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Original article

Green synthesis of gold nanoparticles from *Prunus cerasifera pissardii nigra* leaf and their antimicrobial activities on some food pathogens

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Abstract. In this study, a new and easy method for the biosynthesis of gold nanoparticles (AuNPs) with *Prunus cerasifera pissardii nigra* (PC) leaf extract as a reducing and stabilizing agent was presented. The nanoparticles were demonstrated a characteristic peak at the maximum wavelength of 535 nm with colour change as a result of the ultraviolet (UV)-visible spectrophotometer analysis data. Emission Scanning Electron Microscopy (FE-SEM), Transmission Electron Microscopy (TEM) and energy-dispersive X-ray spectroscopy (EDX) analyzes revealed that the crystal size of the synthesized AuNPs was below 20 nm and the morphological structure was mostly spherical. The size of the crystal structures of AuNPs was calculated as 17.94 nm from the X-ray diffraction (XRD) analysis data. Fourier Transform Infrared (FT-IR) Spectroscopy results confirmed the involvement of various biomolecules in the reduction and stabilization of PC-AuNPs. The zeta potential of the synthesized nanomaterial was measured as -27 mV. The average size of AuNPs was determined as 103.8 nm with Zetasizer. It was determined that AuNPs have strong inhibitory activity against *Escherichia coli, Staphylococcus aureus, Bacillus subtillis* and *Pseudomonas aeruginosa* and *Candida albicans.*

Key words: AuNPs, FE-SEM, TEM, EDX, XRD, MIC, Antimicrobial effect

Introduction

Scientific efforts to synthesize metal nanoparticles from natural sources have increased. For this purpose, many studies have been carried out on metal nanoparticles such as silver (1), platinum (2), iron (3), zinc oxide (4) and gold (5). Especially AuNPs attract great attention due to their oxidation resistance, easy synthesis and biocompatibility (6-9). AuNPs can be used in biomedicine (10), drug delivery, diagnostics, imaging (11), food packaging (12), and cosmetics (13).

AuNPs are generally reduced with stabilizing/reducing reagents such as hydrazine, sodium citrate, sodium borohydride, dimethyl formamide, folic acid and ascorbic acid, and then synthesized by surface modification with suitable capping ligands to prevent spontaneous aggregation (14-16). However, many of the traditional methods use environmentally harmful chemicals and toxic capping agents. In addition, these methods are costly (17). To eliminate these negative situations, "green synthesis" studies in which metal nanoparticles are synthesized from plants (18), algae (19), fungi (20), bacteria (21) and viruses (22) have intensified in recent years.

In this study, a new and easy method for the biosynthesis of AuNPs with PC leaf extract as a reducing and stabilizing agent was introduced. To our knowledge, there is no documentation on the biosynthesis of AuNPs using PC. PC, also known as cherry plum, is a plant native to Southeastern Europe (Balkan Peninsula, Crimea), Western and Central Asia (Caucasus, Iran, Iraq). PC is a thorny shrub tree

with sphere-shaped, yellow/red/purple-colored fruits. Its young leaves are deep purple. When ripe, the leaves turn a dark green color (23-26).

It was presented research on green synthesis, characterization and antimicrobial properties of AuNPs from PC leaf in this article.

Materials and Methods

Materials

Green leaves of PC were collected from Mardin province in southeast Turkey. Tetrachloroauric acid ($HAuCl_4.3H_2O$) was purchased from Alfa Aesar. Vancomycin, fluconazole and colistin antibiotics were purchased from Sigma Aldrich. *Escherichia coli* ATCC 25922, *Pseudomonas aeruginosa* ATCC27853, *Staphylococcus aureus* ATCC 29213, *Bacillus subtilis* ATCC 11774 and *Candida albicans* were also used in the study for the antimicrobial activities of AuNPs.

Preparation of Plant Extract

The green leaves of the PC plant were thoroughly washed with distilled water and dried under room conditions. 25 g of dry leaves were boiled in 500 ml of distilled water for 15 minutes. The extract was cooled to room temperature. Then, it was filtered with Whatman No. 1 filter paper and stored at +4 ˚C.

