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Determination of antioxidant and anti-inflammatory properties of *Salvia aethiopis/sclarea* and synthesized silver nanoparticles

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Abstract

Aim: In the study, the phenolic compounds of *Salvia aethiopis/sclarea* will be determined and the antioxidant and anti-inflammatory activities of the extracts of these plants and the synthesized silver nanoparticles (AgNP) will be examined in vitro.

Materials and Methods: Extracts obtained from *Salvia spp.* (SE) collected from the Kyrenia region of Cyprus were used in the study. Methanol extracts were prepared and total phenolic, flavonoid and alkaloid contents were determined. The amount of phenolic compounds was then quantified and identified using HPLC. The antioxidant properties of *Salvia aethiopis/sclarea* were analyzed using various markers, including lipid peroxidation inhibition activity, 1,1-Diphenyl-2-picrylhydrazyl (DPPH), and ferric reducing antioxidant power (FRAP) scavenging capacity. Additionally, their anti-inflammatory effects were investigated by proteinase inhibitor activity, heat-induced hemolysis and albumin denaturation analyses.

Results: *S. aethiopis* and *S. sclarea* were contained high levels of the phenolic compounds isoquercetin and caffeic acid or naringenin and caffeic acid, respectively. After green synthesis of silver nanoparticles, the antioxidant and anti-inflammatory properties of plant extracts increased significantly ($p<0.05$).

Conclusion: Our study found that silver nanoparticles synthesized using *S. aethiopis/sclarea* extract increased the antioxidant and anti-inflammatory effects of plant extracts and suggested that AgNP/SE could be used as a potential therapeutic agent in medicine.

Introduction

In our country, the plant species called *Salvia aethiopis* and *Salvia sclarea*, woolly sage and musk sage, respectively, belong to the Lamiaceae family and are native to Mediterranean countries such as Türkiye, Greece, Portugal, Spain, Italy and Cyprus [1]. These plants are used to alleviate respiratory tract symptoms in conditions such as colds, flu, bronchitis, and asthma. It also has sedative properties for stomach spasms and can act as a diuretic and pain reliever for gynecological diseases of the urogenital system, such as menopause and galactorrhea. Additionally, it is commonly used to treat rheumatism, heart disease, skin infections, and cancer [1, 2]. These medicinal aromatic plants, known as *Salvia spp.*, have been found to contain rich amounts of polyphenols, phenolics, flavonoids, sterols, terpenoids, and essential oils. Their chemical compositions have been characterized as antioxidant, anticholinesterase, anti-inflammatory, antibacterial, and insecticidal activities [3-5]. *S. aethiopis/sclarea* is an endemic plant of Cyprus. There have been very few in vitro studies conducted on its antioxidant and anti-inflammatory properties. In the vicinity of the village of Degirmenlik (Nicosia) in Northern Cyprus, *S. aethiopis* can be found. On the other hand, *S. sclarea* is a perennial plant known to bloom white and purple flowers, respectively, in rocky areas with streams at an altitude of 1000-1950 m in the Troodos Mountain range (Nicosia) between May and October [5] (Figure 1).

Reactive oxygen species (ROS) are produced during normal cell functions and play critical roles, physiologically. However, if cellular components cannot effectively inactivate ROS at high concentrations, they can damage the structures such as proteins, lipids, and nucleic acids in cells, and the risk of aging, cancer, inflammation, and neurodegenerative diseases increases [6]. Humans ingest bio-

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Figure 1. *S. aethiopis* and *S. sclarea*.

logically active antioxidant compounds, such as polyphenols, carotenoids, flavonoids, ascorbic acid, and fat-soluble vitamins, through plant-based diets and fruits. These compounds are known to have antioxidant activity against free radicals and provide protective effects against oxidative stress [7]. Inflammation occurs as a result of the production of cytokines, which regulate inflammatory reactions of body tissues as a response to negative stimuli, such as irritants, damaged cells or infections. In diseases characterized by chronic inflammation, such as atherosclerosis and cancer, current anti-inflammatory drugs alleviate symptoms and suppress enzyme activities. The anti-inflammatory effects of *Salvia spp.* plant extracts (SEs) in rats were reported that they displayed a significant reduction in edema, peritonitis, myeloperoxidase activity, and NOx-peritoneal lavage concentration induced by carrageenan [4].

Nanoscience has become more prevalent in medicine for disease diagnosis, treatment, and drug development in recent years [8]. The preferred method for obtaining metal nanoparticles, such as Au, Ag, Ce, and Zn, is green synthesis. This method is better than chemical methods that produce dangerous intermediates due to the low cost, decontaminating the environment, easy synthesis stages [9]. In particular, silver nanoparticles (AgNPs) are widely used by researchers due to their unique, size- and shape-dependent optical, electrical, thermal properties, as well as their antioxidant, antimicrobial, antifungal, and highly catalytic activities, and often have a wider range of applications in medicine and health sciences [8, 9].

