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Original article

Rapid green synthesis of gold nanoparticles: synthesis, characterization, and antimicrobial activities

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Abstract. In this research, an easy and environmentally friendly method was presented for the biosynthesis of gold nanoparticles (AuNPs) with *Allium ampeloprasum* (AA) leaf extract as a reducing and stabilizing agent. The maximum absorption peak was found to be around 535 nm with ultraviolet (UV)-visible spectrophotometer. When the Transmission Electron Microscopy (TEM), Emission Scanning Electron Microscopy (FE-SEM) and energy dispersive X-ray (EDX) analyzes were examined, it was seen that the crystal size of the synthesized AuNPs was between 6.02-17.36 nm and their shape was mostly spherical. The size of the crystal structures of AuNPs was calculated as 22.76 nm from the X-ray diffraction (XRD) analysis data. Fourier Transform Infrared (FT-IR) Spectroscopy results showed that phenolics, aromatic compounds and proteins were effective in the reduction and stabilization of AA-AuNPs. The average size of AuNPs was determined as 96.46 nm with Zetasizer. It was determined that AuNPs have a very strong inhibitory effect on pathogenic bacteria (*Escherichia coli, Staphylococcus aureus, Bacillus subtillis* and *Pseudomonas aeruginosa*) and *Candida albicans* by the minimum inhibitory concentration (MIC) method.

Key words: TEM, FE-SEM, FT-IR, XRD, AuNPs, MIC

Introduction

In the last quarter century, the synthesis and characterization of metal nanoparticles gained much attention compared to their larger sized counterparts. During this period, scientific studies on the synthesis of metal nanoparticles from natural sources such as plants (1), fungi (2), algae (3), bacteria (4), and viruses (5) increased. Especially AuNPs are among the important nanomaterials due to their oxidation resistance, stability and biocompatibility (6). AuNPs can be used in food packaging (7), biomedical (8), drug delivery, diagnostics, imaging (9), and cosmetics (10).

AuNPs are generally synthesized by chemical methods using stabilizing/reducing reagents such as sodium citrate, hydrazine, dimethyl formamide, sodium borohydride, ascorbic acid and folic acid (11, 12). However, many of the chemical methods use harmful chemicals and toxic capping agents. Moreover, these methods are costly (13). To eliminate these disadvantageous situations, "green synthesis" studies have intensified. To date, it has been reported that AuNPs have been synthesized by many plants such as *Cannabis sativa* (14), *Zingiber officinale* (15), *Trigonella foenum-graecum* (16), *Scutellaria barbata* (17) and *Artemisia absinthium* (18).

In this study using an environmentally friendly method, aqueous $AuCl4^-$ ions were reduced by the extract of AA leaf. Thus, aqueous synthesis and stabilization of AuNPs were achieved. To our knowledge, there is no documentation regarding the biosynthesis of AuNPs using AA. AA, which belongs to the Alliaceae family, can be cooked and used in meals, as well as for therapeutic purposes due to its lipid-lowering, antidiabetic, anti-asthmatic, antiplatelet, anticancer and anti-atherosclerotic effects (19).

In this article, we presented our research on the green synthesis, characterization and antimicrobial properties of AuNPs from AA leaf.

Materials and Methods

Materials

The AA required for the synthesis of AuNPs was purchased from the market. Tetrachloroauric acid ($HAuCl_4.3H_2O$) was purchased from Alfa Aesar. Fluconasole, vancomycin, and colistin antibiotics were purchased from Sigma Aldrich. *Escherichia coli* ATCC 25922, *Staphylococcus aureus* ATCC 29213, *Pseudomonas aeruginosa* ATCC27833, *Bacillus subtilis* ATCC 11774 and *Candida albicans* were used for the antimicrobial activities of AuNPs in the study.

