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Environmental Factors Related to Climate Change Alter the Chemical Composition and Biological Activity of *Lavandula viridis* L'Hér Essential Oil

Inês Mansinhos [1], Sandra Gonçalves [1,*], Raquel Rodríguez-Solana [1,2,3], José Manuel Moreno-Rojas [3,4] and Anabela Romano [1,*]

[1] MED—Mediterranean Institute for Agriculture, Environment and Development & CHANGE—Global Change and Sustainability Institute, Faculdade de Ciências e Tecnologia, Universidade do Algarve, Campus de Gambelas, 8005-139 Faro, Portugal; ifmansinhos@ualg.pt (I.M.); raquel.rodriguez.solana@juntadeandalucia.es (R.R.-S.)
[2] Department of Agroindustry and Food Quality, Andalusian Institute of Agricultural and Fisheries Research and Training (IFAPA), Rancho de la Merced Center, Carretera Cañada de la Loba (CA-3102) Km 3.1., SN, 11471 Jerez de la Frontera, Cádiz, Spain
[3] Department of Agroindustry and Food Quality, Andalusian Institute of Agricultural and Fisheries Research and Training (IFAPA), Alameda del Obispo Center, Avenida Menendez-Pidal, SN, 14004 Córdoba, Spain; josem.moreno.rojas@juntadeandalucia.es
[4] Foods for Health Group, Instituto Maimónides de Investigación Biomédica de Córdoba (IMIBIC), Avenida Menendez-Pidal, SN, 14004 Córdoba, Spain
* Correspondence: smgoncalves@ualg.pt (S.G.); aromano@ualg.pt (A.R.); Tel.: +351-289800910 (A.R.)

Abstract: Climate change is affecting all regions of the world, and the Mediterranean region is one of the most affected. Plants accumulate secondary metabolites as an adaptive response to stress circumstances. The present study investigated the effect of different abiotic factor conditions (drought, moderate heat, severe heat, salinity, and UV-B radiation) on the essential oil (EO) yield, composition (volatile profile), and biological activity (enzyme inhibition and antioxidant activity) of *Lavandula viridis* L'Hér. In general, the environmental conditions increased the extraction yield of EO. Eighty-two compounds were identified in the EO and environmental factors induced some quantitative changes in EO composition. Severe heat and salinity conditions increased the concentration of the two most abundant compounds, 1,8-cineole and camphor. Severe heat also increased the potential of EO to inhibit the enzymes butyrylcholinesterase and tyrosinase. Drought, salinity, and UV-B radiation promoted the ability of EO to inhibit acetylcholinesterase. In addition, heat and drought enhanced the antioxidant activity of EO. These results are relevant for exploring the potential of this EO for industrial applications, although future studies combining the factors studied are important to understand the influence of synergistic effects on the composition and bioactivity of the plant products obtained.

Keywords: Lamiaceae; environmental factors; GC-MS; terpenes; antioxidants; enzyme inhibition

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1. Introduction

Climate change is the most significant global environmental challenge of this century, with certain regions being more vulnerable to its impact. According to the Intergovernmental Panel on Climate Change (IPCC), a rise in average temperatures and a shift in precipitation patterns are expected, leading to a decrease in soil water availability and an increase in drought and soil salinity [1]. These environmental conditions can have negative effects on plant growth and development, and plants must acquire strategies to cope with these factors. One of the main defense adaptation strategies is the change in the accumulation of secondary metabolites [2], with terpenes, phenolic compounds, and nitrogen-containing compounds being the three main groups [3].

Due to the geographical location of the Mediterranean region, namely between North Africa (with an arid climate) and Central Europe (with a temperate and rainy climate), this basin is particularly vulnerable to changes in climate. Mediterranean-type regions, characterized by warm or hot summers with little or no rainfall (drought summers) and mild or cold winters [4], represent ideal ecosystems for the production of lavender species, which are essentially characterized by their antiseptic and relaxing properties [5]. In addition, *Lavandula* species have other biological properties, such as antimicrobial, neurological, anti-diabetic, anti-parasitic, analgesic, among others [6]. Specifically, *Lavandula viridis* L'Hér is a Mediterranean aromatic species endemic to the Iberian Peninsula [7], present mainly in natural populations, whose existence has been affected in recent years due to climatic changes [8]. This plant produces high-value bioactive phytochemicals such as volatile (essential oil, EO) and phenolic compounds with antioxidant, antifungal, antibacterial, nematocidal, anti-protozoal, and enzyme inhibitory properties [7,9–19].

Lavander EO is traditionally and industrially used in aromatherapy and is a valuable raw material for the food industry (as flavors), cosmetics, and perfumery sectors, especially the EO from *Lavandula angustifolia* Mill. [20]. Due to the growing market demand and widespread use of *L. angustifolia* EO (species distributed worldwide), regulations and standards have been established to maintain the highest quality and safety [21,22]. Lavenders from the Iberian Peninsula are somewhat underexplored, resulting in low recognition in global markets. *L. viridis* EO is very different from those of other lavender species due to its lemon scent [20]. In recent years, its industrial exploitation has increased and it is currently sold in different countries such as Portugal, Spain, and Brazil [23–27]. The main constituent of *L. viridis* EO is the monoterpene 1,8-cineole, also known as eucalyptol [20,28]. This volatile component is often added to cosmetic or fragrance products or used as a food flavoring agent due to its pleasant taste and aroma and its bioactivity. In addition, 1,8-cineole has several pharmacological properties, such as antioxidant and anti-inflammatory, mainly through the regulation of the nuclear factor erythroid-2-related factor 2 (Nrf2) and nuclear factor-kappa B (NF-jB) pathways, respectively, and has entered clinical trials for the treatment of several diseases, such as cardiovascular, digestive, respiratory, pulmonary, and neurological diseases [29].

The applications of EOs are highly dependent on their quantity (yield) and quality, which are not constant as they are affected by various factors, including environmental conditions. For this reason, it is essential to monitor the changes in EO components in order to conclude the potential applications expected depending on the prevailing factors of the climate change scenarios where the plant is growing. This study was carried out to evaluate the effect of drought, heat, salinity, and UV-B radiation on the yield and chemical profile of EO, as well as on its antioxidant capacity and capability to inhibit enzymes (acetylcholinesterase, AChE; butyrylcholinesterase, BChE; and tyrosinase, Tyr) associated with neurodegenerative disorders.

2. Materials and Methods

2.1. Plant Material and Environmental Tests

In order to obtain uniform plants, micropropagated plants of *Lavandula viridis* L'Hér, produced according to Mansinhos et al. [7] and fully acclimatized to ex vitro conditions, were used in the experiments. The environmental experiments were carried out in a growth chamber (750 E, Aralab, Lisbon, Portugal) supplied with four Osram L 18 W/840 lamps (16/8 h, light/dark). Control plants were watered every two days (100 mL/plot) for two weeks and maintained at 25/18 °C (16/8 h, light/dark). Plants exposed to drought (25/18 °C) were watered every two days for one week and one week without watering. For heat experiments, plants were kept at 30/23 °C (moderate heat) or 35/28 °C (severe heat) for two weeks and watered as in the control treatment. Plants exposed to salt conditions (25/18 °C) were watered with 50 mM of NaCl every two days for two weeks. Finally, for the UV-B experiment, the four lamps were replaced by UV-B lamps (Philips TL 20 W/12 RS SLV/25) and the plants were kept at 25/18 °C for two weeks.