There is limited research in the literature on the antioxidant and anti-inflammatory properties of *S. aethiopis/sclarea*, and the synthesis of nanoparticles from these plants has not been studied. This study aims to evaluate the green synthesis of silver nanoparticles using extracts of this plant and assess their antioxidant and anti-inflammatory activities.

Materials and Methods

S. aethiopis and *S. sclarea* plants were collected in May 2023 from the vicinity of Degirmenlik Village and Troodos Mountain range in Nicosia, Cyprus, respectively. *Salvia spp.* according to the simple random sampling method, at least 20 plants/decare, at 20 points per decare, at a distance of 30 cm, were first examined on site, and then they were removed from the soil with their roots and brought to the laboratory. Proportional Stratified Sampling method was used to determine the number of plants to be collected from a 30 decare plant area. Plant specimens were recorded and stored in the Herbarium of Girne American University, Faculty of Pharmacy. For the preparation of samples *S. aethiopis/sclarea* was dried and ground at room temperature. All chemicals and silver nitrate ($AgNO_3$) to be used in the study were purchased from Merck (Germany). For methanol extraction, 100 g of powdered *S. aethiopis/sclarea* was placed in a flask and filtered using 1 L of 95% methanol (1:10 ratio) as solvent for 24 hours and 5-7 times until extraction was complete. The final mixture was filtered with Whatman No. 4 filter papers and concentrated with a special temperature-adjusted evaporator and stored in the refrigerator at +4°C for analysis.

Phenolic compound analysis of Salvia extracts

The extracts were resuspended in sterile phosphate buffered solution (pH 7.2) at a final concentration of 200 mg/mL for analysis. The total phenolic content of SE was determined using the Folin-Ciocalteu colorimetric method described by Singleton et al [10]. 50 µL of each SE was mixed with 5 mL of 10% Folin-Ciocalteu reagent and 5 mL of 7.5% Na_2CO_3 to obtain a blue solution. The solution was incubated at 20°C for 1 hour, and the absorbance was measured at 760 nm using a UV spectrophotometer (Shimadzu UV 1800, Japan) with gallic acid (GA) as the reference material. The amount was specified as mg-gallic acid/g-SE.

The total flavonoid content of SE was determined according to the aluminum chloride colorimetric method of Chang et al [11]. To prepare the solution, 60 µL of SE, 4 mL of distilled water, and 20 µL of aluminum chloride reagents (10% methanol) were combined in a 10 mL measuring glass. The volume was then completed to 5 mL with pure methanol. The solution was incubated at 20°C for 30 minutes, and the absorbance was measured using a UV spectrophotometer at 430 nm. Quercetin was used as the reference material, and the calculation was made as mg-quercetin/g-SE.

The total alkaloid level of SE was determined according to the alkaloid-green bromocresol method, resulting in a yellow-colored product [12]. Each 400 µL of SE was mixed with 2 N HCl, and the final volume was adjusted to 4000 µL. The mixture was then transferred to a separation funnel and washed three times with 10 mL of chloroform. Finally, the solution was neutralized with 0.1 N sodium hydroxide. After 10 minutes, each 10 mL of phosphate buffer and green bromocresol were appropriately added. After thorough shaking, the essence of the formed complex was removed with chloroform. The extracts were diluted with chloroform in 20 mL tubes and its absorbance was determined using a spectrophotometer at 470 nm. To

calculate the results, the formula [alkaloids =(Abs-0.048)/ 0.021] was used and the total amount of alkaloids was expressed as mg-SE /mL.

The analysis of High-Performance Liquid Chromatography (HPLC)

The analysis was used to quantify and identify phenolic compounds in SE by Agilent 1200 HPLC (Agilent Technologies, Palo Alto, CA, USA). First, 200 µg of SE was dissolved in distilled water, and 40 µL of the extract was added into the HPLC column, which had a length of 250 mm, a diameter of 4.6 mm, and a pore size of 5 µm. The mobile phase consisted of two solvents as 2% water/acetic acid and methanol. The flow rate and temperature were adjusted as 10 mL/min and 30°C. The elution gradients were as follows: min/B linear (0-5 / 5-15%; 6-10 / 15-25%; 11-15 / 25-45%; 16-20 / 45-60%; 26-30 / 60-80% and 31-40 / 60-100%). Absorbance measurement was performed in the range of 300 to 400 nm in triplicate [13]. Phytochemical identification in the sample extract was performed using Agilent ChemStation software.

