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Synthesis, characterization, antibacterial, antioxidant activity, and lipoxygenase enzyme inhibition profile of silver nanoparticles (AgNPs) by green synthesis from *Seseli resinosum* Freyn & Sint

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ABSTRACT

In the study, silver nanoparticles (AgNPs) were successfully synthesized by an environmentally friendly synthesis method using *Seseli resinosum* Freyn & Sint extract. The synthesized silver nanoparticles were characterized by ultraviolet/visible light absorption spectrophotometer (UV-Vis), X-ray diffraction (XRD), and scanning electron microscopy (SEM) analysis. As a result of the characterization, it was determined that 33 nm spherical nanoparticles were formed, showing a spectrum at ~420 nm wavelength. Silver nanoparticles showed a bacteriocidal effect against all bacterial strains. DPPH and ABTS methods were used to examine the antioxidant activities of plant extracts and AgNPs. In DPPH removal activity, AgNPs obtained by green synthesis provided a high rate of inhibition removal compared to the extract. According to this percentage, while silver nanoparticles provided 22% removal, the extract provided 15% removal. In ABTS removal activity, when AgNPs were obtained by green synthesis compared to the extract, silver nanoparticles provided 25% removal, while the extract provided 18% removal. The characterization of silver nanoparticles synthesized by the green synthesis method and their antioxidant activity were investigated, and the obtained values indicate the presence of an antioxidant capacity. In addition, the inhibitory effects of the extract and AgNP on lipoxygenase activity, which has an important place in health, were investigated. It was determined that the aqueous extract of *S. resinosum* and the AgNP synthesized from the extract had lipoxygenase enzyme inhibitory activity.

1. Introduction

Nanoparticles; are materials with sizes ranging from 1-100 nm (Ahmed et al., 2016). Nanoparticles are used in many fields such as bio-medical, catalysis, food, clothing, cosmetics, and electronics (Gopinath et al., 2016; Saravanakumar et al., 2018; Selvakumar et al., 2018). Silver (Ag) has been used in antibacterial applications in many fields for many years due to its activity against bacteria and other microorganisms (Yang et al., 2012); silver nanoparticles (AgNPs) have been reported by some researchers to have strong antibacterial activity (Xu et al., 2011; Kung et al., 2018). Various methods are used to obtain silver nanoparticles. Among these methods, the biological method is preferred more than other (physical and chemical) methods because it is environmentally friendly and economical (Chen et al., 2003; Francis et al., 2017). In the synthesis of silver nanoparticles, the use of plant sources is more economical, and the application processes are easier, so it has attracted more attention recently (Pallela et al., 2018). Free radicals are atoms, molecules, or ions with unpaired electrons that are unstable to react chemically with other molecules. Oxygen-centered free radicals are known as reactive oxygen species (ROS) (Han et al., 2018). Oxidative stress is the result of an imbalance between ROS and antioxidant defenses. Oxidative stress disrupts a number of cellular functions and causes various negative effects as it suppresses the organism's antioxidative defense mechanism. Antioxidants are molecules that can prevent or delay the oxidation of an oxidizable substrate (Halliwell, 1990). Therefore, research on natural antioxidant compounds that can be obtained from plants and can efficiently scavenge free radicals has been increasing in recent years (Sindhi et al., 2013). Lipoxygenases (LOX) enzymes are a group of enzymes

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detected in animals, food yeast, plants, algae, fungi, cyanobacteria, and algae. In the pathway of arachidonic acid metabolism carried out by LOX, substances called reactive oxygen species (ROS) are formed. Other arachidonic acid metabolites and ROS substances formed in this way can cause tumor formation or inflammation (Juntachote and Berghofer, 2005). In addition, LOXs and their metabolites are implicated in many types of human cancers, such as prostate, lung, breast, colon, and other cancer cell lines (Samuelsson et al., 1987). It is very important to find new LOX inhibitors to treat human diseases such as cancer, cardiovascular and neurodegenerative disorders (Kelavkar et al., 2000). LOX is a very important enzyme in the food sector as well as in the health sector, and its characterization and inhibition should be investigated. However, the demand for natural antioxidants has increased recently due to the toxicity of these synthetic compounds (Guzel et al., 2017). This study it is aimed to determine the characterization, antibacterial, antioxidant activity, and lipoxygenase enzyme inhibition profile of AgNPs obtained by using the extracts of *Seseli resinosum* Freyn & Sint by green synthesis method without using toxic and expensive chemicals. Characterization was done by SEM, UV-Vis, and XRD analysis. The effects of AgNPs on antibacterial, antioxidant, and lipoxygenase enzyme inhibition were investigated. In conclusion, extracts and AgNPs may be promising antioxidants for the health industry.

