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Article

Synthesis and characterization of *Sophora alopecuroides* L. green synthesized of Ag nanoparticles for the antioxidant, antimicrobial and DNA damage prevention activity

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In this study, it was aimed to investigate the amount of antioxidant, protective properties against DNA damage and antibacterial properties against various pathogens after the interaction of Ag metal (Ag NPs/Sa) of *Sophora alopecuroides* L. (*S. alopecuroides* L) plant seed, which is grown in Iğdır and used in the treatment of many diseases. The DPPH radical quenching activity of Ag NPs/Sa was performed by using Blois method, DNA damage prevention activity by gel electrophoresis and antibacterial property by disk diffusion method. With the green synthesis method, AgNPs obtained as a result of the reaction of the plant and Ag metal are UV visible spectrophotometer (UV-vis), fourier-transformed infrared spectroscopy (FT-IR), X-ray diffraction (XRD) and scanning electron microscope (SEM). DPPH radical quenching activity of Ag NPs/Sa was investigated in the concentration range of 25-250 µg/ml. The radical quenching activity at a concentration of 250 µg/ml was 85,215 ± 0,101%, while this value was 93,018% for the positive control BHA. It has been observed that the protective property of pBR322 plasmid DNA damage against OH radicals originating from H_2O_2 increases with concentration. It has been observed that Ag NPs/Sa has significant antimicrobial properties against some pathogens (*B. subtilis* ATCC 6633 *E. coli* ATCC 25952, *B. cereus* ATCC 10876, *P. aeruginosa* ATCC 27853, *E. faecalis* ATCC 29212, *S. aureus* ATTC 29213 and *C. albicans* ATTC 90028) that cause disease and even some pathogens are more effective than antibiotics.

Keywords: Antimicrobial. Antioxidant. Nanoparticle. Silver, *S. alopecuroides* L.. DNA damage.

INTRODUCTION

Nanotechnology is a rapidly developing method that is effective in all areas of human life (Khan *et al.,* 2018). In the studies conducted, the term Green Nanotechnology has emerged, which is based on the principle of the production of nanoparticles from living cells with low toxic substance content (Duncan, 2011; Chan *et al*., 2020). Nanoparticles with green synthesis are synthesized by different methods. These are physical, chemical and biological methods. Physical and chemical methods have high disadvantages as they contain expensive and toxic chemicals (Bhat, Nayak, Nanda, 2015; Geethalakshmi, Sarada, 2010; Saranya *et al*., 2017). Biological synthesis is a very cheap, environmentally friendly and safe method. Thanks to these advantages, it is preferred more (Latha *et al*., 2018; Valsalam *et al*., 2019).

Increase in free radical formation, decrease in antioxidant enzyme levels and / or defects in DNA repair mechanisms lead to an increase in oxidative DNA damage. Therefore, they cause the emergence of many degenerative diseases, especially cancer (Cooke *et al*., 2003; Kryston *et al*., 2011). Antioxidants are molecules that can be produced in the human body and are found in many plants and foods outside and have protective properties against free radicals. Antioxidants are defined as substances that delay or prevent oxidation of the substrate when it encounters an oxidizable

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substrate such as lipid, protein, DNA and carbohydrate even at very low concentrations (Frankel, Meyer, 2000).

The resistance created by bacteria against antibiotics is increasing day by day. In order to prevent this and especially for the treatment of infections, it is necessary to develop new alternative agents and their combinations with antibiotics. It has been known for a long time that AgNPs have antibacterial properties (Sukdeb, Yu, Joon, 2007; Molina *et al*., 2019). Silver nanoparticles (Ag NPs) are used in many fields as alternative medicine to antibiotics, sensors, spectroscopy and catalysis. These areas can be used in industry such as food and textile, especially in health (Pandit, 2015; Rai, Yadav, Gade, 2009).

S. alopecuroides L. plant is a plant that grows in south west and east Asia, Greece and southern Russia (Chamberlain Sophora, In Davis, 1970). This herb is widely used in Chinese medicine. Seeds of *S. alopecuroides* are used in the treatment of some skin and gynecological diseases such as eczema, dermatitis and colpitis, as well as fever, sore throat and inflammation. The quinolizidine alkaloids (QA) component contained in the plant *S. alopecuroides* L. has many effects; These are sedative, analgesic, antipyretic, anti-inflammatory, anti-tumor and significant antiviral activities (Atta-ur *et al*., 2000; Ding *et al*., 2006).

