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Green synthesis, characterization and biological activity of silver nanoparticles From *Dicranum majus* Turner

O. Tonguc Yayintas[a*], N. Demir, S. Yilmaz[c], M. M. Cicekliyurt[a]
[a]*Department of Medical Biology, Faculty of Medicine, Canakkale Onsekiz Mart University, Canakkale, 17100, Turkey*
[b]*Department of Biology, Faculty of Science and Arts, Canakkale Onsekiz Mart University, Canakkale, 17100, Turkey*
[c]*Department of Chemistry, Faculty of Science and Arts, Canakkale Onsekiz Mart University, Canakkale, 17100, Turkey*

The exploitation of various plant compounds for the biosynthesis of nanoparticles is considered a green technology because it does not involve any harmful chemicals. In the current study, we synthesized and characterized silver nanoparticles from *Dicranum majus* (Dm) Turner, a moss plant (Bryophyte). The occurrence of the visible color change from red to brown confirmed Dm silver nanoparticles (DmAgNPs). The DmAgNPs were characterized based on Ultraviolet-Visible (UV-Vis) Spectroscopy, Fourier Transform Infrared Spectroscopy (FTIR). The morphology, size, and elemental analysis of the prepared silver nanoparticles were examined using Transmission Electron Microscope (TEM) and zeta potential (ZP). The distribution pattern of DmAgNPs particle size and stability were determined with the zeta potential analysis by zeta sizer. The maximum absorption of DmAgNPs was obtained at 422 nm by UV-Vis spectrometer. The presence of carbonyl compounds was demonstrated by FTIR, TEM. Zeta sizer analysis revealed the average size of the nanoparticles as 278.7 nm with -16.7 mV zeta potential demonstrates moderate stability. Considering its antibacterial activities, DmAgNP is more effective on *Enterococcus faecalis* (ATCC 29212), *Listeria monocytogenes* (ATCC 7644), and *Proteus vulgaris* (NRRL B-123) bacteria; it has no mutagenic activity; cleaved DNA as a result of gel electrophoresis; Antioxidant activity was determined by the 2,2-Diphenyl-1-picrylhydrazyl (DPPH) method.

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Keywords: Dicranum majus, Green sythesis, Characterization, Biological activities

1. Introduction

Nanotechnology is exciting and essential science and engineering approach that investigates particles with dimensions ranging from 1 nm to 100 nm, each with its physicochemical properties and the interdisciplinary use potential of these particles [1]. Metal nanoparticle synthesis is the fastest growing area of investigation in bioscience.

The fact that nanotechnology has wide potential use in the medicine and health sector, industrial applications, and agriculture, especially considering the social benefit to be obtained, binds essential investment tools on the wing of the state in developed countries. Comprehensive studies on metal nanoparticles offered researchers the opportunity to explore these particles' antimicrobial and antioxidant effects [2, 3, 4].

The potential of nanoparticles for use in the diagnosis and prevention of cancer diagnosis and treatment methods, medical drug release systems, lung diseases, and infectious diseases Nanoparticle-derived drug delivery systems such as liposomes, dendrimers, nano nets, carbon nanotubes, gold nanoparticles are used as tumor-targeting agents, imaging agents in early diagnosis disease, and targeted gene therapies. For example, when cancer drugs such as amphotericin are administered in liposomal nanoparticles, they can provide more effective and reliable treatment than traditional practice [5].

* Corresponding author: ozlemyayintas@hotmail.com

Biosynthesis is a novel way to synthesize nanoparticles by using biological sources. It is gaining much attention due to its cost-effective, eco-friendly, and large-scale production possibilities. Plants, fungi, microorganisms, and biodegradable polymers can be used as sources of reducing, coating, and stabilizing agents in the green synthesis of nanoparticles [6]. Nanomaterials are the atomic and molecular building blocks (~0.2 nm) of matter [7]. Currently, it is exploited to a vast extent because the plants are widely distributed, easily available, safe to handle, and with a range of metabolites [7-9]. Biosynthesis methods have more compensation over other classical synthesis procedures due to more biological entities and environmental processes. The rich biodiversity and easy availability of plant entities have been highly explored for nanomaterial synthesis. Silver is one of the most commercialized nanomaterials with five hundred tons of silver nanoparticles production per year and is estimated to increase in forthcoming years [7,10]. Since plants contain macromolecules and various reducing substances, they can produce silver nanoparticles without a chemical reducing agents and thus without toxic effects.

