PROSPECTS
IN PHARMACEUTICAL SCIENCES

Prospects in Pharmaceutical Sciences, 23(1), 1-8
https://prospects.wum.edu.pl/

Original Article

DETERMINATION OF THE ANTIMICROBIAL, ANTIOXIDANT ACTIVITIES AND EFFECTS ON OXIDATIVE DNA DAMAGE OF EXTRACTS FROM THREE DIFFERENT *SALVIA* SPECIES GROWN IN TURKEY

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Received: 26.10.2024 / Accepted: 08.12.2024 / Published: 15.01.2025

ABSTRACT

Salvia officinalis L., known as medicinal sage, *Salvia triloba* L., known as Anatolian sage, and *Salvia sclarea* L., known as clary sage, belong to the Lamiaceae family and are species that typically grow in the Mediterranean region. This study aims to investigate the total antioxidant status (TAS), total oxidant status (TOS), oxidative stress index (OSI) values, antioxidant and antimicrobial activities, total flavonoid and total phenolic content, and protective effects against oxidative DNA damage of extracts obtained from the aerial parts of three sage species — *S. officinalis*, *S. triloba*, and *S. sclarea* — which are cultivated using organic farming techniques in Turkey. Ready kits were used to determine TAS, TOS, and OSI values, while the 2,2-diphenyl-1-picrylhydrazyl (DPPH) test was used for antioxidant activity assessment. The broth microdilution method was used to determine the minimum inhibitory concentration (MIC) against microorganisms. To evaluate the protective effects of the extracts on DNA against UV and oxidative damage, pBR322 plasmid DNA was used. In conclusion, among the prepared water, 70% ethanol, and ethyl acetate extracts, the highest TAS was found in *S. triloba* 70% ethanol extract (9.6±1.8 mmol/L). In contrast, the highest TOS was observed in *S. sclarea* water extract (3.9±1.2 µmol/L). The OSI values of all plant species and extracts were found to be very similar. The extract with the highest antioxidant activity was *S. officinalis* 70% ethanol extract (IC_{50} = 0.105±0.056 mg/mL), and all extracts demonstrated low to moderate antimicrobial activity against the tested microorganisms. The highest total phenolic content was determined in *S. triloba* ethyl acetate extract (37.6±8.5 mg GAE/g extract), while the highest total flavonoid content was observed in *S. officinalis* ethyl acetate extract (12.5±2.9 mg QE/g extract). All extracts from the three plant species provided strong protection for plasmid DNA molecules against oxidative damage.

KEYWORDS: Sage, Antimicrobial, Antioxidant, DNA protective effect.

1. Introduction

In addition to being a primary food source essential for the continuation of life, plants contain various phytochemicals, such as phenolics and flavonoids, which are crucial for human health [1]. Their rich phytochemical content has facilitated their use over centuries, and many modern medicines are derived from medicinal plants [2]. Growing concerns about the safety of synthetic and semi-synthetic antioxidants and antimicrobial agents in modern medicine have led to increased research on natural raw materials, particularly medicinal and aromatic plants, due to their high antioxidant and antimicrobial properties [3,4].

Sage (*Salvia*) is a valuable medicinal and aromatic plant from the Lamiaceae family. Medicinal sage is distributed in Mediterranean countries from Spain to the Balkans, from sea level to altitudes of 1500 m. There are over 900 species of sage worldwide, with the most commercially valuable species being medicinal sage (*S. officinalis* L.), Anatolian sage (*S. triloba* L.), apple sage (*S. pomifera* L.), Spanish sage (*S. lavandulaefolia* Vahl.), and clary sage (*S. sclarea* L.) [5]. Turkey is among the countries with the most widespread sage distribution and commercial exploitation. Although 97 sage species (51 of which are endemic) are naturally distributed in Turkey [6], medicinal sage (*S. officinalis* L.) is not one of