Synthesis of AuNPs

For the synthesis of AuNPs, 1 mM gold aqueous solution was prepared from the solid form of $HAuCI_4.3H_2O$. 30 ml of PC leaf extract and 300 ml of $HAuCl_4.3H_2O$ 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 at 10000 rpm for 10 minutes. The solid that remained at the bottom after centrifugation 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 using a mortar and pestle.

Characterization of AuNPs

Agilent CARY 60 UV-visible spectrophotometer was used to confirm the synthesis of AuNPs by scanning the reaction mixture at wavelengths between 250-800 nm. The morphology, size, crystal structure and surface distributions of AuNPs were analyzed by FE-SEM (Quanta FEG240), TEM (Quanta), XRD (Rad B-DMAX II), EDX (Quanta FEG 240) and Zetasizer (Malvern Ins.Ltd.). FT-IR (Agilent Cary 630) was used to determine the functional groups present in the plant extract and the functional groups responsible for the reduction at the end of the reaction.

Antimicrobial Activity of AuNPs

The minimum inhibitory concentrations (MICs) of the resulting particles on gram-negative *(Escherichia coli* ATCC 25922, *Pseudomonas aeruginosa* ATCC27853) and gram-positive bacteria (*Staphylococcus aureus* ATCC 29213, *Bacillus subtilis* ATCC 11774) and *Candida albicans* were determined by microdilution using a 96-well microtiter plate. Mueller Hinton Broth for bacteria and RPMI (Growth Medium Used in Cell Culture) for yeast were added to the 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 (27, 28). Moreover, a 1 mM $HAuCI_43H_2O$ solution with commercial antibiotics vancomycin, colistin and fluconazole was used to compare the antimicrobial effects of AuNPs on *S. aureus, B. subtilis, E. coli, P. aeruginosa* and *C. albicans*.

Results and Discussion

UV-vis. Spectroscopy is an important method to determine the formation and stability of metal nanoparticles in an aqueous solution (29). It is seen that the UV-vis spectra of AuNPs change from light yellow to dark purple (Figure 1). These color changes occur

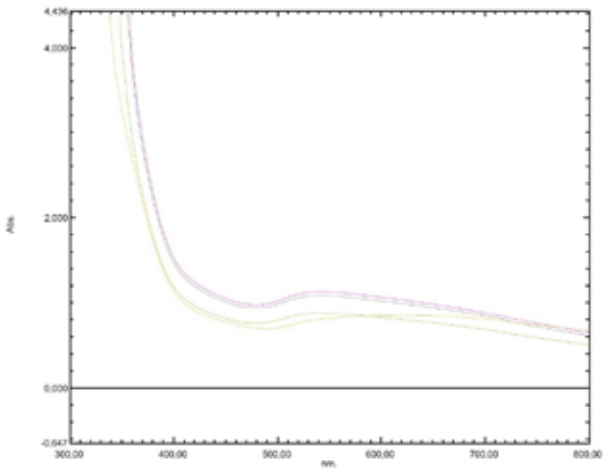

Figure 1. UV-Vis. absorbtion spectra of AuNPs

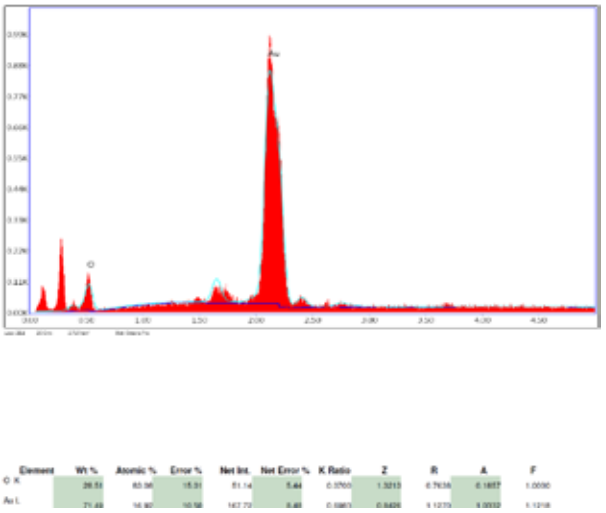

Figure 2. The elemental composition of AuNPs by the EDX analysis

due to surface plasmon vibrations and AuNPs (30). As seen in the figure, AuNPs demonstrated a maximum peak around 535 nm. Daizy (31), Chandran et al. (32) and Kumar et al. (16) also reported peaks in the range of 537-548 nm for AuNPs.