In our present study, it was aimed to synthesize silver nanoparticles by using the extract of *S. alopecuroides* L. plant seed collected in Iğdır region and calculate the antimicrobial activities, total antioxidant amount and DNA damage prevention activity of the obtained silver nanoparticles. Synthesized Ag NPs were characterized by UV-vis, FT-IR, XRD and SEM techniques.

MATERIAL AND METHODS

Synthesis of Ag NPs/Sa

S. alopecuroides L. plant was harvested in Iğdır province, Aralık district in August. The identification of the plant species was made in YYÜ, Faculty of Science, Department of Biology. After *S. alopecuroides* L. (Licorice) plant was brought to the laboratory, it was washed and allowed to dry. After drying at room temperature for 7 days, it was pulverized (Kianbakht, Dabaghian, 2016). For the synthesis of nanoparticles, Okaiyeto *et al*., (2019) was used with some changes in the method it used. 1 mM 500 ml $AgNO_3$ solution was prepared and 100 ml of *S. alopecuroides* L. plant extract was reacted at room temperature in 1000 ml flask. Color change occurred in the solution after 45-50 minutes. The resulting solution was centrifuged at 9,000 rpm for 8 minutes and the upper liquid was removed. The solid was washed twice with distilled water. The solid part obtained was left to dry for 72 hours at 45-50 °C in the oven.

Characterization of synthesized Ag NPs/Sa

The spectrophotometric imaging process of $AgNO_3$ and Ag NPs/Sa was performed on Thermo Fisher UV-Vis spectrophotometers. FT-IR spectra were recorded using Ag NPs/Sa and *S. alopecuroides* L. Perkin Elmar instrument in the range 4000-400 cm^{-1}. Ag NPs/Sa crystal properties were performed on an X-ray diffractometer (Panalytical Emperian Diffractometer). The size and morphological structures of the obtained nanoclusters (Ag NPs/Sa) were visualized with the help of scanning electron microscopy (SEM, Zeiss SmartEDX).

DPPH radical scavening activity

The DPPH quenching activity of the extract (Ag NPs/Sa) used in this study was calculated using the previously found method (Blois, 1958). BHA was used as positive control in this procedure. The experiment was carried out using methanol solutions of 0,1 mg / ml DPPH. DPPH and Ag NPs/Sa extract solutions were prepared at 5 different concentrations of 25, 50, 100, 200 and 250 µg / ml. 3 ml of Ag NPs/Sa extract and positive control were taken and DPPH solution was added on them. The mixtures formed in the tubes were incubated for 30 minutes in the dark and at room temperature. At the end of this period, absorbance values were read at 517 nm. As a result of these processes, a graph of Ag NPs/Sa concentration versus increasing DPPH ethanol concentration was obtained. This graph is obtained using the Eq.1:

$$\% I = [(A_{control}-A_{sample}) / A_{control}] \times 100 \quad (1)$$

DNA Damage Preventive Effect

The effect of silver nanoparticles obtained using the seeds of the *S. alopecuroides* L. plant to prevent damage to plasmid DNA was investigated by agarose gel electrophoresis (Gulbagca *et al*., 2019). Plasmid DNA (pBR322) and loading dye were added to each well. From the second pit, hydrogen peroxide was added to damage the DNA and the nucleic acids were exposed to UV. Silver nanoparticles were added from the third well (50 mg / L Ag NPs/Sa + DNA + H_2O_2 + UV). Fourth well (100 mg / L Ag NPs/Sa + DNA + H_2O_2 + UV) and Fifth well (250 mg / L Ag NPs/Sa + DNA + H_2O_2 + UV) were prepared. Imaging was performed after 45 minutes of electrophoresis at 110 Volts. The effect of Ag NPs/Sa clusters on DNA damage was examined.

Antimicrobial activity

Nanoparticles were obtained using seeds of *S. alopecuroides* L. plant and Silver nitrate. Antibacterial and antifungal activities of Ag NPs/Sa clusters were examined. Disk diffusion method was used for antimicrobial activity (Andrade *et al*., 2016). Neomycin was used as a positive control while applying the method. Six pathogenic bacteria such as *Bacillus subtilis* ATCC 6633 *Escherichia coli* ATCC 25952, *Bacillus cereus* ATCC 10876, *Pseudomonas aeruginosa* ATCC 27853, *Enterococcus faecalis* ATCC 29212, *Staphylococcus aureus* ATTC 29213 and *Candida albicans* ATTC 90028 were used. Strains used in the study were activated in Triptic soy Broth. Müller Hinton Agar medium was used for the disk diffusion method. Microorganisms were obtained from Van Yüzüncü Yıl University, Faculty of Science, Department of Molecular Biology and Genetics.

