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Identification of Bioactive Compounds in Plant Extracts of Greek Flora and Their Antimicrobial and Antioxidant Activity

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Abstract: This study examined five species of the Lamiaceae family, *Origanum vulgare* L., *Origanum dictamnus* L., *Origanum majorana* L., *Mentha spicata* L. and *Lavandula angustifolia* L., and one species of the Hypericaceae family, *Hypericum perforatum* L., for their potential to enhance health and wellbeing or to prevent diseases. An analysis of secondary metabolites was performed by mass spectrometry and HPLC coupled with diode array detection to determine the phenolic compounds included in the plant extracts. The samples were evaluated for their total phenolic content, antioxidant potential, ability to prevent DNA scission caused by peroxyl radicals and antimicrobial activity against pathogens (MIC). All the samples, except *Lavandula angustifolia* L., had a high DPPH radical scavenging activity and showed significant antibacterial activity. *Origanum majorana* L. demonstrated the lowest IC_{50} value (10.31 μg·mL^{-1}). Extracts of medicinal herbs had a remarkably high phenolic content varying between 428 and 1512.74 μg GAE/mg of dry extract, with *Origanum vulgare* L. having the largest amount of polyphenols. Moreover, the plant extracts exhibited a high level of resistance against DNA damage, with *Origanum majorana* L. showing the greatest level of protection with 98.05% inhibition. According to the results of this study, these plants from the Greek flora provide beneficial effects for health as natural antioxidants. A baseline can be established through the analysis of the findings of this survey for future research on the same plant species.

Keywords: natural extracts; Greek flora; chromatographic analysis; phenolic compounds; antioxidant activity; DNA scission; antimicrobial agents

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1. Introduction

Modern lifestyle is associated with several health issues. Humans are daily confronted with the presence of various environmental pollutants including allergens, microbes and various other contaminants. The above and other unhealthy habits, such as smoking and UV radiation, are associated with high levels of free radicals [1]. Their excessive production causes damage to various cellular structures and consequently leads to chronic disorders such as cardiovascular disease, rheumatoid arthritis, respiratory problems, diabetes and cancer [2,3].

Antioxidants play an important role in maintaining a balanced cellular function by neutralizing free radicals through various mechanisms [4]. Food industries widely use synthetic antioxidants to extend shelf life and improve the quality and safety of their food products. However, such synthetic antioxidants are not preferred due to toxicity concerns [5,6]. Therefore, consumer interests have focused on identifying natural extracts

that can be used as natural antioxidants, since they are considered safer [7]. In recent years, the interest in natural extracts from medicinal plants has grown exponentially due to their rich content of phytochemical substances [8,9]. Among these substances, phenolic compounds including phenolic acids and flavonoids comprise the principal categories of natural components in plants. The structure and amount of the hydroxyl groups of phenolic compounds vary, which affects their antioxidant activity [10–12]. Researchers have also turned their focus to medicinal plants for newer antibacterial compounds to replace the ones that are currently on the market, especially nowadays due to constantly emerging antibiotic-resistant bacteria [13,14]. The synergistic action of phytochemical bioactive substances included in several plant extracts is responsible for inhibiting the growth of Gram-positive and Gram-negative bacterial strains [15].

The Lamiaceae family (known as the mint family or Labiatae) is one of the most significant families of herbs. It includes about 236 genera and more than 6000 species [16]. Herbs or shrubs with strong aromatic scents make up the plants in this family. Labiatae are widespread throughout the world, with the Mediterranean region having the highest concentration [17]. Mediterranean countries offer a high level of biodiversity. Secondary metabolites, mainly phenols, found in plants belonging to the Lamiaceae family have been shown to have antimicrobial, antioxidant, antitumor, antispasmodic and antiseptic properties, indicating that they may be a good substitute for synthetic antioxidants [18,19]. Furthermore, Lamiaceae species are valuable in the cosmetic, perfume and fragrance, pesticide and pharmaceutical industries. They are also used as culinary herbs and cultivated for their edible leaves [20].

Plants of the Hypericaceae family have been the main area of interest for many scientists over the past decade. *Hypericum*, the largest of the nine Hypericaceae genera, includes nearly 500 species of herbs, shrubs and small trees [21]. *Hypericum perforatum* L. (St. John's wort) belongs to the *Hypericum* genus. It is indigenous to Madeira, the Azores, West Asia, North Africa and Europe [22]. It is currently one of the most widely used medical plants worldwide [23]. This herb's sales have significantly increased due to its potentially beneficial effect on psychological disorders, such as depression [24]. The extracts of this plant have also a wide range of therapeutic uses including treating burns, eczema, intestinal illnesses and skin wounds [25]. In addition, recent studies have highlighted the potential utility of *Hypericum perforatum* L. in the management of pain disorders [26]. St. John's wort (*Hypericum perforatum* L.) has also been demonstrated to have potent antiviral, anti-inflammatory, antimicrobial and antioxidant properties [27].

The aim of this research was to evaluate the antioxidant and antimicrobial activity of extracts of plants belonging to two different families, the Hypericaceae family and the Lamiaceae family. The evaluated plants included the *Origanum vulgare* L. (oregano), *Origanum dictamnus* L. (dittany), *Mentha spicata* L. (mint), *Origanum majorana* L. (marjoram), *Lavandula angustifolia* L. (lavender) and *Hypericum perforatum* L. (St. John's wort). These plants were obtained from different parts of Greece. Analytical methods, HPLC-DAD analysis and MS analysis, were used to qualitatively and quantitatively assess the bioactive phytochemical compounds of their natural extracts. The antioxidant activity and the total phenolic content of medicinal plants were also determined. Last, the antibacterial activity of selected extracts against Gram-positive and Gram-negative bacteria was evaluated, and the Minimum Inhibitory Concentration (MIC) of the antimicrobial agents was measured.

2. Materials and Methods

2.1. Reagents and Standards

Methanol, acetonitrile, water of HPLC grade and ethanol (analytical grade) were supplied by Merck (Darmstadt, Germany). Standard phenolic compounds such as naringenin, thymol, carvacrol, luteolin, quercetin, kaempferol, apigenin, apigenin-7-glucoside, rutin, eriodictyol, vanillic acid, rosmarinic acid, *p*-coumaric acid, ferulic acid, caffeic acid, hydroxybenzoic acid, benzoic acid and chlorogenic acid were purchased from DR EHRENSTORFER GmbH (Augsburg, Germany). 2,2-Diphenyl-1-picrylhydrazyl (DPPH•), Folin–Ciocalteu's

phenol, sodium carbonate, formic acid, 2,2′-Azobis(2-amidinopropane) dihydrochloride and supercoiled pBR322 DNA were also supplied by DR EHRENSTORFER GmbH.

2.2. Pathogenic Microorganisms

Standard ATCC bacterial strains, *Salmonella enterica* ATCC 14028, *Klebsiella pneumoniae* ATCC 13883, *Escherichia coli* ATCC 25922, *Staphylococcus aureus* ATCC 25923, *Enterococcus faecalis* ATCC 29212 and *Listeria monocytogenes* ATCC 35152 derived from food were collected. All bacteria were cultured on selective nutrient substrates in order to maintain bacterial viability. All strains were stored at −80 °C.

2.3. Extraction Procedure

Dried leaves of oregano (*Origanum vulgare* L.), dittany (*Origanum dictamnus* L.), mint (*Mentha spicata* L.), marjoram (*Origanum majorana* L.) and St. John's wort (*Hypericum perforatum* L.) were collected from Crete, and lavender (*Lavandula angustifolia* L.) was collected from Kozani, during the period June-September 2021. A mechanical blender was used to grind the leaves into a fine powder. The powdered leaves (10 g) were macerated at room temperature for 14 days with frequent agitation, using 10% ethanol and 90% distilled water [28]. The liquid extract was filtered and centrifuged at 8000 rpm for 15 min. The solvent was removed using a rotary evaporator [29]. The residue was dissolved in the appropriate solvent (methanol was used for chromatographic analysis and the evaluation of antioxidant activity, and water was used for the determination of total phenolic content and antimicrobial activity testing), filtered through a 0.22 μm PVDF filter and used for analyses.

