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# Seasonal Variation of Antioxidant Capacity, Phenols, Minerals and Essential Oil Components of Sage, Spearmint and Sideritis Plants Grown at Different Altitudes

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**Abstract:** Medicinal and aromatic plants are well appreciated for their antioxidant and biocidal activities, while great variation on these activities can be related to the species, environmental conditions and harvesting period. In the present study, the seasonal variation of the antioxidant activity, mineral content, yield and chemical composition of the essential oils (EOs) of sage (*Salvia officinalis* L.), sideritis (*Sideritis perfoliata* L. subsp. *perfoliata*) and spearmint (*Mentha spicata* L.) were tested under two different environmental conditions, each with a different altitude (namely mountainous and plain). Season affected total phenolic content and antioxidant capacity with increased values during winter and lower values during summer period. In summer, plants accumulated more Fe and had higher EO yield, while P and Na were accumulated more in winter. Altitude had a lesser effect on antioxidant capacity of the plants; however, increased minerals (N, K, Na and Ca) accumulation was found in plain areas. Sage plants had the highest antioxidant capacity, Zn content and EO yield. Sideritis had increased Fe content and spearmint plants revealed high N, Na and Mg levels. Furthermore, altitude and season had an impact on the content of main EOs components in all species. FRAP and ABTS were variably correlated with total phenols and minerals, depending on the species, season and altitude. In few cases, antioxidant activity was found to be inversely linked to some EO components (e.g., $\alpha$-thujone in sage). Finally, the antioxidant content, minerals and EO yield and composition of the examined MAPs were all altered by season and altitude. These findings can be utilized to implement sage, sideritis and spearmint farming in specific ecosystems, determining the season and areas for harvesting the plants, in order to produce high-value products.

**Keywords:** antioxidant status; ABTS; FRAP; altitude; total phenols; season; volatile compounds



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## 1. Introduction

Medicinal and aromatic plants (MAPs), commonly known as herbs or spices, and their related plant extracts and essential oils (EOs) have been highly valued and widely used for centuries, of regardless the lack of scientific proof for their actual bioactive mechanisms and functions, which are still under research [1,2]. In the present day, dietary patterns recommend MAPs as functional foods, i.e., foods that provide physiological benefits in addition to the standard dietary requirements, preventing or postponing the onset of chronic diseases [3]. The global interest in MAPs is mirrored in the trade of raw material of MAPs, which is estimated to be around 440,000 tons per year, with a total value of 1.3 billion US dollars, 25% of which is marketed in Europe [4].

Antioxidant-rich foods are popular because they can assist in reducing the burden of age-related chronic diseases by scavenging reactive oxygen species (ROS) [5]. As a result of their well-known antioxidant activity, MAPs have been the target of scientific research, food and pharmaceutical industries [1]. Plants produce a wide range of secondary metabolites, such as phenolic compounds, as part of their defense mechanism against oxidative damage caused by ROS and other abiotic and biotic stressors [2]. These compounds may also protect human health when MAPs and/or their components are consumed through diet [6]. Free radical scavenging, hydrogen atom donation, single oxygen quenching, metal ion chelation and activities as an oxidation substrate are all mechanisms involved in phenolic compounds' antioxidant activity [6].

More than 10,000 species of MAPs have been found in the Mediterranean region, as they are commonly used in the Mediterranean diet [7]. Although these species' major bioactive characteristics have been thoroughly described in ethnobotanical and ethnopharmacological studies [8], more research is needed to reveal and indicate their explicit medicinal- and functional-related qualities as food supplements and unique antioxidants [9]. MAPs' biological activity and phytochemicals greatly vary according to their production area, climatic conditions and genetic material [10]. Specific environmental parameters such as the intensity of light and wind, the average temperature, UV-B radiation, ozone density, and partial carbon dioxide pressure may change between altitudes, according to Kofidis and Bosabalidis [11] and influence the quantitative composition of plant compound mixtures as volatiles and EOs [2]. Commonly, MAPs are harvested more than once per year, under favorable climatic conditions. Therefore, the basic knowledge about seasonal influences on the plant secondary metabolites is important to determine the optimal harvesting period, for high quality products [12,13]. Furthermore, the existence of secondary metabolites with antioxidant capacity, such as phenolic compounds, is frequently related with the bioactive properties of MAPs [14]. However, caution should be exercised before suggesting the use of MAPs in human diet, as large dosages of secondary metabolites and potentially dangerous compounds (e.g., heavy metals, anti-nutritional factors) have been shown to induce severe toxicity and significant health effects in some cases [15]. As a result, more research is required to assess potential toxicity and to determine the recommended daily allowance (RDA) limits, particularly for people with medical issues [16].

Apart from using MAPs as herbs and decoctions, their EOs have found applications in the food and pharmaceutical industries [17]. Several researches on EOs indicated substantial antioxidant [18,19] and antimicrobial properties [20,21], piquing interest in using EOs as natural antioxidants and antimicrobial agents rather than synthetic substances, as the latter are currently being chastised for their negative impacts on human health [20–22]. Despite the increased interest and large number of MAPs around the world, only about 10% among the EOs that are previously known have attracted interest because of their wide range of biological activities [1], and they are extensively used in the food, cosmetics and pharmaceutical industries today [23].

MAPs cultivation in Cyprus has excellent prospects because of the crops' low agrochemicals, irrigation water, manpower and energy requirements [24], as well as their resilience to harsh climatic conditions such as high temperatures, winds and drought [25]. All these important characteristics could contribute to the long-term development of rural communities while at the same time reducing the risks associated with MAP harvesting from the wild, reducing native populations [26]. Even though the soil and climatic conditions on the island are suitable for the growth of MAPs, their cultivation is still limited due to the restricted availability of agricultural area and the increased use of land in actions for tourism and building construction. Based on the foregoing, it is suggested that possible regions and/or crop cultivation practices for high quality and added value MAPs must be evaluated [27], so that farmers can switch to these crops and create profitable and successful farms. Due to the rising worldwide demand for high-value MAPs, Cyprus, which has a lengthy history of MAPs cultivation and use, might become a key location for producing and trading high-quality raw materials of MAPs, even to the more industrialized countries for additional processing.

*Salvia* spp. is the largest and the most well-known genus in the Lamiaceae family, with over 900 medicinal and ornamental species distributed all over the world [28]. The genus *Mentha* spp. belongs also to the Lamiaceae family and includes 25 to 30 species that grow in the temperate regions of Europe, Australia, Africa, Asia, and North America [29]. The genus *Sideritis* L. (Lamiaceae) comprises more than 150 species that can be found in the Mediterranean, the Balkans and the Iberian Peninsula [30]. Several researchers have studied the correlation between total phenolics and/or phenolic compounds content and antioxidant capacity of different MAPs products, such as infusions, decoctions and EOs [14,31–33]. As an example, the antioxidant capacity and phenolics components in ten Serbia MAPs demonstrated a positive correlation between phenolics and tannins, as well as a proportional rise in antioxidants with total phenolics [33]. The correlations between the primary EOs constituents and the phenolics content and the antioxidant capacity of leaves from one hand, and the plant mineral content on the other hand, have received little attention [34]. As a result, in order to enrich the existing knowledge, the aim of this study was (i) to compare selected MAPs grown in Cyprus under various environmental conditions (altitudes; mountainous and plain areas) (ii) to examine the effects of the season on three MAPs, in order to uncover possible correlations between leaf antioxidant activity and their mineral content and their essential oil (EO) yield and composition. The plant species selected for this study were chosen based on their popularity and their wide variety of applications.

## 2. Materials and Methods

### *2.1. Plant Material and Growing Conditions*

The medicinal and aromatic plants used in the present study were sage (*Salvia officinalis* L.), sideritis (*Sideritis perfoliata* L. subsp. *perfoliata*) and spearmint (*Mentha spicata* L.) and the parts used from each species are presented in Table 1. The cultivated plants were initially purchased for both studied areas (plain and mountainous) as seedlings for sage and sideritis and as cuttings for spearmint by the Cypriot National Agricultural Department, and the harvested plantations were aged of 4–5 years for sage and sideritis and of 1–2 years for spearmint.

In the current study, two areas with different climatic conditions (for simplicity the term altitude will be used, given the differences in the microclimates as indicated by Kofidis and Bosabalidis [11]) were selected, the mountainous area of ″Gerasa″ village (34°49′37.59″ N; 33°0′26.46″ E) and the plain area of Limassol (“Akrotiri”; 34°38′1.63″ N; 32°56′3.62″ E). The village area of ″Gerasa″ is located at 623 m above sea level. The climate there is characterized by low temperatures, with approx. four freezing days and the lowest recorded temperature of soil surface during winter down to −8.1 °C (February). The soil in the area has sand/silk texture, pH of 8.04, electrical conductivity (EC) of 0.52 mS $cm^{-1}$, and appeared poor in organic matter (<0.5%) and nitrogen-N 0.26 g $kg^{-1}$. On the contrary, the plain area of ″Akrotiri″ is a seaside area, located at 2 m above sea level, with mild winter and dry-hot summer. The physicochemical characteristics of the soil were: sand/clay loam texture, pH 8.28; EC 0.79 mS $cm^{-1}$; total nitrogen-N 0.89 g $kg^{-1}$; potassium-K 0.68 g $kg^{-1}$; phosphorus-P 0.017 g $kg^{-1}$ and organic matter 2.88%. Detailed climatic conditions of the selected areas are described in supplementary material (Figure S1); mean daytime temperatures were averaged at 16.8 °C and 19.9 °C, and air humidity averaged in 56.3% and 66.1%, for mountain and plain areas, respectively. Maximum daytime temperature reached 42.4 °C and 40.2 °C with 9.3–13.7 mm and 0 mm rainfall for mountain and plain areas, respectively, during the mid-summer period.

Both plantations for plain and mountainous areas were farming by the same producer. Plant species from an organic plantation were harvested from the plain area. Common cultivation practices were applied, plants were frequently irrigated (~weekly/biweekly during the growing period) based on the plant needs and common fertilizers and crop protection means were applied as shown in Table S1. Mountain species were harvested also from an organic plantation, and plants were grown under commercial cultivation practices, but plants received conservation practices (periodical irrigation and few fertilizers or pesticides’ application).

Plant tissue (nine samples/area/species) of the above ground parts (leaves/stems or leaves/stems/flowers) (see also Table 1) was collected in four sampling periods, namely summer (June 2018), autumn (October 2018), winter (January 2019) and spring (April 2019), and transferred within an hour to the laboratory. Each sample was divided in two batch samples: one batch was dried at air-forced oven at 42 °C until constant weight for approximately 3–4 days and used for the essential oil extraction (see Section 2.4) and mineral content determination (see Section 2.3), while the other batch was stored at −20 °C for the chemical analyses described in Section 2.2.

**Table 1.** Lamiaceae family plant species and material used.

| Common Name | Latin Name | Plant Material | Reported Medicinal Properties/Indications |
|---|---|---|---|
| Spearmint | *Mentha spicata* L. | Stem/leaves | Anti-inflammatory, sedative, antimicrobial, antioxidant, carminative, antispasmodic, diuretic, insecticidal, vasoconstrictor, decongestant [29,35,36]. |
| Sideritis | *Sideritis perfoliata* L. subsp. *perfoliata* | Stem/Leaves/flowers | Anti-inflammatory, antimicrobial, vulnerary, antioxidant, antispasmodic, analgesic, stomachic, carminative, anti-rheumatic, anti-ulcerative, digestive, vasoprotective [27,30,37] |
| Sage | *Salvia officinalis* L. | Stem/leaves | Antibacterial, antifungal, anticancer, antiviral, antidiabetic, antimutagenic, antiprotozoal, antidementia, antioxidant, anti-inflammatory, anti-nociceptive, antidementia, antiseptic, antispasmoic, astringent, antihidrotic, hypoglycemic, and hypolipidemic effects [12,38,39] |

### *2.2. Total Phenols Content and Antioxidant and Reducing Activity*

Four samples (0.5 g) of freshly cut plants (pooled by three individual plants/sample) from each treatment (four seasons or two altitudes) were milled with 10 mL methanol (50% $v/v$) [40]. The extracts were centrifuged for 30 min at 4000× $g$ at 4 °C (Sigma 3–18 K, Sigma Laboratory Centrifuge, Taufkirchen, Germany), and the supernatant was transferred to a 15 mL falcon tube for the determination of total phenol content and total antioxidant activity.

The total phenol content of the methanolic extracts was determined by using the Folin–Ciocalteu reagent (Merck), based on previous described procedure [40] and results were expressed as gallic acid equivalents (mg GAE per g of fresh weight). The antioxidant activity of the methanolic plant extracts was determined by using the assays of ferric reducing antioxidant power (FRAP), as previously described by Chrysargyris et al. [41], as well as the 2,2′-azino-bis(3-ethylbenzothiazoline-6-sulphonic acid) (ABTS) assay according to the methodology described by Woidjylo et al. [42] by using standard solution of trolox ((±)-6-Hydroxy-2,5,7,8-tetramethylchromane-2-carboxylic acid). The results were expressed as mg trolox $g^{-1}$ Fw.

### *2.3. Mineral Content*

Dried tissue (0.5 g) from the aerial plant parts from each treatment (four biological replications; each replication was a pool of two individual plants) was ashed (490 °C) and acid-digested (2 N HCl) for mineral extraction. Sodium (Na) and potassium (K) were determined with flame photometry (Lasany Model 1832, Lasany International, Haryana, India), phosphorus (P) with the molybdate/vanadate method (yellow method) by spectrophotometry (Multiskan GO, Thermo Fischer Scientific, Massachusetts, MA, USA), nitrogen (N) with the Kjeldahl method (BUCHI, Digest automat K-439 and Distillation Kjelflex K-360, Flawil, Switzerland) and calcium (Ca), magnesium (Mg), iron (Fe), copper (Cu) and zinc (Zn) by an atomic absorption spectrophotometer (PG Instruments AA500 FG, Leicestershire, UK) following the method of Chrysargyris et al. [43]. Plant minerals content was expressed in g $kg^{-1}$ and mg $kg^{-1}$ of dry weight for macronutrients and micronutrients, respectively.