them. However, medicinal sage is successfully cultivated in Turkey, yielding high-quality and high-efficiency plants [7-9]. *Salvia* species have been used from ancient times to the present in cooking as a spice for flavor and aroma and in folk medicine in the form of tea, ointments, tinctures, or extracts as analgesics, expectorants, carminatives, sedatives, diaphoretics, and for external wound healing. They are also used for treating colds, bronchitis, tuberculosis, menstrual disorders, and gastric ailments [10]. The global demand and market for *Salvia* (sage) are steadily increasing. In recent years, different species have been cultivated in various provinces of Turkey, and significant foreign exchange income is generated through their export to many countries worldwide. Studies have shown that secondary metabolites isolated from *Salvia* species possess various biological effects, including antimicrobial, antifungal, antiseptic, analgesic, antioxidant, antispasmodic, antidepressant, antimutagenic, anticholin-esterase, hallucinogenic, antidiabetic, anticancer, antihypertensive, anti-inflammatory, tuberculostatic, vasodilatory, hypoglycemic, and insecticidal activities [11,12].

The aim of this study is to investigate the antimicrobial and antioxidant activities, and protective effects against oxidative DNA damage of water, 70% ethanol, and ethyl acetate extracts obtained from the aerial parts of three different species of sage (*Salvia officinalis*, *Salvia triloba*, and *Salvia sclarea*) cultivated using organic farming techniques in Turkey. Additionally, the total phenolic content and total flavonoid content will be determined and evaluated. The originality of this study lies in the fact that the results will be the first comparative analysis of these three species in the literature.

2. Materials and Methods

2.1. Plant material collection

The aerial parts of *Salvia officinalis*, *Salvia triloba*, and *Salvia sclarea* used in this study were collected in 2021 from the "Temmuz Organic Farm" in Konya, where organic farming of medicinal and aromatic plants is practiced. These pieces were dried in a cool, shaded area and stored for experimental studies.

2.2. Extraction of plants

An infusion process was performed for the aqueous extract. Plant material was weighed to 5 grams and placed in Erlenmeyer flasks. 50 mL of hot distilled water were added, and the flasks were sealed and left to stand at room temperature for 10-15 minutes with occasional shaking. After this time, the extract was filtered, and 50 mL of a new portion was added to the plant material. This process was repeated three times, and the collected extracts were placed in a lyophilizator (freeze-dryer) to remove the water content completely. After four days, the remaining extract in the freeze-dryer was transferred to a dark-colored glass container, and the percentage yield was calculated before storing it in the freezer until further experimental use.

The dried aerial parts of the plant materials were ground into a coarse powder. Plant material was weighed to 5 grams and placed in Erlenmeyer flasks. To each flask, 50 mL of 70% ethanol and ethyl acetate were added, and the flasks were sealed and left to macerate at room temperature. Occasional shaking was done to ensure complete maceration. After 24 hours, the macerates were filtered, and 50 mL of a new portion was added to the plant material. After three days of maceration, the collected macerates were subjected to solvent removal using a rotary evaporator under low heat (40°C) with vacuum [13]. The extracts were combined in dark-colored glass containers, and the percentage yield was calculated before storing them in the freezer until further experimental use.

2.3. Determination of antioxidant activity

The total antioxidant status (TAS), total oxidant status (TOS), and oxidative stress index (OSI) of the extracts obtained with different solvents were evaluated using commercially available Rel Assay Diagnostic kits [14,15]. Trolox and hydrogen peroxide standards were used as references for the TAS and TOS analyses. The oxidative stress index (OSI, Arbitrary Unit = AU) values of each extract were calculated using the following formula [15]:

OSI (AU) = [TOS, μmol H_2O_2 equivalent/L] / [TAS, mmol trolox equivalent/L × 10]