2.4. Analytical Conditions

2.4.1. High-Performance Liquid Chromatography-Diode Array Detection Analysis (HPLC-DAD)

HPLC analysis was carried out using an HPLC-UV system (VWR Hitachi Elite LaChrom system, VWR, Darmstadt, Germany) consisting of an autosampler (L-2200), binary pump (L-2130), column oven (L-2300) and diode array detector (L-2455). The separation of the compounds was achieved on an SVEA C18 reverse-phase column (150 mm × 4.6 mm, 5 μm particle size, Nanologica, Stockholm, Sweden), maintained at 30 °C, with a flow rate of 0.5 mL min^{-1}. The HPLC-DAD analysis was proposed by Kouri et al. [30] with some modifications. The mobile phase consisted of water with 1% formic acid (A), methanol with 1% formic acid (B) and acetonitrile with 1% formic acid (C). The gradient used was 90% A, 6% B, 4% C 0–5 min, 85% A, 9% B, 6% C 5–30 min, 71% A, 17.4% B, 11.6% C 30–60 min, 0% A, 85% B, 15% C 60–63 min, 90% A, 6% B, 4% C 63–65 min. The injection volume was 20 μL, and the spectra were represented at 280 nm. All the analyses were made in triplicate. Standard calibration curves were created for HPLC-DAD quantification.

The concentrations of flavonoids and phenolic acids in the natural extracts were calculated using the calibration curves of the standard compounds. Individual standard solutions (15 mg) were dissolved in methanol (50 mL) at a concentration of 300 μg·mL^{-1} and followed by serial dilutions. A five-point regression curve ($R^2 > 0.98$) was used to quantify each chemical compound separately, ranging from 1 to 100 μg·mL^{-1}. Literature was used to select the standard compounds for this research [31,32]. Thus, the quantitative data were derived from externally calibrated standards, and the identification of phytochemical substances in aromatic herbs was carried out (a) by adding internal standards and (b) by comparing the retention times and the maximal wavelengths of the polyphenols of the extracts with the corresponding external standard.

2.4.2. Mass Spectrometry (MS)

The experiments were performed on a mass spectrometer (Advion, Inc., Ithaca, NY, USA) coupled with an Atmospheric Solid Analysis Probe (ASAP) interface and APCI ion source. This method is a screening process that was used for the identification of

products of commercial herbs and spices [33]. The mass analyzer is a single quadrupole. The conditions used for mass spectrometry were as follows: capillary temperature 200 °C, capillary voltage 180 V, source voltage offset 25 V, source voltage span 20 V, source gas temperature 350 °C, APCI corona discharge 5 µA. The liquid extract (10 µL, 135 µg·mL^{-1}) was spotted into the capillary tip. The spectra were recorded in the positive mode in the range of m/z 50–1000 for full-scan MS analysis [34].

2.5. Evaluation of Antioxidant Activity—DPPH Method

DPPH (1,1-diphenyl-2-picrylhydrazyl) is a stable organic radical with a deep purple color. According to this method, the free radical is reduced by antioxidant compounds or compounds that are donors of a hydrogen atom to the corresponding pale yellow hydrazine [35,36]. The DPPH• solution was prepared in methanol ($6{\cdot}10^{-5}$ M). Various concentrations (8.44, 16.88, 33.75, 67.50, 135 µg·mL^{-1}) of plant extracts (100 µL) were added to 3400 µL of DPPH solution [37]. After 45 min in the darkness, the absorbance was measured with a VIS spectrophotometer (Thermo Spectronic Helios Epsilon, Waltham, MA, USA) at 517 nm. A sample containing 100 µL of methanol in the DPPH• solution was also prepared, and its absorbance was measured (Acontrol). The percentage of DPPH radical scavenging was calculated using the following equation:

$$\%\ \text{radical scavenging activity} = \frac{\text{Acontrol} - \text{Asample}}{\text{Acontrol}} \times 100\%$$

Acontrol is the absorption of the control, and Asample is the absorption of the extract.

2.6. Determination of Total Phenolic Content

Folin–Ciocalteu method was used to measure the total phenolic content of the aqueous natural extracts [38]. Briefly, 200 µL of each extract (8.44–135 µg·mL^{-1}) solution, 0.8 mL Na_2CO_3 (7.5% in deionized water) and 1 mL of the Folin–Ciocalteu reagent (diluted 1:10) were mixed. Before its use and after the addition of deionized water, sodium carbonate was incubated at 50 °C for 5 min with occasional agitation. The absorbance was measured at 765 nm using Thermo Spectronic Helios Epsilon (USA) after 60 min of incubation of the mixtures at room temperature in darkness. Gallic acid was used as the standard compound, and the reference curve was plotted in the same manner as the samples [39]. The results were represented as µg of gallic acid equivalents per mg of dry weight (µg GAE/mg of DW), depending on the gallic acid standard curve (at a linearity range 12.5–200 µg·mL^{-1}, with the equation y = 0.0118x − 0.0819 and R^2 = 0.9953, $p < 0.05$).

2.7. The Inhibition of DNA Scission Caused by Peroxyl Radical

A method described by Chandrasekara and Shahidi [40] was used to determine the effectiveness of the six natural extracts against DNA scission with slight modifications. Gel electrophoresis was used to detect nicking of the DNA strands induced by peroxyl radicals. A phosphate buffer (PBS), 0.5 M, was used to dissolve supercoiled DNA (pBR322 from Escherichia coli RRI) at a concentration of 50 µg·mL^{-1}. In a tube, 4 µL of solution of supercoiled pBR322 DNA, 4 µL of 30 mM 2,2′-Azobis(2-amidinopropane) dihydrochloride (AAHP), 2 µL PBS, 2 µL of the natural extract (135 and 5000 µg·mL^{-1} of *Lavandula angustifolia* L. and 135 and 2 µg·mL^{-1} of the other medicinal plants) were mixed and incubated at 37 °C for 20 min [41]. A control with plasmid DNA and a blank were prepared with each batch of experiments. The blank contained only DNA and the free radical AAHP but no antioxidant substances [42].

All samples were analyzed by electrophoresis in a 0.8% agarose gel. The electrophoresis was accomplished at 100 V for 2 h. The bands were analyzed using the MiniBIS Pro device (DNR Bio-Imaging Systems Ltd., Neve Yamin, Israel) in order to quantify the DNA scission. The results were provided by the software of the device MiniBIS Pro (DNR Bio-Imaging Systems Ltd., Neve Yamin, Israel) and were expressed as percentages.

$$\% \text{ DNA retention} = \frac{\text{Intensity of supercoiled DNA with the oxygen radical and sample}}{\text{Intensity of supercoiled DNA in control}} \times 100\%$$

2.8. In Vitro Evaluation of Antimicrobial Activity

In vitro antimicrobial action of natural extracts was investigated against selected ATCC strains which are mentioned above. A microbial culture was used with an estimated inoculum size of 1.0×10^8 CFU/mL (0.5 McFarland scale). Aqueous extracts of various concentrations (1–800 μg·mL^{-1}) were added to the bacterial suspensions (1.0×10^8 CFU/mL), and 25 μL was inoculated on Chromogenic Agar plates (Bioprepare, Athens, Greece) corresponding to the microorganisms above. The plates were incubated at 37 °C for 24 h. Thus, the antimicrobial activity of the extracts was evaluated by counting the bacterial growth on selective nutrient substrates for each pathogen measuring the MIC values. To determine whether the positive control worked, plates without any plant extracts were inoculated [43].

2.9. Statistical Analysis

The results were represented as mean ± standard deviation of three replicates. A regression analysis was used to analyze the experimental data of DPPH scavenging rate. ANOVA was used to analyze the antioxidant activity of natural extracts. The results were trumpeted as statistically significant when the *p*-value was lower than 0.05. The analysis was interpreted using the Statistica Software (Statistica Release 12, StatSoft Inc., Tusla, OK, USA).