2.2. Essential Oils

2.2.1. Extraction of EOs

Dried plant material of *L. viridis* (50 g) from the different environmental experiments was subjected to hydrodistillation (3 h) with distilled water using a Clevenger-type apparatus. The EO obtained was dried with anhydrous sodium, transferred to a vial, and stored at 4 °C in a sealed tube until it was used for chemical and biological analysis. Yields were calculated based on the dry weight of the plant material.

2.2.2. Gas Chromatography–Mass Spectrometry (GC-MS) Analysis

The EO was analyzed using a CP-3800 gas chromatograph (Varian, Inc., Walnut Creek, CA, USA) equipped with an automated sampler (COMBI PAL) and a 4000 MS mass spectrometer (Varian, Inc., Walnut Creek, CA, USA). Gas chromatography–mass spectrometry analyses were performed on a non-polar DB-5MS fused-silica capillary column (60 m × 0.25 mm × 1 µm). The carrier gas was helium, supplied at a constant flow rate of 1 mL/min, and the samples were injected (1 µL) at a split ratio of 1:50. The oven temperature was set at 40 °C for 2 min, increased at 3 °C /min to 250 °C, and then 5 °C/min to 300 °C [11]. Data were acquired using MS Data Review (Varian, Inc., Walnut Creek, CA, USA). The chromatograms were then analyzed using Xcalibur™ software, version 3.0 (Thermo Fisher Scientific; Waltham, MA, USA). Retention indices were determined experimentally using n-alkanes (C8-C20 and C21-C40). The compounds in the samples were identified by matching their retention indices and mass spectra with those in the National Institute of Standards and Technology (NIST) mass spectra library and with the literature [11,16,20,28,30,31].

2.2.3. Antioxidant Capacity

The antioxidant capacity of the EOs was evaluated following the Mansinhos et al. study [32], using the 2,2-diphenyl-1-picrylhydrazyl (DPPH) and 2,2-azino-bis (3-ethylbenzothiazoline-6-sulfonic acid) (ABTS) methods, with slight modifications. In both methods, the EOs were dissolved in 86% ethanol. For DPPH scavenging, 30 µL of diluted EO was added to 300 µL of DPPH solution (90 µM) and mixed with 570 µL of 80% methanol. After 30 min at room temperature, the absorbance was read at 515 nm. For the ABTS method, the stock solution of ABTS (7 mM) was prepared 12–16 h before use, using potassium persulfate (2.45 mM). After obtaining an absorbance of 0.700 ± 0.02 (734 nm) with the reagent test, 10 µL of diluted EO was added to 190 µL of the reagent test and the absorbance was measured at 734 nm. The results of both antioxidant tests were expressed as micrograms of Trolox equivalents per gram of EO ($\mu g_{TE}/g_{EO}$).

2.2.4. Enzymes' (Tyrosinase and Cholinesterases) Inhibitory Capacity

The evaluation of Tyr, AChE, and BChE's inhibitory activities was performed according to Gonçalves et al. [12] with slight modifications. For Tyr, EOs were dissolved in 86% ethanol and mixed with 20 mM of sodium phosphate buffer (pH 6.8) and Tyr solution (prepared in buffer). L-DOPA (2.5 mM) was added after 10 min and the absorbance was read at 475 nm 10 min later. Kojic acid was used as a positive control. For cholinesterases, EOs were dissolved in 20% ethanol and mixed with DTNB solution (3 mM), acetylthiocholine iodide (15 mM, for AChE) or S-butyrylthiocholine (15 mM, for BChE), and phosphate buffer. AChE or BChE (0.28 U/mL) was added, and the absorbance read at 405 nm. Galantamine was used as a positive control. All results were expressed as IC_{50} (µg/mL).

2.3. Statistical Analysis

Data are expressed as the mean ± standard error (SE) and analyzed by one-way analysis of variance (ANOVA) and Duncan's New Multiple Range Test ($p < 0.05$). Pearson correlation ($p < 0.01$) was performed using IBM SPSS Statistics for Windows (version 26.0, IBM Corporation, Armonk, NY, USA). Principal component analysis (PCA) was performed using OriginPro software, version 2022 (OriginLab Corporation, Northampton, MA, USA).

3. Results and Discussion

3.1. EO Extraction Yield and Chemical Profile

Figure 1 presents the yields of *L. viridis* EO, expressed as the weight of EO in relation to the weight of the starting plant material. The extraction yield of the control plants was higher compared to that obtained in other studies with *L. viridis* [11,20,28], in vitro shoot cultures, or micropropagated plants [28]. When comparing the results of the various environmental treatments, it was found that the highest yield was obtained in the UV-B treatment (2.75% *w*/*w*), followed by moderate and severe heat treatments (10% less) and salinity treatment (13% less).

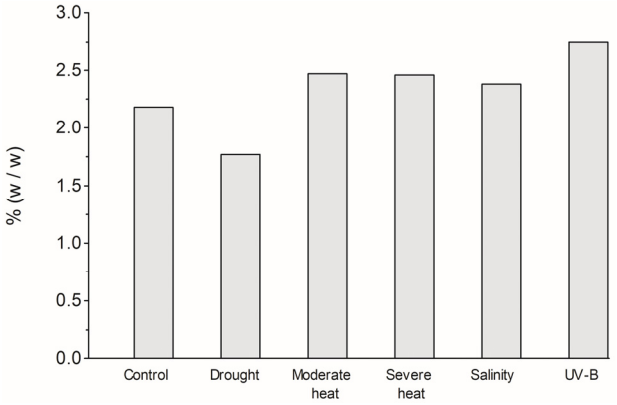

Figure 1. Extraction yield (%, *w*/*w*) of *Lavandula viridis* L'Hér essential oils isolated from plants subjected to different environmental conditions.

In Manukyan's [33] investigation, intensive UV-B radiation had a positive effect on the total EO yield from *Nepeta cataria* L., *Melissa officinalis* L., and *Salvia officinalis* L. of the Lamiaceae family. Additionally, the oil yield of *Ocimum sanctum* L. increased significantly by 42% after UV-B treatment [34]. In 2019, the same author exposed *Thymus transcaucasicus* Ronn. to different temperatures (15, 20, and 25 °C) and found that the highest EO yield was achieved at the highest temperature [35]. Other authors have reported an increase in extraction yield after exposing plants of different Lamiaceae genera to salinity, including *Ocimum basilicum* L. [36,37], *Origanum majorana* L. [38], and *Rosmarinus officinalis* L. [39], which is consistent with our results. In the present study, the lowest extraction yield was obtained when plants were subjected to drought. This resulted in a 19% reduction compared to the control and a 36% reduction compared to the UV-B experiment. Similarly, in *Thymus vulgaris* L. (Lamiaceae), the EO yield also decreased with drought stress [40]. However, in *Sideritis perfoliata* L. (Lamiaceae) and *Melissa officinalis* L., drought had a favorable impact on EO yield.

Lavandula spp. are aromatic shrubs commonly used in alternative medicine and in the cosmetic industry for the production of fragrances. This study analyzed the chemical profile of *L. viridis* EO obtained from both control plants and plants exposed to different abiotic factors (Table 1). To the best of our knowledge, this is the first study to investigate the effects of abiotic factors on the EO of this species. The EO appeared yellow in all treatments, with no discernible differences in color.

Table 1. Chemical composition (relative %) of the *Lavandula viridis* L'Hér essential oils isolated from plants subjected to different environmental conditions.