The free radical DPPH method [16] is a practical and highly reliable technique for determining the free radical scavenging capacity of antioxidants. DPPH is a stable nitrogen radical that is commercially available, and its solution in ethanol is purple, with absorbance measured at 517 nm. When antioxidants are added to the DPPH solution, the antioxidants reduce the DPPH, causing the solution to shift from purple to yellow, and this reaction progress is monitored using a spectrophotometer [17,18]. For this test, the stock solution of each sample was prepared in methanol (MeOH) at a concentration of 1 mg/mL. After filtration, 200 μL of the clear stock solutions were transferred to the first column of a 96-well microtitration plate. Using a multi-channel pipette, eight serial dilutions were made in equal amounts of MeOH, and the mixtures were vortexed for 5 minutes. The DPPH stock solution was prepared by dissolving 2 mg of DPPH in 25 mL of MeOH, yielding a final concentration of 80 μg/mL. To each well, 100 μL of the DPPH solution was added to initiate the reaction, which was then incubated in the dark at room temperature for 30 minutes [19,20]. Ascorbic acid at the same concentration was used as a positive control, DPPH + MeOH as the negative control, and MeOH alone as the blank. The UV absorbance was read at 517 nm using a microplate spectrophotometer (Epoch) at room temperature [20].

The % scavenging value of DPPH• was calculated using the following formula [21,22]:

% Scavenging = [(AControl – ASample) / AControl] x 100

2.4. Antimicrobial activity tests

In this study, the antimicrobial activities of the plant extracts against *Escherichia coli* (ATCC 25922), *Staphylococcus aureus* (ATCC 29213), *Pseudomonas aeruginosa* (ATCC 27853), *Bacillus cereus* (ATCC 11778), and *Candida albicans* (ATCC 10231) were determined using the Microdilution Broth method [23]. Stock solutions of the plant extracts were prepared by dissolving them in 40% dimethyl sulfoxide (DMSO). Mueller Hinton Broth was used for bacteria, and Sabouraud Dextrose Broth was used for *C. albicans*. In 96-well microtiter plates, 90 μl of broth medium was added to the wells in the first column

and 50 µl to the remaining wells. The 11th column served as a sterility control, where 100 µl of broth was added, while the 12th column was used as a growth control. Extract was added to the wells in the first column (10 µl) and then serial dilutions were made. A suspension of microorganisms with a turbidity of McFarland 0.5 was prepared from colonies grown on MHB Blood Agar medium. 50 microliters of microorganism suspension were added to each well, with a concentration of $5x10^5$ CFU/mL for bacteria and 0.5-2.5 $x10^3$ CFU/mL for *C. albicans*. Plates containing bacteria were incubated at 37°C, and plates with *C. albicans* were incubated at 35°C for 16-24 hours. At the end of the incubation period, 50 µl of 2 mg/mL 2,3,5-triphenyl-tetrazolium chloride (TTC) (Merck, Germany) was added to each well to visualize bacterial growth, followed by 2 hours of incubation at 37°C. The tests were conducted in triplicates. The first well in which no color change occurred was considered the MIC value [24,25].

2.5. Total phenolic content

The total phenolic content was determined using the Folin-Ciocalteu method. This method is based on the principle that phenolic compounds dissolved in water or organic solvents form colored complexes with Folin reagents in an alkaline medium. The resulting purple-violet colored complex exhibits maximum absorbance at 760 nm [21,26-32]. The amount of phenolic compounds in the extracts was analyzed quantitatively using UV-VIS (Ultraviolet-visible) spectrophotometry by obtaining absorbance values. Different concentrations of gallic acid, a phenolic compound, were prepared, and a standard curve was plotted to calculate the total phenolic content (Figure 1). The dilution coefficient (dilution factor) was applied as 120 times.

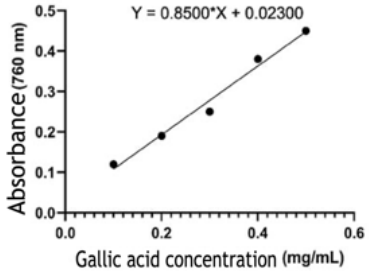

Fig. 1. Gallic acid standard curve.