3. Results and Discussion

3.1. Phytochemical Analysis

3.1.1. HPLC-UV DAD Analysis

Phytochemicals are used as dietary supplements, nutraceuticals, food ingredients, pharmaceuticals and cosmetics by extracting these bioactive compounds from plant materials. A variety of plant samples can be used to extract phenolics, including fresh, frozen or dried samples. These plant samples are usually milled, ground and homogenized in order to obtain the maximum yield of phenolics [44]. Typically, the most usual method for analyzing polyphenols in samples is reversed-phase HPLC on C18 columns. A large number of phenolic compounds in aromatic plants can be identified and quantified by this simple, easy-to-use method [45]. We found that using a mobile phase with 1% formic acid in each solvent provides peak shape and separation of high quality. All compounds examined showed strong linear relationships ($R^2 > 0.98$, $p < 0.05$) at various wavelengths, with the highest response at 280 nm. Chromatograms of natural extracts were recorded at a wavelength of 280 nm, where the majority of the components could be clearly distinguished.

The plant residues after the rotary evaporator were found to weigh 800 μg. This dry matter was dissolved in 1 mL of the solvent, and its concentration was calculated to be 800 ± 10 μg·mL^{-1}. The most abundant phenolic compounds in the *Origanum vulgare* L. extract were the flavonoid glucoside rutin at a concentration of 249.18 μg·mL^{-1} and caffeic acid (79.33 μg·mL^{-1}) (Figure 1A, Table 1). Pandey et al. [46] also found that the leaves of oregano (*Origanum vulgare* L.) were rich in rutin compared to the other phenolics, while caffeic acid was not detected. In this study, *Origanum dictamnus* L. extract was also examined, and it was found that benzoic acid and rosmarinic acid had the highest concentrations (Figure 1B, Table 1). In a previous study, Chatzopoulou et al. [47] evaluated the content of *Origanum dictamnus* L. extract. The authors of this study found that rosmarinic acid, quercetin, apigenin, eriodictyol, naringenin, taxifolin and two alicyclic derivatives (12-hydroxyjasmonic acid, 12-O-β-D glucoside) were the main components. Furthermore, the extract of *Hypericum perforatum* L. was analyzed in our survey, and high quantities of rutin and benzoic acid were identified (Figure 1C, Table 1). Similar to our findings, one previous study by Aybastier et al. [48] reported that the phenolic compounds that were

determined in St. John's wort (*Hypericum perforatum* L.) extract were rutin, quercetin and kaempferol. On the other hand, Aybastier et al. [48] identified chlorogenic and protocatechuic acid, which were not detected in our study.

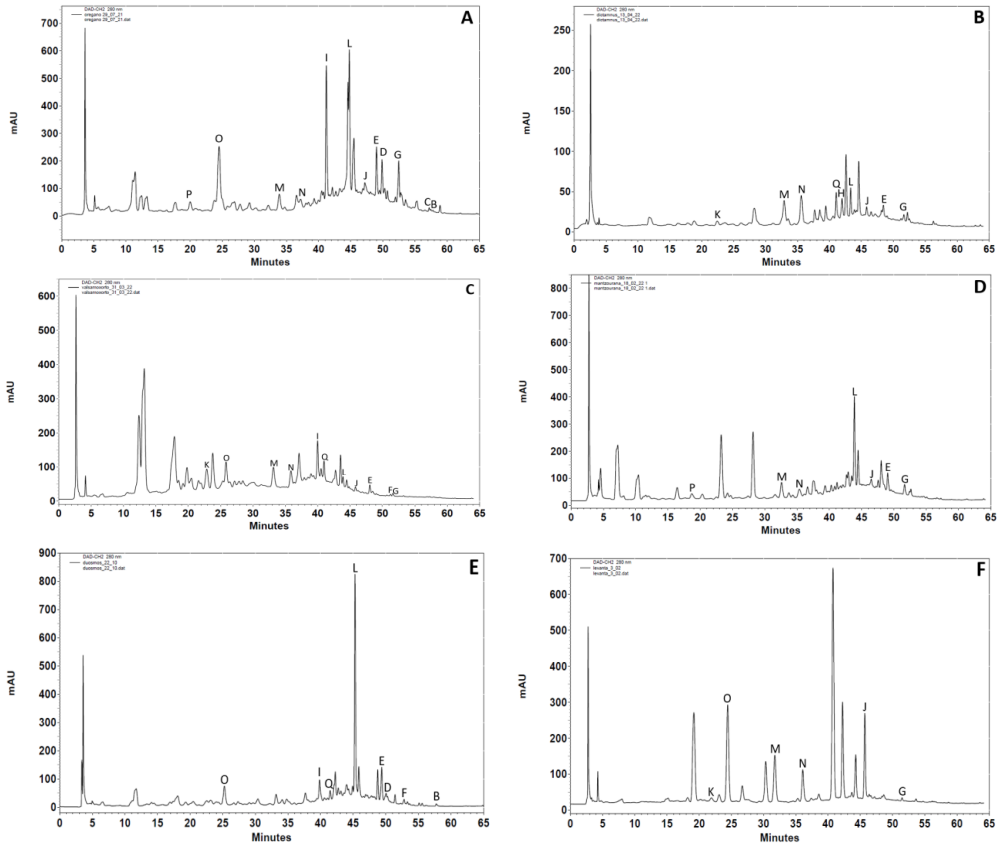

Figure 1. Chromatographic profile of natural extract of (**A**) *Origanum vulgare* L., (**B**) *Origanum dictamnus* L., (**C**) *Hypericum perforatum* L., (**D**) *Origanum majorana* L., (**E**) *Mentha spicata* L. and (**F**) *Lavandula angustifolia* L. under optimum conditions. Scanning at λ = 280 nm.

In our study, *Origanum majorana* L. extract was found to be rich in rosmarinic acid. It also included flavonols such as quercetin, flavones such as apigenin, flavanones such as eriodictyol and other phenolic acids and coumaric, ferulic and hydroxybenzoic acids (Figure 1D, Table 1). Roby et al. [49] demonstrated similar results. In addition, in our study, it was shown that the main components of the extract of *Mentha spicata* L. were rosmarinic acid at a concentration of 126.38 μg·mL^{-1} and quercetin, while there were high quantities of rutin, luteolin and caffeic acid (Figure 1E, Table 1). According to Fatiha et al. [50], the *Mentha spicata* L. extracts were notably rich in rosmarinic acid and other bioactive compounds as shown in our research. The last extract that was studied in this survey was *Lavandula angustifolia* L. The main constituents were caffeic acid at 60.56 μg·mL^{-1} and eriodictyol at 35.09 μg·mL^{-1} (Figure 1F, Table 1). These results are not in line with the study of Adaszyńska-Skwirzyńska et al. [51] who found that rosmarinic acid was the main component of the extract.

Simple phenols, phenolic acids, flavonoids, coumarins, stilbenes, tannins, lignans and lignins are some of the phenolic substances in medicinal herbs. These compounds are all regarded as secondary plant metabolites [52]. Some of these bioactive components were included in the examined samples, as described above. Specifically, rosmarinic acid and rutin were the main phenolics found in aromatic plants. Their identification is an extremely interesting topic, as they are considered to have beneficial biological activities and contribute to human health.

Table 1. Concentration (in $\mu g \cdot mL^{-1}$) of the 17 tested metabolites determined in the extracts of medicinal plants.

