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Profiling of Essential Oils Components and Polyphenols for Their Antioxidant Activity of Medicinal and Aromatic Plants Grown in Different Environmental Conditions

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Abstract: In the present study, the yield, the chemical composition, and the antioxidant activities of the essential oils (EOs) of eight medicinal and aromatic plants (MAPs) cultivated under two environmental conditions characterized by a different altitude (namely mountainous and plain) were evaluated. Cultivation at different environmental conditions resulted in significant differences in the chemical composition and antioxidant activity for most of the studied species. In particular, high altitudes resulted in increased phenolic compounds' content and antioxidant activity for artemisia plants, while specific parameters increased in the case of spearmint (total phenols) and rosemary (flavonoids). In contrast, in pelargonium, all the tested parameters were positively affected in the plain area, whereas, for laurel and sage, only flavanols remained unaffected. EO yield in mountainous pelargonium and spearmint decreased while, in mountainous laurel, pelargonium and spearmint increased when compared to plain areas. In addition, the major EO constituents' content for most of the species were affected by environmental conditions. The 2,2-diphenyl-1-picryl-hydrazyl-hydrate (DPPH) and ferric reducing antioxidant power (FRAP) were variably correlated with total phenols, flavonoids, and flavanols, depending on the species and the altitude. Lastly, in limited cases, antioxidant activity (DPPH or FRAP values) was positively correlated with some EO components (e.g., borneol and β-pinene in artemisia and laurel plants grown in the plain, respectively, or 1,8-cineole in mountainous grown verbena plants). In conclusion, environmental conditions (altitude) affected antioxidants' content and EO yield and composition of the studied MAPs. These findings can be used to introduce cultivation of MAPs in specific ecosystems for the production of high added value products.

Keywords: antioxidant status; DPPH; flavanols; flavonoids; FRAP; altitude; total phenols; volatile compounds

1. Introduction

Medicinal and aromatic plants (MAPs), also known as herbs or spices, and their relevant plant extracts and essential oils (EOs) have been highly appreciated and widely used for centuries despite the lack of scientific evidence for their actual bioactive mechanisms and functions, which are still under investigation [1–3]. Contemporary dietary patterns also prescribe such MAPs as functional foods, i.e., foods that offer additional physiological benefits beyond the usual nutritional requirements such as preventing or delaying the onset of chronic diseases [4]. The global interest in MAPS is reflected in

the trade of MAPs as raw material, which is approximately 440,000 tons per year at a total value of $1.3 billion USD. A total of 25% of this monetary value is marketed in Europe [5].

Food products rich in antioxidants are well appreciated since they can act as scavengers of reactive oxygen species (ROS) and also help decrease the impact of age-related chronic diseases [6]. Therefore, MAPs have been the focus of scientific research and the food and pharmaceutical industry due to their well acknowledged antioxidant capacity [3,7]. A diverse range of secondary metabolites such as phenolic compounds are biosynthesized by plants as part of their protection mechanism toward oxidative damage by ROS and abiotic and biotic stressors, while these compounds may also have protective effects on humans when MAP and/or their components are ingested through diet [8]. The antioxidant capacity of phenolic compounds involves a combination of different mechanisms including free radical scavenging, donation of hydrogen atoms, single oxygen quenching, metal ion chelation, and activities as an oxidation substrate [8].

The Mediterranean basin is abundant in MAPs with more than 10,000 species being identified so far, which have been widely used in the Mediterranean diet [9–11]. The important bioactive properties of these species have been systematically reported in ethnobotanical and ethnopharmacological studies [12–17]. However, further investigation is needed to reveal and define their precise pharmaceutical and functional properties as food additives and novel antioxidants [18]. Biological activity and phytochemicals of MAPs show a great variability depending on the cultivation area, the climatic conditions, and the genetic material [19–21]. According to Kofidis and Bosabalidis [22], altitude was suggested to be one of the most important ecological factors affecting bioactivities of MAPs as specific environmental factors such as light and wind intensity, mean temperature, ozone levels, and partial CO_2 pressure, which may vary between different altitudes. Moreover, MAPs bioactive properties are often associated with the presence of secondary metabolites with antioxidant potential such as phenolic compounds [23,24]. However, special attention should be given prior to recommending the use of MAPs in human diet, since, in several occasions, the intake of high doses of secondary metabolites and potentially harmful substances (e.g., heavy metals and anti-nutritional factors) may cause severe toxicity and adverse health effects [25–28]. Therefore, further research is needed to evaluate possible toxic effects and establish recommended daily allowance (RDA) levels, especially for people with medical conditions [29–31].

Apart from the use of MAPs as herbs and decoctions, their EOs have also found very important uses in the food and pharmaceutical industry [32]. Several studies with EOs revealed significant antioxidant [33,34] and antimicrobial properties [35–38] and further increased the interest to use EOs as natural antioxidants and antimicrobial agents instead of synthetic compounds, which are currently receiving criticism due to harmful effects on human health [36,38,39]. However, despite the increased interest and the great number of MAPs throughout the world, only approximately 10% of the already known EOs have received attention due to their varied biological activities [2,3]. Currently, they are widely used in the food, cosmetics, and pharmaceutical industry [40,41].

MAPs cultivation in Cyprus shows promising prospects as crops have low requirements in agrochemicals, irrigation water, man power, and energy [42]. They also exhibit tolerance to arduous climatic conditions such as high temperatures, winds, and drought [43,44]. All these important features could help the sustainable development of rural areas and also reduce the threats arising from wild harvesting of MAPs [45]. Although the island's soil and climatic conditions are ideal for MAPs growth, their cultivation is not yet widespread because of limited availability of agricultural land due to other uses, i.e., tourism and constructions. Based on the above, it is recommended to evaluate potential areas and/or cultivation practices that may provide high quality and added value products of MAPs [46], so that farmers could shift to these crops and establish economically viable farms. Due to the increased global demands of high value MAPs, Cyprus with a long history on MAPs' cultivation and uses could become a significant spot for producing and exporting high quality raw materials of MAPs to other countries that are more industrially developed for further processing.

So far, several studies examined the correlation of total phenolics and/or phenolic compounds' content with the antioxidant activity of various MAPs products such as infusions, decoctions, and EOs [24,47–50]. For example, the antioxidant activity and phenolics compounds in 10 selected MAPs from Serbia, revealed a positive correlation of phenolics and tannins, but also a proportional increase of antioxidants with total phenolics increase [50]. However, the correlations of the main compounds of EOs with the phenolic compounds content and the antioxidant activity of leaves are scarcely explored. Therefore, in an attempt to contribute to the existing knowledge, the aim of this work was to compare medicinal and aromatic plants grown in Cyprus at different environmental conditions (altitudes: mountainous and plain areas) in view of revealing possible correlations between their leaf antioxidant activity and their essential oil yield and composition. The selection of the studied plant species was based on their popularity and their recommendation for use.

2. Materials and Methods

2.1. Plant Material and Growing Conditions

The studied medicinal and aromatic plants were as follows: artemisia (*Artemisia abrotanum* L.), pelargonium (*Pelargonium roseum* L.), laurel (*Laurus nobilis* L.), rosemary (*Rosmarinus officinalis* L.), spearmint (*Mentha spicata* L.), lavender (*Lavandula angustifolia* L.), lemon verbena (*Aloysia triphylla* L.), sage (*Salvia officinalis* L.), and their parts are presented in Table 1. The plants were identified by staff members of the Cypriot National Agricultural Department.

Table 1. Plant species and material used.

Common Name	Latin Name	Family	Plant Material	Reported Medicinal Properties/Indications
Artemisia	*Artemisia abrotanum* L	Asteraceae	Leaves	Antifungal, anticancer, antiviral antibacterial, antioxidant, anaemia, amenorrhoea, anorexia, chronic fever, hepatitis, splenitis, hysteria [51].
Pelargonium	*Pelargonium roseum* L.	Geraniaceae	Leaves	Antibacterial, antifungal, antioxidant, antitumor, nematocidal, intestinal problems, wounds and respiratory ailments, help hormonal balance, discharge toxins from liver, digestive [52].
Laurel	*Laurus nobilis* L.	Lauraceae	Leaves	Antibacterial, antifungal, cytotoxicity, antioxidant, diuretic, gastrointestinal problems, to treat epilepsy, neuralgia, and parkinsonism [53].
Rosemary	*Rosmarinus officinalis* L.	Lamiaceae	Stem/ leaves	Antibacterial, antihepatotoxic, anti-tumour, anti-inflammatory, anti-trypanosomal, antispasmodic, immune stimulant activity, rheumatic complaints and circulatory disorders, tiredness, defective memory, carminative, rubefacient, promote digestion [54].
Spearmint	*Mentha spicata* L.	Lamiaceae	Stem/ leaves	Anti-inflammatory, sedative, antimicrobial, antioxidant, carminative, antispasmodic, diuretic, insecticidal [55].
Lavender	*Lavandula angustifolia* L.	Lamiaceae	Stem/ leaves	Antibacterial, insecticidal, sedative, analgesic, cytotoxic, anxiolytic, alleviate depression, headaches, and anxiety [56].
Lemon verbena	*Aloysia triphylla* L.	Verbenaceae	Stem/ leaves	Antibacterial, antifungal, antioxidant, treatment of colic, diarrhea, indigestion, insomnia, anxiety, asthma, fever [57].
Sage	*Salvia officinalis* L.	Lamiaceae	Stem/ leaves	Antibacterial, antifungal, anticancer, antiviral, antidiabetic, antimutagenic, antiprotozoal, antidementia, antioxidant, anti-inflammatory [58].

Jacovides, C.P.; Timvios, F.S.; Papaioannou, G.; Asimakopoulos, D.N.; Theofilou, C.M. Ratio of PAR to broadband solar radiation measured in Cyprus. *Agric. For. Meteorol.* **2004**, *121*, 135–140.

In the current study, two areas with different environmental conditions (for simplicity, the term altitude will be used, considering the differences in the microclimates as described by Kofidis and Bosabalidis [22]) were selected, the mountainous area of "Agros" village (34°53′44.10′′ N; 33°01′13.86′′ E) and the plain area of Nicosia ("Athalassa"; 35°08′07.65′′ N; 33°24′07.22′′ E). The village area of "Agros" is located at 880 m above sea level. The climate is dry with low temperatures and snow precipitation during winter. The soil has sand-silk texture. On the other hand, the plain area of "Athalassa" is located 141 m above sea level with mild winter and a dry-hot summer. Detailed climatic conditions of the selected areas are described in supplementary material (Table S1).

Plant species from the plain area harvested from the farm of the Cypriot National Agricultural Department were 2–5 years old (except for laurel where plants were approximately 15 years old). Common cultivation practices were applied and plants were frequently irrigated (~weekly/biweekly during the growing period) and common fertilizers were applied (20-10-10 (N-P-K) once a year in base dressing and 19-19-19 (N-P-K) every second month in side dressing. Mountain species harvested from public green areas/parks of "Agros" village were of a different age and were grown under non-commercial cultivation practices, which means they received conservation practices (periodical irrigation and fertilizer application). Plants for public green area use and landscaping normally originated from the Cypriot National Agricultural Department or from nurseries that collaborate with this department. Plant tissues (six samples/area/species) of the above ground parts (leaves or leaves and stems) (see Table 1) were collected early to mid-October and transferred within an hour to the laboratory. Each sample was divided into two batch samples. One batch was air-dried at room temperature for approximately 7 days and used for the essential oil extraction (see Section 2.4) while the other batch was stored at −20 °C for the chemical analyses described in Sections 2.2 and 2.3.