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

The essential oils were extracted via hydrodistillation using a Clevenger apparatus according to the protocol previously described by the authors [44]. In brief, dried aerial parts (50–60 g for each treatment) of the plants were used for the EO extraction, which 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) [44]. The obtained EOs 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 [44]. 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-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 at 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 through the comparison of their retention indices 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 [44]. The percentage of individual compounds was based on peak area normalization without using correction factors.

*2.5. Statistical Methods*

A three factor (Species, Seasons and Environmental Conditions-namely Altitude) factorial study was carried out. The statistical treatment of the results was carried out using a three-way analysis of variance (ANOVA) by using the IBM SPSS v.22 software for Windows (IBM Corp., Armonk, NY, USA). Duncan's Multiple Range Test was used for the comparison of means in the cases where the effect of Species, Seasons and Altitude and their relevant interactions were significant. Mean values are presented as treatment mean ± SE of four biological measurements ($n$ = 4) for antioxidants and mineral content and of three biological measurements ($n$ = 3) for essential oils analysis. The correlation coefficients between mountainous/plain species and seasons and their antioxidant capacity and essential oil components were also determined. Pairwise metabolite effect correlations were calculated by Pearson's correlation test using the R program.

## 3. Results and Discussion

*3.1. Total Phenols and Antioxidant Capacity*

Phenolic molecules are the most important classes of natural antioxidants, and they are highly correlated with the antioxidant activity in plant tissues [45]. In this study, we determined whether the content of total phenols and the antioxidant activity of three MAP species were affected by the season [46] and by the altitude, as it has been previously reported [32,34]. The light intensity and photoperiod have different effects on the accumulation of plant secondary metabolites in different plants [2]. Table 2 shows the impact of environmental conditions-altitude (mountain *versus* plain), season (summer, autumn, winter and spring) and plant species, on the total phenols content, antioxidant activity, essential oil yield and mineral content of the examined MAP species. The three-way ANOVA revealed that all the examined parameters were affected significantly ($p < 0.001$, $p < 0.01$) by the season, significantly ($p < 0.001$, $p < 0.05$) by the altitude (except for FRAP, leaf Cu and Zn content), and significantly ($p < 0.001$) by the species (except for leaf Cu content). The interaction between the examined factors (seasons, species and altitude) for all the investigated parameters revealed that season * altitude and altitude * species did not affect leaf Zn content (including ABTS levels for the latter). Moreover, the interaction of season * species and the season * altitude * species significantly affected all the other parameters.

**Table 2.** Effects of altitude (mountain vs. plain) and season (summer, autumn, winter and spring) on the content of total phenols (mg GAE $g^{-1}$ Fw), antioxidant and reducing activity (FRAP and ABTS, mg trolox $g^{-1}$ Fw), macronutrient (g $kg^{-1}$) and micronutrient content (mg $kg^{-1}$) content and essential oil (EO) yield (%) in selected medicinal plant species.

| Factors | Season | Altitude | Species | Season * Altitude | Season * Species | Altitude * Species | Season * Altitude * Species |
|---|---|---|---|---|---|---|---|
| Phenols (mg GAE $g^{-1}$) | *** | * | *** | *** | *** | *** | *** |
| FRAP (mg trolox $g^{-1}$) | *** | ns | *** | *** | *** | *** | *** |
| ABTS (mg trolox $g^{-1}$) | *** | *** | *** | *** | *** | ns | *** |
| N (g $kg^{-1}$) | *** | *** | *** | *** | *** | *** | *** |
| K (g $kg^{-1}$) | *** | *** | *** | *** | *** | *** | *** |
| P (g $kg^{-1}$) | *** | *** | *** | *** | *** | *** | *** |
| Na (g $kg^{-1}$) | *** | *** | *** | *** | *** | *** | *** |
| Ca (g $kg^{-1}$) | *** | *** | *** | *** | *** | *** | *** |
| Mg (g $kg^{-1}$) | *** | *** | *** | *** | *** | *** | *** |
| Fe (mg $kg^{-1}$) | *** | *** | *** | *** | *** | ** | *** |
| Zn (mg $kg^{-1}$) | *** | ns | *** | ns | *** | ns | *** |
| Cu (mg $kg^{-1}$) | ** | ns | ns | * | *** | * | ** |
| EO (%) | *** | *** | *** | *** | *** | *** | *** |

ns, *, **, and *** indicate non-significant or significant differences at $p < 0.05$, $p < 0.01$, and $p < 0.001$, respectively, following a three-way ANOVA.

Eco-geographical factors do affect the biosynthesis of secondary metabolites; the effect of collection region on total phenols and antioxidants has previously been reported for *Salvia argentea*, *Salvia officinalis*, *and Salvia verbenaca* [31,47,48]. Altitude and seasonal collection has also been found to affect the levels of phenolics and antioxidant capacity of plant species [34,46,48] among others. Different environmental factors, such as $CO_2$ levels, water availability, temperature and sun radiation, can influence secondary metabolism and stimulate the production of bioactive chemicals [49]. In general, season affected antioxidant status of the examined species. Therefore, plants presented higher total phenols (18.18 ± 1.98 mg GAE $g^{-1}$ Fw) and antioxidant levels based on FRAP (35.31 ± 2.70 mg trolox $g^{-1}$ Fw) and ABTS (24.93 ± 2.76 mg trolox $g^{-1}$ Fw) assays during winter (including spring season for the ABTS levels) in comparison to plants harvested during summer period (Table 3). However, altitude did not affect phenols and the level of antioxidants of the examined species. Interestingly, when comparing all the species regardless the season and the altitude, total phenols levels were higher in sage (21.53 ± 1.25 mg GAE $g^{-1}$ Fw), in comparison to sideritis and spearmint. Similarly, sage revealed the highest antioxidant activity for both FRAP and ABTS assays (34.97 ± 3.08 and 27.94 ± 1.77 mg trolox $g^{-1}$ Fw, respectively), followed by spearmint and then by sideritis. Sideritis also revealed almost half the antioxidant capacity compared to sage. Previous research on the effects of season collection on the total phenols levels in *Rosmarinus officinalis* revealed that during summer, plants had the lowest total phenolics content [46], which is consistent with the current outcomes.

On the other hand, when evaluating the combined effect of the examined factors, mountainous sage plants presented the highest content of total phenols (ranging from 28.13–32.85 mg GAE $g^{-1}$ Fw) during autumn-winter with the highest antioxidant capacity (FRAP; 67.97 ± 4.02 mg trolox $g^{-1}$ Fw and ABTS; 46.29 ± 1.91 mg trolox $g^{-1}$ Fw, respectively) pointed during winter (Table 3). The antioxidant capacity of plants is inversely proportional to overall phenolic content, MAPs with less than 10 mg GAE $g^{-1}$ of the extract having the lowest antioxidant capacity [33], a finding that was also observed in the present study in the case of sideritis (both altitudes) during summer, plain sideritis during autumn, mountainous sideritis during winter and mountainous spearmint during summer (Table 3).

**Table 3.** Effects of altitude (mountain vs. plain) and season (summer, autumn, winter and spring) on the content of total phenols (mg GAE $g^{-1}$ Fw), antioxidant and reducing activity (FRAP and ABTS, mg trolox $g^{-1}$ Fw) and essential oil (EO) yield (%) in selected medicinal plant species.

| Species | Season | Altitude | Total Phenols | FRAP | ABTS | EO |
|---|---|---|---|---|---|---|
| Sage | | | 21.53 ± 1.25 A | 34.97 ± 3.08 A | 27.94 ± 1.77 A | 2.56 ± 0.23 A |
| Sideritis | | | 11.27 ± 1.13 B | 16.73 ± 1.92 B | 14.00 ± 1.95 B | 0.34 ± 0.04 C |
| Spearmint | | | 14.84 ± 0.91 B | 30.66 ± 2.67 A | 18.26 ± 1.85 B | 1.25 ± 0.16 B |
| *Total mean* | | | *15.88 ± 0.81* | *27.45 ± 1.74* | *20.07 ± 1.26* | *1.38 ± 0.14* |
| | Summer | | 12.19 ± 1.37 B | 20.16 ± 3.07 B | 13.29 ± 2.16 B | 1.92 ± 0.31 A |
| | Autumn | | 15.32 ± 1.80 AB | 22.57 ± 2.70 B | 16.95 ± 2.32 AB | 1.93 ± 0.34 A |
| | Winter | | 18.18 ± 1.98 A | 35.31 ± 4.20 A | 24.93 ± 2.76 A | 0.67 ± 0.19 B |
| | Spring | | 17.82 ± 0.69 AB | 31.75 ± 2.70 AB | 25.11 ± 1.68 A | 1.02 ± 0.14 AB |
| | *Total mean* | | *15.88 ± 0.81* | *27.45 ± 1.74* | *20.07 ± 1.26* | *1.38 ± 0.14* |
| | | Mountain | 16.37 ± 1.32 A | 27.37 ± 2.79 A | 21.89 ± 1.97 A | 1.13 ± 0.14 A |
| | | Plain | 15.39 ± 0.93 A | 27.53 ± 2.14 A | 18.24 ± 1.55 A | 1.64 ± 0.24 A |
| | | *Total mean* | *15.88 ± 0.81* | *27.45 ± 1.74* | *20.07 ± 1.26* | *1.38 ± 0.14* |
| Sage | Summer | Mountain | 18.86 ± 0.25 bc [Y] | 21.04 ± 0.56 efghi | 21.17 ± 1.09 defgh | 2.78 ± 0.11 c |
| | | Plain | 18.18 ± 2.01 bc | 30.15 ± 5.92 cdef | 28.65 ± 1.73 bcd | 3.85 ± 0.15 b |
| | Autumn | Mountain | 28.13 ± 1.22 a | 38.63 ± 3.66 bcd | 32.96 ± 2.66 bc | 2.37 ± 0.09 cd |
| | | Plain | 19.28 ± 1.61 bc | 29.83 ± 2.13 cdef | 22.61 ± 2.20 cdefg | 4.76 ± 0.19 a |
| | Winter | Mountain | 32.85 ± 1.56 a | 67.97 ± 4.02 a | 46.29 ± 1.91 a | 1.46 ± 0.05 fg |
| | | Plain | 22.92 ± 0.88 b | 41.51 ± 0.56 bc | 28.62 ± 1.72 bcd | 2.05 ± 0.08 de |
| | Spring | Mountain | 14.83 ± 0.52 cde | 23.15 ± 1.83 efgh | 22.33 ± 2.35 cdefg | 1.48 ± 0.06 fg |
| | | Plain | 17.17 ± 1.25 cd | 27.47 ± 2.93 def | 20.94 ± 1.91 defgh | 1.75 ± 0.07 ef |
| Sideritis | Summer | Mountain | 7.08 ± 0.14 hi | 7.87 ± 0.24 j | 6.31 ± 0.26 ij | 0.35 ± 0.01 hijk |
| | | Plain | 5.70 ± 0.54 i | 9.39 ± 0.90 ij | 3.10 ± 0.18 j | 0.49 ± 0.01 hij |
| | Autumn | Mountain | 12.80 ± 1.59 def | 20.58 ± 2.01 fghi | 17.14 ± 1.31 defghi | 0.67 ± 0.02 h |
| | | Plain | 4.31 ± 1.01 i | 5.27 ± 1.38 j | 3.65 ± 1.10 j | 0.54 ± 0.02 hi |
| | Winter | Mountain | 8.22 ± 0.45 fghi | 11.78 ± 0.62 hij | 11.73 ± 1.57 ghij | 0.09 ± 0.00 jk |
| | | Plain | 19.12 ± 0.39 bc | 32.63 ± 1.37 cde | 26.00 ± 2.09 bcde | 0.12 ± 0.00 jk |
| | Spring | Mountain | 16.31 ± 0.38 cde | 21.34 ± 1.18 efghi | 24.89 ± 6.50 bcdef | 0.21 ± 0.01 ijk |
| | | Plain | 16.65 ± 1.34 cde | 24.97 ± 2.49 efg | 19.23 ± 1.70 defgh | 0.29 ± 0.01 hijk |
| Spearmint | Summer | Mountain | 7.61 ± 0.15 ghi | 11.54 ± 0.39 hij | 9.96 ± 0.92 ghij | 1.39 ± 0.05 fg |
| | | Plain | 15.75 ± 0.44 cde | 40.99 ± 2.66 bc | 10.55 ± 0.47 hij | 2.65 ± 0.10 c |
| | Autumn | Mountain | 12.66 ± 0.86 defg | 26.28 ± 1.88 efg | 14.05 ± 2.42 fghij | 1.35 ± 0.05 fg |
| | | Plain | 14.73 ± 0.31 def | 14.84 ± 1.67 ghij | 11.30 ± 1.57 ghij | 1.90 ± 0.07 e |
| | Winter | Mountain | 14.33 ± 0.21 def | 31.65 ± 0.81 cdef | 20.48 ± 2.00 defgh | 0.23 ± 0.01 ijk |
| | | Plain | 11.67 ± 0.70 efgh | 26.35 ± 1.14 efg | 16.46 ± 1.92 efghi | 0.07 ± 0.00 k |
| | Spring | Mountain | 22.82 ± 0.59 b | 46.65 ± 1.05 b | 35.44 ± 1.11 ab | 1.13 ± 0.04 g |
| | | Plain | 19.18 ± 0.54 bc | 46.95 ± 1.31 b | 27.85 ± 1.49 bcde | 1.29 ± 0.05 g |

[Y] values (means ± SE, $n = 4$) in columns corresponding to the main factors (Altitude, Seasons and Species) followed by the same uppercase letter, and values corresponding to the interaction of the main factors (Altitude, Seasons and Species), which are followed by the same lowercase letter, are not significantly different, $p < 0.05$.