Standard Compounds	*Origanum vulgare* L.	*Origanum dictamnus* L.	*Hypericum perforatum* L.	*Origanum majorana* L.	*Mentha spicata* L.	*Lavandula angustifolia* L.
	Concentration ($\mu g \cdot mL^{-1}$)					
(A) Naringenin	-	-	-	-	-	-
(B) Thymol	0.27 ± 0.10	-	-	-	0.61 ± 0.02	-
(C) Carvacrol	9.17 ± 0.74	-	-	-	-	-
(D) Luteolin	25.97 ± 0.36	-	-	-	19.89 ± 0.25	-
(E) Quercetin	40.01 ± 0.92	10.73 ± 0.13	10.14 ± 0.74	14.52 ± 0.59	46.03 ± 0.13	-
(F) Kaempferol	-	-	3.62 ± 0.46	-	7.42 ± 0.45	-
(G) Apigenin	33.32 ± 0.85	2.45 ± 0.05	1.90 ± 0.96	4.30 ± 0.11	-	2.17 ± 0.13
(H) Apigenin-7-glucoside	-	9.04 ± 0.03	-	-	-	-
(I) Rutin	249.18 ± 1.2	-	57.03 ± 0.32	-	40.58 ± 0.09	-
(J) Eriodictyol	2.14 ± 0.44	4.45 ± 0.11	5.09 ± 0.62	1.72 ± 0.22	-	35.09 ± 1.2
(K) Vanillic acid	-	4.22 ± 0.31	18.68 ± 0.91	-	-	8.50 ± 0.71
(L) Rosmarinic acid	23.80 ± 1.2	15.32 ± 0.34	5.63 ± 0.81	46.63 ± 0.45	126.38 ± 0.23	-
(M) *p*-coumaric acid	10.67 ± 0.81	5.35 ± 0.16	6.06 ± 0.87	8.58 ± 0.56	-	25.29 ± 0.33
(N) Ferulic acid	2.29 ± 0.17	13.99 ± 0.22	7.55 ± 0.88	16.47 ± 0.86	-	24.18 ± 0.97
(O) Caffeic acid	79.33 ± 0.95	-	20.99 ± 1.1	-	19.54 ± 0.56	60.56 ± 0.82
(P) Hydroxybenzoic acid	36.23 ± 0.25	-	-	20.26 ± 0.34	-	-
(Q) Benzoic acid	-	30.79 ± 0.73	45.72 ± 1.06	-	16.41 ± 0.18	-

3.1.2. Mass Spectrometry Analysis

The ionization mechanisms of Atmospheric Solids Analysis Probe (ASAP) and Atmospheric Pressure Chemical Ionization (APCI) are comparable because the corona discharge in a closed reaction chamber for both of them initiates the ionization process. Depending on the amount of water present, the ionization processes for the analyte may either entail proton transfer or charge transfer [53]. Radical cations, protonated molecules and fragment ions of selected phenolic compounds generated under the present ionization conditions in positive ion spectra were studied and explained. The precursor ion of vanillic acid $[M+H]^+$ at m/z 169 was produced, and the fragment ion was observed at m/z 151, which corresponds to the loss of H_2O. The parent ion of rosmarinic acid $[M+H]^+$ was a peak at m/z 361. The fragmentation pathways that were responsible for the formation of its ions at m/z 163 and m/z 181 correspond to caffeoyl and dihydroxyphenyllactic acid fragments, respectively. From the analysis of *p*-coumaric acid, it was proven that the fragment ions were detected at m/z 147 and m/z 119, from the precursor ion $[M+H]^+$ at m/z 165, due to the loss of H_2O and CO, respectively. The same mechanism applies to the compound of ferulic acid, and the resulting fragments were monitored at m/z 177 and m/z 149. The main ions of caffeic acid were observed at m/z 163, 135 and 145 corresponding to the loss of one molecule of H_2O, H_2O-CO and two molecules of water, respectively. The profile fragmentation of benzoic acid and the formation of ions m/z 105 and m/z 77, initiated by proton transfer, were generated by the loss of water and the subsequent loss of carbon monoxide. The detected $[M+H]^+$ of the monoterpenoid thymol, as well as carvacrol, was at m/z 151. The fragment peaks were identified at m/z 135 and m/z 107, which might be due to $C_{10}H_{15}^+$ and $C_7H_7O^+$ or $C_8H_{11}^+$, respectively. In addition, with positive polarity, characteristic ions for rutin were detected at m/z 611 and m/z 303. The signal m/z 611 coincides with the theoretical $[M+H]^+$, and the peak at 303 signifies the loss of the galactose moiety as well as the loss of the rhamnosyl moiety, except for the oxygen atom that forms the bond with galactose. Apigenin-7-O-glucoside had an $[M+H]^+$ ion at m/z 433, and a fragment ion at m/z 271 indicated a loss of the glucone part. Other standard phenolic

compounds, such as quercetin, luteolin and apigenin had an $[M+H]^+$ ion at m/z 303, 287 and 271, respectively. Our findings were similar to the literature [54–61]. MS spectra verified the components of the extracts that had been identified in the previous Section 3.1.1. The characteristic fragment ions, which were studied separately for each compound, were identified and observed in the content of the plant extracts as shown in Figure 2.

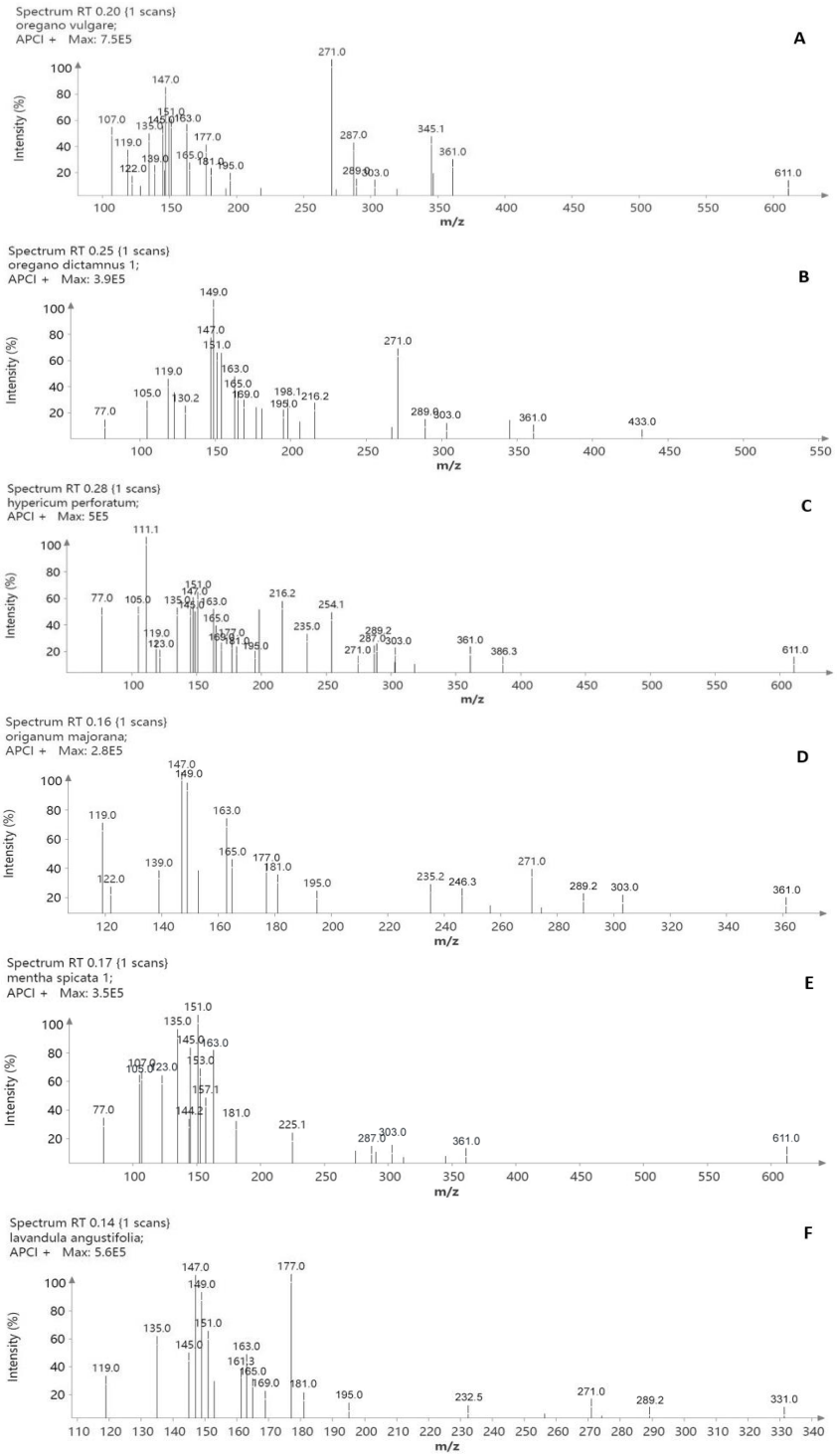

Figure 2. Mass spectra and the representation of the main ions of phenolic compounds included in the extracts of (**A**) *Origanum vulgare* L., (**B**) *Origanum dictamnus* L., (**C**) *Hypericum perforatum* L., (**D**) *Origanum majorana* L., (**E**) *Mentha spicata* L. and (**F**) *Lavandula angustifolia* L.