2.2. Polyphenol Extraction and Analyses

2.2.1. Extract Preparation

Six samples (0.5 g) of freshly cut plants (pooled by two individual plants/sample) from each treatment were milled with 10 mL methanol (80%) [59]. The extracts were centrifuged for 30 min at 4000× g at 4 °C (Sigma 3-18K, Sigma Laboratory Centrifuge, Germany). After centrifugation, the supernatant was transferred to a 15-mL falcon tube, and stored at 4 °C until further analyses (within 24 h) for evaluating total phenolics, flavonoids, and flavanols content and total antioxidant activity.

2.2.2. Total Phenolics

The total phenolic compounds content of the methanolic extracts was determined by using the Folin–Ciocalteu reagent (Merck), according to the procedure described by Tzortzakis et al. [59]. A total of 125 µL of plant extracts were mixed with 125 µL of the Folin–Ciocalteu reagent. The mixture was shaken before the addition of 1.25 mL of 7% Na_2CO_3, adjusted with distilled water to a final volume of 3 mL, and mixed thoroughly. After incubation in the dark for 90 min, the absorbance of extracts at 755 nm was measured in comparison to the prepared blank. Total phenolic compounds content was expressed as µmol of gallic acid equivalents per gram of fresh weight (µmol GAE g^{-1} fw) through a calibration curve prepared with gallic acid. All samples were analysed in triplicate.

2.2.3. Total Flavonoids and Flavanols

The total flavonoid content was determined according to the aluminium chloride colorimetric method [60]. Plant extracts and 0.75 mL of 5% sodium nitrite ($NaNO_2$) were incubated for 6 min. After the incubation, 0.15 mL of $AlCl_3$ solution (10%) was added. After an additional time of 5 min, 0.5 mL of NaOH (1 M) solution was added and the final volume was adjusted to 2.5 mL with the addition of distilled water. The solution was mixed thoroughly, and the absorbance was measured at 510 nm. The total flavonoids content was expressed as rutin equivalents (mg rutin g^{-1} fw).

Total flavanols content was determined according to Tabart et al. [61]. In more details, 1 mL of catechin solution (0–300 μg mL^{-1} in methanol) or test solution (150–250 μg mL^{-1} polyphenols in methanol) were added in test tubes. Then, 2.5 mL of methanol (control) or 1% vanillin solution in methanol and 2.5 mL of 9 M HCl in methanol (test samples) were added. The reaction mixture was incubated for 20 min at 30 °C and the absorbance at 500 nm was measured. The catechin solution was used for preparing the calibration curve. Results were expressed as catechin equivalents (mg catechin g^{-1} fw).

2.3. Antioxidant and Reducing Activity

The antioxidant and reducing activity of plant extracts was evaluated using the 2,2-diphenyl-1-picryl-hydrazyl-hydrate (DPPH) and ferric reducing antioxidant power (FRAP) assays according to Wojdyło et al. [62] with some modifications [63]. The DPPH radical scavenging activity of plant extracts was measured from the bleaching of the purple-colored 0.3 mM solution of DPPH, which consisted of 1 mL of the DPPH solution, 1.98 mL of 50% methanol, and 0.02 mL of plant extract. After shaking, the mixture was incubated at room temperature in the dark for 30 min, and then the absorbance was measured at 517 nm. DPPH radical-scavenging activity was expressed as the inhibition percentage (I %) and was calculated using the following formula.

$$\text{DPPH radical scavenging activity I (\%)} = [100 - 100 \times (\text{Abs} - \text{Abb})/\text{Abc}] \quad (1)$$

where Abb is the absorbance of the blank sample, Abs is the absorbance of the test sample, and Abc is the absorbance of the control with DPPH and 50% methanol.

For the ferric reducing/antioxidant power (FRAP) assay, a sample of 3 mL of freshly prepared FRAP solution (0.3 mol L^{-1} acetate buffer, pH 3.6), containing 10 mmol L^{-1} TPTZ (Tripyridil-s-triazine) and 40 mmol L^{-1} $FeCl_3 \cdot 10H_2O$ and 20 μL of extract (50 mg mL^{-1}) were incubated at 37 °C for 4 min and the absorbance was measured at 593 nm. The changes in absorbance were then converted into a FRAP value by relating the change of absorbance at 593 nm of the test sample to that of the standard solution of trolox ((±)-6-Hydroxy-2,5,7,8-tetramethylchromane-2-carboxylic acid). The results were expressed as mg trolox g^{-1} fw.

2.4. Essential Oil Extraction and Gas Chromatography/Mass Spectrometry Analysis

Extracting essential oils was carried out according to the protocol previously described by the authors [64]. Aerial parts were collected and air-dried at room temperature. The dried tissues (40–50 g for each treatment) were used for the essential oil extraction using a Clevenger apparatus. Each extraction lasted for 3 h, while each treatment was replicated three times. The essential oil (dried over anhydrous sodium sulphate) yield was measured and calculated as percentage of oil per dry weight (dw) [63]. The obtained essential oils were kept in amber glass bottles at −20 °C until GC/MS analysis was performed.

Analytical gas chromatography was carried out with a Shimadzu GC2010 gas chromatograph interfaced Shimadzu GC/MS QP2010 plus mass spectrometer based on the protocol previously described by the authors [63]. An aliquot of 2 μL of each sample was injected in a split mode (split ratio 20:1) into the gas chromatograph fitted with a ZB-5 column (Zebron, Phenomenex, Torrance, CA, USA) coated with 5% pheny to 95% dimethylpolysiloxane with film thickness of 0.25 μm, length of 30.0 m, and a diameter of 0.25 mm. The flow of the carrier gas (helium) was 1.03 mL min^{-1}. The injector temperature was set to 230 °C. Electron impact mass spectra with ionization energy of 70 eV was recorded at the 35–400 m z^{-1}. The column temperature was programmed to rise from 60 °C to 240 °C at a rate of 5 °C min^{-1} with a 5 min hold at 240 °C. The solution of standard alkanes mixture (C8–C20) was also analyzed using the above conditions.

Components were identified by comparing their retention indices (RI) relative to n-alkanes (C8–C20) with those of the literature or with those of authentic compounds when available. Further

identification of compounds was carried out by matching the recorded mass spectra with those stored in the NIST08 mass spectral library of the GC–MS data system and published mass spectra in the literature [63]. The percentage of individual compounds was based on peak area normalization without using correction factors.

2.5. Statistical Methods

A two factor (Species and Environmental Conditions, namely Altitude) factorial experiment was carried out. The statistical treatment of the results was carried out using a two-way analysis of variance (ANOVA) by using the IBM SPSS v.22 software for Windows. The Student's *t*-test ($p < 0.05$) was used for the comparison of means when the effect of altitude was significant, while the Duncan Multiple Range Test was used for comparing means in the cases where the effect of species and the interaction of species × altitude were significant. Mean values are presented as treatment mean ± SE of six biological measurements (n = 6) for antioxidants and for three biological measurements (n = 3) for essential oils analysis. The correlation coefficients between mountainous and plain species and their antioxidant capacity and essential oil components were also determined.

3. Results and Discussion

3.1. Total Phenols, Flavonoids, Flavanols, and Antioxidant and Reducing Activity

Phenolic compounds are one of the most important classes of natural antioxidants and are closely related with the antioxidant activity of plant tissues [49,65]. In this study, we tried to determine whether the content of phenolic compounds (total phenolics, flavonoids, and flavanols) and the antioxidant activity of eight MAP species were affected by the altitude as previously reported [48,66]. Table 2 presents the effects of environmental conditions-altitude (mountain versus plain) and species on the phenolic compounds content as well as on the antioxidant activity of the examined MAP species. The two-way ANOVA revealed a significant ($p < 0.001$) interaction between the tested factors (species and altitude) for all the tested parameters. Moreover, the species factor significantly ($p < 0.001$) affected all the tested parameters ($p < 0.01$), whereas altitude only affected phenolics ($p < 0.01$), DPPH, flavanols ($p < 0.001$), and EO yield ($p < 0.01$).

In general, altitude affected antioxidant capacity of the examined species, as plain plants presented higher flavanols and DPPH (7.16 ± 2.29 mg catechin g^{-1} fw and 25.02 ± 3.72 mg trolox g^{-1} fw, respectively) than mountainous grown plants (Table 2). When comparing all the species regardless of altitude, total phenol levels were higher in laurel and pelargonium (113.02 ± 5.31 and 112.03 ± 17.69 μmol GAE g^{-1} fw, respectively), whereas lemon verbena exhibited the lowest content of total phenols. The highest levels of flavonoids were found in lavender (17.31 ± 1.03 mg rutin g^{-1} fw) while pelargonium, rosemary, lemon verbena, and sage revealed the lowest levels of flavonoids. Moreover, pelargonium revealed the highest levels of flavanols (29.14 ± 5.78 mg catechin g^{-1} fw), which was followed by rosemary (8.48 ± 0.74 mg catechin g^{-1} fw), whereas significantly lower values were found in most of the examined species. Similarly, pelargonium was the species that revealed the higher antioxidant and reducing activity for both DPPH and FRAP assays (47.70 ± 10.41 and 25.28 ± 1.91 mg trolox g^{-1} fw, respectively).

Table 2. Effects of altitude (mountain vs. plain) on the content of total phenols (μmol GAE g^{-1} fw), total flavonoids (mg rutin g^{-1} fw), total flavanols (mg catechin g^{-1} fw), antioxidant and reducing activity (DPPH, FRAP, mg trolox g^{-1} fw), and essential oil (EO) yield (%) in selected medicinal plant species.

