

Antimicrobial Activity of Silver Nanoparticles Synthesized with Extract of Tomato plant Against Bacterial and Fungal Pathogens

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Abstract

Objective: Silver nanoparticles (AgNPs) have a wide range of applications. Environmental-friendly synthesis methods for these nanoparticles are more preferable due to their various advantages. This study aimed to synthesize AgNPs using the extract of the tomato plant in an easy and economical way. and testing this AgNPs against some human pathogens.

Methods: Silver nanoparticles were synthesized using aqueous silver nitrate and reducing tomato plant extract. The characterization of AgNPs was determined by ultraviolet-visible spectrophotometry (UV-Vis), X-ray crystallography (XRD) Scanning electron microscopy (SEM), Fourier transform infrared Spectroscopy (FT-IR), energy dispersive X-ray spectrum (EDAX), thermogravimetric - differential thermal analysis (TGA-DTA) data. The effects of the particles on pathogenic microorganisms were determined by minimum inhibition concentration (MIC).

Results: These data, with a maximum absorbance of 450.51 nm, in the spherical view, with the peaks and values of 111°, 200°, 220° and 311° (38.08, 44.28, 64.42 and 77.34), AgNPs showed a cubic crystal structure and, using the Debye-Scherrer equation, it was determined that they had a crystal size of 21.11 nm AgNPs had an antimicrobial activity on hospital pathogens gram negative, gram positive and *Candida albicans* yeast.

Conclusion: We found that these particles showed antimicrobial activity on various microorganisms even at very high concentrations. As a solution to the antimicrobial search, it can be developed in medical industry.

Key words: Antimicrobial activity, XRD, SEM, TGA-DTA, Silver nanoparticle

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Introduction

Nanoparticles (NPs) are structures smaller than 100 nm. Nanotechnology is a branch of technology that examines the properties of these structures (Beyene et al., 2017). The increasing use of NPs makes these structures more important every day (Tovar-Corona et al., 2018). NPs such as gold, silver, platinum, copper, palladium etc. are widely used in a wide range of areas including personal care, clothing, cosmetics, food industry, catalysis and medical, optic and electronic industries (Lloyd et al., 1998; Song et al., 2010; Gopinath et al., 2016;

Vetchinkina et al., 2016; Prakash et al., 2018). Silver nanoparticles (AgNPs) have large surface areas and a high conductivity capacity and they can be synthesized using various biological sources (Nanda et al., 2018). In addition, AgNPs can be synthesized both physically and chemically, however this may have various disadvantages compared to biological methods. Chemical methods lead to the presence of some toxic chemicals adsorbed on the surface that may have adverse effects in medical applications (Jain et al., 2009; Baran, 2019). Phytochemicals in plant reduce Ag+ to AgO structure and provide the stability of AgNPs. (Ali et al., 2015; Saha et al., 2017). The use of colloidal silver for the treatment of diseases has been the subject of research for more than 50 years. Recent developments in the chemical, biological and material characterization techniques have allowed this subject to be explored better and silver has begun to be used more widely in the medical fields (Brandt et al., 2012). These particles have a strong antimicrobial activity thus, approximately 320 tons of AgNPs are produced every year (Gliga et al., 2014).

In the present study, an AgNP was synthesized in an easy way using the extract of the tomato plant during the autumn period and an inexpensive, simple and environmentally friendly method and the effects of this particle on various microorganisms were examined.

Methods

Preparation of the Tomato Plant Extract and Silver Nitrate Solution

The tomato plants used in this study were collected in the agricultural region of Yenisehir in the district of Diyarbakır, Turkey when they were still unripe during the autumn period. They were washed several times with tap water and then distilled water and were left to dry at room temperature. 50 gr of the plant was mixed with 500 ml distilled water and boiled. After being filtered for several times, it was kept at + 4°C for synthesis. 1 mm aqueous solution was prepared with silver nitrate (AgNO₃) of 99.8% purity was purchased from Alfa Aesar.”

Synthesis and Characterization

The extract and the AgNO₃ solution were mixed with a ratio of 1:4. The color change of the solution was monitored based on time. The formation and presence of AgNPs were observed using Perkin

Elmer UV- Vis spectrophotometer. The FTIR analysis and functional groups responsible for the reduction in the synthesis were examined with Perkin Elmer Spectrum One. After the synthesis, AgNPs were centrifuged at 10,000 rpm for 15 min with OHAUS FC 5706 model device. The bottom precipitate was washed with distilled water and dried at 75°C. The evaluation of content of the particles was carried out with Bruker-125 eV (EDX). The formation of AgNPs was examined with scanning electron microscope (SEM) EVO 40 LEQ data. The crystal structures were examined by RadB-DMAX II computer-controlled X-ray diffractometer (XRD) analysis and the crystal particle size was determined using the Debye-Scherrer equation and TGA-DTA decay temperature values were checked with Shimadzu TGA-50 device data.