3.2. DPPH Radical Scavenging Activity

The 2,2 diphenyl-1-picrylhydrazyl (DPPH) radical scavenging method was used to determine the antioxidant potential of the extracts. This method was developed primarily to determine the antioxidant activity of plants and food extracts [62].

IC_{50} refers to the amount of the antioxidant required to reduce DPPH absorption by 50%. The sample with a lower IC_{50} value has greater antioxidant activity [63]. The IC_{50} value of the standard gallic acid was calculated as 6.96 ± 0.25 $\mu g \cdot mL^{-1}$. Table 2 and Figure 3 illustrate how natural extracts of the selected medicinal plants scavenge DPPH radicals. In addition, as shown in Figure 4, the sigmoidal curve of the studied natural extracts, except for that of *Lavandula angustifolia* L., estimates the potency (IC_{50}) and efficacy (E_{max}) of their antioxidant activity. The IC_{50} values of the methanol extracts of *Origanum vulgare* L., *Origanum dictamnus* L., *Mentha spicata* L., *Origanum majorana* L., *Lavandula angustifolia* L. and *Hypericum perforatum* L. were 12.10 ± 0.14, 37.50 ± 0.23, 16.93 ± 0.19, 10.31 ± 0.33, 3200.00 ± 1.2 and 11.00 ± 0.06 $\mu g \cdot mL^{-1}$, respectively. This means that the methanolic extract of *Origanum majorana* L. showed the best antioxidant activity compared to the other medicinal plants. However, there were no significant differences between the five examined samples. *Lavandula angustifolia* L. was not considered an effective antioxidant aromatic plant. The hydrogen-donating property of natural extracts, attributed to the synergistic action of the phytochemical bioactive compounds, may explain their high ability to scavenge free radicals [64]. According to Kouri et al. [30], the high DPPH activity of herbal extracts was closely correlated with rosmarinic acid concentration, which was detected in the majority of our samples. It is important to note that the values found in this study were comparable to those found in similar research [65,66].

Table 2. Antioxidant activity of six herbal extracts determined by DPPH method.

Herbal Extract	Concentration ($\mu g \cdot mL^{-1}$)	Radical Scavenging Capacity (RSC%)
Origanum vulgare L.	8.44	35.76 ± 0.14
	16.88	59.41 ± 0.14
	33.75	78.82 ± 0.14
	67.50	93.05 ± 0.15
	135.00	93.88 ± 0.16
Origanum dictamnus L.	8.44	13.17 ± 0.09
	16.88	21.53 ± 0.14
	33.75	40.11 ± 0.23
	67.50	66.82 ± 0.28
	135.00	92.82 ± 0.3
Mentha spicata L.	8.44	28.94 ± 0.06
	16.88	52.35 ± 0.19
	33.75	70.59 ± 0.23
	67.50	85.29 ± 0.24
	135.00	92.47 ± 0.26
Origanum majorana L.	8.44	37.76 ± 0.32
	16.88	67.90 ± 0.34
	33.75	77.00 ± 0.35
	67.50	93.88 ± 0.38
	135.00	96.94 ± 0.39

Table 2. *Cont.*

Herbal Extract	Concentration (μg·mL^{-1})	Radical Scavenging Capacity (RSC%)
Lavandula angustifolia L.	8.44	3.50 ± 0.08
	16.88	4.20 ± 0.12
	33.75	9.10 ± 0.23
	67.50	19.40 ± 0.33
	135.00	24.70 ± 0.41
Hypericum perforatum L.	8.44	37.41 ± 0.06
	16.88	66.70 ± 0.11
	33.75	73.76 ± 0.13
	67.50	83.29 ± 0.13
	135.00	94.58 ± 0.15

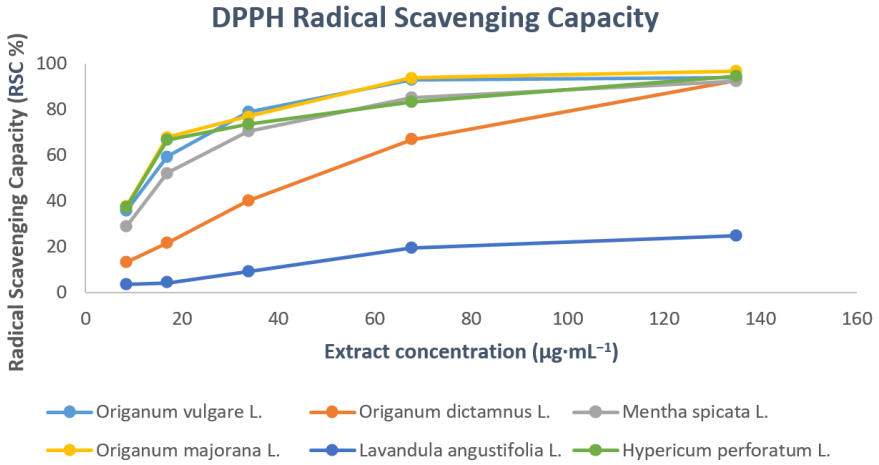

Figure 3. DPPH Radical Scavenging Activity of natural extracts of medicinal plants.

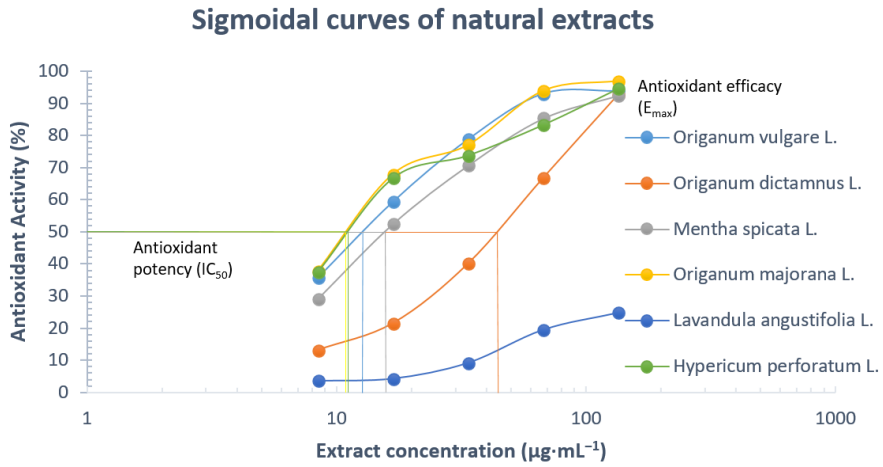

Figure 4. Antioxidant efficacy (IC_{50}) and potency (E_{max}) of natural extracts of medicinal plants.

In addition, the antioxidant activity of different concentrations of plant extracts was measured at different times (t = 0 min, t = 15 min, t = 30 min, t = 45 min, t = 1 h). The maximum values of % RSC of the various concentrations of herb extracts were observed at 45 min, compared to the other studied times, as seen in Table 3.

Table 3. % Radical Scavenging Capacity of various concentrations of plant extracts at different times.