Species	Altitude	Total Phenols	Flavonoids	Flavanols	DPPH	FRAP	EO
	Plain	78.42 ± 6.62A	7.01 ± 0.78A	7.16 ± 2.29A	25.02 ± 3.72A	12.31 ± 1.24A	1.38 ± 0.04A
	Mountain	68.68 ± 4.23A	7.90 ± 1.14A	2.49 ± 0.63B	13.53 ± 0.63B	11.53 ± 0.84A	1.17 ± 0.04B
	Total mean	73.55 ± 3.94	7.46 ± 0.69	4.82 ± 1.20	19.27 ± 1.97	11.92 ± 0.75	1.28 ± 0.03
Artemisia		73.16 ± 12.81BC	13.52 ± 2.71B	0.44 ± 0.13C	12.64 ± 1.63C	9.57 ± 1.48C	0.90 ± 0.08CD
Pelargonium		112.03 ± 17.69A	3.65 ± 0.73D	29.14 ± 5.78A	47.70 ± 10.41A	25.28 ± 1.91A	0.42 ± 0.10D
Laurel		113.02 ± 5.31A	8.15 ± 1.25C	8.48 ± 0.74B	32.51 ± 5.53B	15.59 ± 1.22B	2.68 ± 0.33A
Rosemary		83.89 ± 3.84B	4.66 ± 0.99CD	0.28 ± 0.16C	15.34 ± 1.38C	12.95 ± 0.87B	1.03 ± 0.05C
Spearmint		52.28 ± 4.50CD	8.18 ± 1.13C	0.00 ± 0.00C	11.27 ± 1.02C	6.84 ± 1.22CD	1.89 ± 0.09B
Lavender		61.06 ± 2.77BC	17.31 ± 1.03A	0.02 ± 0.00C	16.06 ± 0.69C	13.24 ± 0.80B	0.63 ± 0.02CD
Lemon verbena		33.47 ± 1.37D	2.24 ± 0.27D	0.22 ± 0.05C	6.13 ± 0.32C	3.66 ± 0.23D	0.76 ± 0.11CD
Sage		59.51 ± 4.37BC	2.06 ± 0.68D	0.04 ± 0.01C	12.54 ± 1.26C	8.23 ± 0.89C	1.90 ± 0.31B
Total mean		73.55 ± 3.94	7.46 ± 0.69	4.82 ± 1.20	19.27 ± 1.97	11.92 ± 0.75	1.28 ± 0.12
Artemisia	Plain	40.6 ± 2.4ijk[Y]	4.7 ± 0.8efghi	0.06 ± 0.02d	7.5 ± 1.0fg	4.8 ± 0.6fg	0.58 ± 0.02g
	Mountain	105.7 ± 17.1c	22.3 ± 1.0a	0.8 ± 0.1d	17.8 ± 0.3c	14.3 ± 0.5c	0.68 ± 0.01efg
Pelargonium	Plain	166.9 ± 12.0a	5.9 ± 0.6defg	47.1 ± 3.8a	80.9 ± 6.0a	29.9 ± 2.1a	0.65 ± 0.07fg
	Mountain	57.1 ± 5.2ghij	1.4 ± 0.2ij	11.1 ± 1.7b	14.5 ± 1.0cde	20.6 ± 1.7b	0.19 ± 0.04e
Laurel	Plain	126.0 ± 6.6b	11.3 ± 1.5c	9.3 ± 1.2bc	49.6 ± 4.1b	18.9 ± 1.0b	2.05 ± 0.39c
	Mountain	100.0 ± 3.5cd	5.0 ± 0.8efgh	7.7 ± 0.8c	15.4 ± 0.8cde	12.3 ± 1.1cd	3.30 ± 0.11a
Rosemary	Plain	87.0 ± 4.7cde	2.7 ± 1.2ghij	0.56 ± 0.02d	14.4 ± 2.7cde	12.8 ± 1.5cd	1.08 ± 0.07de
	Mountain	80.8 ± 6.2def	6.6 ± 1.2def	nd	16.3 ± 1.0cd	13.1 ± 1.0cd	0.99 ± 0.08def
Spearmint	Plain	41.9 ± 3.5ijk	7.4 ± 1.8de	nd	9.0 ± 0.9efg	5.2 ± 0.7fg	2.61 ± 0.04b
	Mountain	62.7 ± 5.8fgh	8.8 ± 1.5cd	nd	13.5 ± 1.3cdef	8.5 ± 2.2ef	1.20 ± 0.06d
Lavender	Plain	58.1 ± 3.3ghi	17.0 ±1.0b	0.90 ± 0.05d	16.4 ± 0.8cd	12.6 ± 0.9cd	0.99 ± 0.13def
	Mountain	64.0 ± 4.4fgh	17.2 ± 1.9b	0.91 ± 0.05d	15.7 ± 1.2cde	13.9 ± 1.4cd	0.54 ± 0.01ge
Lemon verbena	Plain	36.6 ± 1.8jk	2.9 ± 1.6ghij	0.15 ± 0.05d	6.7 ± 0.4g	4.1 ± 0.3g	1.06 ± 0.07de
	Mountain	30.3 ± 1.1k	1.6 ± 0.3hij	0.28 ± 0.09d	5.5 ± 0.4g	3.2 ± 0.2g	0.73 ± 0.07efg
Sage	Plain	70.1 ± 4.9efg	3.8 ± 0.8fghi	0.08 ± 0.04d	15.7 ± 1.5cde	10.14 ± 1.05de	2.06 ± 0.02c
	Mountain	48.9 ± 3.9hijk	0.29 ± 0.03j	nd	9.4 ± 0.9defg	6.32 ± 0.97fg	1.72 ± 0.13c
Species	(S)	***	***	***	***	***	***
Altitude	(A)	**	ns	***	***	ns	**
Interaction	S x A	***	***	***	***	***	***

[Y] values (means ± SE, n = 6) in columns corresponding to the main factors (Altitude and Species) followed by the same uppercase letter, and values corresponding to the interaction of the main factors (Altitude and Species), which is followed by the same lowercase letter, are not significantly different, $p \leq 0.05$. nd: not detected. ns, **, and *** indicate non-significant or significant differences at $p < 0.01$, and $p < 0.001$, respectively, following a two-way ANOVA.

On the other hand, when considering the combined effect of the tested factors, pelargonium plants grown in plain areas presented the highest content of total phenols and total flavanols, and the highest antioxidant activity for DPPH and FRAP (166.9 ± 12.0 μmol GAE g^{-1} fw, 47.1 ± 3.8 mg catechin g^{-1} fw, 80.9 ± 6.0 mg trolox g^{-1} fw, and 29.9 ± 2.1 mg trolox g^{-1} fw, respectively). Moreover, the highest levels of flavonoids (22.3 ± 1.0 mg rutin g^{-1} fw) were found in mountainous artemisia plants.

In previous studies, the effects of collection site on total phenolics and antioxidants has also been reported in *Salvia argentea*, *S. officinalis*, and *S. verbenaca* since eco-geographical characteristics may alter the biosynthetic pathways of secondary metabolites [47,67–69]. The effect of altitude on phenolic compounds content and the antioxidant activity has been reported in various plants species, including *Thalictrum foliolosum* DC. (TF) [67], *Potentilla fruticosa* L. [68], and *Sphagnum junghuhnianum* [69] among others, since several environmental factors such as elevated CO_2, water availability, and differences in temperatures' solar radiation may affect secondary metabolism and trigger the biosynthesis of bioactive compounds [70–72]. Antioxidant activity of plants is positively correlated with the levels of total phenolics content and, according to Žugić et al. [50], MAP species with less than 10 mg GAE g^{-1} of the extract (or <58.78 μmol GAE g^{-1}) exhibited the lowest antioxidant activity. This is a finding that was also observed in our study in the case of artemisia (plain), pelargonium, sage (mountainous), and lemon verbena (both sites) (Table 2). Moreover, Pirbalouti et al. [73] reported flavonoids extracts to vary from 7.63 to 14.52 mg of rutin g^{-1} of tissue in *Echinophora platyloba*, *Heracleum lasiopetalum*, and *Kelussia odoratissima* and suggested the use of MAPs as an alternative preservative and dietary source of antioxidants in the food industry (i.e., pickles). Similarly, a relatively high total flavonoids content was observed in lavender, laurel, and artemisia in the present work under different altitudes.

3.2. Essential Oil Yield and Composition

The two-way ANOVA reveled that EOs yield was affected by altitude ($p < 0.01$), by species ($p < 0.001$), and by the interaction of both factors ($p < 0.001$) (Table 2). Cultivation in the plain area resulted in higher essential oil yield when compared to the mountainous area (1.38 ± 0.04% and 1.17 ± 0.04%, respectively), when the species factor was not considered. When comparing all the species regardless of altitude, laurel had the highest (2.68 ± 0.33%) and pelargonium had the lowest (0.42 ± 0.10 %) EO yield. Similar results were observed when considering the combined effect of the tested factors, where laurel and pelargonium plants grown in the mountainous area resulted in the highest and lowest EO yield (3.30 ± 0.11% and 0.19 ± 0.04% for laurel and pelargonium, respectively), which indicated the significance of the genotype on this parameter, apart from the growing location. In particular, the high altitude of the mountainous area increased by 16.5% and by 60.6% for the EOs yield of artemisia and laurel, respectively, when compared to plants grown in the plain area. In contrast, EOs yield of pelargonium, spearmint, lavender, and lemon verbena decreased significantly (a decrease of 84.1%, 53.9%, 45.3%, and 30.7%, respectively) in plants grown in high altitudes when compared to plants grown in the plain area. Therefore, EOs yield was impacted by the species and altitude in a variable manner for most of the studied MAPs, whereas, in rosemary and sage, EO yield was not affected by altitude and averaged at 1.03% and at 1.89% (for rosemary and sage, respectively) (Table 2). Mahomoodally et al. [74] reported that EO yield can alter during the different months of the year and can be decreased in areas that receive less solar radiation, while Khorshidi et al. [75] reported that plant density and nutrient availability may also affect plant growth and EOs yield as in the case of mountainous plants of our study. Moreover, a different altitude is affecting not only the antioxidant but also the antimicrobial properties of EOs of *Thymus capitata* (L.) due to differences in volatile compounds' profile and in contents of bioactive substances [76]. The lowest EO yield in *Tussilago farfara* (L.) reported at low altitudes (i.e., 229 m) also revealed the highest antioxidant activity when compared to the plants grown in higher ones [48]. These findings agree with the results of our study where a decreased EO yield and increased antioxidants content was found in laurel in the plain area, but not in pelargonium and lemon verbena. This contradiction could be attributed not only to the different altitude and the climatic conditions of each location but also to differences among the studied MAP species. According

to Maurya et al. [77] and Bailen et al. [78], the metabolic pathways related to essential oils composition may be affected by both environmental and genetic factors. Moreover, Formisano et al. [79] reported that chamomile harvested at low altitudes (i.e., 81–89 m) revealed increased EOs yield when compared to plants harvested from higher altitudes (i.e., 640–675 m), which is in accordance with our findings for pelargonium, spearmint, lavender, and lemon verbena (Table 2).

The effect of altitude on the EOs chemical composition of the examined MAP species is given in Tables 3–11. In the case of artemisia, EOs' analysis revealed the presence of 28 and 30 individual compounds, which represent a total percentage of ≥92.01% of the oil profile for the plain and mountainous plants, respectively. The most abundant class (42.69% and 47.75%) was oxygenated monoterpenes, which was followed by oxygenated sesquiterpenes (39.03% and 31.01%), monoterpenes hydrocarbon (10.70% and 11.77%), and sesquiterpenes hydrocarbons (1.35% and 0.98%) for the plain and mountainous plants, respectively (Table 3). The major constituents of the examined artemisia EOs in decreasing order were 1,8-cineole (19.63–27.02%), *cis*-dihydroagarofuran (11.74–13.00%), silphiperfol-5-en-3-one A (9.71–13.06%), borneol (10.88–11.08%), camphor (5.92–8.59%), and p-cymene (5.69–7.26%). Camphene, ascaridole, silphiperfol-5-en-3-one B, silphiperfol-5-en-3-ol A, silphiperfol-5-en-3-ol B, presilphiperfol-8-ol, and caryophylla-4(12),8(13)-dien-5b-ol varied between 1–4%, while the rest of the compounds were identified in amounts lower than 1% of the total volatile components content (Table 3). Artemisia plants grown in the mountain had significantly higher content of 1,8-cineole and p-cymene but lower camphor and camphene content when compared to the plants grown in the plain (Table 3). The essential oil composition of artemisia has been widely studied and the literature reports show a great variability in essential oil composition where the main detected compounds were trans-sabinyl acetate and α-terpineol [80], davanone derivatives, and 4-Methyl-pent-2-enolid [81], whereas Mucciarelli et al. [82] reported 1,8-cineole as the main compound in the case of our study.