### *3.2. Mineral Content*

The impact of season and altitude on nutrient accumulation in sage, sideritis and spearmint plants is presented in Table 4. In general, season affected P, Na and Fe accumulation in plants, as during winter, plants accumulated more P (3.06 ± 0.25 g $kg^{-1}$) and Na (1.40 ± 0.30 g $kg^{-1}$), while Fe was accumulated more in summer-autumn and winter period, and ranged from 336.07 ± 9.77 to 346.38 ± 16.07 mg $kg^{-1}$. The accumulation of N, K, Ca, Mg, Zn and Cu was not affected by the season. Regarding altitude, plain plants accumulated more N, K, Na and Ca comparing to the mountainous plants, while the latter had higher (up to 8.5%) levels of Fe. Among the examined MAPs, spearmint accumulated more N ($27.96 \pm 1.16$ g kg$^{-1}$), P ($3.07 \pm 0.18$ g kg$^{-1}$), Na ($1.22 \pm 0.22$ g kg$^{-1}$) and Mg ($11.42 \pm 0.43$ g kg$^{-1}$), while increased K levels were found in spearmint ($20.77 \pm 1.10$ g kg$^{-1}$) and sideritis ($18.65 \pm 0.81$ g kg$^{-1}$) plants. Additionally, sideritis plants had high Fe content ($345.29 \pm 17.41$ mg kg$^{-1}$), while sage had high Zn content ($42.76 \pm 2.43$ mg kg$^{-1}$).

On the other hand, when evaluating the combined effect of the examined factors, during summer, mountainous spearmint accumulated more Mg and Cu, while mountainous sage accumulated more P, and plain sideritis had the highest Fe content. During autumn, plain sideritis had the highest K content. During winter, mountainous spearmint presented the highest content on N and K while plain spearmint and sage accumulated more Na and Zn, respectively. Moreover, during winter, plain spearmint revealed the highest content of P. During spring, mountainous sage revealed the highest Ca content and plain spearmint the highest K content (Table 4). Nutrient levels play an important role in the growth and development of the plants, while minerals are mainly uptaken by the plants from soil, or from the application of fertilizers, through soil or foliar. The level of nutrients inside plant tissue may not only affect plant growth but also has effects on the production and the concentration of secondary metabolites as antioxidants and essential oils. The concentration of Germacrene D in basil EO, for example, is impacted by the rate of applied N and the interaction between N and K, but not by the rate of applied K [50]. Rosemary plant growth and oil yield were significantly dependent on N and K application [51].

### *3.3. Essential Oil Yield and Composition*

Several aromatic plants have been documented to have their oil content and composition influenced by weather variables such as ambient temperature and rainfall [52]. It should be noted, however, that the time when the more EO is obtained may not be the time when the oil has the greatest production of the chemical constituent(s) of interest [53]. Table 2 presents the effects of environmental conditions-altitude (mountain versus plain), season (summer, autumn, winter and spring) and species on the EOs yield of the examined MAP species. The three-way ANOVA reveled that EOs' yield was affected by season ($p < 0.01$), altitude ($p < 0.01$), species ($p < 0.001$) and by the interaction of the three factors ($p < 0.001$). Both summer ($1.93 \pm 0.32\%$) and autumn ($1.93 \pm 0.34\%$) seasons resulted in higher EO yield compared to winter ($0.67 \pm 0.19\%$) when the species and altitude factors were not considered. In general, winter harvest significantly reduced EO yield in *Cymbopogon winterianus* independently of the actual harvest time [13]. From February to July, the EO content of sage (*S. officinalis*) plants grown in Italy was increased by more than two-fold [54]. During autumn-winter in Cyprus, rainfall was ranged from 73.1–142.6 mm and 52.1–94.5 mm from November till February, in mountainous and plain areas, respectively (Figure S1). Indeed, rainfall exerts effects on the vegetative stage of the plants and can directly influence the production of EO; however, during winter low temperatures slowed down the production of the secondary metabolites, decreasing the EO yield. Similar observations have been reported previously, when harvesting took place in autumn (end October) in the same island, but in different altitudes [34]. However, other researchers reported that low temperature was positively correlated to the EO yield of R. officinalis in Brazil [46]. Since the production and composition of EO and extracts of plants depend on genetics, environmental conditions and plant part [55], this can explain a good portion of the discrepancy among the results obtained by different studies.

**Table 4.** Effects of altitude (mountain vs. plain) and season (summer, autumn, winter and spring) on the macronutrient (g $kg^{-1}$) and micronutrient content (mg $kg^{-1}$) content in selected medicinal plant species.

| Species | Season | Altitude | N | K | P | Na | Ca | Mg | Fe | Zn | Cu |
|---|---|---|---|---|---|---|---|---|---|---|---|
| Sage | | | 15.71 ± 1.09 B | 15.33 ± 0.85 B | 2.25 ± 0.27 B | 0.74 ± 0.08 AB | 8.22 ± 0.64 A | 8.71 ± 0.28 B | 335.56 ± 10.39 AB | 42.76 ± 2.43 A | 41.48 ± 1.78 A |
| Sideritis | | | 17.87 ± 0.53 B | 18.65 ± 0.81 A | 2.44 ± 0.13 AB | 0.46 ± 0.08 B | 7.37 ± 0.47 A | 8.07 ± 0.56 B | 345.29 ± 17.41 A | 12.76 ± 1.08 B | 42.45 ± 2.82 A |
| Spearmint | | | 27.96 ± 1.16 A | 20.77 ± 1.10 A | 3.07 ± 0.18 A | 1.22 ± 0.22 A | 8.06 ± 0.17 A | 11.42 ± 0.43 A | 297.05 ± 9.09 B | 14.86 ± 1.54 B | 41.81 ± 3.72 A |
| *Total mean* | | | *20.51 ± 0.84* | *18.25 ± 0.59* | *2.59 ± 0.12* | *0.81 ± 0.09* | *7.88 ± 0.27* | *9.40 ± 0.30* | *325.97 ± 7.71* | *23.46 ± 1.91* | *41.91 ± 1.64* |
| | Summer | | 18.94 ± 1.52 A | 16.26 ± 0.67 A | 2.66 ± 0.23 AB | 0.77 ± 0.011 AB | 7.23 ± 0.39 A | 9.81 ± 0.80 A | 346.38 ± 16.07 A | 18.66 ± 2.72 A | 49.07 ± 3.72 A |
| | Autumn | | 19.18 ± 1.65 A | 18.11 ± 0.97 A | 1.99 ± 0.13 B | 0.58 ± 0.06 B | 8.32 ± 0.65 A | 8.71 ± 0.66 A | 346.36 ± 11.72 A | 23.72 ± 3.18 A | 41.67 ± 3.54 A |
| | Winter | | 24.39 ± 1.90 A | 18.69 ± 1.74 A | 3.06 ± 0.28 A | 1.40 ± 0.30 A | 7.97 ± 0.21 A | 10.26 ± 0.45 A | 336.07 ± 9.77 A | 26.33 ± 5.31 A | 39.11 ± 1.65 A |
| | Spring | | 19.54 ± 1.38 A | 19.94 ± 1.01 A | 2.64 ± 0.25 AB | 0.48 ± 0.07 B | 8.01 ± 0.77 A | 8.82 ± 0.38 A | 277.05 ± 17.16 B | 25.13 ± 3.64 A | 37.80 ± 3.40 A |
| | *Total mean* | | *20.51 ± 0.84* | *18.25 ± 0.59* | *2.59 ± 0.12* | *0.81 ± 0.09* | *7.88 ± 0.27* | *9.40 ± 0.30* | *325.97 ± 7.71* | *23.46 ± 1.91* | *41.91 ± 1.64* |
| | | Mountain | 18.41 ± 1.31 B | 16.71 ± 0.98 B | 2.45 ± 0.20 A | 0.57 ± 0.07 B | 7.27 ± 0.40 B | 8.90 ± 0.48 A | 340.67 ± 9.26 A | 23.63 ± 2.71 A | 43.42 ± 2.57 A |
| | | Plain | 22.62 ± 0.93 A | 19.79 ± 0.57 A | 2.73 ± 0.14 A | 1.04 ± 0.16 A | 8.50 ± 0.34 A | 9.91 ± 0.35 A | 311.26 ± 11.96 B | 23.29 ± 2.73 A | 40.41 ± 2.05 A |
| | | *Total mean* | *20.51 ± 0.84* | *18.25 ± 0.59* | *2.59 ± 0.12* | *0.81 ± 0.09* | *7.88 ± 0.27* | *9.40 ± 0.30* | *325.97 ± 7.71* | *23.46 ± 1.91* | *41.91 ± 1.64* |
| Sage | Summer | Mountain | 9.55 ± 0.01 l [Y] | 16.13 ± 0.04 fghij | 4.24 ± 0.01 a | 1.59 ± 0.01 b | 4.65 ± 0.18 ij | 8.62 ± 0.27 defgh | 368.52 ± 11.62 bcd | 37.68 ± 1.00 bcd | 38.90 ± 1.13 bcd |
| | | Plain | 16.12 ± 0.24 hi | 14.20 ± 0.24 ijkl | 1.44 ± 0.03 fg | 1.09 ± 0.00 d | 7.76 ± 0.38 efgh | 7.02 ± 0.38 ghi | 301.86 ± 8.22 defg | 26.40 ± 3.92 def | 32.53 ± 0.56 cd |
| | Autumn | Mountain | 11.31 ± 0.01 k | 15.54 ± 0.11 hijk | 0.86 ± 0.00 h | 0.25 ± 0.02 jk | 3.22 ± 0.41 j | 7.05 ± 0.21 ghi | 294.66 ± 9.76 defg | 35.81 ± 1.13 cde | 51.88 ± 0.45 abc |
| | | Plain | 17.01 ± 0.11 gh | 19.71 ± 0.21 def | 2.22 ± 0.08 de | 0.92 ± 0.04 e | 11.29 ± 0.31 ab | 7.61 ± 0.05 fghi | 314.36 ± 10.00 cdef | 43.82 ± 4.06 bc | 36.42 ± 3.90 bcd |
| | Winter | Mountain | 13.52 ± 0.07 j | 7.66 ± 0.52 m | 1.12 ± 0.13 gh | 0.47 ± 0.00 gh | 6.92 ± 0.22 fghi | 9.78 ± 0.08 cde | 384.27 ± 8.18 bc | 47.32 ± 7.07 abc | 41.92 ± 2.79 bcd |
| | | Plain | 23.23 ± 0.02 ef | 15.58 ± 0.26 hij | 3.87 ± 0.02 abc | 0.83 ± 0.00 e | 8.88 ± 0.96 cdefgh | 9.57 ± 0.19 cdef | 347.60 ± 10.88 bcde | 61.76 ± 4.52 a | 34.19 ± 1.51 cd |
| | Spring | Mountain | 10.73 ± 0.13 kl | 11.99 ± 0.11 kl | 0.88 ± 0.01 gh | 0.46 ± 0.00 gh | 12.46 ± 0.81 a | 9.01 ± 0.05 cdefg | 412.43 ± 12.79 ab | 52.11 ± 4.39 ab | 52.38 ± 7.33 abc |
| | | Plain | 24.26 ± 0.06 de | 21.85 ± 0.25 bcd | 3.42 ± 0.12 c | 0.36 ± 0.02 hij | 10.58 ± 0.21 abc | 11.07 ± 0.13 bc | 260.80 ± 3.58 fgh | 37.17 ± 0.20 bcd | 43.62 ± 3.84 abcd |
| Sideritis | Summer | Mountain | 17.96 ± 0.34 g | 12.79 ± 0.39 jkl | 2.01 ± 0.06 ef | 0.27 ± 0.00 jk | 6.60 ± 0.23 hi | 9.31 ± 1.16 cdef | 356.51 ± 34.04 bcd | 14.59 ± 3.07 fgh | 47.61 ± 5.11 abcd |
| | | Plain | 16.17 ± 0.04 hi | 19.29 ± 2.93 defg | 2.36 ± 0.34 de | 0.32 ± 0.00 ijk | 9.65 ± 0.83 bcde | 6.21 ± 1.23 ij | 466.64 ± 4.26 a | 15.65 ± 3.42 fgh | 49.39 ± 6.45 abcd |
| | Autumn | Mountain | 15.11 ± 0.76 i | 17.08 ± 0.27 efghi | 2.17 ± 0.12 e | 0.24 ± 0.02 jk | 8.54 ± 0.23 cdefgh | 4.08 ± 0.11 j | 407.20 ± 15.15 ab | 6.25 ± 1.22 h | 27.31 ± 2.53 cd |
| | | Plain | 16.37 ± 0.04 hi | 24.40 ± 0.42 a | 1.92 ± 0.08 ef | 0.61 ± 0.06 f | 10.51 ± 0.69 abcd | 10.84 ± 0.25 bc | 398.22 ± 18.55 ab | 13.24 ± 0.82 fgh | 64.44 ± 4.89 ab |
| | Winter | Mountain | 18.17 ± 0.05 g | 15.80 ± 0.39 ghij | 2.05 ± 0.06 e | 0.20 ± 0.00 k | 7.67 ± 0.07 efgh | 6.79 ± 0.01 hi | 364.98 ± 3.34 bcd | 7.39 ± 0.30 gh | 37.36 ± 3.45 bcd |
| | | Plain | 23.95 ± 0.02 ef | 18.79 ± 0.31 defgh | 2.79 ± 0.08 d | 1.48 ± 0.04 bc | 7.87 ± 0.38 efgh | 12.15 ± 0.04 b | 276.22 ± 13.39 efg | 11.64 ± 3.29 fgh | 34.89 ± 3.42 bcd |
| | Spring | Mountain | 17.14 ± 0.17 gh | 23.48 ± 0.39 bc | 3.90 ± 0.04 abc | 0.20 ± 0.01 k | 4.29 ± 0.06 j | 6.09 ± 0.05 ij | 297.69 ± 2.85 defg | 19.31 ± 2.96 fgh | 48.19 ± 9.29 abcd |
| | | Plain | 18.09 ± 0.17 g | 17.61 ± 0.04 efghi | 2.30 ± 0.07 de | 0.34 ± 0.00 hij | 3.86 ± 0.10 j | 9.11 ± 0.38 cdefg | 194.84 ± 13.84 h | 14.01 ± 0.09 fgh | 30.44 ± 0.31 cd |