	Compounds	Class	RI *	RI Literature	Composition (%)					
					Control	Drought	Moderate Heat	Severe Heat	Salinity	UV-B
1	Tricyclene	MH	938	938	0.41 ± 0.01 b	0.47 ± 0.03 ab	0.46 ± 0.01 ab	0.49 ± 0.02 a	0.44 ± 0.02 ab	0.34 ± 0.01 c
2	α-Pinene	MH	948	947	5.99 ± 0.11 a	5.25 ± 0.21 b	5.53 ± 0.09 ab	3.57 ± 0.18 cd	3.14 ± 0.17 d	4.09 ± 0.30 c
3	Camphene	MH	967	967	3.61 ± 0.09 a	3.72 ± 0.16 a	3.85 ± 0.04 a	3.93 ± 0.21 a	3.68 ± 0.23 a	3.34 ± 0.26 a
4	Sabinene	MH	986	985	0.81 ± 0.01 a	0.67 ± 0.02 b	0.77 ± 0.00 a	0.53 ± 0.02 c	0.41 ± 0.02 d	0.61 ± 0.03 b
5	Myrcene	MH	992	992	0.28 ± 0.00 a	0.19 ± 0.01 c	0.25 ± 0.00 b	0.13 ± 0.00 d	0.05 ± 0.00 f	0.11 ± 0.00
6	β-Pinene	MH	995	995	2.08 ± 0.03 a	1.76 ± 0.02 b	1.96 ± 0.02 a	1.20 ± 0.04 d	0.95 ± 0.03 e	1.48 ± 0.08 c
7	Dehydro-1,8-cineole	OM	1003	1004	0.51 ± 0.00 bc	0.68 ± 0.00 a	0.55 ± 0.03 b	0.47 ± 0.01 c	0.49 ± 0.02 bc	0.47 ± 0.01 c
8	δ-3-Carene	MH	1024	1022	0.74 ± 0.01 b	0.66 ± 0.01 c	0.84 ± 0.01 a	0.60 ± 0.02 d	0.16 ± 0.01 f	0.23 ± 0.01 e
9	α-Terpinene	MH	1031	1030	0.44 ± 0.01 c	0.58 ± 0.03 ab	0.57 ± 0.01 abc	0.53 ± 0.04 abc	0.61 ± 0.06 a	0.47 ± 0.05 bc
10	p-Cymene	MH	1044	1042	0.72 ± 0.05 a	0.89 ± 0.05 a	0.87 ± 0.01 a	0.87 ± 0.06 a	0.97 ± 0.13 a	0.72 ± 0.03 a
11	1,8-Cineole	OM	1050	1050	24.25 ± 0.03 c	21.56 ± 0.26 d	24.55 ± 0.01 c	28.98 ± 0.88 a	28.37 ± 0.74 ab	26.88 ± 0.76 b
12	γ-Terpinene	MH	1071	1071	0.61 ± 0.01 c	0.71 ± 0.02 ab	0.75 ± 0.01 a	0.68 ± 0.02 abc	0.76 ± 0.04 a	0.65 ± 0.03 bc
13	trans-Linalool oxide	OM	1084	1084	0.48 ± 0.00 b	0.51 ± 0.03 b	0.52 ± 0.01 b	0.69 ± 0.04 a	0.75 ± 0.05 a	0.75 ± 0.05 a
14	cis-p Mentha-2-en-1-ol	OM	1086	1085	0.75 ± 0.03 a	0.42 ± 0.01 c	0.28 ± 0.03 d	0.54 ± 0.01 b	0.34 ± 0.00 d	0.53 ± 0.03 b
15	p-Cymenene	MH	1093	1093	0.05 ± 0.00 c	0.08 ± 0.00 b	0.09 ± 0.00 a	0.08 ± 0.00 b	0.08 ± 0.00 b	0.05 ± 0.00 c
16	6-Camphenol	OM	1100	1097	0.92 ± 0.01 b	1.01 ± 0.02 ab	0.98 ± 0.02 ab	1.07 ± 0.04 a	1.10 ± 0.05 a	1.06 ± 0.05 a
17	Fenchol	OM	1104	1105	2.94 ± 0.18 ab	2.43 ± 0.20 b	1.67 ± 0.15 c	1.22 ± 0.05 c	2.40 ± 0.19 b	3.36 ± 0.15 a
18	α-Pinene oxide	OM	1106	1106	0.20 ± 0.01 c	0.31 ± 0.00 b	0.29 ± 0.00 b	0.24 ± 0.00 c	0.35 ± 0.02 a	0.32 ± 0.02 ab
19	cis-β-Terpineol	OM	1118	1121	0.26 ± 0.00 a	0.19 ± 0.00 d	0.14 ± 0.00 f	0.23 ± 0.01 b	0.16 ± 0.00 e	0.21 ± 0.00 c
20	cis-Limonene oxide	OM	1134	1134	0.31 ± 0.01 a	0.24 ± 0.01 bc	0.21 ± 0.01 c	0.32 ± 0.02 a	0.26 ± 0.01 bc	0.28 ± 0.01 ab
21	α-Campholenal	OM	1146	1136	1.00 ± 0.04 b	1.03 ± 0.04 b	1.07 ± 0.02 ab	1.12 ± 0.03 ab	1.21 ± 0.05 a	1.18 ± 0.05 a
22	trans-Verbenol	OM	1153	1153	0.11 ± 0.01 a	0.11 ± 0.01 a	0.12 ± 0.00 a	0.12 ± 0.00 a	0.12 ± 0.01 a	0.09 ± 0.02 a
23	trans-Limonene oxide	OM	1155	1151	0.12 ± 0.02 a	0.11 ± 0.01 a	0.10 ± 0.00 a	0.10 ± 0.00 a	0.12 ± 0.01 a	0.13 ± 0.01 a
24	3,9-Epoxy-p-menth-1-ene	OM	1159	1178	0.09 ± 0.02 a	0.06 ± 0.00 a	0.05 ± 0.00 a	0.11 ± 0.03 a	0.06 ± 0.01 a	0.09 ± 0.03 a
25	cis-Chrysanthenol	OM	1166	1168	1.17 ± 0.02 b	1.40 ± 0.01 a	1.36 ± 0.09 a	1.47 ± 0.01 a	1.43 ± 0.08 a	1.36 ± 0.02 a
26	cis-Verbenol	OM	1170	1170	4.27 ± 0.10 a	4.10 ± 0.11 a	3.45 ± 0.03 b	4.27 ± 0.09 a	4.05 ± 0.08 a	4.35 ± 0.13 a
27	Camphor	OM	1176	1174	10.74 ± 0.10 d	11.28 ± 0.16 c	11.61 ± 0.06 c	13.21 ± 0.03 a	12.52 ± 0.12 b	10.86 ± 0.10 d
28	Pinocarvone	OM	1189	1181	3.41 ± 0.00 d	4.24 ± 0.08 b	4.21 ± 0.05 b	3.96 ± 0.10 c	4.74 ± 0.06 a	4.06 ± 0.08 bc
29	p-Cymen-8-ol	OM	1195	1194	0.17 ± 0.04 b	0.31 ± 0.03 a	0.29 ± 0.02 ab	0.30 ± 0.04 a	0.30 ± 0.03 ab	0.18 ± 0.02 bc
30	Borneol	OM	1199	1199	2.31 ± 0.05 c	2.75 ± 0.19 a	2.76 ± 0.01 a	2.70 ± 0.12 ab	2.55 ± 0.06 abc	2.36 ± 0.05 bc
31	Terpinen-4-ol	OM	1201	1200	1.29 ± 0.04 c	1.52 ± 0.07 ab	1.56 ± 0.03 ab	1.63 ± 0.06 ab	1.65 ± 0.01 a	1.47 ± 0.03 b
32	p-Methyl-acetophenone	Others	1207	1199	0.32 ± 0.04 c	0.39 ± 0.02 cb	0.38 ± 0.03 bc	0.50 ± 0.01 a	0.43 ± 0.03 ab	0.38 ± 0.03 bc
33	cis-p-Mentha-1(7),8-diene-2-ol	OM	1213	1206	0.26 ± 0.05 b	0.32 ± 0.01 ab	0.31 ± 0.00 ab	0.38 ± 0.02 a	0.38 ± 0.00 a	0.29 ± 0.02 b
34	α-Terpineol	OM	1215	1213	1.79 ± 0.01 a	1.69 ± 0.05 a	1.78 ± 0.02 a	1.18 ± 0.04 c	1.05 ± 0.03 c	1.41 ± 0.05 b
35	Myrtenol	OM	1220	1224	1.08 ± 0.02 b	1.24 ± 0.03 a	1.25 ± 0.02 a	1.08 ± 0.02 b	1.21 ± 0.04 a	1.20 ± 0.00 a
36	Myrtenal	OM	1223	1223	1.01 ± 0.03 ab	1.02 ± 0.05 ab	0.96 ± 0.01 b	0.98 ± 0.03 ab	1.09 ± 0.03 a	1.09 ± 0.02 a
37	Verbenone	OM	1236	1236	2.28 ± 0.02 c	2.79 ± 0.07 ab	2.58± 0.05 bc	3.14 ± 0.20 a	2.80 ± 0.13 ab	2.48 ± 0.11 bc
38	Eucarvone	OM	1244	1245	0.14 ± 0.01 a	0.14 ± 0.00 a	0.15 ± 0.00 a	0.08 ± 0.01 b	0.06 ± 0.00 c	0.08 ± 0.01 bc
39	Carveol	OM	1250	1252	0.31 ± 0.00 ab	0.28 ± 0.01 v	0.23 ± 0.00 c	0.22 ± 0.02 c	0.30 ± 0.01 ab	0.34 ± 0.02 a