Table 3. Chemical composition (%) of essential oils of artemisia plants grown at different altitudes (plain vs. mountain). Data are expressed as means ± SE (n = 3), and significant differences ($p < 0.05$, $p < 0.01$, $p < 0.001$) in bold between treatments are indicated by Student's *t*-test *p*-values.

Components	RI	Plain	Mountain	Student's *t*-Test
α-Pinene	933	0.19 ± 0.097	0.39 ± 0.159	0.343
Camphene	948	3.63 ± 0.151	2.76 ± 0.026	**<0.05**
β-Pinene	977	0.33 ± 0.017	0.60 ± 0.248	0.339
α-Terpinene	1017	0.86 ± 0.159	0.26 ± 0.015	**<0.05**
p-Cymene	1024	5.69 ± 0.466	7.26 ± 0.364	**<0.05**
β-Phellandrene	1029	0.00± 0.000	0.12 ± 0.072	0.163
1,8-Cineole	1031	19.63 ± 1.400	27.02 ± 0.341	**<0.01**
γ-Terpinene	1058	nd	0.37 ± 0.003	-
cis-Sabinene hydrate	1067	0.23 ± 0.123	nd	0.132
trans-Sabinene hydrate	1100	0.27 ± 0.144	nd	0.131
cis-p Menth-2-en-1-ol	1121	nd	0.41 ± 0.017	-
trans-p Menth-2-en-1-ol	1138	0.44 ± 0.02	0.54 ± 0.017	**<0.05**
Camphor	1145	8.59 ± 0.607	5.92 ± 0.12	**<0.05**
Borneol	1166	11.08 ± 0.68	10.88 ± 0.07	0.789
Terpinen-4-ol	1178	0.74 ± 0.026	1.11 ± 0.026	**<0.001**
Ascaridole	1238	1.7 ± 0.536	0.82 ± 0.009	0.174
cis-Piperotone epoxide	1254	nd	0.31 ± 0.012	-
trans-Piperotone epoxide	1257	nd	0.57 ± 0.009	-
Isobornyl acetate	1285	0.59 ± 0.137	0.49 ± 0.020	0.537
Carvacrol	1300	nd	0.16 ± 0.095	-
Silphiperfol-5-ene	1324	0.46 ± 0.070	0.61 ± 0.046	0.155
Presilphiperfol-7-ene	1336	nd	0.08 ± 0.043	-
7-epi Silphiperfol-5-ene	1343	0.21 ± 0.111	0.07 ± 0.040	0.312
Silphiperfol-4.7(14)-diene	1359	0.32 ± 0.035	0.23 ± 0.029	0.108
Germacrene D	1497	0.36 ± 0.180	nd	-
Silphiperfolan-6a-ol	1518	0.67 ± 0.050	0.79 ± 0.038	0.143

Table 3. *Cont.*

Components	RI	Plain	Mountain	Student's *t*-Test
cis-Dihydroagarofuran	1533	13.00 ± 0.767	11.74 ± 0.191	0.187
Silphiperfol-5-en-3-ol B	1544	1.90 ± 0.128	1.56 ± 0.032	0.058
Silphiperfol-5-en-3-one B	1556	2.54 ± 0.074	1.85 ± 0.020	**<0.001**
Silphiperfol-5-en-3-ol A	1562	2.02 ± 0.156	1.55 ± 0.061	**<0.05**
Silphiperfol-5-en-3-one A	1581	13.06 ± 1.320	9.71 ± 0.294	0.068
Spathulenol	1582	0.21 ± 0.207	nd	-
Presilphiperfol-8-ol	1585	3.70 ± 0.199	3.17 ± 0.012	0.056
Caryophylla-4(12),8(13)-dien-5b-ol	1638	1.22 ± 0.152	0.65 ± 0.058	**<0.05**
epi-α-Bisabolol	1685	0.70 ± 0.055	nd	-
Total Identified		94.35 ± 0.981	92.01 ± 0.044	0.075
Monoterpenes hydrocarbons		10.70 ± 0.422	11.77 ± 0.109	0.070
Sesquiterpenes hydrocarbons		1.35 ± 0.321	0.98 ± 0.008	0.319
Oxygenated monoterpenes		42.69 ±1.301	47.75 ± 0.285	**<0.05**
Oxygenated sesquiterpenes		39.03 ± 1.740	31.01 ± 0.326	**<0.05**
Others		0.58 ±0.136	0.49 ± 0.020	0.537

nd: not detected.

Table 4. Chemical composition (%) of essential oils of laurel plants grown at different altitudes (plain vs. mountain). Data are expressed as means ± SE (n = 3), and significant differences ($p < 0.05$, $p < 0.01$, $p < 0.001$) in bold between treatments are indicated by Student's *t*-test *p*-values.

Components	RI	Plain	Mountain	Student's *t*-Test
α-Thujene	926	0.07 ± 0.043	0.10 ± 0.050	0.709
α-Pinene	933	3.13 ± 0.191	4.03 ± 0.102	**<0.05**
Camphene	948	0.03 ± 0.027	0.02 ± 0.017	0.766
Sabinene	973	1.52 ± 0.100	8.72 ± 0.067	**<0.001**
β-Pinene	977	3.00 ± 0.118	3.78 ± 0.044	**<0.01**
Dehydro-1,8-cineole	991	0.56 ± 0.040	0.52 ± 0.070	0.647
α-Terpinene	1017	0.14 ± 0.034	0.34 ± 0.038	**<0.05**
p-Cymene	1024	2.97 ± 0.252	0.70 ± 0.009	**<0.001**
Limonene	1028	0.69 ± 0.067	1.44 ± 0.055	**<0.001**
1,8-Cineole	1031	69.48 ± 1.577	56.63 ± 0.591	**<0.01**
γ-Terpinene	1058	0.36 ± 0.062	0.77 ± 0.052	**<0.01**
cis-Sabinene hydrate	1067	0.06 ± 0.038	0.37 ± 0.038	**<0.01**
Terpinolene	1089	nd	0.14 ± 0.015	-
trans-Sabinene hydrate	1100	0.10 ± 0.052	0.50 ± 0.029	**<0.01**
trans-p Mentha-2,8-dienol	1119	0.15 ± 0.077	0.18 ± 0.096	0.800
trans-Pinocarveol	1139	0.89 ± 0.090	0.42 ± 0.096	**<0.05**
Camphor	1145	0.04 ± 0.037	0.28 ± 0.032	**<0.01**
Pinocarvone	1163	0.80 ± 0.120	0.30 ± 0.085	**<0.05**
p-Mentha-1,5-dien-8-ol	1165	0.59 ± 0.118	0.54 ± 0.084	0.732
Terpinen-4-ol	1178	2.78 ± 0.364	1.98 ± 0.187	0.123
Thuj-3-en-10-al	1184	0.11 ± 0.058	0.12 ± 0.033	0.962
cis-Pinocarveol	1186	0.43 ± 0.060	0.07 ± 0.073	**<0.05**
α-Terpineol	1191	0.78 ± 0.171	1.21 ± 0.32	0.299
Myrtenal	1197	1.14 ± 0.149	0.52 ± 0.115	**<0.05**
trans-Carveol	1219	0.11 ± 0.012	nd	-
cis-Carveol	1231	0.47 ± 0.058	0.13 ± 0.078	**<0.05**
Carvone	1244	0.49 ± 0.107	1.21 ± 0.111	**<0.01**
Bornyl acetate	1285	0.03 ± 0.033	0.02 ± 0.017	0.678
δ-Terpinyl acetate	1316	1.02 ± 0.222	0.95 ± 0.139	0.802
α-Terpinyl acetate	1349	7.19 ± 0.948	13.07 ± 0.359	**<0.01**
Eugenol	1356	nd	0.14 ± 0.137	-
Eugenol methyl	1404	0.07 ± 0.073	nd	-
Caryophyllene oxide	1587	0.11 ± 0.107	0.03 ± 0.027	0.507
β-Eudesmol	1651	0.31 ± 0.043	0.10 ± 0.050	**<0.05**
Total Identified		99.62 ± 0.188	99.34 ± 0.190	0.355
Monoterpenes hydrocarbons		11.91 ± 0.659	20.04 ± 0.274	**<0.001**
Sesquiterpenes hydrocarbons		0.00 ± 0.000	0.00 ± 0.000	-
Oxygenated monoterpenes		78.08 ±1.528	64.70 ± 0.340	**<0.001**
Oxygenated sesquiterpenes		0.41 ± 0.084	0.13 ± 0.068	0.059
Others		8.31 ±1.126	14.04 ± 0.402	**<0.01**

nd: not detected.

Table 5. Chemical composition (%) of essential oils of lavender plants grown at different altitudes (plain vs. mountain). Data are expressed as means ± SE (n = 3), and significant differences ($p < 0.05$, $p < 0.01$, $p < 0.001$) in bold between treatments are indicated by Student's *t*-test *p*-values.

Components	RI	Plain	Mountain	Student's *t*-Test
α-Pinene	933	1.21 ± 0.342	1.01 ± 0.049	0.606
Camphene	948	1.23 ± 0.183	1.12 ± 0.032	0.575
Sabinene	973	0.22 ± 0.015	0.07 ± 0.038	**<0.05**
β-Pinene	977	1.07 ± 0.193	1.15 ± 0.053	0.698
p-Cymene	1024	1.28 ± 0.046	0.96 ± 0.031	**<0.01**
Limonene	1028	1.44 ± 0.362	1.98 ± 0.190	0.254
1,8-Cineole	1031	45.31 ± 2.177	30.82 ± 1.099	**<0.01**
γ-Terpinene	1058	0.04 ± 0.037	0.17 ± 0.015	**<0.05**
cis-Sabinene hydrate	1067	0.31 ± 0.102	nd	-
Linalool	1100	0.30 ± 0.137	7.47 ± 2.038	**<0.05**
α-Campholenal	1127	0.06 ± 0.063	0.08 ± 0.042	0.805
trans-Pinocarveol	1139	0.36 ± 0.160	0.28 ± 0.025	0.647
Camphor	1145	30.48 ± 0.935	34.29 ± 0.946	**<0.05**
Pinocarvone	1163	0.41 ± 0.160	0.30 ± 0.028	0.547
Borneol	1166	5.48 ± 0.520	6.34 ± 0.239	0.206
Terpene-4-ol	1178	0.48 ± 0.052	1.36 ± 0.228	**<0.05**
meta-p-Cymen-8-ol	1181	0.17 ± 0.091	nd	-
p-Cymen-8-ol	1185	0.41 ± 0.058	0.21 ± 0.029	**<0.05**
Cryptone	1187	0.96 ± 0.513	0.90 ± 0.112	0.905
α-Terpineol	1191	0.68 ± 0.083	0.84 ± 0.083	0.244
Myrtenal	1197	0.73 ± 0.397	0.39 ± 0.041	0.446
Verbenone	1211	0.09 ± 0.087	nd	-
trans-Carveol	1219	0.06 ± 0.057	0.15 ± 0.078	0.371
Bornyl formate	1229	nd	0.25 ± 0.010	-
Cumic aldehyde	1241	0.75 ± 0.377	0.93 ± 0.050	0.655
Carvone	1244	1.34 ± 0.003	5.53 ± 1.591	**<0.05**
Linalool acetate	1255	nd	0.61 ± 0.296	-
Bornyl acetate	1285	0.21 ± 0.107	0.07 ± 0.070	0.326
Lavandulyl acetate	1290	0.38 ± 0.010	0.14 ± 0.070	**<0.05**
α-Santalene	1423	0.22 ± 0.111	0.22 ± 0.041	0.979
γ-Cadinene	1525	0.55 ± 0.423	0.11 ± 0.056	0.364
Caryophyllene oxide	1587	1.10 ± 0.213	1.24 ± 0.088	0.576
Cubenol	1616	0.06 ± 0.060	nd	-
tau-Cadinol	1642	1.37 ± 0.794	0.55 ± 0.055	0.357
Bisabolol oxide II	1656	0.24 ± 0.119	0.04 ± 0.043	0.201
α-Bisabolol	1685	0.33 ± 0.167	0.06 ± 0.057	0.191
Muurol-5-en-4-one	1689	0.21 ± 0.207	nd	-
Total Identified		99.49 ± 0.177	99.67 ± 0.068	0.405
Monoterpenes hydrocarbons		5.26 ± 0.150	5.45 ± 0.281	0.590
Sesquiterpenes hydrocarbons		0.76 ± 0.320	0.33 ± 0.028	0.249
Oxygenated monoterpenes		87.39 ±1.279	89.28 ± 0.890	0.293
Oxygenated sesquiterpenes		3.10 ± 0.784	1.88 ± 0.195	0.205
Others		1.76 ±0.421	1.96 ± 0.353	0.730

nd: not detected.

