

Article

The Influence of Abiotic Factors on the Yield and Composition of the Essential Oil of the Mastic Tree (*Pistacia lentiscus* L.) Leaves

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Featured Application

Optimizing the harvest time, phenological stage, and distillation pressure in the production of essential oil from *Pistacia lentiscus* leaves can increase the yield of monoterpene-rich oils with enhanced biological activity. The development of such products has significant potential as an environmentally acceptable alternative for use as natural plant protection agents.

Abstract

This study evaluated the effects of abiotic factors and extraction conditions on the yield, chemical composition, and antimicrobial activity of essential oil (EO) from *Pistacia lentiscus* L. leaves collected at four Adriatic locations during three phenological stages. Steam distillation was performed at 0.3, 0.7, and 1 bar. EO yield increased significantly with pressure, reaching a maximum at 1 bar, while the flowering stage provided the highest yields overall. Leaves from Vela Luka produced the highest EO yield, whereas Pag samples yielded the least. GC–MS analysis identified 56 components, accounting for 99.19–99.99% of total EO, with α -pinene, limonene, myrcene, and β -pinene as the dominant constituents, confirming a monoterpene-rich chemotype. All EO samples showed low but measurable inhibitory activity against *Escherichia coli* AB1157 and *Erwinia amylovora* EaED, as assessed by the disk diffusion method. Pearson correlation and PCA analyses indicated a positive association between monoterpene content and inhibition zone diameter against *E. coli*, and a positive association between monoterpene alcohol content and inhibition against *E. amylovora*. As antimicrobial activity was assessed exclusively by the disk diffusion method, the present findings may serve as an indicative basis for future investigations into the relationship between EO chemical composition and antimicrobial potential, and they require validation through quantitative, standardized antimicrobial testing.



Academic Editor: Antonios E. Koutelidakis

Received: 31 March 2026

Revised: 8 May 2026

Accepted: 9 May 2026

Published: 11 May 2026

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Keywords: *Pistacia lentiscus* L.; essential oil; steam distillation; phenological stage; GC–MS; antimicrobial activity; abiotic factors; monoterpenes

1. Introduction

The mastic tree (*Pistacia lentiscus* L.) is an evergreen shrub belonging to the Anacardiaceae family, widely distributed across Mediterranean regions [1]. The chemovariability of plants, including the mastic tree, is influenced by numerous factors such as environmental conditions and geographical origin. Phenological stage (PS), harvest season, solar

radiation, precipitation, and topography are among the key determinants of plant morphological traits as well as the composition and yield of bioactive compounds.

Beghlal et al. [2] have shown that *P. lentiscus* leaves contain approximately 90.2% dry matter and are rich in phytonutrients, including phenolic compounds, aromatic constituents, and minerals. Due to the complex mixture of chemical constituents present in essential oils (EOs), their applications and biological effects are diverse. EOs have demonstrated antibacterial, antifungal, antiviral, and repellent properties [3] as well as pharmacological effects such as diuretic and spasmolytic activity. In a study conducted by Bouchfara et al., [4] it was shown that mastic tree EOs also possess strong antioxidant properties. Consequently, their major applications today are in the food industry (as natural preservatives), the cosmetic industry (perfume production), and the pharmaceutical industry [5].

Using conventional steam distillation methods, numerous studies have identified and quantified the dominant components of EO obtained from the aerial parts of *P. lentiscus* [6,7]. Recently, in addition to classical methods of essential oil extraction, advanced techniques such as microwave-assisted extraction (MAE), ultrasound-assisted extraction (UAE), and supercritical fluid extraction (SFE) have also been used [8]. The results of the study showed that the extraction technique, in addition to affecting the yield, which was highest with SFE, also significantly influences the composition of the essential oil. Variations in EO composition are primarily associated with the plant organ used for extraction, environmental conditions, harvest period [9], and PS [10]. Depending on the chemotype, *P. lentiscus* EO is mainly composed of monoterpenes (α -pinene, β -pinene, β -myrcene, limonene, terpinen-4-ol) and sesquiterpenes (β -caryophyllene, germacrene D, γ -cadinene) [11–14].

Studies conducted in different Mediterranean countries have identified several chemotypes characterized by dominant constituents such as myrcene (19%, Spain) [11], α -pinene (15%, France) [12], terpinen-4-ol (22%, Sardinia; 33–44%, Morocco) [15,16], δ -3-carene (65%, Egypt) [17], and α -terpineol (13%, Israel) [18].

Fernández et al. [14] reported that sesquiterpenes constitute the dominant fraction (47%) of *P. lentiscus* EO, with *trans*-caryophyllene, τ -cadinene, and germacrene D as major components. In samples collected from Algeria, Djenane et al. [13] identified 57 compounds representing 98.69% of the total oil composition, with β -myrcene (15.8%), 1,8-cineole (15.02%), terpinen-4-ol (6.41%), α -pinene (5.54%), and β -pinene (5.10%) as the most abundant constituents.

The antimicrobial properties of EOs and their individual components have been widely investigated, although their mechanisms of action are not yet fully elucidated [19]. Because EOs can interact with multiple cellular targets in microorganisms, several antibacterial mechanisms have been proposed [20]. In particular, antimicrobial activity is strongly associated with the presence of hydroxyl (OH) groups in phenolic constituents [19].

Several studies have demonstrated the antimicrobial potential of *P. lentiscus* EO against both Gram-positive and Gram-negative bacteria. Aouinti et al. [21] reported that EO obtained by steam distillation from samples collected in the Moroccan region of Jerada showed the strongest antibacterial activity against *Staphylococcus aureus*, followed by *Escherichia coli* and *Klebsiella pneumoniae*. These findings confirm the relevance of *P. lentiscus* EO as a natural antimicrobial agent.

Previous research has predominantly focused either on the chemical composition or on the biological activity of *P. lentiscus* EO, while integrative studies simultaneously evaluating the effects of geographical origin, PS, and technological extraction parameters remain limited. A systematic understanding of the interaction among these factors is essential

for the standardization of EO quality and its targeted application in the pharmaceutical, cosmetic, and food industries.

Therefore, the objectives of this study were to (i) investigate the influence of distillation pressure on *P. lentiscus* leaf EO yield and composition, (ii) determine the effects of location and PS on chemical composition and distribution of compound classes, and (iii) evaluate the antimicrobial activity of the obtained EOs against selected Gram-negative bacteria. The results contribute to a better understanding of the chemical variability of *P. lentiscus* and provide a basis for optimizing extraction conditions to obtain EOs with targeted biological activity.

2. Materials and Methods

2.1. Plant Material

This study used dried and ground samples of wild-growing mastic tree leaves, hand-harvested at 4 locations along the Adriatic coast (Croatia): Barbariga, Pag, Hvar and Vela Luka in three PSs during growing season: in May (1st—flowering stage), August (2nd—early fruit stage), and in October (3rd—late ripe fruiting stage). Geographic coordinates, PSs and bioclimatic characteristics of the growing locations as well as identification of plant material are published in our previous paper [22]. After harvesting, the samples were transferred to a dark room where they were left to dry at room temperature, after which they were cleaned. The cleaned samples were ground (Nutribullet, Capital Brands LLC, Los Angeles, CA, USA), stored in plastic containers and kept in a refrigerator at +4 °C until further analysis.

