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Biological activities of green synthesis silver nanoparticles by *Plantago lanceolata* L. leaves

Karakaş İ [1], Sağır L. B [2] and Hacıoğlu Doğru N [2,*]

[1] *Vocational School of Health Services, Çanakkale Onsekiz Mart University, Çanakkale, Turkey.*
[2] *Department of Biology, Faculty of Science, Çanakkale Onsekiz Mart University, Çanakkale, Turkey.*

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Abstract

In our study, silver nanoparticle (AgNP) was synthesized by green synthesis method using *Plantago lanceolata* L. leaves. The synthesized AgNPs were characterized using energy-dispersive spectra (EDS), scanning electron microscopy (SEM) and transmission electron microscope (TEM). The AgNPs were with an average size of 10–25 nm and mostly spherical. The antimicrobial potential of AgNPs was tested against some bacteria and a yeast culture by disc diffusion and minimum inhibitory concentration (MIC) methods. The AgNPs were significantly inhibited all test culture except *Bacillus subtilis* ATCC 6633. Antioxidant activities were determined by 2,2-diphenyl-1-picrylhydrazil (DPPH) free radical scavenging and 2,2'- Azino-bis (3-ethylbenzothiazolin-6-sulfonic acid) ($ABTS^{\bullet+}$) cation radical scavenging activity. The higher DPPH and ABTS of AgNPs were determined 33.20±0.50% and 39.37±0.54%, respectively. Thus, green synthesis AgNPs may be a new alternative therapeutic agent for infection therapy.

Keywords: Biosynthesis; *Plantago lanceolata* L; Silver nanoparticle; Antimicrobial; DPPH

1. Introduction

The developments in nanotechnology in recent years have increased the use of nanoparticles in fields such as health, medicine, environmental chemistry, nanobiotechnology and biosensors. Importance's of biosynthesized metal oxide nanoparticles has increased in recent years due to the growing demand in sectors such as fillers, opacifiers, disinfectants, antimicrobial agents, catalysts, drug delivery materials, and medical fields [1]. In green synthesis processes, metals such as silver, gold, iron, titanium and thallium are preferred among the metal nanoparticles. Silver nanoparticles (AgNPs) have very strong antimicrobial, antibiofilm, antioxidant, cytotoxic, anticancer, etc. effects [2]. Recently, biological methods for synthesis of AgNPs have been developed because they are eco-friendly and cost effective. Also, these methods do not involve the use of any toxic chemicals [3].

The reasons for the preference of plant extracts in the synthesis of nanoparticles have increased considerably due to their easy applicability as a method, non-toxic effects, economic and availability in different fields. Plant extracts can act as stabilizing agents and reducing agents in the synthesis of nanoparticles. It is also known that the use of plant extract affects the size and morphological properties of nanoparticles [4].

The genus *Plantago* (Plantaginaceae) encompasses approximately 275 species with a cosmopolitan distribution. Recent studies have confirmed that some *Plantago* species have considerable antiviral, anti-inflammatory, and antioxidant activities. Phytochemical studies have also shown that the genus *Plantago* contains a great amount of phenolic compounds (flavonoids and tannins) [5]. Known as the narrow-leaved plantain, *Plantago lanceolata* L. was also used by ancient civilizations (China, Greece and Egypt) for its therapeutic properties. Biologically active glycosides, flavonoids, tannins, polysaccharides and vitamins were found in the leaf parts of *P. lanceolata*, which are still used in many fields

* Corresponding author: Hacıoğlu Doğru N

for medical purposes today [6]. Iridoid glycosides were also determined from *P. lanceolata* leaves, wound healing, antibacterial, anticarcinogenic and NF-kB inhibitory effects on mast cells [7].

The aim of this work is to synthesis the AgNPs using aqueous extract of *P. lanceolata* (APL) leaves as a reducing agent and stabilizer. The characterization, antimicrobial and antioxidant activities of AgNPs were also determined.