Determination of the Antimicrobial Effects of AgNPs

The effects of AgNPs on pathogenic microorganisms were examined by using the micro dilution method to determine the minimum inhibition concentration (MIC). The effects of the particles were investigated on gram negative *Escherichia coli* ATCC 25922, *Pseudomonas aeruginosa* ATCC 27853 and gram positive *Staphylococcus aureus* ATCC 29213, *Streptococcus pyogenes* ATCC 19615 strains and *Candida albicans* ATCC 10231 strains. For the bacteria in the microplate wells, Mueller Hinton broth and for the fungus RPMI broth were added. The solutions prepared from AgNPs were placed on plates at the appropriate concentrations and a mixture of microorganisms was added according to MC Farland standard 0.5 concentration (Nishanthi et al., 2019) and waited for one night at 37 °C. The MIC values of Vancomycin for gram positives, colistin and flucanazol antibiotics for gram negatives and fungi, and the aqueous solution of 1 mM AgNO₃ were also investigated.

Results

After taking 500 ml from the 1 mM AgNO₃ solution and 100 ml from the plant extract and mixing 1000 ml in conical flask, a dark brown discoloration was observed after 75 min. The absorbance data of UV-Visible measurement results are given in Figure 1. The dark brown discoloration is a characteristic data showing the formation of AgNPs due to vibrations on the plasma surface and

the maximum peak value at 450.51 nm and the absorption data also support this fact (Figure 1).

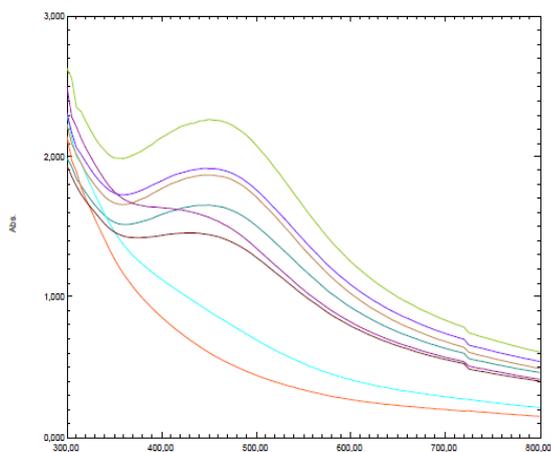


Figure 1A. Time Dependent of AgNPs in UV Visible Spectroscopy

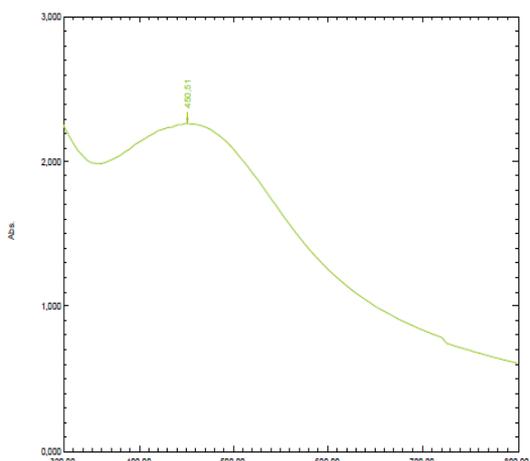


Figure 1B. Synthesis of AgNP at UV Spectrophotometry Maximum Absorbance Value

In this study, when the functional groups participating in the reduction were examined, it was determined that the shifts at 3511, 3206, 2148 and 1618 cm⁻¹ corresponded to -OH, -NH, -CN and C=O respectively (Figure 2.)

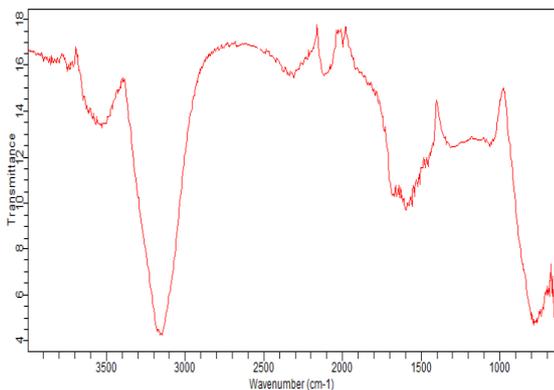


Figure 2A. FT-IR spectrum of extract spectrum.