2.2. Obtaining Essential Oil from Leaves by Steam Distillation

Approximately 20 kg of prepared plant material was loaded into the distillation apparatus (Darkol, Oroslavje, Croatia) in a loose form to prevent excessive compaction and to allow unobstructed steam flow. Each sample was distilled in triplicate at a constant temperature of 100 °C for 90 min, using steam pressures of 0.3, 0.7, and 1 bar.

2.3. Identification and Quantification of Essential Oil Constituents

Qualitative analysis and the quantitative proportion of constituents in the EO samples was carried out using gas chromatography with mass spectrometry on QP 2010 Plus (Shimadzu, Kyoto, Japan) according to method previously described by Adams [23] with certain modifications. The injection volume was 1.0 µL and chromatographic separation was achieved on a ZB-5 MS capillary column (60 m × 0.32 mm i.d., film thickness 0.25 µm, Phenomenex, Torrance, CA, USA). Helium 6.0 was used as the carrier gas at a flow rate of 2.2 mL min^{−1}, with a split ratio of 1:50. The injector temperature was set to 220 °C, the detector temperature to 250 °C, and the transfer line temperature to 280 °C. The oven temperature program was as follows: initial temperature of 60 °C held for 1 min, then increased to 250 °C at a rate of 4 °C min^{−1}. Electron impact ionization was performed at 70 eV. Mass spectra were recorded at a scan rate of 1 scan s^{−1} over *m/z* range of 43–350. In order to calculate the retention index of the separated aromatic compounds, a standard mixture of C₈–C₂₀ alkanes (Sigma-Aldrich Co., Darmstadt, Germany) was prepared and analyzed under the same chromatographic conditions as the samples. The identification of aromatic compounds was carried out by comparing the mass spectra of the extracted aromatic compounds with those in the NIST database, ver. 2.0 (NIST, Gaithersburg, MD, USA) and using the computer program AMDIS, ver. 2.62 (Automated Mass Spectral Deconvolution and Identification System) (NIST, Gaithersburg, MD, USA) and by comparing the obtained retention indices with values in the literature [24]. The

quantitative proportion of the extracted aromatic compounds was expressed as the area fraction in the total aroma of the sample.

2.4. Determining the Antimicrobial Effect of the Essential Oil by the Disk Diffusion Method

Liquid and solid nutrient media Nutrient Broth (NB) and Luria–Bertani (LB) were prepared by dissolving the appropriate components in distilled water with heating on a magnetic stirrer, according to the manufacturer's instructions. The media were sterilized by autoclaving at 121 °C for 15 min. After sterilization, the molten media were transferred to a laminar-flow cabinet, poured into Petri dishes, and allowed to cool to approximately 50 °C for 10 min under sterile airflow to allow the agar to solidify and the surface to dry before closing.

A pure culture of *E. coli* strain AB1157 was grown in a 200 mL glass Erlenmeyer flask containing liquid LB medium at 37 °C for 24 h. A pure culture of *Erwinia amylovora* strain EaED was grown in a 200 mL glass Erlenmeyer flask containing liquid NB medium at 28 °C for 24 h.

The antimicrobial activity of the EOs was evaluated using the disk diffusion method described by Bauer et al. [25]. Solid nutrient media NA and LB were poured into 9 cm diameter glass Petri dishes under sterile conditions. Subsequently, 100 µL of the *E. coli* AB1157 culture was inoculated onto dried LB plates, while 50 µL of the *E. amylovora* EaED culture was inoculated onto dried NA plates. The inoculates were evenly spread over the agar surface using a sterile glass rod.

After absorption of excess moisture, sterile paper filter disks (6 mm diameter; Bio-Rad, Hercules, CA, USA) were placed on the agar surface using sterile tweezers, with eight disks positioned on each Petri dish. Each EO sample was applied to a separate disk at a volume of 5 µL. Chloramphenicol (1 µL) served as the positive control, while distilled water was used as the negative control.

The Petri dishes were incubated at 37 °C, and after incubation, the diameters of the inhibition zones were measured in mm, using the digital vernier caliper (Insize Co., Ltd., Suzhou, China). Inhibition was assessed by measuring the clear halo extending beyond the outer edge of each 6 mm paper disk, excluding the disk diameter. The inhibition zone values are therefore reported as halo width in mm and not as total zone diameter including the disk. All experiments were performed in triplicate.

2.5. Statistical Analysis

Statistical analyses were performed using Statistica software, ver. 14.0 (TIBCO Software Inc., Santa Clara, CA, USA). Descriptive statistics were used to summarize the data, which are presented as mean $\pm$ standard deviation (SD). Data normality and homogeneity of variances were assessed using the Shapiro–Wilk and Levene's tests, respectively.

The effects of location and PSs on chemical groups proportions of *P. lentiscus* leaf EOs and antimicrobial activity were evaluated using factorial analysis of variance (ANOVA), followed by Tukey's honestly significant difference (HSD) test for post hoc comparisons. For data that did not meet parametric assumptions, the non-parametric Kruskal–Wallis test was applied. Relationships between the identified compounds and antimicrobial activity were evaluated using Pearson's correlation coefficients, while potential grouping of samples according to the examined sources of variation (location and PS) was assessed by Principal Component Analysis (PCA). Principal components with an eigenvalue greater than 1 were considered, and variables with a communality value of at least 0.5 were included in the analysis. Statistical significance for all tests was set at $p \leq 0.05$.

3. Results and Discussion

3.1. Effect of Pressure on the Yield of Mastic Tree Leaf Essential Oil

The results in Table 1 show that pressure during distillation significantly affected the yield of mastic tree leaf EO. Increasing the pressure during steam distillation led to progressively higher EO yields, with the maximum yield observed at 1 bar (0.767%) and the lowest at 0.3 bar (0.117%), depending on the location. The yield at 1 bar is comparable to previously reported values for mastic tree leaf EO from Greece (0.4%) and Corsica (0.5%), and exceeds those reported for Morocco (0.2%) and Italy (0.3%) [6,7,26,27]. These findings align with the study by Babu et al. [28], which showed that increasing distillation pressure (0.2–0.8 bar) significantly enhanced the yield of EO from *Rosa damascena* Mill. petals, with the highest yield obtained at the highest pressure. Higher pressure likely promotes the rupture of glandular trichomes and improves the release of volatile compounds, thereby increasing extraction efficiency.

Table 1. Essential oil yields of mastic tree leaves samples harvested at four locations during three phenological stages using steam distillation at different pressures.