RESULTS AND DISCUSSION

Characterization of synthesized Ag NPs/Sa

SEM / SEM-EDX, FT-IR, XRD and UV-vis techniques were used for the structural and morphological characterization of Ag nanoparticles prepared by green synthesis using *S. alopecuroides* L. plant, respectively. Figure 1 (a-c) shows SEM images of Ag NPs / Sa sample taken at different scales and (d) EDX spectrum obtained from one of these images. It is seen from SEM images of different scales that Ag nanoparticles are generally distributed homogeneously. In addition, in the SEM images taken, it is seen that the average size of the silver particles is 3,7 nm, and in some regions it is 8,5 nm. The peaks of Ag, C and O elements in the structure of Ag NPs / Sa in the EDX spectrum are clearly visible.

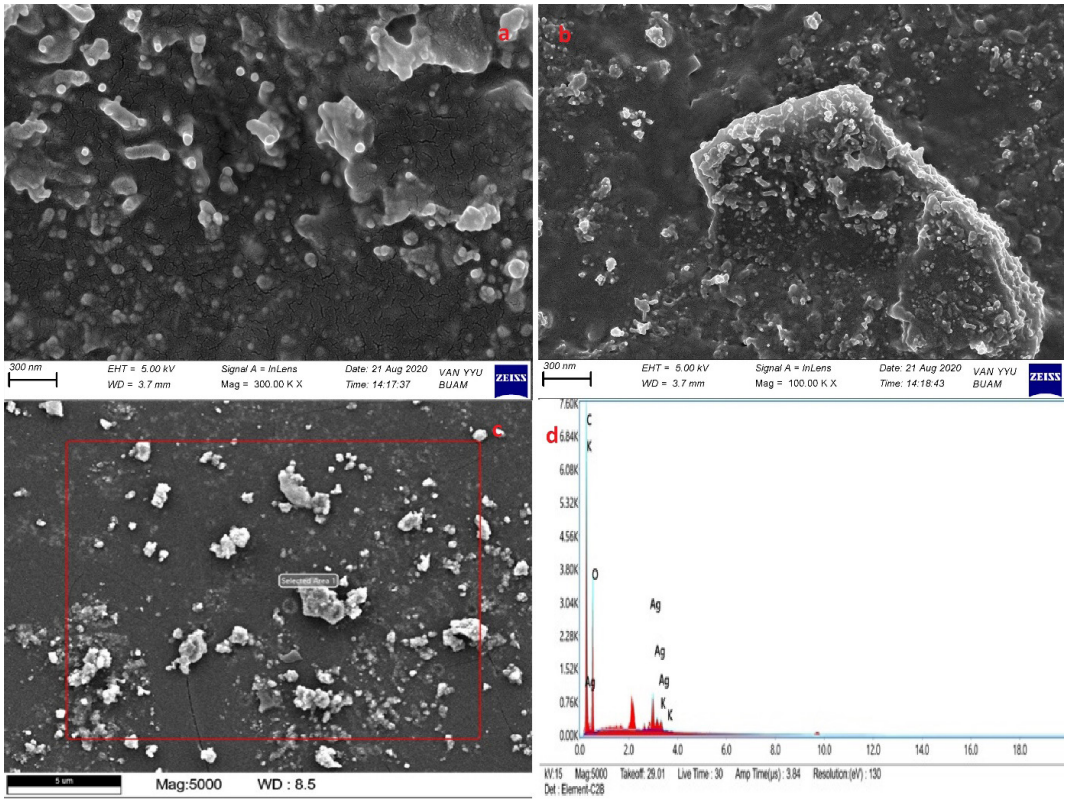


FIGURE 1 - (a-c) SEM images in different scale and corresponding (d) SEM-EDX spectrum of Ag NPs/Sa.