The basis of obtaining nanoparticles by green synthesis is based on reducing metal ions with biomolecules such as enzymes, proteins, amino acids, polysaccharides, and vitamins in organisms. It has long been known that plants have the potential to over-accumulate and reduce metallic ions. Because of these properties, plants are considered a more environmentally friendly tool for the biosynthesis and detoxification of metallic nanoparticles. The green synthesis method enables the production of nanoparticles in large scales, which are cost-effective, do not require toxic chemicals, are simpler, can turn into products in a short time, and are well defined in size and morphology [11,12]. Several prior reports demonstrate that biosynthesized nanoparticles effectively controlled oxidative stress, genotoxicity, and apoptosis-related changes [7,13].

The term bryophyte; is used for a wide range of plants, including liverworts, hornworts, and mosses [14]. Mosses, Ferns, Gymnosperms, and Angiosperms, which originate from green algae, thrive on land. Mosses can be called amphibians of the plant world because their reproduction is dependent on water, but they can continue their development in different environments [15]. Nevertheless, studies on mosses have been limited to a few species until about ten years, and there has not been much focus on them. However, studies on the biosynthesis of nanoparticles from moss sources have gained momentum to discover their existing potential. One of the essential uses of AgNPs is to interact with the HIV1 virus and stop their binding from hosting cells in vitro, targeting cells, and treating the disease [16]. In addition, AgNPs with antimicrobial properties; coating vegetable oil with AgNPs showing excellent antimicrobial properties [17,18]. Furthermore, AgNPs accumulated in carbon filters reduce water-related diseases [19, 20]. The use of Ag for wounds in the treatment of ulcers has provided benefits in different applications [21-23].

In green chemistry, biomolecules have replaced traditional stabilizing and reducing agents. Ag NPs produced using bacteria, yeast, fungi, algae, and plants as reducing and stabilizing agents [24]. For example, *Trichoderma ride* mushroom a precursor for AgNP synthesis from $AgNO_3$ [25]. Also, AgNPs with excellent antimicrobial activity produced by mixing *Fastia japonica* leaf extract with $AgNO_3$ [26].

The study aims to produce a novel and eco-friendly silver nanoparticle by green synthesis, characterization, and determination of biological activity using an aqueous extract of *Dicranum majus* (Dm).

2. Experimental

2.1. Description of plant

Dicranum majus prefers shaded or semi-shaded areas. It is seen at altitudes up to 1500 meters above sea level. It is a green to light green colored, shiny, or slightly dull plant in loose clusters. Leaves are somewhat scattered, falcate, pointed, and regularly toothed on the leaf edge. Also, these species occur in woodland and cliffs (Fig. 1).

Fig. 1. Dicranum majus (Dm) Turner (https://artfakta.se/artbestamning/taxon/dicranum%20majus-2152).

2.2. Plant collection and extraction

The moss plant Dm collected from Mount Ida (Kazdağ), Can district, Canakkale and brought to the laboratory, and then species determinations were made by Dr. Ozlem Yayintas. The plants cleaned by running tap water to remove the debris, and shade dried plants ground into powder. The extract prepared with 10 grams of plant powder was stirred with 100 ml of Milli-Q water and kept at 60^0C for 1 hour. Then the mixture was filtered by Whatman No. 1 filter paper after cooling to room temperature. The extract was preserved in a brown bottle and kept in a dry and cool place for further use.

2.3. Green biosynthesis of silver nanoparticles

Yu et al.'s (2019) method has been modified for synthesizes of silver nanoparticles [27]. First, 10 ml of plant extract were mixed with 90 ml of 1 mM $AgNO_3$ (Merck) aqueous solution in a 250 ml Erlenmeyer flask on a magnetic stirrer for 45 minutes at room temperature. A yellow to reddish-brown turn was observed. Next, the pellet portion was precipitated by centrifugation at 8.000 rpm for 60 minutes (Hettich Universal 320R). It was then purified by washing three times with Milli-Q water at 10.000 rpm. Then it was left to dry at 65 ^{0}C in the oven.