Table 1. *Cont.*

	Compounds	Class	RI *	RI Literature	Composition (%)					
					Control	Drought	Moderate Heat	Severe Heat	Salinity	UV-B
40	Bornyl acetate	Others	1254	1255	0.22 ± 0.00 ab	0.25 ± 0.01 a	0.24 ± 0.00 ab	0.25 ± 0.02 ab	0.25 ± 0.02 ab	0.20 ± 0.01 b
41	(-)-Carvone	OM	1266	1265	0.54 ± 0.02 a	0.60 ± 0.02 a	0.52 ± 0.04 a	0.58 ± 0.02 a	0.60 ± 0.03 a	0.53 ± 0.04 a
42	Myrtenyl acetate	Others	1272	1284	0.24 ± 0.00 bc	0.29 ± 0.01 a	0.26 ± 0.00 ab	0.22 ± 0.02 bc	0.21 ± 0.01 c	0.22 ± 0.01 bc
43	α-Terpinen-7-al	OM	1277	1276	0.19 ± 0.01 ab	0.20 ± 0.00 a	0.15 ± 0.01 b	0.19 ± 0.02 ab	0.15 ± 0.01 b	0.15 ± 0.01 b
44	(R)-Lavandulyl acetate	Others	1281	1282	0.66 ± 0.00 a	0.60 ± 0.02 b	0.57 ± 0.01 bc	0.45 ± 0.01 d	0.54 ± 0.00 c	0.69 ± 0.00 a
45	trans-Carvone oxide	OM	1295	1280	0.14 ± 0.01 a	0.18 ± 0.01 a	0.16 ± 0.03 a	0.20 ± 0.01 a	0.19 ± 0.02 a	0.16 ± 0.01 a
46	Isobornyl acetate	Others	1304	1304	0.44 ± 0.00 d	0.53 ± 0.00 bc	0.51 ± 0.01 c	0.58 ± 0.02 a	0.57 ± 0.01 ab	0.45 ± 0.01 d
47	p-Cymen-7-ol	OM	1308	1308	0.21 ± 0.00 b	0.26 ± 0.01 ab	0.26 ± 0.01 ab	0.26 ± 0.02 ab	0.30 ± 0.02 a	0.26 ± 0.03 ab
48	α-Terpinyl acetate	Others	1313	1314	0.14 ± 0.00 b	0.18 ± 0.01 a	0.17 ± 0.00 ab	0.17 ± 0.01 ab	0.19 ± 0.01 a	0.16 ± 0.01 ab
49	p-Mentha-1,8-dien-7-ol	OM	1319	1313	0.12 ± 0.00 b	0.14 ± 0.01 ab	0.14 ± 0.00 ab	0.13 ± 0.01 ab	0.15 ± 0.00 a	0.13 ± 0.01 ab
50	p-Vinyl guaicol	Others	1328	1328	0.09 ± 0.00 a	0.15 ± 0.05 a	0.17 ± 0.00 a	0.15 ± 0.06 a	0.17 ± 0.06 a	0.15 ± 0.07 a
51	p-Mentha-1,4-dien-7-ol	OM	1345	1333	0.27 ± 0.01 c	0.34 ± 0.00 ab	0.32 ± 0.01 b	0.33 ± 0.00 ab	0.36 ± 0.02 a	0.34 ± 0.00 ab
52	Nerol acetate	Others	1359	1359	0.12 ± 0.03 a	0.15 ± 0.02 a	0.15 ± 0.02 a	0.14 ± 0.06 a	0.13 ± 0.05 a	0.12 ± 0.03 a
53	2-Phenyl ethyl propanoate	Others	1363	1356	0.15 ± 0.01 a	0.17 ± 0.01 a	0.18 ± 0.01 a	0.15 ± 0.02 a	0.16 ± 0.01 a	0.13 ± 0.01 a
54	Eugenol	OM	1369	1369	0.36 ± 0.01 c	0.41 ± 0.00 b	0.43 ± 0.00 b	0.43 ± 0.02 b	0.41 ± 0.01 b	0.49 ± 0.01 a
55	Geranyl acetate	Others	1377	1377	1.51 ± 0.02 a	1.46 ± 0.02 ab	1.27 ± 0.01 c	0.82 ± 0.05 e	1.00 ± 0.03 d	1.34 ± 0.05 bc
56	Octyl ester butanoic acid	Others	1387	1386	0.10 ± 0.00 a	0.14 ± 0.02 a	0.13 ± 0.00 a	0.10 ± 0.02 a	0.14 ± 0.06 a	0.12 ± 0.04 a
57	β-Elemene	SH	1414	1416	0.08 ± 0.00 a	0.13 ± 0.03 a	0.12 ± 0.04 a	0.11 ± 0.03 a	0.11 ± 0.03 a	0.10 ± 0.01 a
58	Octyl 2-methylbutyrate	Others	1433	1436	0.32 ± 0.05 bc	0.38 ± 0.02 ab	0.33 ± 0.01 bc	0.29 ± 0.02 c	0.43 ± 0.01 a	0.44 ± 0.01 a
59	β-Phenylethyl butyrate	Others	1455	1457	0.29 ± 0.00 b	0.37 ± 0.02 a	0.34 ± 0.01 ab	0.35 ± 0.00 ab	0.36 ± 0.02 ab	0.33 ± 0.03 ab
60	*cis*-β-Farnesene	SH	1459	1459	0.10 ± 0.00 b	0.10 ± 0.01 ab	0.10 ± 0.00 b	0.12 ± 0.01 a	0.11 ± 0.00 ab	0.10 ± 0.01 ab
61	γ-Elemene	SH	1485	1482	0.13 ± 0.02 a	0.13 ± 0.01 a	0.10 ± 0.01 a	0.12 ± 0.01 a	0.11 ± 0.02 a	0.16 ± 0.02 a
62	Phenyl ethyl 3-methyl-butanoate	Others	1504	1508	1.50 ± 0.04 b	2.14 ± 0.18 a	1.72 ± 0.02 ab	1.52 ± 0.09 b	1.88 ± 0.16 ab	1.88 ±0.14 ab
63	Geranyl isobutyrate	Others	1509	1511	0.31 ± 0.01 ab	0.30 ± 0.00 ab	0.31 ± 0.00 a	0.19 ± 0.03 d	0.22 ± 0.02 cd	0.26 ± 0.01 bc
64	(Z)-α-Bisabolene	SH	1514	1509	0.77 ± 0.02 a	0.57 ± 0.01 b	0.51 ± 0.01 b	0.26 ± 0.04 c	0.22 ± 0.05 c	0.48 ± 0.06 b
65	Germacrene D	SH	1520	1519	0.15 ± 0.02 a	0.17 ± 0.01 a	0.18 ± 0.03 a	0.18 ± 0.02 a	0.16 ± 0.03 a	0.16 ± 0.03 a
66	β-Selinene	SH	1531	1531	1.38 ± 0.01 a	1.34 ± 0.02 a	1.28 ± 0.01 a	0.99 ± 0.06 b	1.06 ± 0.04 b	1.32 ± 0.08 a
67	α-Selinene	SH	1537	1534	0.51 ± 0.02 a	0.51 ± 0.02 a	0.53 ± 0.01 a	0.45 ± 0.06 a	0.45 ± 0.06 a	0.48 ± 0.05 a
68	β-Bisabolene	SH	1557	1547	0.23 ± 0.01 ab	0.25 ± 0.01 a	0.20 ± 0.00 bc	0.17 ± 0.01 c	0.20 ± 0.01 bc	0.21 ± 0.02 bc
69	β-Himachalene	SH	1563	1550	0.26 ± 0.00 a	0.22 ± 0.00 b	0.21 ± 0.00 b	0.12 ± 0.00 d	0.10 ± 0.01 e	0.18 ± 0.00 c
70	Guaia-3,9-diene	SH	1567	1556	0.80 ± 0.01 a	0.70 ± 0.02 a	0.54 ± 0.01 b	0.30 ± 0.04 c	0.22 ± 0.04 c	0.51 ± 0.04 b
71	Selina-3,7 (11)-diene	SH	1578	1562	0.59 ± 0.01 a	0.50 ± 0.20 ab	0.46 ± 0.01 bc	0.26 ± 0.03 d	0.22 ± 0.03 d	0.38 ± 0.04 c
72	Viridiflorol	OS	1585	1584	2.42 ± 0.03 a	2.05 ± 0.05 b	1.73 ± 0.01 c	1.06 ± 0.05 d	1.01 ± 0.02 d	1.63 ± 0.08 c
73	Geranyl isovalerate	OS	1601	1600	0.74 ± 0.01 a	0.77 ± 0.00 a	0.73 ± 0.01 a	0.57 ± 0.03 b	0.63 ± 0.04 b	0.73 ± 0.01 a
74	(-)-Spathulenol	OS	1604	1619	0.25 ± 0.03 a	0.22 ± 0.01 ab	0.20 ± 0.00 ab	0.17 ± 0.01 b	0.18 ± 0.00 b	0.21 ±0.01 ab
75	Humulane-1,6-dien-3-ol	OS	1623	1619	0.15 ± 0.00 a	0.18 ± 0.01 a	0.18 ± 0.00 a	0.17 ± 0.01 a	0.16 ± 0.02 a	0.17 ± 0.02 a
76	Alloaromadendrene epoxide-(I)	OS	1657	1646	0.94 ± 0.02 a	0.77 ± 0.02 b	0.70 ± 0.04 b	0.49 ± 0.02 c	0.47 ± 0.06 c	0.71 ± 0.08 b