Location	Phenological Stage	Pressure (Bar)		
		0.3	0.7	1
		Yield (%)		
Barbariga	1	0.217 ± 0.01 ^a	0.267 ± 0.03 ^b	0.453 ± 0.02 ^c
	2	0.123 ± 0.03 ^a	0.157 ± 0.02 ^b	0.203 ± 0.03 ^c
	3	0.140 ± 0.03 ^a	0.185 ± 0.01 ^b	0.351 ± 0.02 ^c
Pag	1	0.182 ± 0.02 ^a	0.237 ± 0.02 ^b	0.413 ± 0.03 ^c
	2	0.117 ± 0.03 ^a	0.113 ± 0.02 ^a	0.233 ± 0.02 ^b
	3	0.132 ± 0.03 ^a	0.142 ± 0.01 ^b	0.379 ± 0.01 ^c
Hvar	1	0.348 ± 0.03 ^a	0.490 ± 0.02 ^b	0.633 ± 0.03 ^c
	2	0.163 ± 0.03 ^a	0.303 ± 0.03 ^b	0.426 ± 0.03 ^c
	3	0.215 ± 0.04 ^a	0.396 ± 0.01 ^b	0.517 ± 0.02 ^c
Vela Luka	1	0.365 ± 0.01 ^a	0.513 ± 0.02 ^b	0.767 ± 0.02 ^c
	2	0.173 ± 0.02 ^a	0.411 ± 0.03 ^b	0.492 ± 0.02 ^c
	3	0.243 ± 0.03 ^a	0.485 ± 0.02 ^b	0.559 ± 0.02 ^c

Results are expressed as mean value ± SD; 1 = early flowering stage, 2 = early fruiting stage, 3 = late fruiting stage; Values with different letters within rows are statistically different at $p \leq 0.05$.

3.2. Influence of Location and Phenological Stage on Essential Oil Yield

EO yield is strongly influenced by environmental conditions and the plant's developmental stage. Factors such as temperature, solar radiation, precipitation, wind exposure, and geographical characteristics can induce physiological stress responses that alter secondary metabolite production. Numerous studies have demonstrated that both geographic origin and PS significantly affect EO yield [29,30].

Among the locations investigated (Table 1), the highest yield was recorded for samples collected at Vela Luka (0.767%), followed by Hvar (0.633%), Barbariga (0.453%), and Pag (0.413%). Comparison of EO yields across PSs showed that the highest yields were consistently obtained during PS1 (flowering stage). The same trend was observed during PS2 and PS3, although with lower overall yields. The increased EO yield during PS1 can be explained by enhanced biosynthesis of volatile compounds during flowering, when plants produce aromatic metabolites to attract pollinators [31]. Similar observations have been reported for several aromatic species, including *Thymus vulgaris* [32], *Salvia officinalis* [33], *Hyptis suaveolens* [34], *Satureja rechingeri* [35], and *Origanum majorana* [36]. Although PS1 showed the highest yields overall, slight reductions observed at the Barbariga and Pag locations may be attributed to higher precipitation levels. Increased rainfall can reduce

EO yield by damaging glandular trichomes or diluting metabolite concentrations [37,38]. Across all PSs, similar yield patterns were observed between the Hvar and Vela Luka locations, as well as between Barbariga and Pag, reflecting similarities in climatic conditions and solar exposure [22]. Samples from Hvar and Vela Luka were exposed to direct sunlight, whereas those from Barbariga and Pag experienced partial or full shading.

The results of the statistical analysis of the dependence of EO yield on location and PS (Table 2) showed that both location and PS significantly affected EO yield. The highest EO yield obtained by steam distillation from mastic tree leaves was from samples harvested in Vela Luka, while samples from Pag contained the lowest amount of EO. Considering PS, the early flowering stage (PS1) accumulated the highest levels of EO, while the lowest amounts of EO were isolated from the PS2 samples, i.e., early fruiting-stage samples.

Table 2. The differences in yield of mastic tree leaf EOs due to location and PS at pressure of 1 bar.

Source of Variation	Yield (%)
Location	$p < 0.01$ *
Barbariga	0.16 ± 0.04 ^b
Pag	0.14 ± 0.01 ^a
Hvar	0.24 ± 0.03 ^c
Vela Luka	0.26 ± 0.03 ^d
Phenological stage	$p < 0.01$ *
1	0.28 ± 0.02 ^c
2	0.15 ± 0.01 ^a
3	0.18 ± 0.01 ^b
Grand mean	0.20

1 = early flowering stage, 2 = early fruiting stage, 3 = late fruiting stage; Results are expressed as mean $\pm$ standard error (SE). * Statistically significant variable at $p \leq 0.05$. Values with different letters within column are statistically different at $p \leq 0.05$.

These findings confirm that EO yield is controlled by the combined influence of multiple abiotic factors rather than a single environmental parameter. Response surface modeling further indicated that optimal extraction conditions were achieved at 1 bar during PS1 for samples collected at Vela Luka (Figure 1).

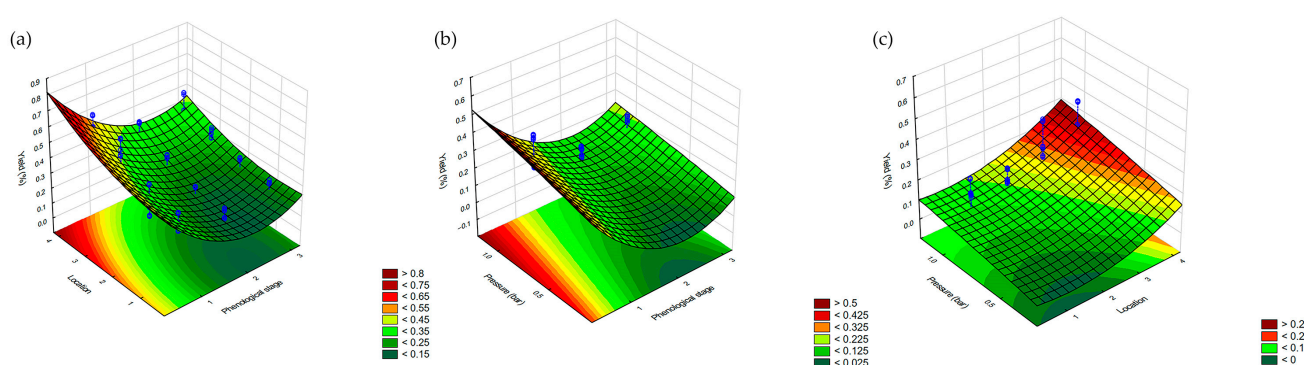


Figure 1. 3D graphs of the evolution of essential oil production by steam distillation (a) yield evolution at a fixed pressure of 1 bar, (b) yield evolution in samples from location Vela Luka (c) yield evolution from samples taken during first phenological phase (Location: 1—Barbariga, 2—Pag, 3—Hvar, 4—Vela Luka).