Herbal Extract	Concentration ($\mu g \cdot mL^{-1}$)	Radical Scavenging Capacity (RSC%)				
		0 min	15 min	30 min	45 min	60 min
Origanum vulgare L.	8.44	2.41	14.70	25.41	35.76	34.76
	16.88	13.76	25.65	57.53	59.41	58.64
	33.75	38.47	43.41	74.12	78.82	68.01
	67.50	55.29	69.53	78.94	93.05	89.12
	135.00	74.11	76.94	90.24	93.88	92.53
Origanum dictamnus L.	8.44	11.53	12.71	12.94	13.17	13.41
	16.88	18.47	19.18	20.00	21.53	24.00
	33.75	30.59	31.76	36.47	40.11	46.12
	67.50	47.29	61.76	64.70	66.82	78.71
	135.00	77.00	84.11	88.82	92.82	92.47
Mentha spicata L.	8.44	13.65	17.41	27.88	28.94	28.94
	16.88	41.41	44.00	48.94	52.35	51.76
	33.75	58.71	64.82	67.88	70.59	70.24
	67.50	71.18	71.76	78.71	85.29	85.06
	135.00	77.41	85.76	91.41	92.47	91.88
Origanum majorana L.	8.44	26.59	31.01	37.65	37.76	37.76
	16.88	62.24	64.71	68.59	67.90	66.71
	33.75	72.71	79.29	78.71	77.00	76.94
	67.50	81.65	88.71	92.82	93.88	93.88
	135.00	88.47	92.47	94.71	96.94	96.47
Lavandula angustifolia L.	8.44	1.19	1.19	2.12	3.50	3.53
	16.88	1.19	1.76	2.47	4.20	3.53
	33.75	2.47	5.53	11.76	9.10	8.24
	67.50	3.50	8.23	16.12	19.40	18.35
	135.00	5.89	11.76	17.65	24.70	23.53
Hypericum perforatum L.	8.44	26.47	28.24	36.00	37.41	36.47
	16.88	37.53	48.71	64.47	66.70	55.18
	33.75	49.65	58.12	69.53	73.76	67.88
	67.50	69.76	76.47	84.12	83.29	83.06
	135.00	84.12	88.47	93.29	94.58	94.11

3.3. Total Phenolic Content

Phenolic compounds are the most widespread and abundant secondary metabolites in plants. The antioxidant properties of plant extracts are attributed to the phenolic sub-

stances [67]. The Folin–Ciocalteu method was used to determine the phenolic content in the samples. The values of phenolic compounds included in the natural extracts ranged between 1512.74 and 428 μg GAE/mg DW. Among the herbs with the highest phenolic content were *Origanum vulgare* L., *Hypericum perforatum* L. and *Origanum majorana* L., with 1512.74 ± 0.27, 1423.60 ± 0.33 and 1097.87 ± 1.5 μg GAE/mg DW, respectively. It was found that the extracts of *Mentha spicata* L. showed a high phenolic content of 820.40 ± 0.7 μg GAE/mg DW. Plants with the lowest phenolic content, among the studied samples, included *Origanum dictamnus* L. and *Lavandula angustifolia* L., with 448.15 ± 0.06 and 428 ± 1.2 μg GAE/mg DW, respectively. Despite the abundant amount of phenolic compounds in *Origanum vulgare* L., extracts of *Origanum majorana* L. were found to have the greatest antioxidant activity. It may be explained by the fact that certain phenolic substances are not considered to be effective antioxidants due to the position and number of primary hydroxyl groups [68,69]. It is believed that the antioxidant activity of the samples is due to the synergistic action of their phytochemical compounds [70].

In terms of other research studies, Sekeroglu et al. [71] found that the total phenolic content of aqueous extracts of *Hypericum perforatum* L. was 125.99 mg GAE/g DW. The survey of Spiridon et al. [65] showed that the total amount of phenolics in the *Origanum vulgare* L. and *Lavandula angustifolia* L. extracts was 67.8 ± 3.41 and 50.6 ± 3.16 mg GAE/g DW. In addition, Kalpoutzakis et al. [72] studied a variety of Greek plants and found that extracts of dittany (*Origanum dictamnus* L.) had a total phenolic content of 172 ± 8.6 mg GAE/g DW. In a 2017 study, the extract of *Mentha spicata* L. showed a total phenolic content of 870.62 ± 45.69 μmol GAE/g DW [69]. The results of another research study conducted by Benslama et al. [64] indicated that the concentration of polyphenols in marjoram (*Origanum majorana* L.) extracts was calculated at 56.08 μg GAE/mg DW. The results of this research regarding the total phenolic content were contradictory to those of the literature. The quantity of phenolics can be affected by various factors, such as the duration and extraction process, cultivation conditions, harvest time, climate and geographical coordinates of the plant's collection point [73].

3.4. The Inhibition of DNA Scission Caused by Peroxyl Radical

Several antioxidant defense mechanisms can protect biomolecules, including deoxyribonucleic acid, from the excessive production of free radicals caused during mitochondrial respiration and lipid peroxidation [74]. The overproduction of reactive oxygen species (ROS) in biological systems leads to oxidative stress. Oxidative stress represents a disturbance in the state of equilibrium of pro-oxidative and antioxidant reactions in living organisms. Thus, damage to the lipids, proteins and DNA of the cells inhibits their normal functions. Because of this, oxidative stress has been implicated in many human diseases [75]. A strand break is usually measured as an indicator of oxidant damage to cellular DNA [76].

In this study, samples were assessed for their ability to prevent DNA strand scission caused by peroxyl radicals in supercoiled plasmid DNA strand inhibition assays. Soluble extracts from the plants of the Lamiaceae and Hypericaceae families were dissolved in PBS at three different concentrations. Precisely, the effectiveness of natural extracts against DNA damage was tested at the IC_{50} values, which were determined in Section 3.2, at higher and lower concentrations of these values, as seen in detail in Table 4. All results were represented in Figures 5–8. Specifically, the protection effect of the natural extract of *Origanum majorana* L. (135 $\mu g \cdot mL^{-1}$) on oxidative damage was determined and found to be the most effective herb with 98.05% inhibition of DNA scission, followed by the natural extract *of Hypericum perforatum* L. (135 $\mu g \cdot mL^{-1}$) with 95.95% inhibition. Soluble extracts of oregano (135 $\mu g \cdot mL^{-1}$ of *Origanum vulgare* L.) and mint (135 $\mu g \cdot mL^{-1}$ of *Mentha spicata* L.) also exhibited a high level of protection against DNA damage (94.60% and 87.20%, respectively). In addition, the extract of dittany (*Origanum dictamnus* L.) (135 $\mu g \cdot mL^{-1}$) showed a high activity with 84.91% inhibition. However, the soluble extract of lavender (*Lavandula angustifolia* L.) at the concentration of 135 $\mu g \cdot mL^{-1}$, showed no inhibition similar to the blank. This concentration was proven to be insufficient to destroy

the open circular DNA (Figure 7). In order to evaluate the antioxidant efficiency of lavender (*Lavandula angustifolia* L.), we used all the extracts with the solvent in two concentrations (5000 and 3200 $\mu g \cdot mL^{-1}$). This indicates that lavender (*Lavandula angustifolia* L.) was not an effective antioxidant, as was demonstrated in Section 3.2 of this research.

Table 4. Percentage of supercoiled DNA retained by extracts of Greek medicinal plants at three different concentrations in studies of DNA strand scission caused by peroxyl radical.