2. Materials and methods

2.1. Preparation of plant sample and extract

After the plant sample used in the study was collected, it was thoroughly washed in tap water, dried in a cool and moisture-free environment, and made ready for grinding (Davis, 1970). Distilled water was used as a solvent to prepare the plant extract, which was dried at room temperature. The leaf part of the plant was ground with the help of liquid nitrogen by crushing in a mortar. 10 g of the ground plant sample was weighed, and 150 ml of distilled water was used as a solvent. The magnetic heater was also set at 80 °C and extracted for 4 hours. At the end of the period, filtration was performed using filter papers. The extract obtained after this process was put at +4 °C to be used (Antony et al., 2013).

2.2. Biological synthesis of AgNPs by green synthesis method

The dried plant leaves were crushed in a mortar and powdered, 10 g were weighed, and distilled water was added to it. The magnetic heater was also subjected to the extraction process for 4-5 hours, and at the end of the period, filtering was done using filter papers. 60 ml of the prepared extract was taken, and 1 mM silver nitrate ($AgNO_3$) solution was added. The mixture was stirred in the magnetic heater for 1 hour until a color change occurred. After the color change was observed, the mixture was centrifuged at 13.000 rpm for 15 minutes, and thus the nanoparticles were purified from other organic molecules. Each time, the supernatant was poured and added from the prepared solution again. Finally, the part remaining at the bottom was dried in an oven at 35 °C (Rather et al., 2013).

2.3. Characterization of synthesized AgNPs

Many methods are used to characterize nanoparticles. In this study, SEM, UV-Vis, and XRD, the basic techniques for the characterization of AgNPs, were used. The analyzes were made by the Eastern Anatolia High Technology Center (DAYTAM), which operates within Atatürk University.

2.3.1. SEM analysis

The characterization of the shape, surface topology, and morphology of the obtained AgNPs was carried out using the Zeiss Sigma 30 model scanning electron microscope (SEM) located at Eastern Anatolia High Technology Center (DAYTAM) Ataturk University.

2.3.2. UV-Vis spectroscopy

1 mg of AgNPs was dissolved in 3 ml of deionized water. The sample was sonicated for a certain time for the AgNPs to show a good distribution in water. This solution was then placed in the cuvette, and the AgNPs were characterized by measuring their UV-Vis spectra at wavelengths between 200 and 875 nm.

2.3.3. XRD analysis

The XRD pattern of the synthesized AgNPs was analyzed with the PANalytical Empyrean XRD device with a step size of 0.02 in the range of 2θ between 10° and 90°. UV-Vis spectra and XRD results were evaluated in the OriginLab data analysis program, and their graphics were drawn.

2.4. Determination of antibacterial activities

2.4.1. Microorganisms used in the study

A total of 6 strains of gram-positive [*Bacillus subtilis* (DSMZ 1971), *Staphylococcus aureus* (ATCC 25923), *Enterococcus faecalis* (ATCC 29212)] and gram-negative [*Pseudomonas aeruginosa* (ATCC 27853), *Salmonella enteritidis* (ATCC 13075), *Escherichia coli* (ATCC 25922)] microorganisms selected by using minimum inhibition concentration (MIC) method. As positive controls, Ertapenem, Tetracycline, Gentamicin (10 mg/ml) antibiotic discs were used.