Preparation of Allium ampeloprasum Leaf Extract

The green leaves of the AA were washed thoroughly with distilled water and dried under room conditions. 25 g of dried leaves were taken and mixed with 250 ml of distilled water and boiled. The exctract was cooled to room temperature. Then, it was filtered with Whatman No. 1 filter paper and stored in the refrigerator for the synthesis of AuNPs.

Synthesis of AuNPs

1 mM gold aqueous solution was prepared from the solid form of tetrachloroauric acid ($HAuCI_4.3H_2O$). 30 ml of AA leaf extract and 300 ml of 1mM $HAuCl_4.3H_2O$ were mixed in a beaker and allowed to react stably at room temperature. The color change was observed within 30 minutes and the resulting dark solution was centrifuged (14000 rpm / 10 min). The solid that remained at the bottom was washed several times with distilled water. The obtained AuNPs were left to dry in an oven at 80 °C for 48 hours. Then the dry part was ground into powder by a mortar and pestle.

Characterization of AuNPs

The Agilent CARY 60 UV-visible spectrophotometer was used to confirm the synthesis of AuNPs by scanning the reaction mixture at wavelengths between 300-800 nm. The morphology, size, crystal structure and surface distributions of AuNPs were analyzed by FE-SEM (Quanta FEG250), TEM (Quanta), EDX (Quanta FEG 250), XRD (Rigaku Ultima IV) and Zetasizer (Malvern Instruments Ltd). The crystal size of AuNPs was calculated according to the Debye-Scherrer equation ($D = K\lambda/(\beta \cos\theta)$) (20). FT-IR (Perkin Elmer Spectrum 100 FT-IR) was used to determine the functional groups in the plant extract that play a role in the reduction at the end of the reaction.

Antimicrobial Activity of AuNPs

MICs of the sythesized particles on gram-negative (*Escherichia coli* ATCC 25922, *Pseudomonas aeruginosa* ATCC27853) and gram-positive (*Staphylococcus aureus* ATCC 29213, *Bacillus subtilis* ATCC 11774) bacteria and *Candida albicans* were determined by microdilution method using a 96-well microtiter plate. Mueller Hinton Broth for bacteria and RPMI (Growth Medium Used in Cell Culture) medium for yeast were added to these wells. AuNPs solution was added to the microplates containing the medium and microorganisms. 100 µL was taken each time and transferred to the next well. Then, a certain amount of microorganism solutions prepared and adjusted according to 0.5 McFarland was added to the microplates. It was incubated at 37 °C for 24 hours. The lowest concentration without growth after incubation was determined as the MIC value (21, 22). In addition, commercial antibiotics fluconazole, vancomycin and colistin and 1 mM $HAuCI_4.3H_2O$ solution were used to compare the antimicrobial effects of AuNPs on *S. aureus, B. subtilis, E. coli, P. aeruginosa* and *C. albicans.*

Results and Discussion

Depending on their size and density, AuNPs exhibit dark purple color in aqueous media due to surface plasmon resonances (23). In this context, UV-vis. Spectroscopy is an important method that reveals the formation and stability of AuNPs in an aqueous solution by color change (23). It is seen that the UV-vis spectra of AuNPs change from black to dark purple (Figure 1).

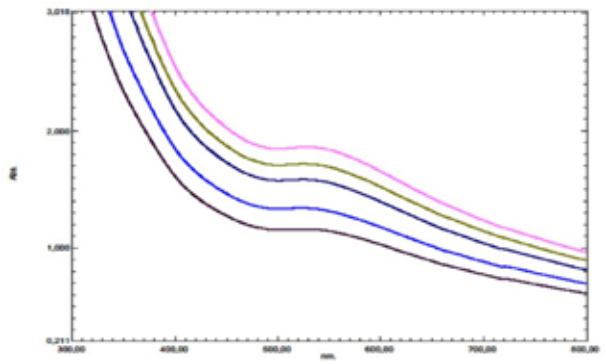

Figure 1. UV-visible absorption spectra of *Allium ampeloprasum* -AuNPs

As seen in the figure, the maximum absorption peak of AuNPs was around 535 nm. Patra et al. (23), Chandran et al. (23) and Kumar et al. (6) also reported peaks in the range of 535-540 nm for AuNPs.