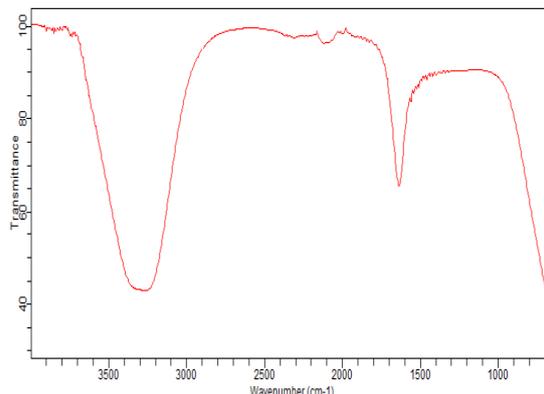


Figure 2B. FT-IR spectrum of synthesised AgNPs.

In the XRD analysis data, the peaks of 111°, 200°, 220° and 311° are the peaks that correspond to the peaks at 2θ which represent the cubic crystal structure of silver (Figure 3). The crystal size of AgNPs was calculated by the Debye-Scherrer equation given below and was found to be 22.11 nm. $D = K\lambda / (\beta \cos\theta)$

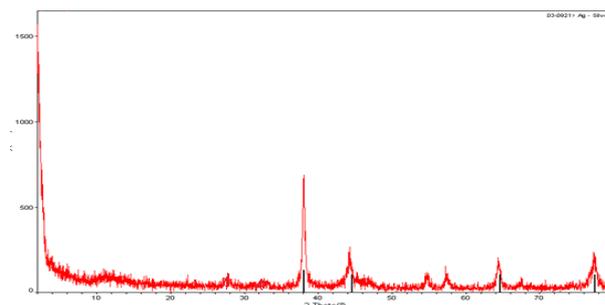


Figure 3. Investigation of crystal structure and silver phases of AgNPs by XRD analysis

The SEM results for examining the morphological views of AgNPs give us that NPs are in spherical view and have an average 52.74 nm of dimensions (Figure 4).

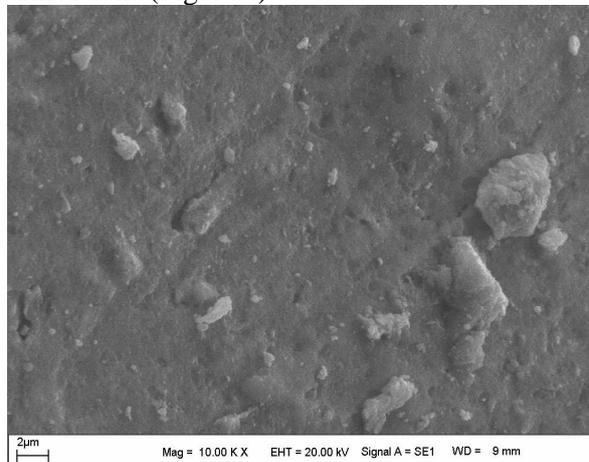


Figure 4A. Evaluation of morphology of AgNPs in SEM results A) 10,000 times magnified view of AgNPs

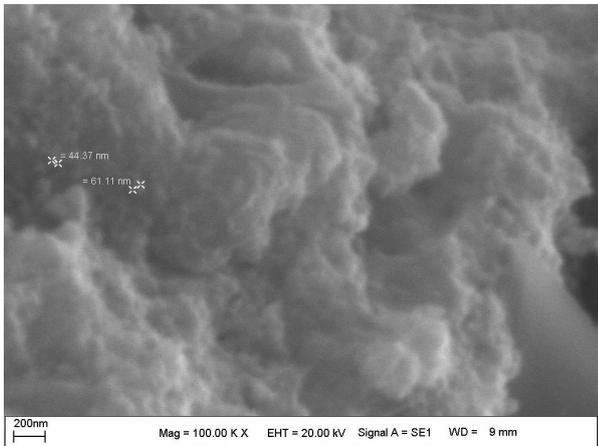


Figure 4B. Evaluation of morphology of AgNPs in SEM results 100,000 times magnified view of AgNPs