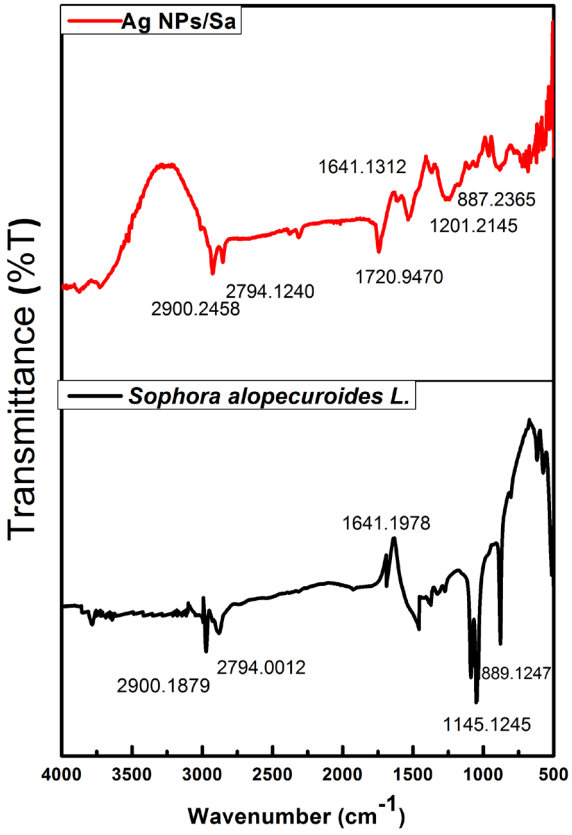


FIGURE 2 - FT-IR spectra of *S. alopecuroides* L. and Ag NPs/Sa.

Figure 2. shows FT-IR spectra of the Ag NPs/Sa sample with *S. alopecuroides* L. The signals observed in the IR spectrum of Sophora alopecuroides L. plant extract between 887-1720 cm^{-1} are due to functional groups in the structure of organic compounds (oxymatrin, sophocarpine, oxysophocarpine, cytisine, sophoramine, sophorodine, nicotine). The peaks observed in the 2794 and 2900 cm^{-1} region of the Sophora alopecuroides L. sample correspond to aromatic CH vibrations. In the FT-IR spectrum of the Ag NPs/Sa sample, it shows a decrease in peak intensities and shifts in some peaks. When the XRD pattern of the Ag NPs/Sa sample is examined in Figure 3 the signals of Ag (111), Ag (200), Ag (220) and Ag (311) surfaces are 37°, 48°, 67° and 78°, respectively. It is understood that these values are quite compatible with the reference data (Liu *et al*., 2018). Figure 4 shows the UV-vis spectra of $AgNO_3$ and Ag NPs / Sa samples. It was observed that the $AgNO_3$ (Ag^{+1}) solution gave two peaks at 670 and 405 nm in the UV-vis spectrum. In the UV-vis spectrum of the Ag NPs / Sa sample, it was observed that the peak signal disappeared completely at 670 nm, and the signal intensity around 405 nm was significantly reduced. This can be interpreted as substantially reducing the Ag^{+1} cation to metallic silver (Lopes, Moreira, Neto, 2020).