2. Material and methods

2.1. Materials

P. lanceolata leaves were obtained from herbalist and kept at −20 °C until further analyses. Silver nitrate (99.98%), were purchased from Merck (Darmstadt, Germany). All chemicals were of analytical reagent grade and were used without further purification. All solutions were freshly prepared using double distilled water and were kept in the dark to avoid any photochemical reactions.

2.2. Biosynthesis of AgNP

The leaves of *P. lanceolata* were washed several times with deionized water. 10 g of leaves was added to 100 mL of deionized water and boiled for 15 min in a water bath. The mixture was then filtered through Whatman filter paper No. 1 to obtain aqueous extract. The filtered extract was stored in refrigerator at 4 °C. These extracts were used as reducing as well as stabilizing agent [3].

AgNPs were synthesized at room temperature in a 250 mL flask using a magnetic stirrer. 1 mM silver nitrate ($AgNO_3$) solution was added to the plant extract at a ratio of 1:10 and mixed in a magnetic stirrer. Stirring was continued until the yellow color of the extract turned burgundy-brown. After the color change was completed, the mixture was centrifuged at 8,000 rpm for one hour. After the first centrifugation, the supernatant part was removed from the pellet part. The washing process was carried out in a micro centrifuge device at 10,000 rpm for 30 minutes with distilled water for 3 repetitions. After washing, the remaining pellet was left to dry in a glass petri dish at a constant temperature of 60°C for one day [8].

2.3. Energy-dispersive spectra (EDS), scanning electron microscopy (SEM) and transmission electron microscope (TEM) analysis

EDS, SEM TEM and analysis was carried out as service procurement at Kastamonu University Central Laboratory (MERLAB). EDS is a method used to determine the superficial element composition of synthesized NPs. SEM analysis is a method that explains the size, shape and surface morphology of NPs that are directly coated with conductive material (3 Å/second thin layer Au) to obtain clear images at the molecular level [8]. TEM is one of the important techniques that provides information about the morphology and chemical content of nanoparticles and explains the bulk formation, size, structural and formal plane of NPs [9]. The AgNP synthesized for size and characterization was imaged from different angles.

2.4. Antimicrobial activity assays

The determination of antimicrobial activity of plant extract and AgNPs was carried out using seven bacteria and one yeast cultures including *Staphylococcus haemolyticus* ATCC 43252, *Acinetobacter baumanii* ATCC 19606, *Staphylococcus aureus* ATCC 6538P, *Escherichia coli* NRRL B-3704, *Bacillus subtilis* ATCC 6633, *Pseudomonas aeruginosa* ATCC 27853, *Proteus vulgaris* ATCC 13315 and *Candida albicans* ATCC 10231.

Standard Kirby-Bauer disc diffusion method and microdilution broth method (MIC) [10] were carried out to detect activity of the APL extract and AgNP against the test cultures. 100 µL of each bacterial suspension was spread on Muller Hinton Agar (MHA) plates for disc diffusion method. 20 µL of AgNP was pipetted into sterile blank paper discs under aseptic conditions. After 24 hours of incubation at 37 °C, the inhibition zone diameters in mm were measured and recorded. Penicillin (10 µg/disk) and Nystatin (100 µg/disk) were used as positive controls. Experiments were repeated three times. Sterile 96-well plates were used for MIC and 50 µL Muller Hinton Broth (MHB) and synthesized AgNP was added to each well and serial dilutions were made. Then, 50 µL of inoculum was added to each well, except for the sterility control. Growth control was performed in the 12th well. After 24 hours of incubation at 37 °C, MIC was determined as the lowest concentration of extract with no visible bacterial growth compared to growth control.

