Green synthesis and antimicrobial effects of silver nanoparticles by pumpkin Cucurbita maxima fruit fiber

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Abstract

In the present study, for the first time, green synthesis of silver nanoparticles was carried out with the extract obtained from the fibrous structure in the pumpkin Cucurbita maxima (CM) fruit. The presence of silver nanoparticles formed by the reduction of Ag+ ions was determined with the maximum absorbance values of 442.89 nm in wavelength scans made by UV-Vis spectroscopy analysis. The functional groups of the phytochemicals involved in the reduction were examined by Fourier-transform infrared (FT-IR) spectroscopy. For the morphology of the synthesized AgNPs, they were found to be uniform in spherical appearance using atomic force microscopy (AFM), transmission electron microscopy (TEM), and field emission scanning electron microscopy (FE-SEM) images. The surface charges of AgNPs were determined to be -16.4 mV by Zeta potential analysis. Their crystal structures and nano-sizes were evaluated by X-ray diffraction (XRD) data to be in cubic pattern, and their size was 21.20 nm using the Debye-Scherrer equation. AgNPs (CM-AgNPs) synthesized via CM provided growth suppression at concentrations of 1.00-0.12, 2.00-1.00, and 0.50 µg/mL on pathogen gram-positive, gram-negative strains and the fungus Candida albicans, respectively. These concentrations were effective against antibiotics and silver solution at a very low concentration.

Keywords: Antimicrobial effect, CM-AgNPs, FE-SEM, TEM, UV-vis

Introduction

"Nano" means "dwarf" and "quite small" in Greek. Nanotechnology is an ever-evolving field for creating products at the nanoscale. A wide range of studies is carried out to synthesize products with dimensions less than 1 mm [1]. Nanotechnology is one of the most active fields in material sciences. It attracts increasing attention due to its contributions to life sciences, especially biomedical devices and biotechnology, in all areas of human life, which is constantly developing [2]. Green nanomedicine is of global interest for the treatment of various diseases. It is a field that deals with the necessity of medicinal plants as bio-reactants in modern nanotechnology [3].

Pumpkin (Cucurbita maxima) is a plant with documented health-promoting properties and is a source of various phytochemicals. It belongs to the Cucurbitaceae family, which consists of about 130 species both grown in the wild and cultivated all over the world [4]. Pumpkin is a vegetable rich in carbohydrates, zinc, antioxidants, and bioactive compounds [5]. C maxima is a highly nutritious fruit that can also be used in traditional medicine in some countries. In some countries, it is used as an antidiabetic, antiparasitic, and against intestinal worms. In addition, other studies have shown that it has antitumor, antihypertension, antibacterial, immune system support, and anticholesterol properties [6]. In their study, Tamer et al. found that pumpkin, along with its fiber, is a vegetable that contributes to a very healthy diet [7].

There are quite a few different methods for obtaining nanoparticles. The interest in this field is increasing day by day thanks to the advantages of green synthesis methods such as not containing toxic chemicals, being environmentally friendly, not harming human health, being simple, inexpensive, not requiring special conditions, obtaining abundant products, biocompatible, and being more stable [8–10].

The application areas of AgNPs obtained from Green synthesis methods are quite wide. They are used in many areas such as
antimicrobial and anticancer agents in biomedical applications, medicine, cellular imaging, cosmetic and food industry, optics, biological labeling, electric batteries, coating processes [11–13]. Resistance to antibiotics in the fight against pathogenic microbial agents is a serious problem on a global scale. For this, research into the search for antimicrobial agents is of great importance [14,15]. There are many studies on the antimicrobial agent uses of AgNPs [16–18].

In this study, for the first time, using the extract obtained through the fibrous structure of the pumpkin (Cucurbita maxima (CM)) fruit, it is possible to synthesize and characterize AgNPs (CM-AgNPs) in an environmentally friendly, easy, and low-cost manner, and to provide antimicrobial effects on pathogenic strains aimed to examine its effects.