**Table 4.** *Cont.*

| Species | Season | Altitude | N | K | P | Na | Ca | Mg | Fe | Zn | Cu |
|---|---|---|---|---|---|---|---|---|---|---|---|
| Spearmint | Summer | Mountain | 28.33 ± 0.16 c | 17.29 ± 0.25 efghi | 3.60 ± 0.12 bc | 0.46 ± 0.01 gh | 7.92 ± 0.32 efgh | 15.28 ± 0.25 a | 273.26 ± 0.28 efg | 5.53 ± 0.34 h | 72.74 ± 12.05 a |
| | | Plain | 25.52 ± 0.05 d | 17.90 ± 0.28 efgh | 2.32 ± 0.11 de | 0.89 ± 0.01 e | 6.83 ± 0.01 ghi | 12.43 ± 0.23 b | 311.51 ± 0.58 cdef | 12.11 ± 2.96 fgh | 53.26 ± 3.63 abc |
| | Autumn | Mountain | 22.92 ± 0.24 f | 11.62 ± 0.10 lkl | 2.49 ± 0.14 de | 0.88 ± 0.00 e | 9.07 ± 0.06 bcdefg | 10.48 ± 0.19 bcd | 356.28 ± 19.78 bcd | 22.88 ± 2.19 defg | 33.21 ± 10.41 cd |
| | | Plain | 32.36 ± 0.14 b | 20.30 ± 0.14 cde | 2.32 ± 0.05 de | 0.58 ± 000 fg | 7.30 ± 0.25 efgh | 12.19 ± 0.19 b | 307.48 ± 1.91 defg | 20.31 ± 2.17 efgh | 36.77 ± 3.18 bcd |
| | Winter | Mountain | 37.95 ± 0.50 a | 30.72 ± 0.03 a | 4.13 ± 0.08 ab | 1.43 ± 0.02 c | 8.19 ± 0.11 efgh | 12.34 ± 0.15 b | 321.87 ± 23.57 cdef | 23.92 ± 3.94 def | 39.33 ± 6.98 bcd |
| | | Plain | 29.55 ± 0.31 c | 23.63 ± 0.27 bc | 4.39 ± 0.01 a | 4.02 ± 0.06 a | 8.27 ± 0.41 defgh | 10.97 ± 0.05 bc | 321.48 ± 17.18 cdef | 5.97 ± 0.68 h | 46.96 ± 0.45 abcd |
| | Spring | Mountain | 18.26 ± 0.05 g | 20.51 ± 0.43 cde | 1.94 ± 0.06 ef | 0.44 ± 0.00 hi | 7.73 ± 0.29 efgh | 7.97 ± 0.07 efghi | 250.34 ± 3.78 fgh | 10.81 ± 0.28 fgh | 30.16 ± 8.03 cd |
| | | Plain | 28.77 ± 0.08 c | 24.22 ± 0.04 a | 3.41 ± 0.02 c | 1.09 ± 0.02 d | 9.17 ± 0.48 bcdef | 9.70 ± 0.32 cdef | 234.19 ± 21.86 gh | 17.37 ± 0.03 fgh | 22.05 ± 3.87 d |

[Y] values (means ± SE, $n$ = 4) in columns corresponding to the main factors (Altitude, Seasons and Species) followed by the same uppercase letter, and values corresponding to the interaction of the main factors (Altitude, Seasons and Species), which are followed by the same lowercase letter, are not significantly different, $p < 0.05$.

Indeed, altitude (mountainous versus plain) did not affect the EO yield, which was averaged at 1.38 ± 0.14%. However, in comparison to plants harvested at higher elevations, Formisano et al. [56] found that chamomile harvested at low altitudes (81–89 m) yielded more EOs in comparison to plants harvested at higher elevations (i.e., 640–675 m). Norani et al. [32] showed that the lowest EO yield in *Tussilago farfara* (L.) was found at low altitudes (i.e., 229 m), exhibiting at the same time the highest antioxidant activity when compared to plants growing at higher altitudes. It has been reported that as the altitude increases, the EO yield of *Tanacetum polycephalum* was found increased [57], but opposed findings were observed in case of *Artemisia absinthium* [58]. Mahomoodally et al. [59] found that EO yield varies throughout the year and was found lower in areas with less solar radiation. Different plant species and environmental conditions may provide diverse results. In the present study, altitude did not affect the EO yield and the antioxidant capacity throughout a calendar year, which is of great importance for farmers, if the species and the season period will not be taken into account.

Among the three examined species, when comparing the EO yield, regardless of the altitude and the collection season, sage had the highest yield (2.56 ± 0.23%), followed by spearmint (1.25 ± 0.16%) and then by sideritis (0.34 ± 0.04%). When considering the combined effect of the tested factors, plain sage in autumn had the highest (4.76 ± 0.19%) and plain spearmint in winter the lowest (0.07 ± 0.00%) EO yield, indicating the significance of the different species on this parameter, apart from the growing location. Specifically, the high altitude of the mountainous area decreased sage EOs yield in all seasons and spearmint EOs yield during summer-autumn (Table 3).

The effect of season and/or altitude on the EOs chemical composition of the examined MAP species is given in Tables 5–7. Sabbahi et al. [60] reported that the major components profile of the EOs was impacted by the altitude gradient and the variation of the EO composition was mostly related to the genetic variables. In the case of sage, EOs analysis revealed the presence of 32 individual compounds, representing a total percentage of ≥99.94% of the oil profile for the mountainous and plain plants. The most abundant class (ranged from 61.79% to 83.75%) was oxygenated monoterpenes, followed by monoterpenes hydrocarbon (ranged from 14.33% to 28.34%), oxygenated sesquiterpenes (ranged from 1.96% to 7.59%), and sesquiterpenes hydrocarbons (ranged from 0.19% to 5.53%) for the mountainous and plain plants throughout the seasons (Table 5). The major constituents of the examined sage EOs in decreasing order were 1,8-Cineole (17.48–29.20%), Camphor (11.50–40.35%), α Thujone (0.09–31.94%), Camphene (2.98–8.82%), β Thujone (0.02–7.51%), Limonene (0.95–7.18%), Viridiflorol (1.67–6.95%) and α Pinene (2.89–4.14%). β Pinene, β Myrcene, β Caryophyllene, Borneol, iso-Bornyl acetate and α Humulene varied between 1–4%, while the rest of the compounds were identified in amounts lower than 1% of the total volatile components content (Table 5). Sage plants grown in the mountain had significantly higher content of 1,8-Cineole during summer but the highest content in plain areas occurred during spring season. Camphor content was greater in summer for mountainous and for plain (including autumn) sage while α Thujone was lower in summer and dominated during the rest of the year (Table 5). Holopainen et al. [61] have suggested that monoterpenes are highly volatile terpenoids emitted with warmer temperature than sesquiterpenes, as both 1,8-Cineole and Camphor belong to the monoterpenes class. The increased levels of 1,8-Cineole are of great importance, as it exhibits insecticidal, antimicrobial, antiallergic and anti-inflammatory, hepatoprotective, antitumoral and gastroprotective action, as has been reviewed by Caldas et al. [62]. In *Lippia gracilis* EOs, the percentages of Myrcene, 1,8-Cineole, and $\gamma$ Terpinene did not vary much between seasons, with the dry season revealing the highest percentage, with the exception of Myrcene, which had higher percentages in the rainy season [53]. In *S. officinallis*, the level of 1,8-Cineole and Camphor were constant until August, and then were slightly decreased [12], and this trend was also observed in the present work. According to Bedini et al. [63], α Thujone, Camphor and 1,8-Cineole were the main components of sage EOs, as they were identified in the present study. Cvetkovikj et al. [64] discovered four unique chemotypes in sage populations from

Balkan countries, based on cis-(β) and trans-(α) Thujone and Camphor content and suggested a significant correlation between EO composition and geographic characteristics. Not only environmental conditions, but cultivation practices as well (biofertilizers and biostimulants) can affect the EOs' composition [65]. Indeed, Thujones are toxic components and their absence from the EO of Thujone-containing species makes it intriguing to be investigated [66]. In this study, the lowest Thujone levels were found during the summer period in both mountainous and plain areas. Mineral application, including N, through compost increased the production of EO in basil plants, increased Linalool and Borneol content but decreased 1,8-Cineole levels [67]; a similar situation was observed in the sage plants of this study, grown in plain areas during autumn (i.e., high EO yield and low 1,8-Cineole levels).

In sideritis, EOs analysis revealed the presence of 35 individual compounds for both plain and mountainous plants, representing $\geq$ 99.95% of the total oil profile (Table 6). The main detected class was that of monoterpenes hydrocarbon (ranged from 58.63% to 93.23%) followed by oxygenated sesquiterpenes (ranged from 5.30% to 39.77%), while both sesquiterpenes hydrocarbons and oxygenated monoterpenes were in lower amounts ($\leq$5.89% and $\leq$3.37%, respectively). Accordingly, the major oil constituents in decreasing order were α Pinene (27.85–42.19%), Valeranone (4.74–37.88%), β Phellandrene (12.40–33.69%), β Pinene (5.17–8.45%) and Sabinene (1.66–4.60%), whereas β Caryophyllene, Caryophyllene-9-epi, Cubenol-1-epi, β Myrcene, 3-Carene and Terpinolene varied between 1–4% (Table 6). Summer was the season that α Pinene content was decreased at both mountainous and plain areas, while β Phellandrene content was increased in mountainous sideritis plants in summer. Botrel et al. [68] examined the seasonal effect on the chemical composition of *Hyptis marrubioide* EO and found that the highest levels of EO were found in summer, but the highest proportions of the major components were found in winter. It should be noted that the period with the maximum active compounds production may not be the same as the period with the highest biomass production. An active component is mainly determined by the degree of stress that the plants are exposed to, which triggers the production of EOs while lowering biomass, or vice versa [69].

Spearmint EOs analysis revealed the presence of 37 different constituents for the plain and mountainous plants throughout the four seasons, representing $\geq$ 99.94% of the total oil profile (Table 7). The most abundant class was oxygenated monoterpenes (ranged from 46.18% to 80.39%), followed by monoterpenes hydrocarbon (ranged from 8.64% to 22.71%), sesquiterpenes hydrocarbons (ranged from 2.09% to 9.95%) and oxygenated sesquiterpenes ($\leq$ 0.285%) (Table 7). The major constituents of the examined spearmint EO in decreasing order were Carvone (34.07–74.79%), Dihydro carveol (0.10–16.25%), Sabinene (0.58–15.54%), D Limonene (4.09–14.60%), cis Carvyl acetate (0.06–14.56%) and 1,8-Cineole (3.79–8.84%), whereas β Pinene, neo Dihydro carveol, cis Carveol, β Caryophyllene and Germacrene D varied between 1–4% (Table 7). During winter time, Carvone was lower in mountainous area but greater in plain areas. In general, spearmint grown in plain areas revealed higher Carvone levels during summer, autumn and winter comparing to the relevant plants grown in mountainous areas. With less sunshine during winter, photosynthesis slows down, resulting in lower amounts of energy available for plant growth and development, resulting in lower production of metabolites as terpenes [68]. Instead, the energy provided by the plant is used to synthesize primary metabolites and maintain growth and development [69]. Several other studies found Carvone to be the most abundant spearmint EO component [70,71], but agronomic conditions, including salinity and water stress, may influence EOs yield and composition [44]. Carvone extracted from spearmint has a wide range of biocidal properties, including antioxidant, insecticidal, antifungal and antibacterial properties, as reviewed by Elmastaş et al. [70]. Talebi et al. [72] reported that the increased oxygenated compounds of EOs from Nepeta species from high altitudes (2290 m versus 1920 m) can be related to the high doses of different UV radiation that lead to the development of oxidative stress in plants. Such trend could be the case for mountainous winter sage and sideritis, but not for spearmint.