3.3. Chemical Composition of Mastic Tree Leaf Essential Oil

In the samples obtained, a total of 56 components were identified, representing 99.19–99.99% of the total composition of the EO extracted from *P. lentiscus* leaves by steam distillation (Table 3). The main components identified were α -pinene (9.19–23.88%),

limonene (6.38–12.45%) myrcene (1.04–16.15%) and β -pinene (2.43–12.17%). Other significant components include sabinene, germacrene D, α -terpinene, γ -terpinene, terpinen-4-ol, β -caryophyllene and δ -cadinene.

The results obtained are consistent with the research conducted by Gardeli et al. [39], who demonstrated that the main components in the EO of Greek mastic tree leaves are α -pinene (9.40–24.9%) and limonene (9.00–17.80%), with other components present in high percentages including germacrene D (2.70–13.50%), terpinen-4-ol (6.80–10.60%), β -pinene (2.00–6.90%), sabinene (1.00–6.70%), γ -terpinene (3.10–3.60%) and α -terpineol (2.50–4.00%). Similar results were also reported by Derwich et al. [40], who identified and quantified 23 constituents of the EO of mastic tree leaves, with the main constituents being α -pinene (24.25%), β -pinene (12.58%), limonene (7.56%), terpinen-4-ol (6.98%), α -terpineol (4.89%) and myrcene (2.09%).

The highest α -pinene contents in the EO samples from mastic tree harvested at Pag, Hvar and Vela Luka are the result of the combined effect of increased insolation and reduced precipitation during PS2 [22]. According to research by Feijó et al. [41], the highest α -pinene contents in the EO of *Varronia curassavica* were also due to insolation, which confirms that α -pinene is a compound dependent on bioclimatic factors. Furthermore, research by Serralutzu et al. (2020) [42] showed that α -pinene, the main component of the EO from *Rosmarinus officinalis* originating from Sardinia, has a significant statistical correlation with air temperature, with its content increasing as temperature rises during PSs. Several factors may cause differences in the contents of EO components. The biosynthesis of EO components is influenced by various environmental factors that significantly affect their content. Some of these factors are light intensity, climatic conditions and the availability of nutrients in the soil [43,44].

Myrcene content exhibited the greatest variability among all identified components of the EO in mastic tree leaves between PS within individual locations, consistent with previous research [45]. The myrcene content ranged from 1.04 to 16.15%, with the highest value observed in samples from the Pag location during PS3 and the lowest in samples from the Vela Luka location during PS1. During the transition from PS1 to PS2, myrcene content decreased in samples from the Barbariga and Hvar locations by 79.45 and 72.19%, respectively, while in samples from the Pag and Vela Luka locations, an increase of 85.48 and 41.44% was recorded between PS2 and PS3. In addition to myrcene, sabinene also showed significant variability depending on PS and location, with a minimum content of 1.33% in samples from the Hvar location during PS1 and a maximum of 19.82% in samples from the Pag location during PS2.

Lakušić et al. [46] stated that differences in the content and quality of EOs depend on various factors, such as differences between plant species, the use of different plant parts for extraction, the stage of development at harvest, and the possibility of growing plants in different locations, which leads to changes in the chemical composition of plants due to their adaptation to different environmental conditions.

In addition to individual compounds (Table 3), Table 4 presents the proportions of eight chemical groups identified in EO samples from mastic tree leaves. The qualitative profiles of all obtained EO samples are characterized by the highest proportions of monoterpenes (52.10–76.67%), which include key components such as α -pinene, limonene, and β -pinene.

Table 3. Components of the mastic tree essential oil leaves (%) harvested at four locations during three phenological stages using steam distillation at 1 bar pressure.