According to the EDX data, the basic composition of the resulting AuNPs was confirmed to be gold (Figure 2). In addition, it was understood that AuNPs were in elemental structure. It can be said that the other peaks seen in Figure 2 were caused by the pollution from the PC leaf pulp. Similar results were obtained in green synthesis studies with extracts of *Pistia stratiotes* (33), *Cymbopogon citratus* (34), and *Artemisia absinthium* (35). From the EDX profile, weak signals such as oxygen and carbon may be due to biomolecules present on the surface of nanoparticles (36).

Surface morphology and particle size distribution of AuNPs obtained by green synthesis were revealed by FE-SEM and TEM measurements (Figures 3a, 3b, 4a and 4b). The FE-SEM and TEM results confirmed that the biosynthesized gold particles had nano dimensions. It was seen that the synthesized AuNPs were in clusters but not in direct contact with each other. This shows the stabilization of AuNPs. TEM images proved that the gold particles were predominantly spherical in shape (Figures 4a and 4b). Similar shaped AuNPs had also been reported in studies

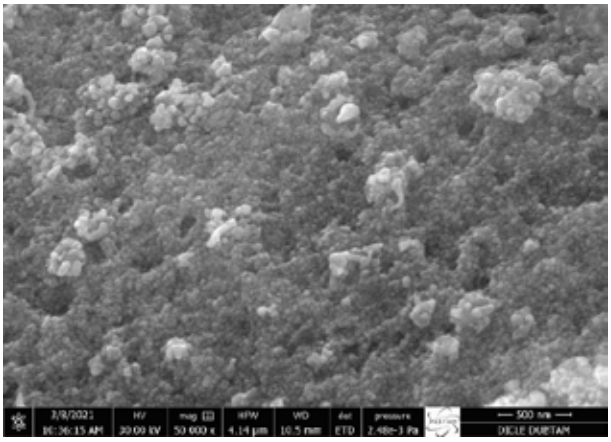

Figure 3a. FE-SEM image of AuNPs

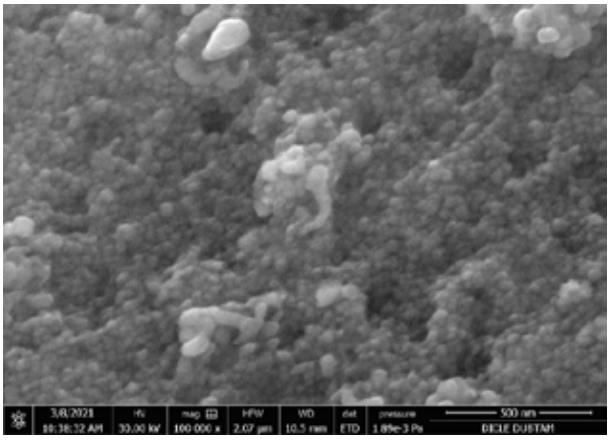

Figure 3b. FE-SEM image of AuNPs

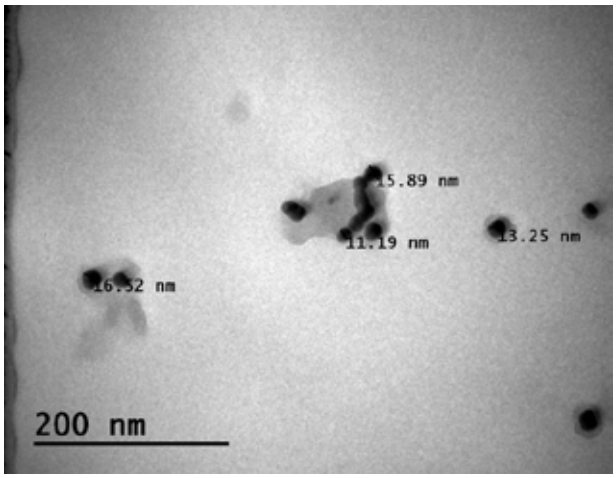

Figure 4a. TEM image of AuNPs

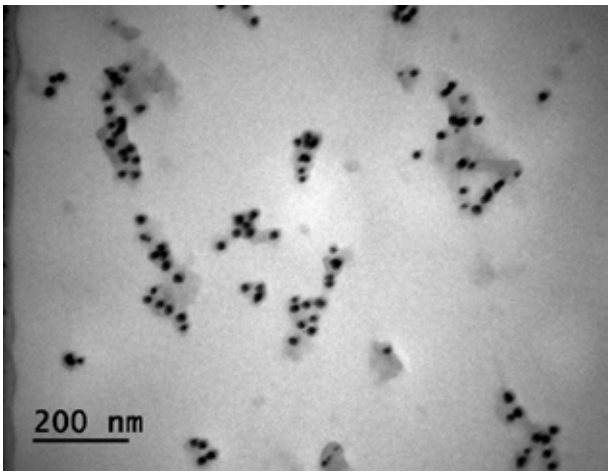