2.4. Characterization of biosynthesized Silver-nanoparticles

Biosynthesized silver nanoparticle exposed to Ultraviolet-Visible (UV-Vis) Spectrometer (Spectro UV-Vis Dual Beam with 8 Auto Cell, Labomed, Inc) range of 250nm-600nm. Fourier Transform Infrared (FTIR) absorption spectra were obtained from a Perkin ElmerBX II spectrometer (USA) in KBr discs and reported in cm^{-1} units at $4000cm^{-1}$ - $400cm^{-1}$. Morphology, size, and element analysis of silver nanoparticles investigated by Transmission Electron Microscope (TEM) (Hitachi HT-7700). The distribution pattern of DmAgNPs particle size and stability was determined with zeta-potential data obtained by zeta-sizer (Zetasizer Nano ZS, Malvern, UK).

2.5. Antibacterial Activity Analysis

Antibacterial activities of the Dm extract and DmAgNP have been carried out using broth microdilution method as suggested by the Institute of Clinical and Laboratory Standards [28]. Antimicrobial activity of NPs *Listeria monocytogenes* (ATCC 7644), *Staphylococcus aureus* (ATCC 25923), *S. aureus (*NRRL-B-767), *Enterococcus faecalis* (ATCC 29212), *Bacillus subtilis* (ATCC 6633), *Pseudomonas aeruginosa* (ATCC 27853), *E. coli* (ATCC 25922) and *Proteus vulgaris* (ATCC 13315) bacteria investigated.

Minimum Inhibition Concentrations (MIC) of nanoparticle extracts were determined by the broth microdilution method. MIC values were evaluated in 96-well microplates by using a serial dilution method. Bacteria inoculum was prepared from 4-6 hours culture in Mueller Hinton Broth (MHB) medium. Densities of microorganism suspensions were adjusted using 0.5 McFarland standard, and the planted microplates were left to incubate for 18-24 hours at 35-37 ºC for bacteria. The lowest substance concentration without visible growth was recorded as the MIC value.

2.6. NP's Interaction with DNA

In DNA cleavage studies, pBR322 plasmid DNA (in 90% super-coil form) was used. Plasmid pBR322 DNA was prepared using Tris-HCl buffer samples (10mM, pH: 7.4). After the prepared samples are incubated at 37 ^{0}C in the incubator for 3 hours, 6X loading dye is added and 1 hour at 60V in 1% agarose gel, 1X TAE buffer (40mM Tris-20 mM acetic acid, 1mM EDTA pH: 8.2) gel conducted in electrophoresis. Later, the bands obtained were photographed using a gel imaging system under UV light [29].

2.7. Determination of Mutagenic Activity

The Ames/Salmonella test will be used to determine the mutagenic activity. The TA98 strain of *Salmonella typhimurium* used in the study determines the frameshift, while the TA100 strain determines the mutations that cause base-pair change [30,31]. The positive control was 4-nitro-*o*-phenylenediamine (NPD) for strain TA98 and sodium azide (SA) for strain TA100; in the negative control, water was used for both stains. To determine whether the NPs have mutagenic activity, the doses to be studied compared with the negative control. To speak of the mutagenic effect, the number of revertant colonies obtained due to applying different concentrations of the tested NPs should be twice as compared to the negative control [31].

2.8. Antioxidant Activity of Silver Nanoparticles

The free radical scavenging activity measured the antioxidant activity of the synthesized nanoparticles by the 2,2-diphenyl-1-picrylhydrazyl (DPPH) assay [32]. One ml of 0.1 mM DPPH solution in methanol was added to 1 mL of AgNPs of different concentrations. After 30 min. incubation, absorbance was measured at 517 nm using a spectrophotometer. % Inhibition was calculated using the following equation: Inhibition (%) = $[(Abs_{control} - Abs_{sample}) / Abs_{control}] \times 100$

3. Results and discussion

3.1. Visual observation of nano synthesis

Dm extract mixed with the 1 mM $AgNO_3$ solution. The color change of pale yellow to reddish-brown color was observed in the mixture of plant extract and $AgNO_3$ solution after one hour of incubation in the direct sunlight (Fig. 2). The color change indicated the reduction of Ag^{+1} ion and confirmed the silver nanoparticle formation. The results were like in [33,34]. They recorded that silver nanoparticle approved with pale-yellow color change into reddish-brown color.