RT	RI	Component	Barbariga			Pag			Hvar			Vela Luka		
			1	2	3	1	2	3	1	2	3	1	2	3
5.86	853	3-Hexen-1-ol	n.d.	0.36 ± 0.01	n.d.	n.d.	0.53 ± 0.04	n.d.	n.d.	n.d.	n.d.	n.d.	0.21 ± 0.04	0.39 ± 0.05
6.12	866	n-Hexanol	n.d.	0.11 ± 0.02	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	0.10 ± 0.02	n.d.
7.52	927	Tricyclene	1.01 ± 0.02	0.3 ± 0.01	0.34 ± 0.01	0.37 ± 0.52	0.40 ± 0.05	0.32 ± 0.05	0.33 ± 0.02	0.82 ± 0.05	0.46 ± 0.03	0.45 ± 0.04	0.87 ± 0.04	0.32 ± 0.06
7.59	931	α-Thujene	0.86 ± 0.02	0.16 ± 0.01	0.20 ± 0.01	0.30 ± 0.01	0.33 ± 0.05	0.14 ± 0.03	0.38 ± 0.04	2.15 ± 0.22	0.32 ± 0.04	0.14 ± 0.03	0.19 ± 0.06	0.70 ± 0.05
7.84	939	α-Pinene	9.26 ± 0.05	19.52 ± 0.17	21.22 ± 0.13	16.25 ± 0.05	19.55 ± 0.13	13.70 ± 0.17	11.71 ± 0.38	23.88 ± 0.19	10.81 ± 0.21	22.51 ± 0.53	23.86 ± 1.01	9.43 ± 0.17
8.24	954	Camphane	2.48 ± 0.06	1.39 ± 0.02	1.84 ± 0.01	1.66 ± 0.02	1.58 ± 0.03	1.55 ± 0.02	1.61 ± 0.09	3.53 ± 0.18	1.91 ± 0.09	1.93 ± 0.05	3.39 ± 0.07	1.24 ± 0.14
8.89	977	Sabinene	5.48 ± 0.05	9.75 ± 0.07	4.01 ± 0.99	8.84 ± 0.05	19.92 ± 0.66	2.85 ± 0.06	1.33 ± 0.04	1.40 ± 0.07	3.94 ± 0.14	8.05 ± 0.80	6.12 ± 0.11	6.64 ± 0.35
9.03	982	β-Pinene	4.93 ± 0.04	9.90 ± 0.03	9.14 ± 0.15	3.23 ± 0.04	2.43 ± 0.06	7.12 ± 0.17	5.99 ± 0.22	11.88 ± 0.49	4.72 ± 0.13	7.53 ± 0.06	12.37 ± 0.45	3.96 ± 0.27
9.33	992	Myrcene	10.23 ± 0.08	1.16 ± 0.02	3.89 ± 0.10	1.07 ± 0.04	1.18 ± 0.05	16.15 ± 0.37	12.71 ± 0.58	2.02 ± 0.06	8.99 ± 0.10	1.04 ± 0.09	1.66 ± 0.07	4.01 ± 0.23
9.64	1004	3-Hexen-1-ol acetate	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	0.33 ± 0.04
9.79	1008	α-Phellandrene	5.42 ± 0.04	2.66 ± 0.02	1.80 ± 0.06	2.69 ± 0.05	0.92 ± 0.08	4.54 ± 0.14	3.71 ± 0.09	2.97 ± 0.11	1.72 ± 0.09	0.58 ± 0.04	3.15 ± 0.06	4.86 ± 0.14
10.04	1018	1,4-Cineole	n.d.	n.d.	0.05 ± 0.01	n.d.	n.d.	n.d.	0.29 ± 0.06	n.d.	n.d.	n.d.	n.d.	n.d.
10.14	1020	α-Terpinene	1.89 ± 0.02	3.43 ± 0.03	6.45 ± 0.07	5.36 ± 0.06	4.96 ± 0.17	4.14 ± 0.08	4.00 ± 0.36	5.11 ± 0.10	6.36 ± 0.09	2.63 ± 0.07	2.34 ± 0.07	4.69 ± 0.14
10.38	1028	p-Cymene	2.58 ± 0.02	0.65 ± 0.02	0.77 ± 0.02	0.91 ± 0.04	1.23 ± 0.07	0.93 ± 0.07	1.91 ± 0.12	0.97 ± 0.04	1.47 ± 0.06	0.91 ± 0.02	0.69 ± 0.06	1.91 ± 0.06
10.57	1034	Limonene	7.83 ± 0.02	9.45 ± 0.02	10.34 ± 0.12	7.58 ± 0.04	6.48 ± 0.27	10.33 ± 0.39	9.92 ± 0.33	12.56 ± 0.15	7.99 ± 0.09	7.50 ± 0.25	10.32 ± 0.86	7.82 ± 0.28
10.64	1038	cis- β-Ocimene	n.d.	n.d.	0.28 ± 0.02	n.d.	n.d.	0.72 ± 0.06	0.33 ± 0.03	0.12 ± 0.06	0.73 ± 0.07	n.d.	0.24 ± 0.05	n.d.
11.03	1049	trans- β-Ocimene	0.27 ± 0.01	0.29 ± 0.03	1.19 ± 0.01	n.d.	0.14 ± 0.01	2.93 ± 0.08	1.44 ± 0.06	0.60 ± 0.06	2.01 ± 0.14	0.22 ± 0.05	1.23 ± 0.05	0.35 ± 0.06
11.18	1056	Pentyl isobutanoate	0.33 ± 0.01	0.29 ± 0.02	0.75 ± 0.01	0.68 ± 0.08	0.56 ± 0.05	0.29 ± 0.07	0.97 ± 0.06	0.19 ± 0.03	0.58 ± 0.07	0.41 ± 0.07	0.29 ± 0.06	0.80 ± 0.06
11.27	1058	Isopentyl butanoate	n.d.	0.08 ± 0.02	0.20 ± 0.01	0.23 ± 0.02	0.12 ± 0.03	0.09 ± 0.02	0.23 ± 0.05	n.d.	0.15 ± 0.01	n.d.	0.28 ± 0.06	n.d.
11.46	1062	γ-Terpinene	2.69 ± 0.04	5.41 ± 0.10	8.99 ± 0.08	7.91 ± 0.06	7.42 ± 0.07	5.70 ± 0.63	5.19 ± 0.10	7.35 ± 0.10	7.79 ± 0.21	4.13 ± 0.06	3.50 ± 0.14	5.89 ± 0.13
11.88	1076	p-Mentha-3,8-dien	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	0.19 ± 0.02	n.d.	n.d.	n.d.	n.d.	n.d.
12.44	1091	Terpinolene	1.7 ± 0.08	1.83 ± 0.01	3.57 ± 0.03	3.26 ± 0.02	2.44 ± 0.08	2.71 ± 0.17	4.37 ± 0.15	3.34 ± 0.10	4.67 ± 0.11	1.81 ± 0.05	1.45 ± 0.11	3.18 ± 0.09
12.73	1099	2-Methylbutyl isovalerate	n.d.	0.08 ± 0.01	0.20 ± 0.02	0.31 ± 0.03	0.15 ± 0.02	0.22 ± 0.03	0.36 ± 0.04	0.08 ± 0.01	0.32 ± 0.05	0.17 ± 0.07	0.11 ± 0.05	0.41 ± 0.05
12.85	1102	Isopentyl isovalerate	0.3 ± 0.08	0.09 ± 0.01	0.32 ± 0.02	0.30 ± 0.01	0.13 ± 0.02	0.13 ± 0.02	0.47 ± 0.06	0.16 ± 0.02	0.40 ± 0.08	0.19 ± 0.02	0.12 ± 0.04	0.34 ± 0.05
15.48	1182	Terpinene-4-ol	1.85 ± 0.02	5.72 ± 0.02	8.40 ± 0.09	8.21 ± 0.09	8.84 ± 0.38	5.52 ± 0.07	6.12 ± 0.39	6.89 ± 0.13	8.51 ± 0.33	3.37 ± 0.12	4.09 ± 0.13	6.90 ± 0.07
15.90	1193	α-Terpineol	0.41 ± 0.01	0.59 ± 0.02	1.57 ± 0.01	0.81 ± 0.05	0.57 ± 0.04	1.21 ± 0.08	3.91 ± 0.08	1.94 ± 0.09	1.64 ± 0.12	0.45 ± 0.08	0.94 ± 0.05	1.49 ± 0.14
16.11	1201	γ-Terpineol	n.d.	n.d.	0.24 ± 0.02	n.d.	n.d.	n.d.	n.d.	n.d.	0.11 ± 0.03	n.d.	0.82 ± 0.06	n.d.
17.78	1251	Pentyl isohexanoate	n.d.	0.16 ± 0.02	0.09 ± 0.01	0.38 ± 0.01	0.36 ± 0.04	n.d.	0.19 ± 0.01	0.05 ± 0.01	0.21 ± 0.05	0.27 ± 0.07	0.20 ± 0.02	0.36 ± 0.08
17.88	1254	Isopentyl hexanoate	n.d.	0.05 ± 0.01	n.d.	n.d.	0.12 ± 0.06	n.d.	0.35 ± 0.04	n.d.	n.d.	n.d.	n.d.	n.d.