Table 1. *Cont.*

	Compounds	Class	RI *	RI Literature	Composition (%)					
					Control	Drought	Moderate Heat	Severe Heat	Salinity	UV-B
77	Ledene oxide-(II)	OS	1660	1678	0.91 ± 0.00 ab	1.11 ± 0.05 a	0.93 ± 0.01 ab	0.79 ± 0.04 b	1.08 ± 0.12 a	1.00 ± 0.03 a
78	Aromadendrene oxide-(II)	OS	1682	1678	0.46 ± 0.02 c	0.62 ± 0.00 a	0.55 ± 0.02 ab	0.50 ± 0.03 c	0.63 ± 0.06 a	0.52 ± 0.03 ab
79	β-selinenol	OS	1705	1691	0.85 ± 0.00 ab	0.96 ± 0.02 a	0.89 ± 0.04 ab	0.74 ± 0.05 b	0.88 ± 0.07 ab	0.85 ± 0.07 ab
80	Juniper camphor	OS	1743	1741	0.80 ± 0.00 a	0.75 ± 0.02 a	0.59 ± 0.02 b	0.39 ± 0.01 c	0.28 ± 0.02 d	0.45 ± 0.02 c
81	β-Vetivone	OS	1795	1808	0.61 ± 0.01 b	0.70 ± 0.00 ab	0.70 ± 0.01 ab	0.72 ± 0.02 ab	0.77 ± 0.08 a	0.70 ± 0.06 ab
82	Benzyl benzoate	Others	1808	1789	0.72 ± 0.00 b	0.88 ± 0.01 a	0.92 ± 0.00 a	0.91 ± 0.03 a	1.01 ± 0.06 a	0.99 ± 0.05 a
	Grouped compounds									
	Monoterpene hydrocarbons (MH)				15.73 ± 0.18 a	14.98± 0.54 a	15.93 ± 0.17 a	12.61 ± 0.61 b	11.24 ± 0.72 b	12.08 ± 0.78 b
	Oxygenated monoterpenes (OM)				64.02 ± 0.05 c	63.90 ± 0.02 c	64.99 ± 0.01 c	71.93 ± 0.39 a	72.05 ± 0.55 a	69.00 ± 0.50 b
	Sesquiterpene hydrocarbons (SH)				4.99 ± 0.06 a	4.61 ± 0.12 ab	4.22 ± 0.03 b	3.08 ± 0.25 c	2.96 ± 0.24 c	4.08 ± 0.33 b
	Oxygenated sesquiterpenes (OS)				8.12 ± 0.07 a	8.11 ± 0.14 a	7.20 ± 0.10 ab	5.59 ± 0.28 d	6.09 ± 0.49 cd	6.97 ± 0.42 bc
	Others				7.13 ± 0.10 ab	8.40 ± 0.30 a	7.65 ± 0.09 ab	6.79 ± 0.46 b	7.69 ± 0.52 ab	7.86 ± 0.52 ab

Notes: * RI, Retention Index relative to C8-C20 n-alkanes on the DB-5MS column. The results were analyzed using one-way analysis of variance (ANOVA) followed by Duncan's new multiple range test. Different letters mean significant differences ($p < 0.05$) among environmental conditions.