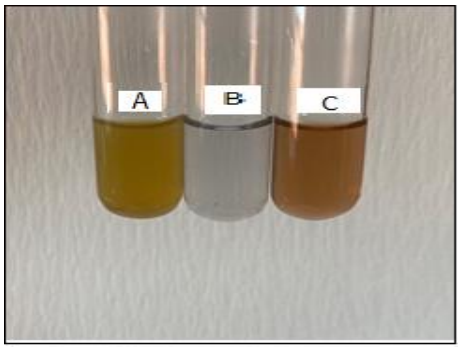

Fig. 2. (A) Dm Extract (Pale-yellow); (B) $AgNO_3$; (C) Dm Extract+ $AgNO_3$ nano particles: color change (reddish brown).

These synthesized DmAgNPs were validated characterization studies such as UV-Vis spectrometry, FTIR, TEM, Zeta potential, and particle size.

3.2. UV-Vis spectroscopic analysis

The UV-Vis spectrum is given in Fig. 3. The UV-Vis spectrum of the Dm is two bands of π–π* transition of the C=C group at 272 nm and n–π* changes of the CO, O-C=O, OH groups at 314 nm are observed. Likewise, when the UV-Vis spectrum of DmAgNP is examined, two bands of the π–π* transition of the C=C group at 272 nm and the n–π* transitions of the CO, O-C=O, OH groups at 314 nm are observed. The peak at 422 nm in the spectrum is attributed to the plasmon absorption of metallic silver nanoparticles. The results were compatible with these studies [33,34]. These studies showed that the color change and the maximum absorbance at 364 nm were caused by the reduction of silver ions to silver metals.

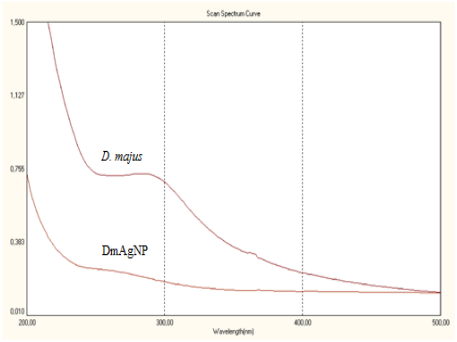

Fig. 3. UV-Vis spectra of the D.majus extract and DmAgNP.

3.3. FTIR analysis

The FTIR spectrum is given in Fig. 4. As seen from the FTIR spectrum of Dm, the characteristic in functional group frequencies; OH, C-H, COOR+C=O+C=C, and C-O vibrations are observed at 3415 wide-spread, 2937 middle, 1624 wide-spread, and 1302 middle cm^{-1}, respectively. COOR+C=O+C=C overlapped in the spectrum, and it was regarded as a vast and spread peak at 1624 cm^{-1}.

As seen from the FTIR spectrum of DmAg, the characteristic in functional group frequencies; OH, C-H, COOR+C=O+C=C, and C-O vibrations are observed at 3427 wide-spread 2916 middle, 1635 wide-spread, and 1294 middle cm^{-1}, respectively. In addition, COOR+C=O+C=C overlapped in the spectrum, and it was marked as a vast and spread peak at 1635 cm^{-1}.

When the FTIR spectra of Dm and DmAg are compared, it is seen that the absorption band at about 3415 cm^{-1} becomes sharper and shifts to a slightly higher frequency 3427 cm^{-1}. This indicated that Ag+ was reduced to Ag^0 with OH in Dm, and OH groups were oxidized to C=O and COOH.

So, the FTIR analysis reveals the carbonyl functional groups of OH, C-H, COOR+C=O+C=C, and C-O were binds to the metal might be behaved as the covering agent to produce silver-nanoparticles. In these studies, an FTIR experiment was carried out to define the biomolecules in charge of reducing the silver ions and covering the AgNPs. By FTIR experiment, they indicated that carbonyl compounds of aldehyde, carboxylic acids, and protein were bind to the metal might be behaved as the covering compound for the fabrication of silver nanoparticles. As a result, it can be said from both UV-Vis and FTIR results that Ag^+ ions are reduced with OH groups in Dm to form Ag nanoparticles.

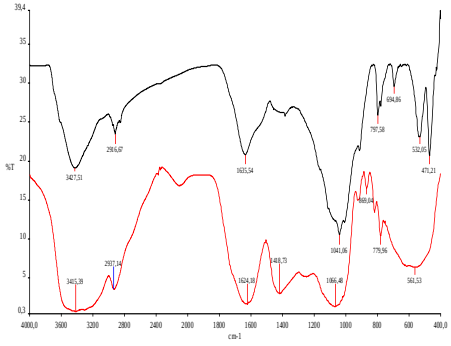

Fig. 4. FTIR spectra of Dm and DmAgNP (Red color: Dm; Black color: DmAgNPs).