Materials and Methods

Extract preparation using the fibrous structure of CM fruit

Cucurbita maxima (CM) fruit was obtained in the middle of October in Yeşil Seren village of Diyarbakır. The CM fruit was cut in half using a knife. The fibrous structure of the inside (waste part) except for the edible was removed and dried under room conditions. Then, 300 g of the dried fibrous structure was weighed and 750 ml of distilled water was mixed and boiled. Then, after cooling at room conditions and filtering, the obtained plant extract was stored in a refrigerator at +4 °C to be used in experimental studies.

Metal solution preparation

A metal solution was prepared to be used in bioreduction with a concentration of 20 millimolar (mM) using Nanokar brand silver nitrate salt.

Green Synthesis of CM-AgNPs

500 ml and 250 ml of 20 mM AgNO₃ solution of the plant extract prepared with the fibers of the CM fruit were added to a 1000 ml glass flask, and mixed. It was left to mix with the heater at 60 °C. After 60 minutes, the color change from yellow to brown was seen. To characterize the formation of CM-AgNPs, maximum wavelength scans were performed using UV-Vis spectrophotometer by sampling the reaction medium according to the intensity of color change with time.

Characterization of CM-AgNPs

CM-AgNPs synthesized from the extract obtained from the fibers of the CM fruit were confirmed on the scale of maximum absorbances in the scans made in the wavelength range of 300-800 nm using UV-Vis spectroscopy (Perkin Elmer). The frequencies of the spectra in the range of 4500-500 cm⁻¹ were evaluated to determine the functional groups of the phytochemicals responsible for the bioreduction in the CM fiber extract using the Agilent Cary 630 FT-IR device. To determine the crystal pattern and nano size of CM-AgNPs, the spectra corresponding to 2θ were examined with the XRD device in the 20-80 range (Rigaku Miniflex 600). Using these data, the crystal nano-size was calculated with the following Debye-Scherrer equation [21].

\[ D = \frac{K\lambda}{\beta \cos \theta} \]

In this equation:
- \( K \) = constant value,
- \( \lambda \) = X-ray wavelength value,
- \( \beta \) = half value of the maximum peak,
- \( \theta \) = Bragg angle of the high peak, and
- "D" indicates the crystal size of AgNPs.

Micrographs of TEM (JEM-2100 Plus, Japan), FE-SEM, (Jeol Jem. 1010), and AFM (JPK, NanoWizard, Germany) devices were examined to define the morphological appearance of synthesized CM-AgNPs. Using AFM, three-dimensional imaging of the physical properties and topographies of the surface distributions of nanostructures of CM-AgNPs with high resolution was also performed. The elemental compositions of the obtained nanoparticles were evaluated with the EDX (RadB-DMAX II) device. Zeta potential (Malvern, UK) analysis data was used to determine the surface charge of CM-AgNPs.

Evaluation of the Effects of Synthesized CM-AgNPs on Pathogen Strains Using Microdilution Technique

Antimicrobial potentials of CM-AgNPs synthesized by fiber extract, Bacillus subtilis ATCC 17714 (B. subtilis), Candida albicans (C. albicans), Staphylococcus aureus (S. aureus) ATCC 29213, Escherichia coli (E. coli) ATCC25922. Pseudomonas aeruginosa ATCC27833 (P. aeruginosa) pathogen strains were investigated by applying the microdilution technique. All microorganism strains used in this study were obtained from Mardin Artuklu University Microbiology Research Laboratory, Mardin, Turkey. Appropriate nutrient media were used for the growth of microorganisms. Nutrient agar media for bacteria and Sabo dextrose agar media for fungus C. albicans were prepared; microorganisms were transferred to these media and incubated for 24 hours at 37 °C. Then, solutions were prepared for each of the microorganisms grown on the medium plates with the Mc Farland standard 0.5 turbidity criterion value [22,23]. Muller Hinton broth for bacteria and C. albicans yeast were transferred from Roswell Park Memorial Institute (RPMI) 1640 medium in appropriate amounts to 96-well microplate wells. Various concentrations of solutions containing CM-AgNPs were prepared and pipetted into the wells. Then, dilution was applied to the microplates, and CM-AgNPs were distributed to the previously left media. After these procedures, appropriate amounts of microorganism solutions prepared for bacteria and fungi were transferred to the wells. Microplates were left to interact with CM-AgNPs in an oven at 37 °C for 24 hours.