19.14	1289	Bornyl acetate	1.09 ± 0.02	0.13 ± 0.01	0.76 ± 0.03	0.50 ± 0.02	0.29 ± 0.07	0.53 ± 0.09	0.26 ± 0.05	0.88 ± 0.08	0.71 ± 0.05	1.03 ± 0.09	1.59 ± 0.09	1.41 ± 0.06
19.25	1292	Undecanone	0.49 ± 0.01	0.12 ± 0.02	0.25 ± 0.04	0.83 ± 0.02	0.44 ± 0.05	0.36 ± 0.06	0.69 ± 0.06	n.d.	0.89 ± 0.08	0.78 ± 0.11	0.25 ± 0.06	1.05 ± 0.08
21.15	1353	α-Terpinyl acetate	n.d.	n.d.	0.05 ± 0.01	n.d.	n.d.	n.d.	0.80 ± 0.05	n.d.	0.63 ± 0.05	1.13 ± 0.07	0.10 ± 0.07	n.d.
21.26	1356	α-Cubabene	0.39 ± 0.01	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	0.10 ± 0.03	n.d.	n.d.	n.d.
22.21	1383	α-Copaene	1.62 ± 0.02	0.50 ± 0.02	0.14 ± 0.02	0.62 ± 0.04	0.29 ± 0.03	0.26 ± 0.03	0.55 ± 0.04	0.12 ± 0.02	0.74 ± 0.04	0.84 ± 0.06	0.58 ± 0.02	0.68 ± 0.06
22.68	1397	β-Cubabene + β-Elementene	1.78 ± 0.02	0.52 ± 0.01	0.15 ± 0.01	0.76 ± 0.04	0.35 ± 0.04	0.25 ± 0.06	0.42 ± 0.04	n.d.	0.55 ± 0.05	0.90 ± 0.10	0.42 ± 0.06	1.12 ± 0.06
23.68	1430	β-Caryophyllene	3.98 ± 0.05	2.05 ± 0.02	1.36 ± 0.01	1.95 ± 0.04	3.26 ± 0.05	2.12 ± 0.03	3.89 ± 0.07	3.66 ± 0.17	3.03 ± 0.17	3.12 ± 0.26	2.86 ± 0.28	4.93 ± 0.17
24.01	1442	Isopentyl benzoate	0.44 ± 0.01	0.11 ± 0.01	0.29 ± 0.02	0.32 ± 0.06	0.07 ± 0.01	0.22 ± 0.09	0.40 ± 0.01	0.11 ± 0.05	0.29 ± 0.06	0.16 ± 0.01	0.12 ± 0.04	0.35 ± 0.03
24.61	1461	E-Murola-3,5-diene	0.62 ± 0.01	0.17 ± 0.04	0.09 ± 0.01	0.29 ± 0.03	0.08 ± 0.01	0.18 ± 0.06	0.27 ± 0.03	0.06 ± 0.02	0.32 ± 0.06	0.33 ± 0.04	0.21 ± 0.08	0.54 ± 0.04
24.74	1466	α-Humulene	1.81 ± 0.03	1.36 ± 0.02	0.48 ± 0.01	1.28 ± 0.08	0.63 ± 0.06	0.70 ± 0.05	1.32 ± 0.03	0.61 ± 0.04	1.29 ± 0.09	1.65 ± 0.06	1.11 ± 0.10	1.63 ± 0.07
24.98	1473	Alloaromadendrene	1.09 ± 0.02	0.52 ± 0.03	0.07 ± 0.01	0.54 ± 0.06	0.19 ± 0.03	0.19 ± 0.06	0.30 ± 0.02	0.07 ± 0.02	0.45 ± 0.08	0.65 ± 0.05	0.39 ± 0.05	0.56 ± 0.05
25.40	1487	γ-Murolene	2.64 ± 0.02	1.14 ± 0.02	0.44 ± 0.01	1.43 ± 0.04	0.60 ± 0.09	0.65 ± 0.07	1.19 ± 0.06	0.37 ± 0.03	1.41 ± 0.06	1.67 ± 0.09	1.09 ± 0.07	1.54 ± 0.09
25.61	1493	Germacrene D	6.32 ± 0.07	11.58 ± 0.02	2.47 ± 0.02	7.84 ± 0.14	9.02 ± 0.09	3.00 ± 0.29	3.17 ± 0.06	1.63 ± 0.04	5.30 ± 0.11	10.38 ± 0.11	6.96 ± 0.11	6.19 ± 0.08
25.94	1504	Murola-4(14),5-diene	1.65 ± 0.03	0.46 ± 0.01	0.14 ± 0.01	0.59 ± 0.02	0.23 ± 0.06	0.29 ± 0.06	0.33 ± 0.05	0.10 ± 0.04	0.53 ± 0.05	0.83 ± 0.09	0.42 ± 0.04	0.86 ± 0.06
26.11	1510	α-Murolene	3.42 ± 0.06	1.66 ± 0.03	0.77 ± 0.02	1.97 ± 0.04	0.98 ± 0.11	1.12 ± 0.02	1.50 ± 0.11	0.47 ± 0.08	1.39 ± 0.07	1.44 ± 0.06	2.67 ± 0.11	n.d.
26.18	1513	Farnesene	0.66 ± 0.04	0.20 ± 0.01	2.40 ± 0.02	0.31 ± 0.05	n.d.	1.77 ± 0.08	0.37 ± 0.03	0.46 ± 0.11	n.d.	n.d.	n.d.	n.d.
26.26	1515	β-Bisabolene	0.6 ± 0.01	0.11 ± 0.02	n.d.	0.23 ± 0.03	n.d.	n.d.	n.d.	0.24 ± 0.05	n.d.	0.44 ± 0.07	0.12 ± 0.05	1.32 ± 0.07
26.55	1525	γ-Cadinene	1.53 ± 0.01	0.35 ± 0.01	0.13 ± 0.01	0.68 ± 0.07	0.20 ± 0.06	0.34 ± 0.04	0.33 ± 0.04	0.06 ± 0.02	0.31 ± 0.06	0.72 ± 0.07	0.27 ± 0.07	0.98 ± 0.11
26.80	1533	δ-Cadinene	4.49 ± 0.05	3.56 ± 0.04	2.92 ± 0.02	4.85 ± 0.12	2.27 ± 0.08	3.86 ± 0.10	3.68 ± 0.08	1.92 ± 0.04	3.73 ± 0.10	4.84 ± 0.16	3.39 ± 0.10	3.55 ± 0.07
26.90	1536	β-Cadinene	0.79 ± 0.01	0.32 ± 0.01	0.22 ± 0.03	0.45 ± 0.08	0.12 ± 0.04	0.37 ± 0.04	0.60 ± 0.05	0.26 ± 0.05	0.44 ± 0.02	0.45 ± 0.05	0.32 ± 0.01	0.46 ± 0.09
27.09	1543	Cadina-1(2),4-diene	0.72 ± 0.01	0.19 ± 0.02	0.10 ± 0.01	0.49 ± 0.05	0.07 ± 0.02	0.19 ± 0.07	0.31 ± 0.06	0.10 ± 0.06	0.34 ± 0.02	0.35 ± 0.05	0.18 ± 0.01	0.39 ± 0.06
27.25	1548	α-Cadinene	0.57 ± 0.05	n.d.	n.d.	0.32 ± 0.04	n.d.	n.d.	n.d.	n.d.	0.11 ± 0.01	0.26 ± 0.06	n.d.	0.57 ± 0.10
28.14	1576	3-Hexene-1-ol benzoate	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	0.22 ± 0.05	0.12 ± 0.04	n.d.	n.d.	n.d.	n.d.
30.30	1651	τ-Cadinol	1.93 ± 0.02	0.71 ± 0.01	0.45 ± 0.01	1.53 ± 0.04	0.31 ± 0.03	1.29 ± 0.06	0.91 ± 0.06	0.47 ± 0.09	0.79 ± 0.06	1.38 ± 0.09	0.49 ± 0.04	1.05 ± 0.09
30.41	1655	δ-Cadinol	0.45 ± 0.01	0.11 ± 0.01	0.06 ± 0.01	0.23 ± 0.02	n.d.	0.20 ± 0.05	0.47 ± 0.05	0.07 ± 0.03	0.12 ± 0.04	0.20 ± 0.05	n.d.	0.18 ± 0.03
30.66	1664	α-Cadinol	1.39 ± 0.04	0.50 ± 0.01	0.29 ± 0.02	1.14 ± 0.02	0.21 ± 0.04	0.72 ± 0.02	n.d.	0.30 ± 0.05	0.46 ± 0.02	0.84 ± 0.06	0.24 ± 0.02	0.81 ± 0.05
31.42	1690	α-Bisabolol	n.d.	n.d.	n.d.	0.20 ± 0.04	n.d.	n.d.	n.d.	n.d.	0.13 ± 0.04	n.d.	n.d.	n.d.
		Total	99.99 ± 0.10	99.80 ± 0.21	99.90 ± 0.39	99.97 ± 0.23	99.90 ± 0.25	99.19 ± 0.20	99.99 ± 0.62	99.85 ± 0.47	99.98 ± 0.41	99.97 ± 0.24	99.99 ± 0.16	99.96 ± 0.04