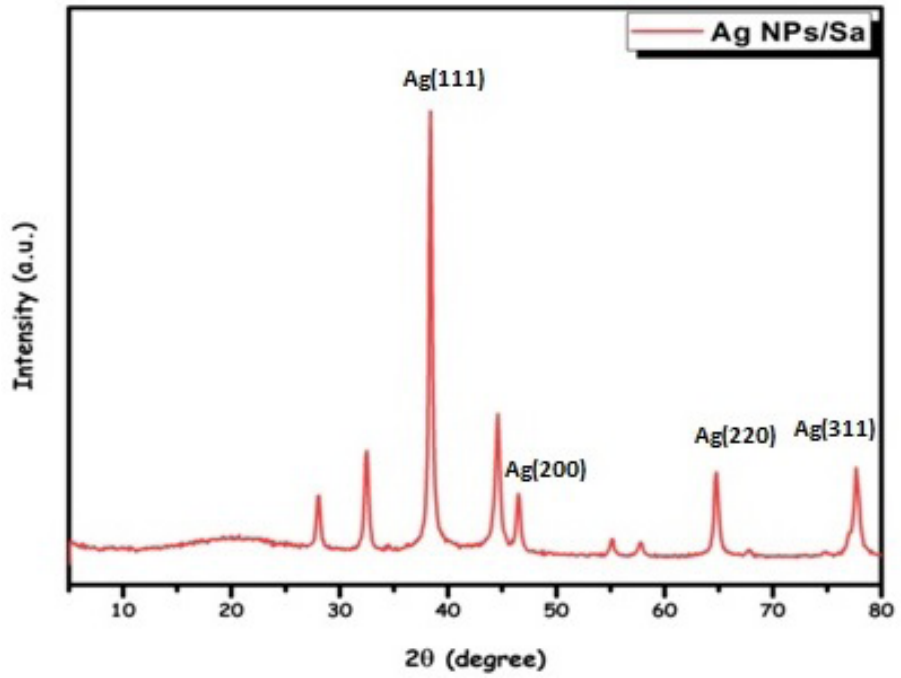


FIGURE 3 - XRD pattern of Ag Nps/Sa.

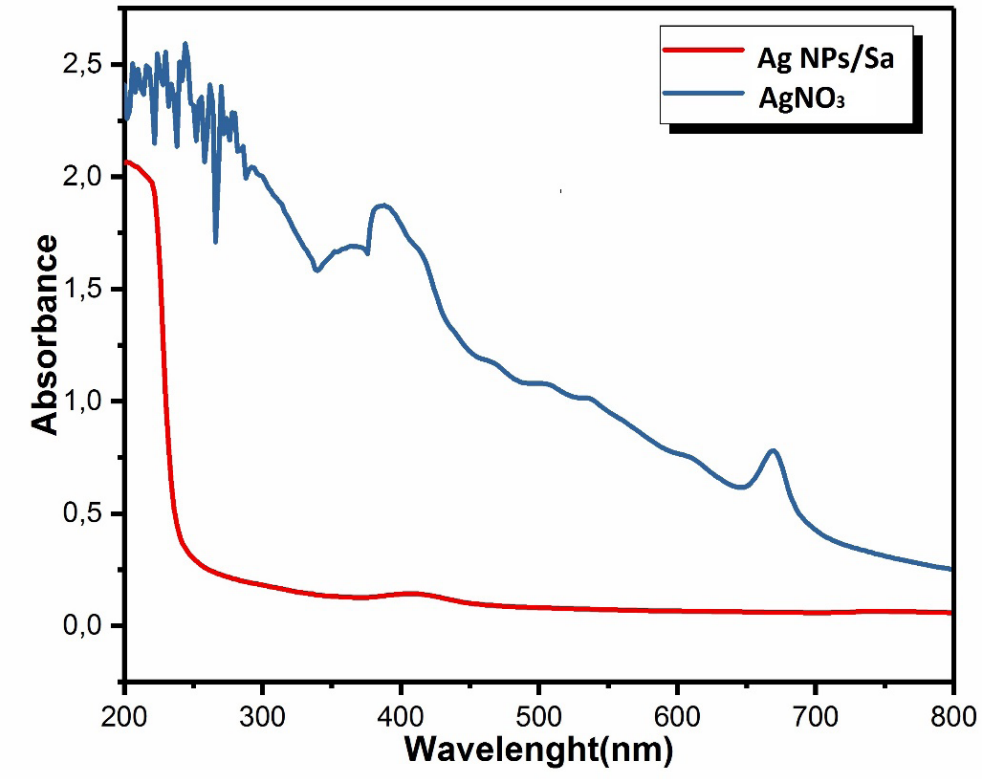


FIGURE 4 - UV-vis spectra of $AgNO_3$ and Ag NPs/Sa samples.

Determination of antioxidant activity

DPPH radical scavening activity

DPPH method is one of the most widely used spectrophotometric methods in antioxidant activity measurement. The antioxidant effect of a substance depends on its ability to scavenge free radicals in the environment (Sharma, Bhat, 2009). DPPH is considered to be a valid, cheap, fast, accurate, easy and economical method to evaluate the activity of antioxidants (Deng, Cheng, Yang, 2011; Kedare, Singh, 2011). In the present study, DPPH radical quenching activity of Ag NPs/Sa in different concentrations was shown in the Figure 5. In Figure 5, it is seen that the DPPH radical quenching activity of Ag NPs/Sa increases from 25 µg / mL to 250 µg / mL. When looking at the values in the graph, it is seen that the radical quenching activity is close to the positive control BHA. It can be said that Ag NPs/Sa sample has a very good antioxidant property and is a powerful preventive agent against radicals that threaten human health. Nano studies of the plant in our current study have not been found in the literature. When compared with similar plant studies, it has been found to be a powerful antioxidant (Vijayakumar *et al*., 2019; Afrah, Fadwa, Jehan, 2018).