2.6. Total flavonoid content

The total flavonoid content in the plant samples was determined using a method optimized from the procedure developed by Dewanto et al. [33]. The total flavonoid amount was measured by modifying the Aluminum chloride ($AlCl_3$) colorimetric method by adding 5% $NaNO_2$ solution [34]. The prepared sample mixtures were left to stand at room temperature for 30 minutes and then measured at a wavelength of 415 nm using a UV spectrophotometer [31,35-37]. A stock solution of quercetin at a concentration of 200 mg/L was prepared, and dilutions were made to obtain standard quercetin solutions, which were used to plot a standard curve (Figure 2) [32,34].

2.7. Determination of protective effect on DNA damage

In this study, pBR322 plasmid DNA (Thermo) was used as the DNA sample to be damaged. Hydrogen peroxide (H_2O_2) solution and ultraviolet (UV) light were applied to induce DNA damage. The method, originally defined by Russo et al. [32], was modified and optimized by Takım (2010) and Berk (2012) [32,38-40]. As shown in Table 1, plant extracts were added to the experimental tubes, while the control tubes received no plant samples, and the results were recorded. A 1.5% agarose gel was prepared to visualize the DNA bands. Electrophoresis was run at 40 V and 500 mA for 3 hours. The agarose gel was then placed on a UV transilluminator to capture images of the DNA bands. Ultraviolet light was passed through the gel from below, allowing the ethidium bromide-bound DNA fragments to be visually observed.

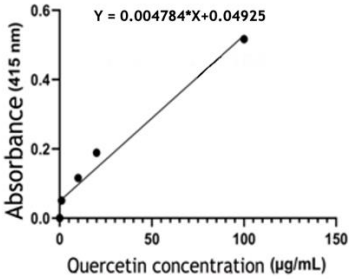

Fig. 2. Quercetin standard curve.

2.8. Statistical method

The results were expressed as mean ± standard deviation, and the statistical evaluation and calculations were performed using the GraphPad Data Analysis program. All tests were performed in triplicate. The findings were presented in graphs and tables. Data were analyzed at a 95% confidence level, and a p-value less than 0.05 was considered statistically significant.

3. Results

In this study, various biological activity properties of three different species of sage, *S. officinalis*, *S. triloba*, and *S. sclarea*, were evaluated. Each plant sample was extracted with three different solvents: water, 70% ethanol, and ethyl acetate. The resulting extracts from these different sage plants were prepared as stock solutions at a concentration of 1 mg/mL. The amounts of the plant material used initially and the extracts obtained after extraction were determined, and the extraction yields were calculated as percentages. It was observed that the ethanol extracts generally had higher yields, while the water extracts had lower yields (Table 2).

3.1. Antioxidant activity evaluations

The antioxidant and oxidant capacities of the plant extracts were determined using Rel Assay Diagnostic kits. The oxidative stress index (OSI) of the plant extracts was assessed by calculating the ratio of the TAS (total antioxidant status) to TOS (total oxidant status) values obtained from the extracts. The results are shown in Table 3. In radical scavenging experiments performed using the DPPH method, IC_{50} values were calculated based on the absorbance measured at 517 nm. The results indicated that 70% ethanol extract had the highest ability to inhibit free radicals, while aqueous extracts had the lowest activity. Ascorbic acid was used as the reference. Overall, it was determined that *S. officinalis* exhibited better antioxidant activity capacity across all extracts than the other species (Table 3).

From the prepared water, 70% ethanol, and ethyl acetate extracts, the highest TAS was found in *S. triloba* 70% ethanol extract (9.6 ± 1.8 mmol/L), and the highest TOS in *S. sclarea* aqueous extract (3.9 ± 1.2 µmol/L). OSI values were found to be quite similar across all plants and extracts. Regarding antioxidant activity, the highest DPPH radical scavenging activity was observed in *S. officinalis* 70% ethanol extract and the lowest in *S. triloba* aqueous extract (Table 3).