**Table 5.** Chemical composition (%) of essential oils of sage plants.

| | | Mountain | | | | Plain | | | |
|---|---|---|---|---|---|---|---|---|---|
| **Compound** | **RI** | **Summer** | **Autumn** | **Winter** | **Spring** | **Summer** | **Autumn** | **Winter** | **Spring** |
| Tricyclene | 921 | 0.123 ± 0.003 b | 0.186 ± 0.005 a | 0.192 ± 0.005 a | 0.071 ± 0.006 d | 0.102 ± 0.003 c | 0.101 ± 0.003 c | 0.181 ± 0.005 a | 0.025 ± 0.001 e |
| $\alpha$ Thujene | 926 | 0.139 ± 0.004 c | 0.128 ± 0.003 c | 0.048 ± 0.001 e | 0.091 ± 0.002 d | 0.079 ± 0.002 d | 0.053 ± 0.002 e | 0.221 ± 0.006 b | 0.289 ± 0.002 a |
| $\alpha$ Pinene | 933 | **3.336 ± 0.081 cd** | **3.67 ± 0.09 bc** | **3.681 ± 0.090 bc** | **2.892 ± 0.009 d** | **4.141 ± 0.101 a** | **3.933 ± 0.096 ab** | **4.088 ± 0.100 ab** | **3.064 ± 0.014 d** |
| Camphene | 948 | **6.562 ± 0.16 c** | **5.911 ± 0.145 c** | **6.448 ± 0.158 c** | **4.812 ± 0.007 d** | **8.824 ± 0.215 a** | **7.58 ± 0.185 b** | **5.757 ± 0.141 c** | **2.989 ± 0.015 e** |
| Sabinene | 973 | 0.039 ± 0.001 b | 0.042 ± 0.001 b | 0 ± 0 d | 0.018 ± 0.004 c | 0 ± 0 d | 0 ± 0 d | 0.034 ± 0.001 b | 0.056 ± 0.001 a |
| $\beta$ Pinene | 977 | **2.364 ± 0.058 c** | **2.205 ± 0.054 c** | **1.533 ± 0.038 ef** | **1.857 ± 0.003 d** | **1.752 ± 0.043 de** | **1.433 ± 0.035 f** | **2.720 ± 0.067 b** | **3.380 ± 0.007 a** |
| $\beta$ Myrcene | 989 | **1.452 ± 0.036 f** | **1.645 ± 0.040 ef** | **1.147 ± 0.028 g** | **1.958 ± 0.006 cd** | **2.183 ± 0.053 c** | **1.858 ± 0.045 de** | **3.167 ± 0.077 b** | **3.791 ± 0.024 a** |
| $\alpha$ Phellandrene | 1004 | 0.052 ± 0.001 c | 0.020 ± 0.001 d | 0.008 ± 0.000 e | 0 ± 0 e | 0.194 ± 0.005 a | 0.105 ± 0.003 b | 0.058 ± 0.002 c | 0.023 ± 0.000 d |
| $\alpha$ Terpinene | 1017 | 0.164 ± 0.004 c | 0.103 ± 0.003 d | 0.038 ± 0.001 e | 0.110 ± 0.009 d | 0.154 ± 0.004 c | 0.159 ± 0.004 c | 0.394 ± 0.01 b | 0.433 ± 0.003 a |
| p Cymene | 1024 | 0.428 ± 0.011 e | 1.482 ± 0.036 b | 1.662 ± 0.041 a | 1.095 ± 0.011 c | 0.243 ± 0.006 f | 0.763 ± 0.019 d | 1.404 ± 0.035 b | 0.402 ± 0.004 e |
| Limonene | 1028 | **3.215 ± 0.078 c** | **1.446 ± 0.035 d** | **1.407 ± 0.034 de** | **1.167 ± 0.022 de** | **7.187 ± 0.176 a** | **5.420 ± 0.132 b** | **1.568 ± 0.038 d** | **0.955 ± 0.014 e** |
| 1,8-Cineole | 1031 | **29.206 ± 0.713 a** | **21.605 ± 0.527 b** | **21.39 ± 0.522 b** | **21.329 ± 0.129 b** | **19.737 ± 0.482 bc** | **17.482 ± 0.426 c** | **21.163 ± 0.517 b** | **27.762 ± 0.005 a** |
| $\gamma$ Terpinene | 1058 | 0.278 ± 0.007 c | 0.146 ± 0.004 e | 0.058 ± 0.002 f | 0.257 ± 0.008 cd | 0.257 ± 0.006 cd | 0.222 ± 0.006 d | 0.604 ± 0.015 b | 0.858 ± 0.000 a |
| Terpinolene | 1089 | 0.754 ± 0.019 c | 0.044 ± 0.001 d | 0 ± 0 d | 0 ± 0 d | 3.226 ± 0.079 a | 1.36 ± 0.033 b | 0.087 ± 0.002 d | 0.114 ± 0.005 d |
| Linalool | 1100 | 0.075 ± 0.002 c | 0.074 ± 0.002 c | 0 ± 0 e | 0.029 ± 0.003 d | 0.253 ± 0.006 a | 0.161 ± 0.004 b | 0.035 ± 0.001 d | 0.065 ± 0.001 c |
| $\alpha$ Thujone | 1105 | **0.274 ± 0.007 d** | **31.286 ± 0.763 a** | **31.948 ± 0.779 a** | **29.678 ± 0.183 a** | **0.093 ± 0.003 d** | **11.079 ± 0.27 c** | **29.977 ± 0.731 a** | **23.103 ± 0.061 b** |
| $\beta$ Thujone | 1122 | **0.023 ± 0.001 e** | **5.802 ± 0.142 c** | **5.41 ± 0.132 c** | **5.503 ± 0.009 c** | **0.045 ± 0.001 e** | **2.866 ± 0.07 d** | **6.623 ± 0.162 b** | **7.506 ± 0.007 a** |
| trans Sabinol | 1138 | 0 ± 0 e | 0.158 ± 0.004 a | 0.017 ± 0.001 de | 0.027 ± 0.007 d | 0 ± 0 e | 0.078 ± 0.002 bc | 0.087 ± 0.002 b | 0.064 ± 0.002 c |
| Camphor | 1145 | **40.358 ± 0.984 a** | **19.794 ± 0.483 bc** | **23.484 ± 0.573 b** | **18.963 ± 0.118 c** | **39.889 ± 0.973 a** | **40.055 ± 0.977 a** | **13.959 ± 0.341 d** | **11.502 ± 0.043 d** |
| Borneol | 1166 | **0.568 ± 0.014 e** | **1.744 ± 0.043 ab** | **1.037 ± 0.026 c** | **1.807 ± 0.041 a** | **0.37 ± 0.009 f** | **0.747 ± 0.019 d** | **1.595 ± 0.039 c** | **0.601 ± 0.01 de** |
| Terpinen-4-ol | 1178 | 0.545 ± 0.014 c | 0.715 ± 0.018 a | 0.418 ± 0.01 d | 0.356 ± 0.022 d | 0.584 ± 0.015 bc | 0.654 ± 0.016 ab | 0.574 ± 0.014 bc | 0.2 ± 0.001 e |
| p-Cymen-8-ol | 1185 | 0.099 ± 0.003 c | 0.032 ± 0.001 d | 0.011 ± 0.001 e | 0 ± 0 e | 0.250 ± 0.006 b | 0.28 ± 0.007 a | 0 ± 0 e | 0 ± 0 e |
| $\alpha$ Terpineol | 1191 | 0.098 ± 0.003 c | 0.074 ± 0.002 cd | 0.019 ± 0.001 e | 0.012 ± 0.012 e | 0.271 ± 0.007 a | 0.178 ± 0.005 b | 0.025 ± 0.001 e | 0.065 ± 0.001 d |
| iso-Bornyl acetate | 1284 | **0.793 ± 0.02 b** | **0.326 ± 0.008 d** | **0.168 ± 0.005 e** | **0.077 ± 0.008 e** | **2.669 ± 0.065 a** | **0.888 ± 0.022 b** | **0.147 ± 0.004 e** | **0.477 ± 0.001 c** |
| trans Sabinyl acetate | 1293 | 0 ± 0 d | 0.051 ± 0.001 c | 0.028 ± 0.001 c | 0 ± 0 d | 0.058 ± 0.002 b | 0.19 ± 0.005 a | 0.050 ± 0.002 b | 0.029 ± 0.002 c |
| $\alpha$ Terpinyl acetate | 1349 | 0.196 ± 0.005 c | 0.215 ± 0.006 c | 0 ± 0 d | 0 ± 0 d | 0.573 ± 0.014 a | 0.266 ± 0.007 b | 0 ± 0 d | 0 ± 0 d |
| $\beta$ Caryophyllene | 1425 | **2.764 ± 0.068 b** | **0 ± 0 e** | **0.032 ± 0.001 e** | **1.371 ± 0.047 c** | **2.741 ± 0.067 b** | **0.95 ± 0.024 d** | **0.813 ± 0.02 d** | **3.105 ± 0.017 a** |

**Table 5.** *Cont.*

| | | Mountain | | | | Plain | | | |
|---|---|---|---|---|---|---|---|---|---|
| Compound | RI | Summer | Autumn | Winter | Spring | Summer | Autumn | Winter | Spring |
| $\delta$ Cadinene | 1522 | 0.039 ± 0.001 c | 0 ± 0 e | 0 ± 0 e | 0 ± 0 e | 0.091 ± 0.002 a | 0.057 ± 0.002 b | 0.024 ± 0.001 d | 0 ± 0 e |
| Caryophyllene oxide | 1587 | 0.519 ± 0.013 a | 0.234 ± 0.006 c | 0.029 ± 0.001 de | 0.043 ± 0.016 de | 0.458 ± 0.0110 b | 0.227 ± 0.006 c | 0.073 ± 0.002 d | 0 ± 0 e |
| Viridiflorol | 1591 | **6.953 ± 0.170 a** | **2.189 ± 0.054 c** | **1.676 ± 0.041 c** | **4.919 ± 0.129 b** | **4.476 ± 0.110 b** | **2.137 ± 0.053 c** | **4.463 ± 0.109 b** | **6.515 ± 0.017 a** |
| Humulene epoxide II | 1608 | 0.121 ± 0.003 c | 0.499 ± 0.012 a | 0.261 ± 0.007 b | 0.234 ± 0.033 b | 0.114 ± 0.003 c | 0.214 ± 0.005 b | 0.515 ± 0.013 a | 0.007 ± 0.007 d |
| Total Identified | | 99.850 ± 0.02 | 99.711 ± 0.02 | 99.881 ± 0.02 | 99.906 ± 0.01 | 99.947 ± 0.02 | 99.881 ± 0.02 | 99.757 ± 0.02 | 99.907 ± 0.005 |
| Monoterpenes hydrocarbons | | 18.904 ± 0.461 cd | 17.025 ± 0.416 de | 16.25 ± 0.396 ef | 14.334 ± 0.043 f | 28.34 ± 0.691 a | 22.985 ± 0.561 b | 20.279 ± 0.495 c | 16.434 ± 0.089 def |
| Sesquiterpenes hydrocarbons | | 3.535 ± 0.086 c | 0.308 ± 0.008 f | 0.196 ± 0.005 f | 2.586 ± 0.085 d | 3.886 ± 0.095 b | 1.764 ± 0.043 e | 2.642 ± 0.065 d | 5.531 ± 0.02 a |
| Oxygenated monoterpenes | | 71.305 ± 1.739 c | 81.338 ± 1.984 ab | 83.75 ± 2.043 a | 77.714 ± 0.323 abc | 61.793 ± 1.507 d | 73.689 ± 1.797 bc | 74.064 ± 1.807 bc | 70.915 ± 0.093 c |
| Oxygenated sesquiterpenes | | 7.593 ± 0.185 a | 2.922 ± 0.072 d | 1.966 ± 0.048 e | 5.196 ± 0.178 c | 5.048 ± 0.123 c | 2.577 ± 0.063 de | 5.051 ± 0.124 c | 6.522 ± 0.024 b |
| Others | | 0.988 ± 0.024 c | 0.592 ± 0.015 d | 0.196 ± 0.005 e | 0.077 ± 0.008 e | 3.359 ± 0.082 a | 1.343 ± 0.033 b | 0.196 ± 0.005 e | 0.507 ± 0.003 d |

Values ($n$ = 3) in rows followed by the same letter are not significantly different, $p \leq 0.05$. In bold indicated EO components > 1%.

**Table 6.** Chemical composition (%) of essential oils of sideritis plants.

| | | Mountain | | | | Plain | | | |
|---|---|---|---|---|---|---|---|---|---|
| Compound | RI | Summer | Autumn | Winter | Spring | Summer | Autumn | Winter | Spring |
| $\alpha$ Thujene | 926 | 0.642 ± 0.019 c | 0.807 ± 0.024 aa | 0.153 ± 0.005 cd | 0.211 ± 0.004 de | 0.710 ± 0.039 ab | 0.701 ± 0.021 ab | 0.341 ± 0.01 cd | 0.474 ± 0.002 c |
| $\alpha$ Pinene | 933 | **27.855 ± 0.811 d** | **37.753 ± 1.100 b** | **31.688 ± 0.923 cd** | **42.196 ± 0.151 a** | **27.985 ± 0.589 d** | **35.378 ± 1.031 bc** | **33.84 ± 0.986 bc** | **32.68 ± 0.151 c** |
| Camphene | 948 | 0.04 ± 0.001 c | 0.105 ± 0.003 a | 0 ± 0 d | 0.023 ± 0.006 c | 0.083 ± 0.005 b | 0.071 ± 0.002 b | 0 ± 0 d | 0.040 ± 0.001 c |
| Sabinene | 973 | **4.005 ± 0.117 a** | **3.944 ± 0.115 a** | **1.663 ± 0.049 c** | **2.531 ± 0.016 b** | **4.602 ± 0.207 a** | **4.326 ± 0.126 a** | **2.610 ± 0.076 b** | **3.975 ± 0.018 a** |
| $\beta$ Pinene | 977 | **5.176 ± 0.151 d** | **8.458 ± 0.247 a** | **5.24 ± 0.153 cd** | **7.291 ± 0.038 b** | **7.136 ± 0.262 b** | **7.173 ± 0.209 b** | **6.292 ± 0.184 bc** | **5.961 ± 0.001 cd** |
| $\beta$ Myrcene | 989 | **2.711 ± 0.079 b** | **3.616 ± 0.106 a** | **0.789 ± 0.023 d** | **0.432 ± 0.010 d** | **2.459 ± 0.12 b** | **2.859 ± 0.083 b** | **0.477 ± 0.014 d** | **1.348 ± 0.010 c** |
| $\alpha$ Phellandrene | 1005 | 0.843 ± 0.025 a | 0.775 ± 0.023 a | 0.056 ± 0.002 d | 0.038 ± 0.008 d | 0.886 ± 0.038 a | 0.807 ± 0.024 a | 0.203 ± 0.006 c | 0.427 ± 0.009 b |
| 3-Carene | 1013 | **2.982 ± 0.087 ab** | **3.153 ± 0.092 a** | **1.036 ± 0.030 d** | **0.569 ± 0.020 e** | **2.294 ± 0.016 c** | **2.775 ± 0.081 b** | **1.126 ± 0.033 d** | **2.148 ± 0.002 c** |
| $\alpha$ Terpinene | 1017 | 0.217 ± 0.007 a | 0.172 ± 0.005 b | 0 ± 0 e | 0 ± 0 e | 0.179 ± 0.005 b | 0.158 ± 0.005 b | 0.112 ± 0.004 c | 0.071 ± 0.000 d |
| p Cymene | 1022 | 0.040 ± 0.001 cd | 0.057 ± 0.002 bc | 0 ± 0 d | 0.028 ± 0.006 cd | 0.155 ± 0.006 a | 0.044 ± 0.002 cd | 0.094 ± 0.003 b | 0.022 ± 0.022 cd |
| *o* Cymene | 1024 | 0.285 ± 0.008 d | 0.494 ± 0.014 b | 0.345 ± 0.010 c | 0.250 ± 0.001 d | 0 ± 0 e | 0.369 ± 0.011 c | 0.634 ± 0.019 a | 0.235 ± 0.002 d |

