldrich).

Synthesis and characterization

The leaf extract and the $AgNO_3$ solution were mixed in a ratio of 1: 4, and after the color change in a few minutes, subsequently the formation and presence of silver nanoparticles were observed with Perkin Elmer one UV Visible spectrophotometer. After centrifugation with OHAUS FC 5706 at 10.000 rpm for 5 min, the resulting nanoparticles were dried. Then the RadB-DMAX II computer controlled X-ray diffractometer for XRD, the scanning electron microscope EVO 40 LEQ for SEM-EDAX, the Perkin Elmer Spectrum One for FTIR, and the Shimadzu TGA-50 for TGA-DTA were used to determine the characterization of the synthesized AgNPs.

Determining antimicrobial effects of silver nanoparticles

The antimicrobial effects of the obtained AgNPs were examined on pathogenic microorganisms, Gram-negative *Escherichia coli* ATCC 25922, Gram-positive *Staphylococcus aureus* ATCC 29213 bacteria and *Candida albicans* yeast. The minimum inhibitory concentration (MIC) was determined by the micro dilution method. In the experiments, Mueller-Hinton medium, the solutions including a certain amount of microorganism, which were prepared according to 0.5 McFarland standard, and appropriate concentrations of the AgNP solution were added to 96-well microplates and wells were incubated at 37 °C overnight (El-Batal et al., 2018; Vishwasrao et al., 2018; Dhand et al., 2016). The next day, the MIC was determined by the well in which the reproduction ended. To compare the effects of AgNPs, commercially available vancomycin, colistin and fluconazole antibiotics and a 1 mM $AgNO_3$ aqueous solution were used on *S. aureus*, *E. coli*, and *C. albicans*.

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Results and discussion

After a few minutes of mixing the grape plant and the $AgNO_3$ solution, a dark coffee color change was observed indicating the formation of AgNPs by vibrations on the plasma surface (Alruqi et al., 2018). Data with the maximum absorbance of 452.47 nm were obtained (*Fig. 1*). The maximum absorbance in the synthesis with green tea and turmeric extracts was found to be 450 nm (Selvan et al., 2018). In another study conducted with the green synthesis, it was stated that the maximum absorbance was at 460 nm (Begum et al., 2009).

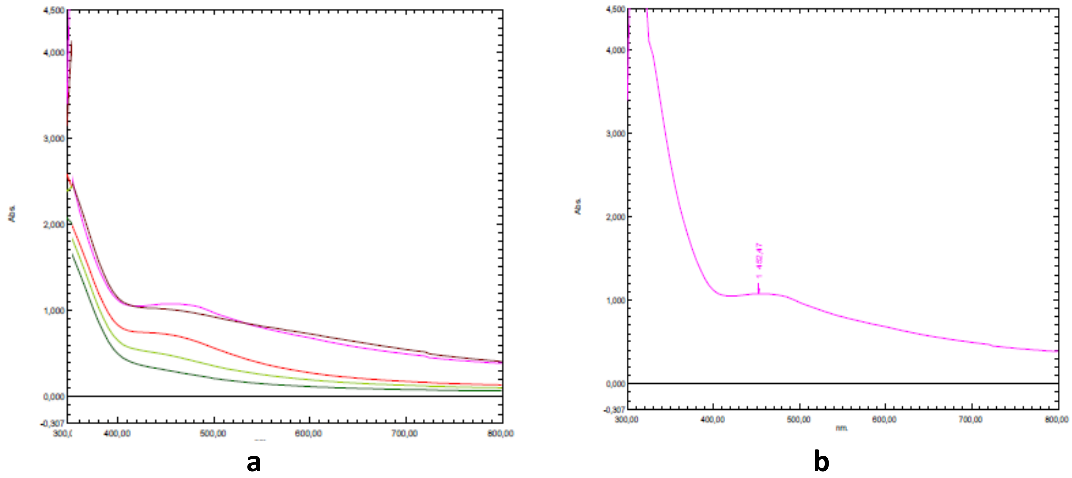

Figure 1. a. *Time-dependent formation of AgNPs in UV spectroscopy.* ***b.*** *Maximum absorbance of synthesized AgNP in UV spectrophotometry*

Functional groups involved in the reduction were evaluated via FTIR analysis. It was thought that the shifts at 3332, 2127 and 1635 cm^{-1} were caused by the active role of -OH, -CN and C = O groups in the reduction (*Fig. 2*). Similar groups were evaluated in the nanoparticle synthesis study with *Matricaria chamomilla* extract (Dadashpour et al., 2018). Other study results also support these findings (Selvakumar et al., 2018).