Results are expressed as mean value ± SD; 1 = early flowering stage, 2 = early fruiting stage, 3 = late fruiting stage; n.d. = not detected.

Sesquiterpenes, mainly germacrene D, α -murolene, and δ -cadinene, represent the second most abundant chemical group (9.53–31.94%). Similar dominance of monoterpenes and sesquiterpenes in mastic tree leaf essential oils from Morocco, Tunisia, and Greece has been reported by Zrira et al. [16], Douissa et al. [47], and Gardeli et al. [39], and later confirmed by El-Sherei et al. [48]. Other quantified chemical groups include monoterpene alcohols (9.53–31.94%), sesquiterpene alcohols (0.88–4.54%), aliphatic esters (0.48–2.57%), monoterpene esters (0.29–2.16%), aromatic esters (0.11–0.62%), and monoterpene oxides (0.05–0.29%).

Statistical analysis of the dependence of EO chemical groups on location (Table 5) showed that the highest yields of monoterpenes ($66.15 \pm 2.53\%$), monoterpene alcohols ($9.50 \pm 0.24\%$), aliphatic esters ($1.59 \pm 0.30\%$), and aromatic esters ($0.39 \pm 0.05\%$) were found in EOs from samples harvested at the Hvar location. The highest proportions of monoterpene esters ($1.76 \pm 0.12\%$) and sesquiterpenes ($25.11 \pm 1.44\%$) were found in EO from samples collected at the Vela Luka location. The highest proportions of sesquiterpene alcohols were found in EOs from mastic tree leaves collected at the Barbariga ($2.35 \pm 0.58\%$) and Pag ($2.33 \pm 0.48\%$) locations, between which there was no significant statistical difference. Regarding monoterpene proportions, the trend of grouping by location is repeated: Barbariga and Pag, between which there is no significant statistical difference, and Hvar and Vela Luka, which is attributed to similar climatic conditions. Specifically, the average precipitation (mm) measured at Barbariga and Pag in the year of sample collection was 2.45 times higher than at Hvar and Vela Luka [22]. According to research by Boira and Blanquer [49], an increase in monoterpenes in *Thymus piperella* L. is a consequence of dry periods, that is, a lack of water.

From the results on the influence of the stage of harvesting of the PS on individual groups of chemical compounds, it can be concluded that the highest proportion of monoterpenes ($69.30 \pm 1.32\%$) was found during PS2, and monoterpene alcohols during PS3 ($8.89 \pm 0.54\%$), while during PS1 the highest proportions were observed for monoterpene esters ($1.19 \pm 0.19\%$), sesquiterpenes ($25.40 \pm 1.69\%$), sesquiterpene alcohols ($3.33 \pm 0.32\%$), aliphatic esters ($1.58 \pm 0.19\%$), and aromatic esters ($0.36 \pm 0.05\%$). There was no significant statistical difference in the proportions of sesquiterpenes between PS2 and PS3 ($17.91 \pm 1.62\%$ and $18.03 \pm 1.72\%$, respectively).

3.4. Antimicrobial Activity of Essential Oil

The results presented in Table 6 indicate that all EO samples exhibited an inhibitory effect on the growth of *E. coli* strain AB1157 and *E. amylovora* strain EaED, and that the antibiotic chloramphenicol was the most effective in inhibiting the growth of the selected bacterial strains. It is evident that mastic tree leaf EO has a more pronounced inhibitory effect on *E. amylovora* compared to *E. coli*.

Chloramphenicol, used as a positive control, achieved inhibition zones of 5.15 mm against *E. coli* and 11.37 mm against *E. amylovora*. Compared to the control antibiotic, the EOs from samples harvested at all locations during three PSs showed low antimicrobial activity against the tested bacteria. However, it should be noted that the disk diffusion assay provides only a preliminary and qualitative indication of antimicrobial potential. Confirmation and quantitative assessment of these findings would require determination of Minimum Inhibitory Concentration (MIC) values using broth microdilution in accordance with CLSI M07 guidelines.

Table 4. Proportions of chemical groups in mastic tree essential oil leaves harvested at four locations during three phenological stages using steam distillation at 1 bar pressure.