The synthesis and analysis of $AgNO_3$ nanoparticles

To synthesize $AgNO_3$ nanoparticles of *S. aethiopis/sclarea* (AgNPs/SaE-SsE), a mixture of 100 mL of extract and 100 mL of 1 mM $AgNO_3$ solution was kept in a beaker (1 h and 20°C). The mixture was then continuously heated and stirred (100 g x 4 h). The color of the mixture changed from colorless to brown, indicating the formation of AgNPs/SE. The solution was then centrifuged with distilled water (10 000 g x 20 min) and purified [14]. In order to obtain the nanoparticles, the supernatant was separated with distilled water. Then Alpha 1-2 plus was added and stored at -80°C. The absorbance of AgNPs/SE was characterized by its maximum absorbance using a UV spectrophotometer at 200-800 nm. The nanoparticles underwent structural characterization and particle size examination using Electron Microscopy (SEM) (Hitachi, Japan). Additionally, spectrum characteristics were analyzed using Fourier Transmission Infrared Spectroscopy (FTIR) (Bruker, Germany) in the spectrum range of 4000-400 cm^{-1}. X-ray diffraction (XRD) of AgNPs/SE was analyzed using XPert Pro diffractometer (Netherlands) in the range of 20-80° 2Θ [14].

The determination of antioxidant status

The antioxidant analyses of *S. aethiopis/sclarea* extracts (SEs) and AgNPs/SE were performed by lipid peroxidation inhibition, 1,1-Diphenyl-2-picrylhydrazyl (DPPH) and ferric reducing antioxidant power (FRAP) tests.

The inhibitory activity of SEs and AgNPs/SE on lipid peroxidation was measured by the thiobarbituric acid (TBA) test [15]. The samples were dissolved in 97% ethanol at 100 mg/L (w/v). Then, 400 µL of SE or AgNPs/SE, 400 µL of $FeCl_3$, 400 µL of EDTA, 400 µL of H_2O_2, and 400 µL of ascorbic acid were added to the tubes and vortexed. After 1.5 hours of incubation at 37°C, 2.4 mL of 28% TBA was added, and centrifuged (3000 g x 15 min). The supernatant was then discarded, and 2.4 mL of TBA was added to the remaining pellet. The samples were boiled and cooled in ice. Its absorbance was determined at 532 nm in a UV spectrophotometer. Lipid peroxidation was expressed as pmol/mg-SE or AgNPs/SE.

The DPPH test was performed following the method of Clarke et al [16]. A solution of SE or AgNPs/SE 200 mg/L (w/v) or 0.2 mL standard solution in ethanol was mixed with 7.8 mL of DPPH. The mixture was vortexed for 1 min and incubated at 20°C for 1 h. Its absorbance was determined at 517 nm range in a UV spectrophotometer. Vitamin C was used as a reference to analysis of the DPPH capacity of SE or AgNPs/SE. The percent inhibitions of DPPH were calculated using the equation below, and a graph was plotted against the concentration of SE or AgNPs/SE for the percent inhibition values: % inhibition = [(Acontrol-Asample)/Acontrol] × 100 (Eq-1), where Asample is the absorbance of SE or AgNPs/SE, and Acontrol is the absorbance of control.

The FRAP test for SE or AgNPs/SE samples was performed following the method of Clarke et al [16]. The FRAP reagent was composed of 10 mM tripyryltriazine at 40 mM HCl and 20 mM $FeCl_3$ in acetate buffer (pH 3.6). A total of 7.8 mL of FRAP reagent was mixed with 0.1 mL of sample (100 mg/L) or standard solution. It was then incubated (20°C, 40 min), and its absorbance was determined at 590 nm in a UV spectrophotometer. Trolox was used as reference standard, and the FRAP activity was specified as mg-trolox/g-SE or AgNPs/SE.

Anti-inflammatory analysis

In vitro, the study investigated the anti-inflammatory effects of the samples using proteinase inhibitory activity, heat-induced hemolysis, and albumin denaturation tests. Acetylsalicylic acid and phosphate buffer solution were used as the reference drug or control. The percentages of inhibition were calculated using Eq-1.

The proteinase inhibitor activity test was performed using the following procedure: 2 mL of the sample was mixed with 0.12 mg trypsin and 2 mL of Tris-HCl buffer (20 mM, pH 7.4) and incubated (37°C, 5 min). Then, 2 mL of casein was added to them and centrifuged (2,000 g x 20 min). The supernatant was then mixed with 4 mL of perchloric acid (70%), and its absorbance was determined at 210 nm in UV spectrophotometer [17].