3.4. TEM studies

The size of the green synthesized silver-nanoparticles was investigated by TEM (Fig. 5). TEM image showed the size of the DmAgNPs mediated synthesized silver nanoparticles.

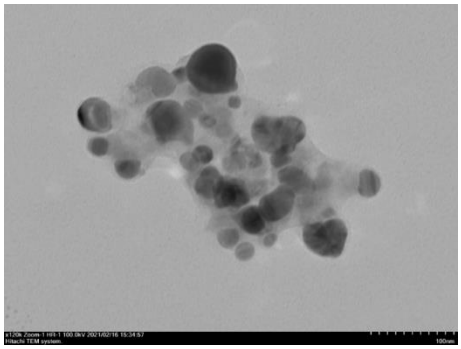

Fig. 5. TEM image of AgNPs synthesized from Dm.

3.5. Zeta potential analysis

The stability, mobility, and dispersion of synthesized silver-nanoparticles were determined by the measurement of zeta potential studies. The zeta potential value for the DmAgNPs was found to be –16.7±6.50 mV (Table 1 and Fig. 6). So, this result indicated that the green synthesized DmAgNPs were moderately stable and having a middle-level electrostatic repulsion among the particles. These results are in agreement with the values in the literature [26,33-36]. In these studies, some parameters such as stability, dispersion, and mobility of particle surface were determined with zeta-potential measurements. Vimala and Sathis 2017; Vimala et al. 2017 stated that the zeta potential value for *Campylopus flexuosus* silver nanoparticles (CfAgNPs) is −25mV, confirming the repulsion between the negatively charged particles; hence the negative values demonstrate the strong stability of the synthesized silver nanoparticles [34,35].

Ateş et al. [36] emphasized that the magnitude of the zeta potential provides information about particle stability; the higher potentials indicate increased electrostatic repulsion and hence increased stability. For instance, 0-5 mV particles tend to aggregate whereas 5-20 mV particles are minimally stable, and 20-40 mV particles are moderately stable. If they are bigger than 40+ mV, the particles are extremely stable. Accordingly, the zeta potential value of the AgNPs produced in their study is 30.1 ± 6.8 (mV); it indicated that they are moderately stable.

Table 1. Zeta potential analysis data of DmAgNPs by zeta sizer.

Results quality:	**Mean zeta potential* (mV)**	**Zeta standart deviation (mV)**	**Area (%)**	**Conductivity (mS/cm)**
Good	-16.7	6.50	100.0	0.0156

*Mean of the triplicate

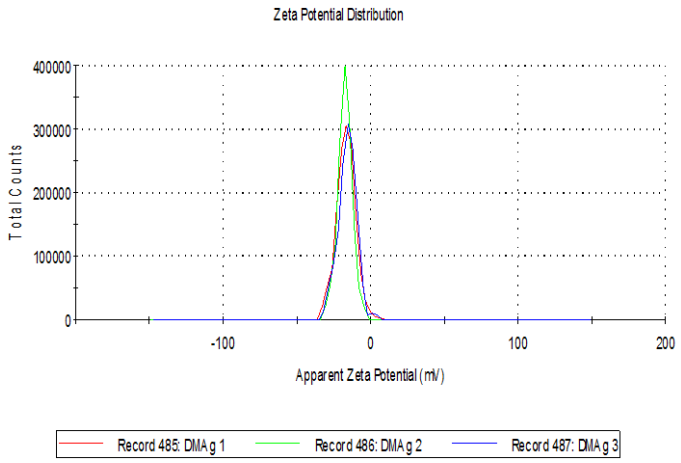

Fig. 6. Zeta potential distribution graph of green synthesized DmAgNPs for triplicate measurements is given a different color.

3.6. Particle size studies

The particle sizes were investigated with the zeta size studies (Table 2 and Figure 7). As a result, the dispersion of the particle size of the synthesized DmAgNPs is obtained as 268.6± 8.86 nm mean with standard deviation values (Table 2).

Table 2. Size exclusion analysis data of DmAgNPs at 25 ^{o}C by zeta sizer.