Numerous factors such as the genotype, plant phenological stage of the plant, botanical organ, geographical location, environmental conditions, propagation method, harvesting time, processing of the plant material, nature (fresh or dried), and extraction techniques influence the quality and chemical composition of EOs [41,42]. Table 1 shows that 82 compounds were identified in the *L. viridis* EO. Some of these compounds were identified for the first time in this species [11,16,20,28,30,31]. Significant differences in the concentration of volatile compounds were observed after subjecting plants to different environmental stresses. The monoterpene fraction was predominant in all six EOs studied, ranging from 78.88 to 84.54%. The main components were oxygen-containing monoterpenes, which accounted for 63.90 to 72.05%. The oxygenated monoterpenes 1,8-cineole (eucalyptol) (21.56–28.98%) and camphor (10.74–13.21%) were the primary components of all *L. viridis* EOs, consistent with previous studies on this species [11,16,20,28,30,31]. The valuable scent of lavender is attributed to the sensory characteristics of oxygenated terpenes [41]. A study conducted by Xiao et al. [43] used gas chromatography–olfactometry (GC-O) and descriptive sensory analysis to identify the characteristic aroma components of five different lavender EOs. The study found that woody and camphor odors were related to two specific compounds. Camphor was identified as one of the characteristic aroma components of the lavender EOs, conferring "musty, penetrating, slightly minty notes" [41]. According to Aprotosoaie et al. [41], EOs from stems and leaves have a high content of 1,8-cineole and camphor, which results in a harsher note and consequently reduces the quality of true lavender flower EO. In fact, the most valued lavender EOs in the perfumery and cosmetics are those with a high concentration of linalool (and its esters) and a low concentration of camphor. On the other hand, EOs with high camphor content are mainly valued for their therapeutic properties and are used in aromatherapy and phytotherapy [42]. The levels of 1,8-cineole and camphor increased after exposure to severe heat and salinity conditions. Aćimović et al. [44] demonstrated a positive correlation between high temperature and the accumulation of 1,8-cineole, the main component of *Nepeta nuda* L. However, salinity, the most studied abiotic stress in the Lamiaceae family, has produced highly variable results. For instance, in *Mentha* x *piperita* L. [45], *O. basilicum* [46,47], *R. officinalis* [48,49], and *S. officinalis* [50], the concentration of 1,8-cineole decreased after the plants were exposed to salt stress. In contrast, this stress had a positive effect on the production of this compound in *O. basilicum* [51], *S. officinalis* [52], and *Salvia mirzayanii* Rech. [53,54]. Drought had a

negative impact on 1,8-cineole production in *L. viridis*, which is consistent with different Lamiaceae species, such as *R. officinalis* [55], *Salvia reuterana* [56], and *Lavandula angustifolia* [57]. However, several authors have found that drought is an effective strategy for increasing the production of 1,8-cineole in other species [55–61]. In accordance with our results, salinity improved the concentration of camphor in other Lamiaceae species, such as *R. officinalis* [48] and *S. officinalis* [62].

As previously observed [11,16,20,28,30,31], α-pinene (monoterpene hydrocarbon) was the third most abundant compound in the control plants. The concentration of this compound, which has a major influence on the aroma of *Lavandula officinalis* L. [43], decreased in response to the environmental factors tested. Other authors found distinct results showing that UV-B radiation [33,63], drought [55], and salinity [62] positively influenced α-pinene production. Cis-verbenol was the fourth most abundant compound in control plants, with a concentration of 4.27 ± 0.10%. This concentration was maintained after stress exposure, with the exception of moderate heat, where a decrease was observed (3.45 ± 0.03%). At comparable levels, pinocarvone is also produced and its accumulation is significantly stimulated by all abiotic factors, especially by salinity (Table 1). In fact, the abiotic factors tested led to an improvement in the accumulation of 44% of the identified compounds compared to the control.

The sesquiterpene fraction, which ranged from 8.67 to 13.12%, was predominantly composed of oxygenated compounds (5.59–8.12%) (Table 1). Viridiflorol (identified for the first time in *L. viridis*) was the predominant oxygenated sesquiterpene (1.01–2.42%) present in the six EOs. However, its concentration decreased significantly after exposure to different abiotic treatments, especially salinity and severe heat. Viridiflorol concentration increased in response to drought conditions [58] but decreased in response to salt stress [52] in *S. officinalis*. The sesquiterpene hydrocarbons were found in small amounts (2.96–4.99%), with β-selinene being the main compound of this fraction. A significant decrease in the concentration of this compound was observed in plants exposed to severe heat and salinity.

In order to assess the impact of the five distinct environmental factors on the individual volatile components of *L. viridis* EOs, a biplot PCA was constructed utilizing the sample scores and the variable loadings derived from GC-MS results (Figure 2).

The first two PCs (PC1 and PC2) were found to account for 72.05% of the total variation within the dataset. The results indicate that moderate heat and drought exert a comparable influence on EOs' composition, with the content of borneol, dehydro-1,8-cineole, myrtenyl acetate, eucarvone, 2-phenyl ethyl propanoate, and nerol acetate being particularly noteworthy. Furthermore, drought and moderate heat also exerted a significant influence on the monoterpene hydrocarbons (α-pinene, camphene, sabinene, β-pinene, δ-3-carene, p-cymene, γ-terpinene, tricyclene, myrcene, and p-cymenene), all of which were positioned at positive PC2 values (third and fourth quadrants). Similarly, salinity and severe heat (located at the first quadrant, positive PC1 and negative PC2) demonstrated a comparable impact on the EOs' profiles, with the most representative compounds belonging to the oxygenated monoterpene family (pinocarvone, verbenone, terpinene-4-ol, cis-chrysanthenol, α-campholenal, 6-camphenol, trans-linalool oxide, (-)-carvone, cis-p-mentha-1(7),8-diene-2-ol, p-mentha-1,4-dien-7-ol, α-pinene oxide, p-cymen-7-ol, trans-carvone oxide, and p-mentha-1,8-dien-7-ol), including the two most abundant compounds (1,8-cineole and camphor). Conversely, control and UV-B, despite being in the same quadrant, exhibited a notable disparity in their chemical response. Abiotic factors exhibited a minimal impact on the majority of the sesquiterpenes (both hydrocarbons and oxygenated compounds), which were primarily situated in the third quadrant (negative PC1; positive PC2). UV-B was the treatment that exerted the least influence on the chemical profile of EO *L. viridis*.