2.5. Antioxidant activity assays

The free radical scavenging activity of the extract and AgNP was determined by modifying the 1,1-diphenyl-2-picrylhydrazil (DPPH) method. Extract and AgNP samples prepared at different concentrations were mixed with DPPH solution at a ratio of 1:4. After incubation at 25°C in the dark for 30 minutes, absorbances were read 3 times at 517 nm versus the blank. The % inhibition of the samples was calculated and synthetic antioxidant butylated hydroxytoluene (BHT) was used as a standard [11].

Cation radical scavenging activity analysis was performed via 2,2'-Azino-bis (3-ethylbenzothiazolin-6-sulfonic acid) ($ABTS^{\bullet+}$) method [12]. Samples prepared at different concentrations were treated with ABTS reagent. ABTS reagent mixed with the samples was left to incubate at room temperature for 6 minutes. The absorbances of the samples were then taken at 734 nm versus 36 (ethanol) blank. The experiment was done in three repetitions. The % $ABTS^{\bullet+}$ radical scavenging inhibition of the obtained absorbance values was calculated and these results were evaluated by comparing with BHT used as positive control.

3. Results and discussion

3.1. Determination of synthesized AgNP

Reduction of Ag+ into AgNPs during exposure to the leaves of *P. lanceolata* extract could be seen by the color change. The color of fresh extract of *P. lanceolata* extract was yellow. However, after addition of $AgNO_3$ and incubation for 2 h in rotary shaker at 120 rpm, the emulsion turned dark brown (Figure 1). The color changes in aqueous solutions are due to the surface plasmon resonance phenomenon [3]. The result obtained is evidence that *P. lanceolata* is a good reducing agent for AgNPs.

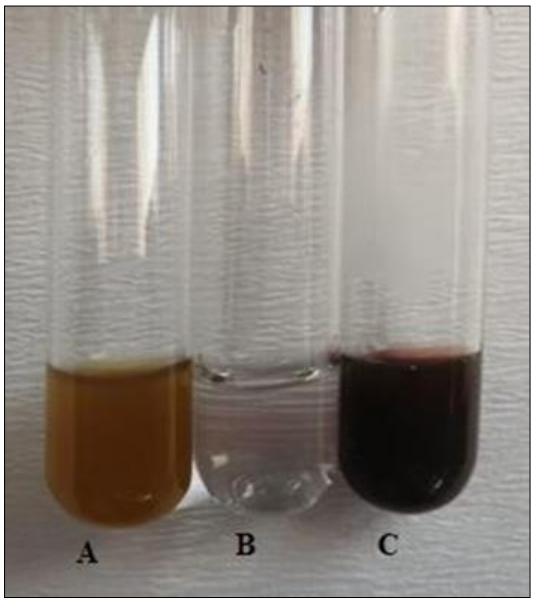

Figure 1 Colour changes of synthesized AgNP (A: Aqueous extract of *P. lanceolata*; B: Extract of $AgNO_3$; C: Synthesized AgNP)

The presence of elements in the nanoparticle was confirmed by EDS analysis. It was determined that the peak at 3 keV in the EDS spectrum belonged to silver atoms [13]. It was observed that the other peaks in the EDS spectrum belonged to chlorine (Cl) (Figure 2). It is reported that these peaks are caused by phenolic and flavonoid components in the plant [14].

SEM and TEM analysis confirmed the existence of AgNPs of almost identical shape and size, respectively. The results of SEM and TEM analysis revealed that the biosynthesized AgNPs were spherical (Figure 3) with sizes ranging from 10 nm to 25 nm (Figure 4). This data is similar to Sukweenadhi et al., 2021 [14] which was found that that the *Plantoga major*-Ag NPs have a spherical shape.