Figure 4b. TEM image of AuNPs

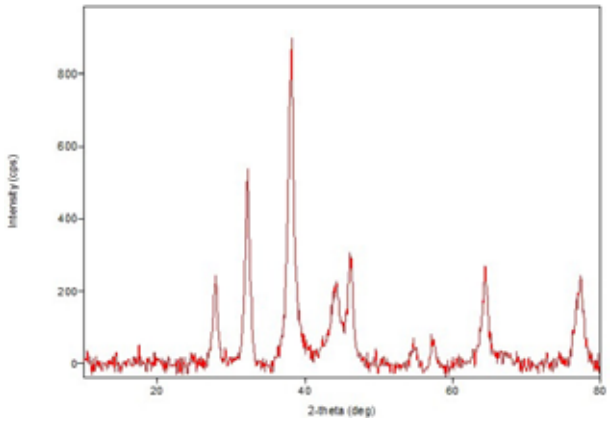

Figure 5. X-ray diffraction patterns of PC-AuNPs

of other researchers (18, 37, 38). The sizes of AuNPs were between 11.19-16.32 nm, with an average of 14.16 nm (Figure 4a).

The XRD spectrum for the synthesized AuNPs is shown in Figure 5. The peaks of 38.17°(111), 44.06°(200), 64.31°(220) and 77.24°(311) corresponding to 2θ in the XRD spectrum pattern show that metallic gold has face-centered cubic crystal geometry (16, 39). The size of the nanoparticles was calculated as approximately 17.94 by the Debye-Scherrer equation.

When the biomolecules involved in the reduction during the formation of AuNPs are examined (Figures 6a and 6b), the absorption peak at 1636 cm-1 can be defined as amide I, which arises due to carbonyl (C = O) stretching vibrations in the amide (–C(=O) N=) linkages of the proteins (37). It can be said that the absorption peak at 3326 cm-^{1} belongs to O-H and N-H stretching. Presumably, these functional groups are responsible for the reduction of metal ions. 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 (35, 40).

The average size of AuNPs with Zetasizer was determined as 103.8 nm (Figure 7a). The zeta potential (surface charge) of the biosynthesized AuNPs was found to be -27 mV (Figure 7b). Researchers who conducted similar studies with different plants reported that the zeta potential values of AuNPs were