According to the EDX data, it was confirmed that the sythesized nanoparticles had gold in their composition (Figure 2). In other words, the strong signal from gold atoms indicates successful sythesis of AuNPs. It can be said that the other peaks seen in Figure 2 are caused by phytochemicals from the AA leaf extract. It was reported that similar results were obtained in green synthesis studies with *Croton Caudatus Geisel*, *Cymbopogon citratus* and *Pistia stratiotes* leaf extracts (6, 25, 26). In this EDX profile, signals such as oxygen and carbon may have originated from organic biomolecules or phenolic compounds on the surface of nanoparticles (27, 28).

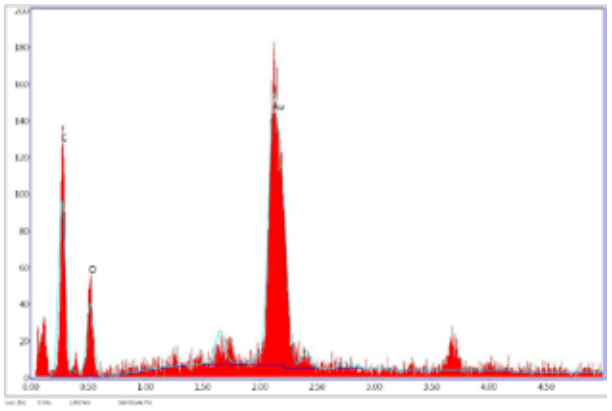

Figure 2. The elemental composition of AuNPs by the EDX analysis

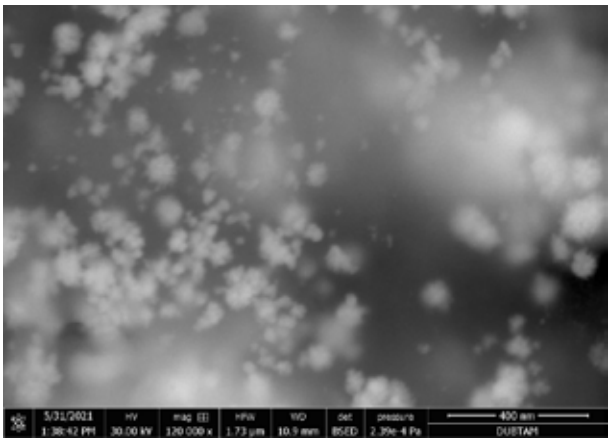

Figure 3. FE-SEM image of AuNPs capped with *Allium ampeloprasum* leaf extract

Particle size distribution and surface morphology of AuNPs were revealed by FE-SEM and TEM measurements (Figure 3 and Figure 4). Images from both devices confirmed that the biosynthesized gold particles had nanoscales. It was seen that AuNPs were in clusters but not in direct contact with each other. This indicates the stabilization of AuNPs. TEM images proved that the gold particles were predominantly