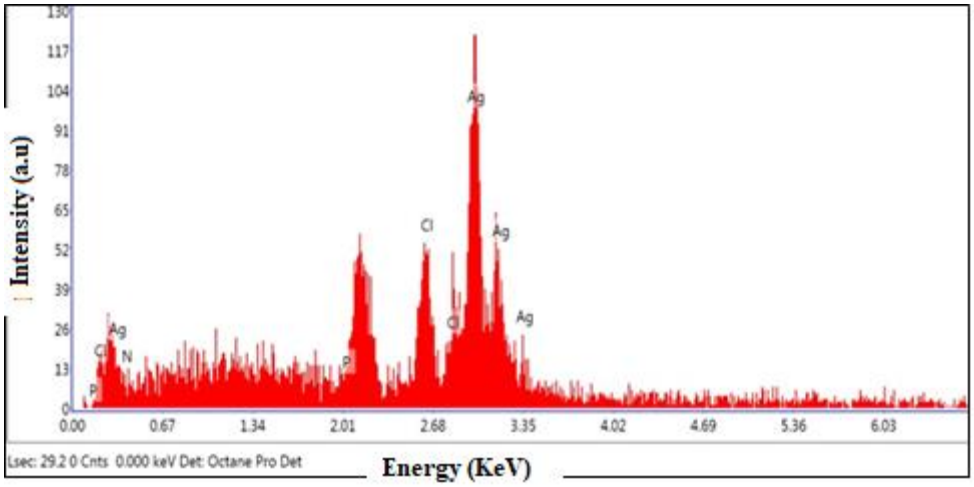

Figure 2 EDS analysis of AgNPs

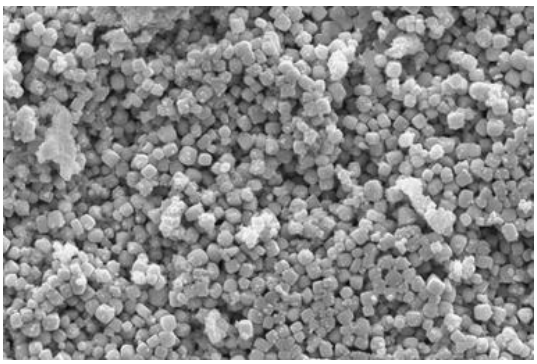

Figure 3 The SEM images of synthesized AgNP

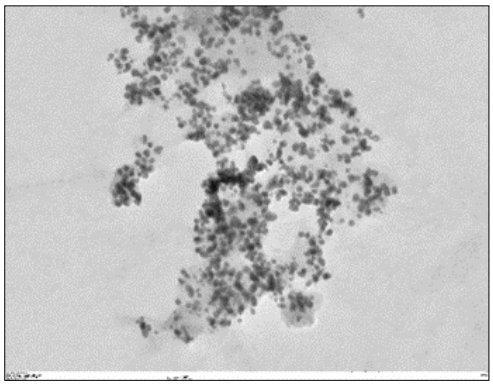

Figure 4 The TEM images of synthesized AgNP

Singh et al. (2016) [1] reported that in biosynthesized AgNPs by plant extracts; nanoparticles exhibited that spherical, hexagonal, irregular shapes and sizes ranged from 10 to 90 nm. Dehvari and Ghahghaei (2018) [15] and Pallela et al. (2018) [16] reported that AgNPs obtained from the *Pulicaria undulata* and *Sida cordifolia* extract also had a spherical appearance, respectively.

3.2. The antimicrobial activities of APL and AgNPs

Silver ions as well as AgNPs were known to have strong antimicrobial activities The Ag+ ion from the nanoparticles is predicted to bind to the negatively charged bacterial cell wall and disintegrate it, leading to cell death and protein denaturation [13]. The antimicrobial activity results of APL and AgNPs were given in Table 1. The highest inhibition

zone diameter for APL extract was 12.00 mm against *B. subtilis* ATCC 6633 and for AgNP; 23.00 mm was found against *A. baumanii* ATCC 19606 bacteria. The highest MIC value was found 75 µg mL^{-1} from *A. baumanii* ATCC 19606 bacteria for *P. lanceolata* extract, and 9.375 µg mL^{-1} from *S. haemolyticus* ATCC 43252 and *A. baumanii* ATCC 19606 bacteria for AgNP (Table 1). Especially AgNP extracts showed higher antimicrobial effect against *S. haemolyticus* ATCC 43252, *A. baumanii* ATCC 19606, *S. aureus* ATCC 6538P, *E. coli* NRRL B-3704 and *C. albicans* ATCC 10231 than comparison antibiotic P10 and NY100.