Results quality: Good	**Z-Average of size (nm)**	**Mean intensity (%)**	**Standard deviation (d.nm)**
Peak 1	278.7	57.10	9.50
Peak 2	262.3	36.10	7.30
Peak 3	264.7	6.80	2.30
Mean size (d.nm)*	**268.6**	-	-
The standard deviation of mean size d.nm)	8.86	-	-
Polydispersity index (Pdi) (d.nm)	0.523	-	-
Standard devition of polydispersity index (Pdi) (d.nm)	0.071	-	-

*Mean of the triplicate.

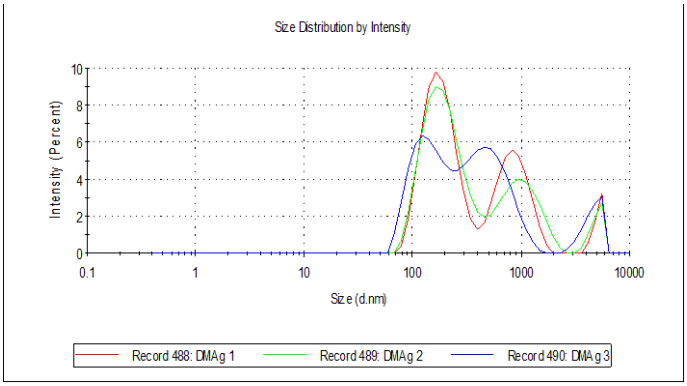

Fig. 7. Particle size distribution graph of green synthesized DmAgNPs for triplicate measurements is given a different color.

3.7. Antibacterial activity

MIC values of the DmAgNP were determined for antibacterial activity. The studied DmAgNP was found to be more effective against Gram (+) *E. faecalis* (ATCC 29212) and *L. monocytogenes* (ATCC 7644) bacteria and Gram (-) *P. vulgaris* (NRRL B-123) bacteria (Table 3).

Table 3. MIC values of DmAgNP.

Microorganisms		DmAgNP (µg/mL)
Gram (+)	*L. monocytogenes* ATCC 7644	0.078
	B. subtilis ATCC 6633	1,25
	S. aureus NRRL-B-767	>5
	S. aureus ATCC 25923	>5
	E. faecalis ATCC 29212	0.004
Gram (-)	*P. aeruginosa* ATCC 27853	2,5
	E. coli ATCC 25922	5
	P. vulgaris NRRL B-123	0.009

3.8. DNA Cleavage Activity

The pBR322 DNA cleavage activity of silver nanoparticles was determined at five different concentrations (1.25, 2.5, 5, 10, and 20 ppm). As a result of the research, it was defined that DmAgNP breaks DNA at all concentrations. It forms the circular form of DNA (Form II) by creating a single strand cut in DNA (Fig. 8).

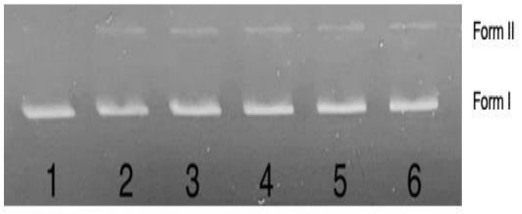

Fig. 8. DmAgNP DNA cleavage activity, 1. DNA control, 2. DNA+1.25 ppm, 3. DNA+2.5 ppm, 4. DNA+5 ppm, 5. DNA+10 ppm, 6. DNA+20 ppm.

3.9. Mutagenic Activity

The mutagenic activities of Dm and DmAgNPs were investigated with the Ames test system using TA98 and TA100 mutant strains of *S. typhimurium*. It was determined that the tested concentrations in the study did not have a mutagenic effect both Dm and DmAgNPs (Table 4).

Table 4. The mutagenic activities of Dm and DmAgNPs.