3.2. Results of antimicrobial activity

The antimicrobial activity of different sage extracts is shown in Table 4. MIC values ≤ 0.1 mg/mL were classified as highly effective, MIC values between 0.1 < MIC ≤ 0.625 mg/mL as moderately effective, and MIC values > 0.625 mg/mL as weakly effective [41,42]. The findings suggest that the ethanol extracts of *S. officinalis*, *S. triloba*, and *S. sclarea* showed moderate to weak antimicrobial activity against the microorganisms tested. The samples were found to have moderate and weak activity against *E. coli*, *S. aureus*, and *P. aeruginosa*, weak activity against *B. cereus*, and moderate to weak antimicrobial activity against *C. albicans*.

3.3. Results of total phenolic content determination

The total phenolic content of sage extracts was determined using the Folin-Ciocalteu method and expressed as gallic acid equivalents (mg GAE/g extract). It was observed that the *S. officinalis* and *S. triloba* ethyl acetate extracts, along with *S. sclarea* 70% ethanol extract, had the highest total phenolic content, while the aqueous extracts of all plants had the lowest phenolic content. The total phenolic content of the extracts is provided in Table 5.

3.4. Results of total flavonoid content determination

The total flavonoid content of the samples was expressed as quercetin equivalents (mg QE/g extract). The results revealed that the ethyl acetate extracts of all three *Salvia* species had the highest total flavonoid content, while the aqueous extracts had the lowest. The total flavonoid content values of the extracts are provided in Table 5.

3.5. Results of protective activity against DNA damage

Within the scope of this study, pBR322 plasmid DNA was treated with extracts of 3 different sage plant species obtained with 3 different solvents. In this way, the abilities of the extracts to modify the supercoil of the closed circular pBR322 plasmid DNA were investigated. According to this method, the ability of plant extracts to prevent DNA damage was evaluated in the presence of hydrogen peroxide (H_2O_2) and UV light, which are factors that damage DNA. In the images where the protective effect of plant extracts against DNA damage is seen, it is seen that 2 bands of plasmid DNA are clearly present on the gel (Figure 3). It was found that the protective activity of the extracts of all 3 species of sage plants (water, 70% ethanol and ethyl acetate) on plasmid DNA were very close to each other and that they protected plasmid DNA well from damage (Figure 3). The ability of sage plant extracts to protect the supercoiled DNA structure of plasmid DNA was shown by the presence of Form 1 bands on the gel.

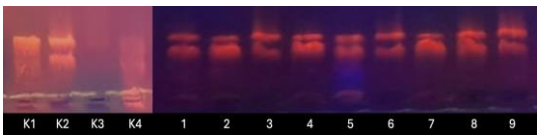

Fig. 3. Protective effect of sage extracts on plasmid DNA against damage induced by H_2O_2 and UV light.

Table 1. Contents of control and experimental tubes.

Number	Content	Plasmid DNA	dH_2O	UV	H_2O_2	Extract
K1	Control 1	3 µl	6 µl	-	-	-
K2	Control 2	3 µl	6 µl	+	-	-
K3	Control 3	3 µl	6 µl	-	1 µl	-
K4	Control 4	3 µl	6 µl	+	1 µl	-
1	*S. officinalis* aqueous extract	3 µl	-	+	1 µl	5 µl
2	*S. officinalis* 70% ethanol extract	3 µl	-	+	1 µl	5 µl
3	*S. officinalis* ethyl acetate extract	3 µl	-	+	1 µl	5 µl
4	*S. sclarea* aqueous extract	3 µl	-	+	1 µl	5 µl
5	*S. sclarea* 70% ethanol extract	3 µl	-	+	1 µl	5 µl
6	*S. sclarea* ethyl acetate extract	3 µl	-	+	1 µl	5 µl
7	*S. triloba* aqueous extract	3 µl	-	+	1 µl	5 µl
8	*S. triloba* 70% ethanol extract	3 µl	-	+	1 µl	5 µl
9	*S. triloba* ethyl acetate extract	3 µl	-	+	1 µl	5 µl

Table 2. Yield values obtained from extraction.