In the heat-induced hemolysis test, a healthy individual, who had obtained approval from the ethics committee, provided whole blood for the preparation of an erythrocyte suspension. The blood was mixed with 0.9% NaCl and centrifuged (2000 g x 5 min). The supernatant was then removed, and added sodium phosphate buffer (10 mM, pH 7.4). 0.12 mL sample was mixed with 0.12 mL of blood cell suspension and 5.86 mL of phosphate buffer. The tube of mixture was putted in a water bath (54°C), and centrifuged at 2000 rpm, 3 min. Its absorbance was determined at 540 nm, spectrophotometrically [18].

In the albumin denaturation test, 2 mL sample was prepared by adding 0.4 mL of 1% bovine albumin and 7.6 mL of sodium phosphate buffer (10 mM, pH 6.4). It was then incubated (37°C, 15 min and 70°C, 5 min, respectively). The mixture was cooled, and its absorbance was determined at 660 nm, spectrophotometrically [18].

Statistical analysis

SPSS 26.0 program was preferred in the statistical analysis for the findings obtained in the study. One-Way ANOVA was used for statistical comparison of the data after antioxidant and anti-inflammatory analysis, followed by Tukey test for bilateral comparisons. IC_{50} values were calculated by linear regression analysis. The data were indicated in the tables as mean ± standard deviation (SD) at a 95% confidence level. To evaluate the significance of the observed differences, the least significant difference (LSD) test was used. The conclusions were expressed as mean ± SD and $p<0.05$ has been conceived significant.

Results

The Folin-Ciocalteu colorimetric method, aluminum chloride colorimetric test, and alkaloid-green bromocresol method were used to determine the total phenolic, flavonoid, and alkaloid contents of *S. aethiopis/sclarea* extract (SE), respectively. As a result of the analysis of the total phenolic/flavonoid/alkaloid compounds in the extracts of *S. sclarea aethiopis* (SaE) and *S. sclarea* (SsE), it was determined that the values of the SaE group were statistically significantly higher ($p \leq 0.05$) (Table 1).

Table 1. Quantitative phytochemical constituents of *Salvia aethiopis/sclarea* extract (SE)*

Parameter	SaE	SsE
Total phenol (mg-GA/g-SE)	90.75±0.35[a]	86.42±0.58
Total flavonoid (mg-quercetin/g-SE))	121.76±2.14[a]	112.45±1.82
Total alkaloid (mg-SE/mL	322.48±4.82[a]	285.52±5.21

*Mean values were presented as x ± SD after performing the analysis triplicate. [a] Significant against the SsE group were determined ($p \leq 0.05$).

HPLC analysis was used to quantify and identify phenolic compounds in SE. The phytochemical identification in SE was highlighted by retention times (40 min) and UV spectra ranging 300-400 nm. The calculated amount was expressed in milligrams per gram of SE. Phytochemical identification in the sample extract was performed using standard/molecular weight/mass spectrum/retention time, and 16 phenolic compounds were identified (Figure 2 and Table 2).

In our study, UV, FTIR, XRD and SEM techniques were used to control the synthesis and catalytic activity of AgNPs/SE. Their absorbance was scanned in the wavelength range of 400-800 nm on a UV spectrophotometer.

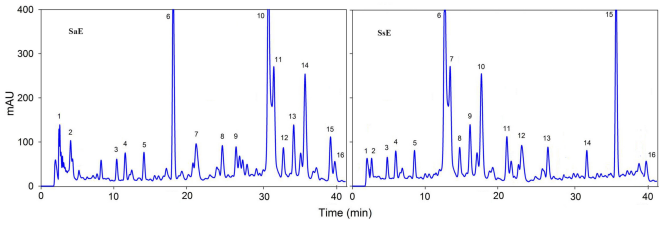

Figure 2. The chromatogram of *S. aethiopis/sclarea* extracts in HPLC (300-400 nm).