Table 6. Chemical composition (%) of essential oils of lemon verbena plants grown at different altitudes (plain vs. mountain). Data are expressed as means ± SE (n = 3), and significant differences ($p < 0.05$, $p < 0.01$, $p < 0.001$) in bold between treatments are indicated by Student's *t*-test *p*-values.

Components	RI	Plain	Mountain	Student's *t*-Test
α-Pinene	933	1.21 ± 0.120	1.82 ± 0.699	0.440
Camphene	948	0.42 ± 0.118	2.00 ± 1.155	0.244
Sabinene	973	1.87 ± 0.119	0.68 ± 0.199	**<0.01**
Oct-1-en-3-ol	975	nd	0.19 ± 0.110	-
β-Pinene	977	0.30 ± 0.055	0.70 ± 0.401	0.379
6-methyl-5 Hepten-2-one	984	0.36 ± 0.045	0.06 ± 0.038	**<0.01**
β-Myrcene	989	0.27 ± 0.023	0.31 ± 0.124	0.767
p Cymene	1006	nd	0.28 ± 0.162	-
Limonene	1026	15.67 ± 0.616	8.81 ± 1.091	**<0.01**
1,8-Cineole	1031	6.85 ± 0.320	8.41 ± 2.327	0.543
cis-Sabinene hydrate	1067	0.30 ± 0.006	0.09 ± 0.052	**<0.05**
Linalool	1100	nd	0.11 ± 0.066	-
α-Thujone	1106	1.13 ± 0.312	5.25 ± 2.921	0.233
β-Thujone	1116	0.23 ± 0.119	1.29 ± 0.745	0.231

Table 6. *Cont.*

Components	RI	Plain	Mountain	Student's *t*-Test
Camphor	1145	1.35 ± 0.341	5.87 ± 3.230	0.236
Borneol	1166	0.11 ± 0.113	0.87 ± 0.358	0.114
Isocitral	1177	0.20 ± 0.101	0.27 ± 0.101	0.665
α-Terpineol	1191	1.02 ± 0.035	0.45 ± 0.139	**<0.05**
Neral (β *cis* Citral)	1240	16.03 ± 0.642	17.72 ± 3.014	0.612
Carvone	1244	0.08 ± 0.080	0.06 ± 0.032	0.800
Geranial (α *trans* Citral)	1271	22.42 ± 0.866	29.06 ± 3.398	0.131
α-Copaene	1376	0.37 ± 0.025	nd	-
Geranyl acetate	1383	0.81 ± 0.029	0.82 ± 0.228	0.967
β-Bourbonene	1414	0.52 ± 0.018	0.08 ± 0.046	**<0.001**
α-Cedrene	1422	nd	0.10 ± 0.055	-
β-Caryophyllene	1425	2.20 ± 0.226	0.51 ± 0.188	**<0.01**
Alloaromadendrene	1464	0.37 ± 0.030	0.13 ± 0.043	**<0.05**
ar-Curcumene	1496	7.42 ± 0.451	5.46 ± 1.103	0.176
Cubebol	1527	0.28 ± 0.181	nd	-
Nerolidol E	1568	0.32 ± 0.006	0.16 ± 0.092	0.159
Spathulenol	1581	6.45 ± 0.264	3.08 ± 0.921	**<0.05**
Caryophyllene oxide	1587	9.38 ± 0.702	4.67 ± 0.990	**<0.05**
Humulene epoxide II	1608	0.28 ± 0.024	nd	-
epi-α-Cadinol	1641	0.79 ± 0.047	0.24 ± 0.136	**<0.05**
Total Identified		98.99 ± 0.206	99.53 ± 0.089	0.075
Monoterpenes hydrocarbons		19.73 ± 0.833	14.60 ± 1.125	**<0.05**
Sesquiterpenes hydrocarbons		10.87 ± 0.743	6.28 ± 1.431	**<0.05**
Oxygenated monoterpenes		49.72 ±1.006	69.45 ± 2.782	**<0.01**
Oxygenated sesquiterpenes		17.50 ± 1.116	8.13 ± 2.139	**<0.05**
Others		1.16 ±0.069	1.07 ± 0.375	0.813

nd: not detected.

Table 7. Chemical composition (%) of essential oils of pelargonium plants grown at different altitudes (plain vs. mountain). Data are expressed as means ± SE (n = 3), and significant differences ($p < 0.05$, $p < 0.01$, $p < 0.001$) in bold between treatments are indicated by Student's *t*-test *p*-values.

Components	RI	Plain	Mountain	Student's *t*-Test
α-Pinene	933	0.23 ± 0.015	6.64 ± 0.372	**<0.001**
β-Pinene	977	nd	0.67 ± 0.015	-
β-Myrcene	989	nd	0.26 ± 0.006	-
Limonene	1028	nd	0.41 ± 0.009	-
Artemisia ketone	1059	nd	2.11 ± 0.041	-
Linalool	1100	1.02 ± 0.225	0.88 ± 0.018	0.578
cis-Rose oxide	1110	1.89 ± 0.288	5.79 ± 0.116	**<0.001**
trans-Rose oxide	1126	0.76 ± 0.132	1.88 ± 0.038	**<0.001**
Camphor	1145	nd	0.25 ± 0.003	-
Menthone	1153	0.08 ± 0.040	0.16 ± 0.007	0.132
Isomenthone	1164	5.78 ± 0.218	10.61 ± 0.217	**<0.001**
Citronellol	1227	36.69 ± 1.577	24.25 ± 0.495	**<0.01**
Neral (β *cis* Citral)	1240	0.27 ± 0.028	0.00 ± 0.000	**<0.001**
Carvone	1244	nd	0.74 ± 0.015	-
Geraniol	1253	15.45 ± 1.697	11.13 ± 0.142	**<0.05**
Geranial	1271	0.86 ± 0.020	0.44 ± 0.009	**<0.001**
Citronellyl formate	1275	13.29 ± 0.212	14.11 ± 0.290	0.085
p-Menth-1-en-9-ol	1299	0.09 ± 0.043	0.70 ± 0.015	**<0.001**
Geranyl formate	1302	4.21 ± 0.353	4.75 ± 0.099	0.215
Citronellyl acetate	1352	0.21 ± 0.015	nd	-
Geranyl acetate	1383	0.39 ± 0.048	nd	-
β-Bourbonene	1386	0.97 ± 0.160	nd	-
β-Caryophyllene	1425	0.35 ± 0.009	nd	-
Citronellyl propanoate	1450	0.35 ± 0.009	nd	-
Geranyl proponoate	1487	0.86 ± 0.099	0.49 ± 0.012	**<0.05**
Germacrene D	1497	1.35 ± 0.060	0.34 ± 0.009	**<0.001**
Viridiflorene	1509	0.88 ± 0.020	nd	-
δ-Cadinene	1534	0.45 ± 0.031	0.42 ± 0.009	0.354
Citronellyl butanoate	1537	0.51 ± 0.018	nd	-
Geranyl butanoate	1565	0.60 ± 0.084	0.89 ± 0.020	**<0.05**
Phenethyl tiglate	1588	1.90 ± 0.165	2.50 ± 0.049	**<0.05**
γ-Eudesmol	1621	6.92 ± 0.079	5.92 ± 0.122	**<0.01**
β-Eudesmol	1651	0.39 ± 0.021	0.64 ± 0.015	**<0.001**

Table 7. *Cont.*

Components	RI	Plain	Mountain	Student's *t*-Test
Citronellyl tiglate	1665	0.44 ± 0.083	nd	-
Geranyl tiglate	1700	2.43 ± 0.052	1.58 ± 0.019	**<0.001**
Farnesyl acetone	2005	nd	1.22 ± 0.023	-
Total Identified		99.65 ± 0.208	99.75 ± 0.128	0.713
Monoterpenes hydrocarbons		0.23 ± 0.015	7.97 ± 0.364	**<0.001**
Sesquiterpenes hydrocarbons		4.01 ± 0.131	0.75 ± 0.014	**<0.001**
Oxygenated monoterpenes		76.20 ±0.508	73.04 ± 0.620	**<0.05**
Oxygenated sesquiterpenes		10.19 ± 0.032	8.13 ± 0.104	**<0.001**
Others		9.03 ± 0.389	9.85 ± 0.095	0.111

nd: not detected.

Table 8. Chemical composition (%) of essential oils of rosemary plants grown at different altitudes (plain vs. mountain). Data are expressed as means ± SE (n = 3), and significant differences ($p < 0.05$, $p < 0.01$, $p < 0.001$) in bold expressed between treatments are indicated by Student's *t*-test *p*-values.