Table 1 Antimicrobial activity results of APL and AgNPs

Test microorganisms	**Methods**							
	***Disc Diffusion (mm)**				**MIC (µg mL^{-1})**			
	Extracts		**Control antibiotics**		**Extracts**		**Control antibiotics**	
	APL	**AgNP**	**P10**	**NY100**	**APL**	**AgNP**	**ST**	**NY100**
S. haemolyticus ATCC 43252	11.00	20.00	16.00	NT	150	9.375	5.0	NT
S. aureus ATCC 6538P	9.00	18.30	13.00	NT	300	18.75	4.0	NT
B. subtilis ATCC 6633	12.00	11,60	15.00	NT	150	150	4.0	NT
A. baumanii ATCC 19606	11.00	23.00	8.00	NT	75	9.375	2.0	NT
E. coli NRRL B-3704	9,00	18.30	12.00	NT	300	18.75	4.0	NT
P. aeruginosa ATCC 27853	10,0	13.00	14.00	NT	300	75	1.0	NT
P. vulgaris ATCC 13315	11,00	15.00	16.00	NT	150	37.5	4.0	NT
C. albicans ATCC 10231	9.00	13.00	NT	9.00	300	75	NT	5.0

APL: aqueous extract of *P. lanceolata*; P10 = penicillin (10 ug/disc); ST: Streptomycin (10 ug/disc); NY100: Nystatin (100 ug/disc), Nt: not tested

It has been reported that 25 µg mL^{-1} concentrations of AgNPs obtained with *Carica papaya* leaf extract has the MIC for gram positive and gram negative bacteria [8]. In a study conducted in 2022, the antibacterial activity of AgNP obtained from *P. major* L. plant against *S.aureus* ATCC 25923, *P. aeruginosa* ATCC 9027 and *E. col*i ATCC 25922 bacteria was investigated. The highest inhibition zone value was determined as 9.91 mm from *E. coli* ATCC 25922 bacteria [14]. In another study, the antibacterial activity of AgNP synthesized from the *Ferula pseudalliacea rech*. F. was investigated against *A. baumannii*, *B. cereus, E. faecalis* ATCC 29212, *E. faecium, S. aureus* ATTC 29213, *K. pneumoniae, E. coli* and *C. albicans* ATTC 90028. The inhibition zone values obtained were between 9.00 mm and 11.00 mm [17]. Contrast to literature, our findings showed that higher antagonistic activity of AgNPs against to test culture [14,17].

3.3. The Antioxidant activities of APL and AgNPs

Antioxidant activity of APL and AgNPs were shown in Figure 5 and Figure 6. The maximum inhibition values of DPPH activity at 1000 µg mL^{-1}, which is the highest concentration of the APL and AgNP, were determined to be 80.94±0.66% and 33.20±0.50%, respectively. The DPPH activity of AgNPs obtained from *P. major* extract was reported as 70.48% [6]. It was determined that the DPPH activity of AgNPs obtained from *F. pseudalliacea rech*. F. extract was 78.123% [17].

For ABTS activity, the maximum inhibition values at 1000 µg mL^{-1}, which is the highest concentration of the extract and nanoparticle, were 63.89±0.39% and 39.37±0.54%, respectively (Figure 6).