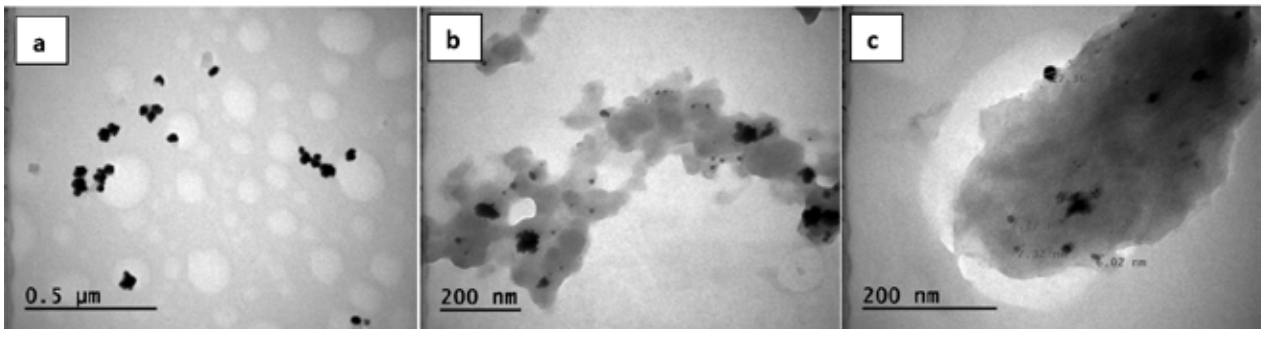

Figure 4. TEM images of AuNPs capped with *Allium ampeloprasum* leaf extract

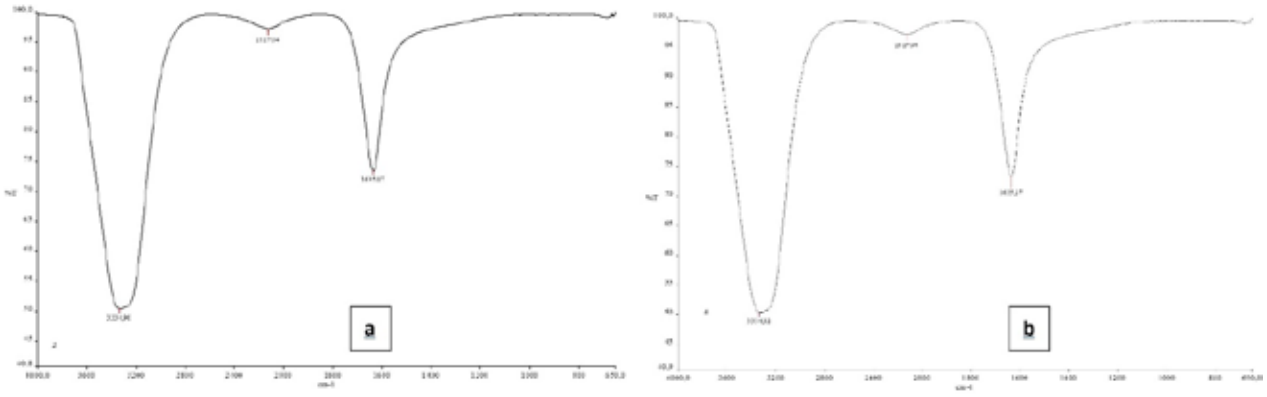

Figure 5. FT-IR spectra of *Allium ampeloprasum* extract (a) and (b) synthesized *Allium ampeloprasum*-AuNPs

spherical in shape (Figure 4). Similar results were reported in the studies of other researchers (1, 12, 29, 30). The sizes of AuNPs ranged from 6.02 to 17.36 nm, with an average of 9.49 nm (Figure 4).

Figure 5 presents a comparison of the FT-IR spectra for the aqueous AA extract (Figure 5a) and the synthesized AuNPs (Figure 5b). The FT-IR spectra of biosynthesized AuNPs demonstrated peaks at 3334.82 cm^{-1}, 2127.85 cm^{-1} and 1635.36 cm^{-1} belonging to the OH/NH, C-C and CAC/NAH groups, respectively. This suggests that functional groups such as proteins, phenolics and aromatic compounds in the plant extract are probably responsible for the reduction and stabilization of AuNPs (12). In some studies supporting the data of this research, NH and/or OH, (C=O)NH2 groups were reported to be responsible for the reduction (18, 31, 32, 33).