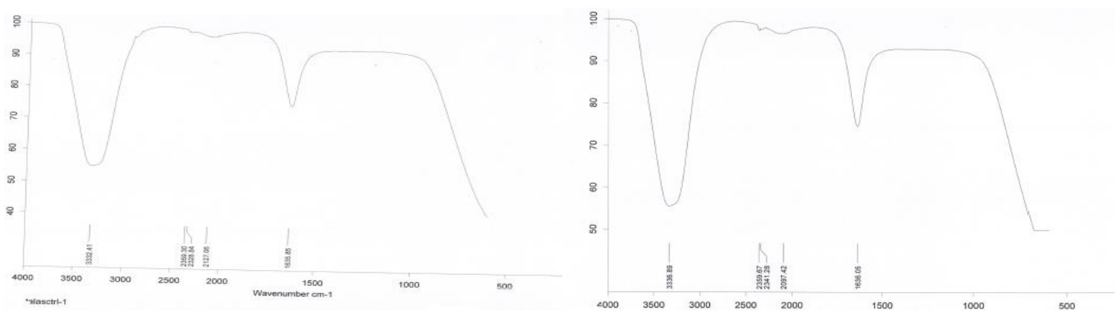

Figure 2. *UV-vis spectrum of plant extract and UV-vis spectrum of synthesized AgNPs*

The crystal structure of the obtained AgNPs particles was found by the 2θ values of 38.52°, 44.71°, 64.85° and 77.72° from the peaks which were located at (111), (200), (220) and (311) (*Fig. 3*). In the study with *Melissa officinali*, the peaks at (111), (200), (220) and (311) were associated with AgNPs (de Jesús Ruíz-Baltazar et al., 2017). The

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orientation at (111), (200), (220) and (311) were also connected to AgNPs in the nanoparticle synthesis research of *Coffea arabica* extract (Dhand et al., 2016).

The crystal dimensions of AgNPs were determined as 18.53 nm using Debye-Scherrer equation [D = Kλ / (β cos Sch)].

In other studies on the synthesis of AgNPs, the crystal dimensions of the nanoparticles were calculated using Debye-Scherrer equation (Pugazhendhi et al., 2018; Rajesh et al., 2018; Jogaiah et al., 2017).

It was seen that AgNPs were spherical in SEM images, and according to EDAX, there were peaks of Ag metal in the elemental composition (*Fig. 4*). It was reported that the AgNPs obtained in a study with *Physalis angulata* extract also have a spherical shape (Nishanthi et al., 2018). Many researchers have reported having synthesized AgNPs in spherical shapes (Premkumar et al., 2018; Alam et al., 2018; Ibrahim et al., 2016).