2.4.2. Disc diffusion method

Sterile, disposable, MH (Mueller Hinton) agar with a 4 mm height medium was used on petri dishes with a diameter of 15 cm. A sterile loop was taken from the bacterial colonies that grew as pure colonies on the culture plates and inoculated into MH broth, incubated for 1-2 hours at 37 °C. After turbidity was formed, standard turbidity was established by adjusting McFarland 0.5 (10^8 microorganisms/ml). Widespread cultivation was performed from this suspension on MH agar medium with a sterile swab. Dilutions of the substance to be investigated with distilled water at a certain concentration were prepared. Paper disks impregnated with 20 µl of these dilutions were placed in the medium with sterile forceps. 3 were run in parallel. Three different antibiotics [Ertapenem, Tetracycline, and Gentamicin (10 mg/ml)] were used for control purposes. After incubation of petri dishes at 35-37 °C for 18-24 hours, inhibition zone diameters were measured (Hudzicki, 2009).

2.5. MIC analysis

The antibacterial activities of silver nanoparticles were determined by the minimum inhibition concentration (MIC) method (Wang et al., 2017). For the microdilution method, 96-well microplate wells were used. In applications carried out with the microdilution method, an MHB medium for bacteria was added to the wells of 96 microplates. After preparing a series of dilutions from the adjusted concentrations of silver nanoparticles, AgNPs solutions were added to the microplates and diluted. Then, a certain amount of microorganism solutions prepared and adjusted according to 0.5 McFarland (1.5×10^8 CFU/ml) were added to the microplates and

incubated overnight at 37 °C for 24 hours. At the end of the period, the absorbance value was measured at 600 nm in the spectrophotometer device. Obtained results were schematized as multiples of MIC, and % inhibition was calculated.

2.6. Methods applied in Determining antioxidant capacity

2.6. Identification of radical scavenging potential

2.6.1. DPPH method

To prepare a 0.1 mM DPPH solution, 4 mg of DPPH was weighed. It was dissolved by adding 200 ml of methanol. 50µl of extract and AgNPs sample was added to eppendorf tubes. It was vortexed by adding 200µl of DPPH solution to them. The absorbance was measured at 517 nm after being kept in the dark for 60 minutes and added to the 96 well plate wells (Almeida et al., 2020).

2.6.2. ABTS method

To prepare a 7 mM ABTS solution, 0.192 g ABTS and 0.0324 g potassium persulfate were added to 50 ml distilled water. The volume was completed to 100 ml and kept in the dark overnight by mixing these mixtures. Then, 1 ml of this mixture was taken, and 39 ml of methanol was added to it and diluted to 40 ml. After dilution, 15µl of plant extract and AgNPs were taken. 285 µl of ABTS solution was added to them and vortexed. After 2 hours in the dark, absorbance was measured at 734 nm (Almeida et al., 2020).

2.7. Lipoxygenase enzyme inhibition

In the determination of lipoxygenase activity of the samples, the spectrophotometric method is given by Anthon and Barrett (2012) was used with some modifications. To determine the lipoxygenase activity, 0.2% Triton X-100 was added to 0.1 M phosphate buffer (pH 6.5) to free the enzyme, and a homogenization buffer was prepared. Linoleic acid was used as a substrate for the activity assay. To prepare 25 ml of the linoleic acid substrate, 280 mg of Tween 20 and 140 mg of linoleic acid were added to 5 ml of distilled water. 0.6 ml, 1 N NaOH was added to clarify the solution. The volume was made up to 25 ml with distilled water and stored at -18 °C as 1 ml aliquots. At the extraction stage, approximately 5 g of the sample was weighed, and the same amount of cold (4 °C) homogenization buffer was added and centrifuged at 9000 g for 10 minutes. After centrifugation, some of the supernatants were transferred to a clean centrifuge tube. The enzyme obtained from soybean [Glycine max (L.) Merr.] was used. The enzyme solution was prepared by weighing 5 mg enzyme and dissolving it in a 4.3 ml phosphate buffer. The prepared enzyme solution was stored in eppendorf tubes in 250 µl portions at -80 °C until the experiments were carried out. Nordihydroguaiaretic acid (NDGA) was used as the reference inhibitor. 2 mg of NDGA was weighed and first dissolved in 2 ml of methanol. Pure water, buffer solution, substrate solution, and inhibitor mixture was used as blank. The reaction started with the addition of 0.2 ml of the enzyme extract, and the increase in absorbance at 234 nm wavelength was observed for 3 minutes. The amount of enzyme was calculated as nmol/GFW/min from the slope of the absorbance curve at 234 nm wavelength versus time.