Results

UV-Vis Spectrophotometry Data of CM-AgNPs

One hour after mixing the fiber extract of CM fruit and 20 millimolar (mM) AgNO₃ solutions, a color change from yellow to dark brown was observed in the color of the solution. The presence of maximum absorbance bands at a wavelength of 442.89 nm was evaluated in the UV-vis. measurements made with the samples taken due to the color change (Figure 1).
Morphological structures of CM-AgNPs were determined in the images taken on FE-SEM and TEM devices (Figure 2A-B). Micrographs showed that CM-AgNPs were spherical in appearance and exhibited a monodisperse. In the particle measurement of the TEM images, it is shown in the TEM micrograph in Figure 2A that the CM-AgNPs have an average size of 4.41-9.26 nm.

When the EDX profile element compositions of the nanoparticles synthesized with the extract obtained from the fibers of the CM fruit were evaluated (Figure 2B), it was determined that 98% of the nanoparticles belonged to the element silver with strong silver peaks. It was observed that the 1.91% low chlorine (Cl) peak was reflected in the profile in the presence of it.

**XRD Data of CM-AgNPs**

In the XRD analysis performed to evaluate the crystal nano sizes and patterns of CM-AgNPs synthesized by bioreduction, peaks taken at 111°, 200°, 220°, and 311° in measurements made at 2θ and their values of 38.23, 44.05, 64.58, and 77.68 were determined, respectively (Figure 3). The crystal nanosize of CM-AgNPs synthesized by the Debye sherer formula was calculated as 21.20 nm.
Figure 3. XRD diagram of crystal patterns of synthesized CM-AgNPs

Zeta Potentials of Surface Charges of CM-AgNPs

Zeta potential analysis data were used to determine the mobility, distribution, and stability of CM-AgNPs synthesized with the fiber extract of CM fruit due to surface charges. It is shown in figure 4 that the synthesized CM-AgNPs exhibited a surface charge distribution of -16.4 mV.

Figure 4. Zeta potential graph showing the surface charge distributions of CM-AgNPs

FT-IR Spectrophotometry data of Groups Responsible for Bioreduction of CM-AgNPs

The FT-IR diagram of the functional groups responsible for the bioreduction of the Ag+ form to the Ago state of the phytochemicals in the CM fruit fiber extract is given in Figure 5. When the FTIR spectra were evaluated, it was observed that there were changes due to frequency shifts on three main functional groups. These three points belong to the frequencies at 3365-3243 cm⁻¹, 2306-2119 cm⁻¹, and 1636-1635 cm⁻¹, and these groups showed hydroxyl groups, amine groups, and carbonyl groups, respectively (Figure 5).

Figure 5. FT-IR spectrophotometry data, the fiber extract of CM fruit (A), and the dark liquid taken at the end of the synthesis (B)

Morphologies and Topographic Distributions of CM-AgNPs

Topography and phase-contrast images of the synthesized CM-AgNPs were acquired using an Innova SPM Atomic Force Microscope. In the image given in Figure 6, it was seen that CM-AgNPs exhibited spherical morphology, monodisperse, and size distribution below 50 nm.