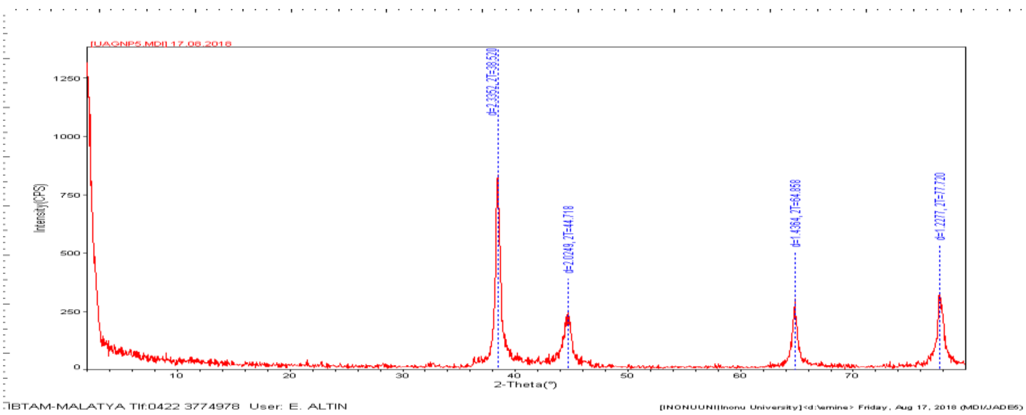

Figure 3. *X-ray diffraction (XRD) analysis*

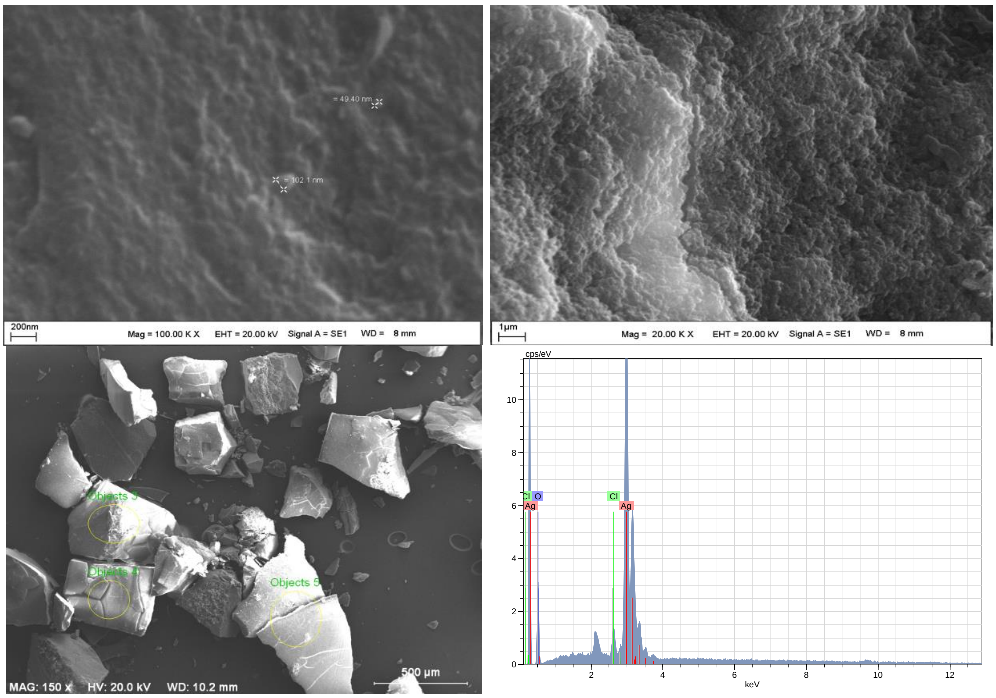

Figure 4. *SEM-EDAX results of AgNPs*

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The TGA and DTA analyses of the AgNPs were evaluated between 30-900 °C with a heating rate of 10 °C min^{-1} and a flow rate of 20 mL min^{-1} in N_2 (g) atmosphere. The TGA curve indicates the mass loss of the nanoparticles versus the temperature, and the DTA curve shows the highest decomposition temperature at all levels of the degradation (Baran et al., 2018).

The loss of mass at 30-201 °C was caused by moisture, while the loss of mass at 201-515 °C was due to phytochemicals in the plant extract and the green synthesized AgNPs were gradually degraded between 515-810 °C (*Fig. 5*).

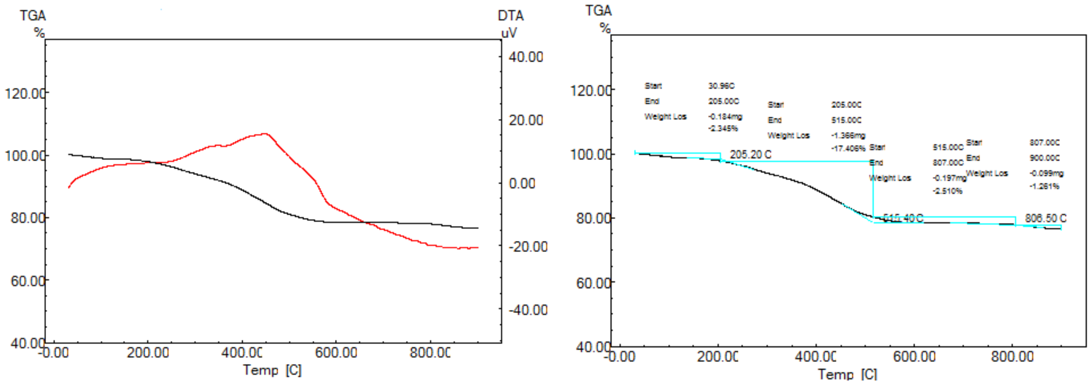

Figure 5. *TGA-DTA results of synthesized AgNPs*

The minimum inhibitory concentrations (MIC) of AgNPs on Gram-negative *Escherichia coli* ATCC 25922, Gram-positive *Staphylococcus aureus* ATCC 29213 bacteria and *Candida albicans* microorganisms were found to be 0.078, 0.314 and 0.331 µg mL^{-1}, respectively (*Table 1*). In the green synthesis and characterization of AgNPs from *Abelia grandiflora*, MIC values of Gram-negative and Gram-positive bacteria were found to be 3.12 µg mL^{-1} for *E. coli* and 12.5 µg mL^{-1} for *S. aureus* (Sharma et al., 2014).

Table 1. *MIC values of synthesized silver nanoparticles (AgNPs), silver nitrate, vancomycin, fluconazole and colistin antibiotics on S. aureus, C. albicans and E. coli*

Organism	**AgNP (µg mL^{-1})**	**Antibiotic (µg mL^{-1})**	**Silver nitrate (µg mL^{-1})**
S. Aureus ATCC 29213	0.314	0.50	0.50
E. coli ATCC 25922	0.078	0.12	1.00
C. albicans	0.331	0.06	0.50

Conclusion

In this research, synthesis of AgNPs was performed using the common grape (*Vitis vinifera*) leaf extract. It was determined that AgNPs had spherical shapes with sizes around 75 nm according to the data obtained from the SEM images, and also had a crystal size of 18.53 nm according to XRD results. FTIR results showed that the functional groups involved in the reduction were -OH, -CN and C=O. The antimicrobial effect of nanoparticles was determined to be significant on *E. coli* ATCC 25922 and *S. aureus* ATCC 29213 strains. In the light of these results, the usage of the nanoparticles

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obtained by an environmentally friendly synthesis can be improved for the medical industry due to their biocompatibility as antimicrobial agents. With their antimicrobial effect, AgNPs are thought to be effective in bioremediation of wastewater due to their rapid and efficient synthesis, as well as their being used in many areas such as shelf life extension, cosmetics, and others.

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