$ABTS^{\bullet+}$ values of AgNP synthesized from *Asphodelus aestivus* extract and silver nanoparticle were determined as 39.62±0.02% and 79.94±0.02%, respectively [18]. Narayanan et al. (2021) [19] determined the antioxidant activities of silver nanoparticles synthesized with *Ctenolepis garcini* extract by $ABTS^{\bullet+}$ cation removal method. They reported % inhibitions for extract, silver nanoparticles and ascorbic acid 64.15, 71.42 and 36.15, respectively. In the results they obtained, they stated that silver nanoparticles had more effective $ABTS^{\bullet+}$ removal activity compared to the extract. DPPH activity of *P. lanceolata* L. extract and FeNP was calculated as 78.86 ± 0.65% and 89.79 ± 0.21%, respectively [20]. Unlike other studies, low percentages of AgNP antioxidant activity are thought to be related to the secondary metabolite potential of the plant as a reducing agent.

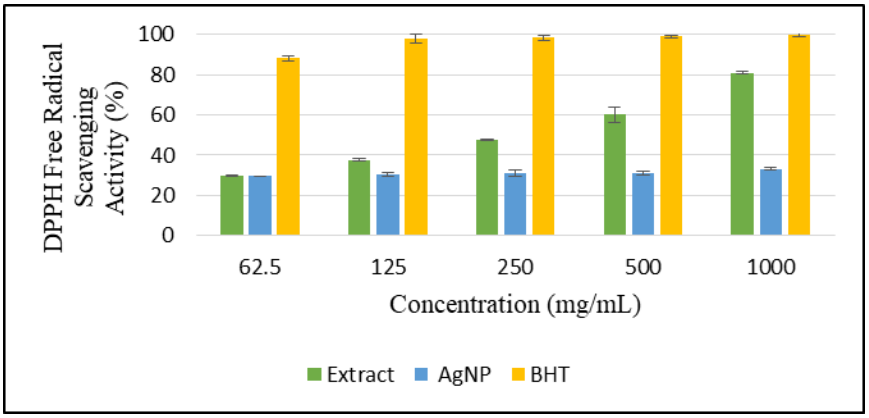

Figure 5 DPPH Activity (%) of APL and AgNPs

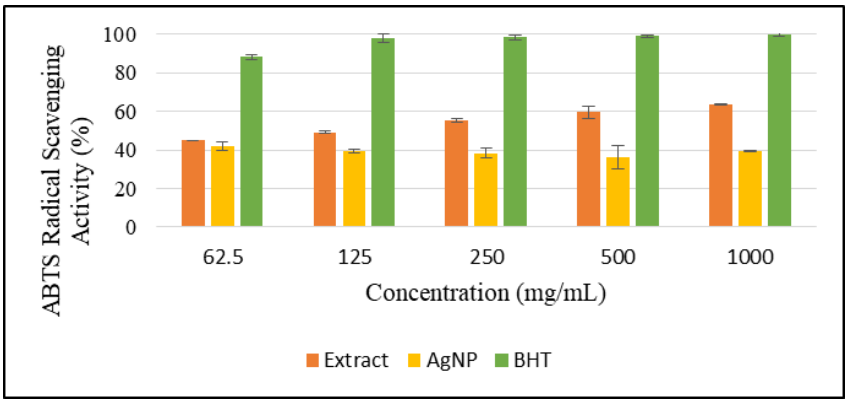

Figure 6 ABTS Activity (%) of APL and AgNPs

4. Conclusion

In this work it was showed that, green synthesis of AgNPs using *P. lanceolata* is cost-effective, environmentally friendly and compatible with human health due to less waste and safer products so this method can be considered as an alternative synthesis method. Due to the antimicrobial and antioxidant activities of AgNPs synthesized using the *Plantago* species, the product may be used in various industries in the future. Especially AgNP of *P. lanceolata* should be explored further for antimicrobial applications such pharmaceutical industry with its high antimicrobial activity against important pathogens.

Compliance with ethical standards

Acknowledgments

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Disclosure of conflict of interest

On behalf of all authors, the corresponding author states that there is no conflict of interest.

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