Figure 6. AFM micrograph showing the morphological and topographic distributions of CM-AgNPs

Antimicrobial Effects of CM-AgNPs

The effects of CM-AgNPs synthesized with the extract of the fibrous structure in the CM fruit on pathogenic microorganisms were determined by using the micro-dilution technique to determine the effective MIC on their growth. MIC values effective on pathogenic strains were defined as 0.12-2.00 µg mL⁻¹. The effect of CM-AgNPs at the lowest concentration was determined by the MIC value of 0.12 µg mL⁻¹ on the growth of gram-positive *B. subtilis*. This MIC value showed that CM-AgNPs were more effective at a very low concentration than the antibiotic and AgNO₃ solution. The highest concentration was on gram-negative *E. coli* growths with a MIC value of 2.00 µg mL⁻¹. It was determined that the MIC value effective on *E. coli* was two times lower than the antibiotic and AgNO₃ solution. It was determined that CM-AgNPs were effective on *P. aeruginosa* bacteria at four times lower concentrations than the antibiotic and at two times lower concentrations than the AgNO₃ solution. It was observed that CM-AgNPs had a higher effect on *S. aureus* than the antibiotic and two times lower concentration than the AgNO₃ solution, and also showed that a concentration of 0.50 µg mL⁻¹ of CM-AgNPs on the growth of *C. albicans* was lower than the antibiotic and had the same effect as the AgNO₃ solution (Table 1 and Figure 7).

Figure 7. Shape view of MIC values of CM-AgNPs
Table 1. MIC values of CM-AgNPs, AgNO₃ solution, and antibiotics (Fluconazole, Vancomycin, and Colisitin for fungi, gram-positive and negative microorganisms, respectively) synthesized on the growth of pathogenic strains

<table>
<thead>
<tr>
<th>ORGANISM</th>
<th>CM-AgNPs µg/mL</th>
<th>AgNO₃ Solution µg/mL</th>
<th>Antibiotic µg/mL</th>
</tr>
</thead>
<tbody>
<tr>
<td>B. subtilis</td>
<td>1.00</td>
<td>2.00</td>
<td>0.50</td>
</tr>
<tr>
<td>S. aureus</td>
<td>0.12</td>
<td>1.00</td>
<td>2.00</td>
</tr>
<tr>
<td>P. aeruginosa</td>
<td>2.00</td>
<td>4.00</td>
<td>4.00</td>
</tr>
<tr>
<td>E. coli</td>
<td>1.00</td>
<td>2.00</td>
<td>4.00</td>
</tr>
<tr>
<td>C. albicans</td>
<td>0.50</td>
<td>0.50</td>
<td>1.00</td>
</tr>
</tbody>
</table>

Discussion

In Figure 1, the formation of yellow-brown color transformation 60 minutes after the combination of the fiber extract and the metal solution of the CM fruit [24–26], and absorbance values of 442.89 nm obtained in UV-vis spectrophotometry maximum wavelength scans, the formation of CM-AgNPs synthesized and showed its presence. In a study conducted, the extracts of Punica granatum, Cydonia oblonga, Castanea sativa, Ficus carica, Juglans cinerea, and Morus nigra were determined at the maximum absorbances of 422.0 nm, 430.5 nm, 440.0 nm, 442.5 nm, 442.5 nm, 445.0 nm, and 451 nm, respectively. It has been noted that characteristic data are indicating the presence of AgNPs [27]. In a green synthesis study using Euphorbia longana Lam. extract, the maximum absorbance measured at 445 nm showed the presence of AgNPs [28]. The color change from yellow to dark brown and the maximum absorbances taken in the wavelength range of 400-500 nm in the UV-vis spectra depend on the Surface Plasma Resonance (SPR) bands formed by the vibrations on the plasma surface due to the formation of AgNPs [29].

In Figure 5, the FT-IR spectra of the functional groups responsible for the bioreduction of the Ag⁺ form to the Ag⁻ state were evaluated. It was observed that there were changes due to frequency shifts on three main functional groups. These three points showed that hydroxyl groups, amine groups, and carbonyl groups at 3365-3243 cm⁻¹, 2306-2119 cm⁻¹ and 1636-1635 cm⁻¹, respectively, cause bioreduction [1,26,30].