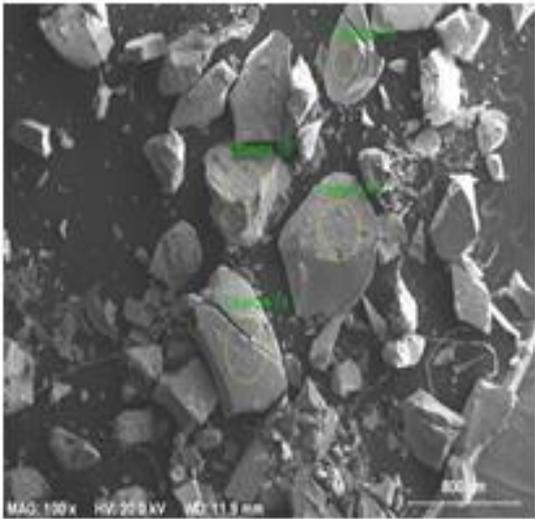


Figure 5 A. Analysis of the elemental composition by the EDX analysis of AgNPs. EDX measurement with three different points,

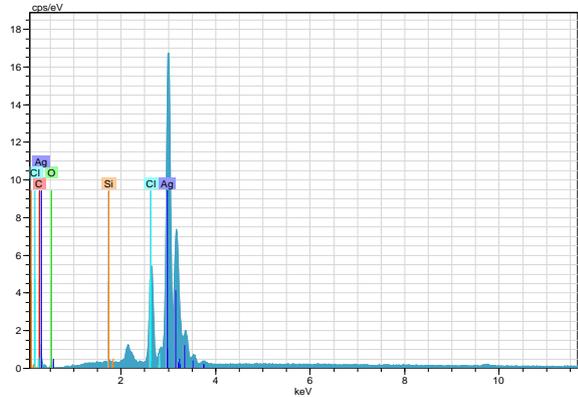


Figure 5B. Analysis of the elemental composition by the EDX analysis of AgNPs. EDX pattern showing the presence of silver

When the element content was examined according to the results of EDX (figure 5), it was found that it was mostly composed of silver the TGA-DTA curve was examined, it was thought that the mass loss of 2.24% at 12-227°C was due to the hydration by moisture and water, the mass loss of 3.8% at 227-355°C was due to phytochemicals present in the plant extract, and the mass loss of 23% at 355-846°C was due to the structure of the substance deteriorating (Figure 6). AgNPs showed a suppressive effect against microorganisms. When the AgNPs obtained in this study were compared with the antibiotic and 5 mM AgNO₃ solution, the effect of Ag NPs was examined in low concentrations (Table 1).

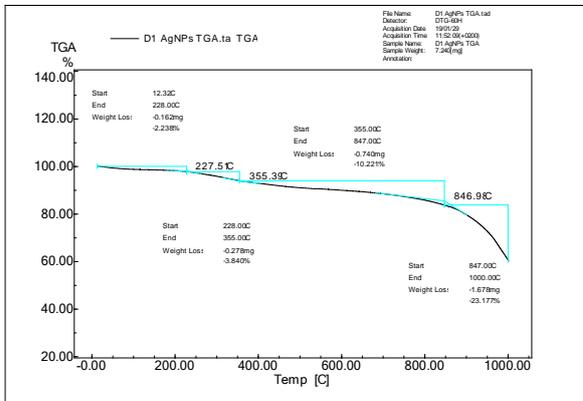


Figure 6A. TGA-DTA data of the synthesized nanoparticle. Nanoparticle TGA curve at different temperatures

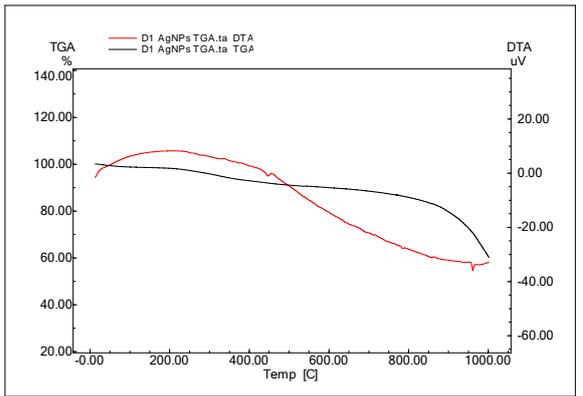


Figure 6B. TGA-DTA data of the synthesized nanoparticle. Nanoparticle DTA curve at different temperatures

Table 1. MIC values of Synthesized silver nanoparticles (AgNP) (mg mL⁻¹) on Silver nitrate solution and vancomycin, fluconazole, colistin antibiotics, *S. Aureus*, *S.pyogenes*, *S. albicans* and *E. Coli*, *P.aeruginosa* microorganisms.