Extract	% Inhibition
Origanum vulgare L. (135 $\mu g \cdot mL^{-1}$)	94.60 ± 1.51
Origanum vulgare L. (IC_{50} = 12.10 $\mu g \cdot mL^{-1}$)	89.42 ± 0.85
Origanum vulgare L. (2 $\mu g \cdot mL^{-1}$)	57.40 ± 1.23
Origanum dictamnus L. (135 $\mu g \cdot mL^{-1}$)	84.91 ± 1.82
Origanum dictamnus L. (IC_{50} = 37.50 $\mu g \cdot mL^{-1}$)	77.83 ± 1.97
Origanum dictamnus L. (2 $\mu g \cdot mL^{-1}$)	40.16 ± 0.91
Hypericum perforatum L. (135 $\mu g \cdot mL^{-1}$)	95.95 ± 0.74
Hypericum perforatum L. (IC_{50} = 11.00 $\mu g \cdot mL^{-1}$)	88.83 ± 1.66
Hypericum perforatum L. (2 $\mu g \cdot mL^{-1}$)	81.80 ± 1.14
Mentha spicata L. (135 $\mu g \cdot mL^{-1}$)	87.20 ± 0.35
Mentha spicata L. (IC_{50} = 16.93 $\mu g \cdot mL^{-1}$)	81.15 ± 1.21
Mentha spicata L. (2 $\mu g \cdot mL^{-1}$)	80.73 ± 0.83
Origanum majorana L. (135 $\mu g \cdot mL^{-1}$)	98.05 ± 0.75
Origanum majorana L. (IC_{50} = 10.31 $\mu g \cdot mL^{-1}$)	94.42 ± 1.52
Origanum majorana L. (2 $\mu g \cdot mL^{-1}$)	82.98 ± 1.33
Lavandula angustifolia L. (135 $\mu g \cdot mL^{-1}$)	-
Lavandula angustifolia L. (IC_{50} = 3200 $\mu g \cdot mL^{-1}$)	47.08 ± 0.93
Lavandula angustifolia L. (5000 $\mu g \cdot mL^{-1}$)	61.00 ± 1.42

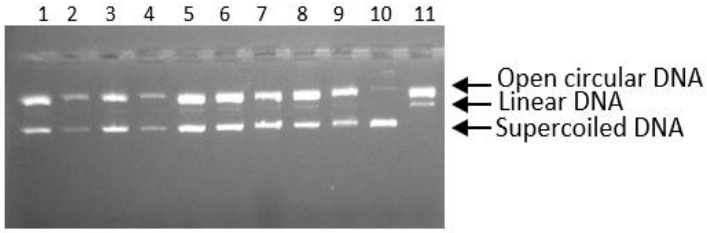

Figure 5. Effect of adding three different natural extracts, at various concentrations, to peroxyl-radical-treated DNA. Lane 1: DNA + 30 mM AAHP + 37.50 $\mu g \cdot mL^{-1}$ (IC_{50}) of *Origanum dictamnus* L., Lane 2: DNA + 30 mM AAHP + 2 $\mu g \cdot mL^{-1}$ of *Origanum dictamnus* L., Lane 3: DNA + 30 mM AAHP + 135 $\mu g \cdot mL^{-1}$ of *Origanum dictamnus* L., Lane 4: DNA + 30 mM AAHP + 2 $\mu g \cdot mL^{-1}$ of *Origanum vulgare* L., Lane 5: DNA + 30 mM AAHP + 12.10 $\mu g \cdot mL^{-1}$ (IC_{50}) of *Origanum vulgare* L., Lane 6: DNA + 30 mM AAHP + 135 $\mu g \cdot mL^{-1}$ of *Origanum vulgare* L., Lane 7: DNA + 30 mM AAHP + 11 $\mu g \cdot mL^{-1}$ (IC_{50}) of *Hypericum perforatum* L., Lane 8: DNA + 30 mM AAHP + 135 $\mu g \cdot mL^{-1}$ of *Hypericum perforatum* L., Lane 9: DNA + 30 mM AAHP + 2 $\mu g \cdot mL^{-1}$ of *Hypericum perforatum* L., Lane 10: control (DNA only), Lane 11: blank (DNA + 30 mM AAHP).

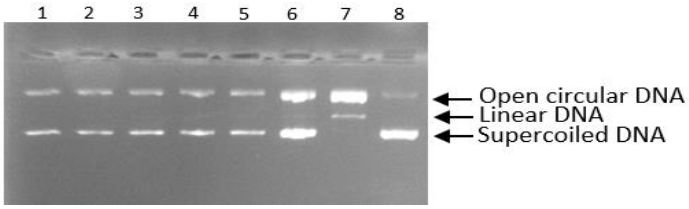

Figure 6. Effect of two natural extracts at various concentrations in preventing peroxyl-radical-induced DNA scission. Lane 1: DNA + 30 mM AAHP + 2 μg·mL^{-1} of *Mentha spicata* L., Lane 2: DNA + 30 mM AAHP + 16.93 μg·mL^{-1} (IC_{50}) of *Mentha spicata* L., Lane 3: DNA + 30 mM AAHP + 135 μg·mL^{-1} of *Mentha spicata* L., Lane 4: DNA + 30 mM AAHP + 10.31 μg·mL^{-1} (IC_{50}) of *Origanum majorana* L., Lane 5: DNA + 30 mM AAHP + 2 μg·mL^{-1} of *Origanum majorana* L., Lane 6: DNA + 30 mM AAHP + 135 μg·mL^{-1} of *Origanum majorana* L., Lane 7: blank (DNA + 30 mM AAHP), Lane 8: control (DNA only).

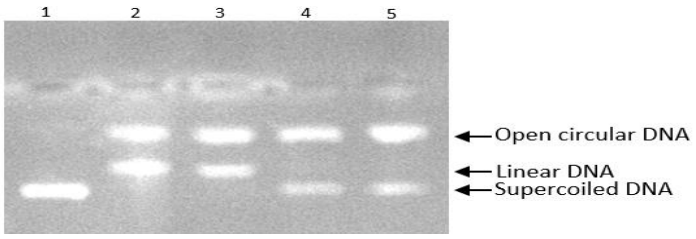

Figure 7. Protective effect against pBR322 plasmid DNA damage by extract of *L. angustifolia* in different concentrations. Lane 1: control (DNA only), Lane 2: blank (DNA + 30 mM AAHP), Lane 3: DNA + 30 mM AAHP + 135 μg·mL^{-1} of *Lavandula angustifolia* L., Lane 4: DNA + 30 mM AAHP + 5000 μg·mL^{-1} of *Lavandula angustifolia* L., Lane 5: DNA + 30 mM AAHP + 3200 μg·mL^{-1} (IC_{50}) of *Lavandula angustifolia* L.

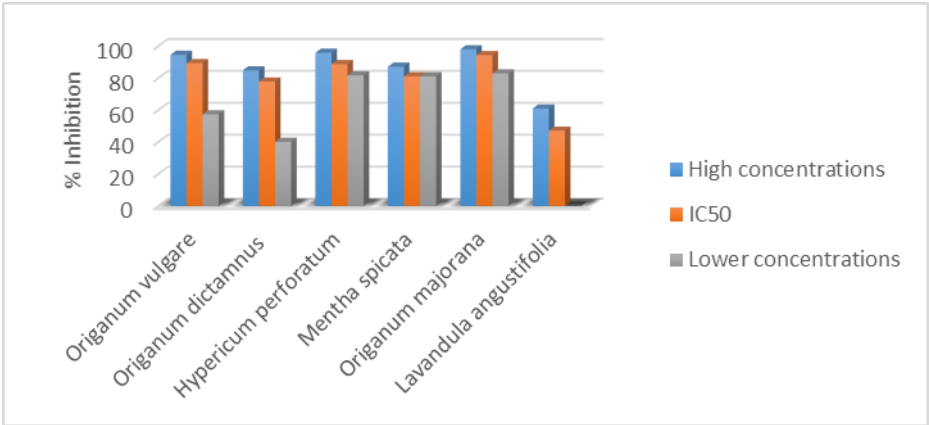

Figure 8. Correlation of the protective activity of the six phenolic extracts in different concentrations against the scission of the DNA strands.