Table 2. Quantification and identification of phenolic compounds of *S. aethiopis/sclarea* extract*

Compounds	Peak	Retention time	Quantity- (mg/g SaE)	Peak	Retention time	Quantity- (mg/g SsE)
Quinic acid	1	4.2	1.76±0.34	1	2.1	0.94±0.32
Fumaric acid	2	5.9	9.86±0.9	2	3.9	5.42±0.7
Ferulic acid	3	10.8	4.21±0.8	3	5.3	2.37±0.6
Gallic acid	4	12.4	0.27±0.04	4	7.6	0.42±0.07
Rosmarinic acid	5	16.5	4.18±0.9	5	8.2	1.29±1.8
Caffeic acid	6	20.1	18.28±2.3	6	13.4	17.13±0.3
Luteolin	7	31.6	5.56±0.2	7	14.9	10.27±0.4
Tannic acid	8	33.7	2.25±0.4	8	16.3	2.29±0.3
Trans-sinnamic asid	9	34.9	3.34±0.13	9	17.4	5.9±0.14
Isoquercetin	10	35.5	20.52±1.7	10	18.5	9.05±0.8
Apigenin	11	25.7	9.32±0.4	11	22.7	4.12±0.5
Catechin	12	30.3	2.81±0.2	12	24.3	3.14±0.5
Vanillic acid	13	30.5	9.73±0.3	13	27.5	3.21±0.7
Acacetin	14	35.6	11.34±0.8	14	37.6	8.57±0.7
Naringenin	15	35.8	7.35±0.3	15	35.9	20.76±3.8
Synapic acid	16	36.0	1.01±0.1	16	38.3	1.94±0.6

* The mean values (x) obtained after 3 parallel measurements were expressed as x±SD.

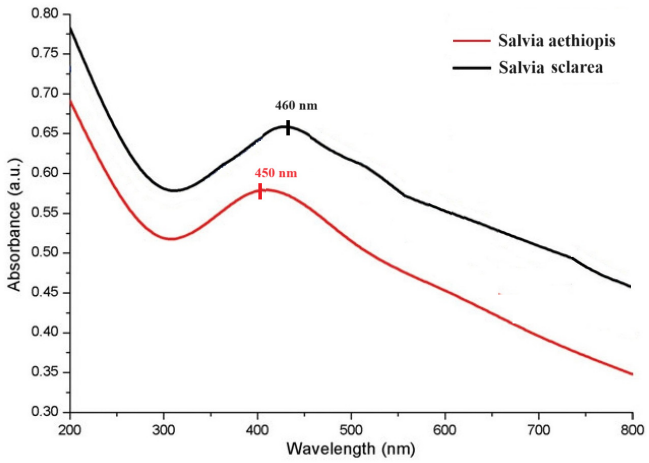

Figure 3. The UV spectrum of *S. aethiopis/sclarea* silver nanoparticles.

The absorbance in AgNP/SE was highlighted in spectra ranging 300-400 nm. The UV spectra of AgNP/SaE and AgNP/SsE give absorbance peaks at 450 nm and 460 nm (Figure 3).

Fourier transform infrared spectroscopy (FTIR) was performed to identify secondary metabolites involved in AgNP/SaE and AgNP/SsE, and exhibited major absorbance peaks at specific wavelengths (Figure 3). The FTIR spectra of AgNPs/SE exhibit absorbance peaks at 3215 and 3326 cm^{-1} (O–H / N–H bonds in the phenolic compounds of SE), 2908 and 2927 cm^{-1} (C–H bonds in the methylene or aliphatic groups), 1725 and 1683 cm^{-1} (C–C bond in the alkenyl or aromatic groups), 1212 and 1324 cm^{-1} (–C–O bond in phenol or tertiary alcohols), 987 and 1205 cm^{-1} (O–H bond in the phenol groups), 905 and 922 cm^{-1} (C–O and C–S bonds or aliphatic chloral

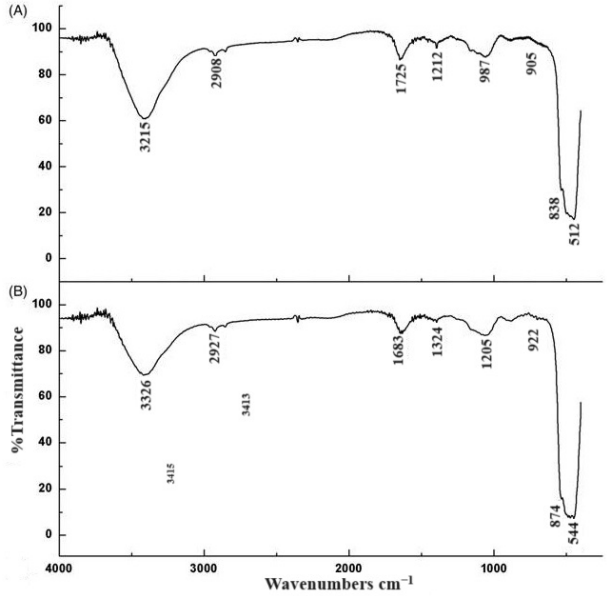

Figure 4. The spectrums of *S. aethiopis/sclarea* silver nanoparticles in FTIR analysis.