3. Results and discussion

3.1. Characterization of AgNPs

3.1.1. SEM results

SEM images of AgNPs obtained from *S. resinosum* by the green synthesis method are given in Figure 1. From the SEM images, it is seen that the particles have different diameters and sizes. SEM images of AgNPs were evaluated, and they were found to be spherical. With the *Trianthema decandra* extract, it was determined that the particles were 33 nm on average (Geethalakshmi and Sarada, 2010). They synthesized spherical nanoparticles with sizes ranging from 16-50 nm with *Sterculia foetida* extract (Premkumar et al., 2018). In another study with orchid extract, spherical-looking AgNPs varying between 15-40 nm were obtained (Gopinath et al., 2017).

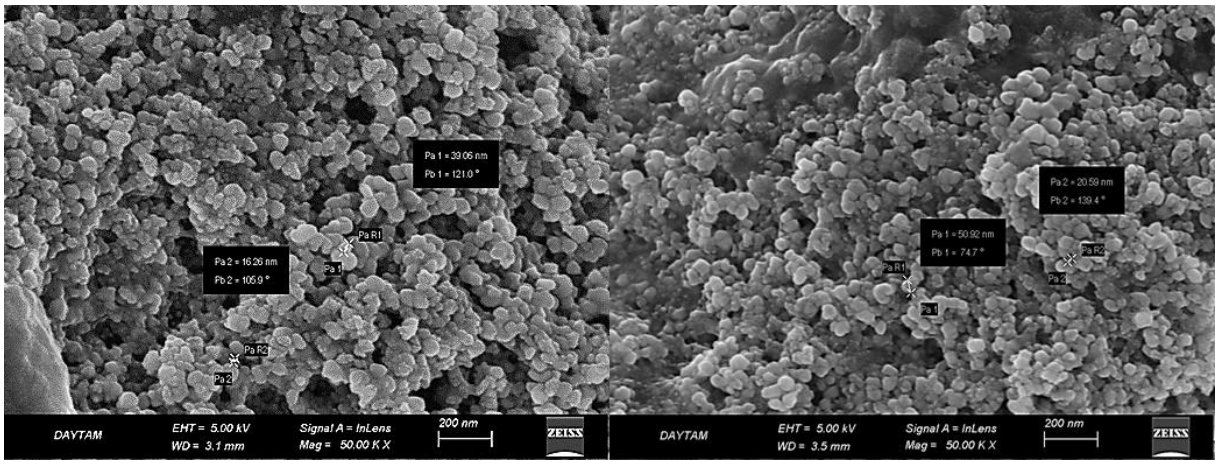

Figure 1. Images of SEM analyzes of synthesized silver nanoparticles

3.1.2. UV-Visible absorption analysis

The formation of silver nanoparticles causes vibrations on the plasma surface to change the color of the solution from yellow to brown. This indicates the formation of nanoparticles (Geethalakshmi and Sarada, 2010; Al-Ogaidi et al., 2017). The color change observed in the study and the data with a maximum absorbance of 420 nm in UV-Vis measurements supports this formation (Figure 2). Similar absorbances are observed in the study with green coffee (Wang et al., 2017). Alsammarraie et al. (2018) stated that they found a maximum value of 435 nm due to UV-Vis analysis using turmeric (*Curcuma longa*) plant extract. In another study, the maximum

absorbance of 461 nm was determined in the synthesis of AgNPs with the plant extract of corn leaves (Eren and Baran, 2019).

3.1.3. XRD analysis

The X-ray diffraction of the obtained AgNPs was evaluated (Figure 3), and the characteristic peaks of silver at 111, 200, 220, and 311 (with values of 27.81o, 32.22o, 46.27o and 54.89o at 2θ) showed the crystal structure of silver. It was observed that similar data were obtained due to the synthesis made with *Sida cordifolia* plant extract (Pallela et al., 2018).