Extract	Yield %
S. officinalis aqueous extract	2.9%
S. officinalis 70% ethanol extract	12.6%
S. officinalis ethyl acetate extract	5.4%
S. triloba aqueous extract	2.6%
S. triloba 70% ethanol extract	10.8%
S. triloba ethyl acetate extract	5.1%
S. sclarea aqueous extract	2.2%
S. sclarea 70% ethanol extract	13.4%
S. sclarea ethyl acetate extract	4.4%

Table 3. TAS, TOS, OSI, and DPPH radical scavenging activity of plant extracts (values expressed as Mean ± SD).

Sample	Plant Name	Extract	TAS (mmol/L)	TOS (µmol/L)	OSI	IC_{50} (mg/mL)
1		Aqueous	8.0 ± 2.0	3.3 ± 0.7	0.04 ± 0.01	0.414 ± 0.231
2	*Salvia officinalis*	70% Ethanol	9.2 ± 2.3	3.6 ± 0.8	0.04 ± 0.01	0.105 ± 0.056
3		Ethyl acetate	8.1 ± 2.0	3.2 ± 0.5	0.04 ± 0.01	0.156 ± 0.072
4		Aqueous	7.6 ± 1.5	3.5 ± 0.5	0.05 ± 0.02	0.423 ± 0.102
5	*Salvia triloba*	70% Ethanol	9.6 ± 1.8	3.1 ± 0.6	0.03 ± 0.01	0.128 ± 0.089
6		Ethyl acetate	8.3 ± 2.1	3.7 ± 0.9	0.04 ± 0.01	0.228 ± 0.108
7		Aqueous	7.0 ± 1.2	3.9 ± 1.2	0.06 ± 0.03	0.321 ± 0.114
8	*Salvia sclarea*	70% Ethanol	8.3 ± 2.0	3.7 ± 0.8	0.04 ± 0.02	0.158 ± 0.072
9		Ethyl acetate	7.3 ± 2.4	3.6 ± 0.9	0.05 ± 0.03	0.223 ± 0.102
Ascorbic acid						0.005 ± 0.001

Table 4. MIC results of different sage extracts (mg/mL).

No	Plant Name	Extracts	*E. coli*	*S. aureus*	*P. aeruginosa*	*B. cereus*	*C. albicans*
1		Aqueous	0.31	0.16	0.16	1.25	0.63
2	*Salvia officinalis*	70% Ethanol	0.16	0.16	0.16	0.63	0.31
3		Ethyl acetate	0.16	0.16	0.16	0.63	0.31
4		Aqueous	0.31	0.31	0.16	1.25	1.25
5	*Salvia triloba*	70% Ethanol	0.16	0.31	0.16	0.63	0.63
6		Ethyl acetate	0.16	0.31	0.31	1.25	0.63
7		Aqueous	0.63	0.31	0.16	1.25	1.25
8	*Salvia sclarea*	70% Ethanol	0.31	0.16	0.16	0.63	0.31
9		Ethyl acetate	0.31	0.31	0.31	1.25	0.63

Table 5. Total phenolic content (mg GAE/g extract) and total flavonoid content (mg QE/g extract) of sage plant extracts.

No	Plant Name	Extracts	Total Phenolic Content (mg GAE/g extract)	Total Flavonoid Content (mg QE/g)
1		Aqueous	24.5 ± 3.8	10.5 ± 2.1
2	*Salvia officinalis*	70% Ethanol	34.4 ± 4.2	11.1 ± 2.8
3		Ethyl acetate	36.9 ± 9.2	12.5 ± 2.9
4		Aqueous	22.8 ± 2.7	9.3 ± 1.5
5	*Salvia triloba*	70% Ethanol	35.1 ± 5.3	9.8 ± 2.1
6		Ethyl acetate	37.6 ±8.5	10.6 ± 2.3
7		Aqueous	26.8 ± 3.3	10.8 ± 2.4
8	*Salvia sclarea*	70% Ethanol	36.1 ± 5.6	10.9 ± 2.5
9		Ethyl acetate	35.2 ± 7.4	12.4 ± 2.6

4. Discussion

Most of the studies conducted to date on *S. officinalis*, *S. triloba*, and *S. sclarea* have primarily focused on *S. officinalis*, with the samples typically being wild and, occasionally, cultivated forms. In this study, for the first time in Turkey, a comparative investigation was carried out on the antimicrobial, antioxidant activities, and protective effects on oxidative damage-induced plasmid DNA molecules of extracts obtained from *S. officinalis*, *S. triloba*, and *S. sclarea* species grown using organic farming techniques, utilizing three different solvents. Furthermore, the total flavonoid and total phenolic content of the plant samples were also determined.