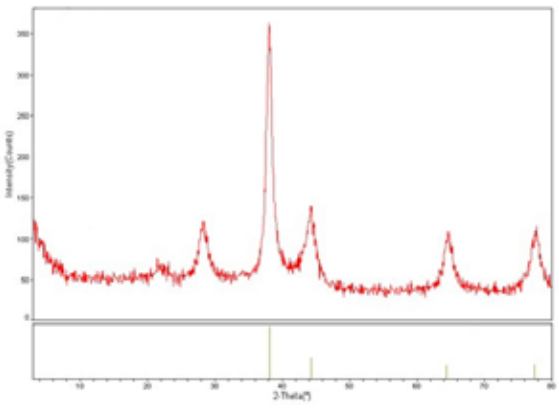

Figure 6. X-ray diffraction patterns of *Allium ampeloprasum*-AuNPs

According to the XRD spectrum pattern for the synthesized AuNPs (Figure 6), the peaks of 38.17°(111), 44.06°(200), 64.31°(220) and 77.24°(311) corresponding to 2θ were found to have face-centered cubic crystal geometry of metallic gold (6, 29). The size of the nanomaterials was calculated as approximately 22.76 nm using the Debye-Scherrer equation. On the other hand, the average size of AuNPs was determined as 96.46 nm by Zetasizer (Figure 7).

The antimicrobial effects of AuNPs become more important as pathogenic microorganisms develop resistance to the antibiotics used. According to the results of the study (Table 1), AA-AuNPs were found to be highly effective on *Escherichia coli, Bacillus subtilis, Staphylococcus aureus, Pseudomonas aeruginosa* and *Candida albicans*, which are common in foodborne disease (34-38). AuNPs showed a significant inhibition effect on pathogens at lower concentrations compared to $HAuCl_4 3H_2O$ and antibiotics. AuNPs (0.0612 mg/mL) were found to be much more effective than $HAuCl_4 3H_2O$ and antibiotics, especially on *Staphylococcus aureus*. This study revealed that the synthesized AuNPs

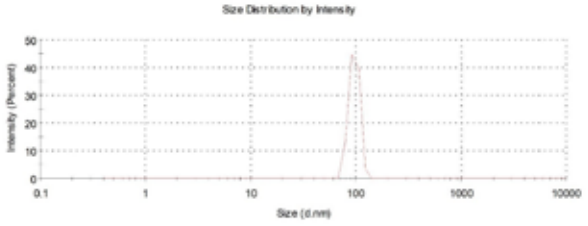

Figure 7. Size distribution of AuNPs by density

Table 1. MIC values of synthesized AA-AuNPs, HAuCI4.3H2O and antibiotics (mg mL-1)

Microorganisms	AA-AuNPs	$HAuCI_4.3H_2O$	Standard Antibiotics
Staphylococcus aureus ATCC 29213	0.0612	0.5	1
Bacillus subtilis ATCC 11774	0.25	0.25	1
Escherichia coli ATCC25922	0.50	1.0	2
Pseudomonas aeruginosa ATCC27833	1.0	1.0	1
Candida albicans	0.125	0.5	2

were effective not only at lower concentrations but also in a wider bacterial spectrum range. The antimicrobial mechanism of AuNPs is explained as membrane, cell wall, mitochondrial and ribosome damage and causing cell death by forming a thiol group in bacterial cells (39). Although AuNPs significantly suppress the proliferation of these bacteria (40), it is reported that AuNPs are more resistant to fungal species than bacteria (41).

Conclusion

In this research, stable gold colloids, most with spherical NPs, were synthesized using an aqueous extract of AA leaf. This method is simple, economical, environmentally friendly and efficient. UV-vis absorption, EDX and XRD analyzes confirmed the synthesis of AuNPs. FE-SEM and TEM studies revealed that AuNPs were predominantly spherical in shape and had an average size of about 9.49 nm. From the FT-IR spectrum, it was found that the capping material responsible for reduction and stabilization were the proteins, phenolics and aromatic compounds present in the extract. It was determined that the obtained particles had strong antibacterial and anticandidal activity even at low concentrations.

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