**Table 6.** *Cont.*

| | | Mountain | | | | Plain | | | |
|---|---|---|---|---|---|---|---|---|---|
| **Compound** | **RI** | **Summer** | **Autumn** | **Winter** | **Spring** | **Summer** | **Autumn** | **Winter** | **Spring** |
| $\beta$ Phellandrene | 1029 | **33.696 ± 0.982 a** | **30.551 ± 0.890 ab** | **17.235 ± 0.502 d** | **12.400 ± 0.054 e** | **26.659 ± 0.644 c** | **30.900 ± 0.900 ab** | **13.75 ± 0.401 de** | **27.392 ± 0.033 bc** |
| $\gamma$ Terpinene | 1058 | 0.495 ± 0.015 a | 0.465 ± 0.014 a | 0 ± 0 d | 0.038 ± 0.008 d | 0.376 ± 0.013 b | 0.371 ± 0.011 b | 0.261 ± 0.008 c | 0.246 ± 0.003 c |
| Terpinolene | 1089 | **3.365 ± 0.098 a** | **2.889 ± 0.084 b** | **0.431 ± 0.013 f** | **0.129 ± 0.010 g** | **2.481 ± 0.036 c** | **2.890 ± 0.084 b** | **0.854 ± 0.025 e** | **1.535 ± 0.005 d** |
| trans Sabinene hydrate | 1100 | 0.115 ± 0.003 c | 0.110 ± 0.003 c | 0.341 ± 0.01 b | 0.008 ± 0.008 d | 0.472 ± 0.014 a | 0.115 ± 0.004 c | 0.120 ± 0.004 c | 0.088 ± 0.005 c |
| $\alpha$ Thujone | 1106 | 0 ± 0 b | 0.739 ± 0.022 a | 0.77 ± 0.023 a | 0.057 ± 0.015 b | 0 ± 0 b | 0 ± 0 b | 0 ± 0 b | 0 ± 0 b |
| $\alpha$ Campholenal | 1127 | 0.042 ± 0.001 f | 0.069 ± 0.002 ed | 0.418 ± 0.013 c | 0.773 ± 0.023 b | 0 ± 0 f | 0.204 ± 0.006 d | 1.413 ± 0.041 a | 0.141 ± 0.002 de |
| Camphor | 1145 | 0.173 ± 0.005 b | 0.185 ± 0.006 b | 0 ± 0 c | 0.638 ± 0.044 a | 0 ± 0 c | 0.049 ± 0.001 c | 0 ± 0 c | 0.024 ± 0.000 c |
| pinocarvone | 1163 | 0 ± 0 d | 0 ± 0 d | 0 ± 0 d | 0.172 ± 0.012 b | 0 ± 0 d | 0.059 ± 0.002 c | 0.429 ± 0.013 a | 0.013 ± 0.001 d |
| Terpinen-4-ol | 1178 | 0.069 ± 0.002 cd | 0.345 ± 0.011 a | 0 ± 0 e | 0 ± 0 e | 0.225 ± 0.018 b | 0.120 ± 0.004 c | 0.222 ± 0.007 b | 0.034 ± 0.002 de |
| Myrtenal | 1193 | 0 ± 0 d | 0 ± 0 d | 0.066 ± 0.002 c | 0.138 ± 0.013 b | 0 ± 0 d | 0.069 ± 0.002 c | 0.588 ± 0.018 a | 0.015 ± 0.001 d |
| Decanal | 1204 | 0.435 ± 0.013 a | 0.167 ± 0.005 c | 0 ± 0 d | 0 ± 0 d | 0.174 ± 0.002 c | 0.224 ± 0.007 b | 0 ± 0 d | 0 ± 0 d |
| Carvone | 1244 | 0.806 ± 0.024 a | 0.176 ± 0.005 b | 0.318 ± 0.009 b | 0 ± 0 b | 0.368 ± 0.139 b | 0.090 ± 0.003 b | 0.198 ± 0.006 b | 0.024 ± 0.001 b |
| $\beta$ Bourbonene | 1386 | 0.169 ± 0.005 c | 0.140 ± 0.004 c | 0 ± 0 d | 0.229 ± 0.006 b | 0.169 ± 0.008 c | 0.154 ± 0.004 c | 0.3 ± 0.009 a | 0.158 ± 0.001 c |
| $\beta$ Caryophyllene | 1425 | **2.354 ± 0.069 c** | **0.542 ± 0.016 e** | **0.816 ± 0.024 e** | **2.821 ± 0.069 b** | **2.900 ± 0.122 b** | **1.406 ± 0.041 d** | **2.143 ± 0.063 c** | **3.403 ± 0.021 a** |
| Caryophyllene-9-epi | 1479 | **1.350 ± 0.039 a** | **0.400 ± 0.012 c** | **0 ± 0 d** | **0 ± 0 d** | **0.974 ± 0.071 b** | **0.869 ± 0.025 b** | **0 ± 0 d** | **0 ± 0 d** |
| Germacrene D | 1495 | 0.453 ± 0.013 d | 0.223 ± 0.007 d | 0.200 ± 0.006 d | 1.276 ± 0.023 b | 1.759 ± 0.090 a | 0.972 ± 0.028 c | 0.974 ± 0.029 c | 1.050 ± 0.032 bc |
| Germacrene B | 1559 | 0.756 ± 0.022 bc | 0.313 ± 0.009 e | 0.155 ± 0.005 f | 0.844 ± 0.052 b | 0 ± 0 g | 0.474 ± 0.014 d | 1.542 ± 0.045 a | 0.671 ± 0.019 c |
| Caryophyllene oxide | 1587 | **0.467 ± 0.014 d** | **0 ± 0 e** | **1.592 ± 0.046 b** | **1.590 ± 0.029 b** | **0.127 ± 0.005 e** | **0.103 ± 0.003 e** | **3.435 ± 0.100 a** | **0.699 ± 0.006 c** |
| Viridiflorol | 1592 | 0.242 ± 0.007 b | 0 ± 0 c | 0 ± 0 c | 0 ± 0 c | 0.206 ± 0.020 b | 0 ± 0 c | 0.309 ± 0.009 a | 0 ± 0 c |
| Cubenol-1-epi | 1617 | **1.171 ± 0.035 b** | **0.557 ± 0.016 cd** | **0.297 ± 0.009 d** | **1.275 ± 0.006 b** | **1.834 ± 0.113 a** | **0.756 ± 0.022 c** | **1.456 ± 0.043 b** | **1.229 ± 0.023 b** |
| Valeranone | 1673 | **10.393 ± 0.303 de** | **4.745 ± 0.138 f** | **37.884 ± 1.104 a** | **21.853 ± 0.579 b** | **11.242 ± 0.848 d** | **6.972 ± 0.203 ef** | **24.593 ± 0.717 b** | **14.953 ± 0.050 c** |
| $\delta$ Dodecalactone | 1704 | 0.222 ± 0.007 cd | 0.158 ± 0.005 d | 0.484 ± 0.014 b | 0.913 ± 0.059 a | 0.407 ± 0.068 bc | 0.235 ± 0.007 cd | 1.062 ± 0.031 a | 0.394 ± 0.031 bcd |
| Isokaurene | 1990 | 0.465 ± 0.014 a | 0.317 ± 0.009 b | 0 ± 0 c | 0 ± 0 c | 0.292 ± 0.045 b | 0.505 ± 0.015 a | 0 ± 0 c | 0.011 ± 0.011 c |
| Sclareol | 2135 | 0.666 ± 0.02 a | 0.103 ± 0.003 cd | 0 ± 0 d | 0.106 ± 0.012 cd | 0.302 ± 0.082 bc | 0.502 ± 0.015 ab | 0 ± 0 d | 0 ± 0 d |
| Total Identified | | 99.764 ± 0.020 | 99.951 ± 0.020 | 99.021 ± 0.020 | 99.003 ± 0.028 | 98.04 ± 0.297 | 99.863 ± 0.0020 | 97.529 ± 0.020 | 99.444 ± 0.057 |
| Monoterpenes hydrocarbons | | 82.392 ± 2.400 ab | 93.234 ± 2.716 a | 58.633 ± 1.708 d | 66.191 ± 0.331 cd | 76.042 ± 1.999 bc | 88.82 ± 2.587 a | 60.808 ± 1.771 d | 76.525 ± 0.159 bc |
| Sesquiterpenes hydrocarbons | | 5.081 ± 0.148 a | 1.616 ± 0.047 c | 1.171 ± 0.034 c | 5.170 ± 0.149 a | 5.892 ± 0.294 a | 3.875 ± 0.113 b | 4.957 ± 0.144 a | 5.298 ± 0.072 a |

**Table 6.** *Cont.*

| | | Mountain | | | | Plain | | | |
|---|---|---|---|---|---|---|---|---|---|
| **Compound** | **RI** | **Summer** | **Autumn** | **Winter** | **Spring** | **Summer** | **Autumn** | **Winter** | **Spring** |
| Oxygenated monoterpenes | | 1.640 ± 0.048 bc | 2.116 ± 0.062 b | 1.912 ± 0.056 b | 1.844 ± 0.136 b | 1.521 ± 0.234 bc | 0.971 ± 0.029 cd | 3.374 ± 0.098 a | 0.338 ± 0.010 d |
| Oxygenated sesquiterpenes | | 12.273 ± 0.358 e | 5.302 ± 0.154 f | 39.772 ± 1.159 a | 24.718 ± 0.544 c | 13.409 ± 0.986 de | 7.832 ± 0.228 f | 29.793 ± 0.868 b | 16.879 ± 0.021 d |
| Others | | 1.352 ± 0.040 a | 0.663 ± 0.020 bcd | 0.484 ± 0.014 cd | 1.081 ± 0.045 abc | 1.177 ± 0.188 ab | 1.340 ± 0.039 a | 1.502 ± 0.044 a | 0.405 ± 0.042 d |

Values ($n$ = 3) in rows followed by the same letter are not significantly different, $p \leq 0.05$. In bold indicated EO components > 1%.