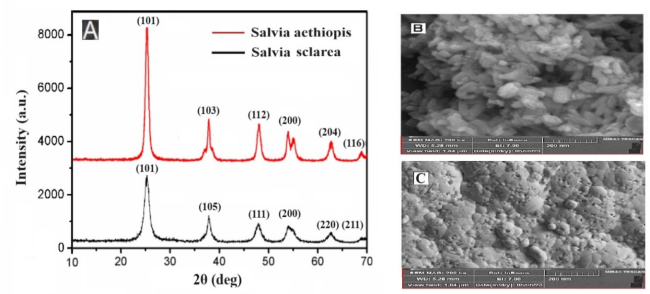

Figure 5. A) The pattern of *S. aethiopis/sclarea* silver nanoparticles in XRD analysis, B) The image *S. aethiopis* in SEM C) The image of *S. Sclarea* in SEM.

compounds), 838 and 874 cm^{-1} (C–H bond in the aromatic groups), and 512 and 544 cm^{-1} (OH group in phenols) (Figure 4).

In the confirmation of the crystal structures of *S. aethiopis/sclarea* silver nanoparticles, X-ray diffraction (XRD) analysis was performed. The crystal structure of AgNPs/SE was confirmed by the presence of five peaks of AgNP/SaE and AgNP/SsE at 2Θ = 32.84°, 34.98°, 38.27°, 44.27°, 55.48° and 2Θ = 33.77°, 35.12°, 39.54°, 45.16°, 56. 25°; for AgNP/SaE and AgNP/SsE, (101), (103), (112), (200), (204), (116) to (101), (105), (111), (200), (220), (211) due to the Bragg reflection planes (Figure 5). The morphology of AgNPs/SE synthesized using green methods was investigated using scanning electron microscopy (SEM). The SEM analysis images indicated that the AgNPs/SE were uniformly distributed, shaped spherical, and sized 40 nm.

Lipid peroxidation inhibition and determination of DPPH scavenger and FRAP activities have long been used as antioxidant markers in studies on oxidative stress. The results determined for the antioxidant activities of *S. aethiopis/sclarea* extracts and synthesized silver nanoparticles were presented in Table 3. MDA values, which were lipid peroxidation markers, of SaE and SsE extracts were found to be significantly higher than Vitamin C. The MDA values of AgNP/SaE and AgNP/SsE were significantly lower than those of *S. aethiopis/sclarea* extracts ($p \leq 0.05$). The DPPH scavenger and FRAP activities had significantly lower levels for SaE and SsE extracts than the Vitamin C and Trolox standards. However, these reduction values were found to be significantly higher in AgNP/SaE and AgNP/SsE compared to *Salvia spp.* extracts ($p \leq 0.05$). Thus, green synthesis of AgNP/SaE and AgNP/SsE was shown to increase antioxidant levels.

Table 3. The effects of lipid peroxidation (MDA) inhibition, DPPH and FRAP of AgNPs/SE synthesized with SEs*

Antioxidant test			
Group	MDA (pmol/mg)	%DPPH	FRAP (mg-trolox/g)
Vitamin C/Trolox	105.2±15.8	80.23±5.8	91.84±6.5
SaE	507.3±11.6^{a}	26.54±3.8^{a}	28.78±5.2^{a}
SsE	521.5±16.2^{a}	29.52±4.7^{a}	26.23±3.8^{a}
AgNP/SaE	326.2±14.1^{ab}	47.16±6.1^{ab}	37.42±3.7^{ab}
AgNP/SsE	336.8±18.6^{ab}	48.32±4.8^{ab}	36.82±4.1^{ab}

*Mean values were presented as x ± SD after performing the analysis triplicate. ^{a} Significant against the standard, and ^{b} significant against the extract group were determined ($p \leq 0.05$).

Table 4. In vitro determination of anti-inflammatory inhibitory activities of AgNPs/SaE and AgNPs/SsE synthesized with SEs*

Anti-inflammatory test (IC_{50} mg/mL)			
Group	Heat-induced hemolysis %	Proteinase inhibitory activity	Albumin denaturation
Acetylsalicylic acid	2.18±0.72	2.32±0.64	2.86±0.92
SaE	3.82±0.64^{a}	3.67±0.82^{a}	4.52±0.78^{a}
SsE	3.96±0.86^{a}	3.75±0.71^{a}	4.69±0.96^{a}
AgNP/SaE	3.04±0.46^{ab}	2.94±0.43^{ab}	3.54±0.85^{ab}
AgNP/SsE	2.86±0.79^{ab}	2.81±0.51^{ab}	3.62±0.36^{ab}

*Mean values were presented as x ± SD after performing the analysis triplicate. ^{a} Significant against the standard, and ^{b} significant against the extract group were determined ($p \leq 0.05$).