Components	RI	Plain	Mountain	Student's *t*-Test
Tricyclene	922	0.22 ± 0.003	0.20 ± 0.006	**<0.05**
α-Thujene	926	0.01 ± 0.003	0.04 ± 0.034	0.341
α-Pinene	933	12.01 ± 0.255	13.05 ± 0.625	0.196
Camphene	948	8.29 ± 0.135	8.12 ± 0.273	0.614
β-Pinene	977	1.67 ± 0.02	1.71 ± 0.513	0.942
n-Octanone	984	0.01 ± 0.007	0.01 ± 0.007	0.519
β-Myrcene	989	1.07 ± 0.007	1.18 ± 0.134	0.460
3-Octanol	1003	0.03 ± 0.000	0.03 ± 0.030	1.000
α-Phellandrene	1004	0.09 ± 0.000	0.14 ± 0.030	0.146
α-Terpinene	1017	0.32 ± 0.000	0.53 ± 0.150	0.234
p-Cymene	1024	3.03 ± 0.061	3.03 ± 0.248	0.990
Limonene	1028	3.84 ± 0.050	3.94 ± 0.145	0.537
1,8-Cineole	1031	32.94 ± 0.703	32.94 ± 0.927	0.996
γ-Terpinene	1058	0.23 ± 0.018	0.63 ± 0.325	0.286
Terpinolene	1089	0.22 ± 0.007	0.38 ± 0.084	0.118
Linalool	1100	0.70 ± 0.043	0.63 ± 0.187	0.734
β-Thujone	1116	0.02 ± 0.009	0.09 ± 0.035	0.100
Camphor	1145	20.86 ± 0.401	19.21 ± 0.445	**<0.05**
Borneol	1166	8.94 ± 0.143	7.88 ± 1.071	0.385
Terpinen-4-ol	1178	0.92 ± 0.035	0.97 ± 0.041	0.465
p-Cymen-8-ol	1185	0.06 ± 0.006	0.03 ± 0.018	0.224
α-Terpineol	1191	3.05 ± 0.107	2.58 ± 0.141	**<0.05**
Bornyl acetate	1285	0.56 ± 0.103	0.85 ± 0.254	0.355
Methyl eugenol	1404	0.19 ± 0.035	0.19 ± 0.078	0.941
β-Caryophyllene	1425	0.46 ± 0.059	1.14 ± 0.212	**<0.05**
α-Caryophyllene	1462	0.03 ± 0.013	0.12 ± 0.012	**<0.01**
δ-Cadinene	1534	0.03 ± 0.009	0.10 ± 0.017	**<0.05**
Caryophyllene oxide	1587	0.06 ± 0.015	0.04 ± 0.026	0.678
α-Bisabolol	1685	0.05 ± 0.025	0.09 ± 0.019	0.238
Total Identified		99.87 ± 0.050	99.87 ± 0.030	0.915
Monoterpenes hydrocarbons		30.97 ± 0.409	32.96 ± 1.774	0.335
Sesquiterpenes hydrocarbons		0.51 ± 0.078	1.36 ± 0.239	**<0.05**
Oxygenated monoterpenes		67.48 ±0.233	64.33 ± 2.151	0.219
Oxygenated sesquiterpenes		0.11 ± 0.039	0.13 ± 0.044	0.638
Others		0.79 ± 0.133	1.07 ± 0.368	0.519

Table 9. Chemical composition (%) of essential oils of sage plants grown at different altitudes (plain vs. mountain). Data are expressed as means ± SE (n = 3), and significant differences ($p < 0.05$, $p < 0.01$, $p < 0.001$) in bold between treatments are indicated by Student's *t*-test *p*-values.

Components	RI	Plain	Mountain	Student's *t*-Test
Salvene	865	0.11 ± 0.015	nd	-
Tricyclene	922	0.23 ± 0.012	0.23 ± 0.032	0.926
α-Thujene	926	0.18 ± 0.006	0.13 ± 0.067	0.525
α-Pinene	933	4.33 ± 0.133	5.62 ± 0.479	**<0.05**
Camphene	948	7.10 ± 0.258	8.13 ± 0.493	0.138
β-Pinene	977	2.90 ± 0.101	3.31 ± 0.227	0.179
β-Myrcene	989	1.69 ± 0.110	1.47 ± 0.043	0.136
α-Terpinene	1017	0.12 ± 0.009	0.05 ± 0.050	0.222

Table 9. *Cont.*

Components	RI	Plain	Mountain	Student's *t*-Test
o-Cymene	1024	1.42 ± 0.115	0.71 ± 0.052	**<0.01**
Limonene	1028	2.15 ± 0.059	2.46 ± 0.055	**<0.05**
1,8-Cineole	1031	16.69 ± 0.524	15.28 ± 0.359	0.091
γ-Terpinene	1058	0.20 ± 0.021	0.29 ± 0.010	**<0.05**
Terpinolene	1089	0.03 ± 0.030	0.18 ± 0.021	**<0.05**
Linalool	1100	0.14 ± 0.003	0.06 ± 0.057	0.202
α-Thujone	1106	23.83 ± 0.071	5.34 ± 0.426	**<0.001**
β-Thujone	1116	5.23 ± 0.176	13.32 ± 0.665	**<0.001**
iso-3-Thujanol	1133	nd	0.33 ± 0.031	-
trans-Sabinol	1140	0.30 ± 0.018	0.47 ± 0.006	**<0.001**
Camphor	1145	22.26 ± 1.025	16.98 ± 0.474	**<0.01**
Borneol	1166	3.54 ± 0.125	13.01 ± 0.656	**<0.001**
Terpinen-4-ol	1178	0.81 ± 0.049	0.41 ± 0.023	**<0.001**
α-Terpineol	1191	0.23 ± 0.024	0.15 ± 0.078	0.417
Estragol	1197	0.16 ± 0.009	nd	-
Bornyl acetate	1285	0.59 ± 0.049	2.21 ± 0.421	**<0.05**
trans-sabinyl acetate	1292	0.11 ± 0.009	nd	-
Copaene	1349	0.02 ± 0.023	0.41 ± 0.067	**<0.01**
β-Caryophyllene	1425	0.48 ± 0.141	3.49 ± 0.360	**<0.001**
α-Caryophyllene	1462	0.86 ± 0.179	0.48 ± 0.052	**0.111**
γ-Cadinene	1525	nd	0.29 ± 0.047	-
δ-Cadinene	1534	nd	0.71 ± 0.115	-
Caryophyllene oxide	1587	0.15 ± 0.033	0.27 ± 0.035	0.062
Viridiflorol	1594	2.77 ± 0.297	0.88 ± 0.032	**<0.01**
Humulene epoxide II	1608	0.64 ± 0.065	0.00 ± 0.000	**<0.001**
Cubenol	1643	nd	0.42 ± 0.069	-
neo-5-Cedranol	1699	nd	0.66 ± 0.174	-
Manool	2055	0.72 ± 0.184	2.25 ± 0.514	**<0.05**
Total Identified		99.97 ± 0.030	100.00 ± 0.000	0.374
Monoterpenes hydrocarbons		20.35 ± 0.426	22.58 ± 1.284	0.174
Sesquiterpenes hydrocarbons		1.36 ± 0.304	5.38 ± 0.572	**<0.01**
Oxygenated monoterpenes		73.20 ± 0.368	65.53 ± 0.228	**<0.001**
Oxygenated sesquiterpenes		3.55 ± 0.324	2.23 ± 0.270	**<0.05**
Others		1.53 ± 0.178	4.45 ± 0.840	**<0.05**

nd: not detected.

Table 10. Chemical composition (%) of essential oils of spearmint plants grown at different altitudes (plain vs. mountain). Data are expressed as means ± SE (n = 3), and significant differences ($p < 0.05$, $p < 0.01$, $p < 0.001$) in bold between treatments are indicated by Student's *t*-test *p*-values.

Components	RI	Plain	Mountain	Student's *t*-Test
α-Pinene	933	1.01 ± 0.052	0.81 ± 0.040	**<0.05**
Camphene	948	0.08 ± 0.006	0.09 ± 0.006	0.288
Sabinene	973	0.67 ± 0.032	1.26 ± 0.061	**<0.001**
β-Pinene	977	1.32 ± 0.046	0.88 ± 0.380	0.311
β-Myrcene	989	0.63 ± 0.032	0.55 ± 0.026	0.123
3-octanol	995	0.15 ± 0.009	0.30 ± 0.024	**<0.01**
Limonene	1028	12.07 ± 0.302	6.65 ± 0.055	**<0.001**
1,8-Cineole	1031	4.98 ± 0.234	5.68 ± 0.150	**<0.05**
cis-β-Ocimene	1036	0.13 ± 0.006	0.18 ± 0.009	**<0.05**
trans-β-Ocimene	1046	nd	0.06 ± 0.003	-
γ-Terpinene	1058	0.04 ± 0.003	0.09 ± 0.003	**<0.001**
cis-Sabinene hydrate	1067	0.19 ± 0.012	0.30 ± 0.017	**<0.01**
3-Octanol acetate	1121	nd	0.10 ± 0.003	-
iso-Menthone	1164	0.08 ± 0.003	nd	-
Borneol	1166	0.26 ± 0.012	0.25 ± 0.009	0.670
Terpinen-4-ol	1178	0.12 ± 0.009	0.23 ± 0.012	**<0.001**
α-Terpineol	1191	0.19 ± 0.009	0.07 ± 0.006	**<0.001**
cis-Dihydro carvone	1198	0.65 ± 0.033	12.97 ± 0.613	**<0.001**
neo-Dihydro carveol	1194	0.38 ± 0.017	1.34 ± 0.042	**<0.001**
trans-Carveol	1219	nd	0.29 ± 0.015	-
cis-Carveol	1231	0.13 ± 0.009	3.60 ± 0.159	**<0.001**
Pulegone	1240	0.70 ± 0.026	0.44 ± 0.020	**<0.001**
Carvone	1244	72.12 ± 0.911	50.12 ± 1.203	**<0.001**
cis-Carvone oxide	1262	0.07 ± 0.006	nd	-
trans-Carvone oxide	1276	0.13 ± 0.006	nd	-
iso-Bornyl acetate	1285	nd	0.08 ± 0.003	-

Table 10. *Cont.*

Components	RI	Plain	Mountain	Student's *t*-Test
Dihydrocarveol acetate	1325	0.43 ± 0.020	5.67 ± 0.278	**<0.001**
trans-Carvyl acetate	1335	nd	0.24 ± 0.012	-
cis-Carvyl acetate	1360	0.27 ± 0.015	4.92 ± 0.187	**<0.001**
β-Bourbonene	1386	0.61 ± 0.019	0.61 ± 0.019	1.000
β-Elemene b	1393	0.21 ± 0.009	0.19 ± 0.028	0.539
β-Caryophyllene	1425	0.92 ± 0.068	0.91 ± 0.031	0.900
cis-Muurola-3,5-diene	1456	0.42 ± 0.020	0.42 ± 0.018	1.000
Germacrene D	1497	0.38 ± 0.023	0.31 ± 0.024	0.116
Bicyclogermacrene	1512	0.16 ± 0.006	0.10 ± 0.038	0.179
Germacrene A	1519	0.07 ± 0.003	0.07 ± 0.012	0.621
trans-Calamene	1531	0.24 ± 0.015	0.20 ± 0.007	0.105
1,10-di-epi Cubenol	1642	0.10 ± 0.000	nd	-
Total Identified		99.91 ± 0.003	100.00 ± 0.000	**<0.001**
Monoterpenes hydrocarbons		15.95 ± 0.429	10.58 ± 0.463	**<0.001**
Sesquiterpenes hydrocarbons		3.01 ± 0.163	2.81 ± 0.087	0.034
Oxygenated monoterpenes		79.59 ± 0.640	74.03 ± 0.360	**<0.01**
Oxygenated sesquiterpenes		0.10 ± 0.000	nd	-
Others		1.23 ± 0.057	12.56 ± 0.076	**<0.001**

nd: not detected.

In laurel, EOs analysis resulted in 32 individual compounds in total for both the plain and mountainous plants, which represent ≥99.34% of the total oil profile (Table 4). Oxygenated monoterpenes were the most abundant class (78.08% and 64.70%), which is followed by monoterpenes hydrocarbon (11.91% and 20.04%), and by oxygenated sesquiterpenes (0.41% and 0.13%), as sesquiterpenes hydrocarbons were not detected in either plain or mountainous plants (Table 4). The main laurel component was 1,8-cineole (56.63–69.48%), which was followed by the α-terpinyl acetate (7.19–13.07%) while α-pinene, sabinene, β-pinene, p-cymene, terpinen-4-ol, myrtenal, carvone, and δ-terpinyl acetate that varied between 1–4% (Table 4). Laurel plants grown in the mountain had significantly higher α-pinene, sabinene, β-pinene, and α-terpinyl acetate but lower 1,8-cineole and p-cymene compared to the relevant plants grown in the plain area (Table 4). 1,8-cineole and α-terpinyl acetate was also reported as the major volatile compound in laurel essential oils by Ordoudi et al. [83] and Fidan et al. [84], while other studies refer to 1,8-cineole and linalool [85], eucalyptol, and terpinyl acetate [86], since extraction methods and sample preparation may affect the essential oils' composition [87,88].