**Table 7.** Chemical composition (%) of essential oils of spearmint plants.

| | | Mountain | | | | Plain | | | |
|---|---|---|---|---|---|---|---|---|---|
| **Compound** | **RI** | **Summer** | **Autumn** | **Winter** | **Spring** | **Summer** | **Autumn** | **Winter** | **Spring** |
| $\alpha$ Pinene | 933 | 0.892 ± 0.026 bc | 0.835 ± 0.025 c | 0.705 ± 0.021 d | 1.003 ± 0.022 ab | 0.999 ± 0.010 ab | 1.039 ± 0.030 a | 0.918 ± 0.027 ab | 0.837 ± 0.004 c |
| Camphene | 948 | 0.087 ± 0.003 bc | 0.095 ± 0.003 bc | 0.079 ± 0.003 bc | 0.158 ± 0.017 a | 0.068 ± 0.001 c | 0.082 ± 0.003 bc | 0.063 ± 0.002 c | 0.104 ± 0.001 b |
| Sabinene | 973 | **0.586 ± 0.017 d** | **1.291 ± 0.038 d** | **15.547 ± 0.453 a** | **3.608 ± 0.033 c** | **0.687 ± 0.018 d** | **0.685 ± 0.020 d** | **4.798 ± 0.140 b** | **1.032 ± 0.008 d** |
| $\beta$ Pinene | 977 | **1.200 ± 0.035 cd** | **1.238 ± 0.036 bcd** | **1.165 ± 0.034 d** | **1.592 ± 0.001 a** | **1.39 ± 0.010 b** | **1.358 ± 0.040 bc** | **1.336 ± 0.039 bc** | **1.299 ± 0.004 bcd** |
| $\beta$ Myrcene | 989 | 0.427 ± 0.013 d | 0.570 ± 0.017 c | 0.335 ± 0.010 de | 0.739 ± 0.031 ab | 0.768 ± 0.045 a | 0.652 ± 0.019 abc | 0.269 ± 0.008 e | 0.634 ± 0.004 bc |
| 3-Octanol | 1003 | 0.067 ± 0.002 cd | 0.277 ± 0.008 a | 0.075 ± 0.003 cd | 0.027 ± 0.027 d | 0.139 ± 0.005 b | 0.158 ± 0.005 b | 0.111 ± 0.004 bc | 0.065 ± 0.004 cd |
| $\alpha$ Terpinene | 1005 | 0.062 ± 0.002 c | 0.047 ± 0.001 cd | 0.231 ± 0.007 a | 0.129 ± 0.010 b | 0.043 ± 0.005 cd | 0.022 ± 0.001 cd | 0.134 ± 0.004 b | 0.018 ± 0.018 d |
| D Limonene | 1028 | **5.185 ± 0.151 b** | **6.842 ± 0.199 b** | **4.091 ± 0.119 b** | **5.171 ± 0.030 b** | **14.603 ± 1.313 a** | **11.913 ± 0.347 a** | **4.731 ± 0.138 b** | **6.555 ± 0.039 b** |
| 1,8-Cineole | 1031 | **5.034 ± 0.147 cd** | **6.157 ± 0.180 b** | **3.799 ± 0.111 e** | **8.844 ± 0.002 a** | **6.516 ± 0.417 b** | **5.097 ± 0.149 c** | **3.995 ± 0.117 de** | **8.206 ± 0.039 a** |
| cis Ocimene | 1036 | 0.088 ± 0.003 def | 0.183 ± 0.006 bc | 0.076 ± 0.002 ef | 0.249 ± 0.026 a | 0.129 ± 0.005 cde | 0.133 ± 0.004 cd | 0.073 ± 0.002 f | 0.228 ± 0.002 ab |
| $\gamma$ Terpinene | 1058 | 0.124 ± 0.004 c | 0.097 ± 0.003 c | 0.486 ± 0.014 a | 0.268 ± 0.017 b | 0.094 ± 0.009 cd | 0.045 ± 0.001 d | 0.281 ± 0.008 b | 0.088 ± 0.004 cd |
| cis Sabinene hydrate | 1067 | 0.483 ± 0.014 a | 0.313 ± 0.01 b | 0.098 ± 0.003 d | 0.467 ± 0.023 a | 0.334 ± 0.030 b | 0.199 ± 0.006 c | 0 ± 0 e | 0.363 ± 0.004 b |
| Isovaleric acid, 2-methylbutyl ester | 1102 | 0.098 ± 0.003 bc | 0 ± 0 c | 0.122 ± 0.004 b | 0.05 ± 0.050 bc | 0 ± 0 c | 0 ± 0 c | 0.334 ± 0.010 a | 0.014 ± 0.014 c |
| Borneol | 1166 | 0.220 ± 0.006 abc | 0.251 ± 0.007 ab | 0 ± 0 c | 0.212 ± 0.119 abc | 0.334 ± 0.005 a | 0.272 ± 0.008 ab | 0.082 ± 0.003 bc | 0.320 ± 0.011 a |
| Terpinen-4-ol | 1178 | 0.297 ± 0.009 bc | 0.235 ± 0.007 bcde | 0.331 ± 0.010 b | 0.539 ± 0.070 a | 0.295 ± 0.007 bcd | 0.120 ± 0.004 e | 0.155 ± 0.005 de | 0.175 ± 0.004 cde |
| $\alpha$ Terpineol | 1191 | 0.061 ± 0.002 c | 0.072 ± 0.002 c | 0 ± 0 d | 0.016 ± 0.016 d | 0.251 ± 0.005 a | 0.193 ± 0.006 b | 0 ± 0 d | 0.080 ± 0.001 c |
| Dihydro carveol | 1194 | **16.250 ± 0.474 a** | **13.272 ± 0.387 b** | **5.493 ± 0.160 c** | **0.265 ± 0.265 d** | **0.292 ± 0.076 d** | **0.651 ± 0.019 d** | **6.887 ± 0.201 c** | **0.101 ± 0.008 d** |

**Table 7.** *Cont.*

| | | Mountain | | | | Plain | | | |
|---|---|---|---|---|---|---|---|---|---|
| **Compound** | **RI** | **Summer** | **Autumn** | **Winter** | **Spring** | **Summer** | **Autumn** | **Winter** | **Spring** |
| neo Dihydro carveol | 1195 | **1.882 ± 0.055 a** | **1.300 ± 0.038 b** | **0.789 ± 0.023 cd** | **0.450 ± 0.214 de** | **0.409 ± 0.034 de** | **0.391 ± 0.011 de** | **1.104 ± 0.033 bc** | **0.197 ± 0.004 e** |
| trans Carveol | 1220 | 0.091 ± 0.003 c | 0.294 ± 0.009 a | 0 ± 0 d | 0 ± 0 d | 0.236 ± 0.013 b | 0 ± 0 d | 0.063 ± 0.002 c | 0 ± 0 d |
| cis Carveol | 1231 | 0.697 ± 0.021 c | 3.674 ± 0.107 a | 2.546 ± 0.074 b | 0.853 ± 0.175 c | 0.384 ± 0.075 cd | 0.131 ± 0.004 d | 2.521 ± 0.074 b | 0.183 ± 0.028 d |
| Pulegone | 1240 | 0.483 ± 0.014 b | 0.448 ± 0.013 b | 0.063 ± 0.002 c | 0.085 ± 0.027 c | 0.510 ± 0.040 b | 0.724 ± 0.021 a | 0.127 ± 0.004 c | 0.111 ± 0.002 c |
| Carvone | 1244 | **49.503 ± 1.442 c** | **51.190 ± 1.491 c** | **34.07 ± 0.992 d** | **64.525 ± 0.688 b** | **67.626 ± 0.912 b** | **74.794 ± 2.179 a** | **44.937 ± 1.309 c** | **67.042 ± 0.139 b** |
| Isobornyl acetate | 1285 | 0.077 ± 0.003 c | 0.087 ± 0.003 c | 0.367 ± 0.011 b | 0 ± 0 d | 0 ± 0 d | 0 ± 0 d | 0.453 ± 0.013 a | 0 ± 0 d |
| iso Dihydro carveol acetate | 1325 | **12.913 ± 0.376 a** | **6.076 ± 0.177 d** | **10.846 ± 0.316 b** | **0.547 ± 0.05 e** | **0.028 ± 0.028 e** | **0.441 ± 0.013 e** | **8.088 ± 0.236 c** | **0.103 ± 0.004 e** |
| trans Carvyl acetate | 1335 | 0 ± 0 d | 0.247 ± 0.008 c | 0.613 ± 0.018 a | 0 ± 0 d | 0 ± 0 d | 0 ± 0 d | 0.495 ± 0.015 b | 0 ± 0 d |
| cis Carvyl acetate | 1360 | **2.665 ± 0.078 d** | **4.982 ± 0.146 c** | **14.56 ± 0.424 a** | **1.685 ± 0.109 d** | **0.06 ± 0.022 e** | **0.279 ± 0.008 e** | **12.514 ± 0.365 b** | **0.340 ± 0.006 e** |
| $\beta$ Bourbonene | 1386 | 0.634 ± 0.019 d | 0.614 ± 0.018 d | 0.913 ± 0.027 c | 1.114 ± 0.059 b | 0.684 ± 0.004 d | 0.617 ± 0.018 d | 1.403 ± 0.041 a | 1.534 ± 0.007 a |
| $\beta$ Elemene | 1393 | 0.219 ± 0.007 d | 0.162 ± 0.005 d | 0.248 ± 0.007 cd | 0.399 ± 0.044 b | 0.216 ± 0.018 d | 0.219 ± 0.007 d | 0.340 ± 0.010 bc | 0.596 ± 0.018 a |
| $\beta$ Caryophyllene | 1425 | **0.863 ± 0.025 d** | **0.876 ± 0.026 d** | **1.665 ± 0.049 c** | **2.648 ± 0.088 b** | **1.094 ± 0.034 d** | **0.911 ± 0.027 d** | **1.781 ± 0.052 c** | **3.485 ± 0.024 a** |
| cis Muurola-3,5-diene | 1456 | 0.054 ± 0.002 c | 0.051 ± 0.002 c | 0 ± 0 d | 0.147 ± 0.002 b | 0 ± 0 d | 0.044 ± 0.001 cd | 0 ± 0 d | 0.293 ± 0.025 a |
| cis Cadina-1(6),4-diene | 1476 | 0.456 ± 0.014 c | 0.405 ± 0.012 c | 0.731 ± 0.022 b | 0.703 ± 0.059 b | 0.384 ± 0.021 c | 0.434 ± 0.013 c | 0.793 ± 0.023 b | 0.968 ± 0.011 a |
| Germacrene D | 1497 | **0.454 ± 0.014 c** | **0.287 ± 0.009 cd** | **0.153 ± 0.005 de** | **1.512 ± 0.082 b** | **0.476 ± 0.053 c** | **0.394 ± 0.012 c** | **0 ± 0 e** | **2.676 ± 0.031 a** |
| Bicyclogermacrene | 1512 | 0.177 ± 0.005 c | 0.129 ± 0.004 cd | 0 ± 0 d | 0.884 ± 0.057 b | 0.237 ± 0.023 | 0.160 ± 0.005 c | 0 ± 0 d | 1.398 ± 0.025 a |
| Germacrene A | 1519 | 0.051 ± 0.002 bc | 0.055 ± 0.002 bc | 0 ± 0 c | 0.078 ± 0.038 b | 0.072 ± 0.01 bc | 0.075 ± 0.002 bc | 0.123 ± 0.004 ab | 0.165 ± 0.002 a |
| trans Calamene | 1534 | 0.273 ± 0.008 d | 0.195 ± 0.006 e | 0.478 ± 0.014 b | 0.304 ± 0.012 d | 0.205 ± 0.009 e | 0.244 ± 0.007 de | 0.588 ± 0.017 a | 0.375 ± 0.010 c |
| Cubenol-1,10-di-epi | 1617 | 0.097 ± 0.003 bc | 0 ± 0 c | 0.152 ± 0.004 b | 0.198 ± 0.053 ab | 0.104 ± 0.008 bc | 0.099 ± 0.003 bc | 0.293 ± 0.009 a | 0.172 ± 0.012 b |
| $a$ Cadinol | 1657 | 0 ± 0 b | 0 ± 0 b | 0 ± 0 b | 0.088 ± 0.031 a | 0.100 ± 0.006 a | 0 ± 0 b | 0 ± 0 b | 0.076 ± 0.005 a |
| Total Identified | | 99.893 ± 0.020 | 99.766 ± 0.002 | 98.165 ± 0.020 | 99.784 ± 0.055 | 99.896 ± 0.003 | 99.805 ± 0.020 | 97.4 ± 0.020 | 99.944 ± 0.017 |
| Monoterpenes hydrocarbons | | 8.649 ± 0.252 e | 11.261 ± 0.328 de | 22.715 ± 0.662 a | 12.915 ± 0.185 cd | 18.822 ± 1.344 b | 15.961 ± 0.465 bc | 12.602 ± 0.367 d | 10.793 ± 0.066 de |
| Sesquiterpenes hydrocarbons | | 2.473 ± 0.003 de | 2.096 ± 0.003 e | 3.181 ± 0.004 cd | 6.673 ± 0.380 b | 2.682 ± 0.166 cde | 2.41 ± 0.003 de | 3.523 ± 0.004 c | 9.953 ± 0.143 a |
| Oxygenated monoterpenes | | 73.197 ± 0.073 c | 75.124 ± 0.075 bc | 46.179 ± 0.047 e | 76.303 ± 0.976 bc | 77.28 ± 1.477 ab | 80.391 ± 0.081 a | 58.836 ± 0.059 d | 76.789 ± 0.113 b |
| Oxygenated sesquiterpenes | | 0.094 ± 0.000 bc | 0 ± 0 c | 0.148 ± 0.000 abc | 0.285 ± 0.083 a | 0.203 ± 0.013 ab | 0.096 ± 0.000 bc | 0.284 ± 0.000 a | 0.248 ± 0.018 ab |
| Others | | 15.317 ± 0.016 c | 11.34 ± 0.012 d | 25.716 ± 0.026 a | 2.402 ± 0.166 e | 0.226 ± 0.045 g | 0.854 ± 0.001 f | 21.05 ± 0.021 b | 0.508 ± 0.014 fg |

Values ($n$ = 3) in rows followed by the same letter are not significantly different, $p \leq 0.05$. In bold indicated EO components > 1%.

*3.4. Correlation of Antioxidant and Reducing Activity with Polyphenols, Minerals and Essential Oils Components*

MAP species are valued as a natural source of antioxidants, with phenolic compounds and EO constituents playing an important role in this capacity [19,27]. Linear correlation coefficients were calculated and reported in detail in Tables S2–S4, to analyze the contribution of phenols, mineral content and EOs yield and components (only the three most prevalent elements of each EO were evaluated) to their total antioxidant capacity. The correlation coefficient (*r*) and *p*-values between the examined EO compounds, the mineral content and the antioxidant capacity of sage are given in Table S2. In mountainous areas, in summer ABTS was negatively correlated with N content. EO yield was positively correlated with Zn but negatively with Ca, Mg and Fe. In autumn, EO yield was positively correlated with Mg and Fe but negatively with P, Na, Ca and Zn. In winter, phenols were positively correlated with ABTS, EO yield was positively correlated with Fe but negatively with K, P, Ca, Zn and Cu. In spring, FRAP was positively correlated with P, EO yield was positively correlated with N, Ca and Cu but negatively with Fe and Zn (Table S2). Essential oil yield of *Thymus migricus* was fairly strongly related to the concentrations of minerals as Ca and K in soil, along with a series of soil properties such as the percentage of organic matter and soil texture, altitude and temperature [73].

In plain areas for summer sage, phenols were negatively correlated with 1,8-Cineole and Camphor, while EO yield was positively correlated with N, Mg and Fe but negatively with nutrients as K, Ca, Zn and Cu. In autumn, phenols were positively correlated with ABTS, and the EO yield was positively correlated with P, Na, Fe, Zn and Cu but negatively with Ca. In winter, sage EO yield was positively correlated with K, Ca, Fe and Zn but negatively with Na, Mg and Cu. In spring, FRAP was positively correlated with ABTS, while both FRAP and ABTS were negatively correlated with α Thujone. Antioxidant activity was also negatively linked to 1,8-Cineole and α Thujone in laurel and sage plants growing in Cyprus' plains, as well as to Neral, Geranial and Caryophyllene oxide in lemon verbena plants grown Cyprus' mountains [34]. EO yield was positively correlated with Fe and Cu but negatively with P, Na and Ca (Table S2). The low antioxidant activity of 1,8-Cineole [74] may justify the negative correlation with phenols values observed in our study.