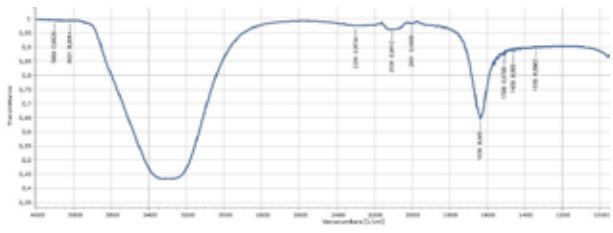

Figure 6a. FT-IR spectra of PC leaf extract

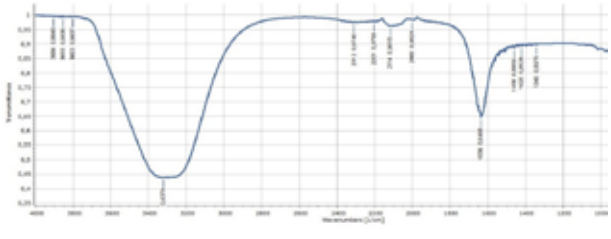

Figure 6b. FT-IR spectra of sythesized PC-AuNPs

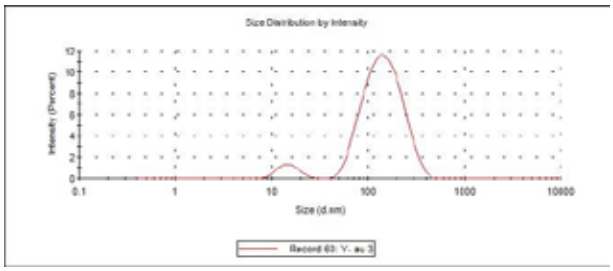

Figure 7a. Zeta size of AuNPs

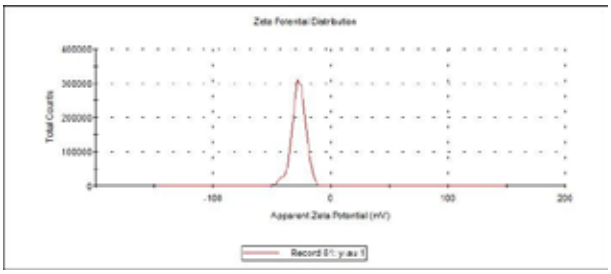

Figure 7b. Zeta potential of AuNPs

Table 1. MIC values of synthesized PC-AuNPs, $HAuCI_4.3H_2O$ and antibiotics (mg / mL)

Microorganisms	PC-AuNPs	$HAuCI_4.3H_2O$	Antibiotics
Staphylococcus aureus ATCC 29213	0.25	0.5	1
Bacillus subtilis ATCC 11774	0.125	0.25	1
Escherichia coli ATCC25922	1.0	1.0	2
Pseudomonas aeruginosa ATCC27853	0.5	1.0	1
Candida albicans	0.5	0.5	2

between -12 mV and -37 mV (41-45). The high negative value of the zeta potential is indicative of the high electrical charge on the surface of the particles in question due to the strong repulsion between the AuNPs. The high negative electric charge prevents aggregation and demonstrates the stability of the AuNP colloid (46).

Escherichia coli, Staphylococcus aureus, Bacillus subtillis, Pseudomonas aeruginosa, and Candida albicans are pathogenic microorganisms frequently encountered in foodborne diseases (47-51). In the study, it was observed that biosynthesized AuNPs significantly inhibited the growth of these microorganisms (Table 1). It was determined that AuNPs suppressed *Bacillus subtillis* more than the others. The antimicrobial mechanism of AuNPs is explained as cell wall, membrane, ribosome and mitochondrial damage and causing cell death by forming a thiol group in bacterial cells (52). Vijayakumar et al. (45) reported that different AuNP concentrations (12.5 mg/mL-100 mg/mL) significantly inhibited the proliferation of *Escherichia coli, Staphylococcus aureus, Bacillus subtillis,* and *Pseudomonas aeruginosa*. Bhau et al. (53), on the other hand, reported that AuNPs were more resistant (effective) against fungal species than bacteria.

Conclusion

With this research, aqueous green synthesis of AuNPs from PC leaf extract was reported for the first time. Therefore, economical, simple, fast and environmentally friendly AuNPs were synthesized using PC leaf extract. In addition, no toxic substance was used during this synthesis. EDX, XRD and UV-vis absorption confirmed the synthesis of AuNPs. FE-SEM and TEM studies revealed that AuNPs were generally spherical in shape and had an average size of about 14.16 nm. From the FT-IR spectra, the biomolecules responsible for the reduction and stabilization of AuNPs were found to be proteins present in the extract. In this study, it was determined that AuNPs had strong antimicrobial effects against foodborne pathogens even at low concentrations. However, new studies are recommended to reveal the effects of biosynthesized AuNPs on other microorganisms.

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