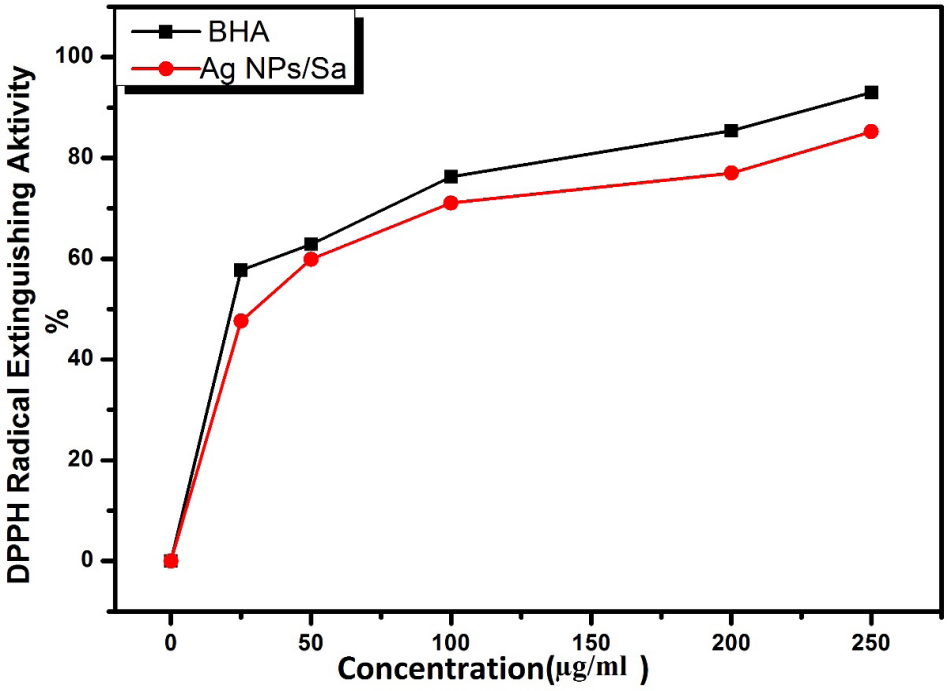
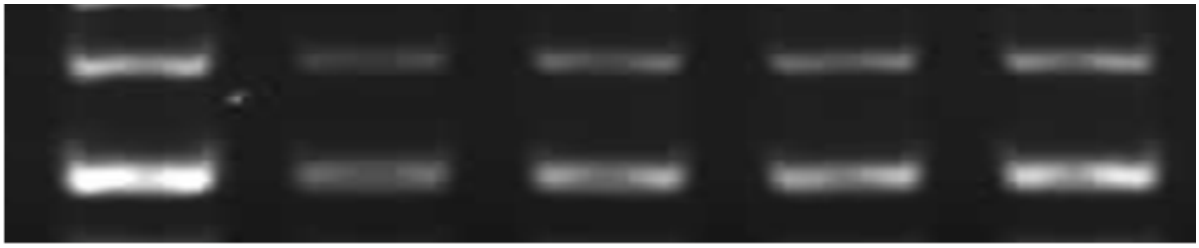


FIGURE 5 - DPPH radical scavenging activity of the ethanol extract of *S. alopecuroides* L. green synthesized of Ag NPs.

DNA damage prevention effect

Over production of free radicals in the human body can damage a wide variety of essential cellular biomolecules such as proteins, enzymes, DNA, RNA, lipids, and carbohydrates (Breen, Murphy, 1995). Damage to DNA and other biomolecules causes many diseases to occur. These diseases; carcinogenesis, aging, gastric ulcer, diabetes, neurodegenerative diseases, rheumatic joint in inamination and pathological conditions such as AIDS (Moskovitz, Yim, Chock, 2002; Temple, 2000). It was observed that silver nanoparticles prepared with *Bergenia ciliata* root extract had DNA protection effect (Zia *et al*., 2018). Looking at the image obtained as a result of agarose gel electrophoresis, it is seen that the anti-damage effect is low at the beginning in the wells with Ag NPs/Sa, and the activity of preventing DNA damage that may occur when the concentration is increased. With the increase in the ratio of nano clusters, it was observed that the DNA's moved at the desired level. It was observed that the protective effect of plasmid DNA was very good especially in the well prepared as 250 mg / L Ag NPs/Sa + DNA + H_2O_2 + UV. Gel electrophoresis image of Ag NPs/Sa clusters and substances added to the wells (Figure 6).