	ORGANISM	AgNPs	Silver Nitrat	Antibiotic
Gram Positive	<i>S. aureus</i> ATCC 29213	0.035	2.65	1
	<i>S.pyogenes</i> ATCC 19615	0.035	1.32	1
Gram Negative	<i>E. coli</i> ATCC25922	0.017	0.66	2
	<i>P.aeruginosa</i> ATCC 27853	0.009	0.66	2
Fungi	<i>C. albicans</i>	0.018	0.66	2

Discussion

The presence of AgNPs was determined by detecting dark brown color changes by UV-Vis. The maximum absorbance values at the 345 nm wavelength in the results are the data showing the character of AgNPs. The dark brown color changes are a characteristic data showing the formation of AgNPs due to vibrations on the plasma surface (Begum et al., 2009; Prakash et al., 2013; Sinsinwar et al., 2018). Similar values found as a result of other studies conducted regarding the synthesis of AgNPs using plants are also in correlation with the results of the present study (Ferreira et al., 2018; Shao et al., 2018). The XRD results show us the peaks: 111°, 200°, 220°, 311° and 38.08, 44.28, 64.42 and 77.34. The values of these peaks indicate the characteristic of AgNPs. The results of different studies in literature interpreted these peaks as AgNPs (Sengottaiyan et al. 2016; Khan et al., 2018). In accordance with this data, the crystal size of AgNPs was calculated 22.11 nm. In similar studies, NPs were evaluated using Debye-Scherrer equation (Sagar and Ashok, 2012; Pugazhendhi et al., 2018). In this study, when the functional groups participating in the reduction were examined, it was determined that the shifts at 3511, 3206, 2148 and 1618 cm⁻¹ corresponded to -OH, -NH, -CN and C = O respectively. It was observed that the reduction of the tension in 3511 cm⁻¹ did not occur at the end of the reaction, and other functional groups were also

involved in the reduction when the FTIR images in Figure 2 were examined. These groups were evaluated in studies for the synthesis of other AgNPs (Baran, 2019). The SEM results showed that AgNPs were global in appearance. Some studies mention that AgNPs are in spherical view and have an average 52.74 nm of dimensions (Hemmati et al., 2019; Singh et al., 2017). Other studies also state that AgNPs are in global view (Kumar et al., 2016; Kobashigawa et al., 2018). When the TGA-DTA curve was examined, it was thought that the mass loss of 2.24% at 12-227°C was due to the hydration by moisture and water, the mass loss of 3.8% at 227-355°C was due to phytochemicals present in the plant extract, and the mass loss of 23% at 355-846°C was due to the structure of the substance deteriorating. In one study, the results are almost identical (Baran, 2019). Various studies in literature showed that AgNPs had a repressive effect on the reproduction of various microorganisms (Singh et al., 2017; Alsammarraie et al., 2018; Baran, 2019). AgNPs increase the formation of reactive oxygen species (ROS) by disrupting the membrane structure of the microorganisms, and negatively affect the functions of structures such as DNA and proteins, which provide other vital activities (Ahmed et al., 2018 ; Sarkar et al., 2018).

Conclusions

AgNPs are used in a wide range of areas. Due to their antibiotic resistance properties, these nanomaterials can play a key role in the search for antimicrobial agents. Due to the advantages of environmentally friendly methods, the interest in AgNPs increases every day. In this study, AgNPs were synthesized from the green parts of the tomato plant using simple, inexpensive and environmentally friendly methods. AgNPs were characterized and it was determined that, these particles had a maximum absorbance of 450.51 nm, a spherical size of 52.74 nm, a cubic crystal structure and a crystal size of 21.11 nm

It was determined that AgNPs had an antimicrobial activity on various microorganisms even at very high concentrations. In conclusion, AgNPs can be developed in the medical industry for the antimicrobial search.

Peer-review: Externally peer-reviewed.

Author Contributions: Concept - MFB, HA
Design - MFB, HA Supervision- MFB, HA ;
Materials MFB, HA ; Data Collection and/or
Processing - MFB, HA ; Analysis and/or
Interpretation - MFB, HA Literature Review -
MFB, HA Writing - MFB, HA ; Critical Review -
MFB, HA

Conflict of Interest: No conflict of interest was declared by the authors.

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