Treatment		Concentration (µg/plate)	Number of His^+ Revertant Colony/ Plate	
			TA98	TA100
			Mean±SD	Mean±SD
Positive Control	NPD	10^{-2}	779.34±23.86	
	SA	10^{-3}		1247.67±39.32
DmAgNP		6.25	12.67±1.53	165.34±14.29
		25	20.00±3.60	122.00±12.49
		100	13.67±5.50	134.34±8.62
		400	27.67±8.50	130.00±15.09
D. majus		6.25	10.67±1.53	148.67±02.52
		25	15.67±1.53	176.67±13.05
		100	16.34±3.79	179.34±8.33
		400	16.00±2.64	161.67±3.52
Negative Control			32.67±2.08	116.67±10.59
Spontaneous Control			28.00±4.58	115.34±11.93

NPD: 4-nitro-*o*-phenylenediamine, SA: sodium azide (SA)

3.10. 2,2-Diphenyl-1-picrylhydrazyl (DPPH) Activity

The antioxidant activity of silver NPs tested by the DPPH method. Ascorbic acid (AA) was used as positive controls. There is a decrease in the activity of silver NPs compared to *D. majus* extract. Inhibition percentages at the highest concentration (400 ppm) were determined as 65.36 and 59.70, respectively (Fig. 9).

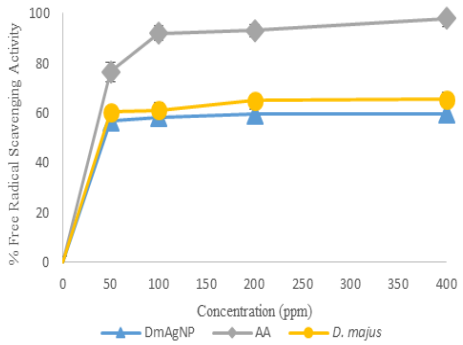

Fig. 9. Free radical scavenging activity of DmAgNP and D. majus aqueous extract

There are many ways to synthesize silver nanoparticles in various literature. These include physical, chemical, and biological methods. Unfortunately, the physical and chemical methods are numerous, and many of these methods are expensive or use toxic substances, the main factors that make them 'undesirable' synthesis methods. An alternative and viable method to synthesize silver nanoparticles are to use biological methods to utilize microbes and plants [37]. Since control of particle size and shape is an essential factor for various biomedical applications, using biological methods to synthesize Ag nanoparticles is an environmentally friendly alternative.

Significant application in the medical field, an antimicrobial, and an anticancer agent. The biosynthesis of silver nanoparticles using higher plants such as angiosperms, gymnosperms, and pteridophytes. Fewer trials were conducted in sub-cryptogamic plants such as *Riccia* sp. and *Fissidens* sp. [9,35,38-42]. Thus; Dm has selected to synthesize silver nanoparticles for this study.

Silver nanoparticles (AgNPs) have gained importance in medical applications with their significance and local surface plasmon resonance properties. They also have unique broad-spectrum antimicrobial properties against fungi, viruses, and bacteria [43-46].

In the study conducted by Coşkunçay (2017), the antimicrobial and antioxidant effects of spherical and 50±5 nm-sized silver NPs synthesized from the leaves of *Aesculus hippocastanum* (Horse Chestnut) plant were investigated [47]. While antibacterial activity was observed against fourteen bacteria (7 gram-positive, 7 gram-negative), no antifungal effect was observed against the three yeast strains tested. The antioxidant activity of silver NPs measured by the DPPH method increased in free radical removal activity with an increase in concentration (1.56-100 ppm). The % inhibition value was calculated as 54.72% at a concentration of 100 ppm. The interaction of 5 different NPs (6.25-100 ppm) with pBR322 plasmid DNA was examined by agarose gel electrophoresis, and no effect of NPs on DNA was observed.

In gel electrophoresis of plasmid DNA, when the original superhelix form (Form I) is opened with damage, an annular form (Form II) is formed, and more fractures can be found in the linear form (Form III). When conducted in DNA gel electrophoresis, Form I progress faster in the gel than others, while Form II goes more slowly, and Form III moves between Form I and Form II. Within the scope of the study, the cleavage activity of NP was examined. In our research showed that biologically synthesized AgNPs cleave plasmid DNA. Furthermore, Duman et al. (2016) found that CuO nanoparticles both disrupt and break the double helix structure of DNA [50].

Shah et al. (2018) synthesized 12 nm silver NPs from *Daphne mucronata* water extract [48]. The free radical scavenging capacity was investigated by the DPPH method, and silver NPs (85.4%) showed the highest antioxidant activity in water extract (91.99%) and 600 µg/ml concentration. The mutagenic effects of the water extract and the synthesized silver NPs were tested on TA98 and TA100 strains by the Ames method, and it was determined that the water extract and silver NPs were not mutagenic in the two strains.