DNA	+	+	+	+	+
H_2O_2	-	+	+	+	+
UV	-	+	+	+	+
Ag NPs/Sa	-	-	50 mg/L	100 mg/L	250 mg/L

FIGURE 6 - Gel electrophoresis image of Ag NPs/Sa samples and substances added to the wells.

Antimicrobial activity

Extract obtained from seeds of *S. alopecuroides* L. plant was dissolved in H_2O. There was no antimicrobial effect of the extract absorbed on blank discs against pathogenic microorganisms. Flavonostilbens from *S. alopecuroides* L. exhibited antibacterial activities against *Staphylococcus epidermidis* (Wan *et al*., 2015). Alkaloids obtained from *S. alopecuroides* plant showed antimicrobial activity against pathogens *Staphylococcus aureus*, *Bacillus subtilis*, *Pseudomonas aeruginosa* and *Candida krusei* (Küçükboyacı *et al*., 2011). Das *et al*. (2019) applied the AgNPs they obtained to five different pathogens and obtained zones between 8.74 and 11.52 mm. In our study, it was found that Ag NPs / Sa has activation against *Bacillus cereus* ATCC 10876, *Candida albicans* ATTC 90028, *Bacillus subtilis* ATCC 6633, *Escherichia coli* ATCC 25952, *Enterococcus faecalis* ATCC 29212, *Pseudomonas aeruginosa* ATCC 27853 and *Staphylococcus aureus* ATCC 27853 and *Staphylococcus aureus* has been seen. It has been determined that silver nano clusters have an inhibitory effect against pathogens between 8.1-14.5 mm. In addition, silver nanoparticles were found to be more effective than neomycin antibiotics against *Enterococcus faecalis* ATCC 29212 and *Pseudomonas aeruginosa* ATCC 27853 pathogens. The zone diameters of the extract obtained from the seeds of the *S. alopecuroides* L. plant and Ag NPs/Sa against pathogens are given in Table I Some images obtained by disk diffusion method are given in Figure 7.

TABLE I - Antimicrobial activity results

Test Mikroorganisms	**Zone of Inhibition (mm)**		
Bacteria	**Extract-1**	**Ag NPs/ Sa-2**	**Neomycin-3**
Bacillus cereus ATCC 10876	-	12.2	20.4
Bacillus subtilis ATCC 6633	-	9.3	20.4
Escherichia coli ATCC 25952	-	8.1	13.1
Enterococcus faecalis ATCC 29212	-	10.3	
Pseudomonas aeruginosa ATCC 27853	-	14.3	8.0
Staphylococcus aureus ATTC 29213	-	14.5	17.7
Fungus			
Candida albicans ATTC 90028	-	12.1	21.6