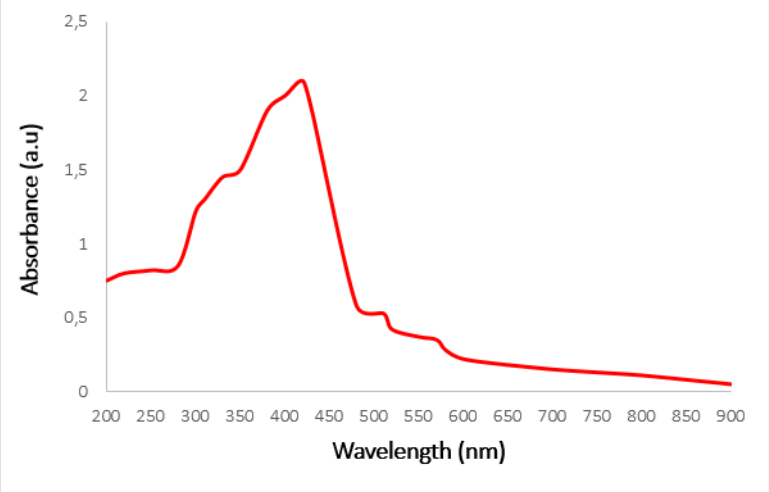

Figure 2. UV-Vis spectrum of synthesized AgNPs

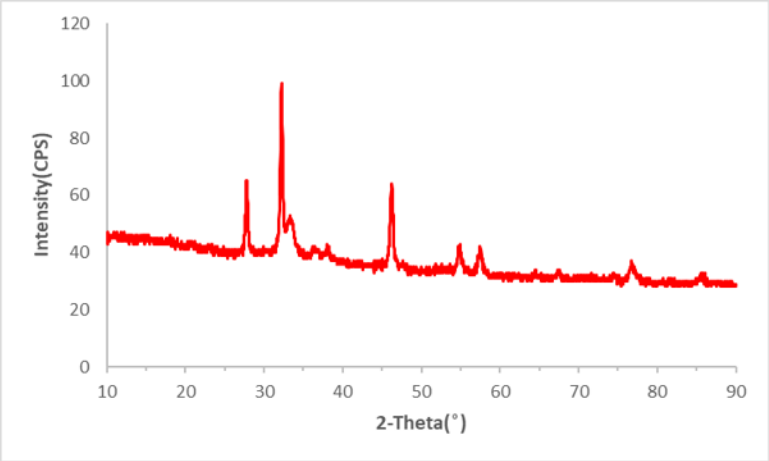

Figure 3. XRD results of synthesized AgNPs

3.2. Antibacterial activity results

In this study, antibacterial activities of AgNPs were obtained using *S. resinosum* plant; disc diffusion was evaluated against a total of 6 strains of gram-positive (*B. subtilis*, *S. aureus*, *E. faecalis*) and gram-negative (*P. aeruginosa*, *S. enteritidis*, *E. coli*) microorganisms selected by minimum inhibition concentration (MIC) methods. As positive controls, Ertapenem, Tetracycline, Gentamicin (10 mg/ml) antibiotic discs were used. It was determined that the antibiotics showed an inhibition zone in the range of 7-13 mm against the test microorganisms. Disc diameters were included when calculating the results. The minimum inhibitory concentration value of the plant extract and AgNPs showing antibacterial effect by the disc diffusion method was determined using 96-well microplates. After the incubation period was complete, the spectrophotometer (600nm) was measured. The minimum inhibitory values (mg/ml) of the tested compounds against bacteria are given in Table 1. The MIC range for *E. coli* ATCC 25922, *P. aeruginosa* ATCC 27853, *S. aureus* ATCC 25923, *B. subtilis* DSMZ 1971, *E. faecalis* ATCC 29212, and *S. enteritidis* ATCC 13075 was between 0.625-0.001 mg/ml, and the MIC_{90} values were 0.009, 0.009, 0.019, 0.019, 0.019, and 0.009 mg/ml, respectively. 95.83% of *E. coli* (ATCC 25922), 94.76% of *P. aeruginosa* (ATCC 27853), 84.14% of *S. aureus* (ATCC 25923), 83.07% of *B. subtilis* (DSMZ 1971), 80.93% of *E. faecalis* (ATCC 29212), and 92.62% of *S. enteritidis* (ATCC 13075) were found to be sensitive to the substance (Table 1). Dipankar and Murugan (2012) reported that silver nanoparticles obtained from *Iresine herbstii* leaf extract showed strong antibacterial activity against *S. aureus*, *P. aeruginosa*, *E. coli*, *E. faecalis*, and *Klebsiella pneumoniae* bacteria. Singhal et al. (2011) analyzed the AgNPs obtained from the *Ocimum sanctum* plant extract, a basil species, on *E. coli* and *S. aureus* bacteria and stated that nanoparticles had an antibacterial effect even at lower concentrations than standard antibiotics. According to the results obtained, AgNPs showed bacteriocidal effects against all bacteria. It was observed that the nanoparticles we used in our study also showed antibacterial effects at low concentrations. They