The relevant correlation coefficient (*r*) and *p*-values between the analyzed EO compounds, and the mineral content and the antioxidant capacity of sideritis are given in Table S3. In mountainous areas, in summer, the minerals (N, K, P, Ca, Mg, Fe, Zn and Cu) were positively correlated with the sideritis EOs major components, namely α Pinene, Valeranone and β Phellandrene. Additionally, the EO yield was positively correlated with N, K and Ca. In autumn, Ca, Mg, and Zn and the EO yield were positively correlated but N, K, P, Fe and Cu were negatively correlated with the EO components such as α Pinene, Valeranone and β Phellandrene. Additionally, the sideritis EO yield was positively correlated with Zn but negatively with N, K, P, Na, Fe and Cu. In winter, ABTS was positively correlated with N, while K, P, and Zn and the EO yield were positively correlated but Cu was negatively correlated with α Pinene, Valeranone and β Phellandrene. Moreover, the EO yield was positively correlated with K, P and Zn but negatively with Cu. In spring, FRAP was positively correlated with ABTS. K, Na, Ca and Zn were positively correlated with α Pinene and β Phellandrene, and negatively with Valeranone. However, the opposite was noticed in the case of Cu, which was positively correlated with Valeranone and negatively with α Pinene and β Phellandrene. EO yield was positively correlated with P (Table S3). In thyme, the content of Linalool was positively correlated with the percentages of silt and clay, and negatively with the concentrations of Ca, K and P, the percentages of sand and organic matter, and temperature. The opposite trend was evidenced for Thymol content [73], indicating the interaction of the EOs composition with nutrients availability of the growing substrate and the environmental conditions of plant growth.

In plain areas for sideritis, in summer, phenols were positively correlated with FRAP and ABTS, while positive correlation was found between FRAP and ABTS. K, P, Mg and Zn were negatively correlated α Pinene, and β Phellandrene and positively with Valeranone. However, the opposite was found in the case of Ca, Cu and EO yield, which were positively correlated with α Pinene and β Phellandrene. EO yield was positively correlated with Ca and Cu, but negatively with K, P and Mg. In autumn, phenols were positively correlated with FRAP. Ca, Mg, Fe, Zn, and Cu and EO yield were positively correlated but K, P, and Na were negatively correlated with α Pinene, Valeranone and β Phellandrene. Similar to the individual EO components, EO yield was also positively correlated with Ca, Mg, Fe, Zn and Cu and negatively correlated with K, P and Na. In winter, FRAP was positively correlated with ABTS. P and Ca were positively but K, Fe, Zn and Cu were negatively correlated with α Pinene, Valeranone and β Phellandrene. In spring, FRAP was positively correlated with ABTS. P and Mg as well as EO yield were positively but Ca and Fe were negatively correlated with α Pinene, Valeranone and β Phellandrene. EO yield was positively correlated with P and Mg and negatively with Ca and Fe (Table S3).

In spearmint, in mountainous areas in summer, K and Na content in plants were positively correlated with Carvone, Dihydro carveol and Sabinene, N was positively correlated with Sabinene, Zn and Cu were positively correlated with Carvone and Dihydro carveol, while EO yield was positively correlated with Dihydro carveol levels. EO yield were positively correlated K, Zn and Cu but negatively correlated with P, Ca and Mg. In autumn, phenols were positively correlated with ABTS and Na. Moreover, K and Zn were positively correlated with Carvone, Dihydro carveol and Sabinene, while Cu was negatively correlated with Sabinene. Considering EO yield, Zn was positively but N, P, Mg, Fe and Cu were negatively correlated with spearmint EO yield. In winter, Carvone, Dihydro carveol and Sabinene were positively correlated with the levels of N, P, Mg and Fe. In spring, Dihydro carveol was positively correlated with K, P, Fe, Zn and Cu but negatively with Ca (Table S3).

Spearmint grown in plain area, in summer revealed a negative correlation between ABTS and N levels. Moreover, N, K and Fe were positively correlated with Sabinene and negative correlated with Dihydro carveol, while Ca, Cu and EO yield were positive correlated with Dihydro carveol. Mg and Cu were positively but P and Zn were negatively correlated with EO yield. In summer, phenols were positive correlated with EO yield but negatively correlated with K, P and Zn. FRAP was positively correlated with Fe. Ca, Mg and Cu were positively corelated with Carvone, Dihydro carveol and Sabinene, while Dihydro carveol was negatively correlated with Zn but positively with the EO yield. Moreover, EO yield was positively correlated with Ca, Mg and Cu but negatively with Zn. No correlation among phenols, antioxidants, EO yield and components could be found during winter in plain area for spearmint. In spring, Na, Ca, Mg and Fe were positively correlated but Cu and EO yield were negatively correlated with Dihydro carveol. EO yield was positively correlated with Na and Cu and negatively correlated with Ca, Mg and Fe (Table S3).

Depending on the species and their growing environment, EOs may contribute to the total antioxidant activity. Figure 1 summarizes the most relevant correlations between antioxidant activity and chemical composition, as well as EO yield and component content. Total phenols and antioxidant activity (FRAP and ABTS) were stimulated in winter and spring in sage and spearmint, respectively, in mountainous areas (Figure 1). In sideritis, total phenols and antioxidants were stimulated mainly in winter in plain areas.

Increased EO yield in sage was observed in the summer-autumn period in plain areas, which was positively correlated with the increased levels of Camphor (Figure 1). For sage plants grown in plain areas in spring, FRAP and ABTS were negatively correlated with α Thujone, being in agreement with previous reports on the negative correlation of α Thujone and antioxidants (assayed DPPH) [34]; however, α Thujone alone is reported to possess antibacterial, cytotoxic and antiviral activities [75]. Higher temperatures may also favor the activity of the enzymes responsible for the synthesis of the terpenes, the main compounds of EOs [76]. It is widely known that high levels of Camphor are toxic [77] while EO with high proportions of Camphor are used in the phytosanitary industry [78]. Sideritis increased EO yield in autumn in the mountainous areas. According to Salehi et al. [74], Pinenes have considerable bioactive properties and their increased content in mountainous-spring and plain-winter cultivated sideritis plants could partially justify the increased antioxidant activity (See Table 3). In spearmint, increased EO yield was found in summer, in plain areas (Figure 1). Spearmint plants, when subjected to several stress factors such as salinity and Cu toxicity, revealed oxidative damage and had lower levels of antioxidants and 1,8-Cineole in their leaves than plants that were not stressed [79], while the levels of 1,8-Cineole were positively correlated to flavanols [34].

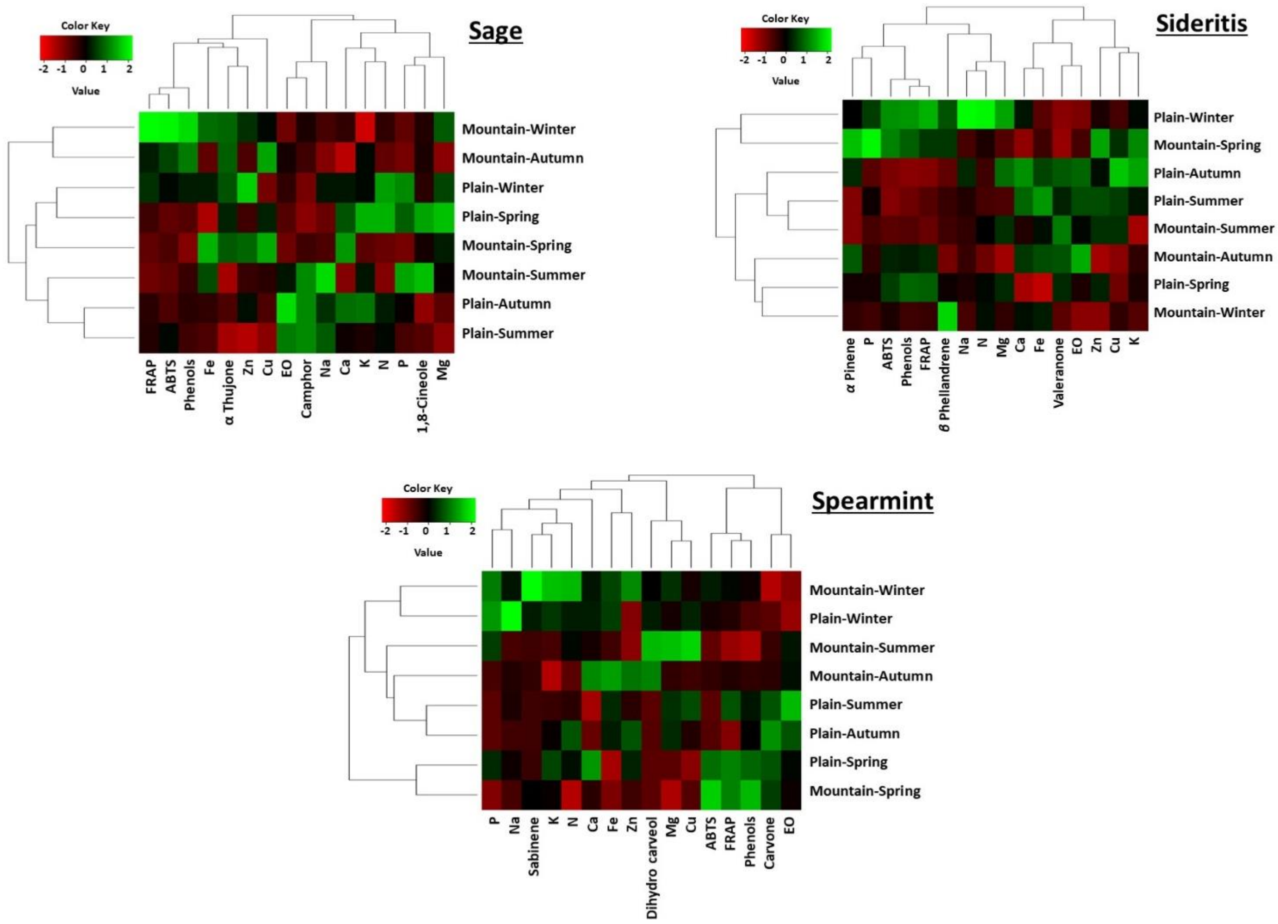


**Figure 1.** Relative changes (Heat maps) in total phenols (mg GAE $g^{-1}$ Fw), antioxidant and reducing activity (FRAP, ABTS, mg trolox $g^{-1}$ Fw), macronutrient (g $kg^{-1}$) and micronutrient content (mg $kg^{-1}$) content, and essential oil (EO) yield (%) and three major EO components in sage, sideritis and spearmint as affected by the altitude (mountain vs. plain) and season (summer, autumn, winter, spring). Red shades indicate the lower level (less than −2.0 fold), deep red corresponds to −1.0 fold, black signifies that the level is not different from the mean value, deep green corresponds to 1.0 fold, clear green indicates that the level is more than 2.0 fold higher than the mean value.

These data suggest that, depending on the species and the environment where plants grow, EOs may add to total antioxidant activity. However, it should be noted that in our study, correlation analysis was performed only using data from the most abundant components of each species' EOs, which reduces the effect of the compounds with lower participation in the essential oil profile, in terms of antioxidant activity. It is well recognized that synergistic effects can exist among the components of natural matrices, and minor components may become critical for plant tissue antioxidant capability [80,81]. As a result, EOs may have greater antioxidant activity than isolated components, as Wang et al. [82] discovered for rosemary EO. Conjugated double bonds and phenolic groups, both of which have functional and antioxidant properties, may be found in essential oils [80].

## 4. Conclusions

In the present study, the levels of total phenols, antioxidant activity and mineral content of sage, sideritis and spearmint were investigated in terms of the impact of environmental condition-altitude (mountainous *versus* plain areas) and season (summer, autumn, winter and spring) as well as their correlation to the EO yield and composition. Season affected the total phenolic compounds content and antioxidant capacity, revealing increased values during winter and lower values during summer. Moreover, in winter, P and Na were the most accumulated minerals in the plant tissue but in summer, Fe was found to be accumulated more and the EO yield was increased. Altitude did not affect total phenolics and antioxidant compounds in a great extent, throughout a year period, but it did increase Fe content in mountainous plant species, while high levels of N, K, Na and Ca were obtained from plants grown in the plain areas. EO yield was varied in the different altitudes; EO composition was significantly affected as well. The highest antioxidant capacity, Zn content and EO yield were observed in sage, increased Fe content was found in sideritis, while spearmint plants revealed high N, Na and Mg levels. The EO yield and composition were correlated with various minerals, depending on the species. Plant antioxidant activity was positively linked to the total phenolic content, and in some cases with particular minerals (i.e., P in sage, N in sideritis), but negatively in some other cases (i.e., N in sage and spearmint) or constituents of the EO of the species (i.e., $\alpha$ Thujone in sage), although a varied response was observed depending on the species, season and altitude. In conclusion, the effect of different climatic conditions-altitudes and season on the antioxidant capacity, mineral content, EO yield and oils' composition of the researched MAPs in a species-dependent way may considerably alter plants secondary metabolites composition. These findings can be utilized to pinpoint certain sites and ecosystems but also to provide the appropriate fertilization on the cultivated species, where selected MAP cultures could be used to produce high-value crops with better quality and higher bioactive properties.

**Supplementary Materials:** The following are available online at https://www.mdpi.com/article/10.3390/agronomy11091766/s1, Table S1: Fertilizers and crop protection means applied during the 1 year cultivation period. Table S2: Correlations coefficients and ($p$-values) between the antioxidant activity and essential oils components of sage. Table S3: Correlations coefficients and ($p$-values) between the antioxidant activity and essential oils components of Sideritis. Table S4: Correlations coefficients and ($p$-values) between the antioxidant activity and essential oils components of spearmint. Figure S1: Meteorological data of the last 40 years (1972–2012 for the mountain and plain areas of the study. Meteorological data were obtained by the Department of Meteorology of Cyprus.

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