The EOs' composition of lavender aerial parts is presented in Table 5 with 35 and 32 compounds identified in plain and mountainous plants, respectively, which represent ≥99.49% of the total oil profile. The classification of individual components revealed the oxygenated monoterpenes (87.39% and 89.28%) as most abundant, which is followed by monoterpenes hydrocarbon (5.26% and 5.45%), oxygenated sesquiterpenes (3.10% and 1.88%), and, lastly, by sesquiterpenes hydrocarbons (0.76% and 0.33%) for the plain and mountainous plants, respectively (Table 5). The main constituents of lavender EOs in decreasing order were 1,8-cineole (30.82–45.31%), which is followed by camphor (30.48–34.29%), linalool (0.30–7.47%), borneol (5.48–6.34%), and carvone (1.34–5.53%), whereas α-pinene, camphene, β-pinene, p-cymene, limonene, terpene-4-ol, caryophyllene oxide, and tau-cadinol levels varied between 1–4% (Table 5). In mountainous conditions, lavender EOs had higher camphor, linalool, and terpene-4-ol, but lower 1,8-cineole and p-cymene content compared to plants harvested in the plain area (Table 5). Contrasting reports exist in the literature regarding the essential oil composition of lavender, where linalyl acetate and linalool [89,90] or terpinene-4-ol [91] were identified as the major compounds, since agronomic practices may affect essential oil composition [89]. Moreover, Łyczko et al. [92] reported that camphor is an important quality marker for lavender essential oil. The higher the ratio of linalool and linalyl acetate is to camphor, the better the quality is. They also suggested that drying methods may significantly affect the essential oil composition. Similarly to our study, Oroian et al. [93] reported that geographical origin of lavender samples has a significant effect on essential oils composition, even though they reported linalool and linalyl acetate as the major compounds.

Table 11. Correlations between antioxidant activity, phenolic compounds, and essential oil components.

Species	Altitude	Antioxidant Activity Assay	Phenols	Flavonoids	Flavanols	Essential Oils (EO)	1,8 Cineole	Camphor	Borneol	*cis*-Dihydro Agarofuran	Silphiperfol-5-En-3-One A
Artemisia	P	FRAP									
		DPPH		+					+		
	M	FRAP	+								
		DPPH	+								
			Phenols	**Flavonoids**	**Flavanols**	**EO**	**Isomenthone**	**Citronellol**	**Geraniol**	**Citronellyl formate**	**γ-Eudesmol**
Pelargonium	P	FRAP		+	+						
		DPPH	+								
	M	FRAP		+	+						
		DPPH	+		+						
			Phenols	**Flavonoids**	**Flavanols**	**EO**	**α-Pinene**	**Sabinene**	**β-Pinene**	**1,8-Cineole**	**Terpinyl acetate a**
Laurel	P	FRAP	+	+	+				+	-	
		DPPH	+	+	+					-	
	M	FRAP	+								
		DPPH	+	+	+						
			Phenols	**Flavonoids**	**Flavanols**	**EO**	**1,8 Cineole**	**Linalool**	**Camphor**	**Borneol**	**Carvone**
Lavender	P	FRAP	+	+							
		DPPH	+	+							
	M	FRAP	+	+							
		DPPH	+								
			Phenols	**Flavonoids**	**Flavanols**	**EO**	**D-Limonene**	**1,8-Cineole**	**Neral**	**Geranial**	**Caryophyllene oxide**
Lemon verbena	P	FRAP		+							
		DPPH	+	+							
	M	FRAP	+	+			-	+	-	-	-
		DPPH									
			Phenols	**Flavonoids**	**Flavanols**	**EO**	**α-Pinene**	**Camphene**	**1,8-Cineole**	**Camphor**	**Borneol**
Rosemary	P	FRAP	+	+							
		DPPH									
	M	FRAP	+	+							
		DPPH	+	+							
			Phenols	**Flavonoids**	**Flavanols**	**EO**	**1,8-Cineole**	**α-Thujone**	**β-Thujone**	**Camphor**	**Borneol**
Sage	P	FRAP	+	+							
		DPPH						-			
	M	FRAP	+	+							
		DPPH									
			Phenols	**Flavonoids**	**Flavanols**	**EO**	**D-limonene**	**1,8-Cineole**	**cis-dihydro carvone**	**Carvone**	**Dihydrocarveol acetate**
Spearmint	P	FRAP	+								
		DPPH									
	M	FRAP	+	+							
		DPPH		+							

The plus (+) and minus (−) symbols indicate positive and negative correlations, respectively. The P and M indicate the plain and mountain areas, respectively.

In lemon verbena, EOs' analysis resulted in 30 and 31 individual compounds for the plain and mountain grown plants, respectively, which represented ≥98.99% of the total oil profile (Table 6). The analysed oils were dominated by the monoterpenes fraction. In particular, the oxygenated monoterpenes were the most represented class with percentages of 49.72% and 69.45%, which were followed by monoterpenes hydrocarbon (19.73% and 14.60%), oxygenated sesquiterpenes (17.50% and 8.13%), and sesquiterpenes hydrocarbons (10.87% and 6.28%) for the plain and mountainous plants, respectively. The main constituents for lemon verbena were geranial (α *trans* citral) (22.42–29.06%), neral (β *cis* citral) (16.03–17.72%), limonene (8.81–15.67%), caryophyllene oxide (4.67–9.38%), 1,8-cineole (6.85–8.41%), ar-curcumene (5.46–7.42%), spathulenol (3.08–6.45%), camphor (1.35–5.87%), α-thujone (1.13–5.25%), while the α-pinene, sabinene, β-thujone, and β-caryophyllene varied between 1–4% (Table 6). Lemon verbena grown in the mountain had significantly lower limonene, sabinene, β-caryophyllene, spathulenol, and caryophyllene oxide when compared to the relevant plants grown in the plain area (Table 6). The same compounds have been identified as the major constituents of lemon verbena EOs in various reports [94]. However, a seasonal effect on EOs was also suggested [95,96].

Pelargonium EOs' analysis is presented in Table 7 and it revealed the identification of 29 and 27 components in plain and mountainous plants, respectively, that represented ≥99.65% of the total oil profile. Following constituents' classification, the oxygenated monoterpenes predominated (76.20% and 73.04%) and was followed by oxygenated sesquiterpenes (10.19% and 8.13%), monoterpenes hydrocarbon (0.23% and 7.97%), and sesquiterpenes hydrocarbons (4.01% and 0.75%) for the plain and mountainous plants, respectively. The most abundant oil component was citronellol (24.25–36.69%), which was followed by geraniol (11.13–15.45%), citronellyl formate (13.29–14.11%), isomenthone (5.78–10.61%), geranyl formate (4.11–4.75%), γ-eudesmol (5.92–6.92%), *cis*-rose oxide (1.89–5.79%), and α-pinene (0.23–6.64%). The oil components of artemisia ketone, linalool, *trans*-rose oxide, germacrene D, phenethyl tiglate, geranyl tiglate, and farnesyl acetone varied from 1–4%, while other compounds were identified in amounts lower than 1% of the total volatile components content (Table 7). Compared to the plain area, mountainous plants contained significantly higher levels of α-pinene, artemisia ketone, *cis*-rose oxide, *trans*-rose oxide, isomenthone, phenethyl tiglate, and lower levels of citronellol, geraniol, γ-eudesmol, and geranyl tiglate (Table 7). Similarly to our study, citronellol and geraniol were identified as the major compounds of pelargonium EOs in many other reports, which also correlate with the bioactive properties of the EOs of the species with the presence of these compounds [97–100].

In rosemary, EOs analysis revealed the presence of 29 individual compounds for both plain and mountainous plants, which represent ≥99.87% of the total oil profile (Table 8). The main detected class was that of oxygenated monoterpenes (67.48% and 64.33%), which was followed by monoterpenes hydrocarbon (30.97% and 32.96%) for the plain and mountainous plants, respectively, whereas very low amounts of sesquiterpenes (oxygenated up to 0.13% and hydrocarbons up to 1.36%) were identified (Table 8). Accordingly, the major oil constituents in decreasing order were 1,8-cineole (32.94%), camphor (19.21–20.86%), α-pinene (12.01–13.05%), camphene (8.12–8.29%), and borneol (7.88–8.94%), whereas β-pinene, β-myrcene, p-cymene, limonene, and α-terpineol varied between 1–4% (Table 8). Rosemary plants grown in the mountain had significantly lower camphor, and α-terpineol content compared to the plants grown in the plain. The detected profile of volatile compounds is typical for the species based on several literature reports [101,102], whereas Contini et al. [103] reported a-pinene and 1,8-cineole as the most abundant compounds. Moreover, according to Sabbahi et al. [104], the profile of the major compounds was not affected by the elevation gradient. Compositional variability is attributed mostly to genetic factors.

Sage EOs' analysis revealed the identification of 31 and 32 individual components for the plain and mountainous plants, respectively, which represent ≥99.97% of the total oil profile (Table 9). The most abundant class was oxygenated monoterpenes (73.20% and 65.53%), which was followed by monoterpenes hydrocarbon (20.35% and 22.58%), sesquiterpenes hydrocarbons (1.36% and 5.38%), and oxygenated sesquiterpenes (3.55% and 2.23%) for the plain and mountain plants, respectively (Table 9). The main oil constituents in decreasing order were camphor (22.26% and 16.98%), 1,8-cineole (16.69%

and 15.28%), α-thujone (23.83% and 5.34%), β-thujone (5.23% and 13.32%), borneol (3.54% and 13.01%), camphene (7.10% and 8.13%), and α-pinene (4.33% and 5.62%) (Table 9). Moreover, EOs of sage plants grown in the mountain had significantly higher content of α-pinene, limonene, β-thujone, borneol, and manool, but lower α-thujone, camphor, and viridiflorol content when compared to the plants grown in the plain area. According to Bedini et al. [105], a-thujone was the major compound of sage EOs, while they also detected significant amounts of camphor and 1,8-cineole. Moreover, EOs' composition may be affected by several factors such as environmental stressors [106], or the application of biofertilizers and bio-stimulants [107], which indicated the importance of exogenous factors on EOs' biosynthesis. In contrast, Cvetkovikj et al. [108] who studied several sage populations from Balkan countries identified four distinct chemotypes, which differ in *cis*-thujone, *trans*-thujone, and camphor content and suggested a significant correlation of essential oil composition with geographic variables.