reported that smaller-sized AgNPs might cause more toxicity in bacteria, and they may have a better bactericidal effect than larger particles because they have a larger surface area (Zhang et al., 2016). Agnihotri et al. (2014) found that AgNPs smaller than 10 nm increased their antibacterial activity. Banala et al. (2015) reported that a 25 µg/ml concentration of AgNPs obtained with *Carica papaya* leaf extract has the minimum inhibition concentration for gram-positive and gram-negative bacteria. It was observed that the nanoparticles we used in our study also showed antibacterial effects at low concentrations (Table 2).

Table 1. MIC ranges, MIC_{90} values, and % inhibition values of the chemical according to different species

Bacterial isolates	MIC range (mg/ml)	MIC_{90} (mg/ml)	% Inhibition
E. coli	0.625-0.001	0.009	95.83
P. aeruginosa	0.625-0.001	0.009	94.76
S. aureus	0.625-0.001	0.019	84.14
B. subtilis	0.625-0.001	0.019	83.07
E. faecalis	0.625-0.001	0.019	80.93
S. enteritidis	0.625-0.001	0.009	92.62

Table 2. Zone diameters of AgNPs in mm against bacterial strains by disc diffusion method*

Bacterial isolates	Amount(mg/ml)	Zone diameters (mm)	Control antibiotic zone diameters		
			Ertapenem	Tetracycline	Gentamicin
S. aureus	5.0	13 ± 0	28 mm	34 mm	25 mm
	2.5	12.33 ± 0.57			
	1.25	12 ± 0			
	0.625	11 ± 0			
E. coli	5.0	11.33 ± 0.57	35mm	28 mm	25 mm
	2.5	10.66 ± 0.57			
	1.25	10 ± 0			
	0.625	10 ± 0			
P. aeruginosa	5.0	12 ± 0	19 mm	16 mm	25 mm
	2.5	11.66 ± 0.57			
	1.25	10.66 ± 0.57			
	0.625	10.66 ± 0.57			
B. subtilis	5.0	11.5 ± 0.57	25 mm	18 mm	25 mm
	2.5	11 ± 0			
	1.25	11 ± 0			
	0.625	10.66 ± 0.57			
E. faecalis	5.0	7 ± 0	11 mm	16.5 mm	25 mm
	2.5	9.3 ± 0.57			
	1.25	10 ± 0			
	0.625	10 ± 0			
S. enteritidis	5.0	13 ± 0	19 mm	14.5 mm	25 mm
	2.5	12.6 ± 0.57			
	1.25	12 ± 0			
	0.625	11.33 ± 0.57			

*In the table, the inhibition zone diameters are given in mm and the mean ± SE.

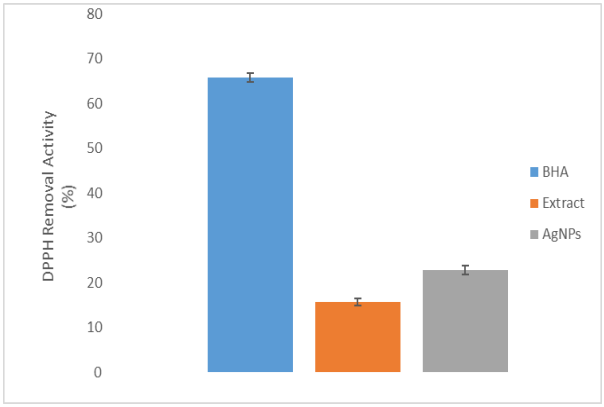

Figure 4. Plant extract and AgNPs DPPH removal activity (%)