We performed the assays, heat-induced hemolysis, proteinase inhibitory activity and albumin denaturation in vitro to determine the antiinflammatory effects of AgNP/SaE and AgNPs/SsE or SaE and SsE extract. Acetylsalicylic acid was used as the reference drug for the experiment. In these tests, we found that the results of SaE and SsE extracts were significantly lower compared to the anti-inflammatory IC_{50} value of acetylsalicylic acid, but this decrease was slightly in AgNP/SaE and AgNPs/SsE (Table 4).

Discussion

The use of herbal extracts in alternative medicine for disease treatment has become an integral part of the health sector. However, it is important to ensure that they are scientifically researched for the appropriate disease, dosage, and method of use. It is worth noting that plants contain various active substances, such as flavonoids, alkaloids, carotenoids, and phenols, collectively known as phytochemicals, which may be beneficial in treating numerous diseases. According to research, it has been found that these compounds can help reduce cell damage by binding to free oxygen radicals. These radicals are known to play a crucial role in the development of chronic diseases [6, 7]. The compounds achieve this by causing lipid peroxidation and effectively binding hydroxyl radical, hydrogen peroxide, superoxide anion radical, and singlet oxygen. It has been observed that nanoparticles synthesized from medicinal plants with high levels of antioxidant phytochemicals, such as phenols, tannins, and flavonoids, may enhance the inhibitory effects of lipid peroxidation in plants [8, 9]. Furthermore, according to reports, plant extracts from *Salvia spp.* have high antioxidant capacity and may provide protection against cancer, diabetes, cardiovascular disease, cerebrovascular disease, and inflammatory chronic diseases [3, 4]. This study aimed to investigate the effect of silver nanoparticles synthesized from *S. aethiopis/sclarea.*

When the phenolic amounts of SE were evaluated, the phenolic compounds of *S. aethiopis* contained isoquercetin (20.52±1.7 mg/g) and caffeic acid (18.28±2.3 mg/g); On the other hand, naringenin (20.76±3.8 mg/g) and caffeic acid (17.13±0.3 mg/g) levels were found to be high in *S. sclarea.* In addition, high levels of acecetin (11.34±0.8 mg/g), fumaric acid (9.86±0.9 mg/g), luteolin (10.27±0.4 mg/g) and isoquercetin (9.05±0.8 mg/g) were also determined in SaE. Bakir et al [21] examined *S. hypargeia* leaf extract and found 38.04 mg/g of rosmarinic acid and 4.14 mg/g of isoquercetin. Bursal et al. [22] identified fumaric acid (0.555±0.038 mg/g), caffeic acid (0.103±0.02 mg/g), and epicatechin (0.083±0.008 mg/g) as the main compounds in the water extract of *S. eriophora Boiss & Kotschy.* Yilmaz et al [3] detected high levels of phenolic compounds, such as rosmarinic acid and caffeic acid, as well as flavonoids like luteolin, naringenin, and acacetin in *S. aytachii.* Our findings are consistent with them.

In our study, we used UV, FTIR, XRD, and SEM techniques to control the synthesis and catalytic activity of AgNPs/SE. UV spectroscopy is used to characterize the structure of nanoparticles, and the absorbance peaks measured at 450 nm and 460 nm are determined due to plasmon resonance electrons located on the nanoparticle surface, which consequently indicate the formation of AgNPs in solution [14]. The FTIR could identified the secondary metabolites involved in the bio-reduction of Ag^{+} and AgNP/SaE and AgNP/SsE showed major absorbance peaks at wavelengths of 3215, 2908, 1725, 1212, 987, 905, 838, 512 and 3326, 2927, 1683, 1324, 1205, 922, 874, 544 cm^{-1}, respectively. The results are in line with previous phytochemical analyses of *Salvia spp.* [23, 24]. X-ray diffraction (XRD) analysis was performed to confirm the crystal and nanostructures of silver nanoparticles from *S. aethiopis/sclarea.* The crystal structure of AgNPs/SE was confirmed by the presence of five peaks of AgNP/SaE and AgNP/SsE, or Bragg reflection planes. The position, height, and width of XRD peaks [23, 24] determine the nanocrystal structure and purity of nanoparticles. Scanning electron microscopy (SEM) was used to investigate the morphology of green-synthesized AgNPs/SE. The AgNPs/SE appeared as polymorphic granular clusters. This is consistent with previous studies on biosynthesis of silver nanoparticles using *Salvia spp.* [23, 24]. Dehydration during sample preparation for SEM analysis likely induced the high agglomeration of biosynthesized AgNPs/SE.