Extractions were performed using water, 70% ethanol, and ethyl acetate as solvents. When all results were evaluated comparatively, it was determined that the best species in terms of activity was *S. officinalis* and the most effective solvent was 70% ethanol.

With the continuous advancement of technology, studies have shown that secondary metabolites produced by plants under stress contain a wide variety of phytochemicals, which can serve as therapeutic agents in the treatment of numerous diseases [11]. Oxidative stress, caused by free radicals, plays a role in the pathogenesis of various diseases and disorders. Antioxidants play a crucial role in protecting the body against this oxidative stress. Research over the past few decades has contributed to evidence supporting the enrichment of the body with antioxidant principles derived from plants. In a study evaluating the in vitro antioxidant activity of methanol extracts of *S. coccinea* and *S. officinalis* L. leaves, it was revealed that the extracts exhibited strong antioxidant and free radical scavenging activity. The IC_{50} (half-maximal inhibitory concentration) values of the extracts ranged from 7.34 mg/mL to 8.79 mg/mL, suggesting their potential use as natural sources for the development of free radical scavengers and antioxidant agents [43]. In another study evaluating the antioxidant activities of chloroform and acetone extracts of *S. sclarea* (clary sage), it was observed that the chloroform extract exhibited higher total antioxidant activity than the acetone extract [44]. Additionally, the antioxidant capacities of ethanol and methanol extracts of *S. sclarea* L. were also investigated, revealing that both extracts had high antioxidant activity, with the methanolic extract (0.81±0.041 mg GAE/g extract) demonstrating better activity compared to the ethanolic extract (0.87±0.005 mg GAE/g extract) [45]. In experiments evaluating the antioxidant potential of water extracts of *S. officinalis* and *S. triloba* using the ABTS method, it was determined that the ABTS radical scavenging effect of both extracts increased in a dose-dependent manner, with *S. triloba* (Inhibition% = 87.41 ± 0.86) showing a higher antioxidant potential compared to *S. officinalis* (Inhibition% = 73.30 ± 0.60) [46]. Similarly, in our study, it was observed that *S. triloba* exhibited greater antioxidant potential. The radical scavenging activity of ethanolic extracts obtained from five Salvia species including *S. hypoleuca* Benth., *S. reuterana* Boiss., *S. verticillata* L., *S. virgata* Jacq. and *S. officinalis* L. was evaluated in vitro by spectrophotometric method based on the reduction of DPPH free radical. As a result of the study, all extracts showed radical scavenging activity, especially *S. verticillata* [IC_{50} = 23.53 µg ml^{-1}] and *S. virgata* [IC_{50} = 27.01 µg ml^{-1}] were determined as the most active species, while *S. officinalis* L. [IC_{50} = 30.67 µg ml^{-1}] was found the least active [47].