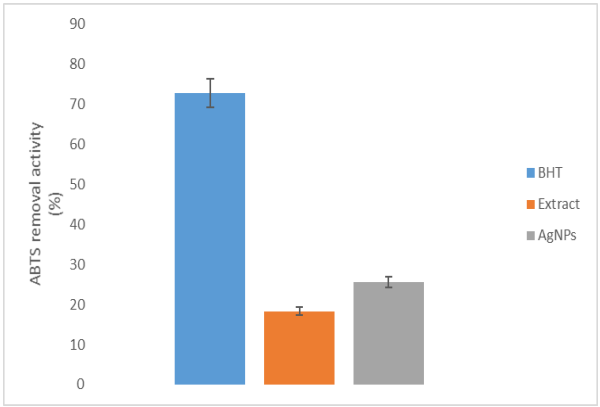

Figure 5. Plant extract and AgNPs ABTS removal activity(%)

3.3. Results of antioxidant capacity tests

DPPH (2,2-diphenyl-1-picrylhydrazil) is organic nitrogen radical commercially available product. It is a simple and fast method used to measure the antioxidant capacity of plant extracts. In DPPH removal activity, AgNPs obtained by green synthesis provided a high percentage of inhibition removal than extract. Silver nanoparticles provided 22% removal based on this percentage, while extract caused 15% removal (Figure 4). It shows that the flavonoid and phenolic groups in *S. resinosum* have a synergistic effect on the silver nanoparticle, and besides, the physical and chemical properties of the silver nanoparticle were also effective in DPPH removal. DPPH removal activity of butylated hydroxyl anisole (BHA) was 65%, and it was higher than extract and silver nanoparticle obtained by green synthesis. Silver nanoparticles obtained by green synthesis provided 25% ABTS removal. The extract showed an 18%

removal rate (Figure 5). BHT (Butylated hydroxytoluene) provided 72% ABTS removal compared to the nanoparticle and extract obtained by the biological method. According to the results, it was observed that the best ABTS removal was in the nanoparticle obtained by biological synthesis (25%). This value is compatible with previous studies (Genc, 2021; Genc et al., 2020), and the free radical scavenging power of silver nanoparticles is related to different types of functional groups responsible for the reduction and coating of silver nanoparticles. Our results showed that these biological components increased the antioxidant activity of silver nanoparticles.

Table 3. Lipoxygenase inhibition of plant extract and AgNPs

Plant Name	Extract/AgNPs	LOX inhibition (IC_{50} µg/mL)
S. resinosum	Extract	6.83 ± 1.1
	AgNPs	3.67 ± 0.5

3.4. Results of lipoxygenase enzyme inhibition

The inhibition potential of aqueous and methanolic extracts of *S. resinosum* on LOX enzyme was measured *in vitro*. NDGA was used as standard. Concentrations of 1, 1.3, 1.6, 2.3, and 2.6 µg/mL of NDGA have been tested. The NDGA substance inhibited the LOX enzyme by 66.6%, even at a 1 µg/mL concentration. The concentration showing the IC_{50} value of NDGA (which inhibits the enzyme by 50%) was calculated as 1.09 µg/mL. The effect of extract and AgNPs of plants on lipoxygenase activity is shown in Table 3. *S. resinosum* extract and AgNPs inhibited LOX less than NDGA (Figures 6, 7, and 8). *S. resinosum* was found to have LOX inhibitory activity. The IC_{50} value of the AgNPs was 3.67 ± 0.5 mg/ml, and the IC_{50} value of the extract was 6.83 ± 1.1 µg/ml. The higher inhibitory activity is associated with a lower IC_{50} value. This plant extract is the most active compound against the enzyme. Inhibition of lipoxygenase enzyme activity by various plants has been studied. They showed that *Epilobium angustofolium* extract inhibited lipoxygenase activity, and they found the IC_{50} value as 0.57 ± 0.06 µg/ml (Onar et al., 2012). Water extract of *Pituranthos chloranthus* showed strong lipoxygenase inhibition with an IC_{50} value of 0.02 mg/ml. Soybean lipoxygenase was successfully inhibited by *Lavatera cretica* leaf and flower water extracts with an IC_{50} value of 0.01 µg/ml (Lončarić et al., 2021). The results suggest that plant extracts have a potentially high anti-inflammatory effect (antilipoxygenase activity), related to polyphenolic content and other antioxidant substances.