As expected, the protection against the nicking of supercoiled DNA increased along with the concentration of antioxidant extracts. Supercoiled DNA strands were protected in the presence of the majority of plant extracts. This is probably explained by the fact that the studied samples contain a large amount of polyphenols, as demonstrated by the use of the Folin–Ciocalteu method. It has been established that the hydroxyl groups of phenolic bioactive compounds included in the natural extracts could donate electrons or hydrogen [74]. Thus, polyphenols can be characterized as chain-breaking antioxidants and contribute to the avoidance of DNA damage [77]. All the results are visible in the agarose gel electrophoresis (Figures 5–7).

Extracts of *Hypericum perforatum* L., *Mentha spicata* L. and *Origanum majorana* L. at the three different concentrations showed no significant difference as inhibitors of DNA

strand breakage induced by AAHP (Table 4, Figure 8). The findings of this research proved that five of the examined extracts are considered effective against pBR322 plasmid DNA damage, while the extract of *Lavandula angustifolia* L. from the Lamiaceae family showed a lower value of the % inhibition of DNA scission. According to previous studies, phenolic extracts are determined to have antimutagenic properties as they can contribute to protection against oxidative DNA damage [46,78–80].

3.5. In Vitro Evaluation of Antimicrobial Activity

The antimicrobial assay used in this study measured the Minimum Inhibitory Concentration (MIC) of the antimicrobial substances on selective nutrient substrates. The lowest concentration that can prevent any discernible bacterial growth on culture plates under suitable incubation conditions is known as the MIC [81]. The estimation of the MIC values is an extremely significant factor in laboratories as it can be used to treat various bacterial infections [82]. Aqueous extracts of the six medicinal herbs were evaluated for their antimicrobial properties against six pathogenic microorganisms, three Gram-positive bacteria, such as *Staphylococcus aureus*, *Enterococcus faecalis* and *Listeria monocytogenes*, and three Gram-negative bacteria, such as *Salmonella enterica*, *Escherichia coli* and *Klebsiella pneumoniae*, using the dilution assay (Table 5). The MIC values for bacteria were found to be between 40 and 135 $\mu g \cdot mL^{-1}$ for *Origanum dictamnus* L. extracts and 135–500 $\mu g \cdot mL^{-1}$ for *Origanum vulgare* L. extracts. Meanwhile, *Origanum dictamnus* L. extracts did not appear to have any antimicrobial activity against *Listeria monocytogenes*, *Escherichia coli* and *Klebsiella pneumoniae*. Furthermore, the extracts of *Origanum majorana* L. exhibited a strong antibacterial activity against all the examined pathogenic bacteria with MIC values ranging from 1 $\mu g \cdot mL^{-1}$ to 135 $\mu g \cdot mL^{-1}$. *Mentha spicata* L. extracts showed a good antimicrobial effect with MIC values ranging from 5 to 500 $\mu g \cdot mL^{-1}$, and the MIC values of *Hypericum perforatum* L. extracts were between 34 $\mu g \cdot mL^{-1}$ and 650 $\mu g \cdot mL^{-1}$. The majority of the assayed plant extracts were considered to be effective antimicrobial agents. Their strong antibacterial activity is attributed to the high concentrations of the phenolic compounds and their synergistic action. Specifically, the most abundant phytochemical substances in the plant extracts were rutin, rosmarinic acid, benzoic acid and caffeic acid. These phenolic acids and flavonoids have been promised potential antioxidant activity and were found to be great bacteriostatic agents [83]. The extract of *Origanum majorana* L. showed the strongest antimicrobial activity against *Staphylococcus aureus*, with an MIC value of 1 $\mu g \cdot mL^{-1}$, and the extract of *Hypericum perforatum* L. showed the weakest antibacterial action against *Klebsiella pneumoniae*, while the *Lavandula angustifolia* L. extract appeared to show no inhibition against the pathogenic microorganisms. The data showed that the studied plant extracts had various mechanisms of action and demonstrated better inhibitory activity against Gram-positive bacteria. This means that Gram-positive bacteria are more susceptible, in contrast to Gram-negative bacteria. According to the literature, this can be explained by studying the membrane potential disruption of microorganisms and the modifications in the cytoplasmic pH [84].

The antibacterial effects of the tested extracts were in accordance with other studies when comparing their MIC values [22,85,86]. However, the interest of the previous research studies has been mainly dedicated to the antimicrobial activity of essential oils of medicinal plants rather than extracts. The essential oils include very high concentrations of substances, such as carvacrol, thymol and hypericin (*Hypericum perforatum* L.), which are deemed to have hazardous and toxic effects on human health and food quality [87,88]. In conclusion, the experimental studies of plant extracts could highlight their commercial uses and their function as antioxidant, flavoring and antibacterial agents and nutrient enhancers.

Table 5. Antimicrobial activity of natural extracts (MIC $\mu g \cdot mL^{-1}$).

	MIC (Minimum Inhibitory Concentration) $\mu g \cdot mL^{-1}$					
Aqueous Natural Extracts	***Staphylococcus aureus* ATCC 25923**	***Enterococcus faecalis* ATCC 29212**	***Listeria monocytogenes* ATCC 35152**	***Salmonella enterica* ATCC 14028**	***Escherichia coli* ATCC 25922**	***Klebsiella pneumoniae* ATCC 13883**
Origanum vulgare L.	135	300	135	300	500	135
Origanum dictamnus L.	135	nd	80	40	nd	nd
Hypericum perforatum L.	34	34	30	400	135	650
Origanum majorana L.	1	30	5	135	67.5	135
Mentha spicata L.	135	500	5	135	135	60
Lavandula angustifolia L.	nd	nd	nd	nd	nd	nd

nd: not detectable.

4. Conclusions

The multifaceted study of natural extracts is a contemporary topic. Medicinal herbs have a wide range of uses in pharmacology and food industries. In this current study, extracts of five species of the Lamiaceae family, *Origanum vulgare* L., *Origanum dictamnus* L., *Origanum majorana* L., *Mentha spicata* L. and *Lavandula angustifolia* L., and one species of the Hypericaceae family, *Hypericum perforatum* L., were examined and evaluated as functional food products that are intended to enhance health and wellbeing or prevent various diseases. These aromatic plants contain certain amounts of polyphenols which are responsible for neutralizing the adverse effects of ROS as they are considered natural antioxidants. The phenolic profile of methanol plant extracts was assessed by RP-HPLC-DAD analysis with the most abundant constituents being rosmarinic acid, benzoic acid, caffeic acid and rutin. Mass spectrometry analysis confirmed the presence of the bioactive compounds with their characteristic m/z ratio. The antioxidant capacity of these extracts was determined by the free-radical scavenging method DPPH. It was proven that all the samples had significant antioxidant activity, except for lavender (*Lavandula angustifolia* L.) which was the less effective antioxidant, and they showed a high phenolic concentration. In addition, the samples showed a great ability in counteracting DNA damage, which is due to the presence of the detected phytochemical substances.

The majority of the studied extracts, as a rich source of phenolic compounds and other secondary metabolites with antimicrobial activity, are considered to be effective antibacterial agents against selected pathogenic microorganisms. Specifically, Gram-positive bacteria were found to be more susceptible to the extracts than Gram-negative bacteria. Thus, the use of antimicrobial medicinal plants is a new approach to combating the growing threat of antimicrobial resistance. Therefore, the quantitative and qualitative determination of new bioactive compounds from medicinal plants is urgently needed.

Our results showed no significant difference regarding the species of the two plant families. According to the various experimental methods in this research, St. John's wort (*Hypericum perforatum* L.) is as effective as species belonging to the Lamiaceae family. The findings of this survey can be used as a benchmark for future research on the same plant species. For example, in future studies, it would be desirable to investigate the exact mechanism of the antimicrobial action of plant extracts against pathogens. Food, pharmaceutical and cosmetic industries can benefit from the above investigations which highlight the advantages of plant extracts for human health.

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editing, A.T., D.H., A.C., A.G.T. and E.T.; visualization, A.T., D.H., A.C., A.G.T. and E.T.; supervision, D.H.; project administration, A.T. and D.H.; funding acquisition, all the authors. All authors have read and agreed to the published version of the manuscript.

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