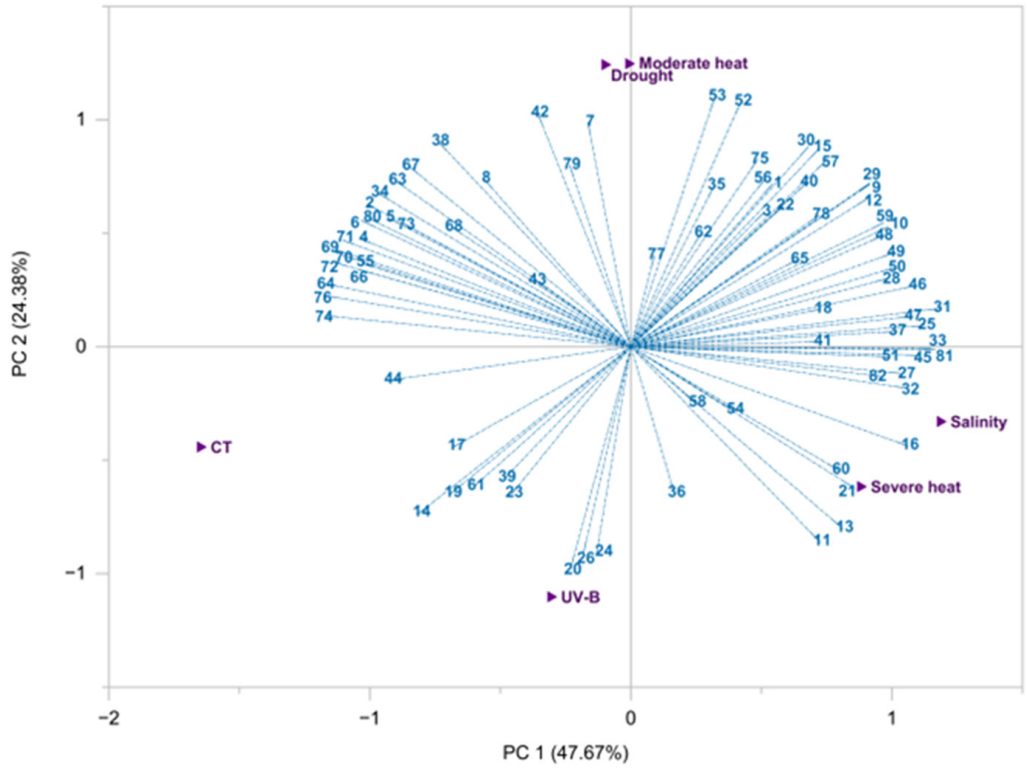

Figure 2. Principal component analysis (PCA) biplot of the eighty-two compounds identified and quantified in the *Lavandula viridis* L'Hér essential oils (Table 1) isolated from plants subjected to different environmental conditions.

3.2. Antioxidant Potential of EOs

Due to the abundance of highly bioactive chemicals in EOs, including monoterpenes and sesquiterpenes, they have been increasingly used as natural antioxidants and antibacterial agents [64]. In this study, the antioxidant activity of *L. viridis* EOs derived from plants subjected to five distinct environmental factors was evaluated using two distinct analytical methodologies (DPPH and ABTS) (Figure 3).

The results indicate that EOs from plants grown under control conditions exhibited a higher potential to scavenge the DPPH radical (943.23 ± 65.82 $\mu g_{TE}/g_{EO}$) than the radical ABTS (693.92 ± 69.22 $\mu g_{TE}/g_{EO}$). The results were different for EOs from other *Lavandula* species, such as *Lavandula coronopifolia* Poir. [65], *L. angustifolia*, *Lavandula latifolia* Medik., and *Lavandula hybrida* L. [66], that displayed a higher anti-ABTS activity. The *L. viridis* EO demonstrated superior performance in scavenging DPPH radicals in comparison to *L. coronopifolia* [65], *L. angustifolia*, *L. latifolia*, and *L. hybrida* EOs [66] and ABTS radicals in comparison to *L. latifolia* and *L. hybrida* EOs [66].

Overall, EOs from *L. viridis* plants exposed to abiotic factors exhibited higher antioxidant potential than the EO from control plants (Figure 3). This is not surprising since EOs are rich in compounds with antioxidant potential, which play an important role in combating abiotic stress in plants. The highest results in both DPPH (1444.37 ± 77.34 $\mu g_{TE}/g_{EO}$) and ABTS (1181.96 ± 118.86 $\mu g_{TE}/g_{EO}$) assays were observed in the EO from plants subjected to moderate heat, with a 41 and 34% increase in the capacity to scavenge ABTS and DPPH free radicals, respectively, compared to the control. Furthermore, drought, severe heat, and UV-B radiation also enhanced the antioxidant capacity of the EO.

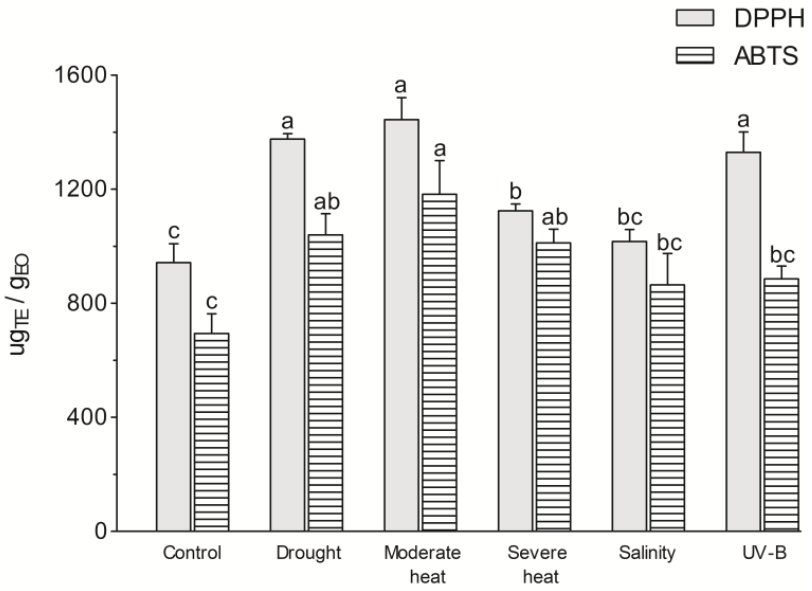

Figure 3. Antioxidant activity (DPPH, 2,2-diphenyl-1-picrylhydrazyl; ABTS, 2,2-azino-bis(3-ethylbenzothiazoline-6-sulfonic acid) of *Lavandula viridis* L'Hér essential oils isolated from plants subjected to different environmental conditions. Values are presented as mean ± SE. Different letters in each method indicate significant differences ($p < 0.05$), (Duncan's new multiple range test).

The antioxidant capacity of plant extracts is typically higher than that of EOs, mainly due to the chemical structure and redox properties of the phenolic compounds usually present in the extracts, which are essential for neutralizing reactive oxygen species (ROS), such as free radicals [60]. This is consistent with our findings, in which *L. viridis* extracts rich in phenolic compounds exhibited a greater antioxidant activity (approximately 70–180 $mg_{TE}/g_{extract}$) [7] than the EOs evaluated in this study (approximately 0.7–1.4 mg_{TE}/g_{EO}). Only one study was identified in the literature that examined the influence of abiotic factors on the antioxidant activity of Lamiaceae EOs. In contrast to our findings, the aforementioned study demonstrated that the antioxidant capacity of *Thymus daenensis* Celak EO was higher under normal irrigation conditions than under drought [60]. Interestingly, salinity, despite being a favorable stimulus for enhancing the production of the main compounds of *L. viridis* EO (1,8-cineole and camphor), did not demonstrate a pronounced effect on the antioxidant capacity. These findings may indicate the potential involvement of minor components of the EO in the antioxidant activity. Pearson's correlation (Figure 4) indicates that borneol (identified in a range of 2.31–2.76%) was the compound that most contributed to ABTS scavenging (0.745, $p \leq 0.01$), followed by p-cymenene (0.643, $p \leq 0.05$) (0.05–0.09%), 2-phenyl ethyl propanoate (0.622, $p \leq 0.05$) (0.13–0.18%), p-cymen-8-ol (0.613, $p \leq 0.05$) (0.17–0.31%), humulane-1,6-dien-3-ol (0.579, $p \leq 0.05$) (0.15–0.18%), and germacrene D (0.578, $p \leq 0.05$) (0.15–0.18%). Eugenol (0.637, $p \leq 0.05$) (0.36–0.49%) and myrtenol (0.599, $p \leq 0.05$) (1.08–1.25%) were the most important components responsible for the DPPH scavenging capacity. Moreover, numerous other compounds demonstrate a significant or moderate correlation with ABTS and DPPH scavenging potential. This suggests that the antioxidant efficacy of the investigated EOs may depend on the collective action of multiple compounds, rather than a single component.