TEM, FE-SEM, and AFM micrographs were evaluated to evaluate the morphological appearance of the synthesized CM-AgNPs. In Figure 2A-B, CM-AgNPs showed spherical appearance and mono size distribution below 50 nm in TEM and FE-SEM images, in addition, in figure 6 the same morphological structure and size distribution were observed in AFM micrography. In the green synthesis studies, it was observed that AgNPs exhibited spherical morphology in TEM and SEM images [2,10,31–35]. AFM studies for the evaluation of topographic structures and morphologies of AgNPs revealed similar findings for unidirectional distribution, spherical morphology, and size distribution [29,35,36].

Zeta potential analysis data for the evaluation of the surface structure and stability of the synthesized CM-AgNPs showed an average surface charge of -16.4 mV (Figure 4). In the studies, it was seen that the surface charges of AgNPs were -13.1 mV [22], -19.9 mV [37] -14 mV [38], and -23 mV [34]. The negative surface charges of AgNPs are due to phytochemicals The fact that the surface charge of AgNPs is only negative enables them to exhibit a stable structure by getting ahead of the conditions that negatively affect the stability such as aggregation and fluctuation [13,17,26]. In addition, having only a negative surface charge is effective in interacting with living organisms such as positively charged bacteria and fungi. This contributes to their biocompatibility.

The crystal pattern of the synthesized CM-AgNPs was determined by the XRD obtained from the 2θ given in figure 3. It was seen that the four faces of the face-centered cubic (FCC) crystal structure of CM-AgNPs correspond to 111° 200°, 220°, and 311° Bragg’s angles on the reflection plane. The FCC characteristic cubic pattern structure of AgNPs was revealed in synthesis studies with extracts of Acanthophora spicifera, Morus indica L. [38], chitosan [39], and Raphanus sativus [17].

Table 1 and figure 7 showed that CM-AgNPs synthesized with the pulp of the fibrous structure in CM fruit had a significant effect on the growth of pathogenic microorganisms. Using the microdilution method, the MIC values on the growth of pathogenic strains were determined to be in the range of 0.12-2.00 µg mL⁻¹. Effective suppression at the lowest concentration was on B. subtilis, and effective suppression at the highest concentration was on E. coli. MIC values showed that CM-AgNPs was effective at a very low concentration than antibiotic and AgNO₃ solution. It also showed a lower amount of inhibition on the growth of C. albicans with a concentration of 0.50 µg mL⁻¹ than the antibiotic. In a green synthesis study, AgNPs synthesized using Fritillaria flower extract had a suppressive effect on the growth of E. coli, P. aeruginosa, and S. aureus, and B. subtilis bacteria at concentrations of 4.00, 1.00, 4.00, and 1.00 µg mL⁻¹, respectively. In another similar study, it was stated that AgNPs synthesized with Camellia sinensis extract were effective in suppressing the growth of E. coli, P. Aeruginosa, and S. aureus bacteria at concentrations of 250, 15, and 30 µg mL⁻¹ [40].

Conclusion

In this study, for the first time, CM-AgNPs were synthesized in an environmentally friendly, low-cost, and easy way by using the extract obtained from the non-edible fibrous part of Cucurbita moschata fruit. In the FTIR spectrum, where the functional groups of phytochemicals responsible for bioreduction are alcohol, amines, and carbonyl groups, stretching vibrations of the peaks occurring in these groups are shown. The properties of CM-AgNPs were characterized by UV-vis, TEM, FES-EM, AFM, EDX, and zeta potential analyses. It was determined that CM-AgNPs have spherical morphology, maximum absorbance at 442.89 nm wavelength, 21.20 nm crystal nano size, -e MV surface charge. It was determined by the microdilution method that CM-AgNPs were effective on the growth of pathogenic strains at very low concentrations between 0.12-2.00 µg mL⁻¹. It is thought that when the synthesis steps of synthesized environmentally friendly CM-AgNPs with biocompatible properties are developed, they will greatly contribute to the search for antimicrobial agents for antibiotic resistance on a global scale.

Conflict of interests
The authors declare that there is no conflict of interest in the study.

Financial Disclosure
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No ethics committee is required for this research article. Because the study does not require an ethics committee.

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