Plant extracts were studied in vitro for their ability to inhibit lipid peroxidation and scavenge DPPH and FRAP radicals. The results showed that these extracts have remarkable antioxidant potential. Previous studies have demonstrated a positive correlation between the antioxidant activity of plant extracts and their radical scavenging activity [19, 20]. Ercetin et al [25] investigated the antioxidant activity of *S. willeana* and found that the methanol extracts of the leaves and flowers of the plant exhibited the highest antioxidant activity in terms of DPPH radical scavenging activity (67.94±0.003% and 45.07±0.001%, respectively) at a concentration of 0.5 mg/mL. The FRAP activity of the extracts was generally weak. This result can be explained by the increased presence of phenolic and flavonoid compounds in *Salvia spp.* extracts in the samples. Plant extracts containing phenolics and flavonoids have been found to effectively scavenge oxidizing molecules, including singlet oxygen and various ROS, while also suppressing ROS formation. Additionally, these extracts chelate trace elements involved in ROS production and increase and maintain antioxidant defenses [13, 19, 20, 26, 27].

We investigated the potential anti-inflammatory effects of the medicinal plant *S. aethiopis/sclarea.* This plant is commonly used in the treatment of various diseases, particularly in Türkiye and Nortern Cyprus. *Salvia spp.* inhibits enzymes such as serine proteinase released from the lysosomal granules of neutrophils, exerting anti-inflammatory effects in protein and albumin denaturation [27]. Leukocyte proteinase has been documented to play an important role in the development of tissue damage during inflammatory reactions. Protein denaturation occurs in various inflammatory disorders, including rheumatoid arthritis, diabetes, and cancer. Inflammatory disorders can be minimized by preventing protein denaturation. Anti-inflammatory drugs can utilize chemical compounds that prevent protein denaturation. Studies have been conducted on the anti-inflammatory activities of *Salvia spp.* [27, 28].

The literature has also shown that *Salvia spp.* extract has anti-inflammatory potential due to its content of phenolics, flavonoids, tannins, saponins, terpenoids, and alkaloids. Alkaloids are used in the treatment of asthma due to their anti-inflammatory and analgesic effects. They can also help strengthen the immune system and relieve skin disorders. Phenols and flavonoids have important antioxidant effects, which can help prevent viral infections, allergic reactions, and the body's response to carcinogens. The literature and current results indicate that AgNP/SaE and AgNP/ SsE possess in vitro anti-inflammatory and antiox-

idant activities. These findings are supported by the use of SaE and SsE [19, 25].

Conclusion

The sage plant, *S. aethiopis/sclarea*, is traditionally used among people for its soothing effects on respiratory, digestive, rheumatism, heart, skin, and gynecological diseases, as well as cancer. In vitro studies on *Salvia spp.* have shown that the plant extract contains many compounds, such as polyphenols, phenolics, flavonoids, sterols, terpenoids, and volatile fatty acids, which have antioxidant activity against ROS in the body. In the past decade, there has been a growing interest in the use of nanoparticles for medicinal purposes. Specifically, silver nanoparticles (AgNPs) obtained through green biosynthesis using medicinal plant extracts have gained attention due to their non-toxic, biosafe, and biocompatible properties. An advantage of using plant extracts to synthesize nanoparticles is the avoidance of toxic chemicals. Additionally, polyphenols found in plant extracts can enhance the reduction effect of metal ions or free radicals. Our study demonstrated that silver nanoparticles synthesized using *S. aethiopis/sclarea* plant extracts (AgNPs/SE) possess antioxidant and anti-inflammatory properties in vitro. It is proposed that nanoparticles synthesized in an environmentally friendly manner can be utilized as an effective agent in the prevention and treatment of diseases due to their antioxidant activities and radical scavenging effect that protect cells. Therefore, silver nanoparticles, whose production and consumption potential is increasing, are believed to offer numerous benefits to humanity in the prevention and treatment of diseases and cancer. Finally, we propose conducting new studies to examine the potential health benefits of different types of Salvia, including their anti-aging, anti-Alzheimer's, anti-obesity, and anti-diabetic properties.

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CRediT authorship contribution statement

Emrah Caylak: Conceptualization, Investigation, Writing – original draft, review & editing, Supervision.

Declaration of competing 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.

Data availability

Research data is available upon request. Data can be obtained from the corresponding author via email.

Ethical approval

We used all reagents in our study or disposed of in accordance with laboratory guidelines and material safety (MSDS). As no animals or humans were involved in the study, approval from the ethics committee was deemed unnecessary by the university ethics committee.

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