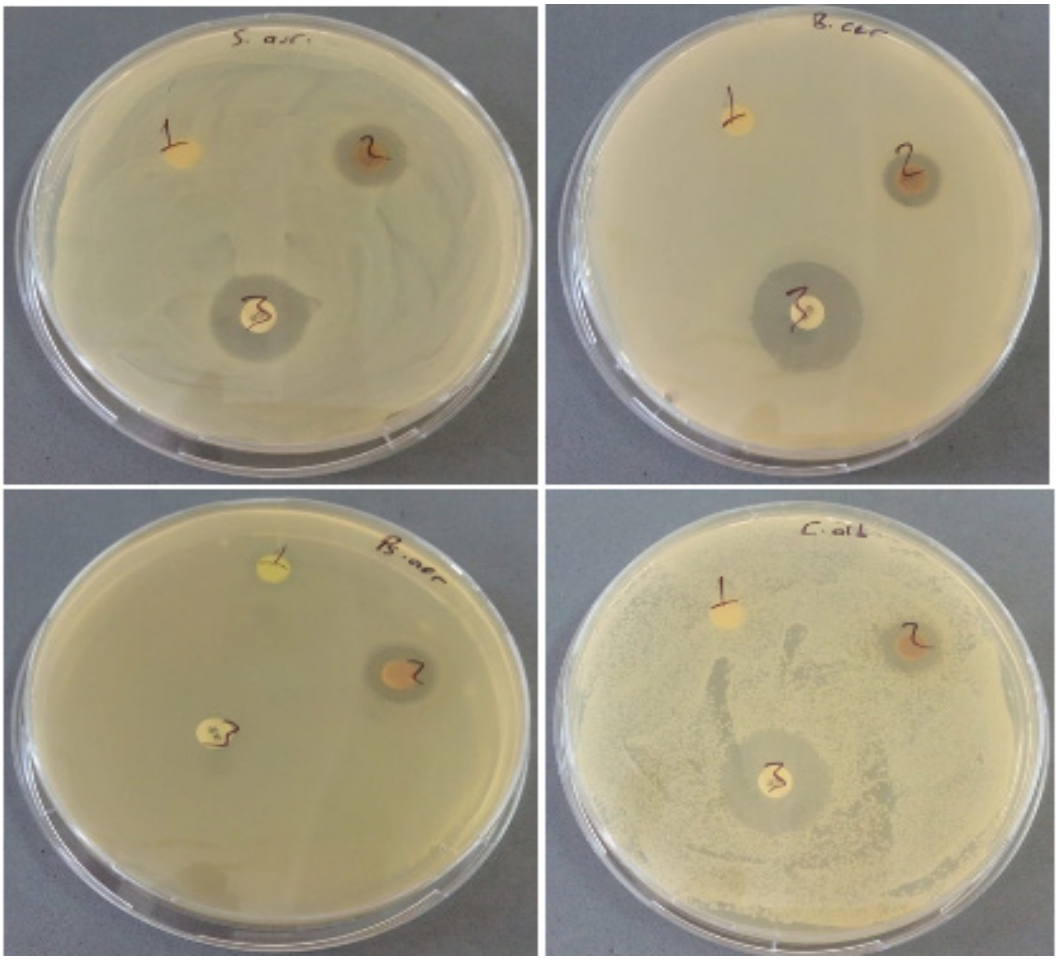


FIGURE 7 - Some images obtained by the disk diffusion method.

CONCLUSION

Until today, many articles have been written about the synthesis of silver nanaparticles using plant extracts. It is known that there are still numerous plant-silver nanoparticles that have not been synthesized in this field. Ag NPs/Sa clusters are the a new material produced as result of green synthesis. It is seen that some biochemical and microbiological analyzes of this nanomaterial have important results. Synthesized Ag NPs may be more environmentally compatible and economical and, as in many areas, be a promising candidate for the development of new antibacterial drugs. It is understood that the silver nanoparticles formed using the *S. alopecuroides* L. plant are successfully used by the organic components found in the plant. This means that Ag nano clusters that appear to be active in biological applications bind or successfully stabilize structures such as matrin, sophoridine and cytisine found in the plant *S. alopecuroides* L. The presence of a large number of silver metals on the surface or structure of the bioactive Ag NPs/Sa material synthesized as a result of this stabilization explains the structure-activity relationship. The findings from these studies could provide a basis for future studies of synthesized Ag NPs. In our current study, it is seen that Ag NPs/Sa is a good antioxidant when DPPH radical quenching activity is examined. At the same time, when the DNA damage protection activity is examined in the gel electrophoresis image, it is seen that the concentration of AgNPs/Sa is increased. The antimicrobial properties demonstrated by the Ag NPs/Sa reported here are against *Bacillus cereus* ATCC 10876, *Candida albicans* ATTC 90028, *Bacillus subtilis* ATCC 6633, *Escherichia coli*

ATCC 29952, especially *Pseudomonas aeruginosa* ATCC 27853 and *Staphylococcus aureus* ATTC 29213. It has been observed that they form an inhibitory zone between 8.0-14.0. In addition, silver nanoparticles were found to be more effective than neomycin antibiotics against *Enterococcus faecalis* ATCC 29212 and *Pseudomonas aeruginosa* ATCC 27853 pathogens. The results reported in this study are thought to contribute to the development of more efficient and non-toxic alternatives for pharmacological treatments by utilizing the tools developed by nanotechnology. However, more research needs to be done on this topic to fully elucidate the mechanism of interaction between Ag NPs/Sa and bacteria. It is also essential for pharmacological and toxicological studies, particularly in vivo research and the development and design of future antimicrobial therapeutic agents.

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