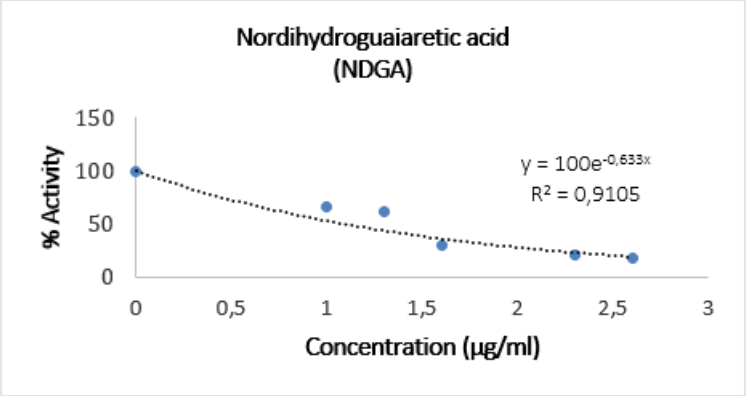

Figure 6. The IC_{50} value of NDGA to LOX enzyme

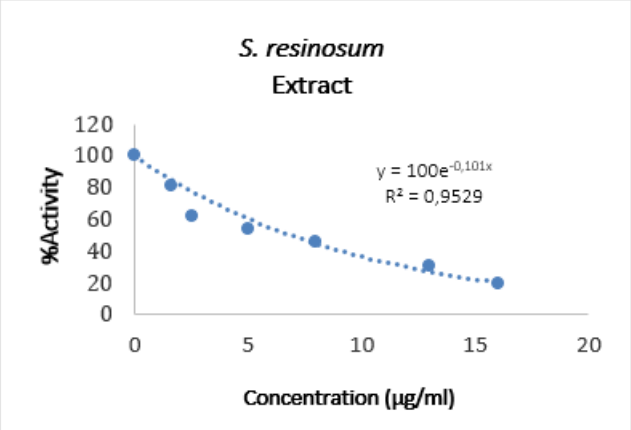

Figure 7. The IC_{50} value of plant extract to LOX enzyme

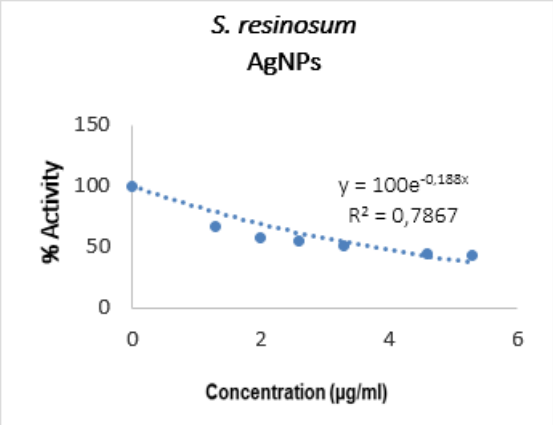

Figure 8. The IC_{50} value of AgNPs to LOX enzyme

4. Conclusions

The interest in the green synthesis method used to obtain nanoparticles increases day by day. It has been determined that these particles are effective at lower concentrations against commercial antibiotics, and AgNPs synthesized by the green method has a good antibacterial effect. With the increase in nanotechnological research, synthesized AgNPs will open a new field in producing pharmaceutical products; in the pharmaceutical industry, biomedical and industrial products can become more useful. Also, in this study, it has been shown that *S. resinosum* extract and AgNPs synthesized from the extract have LOX inhibitory

activity, indicating that they may be useful in treating various inflammatory diseases such as cancer, allergic disease, asthma, aging, and atherosclerosis. In addition, the biochemical examination of the effect of the plant extracts on the inhibition of target enzymes may pave the way for their presentation to the biotechnology market as alternative drug molecules to be used in different fields of pharmaceutical chemistry and industry.

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None.

Conflict of interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

CRediT authorship contribution statement

Ozlem Bakir: Conceptualization, Investigation, Data curation, Writing - original draft, Visualization
Pınar Güller: Formal analysis, Investigation, Methodology
Esabi Basaran Kurbanoglu: Conceptualization, Formal analysis

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Supplementary File

None.

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