Figure 4. Heatmap representing the Pearson's correlation coefficients between the 82 compounds identified in the *Lavandula viridis* L'Hér essential oils and the inhibition of three enzymes involved in neurodegenerative diseases (AChE, BChE, and Tyr), as well as antioxidant activity (DPPH and ABTS). Correlation is significant at $p \leq 0.01$ (**) and $p \leq 0.05$ (*).

3.3. Enzyme Inhibitory Assays

The ability of *L. viridis* EOs to inhibit the activity of three enzymes (AChE, BChE, and Tyr) is shown in Table 2. This is the first report on the ability of *L. viridis* EOs to inhibit Tyr. Furthermore, to the best of our knowledge, this is also the first study reporting the influence of abiotic factors on the inhibitory activities of EOs against the three enzymes. In vitro inhibition assays of AChE and BChE, which are responsible for the hydrolysis of the neurotransmitter acetylcholine, represent a valuable approach to identify new inhibitors of these enzymes from natural sources with potential applications in the treatment of Alzheimer's disease [67]. In humans, excessive melanin production can cause hyperpigmentation pathologies, such as melanoma [68], as well as neurodegenerative processes leading to Parkinson's disease [69]. Consequently, the identification of novel Tyr inhibitors may facilitate the development of novel cosmetic products and innovative therapeutic strategies for the treatment of Parkinson's disease and cancer. The neuroprotective effects of EOs derived from Lamiaceae species and their components have been documented [70,71]. Furthermore, EOs have been employed in aromatherapy to mitigate the symptoms of dementia and improve memory and cognition in patients [72]. The present study demonstrated that *L. viridis* EOs exhibited greater efficacy in inhibiting AChE than BChE and Tyr (Table 2).

Table 2. Acetylcholinesterase (AChE), butyrylcholinesterase (BChE), and tyrosinase (Tyr) inhibitory activities of the *Lavandula viridis* L'Hér essential oils isolated from plants subjected to different environmental conditions.

Treatment	AChE Inhibition (IC50, µg/mL)	BChE Inhibition (IC50, µg/mL)	Tyr Inhibition (IC50, µg/mL)
Control	781.33 ± 31.70 a	4479.75 ± 94.99 ab	6303.13 ± 952.03 a
Drought	515.56 ± 117.51 bc	3919.30 ± 182.36 b	5991.08 ± 665.98 ab
Moderate heat	626.07 ± 8.53 ab	6027.41 ± 825.47 a	5478.96 ± 994.46 ab
Severe heat	566.21 ± 96.06 abc	2250.22 ± 205.42 c	3551.01 ± 315.98 b
Salinity	406.56 ± 53.43 bc	5660.39 ± 505.35 a	7332.91 ± 682.69 a
UV-B	338.80 ± 9.11 c	4799.53 ± 293.32 ab	5612.41 ± 712.80 ab
Kojic acid			6.88 ± 0.77
Galantamine	10.05 ± 0.03	153.13 ± 8.82	

The results were analyzed using one-way analysis of variance (ANOVA) followed by Duncan's new multiple range test. Different letters mean significant differences ($p < 0.05$) among environmental conditions.

EOs derived from plants exposed to drought, salinity, and UV-B radiation showed superior inhibitory activity against AChE compared to the control EO. The most effective result was observed for UV-B radiation (338.80 ± 9.11 µg/mL), which caused a twofold higher inhibition compared to the control (781.33 ± 31.70 µg/mL). Furthermore, the UV-B result was superior to that reported by Costa et al. [11], in which the EO of *L. viridis* exhibited an IC_{50} of 411.33 ± 72.73 µg/mL. Furthermore, the *L. viridis* EOs showed greater AChE inhibitory activity than that shown by other *Lavandula* species, including *L. angustifolia* [64]. The highest inhibition results of BChE and Tyr were observed when severe heat was applied (IC_{50} = 2250.22 ± 205.42 µg/mL for BChE and IC_{50} = 3551.01 ± 315.98 µg/mL for Tyr). Similarly to our results, *L. angustifolia* EOs also exhibited a low anti-tyrosinase activity [64,73], with *Lavandula stoechas* L. EO [74] being more effective in inhibiting this enzyme.

The results presented in the heatmap (Figure 4) indicate that α-campholenal was the compound with the strongest correlation with AChE inhibition (−0.813, $p \leq 0.01$), followed by 6-camphenol (−0.799, $p \leq 0.01$), trans-linalool oxide (−0.787, $p \leq 0.01$), p-mentha-1,4-dien-7-ol (−0.779, $p \leq 0.01$), α-pinene oxide (−0.759, $p \leq 0.01$), octyl 2-methylbutyrate (−0.731, $p \leq 0.01$), benzyl benzoate (−0.711, $p \leq 0.01$), myrtenal (−0.656, $p \leq 0.05$), and terpinen-4-ol (−0.589, $p \leq 0.05$). The literature indicates that benzyl benzoate has the potential to reduce the activity of AChE in *Haemaphysalis longicornis* [75]. Myrtenal has been shown to possess neuroprotective properties [76], while terpinene-4-ol has demonstrated

a high binding affinity towards the active binding site of AChE in a molecular docking study [77]. The compounds with the strongest correlations with BChE were cis-β-terpineol (-0.610, $p \leq 0.05$), 3,9-epoxy-p-menth-1-ene (-0.607, $p \leq 0.05$), α-terpinen-7-al (-0.583, $p \leq 0.05$), and cis-verbenol (-0.582, $p \leq 0.05$). Furthermore, the results demonstrated that germacrene D (-0.751, $p \leq 0.01$), cis-β-farnesene (-0.722, $p \leq 0.01$), and ρ-methyl-acetophenone (-0.597, $p \leq 0.05$) exhibited a high degree of correlation with Tyr inhibition. Recently, Tran-Trung et al. [78] demonstrated that germacrene D interacts with the residue His381 within the active site of the Tyr enzyme, suggesting that this compound may be a potential candidate for Tyr inhibitors. Similarly, all these correlated compounds are present in *L. viridis* EOs in lower concentrations (0.05–4.07%), indicating that synergism may also be related to the potential of EOs to inhibit different enzymes.

4. Conclusions

This is the first report studying the effect of abiotic factors on the EOs' composition of *L. viridis*. In general, the environmental conditions tested resulted in enhanced EO extraction yield and caused some quantitative changes in the chemical composition. The oxygenated monoterpenes were the most abundant components of the essential oils studied, with 1,8-cineole being the major compound. Furthermore, the application of heat and salinity resulted in an enhanced production of 1,8-cineole, which could be of significant interest to the cosmetic and biomedical industries in the context of climate change. Furthermore, the environmental conditions tested in this study demonstrated an increase in the biological properties of *L. viridis* EO, including its capacity to inhibit AChE, BChE, and Tyr, as well as its antioxidant activity. Nevertheless, since EOs are composed of numerous chemical compounds with diverse structures and modes of action, it is crucial to investigate the influence of the synergistic mechanisms on the composition and bioactivity of the plant products in future studies.

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