Spearmint EOs analysis resulted in the presence of 33 and 34 individual compounds for the plain and mountainous plants, respectively, which represented ≥99.91% of the total oil profile (Table 10). The most abundant class was oxygenated monoterpenes (79.59% and 74.03%), which were followed by monoterpenes hydrocarbon (15.95% and 10.58%), sesquiterpenes hydrocarbons (3.01% and 2.81%), and very low amounts of oxygenated sesquiterpenes for the plain and mountainous plants, respectively (Table 10). The major constituents of the examined spearmint EO in decreasing order were carvone (72.12% and 50.12%), limonene (12.07% and 6.65%), 1,8-cineole (4.98% and 5.68%), *cis*-dihydro carvone (0.65% and 12.97%), dihydrocarveol acetate (0.43% and 5.67%), and *cis*-carvyl acetate (0.27% and 4.92%). Spearmint plants grown in the mountain had significantly higher content of 1,8-cineole, *cis*-dihydro carvone, *neo*-dihydro carveol, *cis*-carveol, dihydrocarveol acetate, and *cis*-carvyl acetate but lower carvone, and limonene when compared to the plants grown in a plain (Table 10). Carvone was the most abundant spearmint EO constituent in several other reports [109,110], while agronomic factors such as salinity and water stress may affect EOs yield and composition [64,111].

3.3. Correlation of Antioxidant and Reducing Activity with Polyphenols and Essential Oils Components

MAP species are highly appreciated as a natural source of antioxidants, while phenolic compounds and essential oils components are involved in such antioxidant capacity [34,46]. To evaluate the contribution of phenols, flavonoids, flavanols, and essential oils components, only the five most abundant constituents of each EOs were considered. For their total antioxidant capacity, linear correlation coefficients were determined and presented in detail in Tables S2–S9. The correlation coefficient (r) and p-values between the analysed EO compounds and the antioxidant capacity of artemisia are given in Table S2. In the plain area, phenols content was strongly and positively correlated with flavanols. DPPH was correlated with borneol, while flavonoids were correlated with DPPH and *cis*-dihydroagarofuran. Accordingly, for plants grown in the mountainous area, a positive correlation was found with phenols and antioxidant activity of DPPH and FRAP. Borneol has been associated with antioxidant activity as well as with antihypertensive properties in animal models [112,113]. Its increased content could increase the overall antioxidant capacity of artemisia. Moreover, in the study of Wang et al. [114], it was observed that thyme borneol essential oils presented the highest antioxidant activities (reducing power and β-carotene bleaching) and the highest total phenols content among 26 essential oils.

In pelargonium in the plain area, phenols were positively correlated with DPPH, flavonoids, and flavanols. FRAP was correlated to flavonoids and flavanols, while flavonoids were correlated to flavanols (Table S3). In the mountainous area, phenols were correlated to DPPH, FRAP, flavonoids, flavanols, and EO yield. DPPH was correlated to FRAP and flavanols while FRAP was correlated to flavonoids and flavanols. Essential oil yield was correlated to the geraniol content (Table S3), as decreased EO and geraniol content were observed in the mountainous areas.

In laurel grown in plain areas, phenols were positively correlated to DPPH, FRAP, flavonoids, and flavanols. DPPH was positively correlated with flavonoids and flavanols and negatively correlated with 1,8-cineole. FRAP was positively correlated with flavonoids, flavanols, and β-pinene and

negatively correlated with 1,8-cineole. Flavonoids were correlated with flavanols, while flavanols were positively correlated with α-pinene (Table S4). In the mountainous area, phenols were positively correlated with DPPH and FRAP. FRAP was positively correlated to flavonoids and flavanols. Flavonoids were correlated with flavanols and 1,8-cineole. It has been reported that lavender plants subjected to salinity stress in hydroponics showed increased levels of α-pinene (4.45% and 3.86% 100 mM NaCl) in conjunction with the increased levels of antioxidants [(DPPH, FRAP, ABTS (2,2′-azino-bis-3-ethylbenzthiazoline-6-sulphonic acid), and phenols)] [115]. Additionally, *Sideritis perfoliata* plants that were cultivated under organic cultivation system appeared to have higher antioxidant activity in terms of DPPH, ABTS, FRAP, and total phenolic content and higher content in α-pinene and β-pinene (25.35% and 27.98% for α-pinene and 6.51% and 7.13% for β-pinene) [46,116]. According to Salehi et al. [117], pinenes possess significant bioactive properties and the increased content in mountainous grown plant of laurel observed could partially justify the increased antioxidant activity. Moreover, the low antioxidant activity of 1,8-cineole [117,118] may justify the negative correlation with FRAP and DPPH values observed in our study.

Correlation analysis in lavender revealed that, in plain grown plants, phenols were positively correlated with DPPH, FRAP, and flavonoids. DPPH was positively correlated with flavonoids and FRAP was positively correlated with flavonoids. Flavonoids were positively correlated with 1,8-cineole. Flavanols were positively correlated with borneol (Table S5). In the case of mountainous grown lavender, phenols were positively correlated with DPPH, FRAP, and flavonoids. DPPH was positively correlated with FRAP and FRAP was positively correlated with flavonoids. However, flavonoids were negatively correlated with carvone. Carvone isolated from spearmint exhibited diverse biocidal activities including antioxidant, insecticidal, antifungal, and antibacterial as reviewed by Elmastaş et al. [109].

In lemon verbena grown in plain areas, phenols were positively correlated to DPPH, and flavonoids. DPPH was positively correlated with FRAP and flavonoids. FRAP was positively correlated to flavonoids. Flavonoids were positively correlated with neral (Table S6). In the mountainous area, lemon verbena's phenols were positively correlated with FRAP. FRAP was positively correlated with flavonoids, 1,8-cineole, and negatively correlated with D-limonene, neral, geranial, and caryophyllene oxide. 1,8-cineole exhibited insecticidal and antimicrobial, anti-allergic and anti-inflammatory, hepatoprotective, antitumoral, and gastroprotective action as reviewed by Caldas et al. [119]. In contrast to our study, D-limonene has been associated with significant antioxidant activities [120,121], which is a finding that could be associated with possible interactions among the essential oil components as already reported for rosemary essential oils by Wang et al. [122]. Similar assumptions could be made for geranial, neral, and caryophyllene oxide [123,124].

In rosemary grown in plain areas, phenols were positively correlated with FRAP, flavonoids, and camphor and negatively correlated with α-pinene. FRAP was positively correlated with flavonoids, while flavonoids were negatively correlated with 1,8-cineole (Table S7). In the mountainous area, rosemary phenols were positively correlated with DPPH, FRAP, and flavonoids, but negatively correlated with borneol. DPPH was positively correlated with FRAP and flavonoids. FRAP was positively correlated with flavonoids, while flavonoids were negatively correlated with borneol. The essential oil yield was negatively correlated with 1,8-cineole.

In sage grown in plain areas, phenols were positively correlated with FRAP and flavonoids. DPPH was negatively correlated with α-thujone. FRAP was positively correlated with flavonoids (Table S8). In the mountainous area, sage phenols were positively correlated with FRAP and flavonoids. FRAP was positively correlated with flavonoids. Flavanols were positively correlated with 1,8-cineole. EOs were positively correlated with camphor. When sage subjected to drought stress, total phenols, DPPH, FRAP, ABTS, flavonoids, and EO yield increased and this was reflected to the increased levels of camphor (45.91% full irrigation and 47.93% water deficit) and a-thujone (5.40% water deficit and 1.90% full irrigation) when compared with the plants subjected to full irrigation [125]. Similarly, increased camphor levels were found in *Artemisia herba-alba* plants as well as increased levels of total phenols and

antioxidants [126]. It is widely known that high levels of camphor are toxic, whereas natural borneol is non-toxic [127]. 1,8-cineole also appeared to increase (from 18.61% to 35.42%) along with the increase in salinity levels, while the antioxidant activity and total phenol content of the methanolic extracts of *Salvia mirzayanii* plants also increased in saline conditions [128]. α-thujone exhibited antibacterial, cytotoxic, and antiviral activities [129]. Regarding the negative correlation of DPPH values with α-thujone, it has been previously reported that the specific compounds show low antioxidant activity [130], while it is also considered harmful to human health [131].

In the case of spearmint, correlation analysis in plain revealed that phenols were positively correlated with DPPH and FRAP, while DPPH was positively correlated with FRAP (Table S9). In the mountainous area, phenols were positively correlated with FRAP and flavonoids, while DPPH and FRAP were positively correlated with flavonoids. Flavonoids were positively correlated with flavanols, while flavanols were positively correlated with 1,8-cineole (Table S9). Spearmint plants exposed to multiple stress of salinity and copper toxicity revealed oxidative damage and decreased the levels of antioxidants and the levels of 1,8-cineole (4.46% and 3.55% sal and Cu) in leaves when compared with plants grown without stress [132].

The most important correlations related to antioxidant activity and chemical composition and essential oil compounds content are summarized in Table 11. The correlation analysis of antioxidant activity (DPPH and FRAP) with the major essential oil components and phenolic compounds (total phenols, flavonoids, and flavanols) showed a strong positive correlation of FRAP and DPPH values with total phenols, flavonoids, and flavanols content. However, this correlation varied depending on the species and the growing conditions (plain or mountainous area), which was also reflected in the results presented in Table 2. Moreover, in limited cases, antioxidant activity (DPPH or FRAP values) was positively correlated with essential oil components, as in the case of borneol and β-pinene in artemisia and laurel plants grown in the plain, respectively, or 1,8-cineole in verbena plants grown in the mountainous area. Similarly, antioxidant activity was negatively correlated with 1,8-cineole and α-thujone in laurel and sage plants grown in the plain as well as with neral, geranial, and caryophyllene oxide in lemon verbena plants grown in the mountainous area. These findings indicate that essential oils may contribute to the overall antioxidant activity depending on the species and its growing environment. Correlation analysis in our study was performed only with data from the most abundant compounds of the essential oils of each species, which eliminates the effect of minor compounds in terms of their antioxidant capacity. It is well known that, in natural matrices, synergistic effects may exist among their components and minor compounds can become essential for the antioxidant capacity of plant tissues [40,133–136]. Therefore, essential oils may exhibit higher antioxidant activity than the isolated components, as already reported by Wang et al. [117] for rosemary essential oils. In addition, essential oils may contain conjugated double bonds and phenolic groups with associated functional and antioxidant properties [135].

4. Conclusions

In the present study, we examined the levels of total phenolics, flavonoids, and flavanols content and antioxidant activity of eight MAP species, as affected by the environmental condition-altitude (mountainous versus plain areas) and their interconnection with the essential oil yield and composition. Altitude increased the phenolic compounds content and the antioxidant capacity of specific species such as artemisia, spearmint (total phenols only), and rosemary (flavonoids only), but higher antioxidant capacity was observed in plants grown in the plain compared to the mountainous area. EO yield was also affected by high altitude, by causing increased EO yield in laurel and decreased EO yield in pelargonium, and spearmint in mountainous areas. EO composition was also altered by the altitude. Plant antioxidant activity was positively correlated with total phenols, flavonoids, and flavanols and, in some cases, with specific constituents of the species (i.e., 1,8-cineole in laurel, α-thujone in sage, etc.), even though a varied response was observed depending on the species and the altitude. In conclusion, the effect of growing conditions defined by different environmental conditions-altitudes

may significantly affect antioxidant compounds content and EO yield as well as composition of the studied MAPs in a species-dependent manner. These findings can be used to identify specific locations and ecosystems in which cultivation of MAPs could be introduced for producing high added value products with improved quality and bioactive properties.

Supplementary Materials: The following are available online at http://www.mdpi.com/2073-4395/10/5/727/s1. Tables S1–S9.

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