Yıldız et al. (2011) carried out the biological synthesis of Ag nanoparticles, which are widely researched in the field of nanotechnology, using *Cetraria islandica* lichen extract [49]. As a result of the study, UV-Vis analyzed the samples synthesized under different conditions performed, and peaks in the range of 405-438 nm were obtained. By TEM analysis, the size of the particles was measured as 5.6-28.6 nm on average. Furthermore, to determine which bonds the synthesis of Ag nanoparticles proceeds with, the obtained lichen extract was analyzed with FTIR before and after interacting with $AgNO_3$. In the FTIR spectra before and after the interaction, the band at 1750 cm^{-1} wave number weakened, and the band at 1650 cm^{-1} wavelength determined that the band became stronger [49].

Duman et al. (2016) investigated the antioxidant activity of CuO nanoparticles obtained from chamomile flower extract and their interaction with plasmid DNA (pBR322) [50]. First, CuO nanoparticles were characterized by UV-Vis spectroscopy, FTIR, DLS, X-ray diffraction, EDX spectroscopy, and scanning electron microscopy techniques. Then, the antioxidant activity of this compound is determined by the DPPH method. They found the mean percent inhibition of CuONPs to be 87.27%. Mumtaz et al. (2019) synthesized zinc oxide and silver nitrate NPs using *Mimosa pudica* and applied the Ames test using *Salmonella typhimurium* TA98 and TA100 strains to determine their mutagenic activities. It was determined that NPs did not show any mutagenic effect [51]. Lakshmanan et al. (2018) synthesized 20-50 nm silver NPs using *Cleome viscosa* plant extract and investigated the antibacterial activities of the synthesized particles. Silver NPs were

found to have antibacterial activity against *Bacillus subtilis*, *Staphylococcus aureus*, *Escherichia coli*, and *Klebsiella pneumonia* [52]. MubarakAli et al. (2011) synthesized 150 nm gold and 90 nm silver NPs from the leaf extract of *Mentha piperita*. They determined that silver NPs showed potent antibacterial activity against clinically isolated human pathogens *S. aureus* and *E. coli*, gold NPs did not show activity against *S. aureus* [53].

They stated that the reason for this situation is that the thicker peptidoglycan layer in gram-positive bacteria may be a more substantial barrier and may cause reductions in the entry of nanoparticles into the cell [54]. At the same time, it was stated that teichoic acid and lipoteichoic acid found in gram-positive bacteria might also inhibit the entry of silver nanoparticles [55].

4. Conclusion

The basis of obtaining nanoparticles by green synthesis is based on reducing metal ions with biomolecules such as enzymes, proteins, amino acids, polysaccharides, and vitamins in organisms. It has long been known that plants have the potential to over-accumulate and reduce metallic ions. Because of these properties, plants are considered a more environmentally friendly tool for the biosynthesis and detoxification of metallic nanoparticles. The green synthesis method enables the production of nanoparticles on large scales, which are cost-effective, do not require toxic chemicals, are simpler, can turn into products in a short time, and are well defined in size and morphology [11,48,56].

Mosses are also organisms that can achieve high success in nanoparticle synthesis due to their biodiversity, growth rate, biomass efficiency, and especially heavy metal accumulation capabilities. In this present study, a novel and eco-friendly DmAgNPs was successfully green synthesized, characterized, and determined by its biological activity, including antibacterial, antimutagenic, and antioxidant. Changing in color is the primary confirmation of silver nanoparticle formation. Silver nanoparticles were characterized by UV-Vis, FTIR, TEM, Zeta potential, and particle size. Maximum absorption in UV-Vis is at 422 nm and indicated formation of DmAgNPs FTIR analysis reveals that the carboxyl functional groups of OH, C-H, COOR+C=O+C=C, and C-O were bound to the metal and acted as the capping agent to produce silver nanoparticles. The average particle size of the synthesized DmAgNPs is obtained as 268.6±8.86 nm means with standard deviation values by TEM. Our results demonstrated the nanoparticles were successfully synthesized. Thus, the present investigation proved that *D. majus* could fabricate the nanoparticles.

It is concluded that *D. majus* could be a good source for the synthesis of silver nanoparticles and acted as a promising antibacterial, non-mutagenic and antioxidant agent. Hence this plant may be used in the pharmaceutical field to develop a new drug.

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