The potential side effects of synthetic and semi-synthetic drugs used in modern medicine, the economic burden they place on countries, the resistance microorganisms have developed against these antimicrobial drugs, and concerns over the use of chemical agents for food preservation have significantly increased interest in natural resources in recent years. In one study, an extract obtained from *S. officinalis* leaves demonstrated antimicrobial activity against vancomycin-resistant enterococci. The compounds also exhibited antimicrobial activity against *Streptococcus pneumoniae* and methicillin-resistant *Staphylococcus aureus*. These compounds (oleanolic acid, ursolic acid, uvaol, betulinic acid and betulin) showed bactericidal activity against vancomycin-resistant enterococci for at least 48 hours in a dose-dependent manner [48]. In an article investigating the antimicrobial effects of chloroform and acetone extracts of *S. sclarea* on *Bacillus megaterium*, *Proteus vulgaris*, *Listeria monocytogenes*, *Bacillus cereus*, *Staphylococcus aureus*, *Bacillus brevis*, *Klebsiella pneumoniae*, *Micrococcus luteus*, *Pseudomonas aeruginosa*, *Escherichia coli*, and *Mycobacterium smegmatis* bacteria, it was observed that the extracts were effective against all bacteria except *E. coli* [1]. In a similar manner to this study, our research determined that the ethanol extracts of three different *Salvia* species exhibited moderate to weak antimicrobial activity against *E. coli*, *S. aureus*, and *P. aeruginosa* bacteria. In one study, the total phenol content of water extracts from *S. officinalis* and *S. triloba* species was calculated as 193.50 ± 8.22 and 203.01 ± 7.85 GAE mg/g, respectively, while the total flavonoid content was 71.51 ± 1.88 and 78.84 ± 8.76 QE mg/g, respectively [46]. In another study, the total phenolic compound content in the methanol extract of *S. officinalis* was determined to be 31.25 mg/g gallic acid equivalent. In comparison, the total flavonoid content was 18.46 mg/g gallic acid equivalent [49].

It was observed that these approximate values are consistent with the results obtained in our current study. DNA, a stable molecule like carbohydrates, lipids, and proteins, undergoes chemical oxidative damage, which is repaired by DNA repair systems. In cases where the damage is severe or cannot be repaired, the resulting DNA damage can lead to cell death or mutation. Additionally, DNA damage has been reported to play a critical role in the etiology of various diseases and aging. Oxidative DNA damage is not only considered a factor in the normal aging process but is also recognized as a significant trigger for cancer. In a study, the aqueous extracts of *S. fruticosa*, *S. officinalis*, and *S. lavandulifolia*, along with rosmarinic acid and luteolin-7-glucoside, were investigated for their effects on DNA repair in Caco-2 and HeLa cells exposed to oxidative agents. The study concluded that the *Salvia* extracts and rosmarinic acid, along with luteolin-7-glucoside, protected cells from oxidative DNA damage and stimulated DNA repair [50]. Similarly, in another study, the aqueous extract of *S. fruticosa* was found to protect human embryonic kidney cell lines from both intrinsic and H_2O_2-induced DNA oxidation [51]. In our study, it was also demonstrated that the water, 70% ethanol, and ethyl acetate extracts of all three *Salvia* species exhibited good protective activity against DNA oxidation.

5. Conclusions

It has been found that *Salvia* species, commonly used as tea infusions among the public, may be a potent antioxidant source capable of reducing oxidative damage in the body. Additionally, *S. officinalis*, *S. triloba*, and *S. sclarea* species should be considered among the plants that need to be examined more thoroughly for the extraction and production of active compounds that can be used in repairing the damage caused by free radicals. This study is thought to contribute to the existing literature by comparatively determining the antimicrobial, antioxidant, and oxidative DNA damage protective effects

of three distinct species of sage, which hold a significant position in both domestic and international markets. The results are expected to provide a foundation for the introduction of innovative new products from the most effective species and solvents.

Author Contributions: Conceptualization, M.K. and E.S.G.; methodology, M.K. and E.S.G.; validation, M.K. and E.S.G.; investigation, M.K. and E.S.G.; resources, M.K. and E.S.G.; data curation, M.K. and E.S.G.; writing—original draft preparation, M.K. and E.S.G.; writing—review and editing, M.K. and E.S.G.; visualization, M.K. and E.S.G.; supervision, M.K. and E.S.G.; project administration, M.K. and E.S.G.; funding acquisition, M.K. and E.S.G., All authors have read and agreed to the published version of the manuscript.

Funding: This work was supported by the TUBITAK 2209-A project 'University Students Research Project Support Program' (Project No: 1919B012207846).

Acknowledgments: We would like to thank the Temmuz Organic Farm for providing organically grown plants.

Conflicts of Interest: The authors declare no conflict of interest.

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