Chemical Group	Barbariga			Pag			Hvar			Vela Luka		
	1	2	3	1	2	3	1	2	3	1	2	3
Monoterpenes	54.67 ± 0.20	64.43 ± 0.20	71.91 ± 0.17	56.99 ± 0.15	67.30 ± 0.23	66.15 ± 0.15	62.09 ± 0.21	76.67 ± 0.43	60.66 ± 0.44	57.41 ± 0.29	69.66 ± 0.36	52.10 ± 0.13
Monoterpene alcohols	2.16 ± 0.03	6.31 ± 0.14	10.21 ± 0.26	9.02 ± 0.08	9.20 ± 0.61	5.93 ± 0.05	9.83 ± 0.15	8.63 ± 0.06	10.06 ± 0.07	3.82 ± 0.05	4.97 ± 0.05	9.21 ± 0.08
Monoterpene oxides	n.d.	n.d.	0.05 ± 0.01	n.d.	n.d.	n.d.	1.01 ± 0.02	n.d.	n.d.	n.d.	n.d.	n.d.
Monoterpene esters	1.09 ± 0.04	0.13 ± 0.03	0.81 ± 0.03	0.50 ± 0.04	0.29 ± 0.03	0.53 ± 0.03	1.06 ± 0.05	0.88 ± 0.02	1.34 ± 0.03	2.16 ± 0.05	1.69 ± 0.03	1.41 ± 0.05
Sesquiterpenes	31.94 ± 0.23	24.37 ± 0.11	11.66 ± 0.05	23.83 ± 0.19	18.13 ± 0.08	14.12 ± 0.08	17.16 ± 0.07	9.53 ± 0.05	19.73 ± 0.16	29.32 ± 0.14	19.44 ± 0.09	26.44 ± 0.19
Sesquiterpenes alcohols	4.54 ± 0.09	1.49 ± 0.04	0.91 ± 0.02	3.60 ± 0.18	0.50 ± 0.42	2.63 ± 0.06	1.78 ± 0.05	0.94 ± 0.04	1.78 ± 0.04	3.12 ± 0.04	0.88 ± 0.04	2.37 ± 0.07
Aliphatic esters	0.63 ± 0.01	0.72 ± 0.04	1.56 ± 0.06	1.90 ± 0.04	1.44 ± 0.03	0.73 ± 0.03	2.57 ± 0.05	0.48 ± 0.04	1.66 ± 0.02	1.04 ± 0.03	0.72 ± 0.02	2.52 ± 0.03
Aromatic esters	0.44 ± 0.01	0.11 ± 0.02	0.29 ± 0.03	0.32 ± 0.03	0.07 ± 0.01	0.22 ± 0.04	0.62 ± 0.03	0.23 ± 0.04	0.29 ± 0.04	0.16 ± 0.03	0.12 ± 0.02	0.35 ± 0.04
Other	4.52 ± 0.05	2.25 ± 0.05	2.505 ± 0.09	3.81 ± 0.17	2.92 ± 0.14	8.88 ± 0.05	4.60 ± 0.12	2.49 ± 0.04	4.47 ± 0.04	2.95 ± 0.09	2.52 ± 0.06	5.55 ± 0.08
Total identified	99.88 ± 0.11	99.71 ± 0.10	99.93 ± 0.04	99.95 ± 0.02	99.94 ± 0.04	99.11 ± 0.08	99.94 ± 0.06	99.41 ± 0.39	99.42 ± 0.49	99.43 ± 0.48	99.75 ± 0.21	99.96 ± 0.03

Results are expressed as mean value ± SD; 1 = early flowering stage, 2 = early fruiting stage, 3 = late fruiting stage; n.d. = not detected.

Table 5. The differences in chemical groups proportions of mastic tree leaf essential oil due to location and PS.

Source of Variation	Monoterpenes	Monoterpene Alcohols	Monoterpene Oxides	Monoterpene Esters	Sesquiterpenes	Sesquiterpene Alcohols	Aliphatic Esters	Aromatic Esters	Other	Total Identified
Location	$p = 0.05$	$p < 0.01^*$	$p = 0.04$	$p < 0.01^*$	$p < 0.01^*$	$p = 0.05$	$p = 0.05$	$p < 0.01^*$	$p = 0.05$	$p = 0.93$
Barbariga	63.55 ± 2.46 ^b	6.33 ± 1.18 ^b	0.01 ± 0.01 ^a	0.67 ± 0.14 ^b	22.62 ± 2.92 ^c	2.35 ± 0.58 ^c	0.93 ± 0.14 ^a	0.26 ± 0.05 ^b	3.10 ± 0.36 ^a	99.84 ± 0.04 ^a
Pag	63.56 ± 1.62 ^b	8.07 ± 0.54 ^c	n.d.	0.43 ± 0.04 ^a	18.63 ± 1.37 ^b	2.33 ± 0.48 ^c	1.35 ± 0.18 ^b	0.20 ± 0.04 ^a	5.08 ± 0.95 ^d	99.67 ± 0.14 ^a
Hvar	66.15 ± 2.53 ^c	9.50 ± 0.24 ^d	0.09 ± 0.05 ^b	1.08 ± 0.08 ^c	15.41 ± 1.50 ^a	1.48 ± 0.15 ^a	1.59 ± 0.30 ^d	0.39 ± 0.05 ^c	3.89 ± 0.34 ^c	99.59 ± 0.14 ^a
Vela Luka	59.43 ± 2.56 ^a	5.95 ± 0.82 ^a	n.d.	1.76 ± 0.12 ^d	25.11 ± 1.44 ^d	2.17 ± 0.33 ^b	1.43 ± 0.27 ^c	0.19 ± 0.03 ^a	3.67 ± 0.50 ^b	99.71 ± 0.12 ^a
Phenological stage	$p < 0.01^*$	$p < 0.01^*$	$p = 0.05$	$p = 0.17$	$p < 0.01^*$	$p < 0.01^*$	$p < 0.01^*$	$p < 0.01^*$	$p < 0.01^*$	$p = 0.45$
1	57.77 ± 0.81 ^a	6.20 ± 0.99 ^a	0.07 ± 0.04 ^b	1.19 ± 0.19 ^c	25.40 ± 1.69 ^b	3.33 ± 0.32 ^c	1.56 ± 0.23 ^b	0.36 ± 0.05 ^c	3.93 ± 0.23 ^b	99.80 ± 0.09 ^a
2	69.30 ± 1.32 ^c	7.31 ± 0.52 ^b	n.d.	0.74 ± 0.19 ^a	17.91 ± 1.62 ^a	0.97 ± 0.11 ^a	0.84 ± 0.10 ^a	0.14 ± 0.02 ^a	2.50 ± 0.06 ^a	99.70 ± 0.08 ^a
3	62.46 ± 2.20 ^b	8.89 ± 0.54 ^c	0.01 ± 0.01 ^a	1.03 ± 0.11 ^b	18.03 ± 1.72 ^a	1.95 ± 0.21 ^b	1.58 ± 0.19 ^b	0.29 ± 0.01 ^b	5.38 ± 0.68 ^c	99.61 ± 0.12 ^a
Grand mean	63.17	7.46	0.03	0.99	2.44	2.08	1.33	0.26	3.94	99.70

Results are expressed as mean ± SE. * Statistically significant variable at $p \leq 0.05$. Values with different letters within column are statistically different at $p \leq 0.05$.

Table 6. Antimicrobial activity of essential oils of mastic tree leaves obtained by steam distillation from samples harvested at four locations during three phenological stages determined by the disk diffusion method against Gram-negative bacteria *E. coli* strain AB1157 and *E. amylovora* strain EaED.

Location	Phenological Stage	<i>E. coli</i> Strain AB1157 Inhibition Zone (mm)	<i>E. amylovora</i> Strain EaED Inhibition Zone (mm)
Barbariga	1	0.41 ± 0.01	0.42 ± 0.01
	2	0.43 ± 0.02	0.86 ± 0.02
	3	0.91 ± 0.01	1.29 ± 0.02
Pag	1	0.52 ± 0.01	1.29 ± 0.02
	2	0.95 ± 0.01	0.89 ± 0.02
	3	0.24 ± 0.01	0.42 ± 0.01
Hvar	1	0.59 ± 0.02	1.29 ± 0.02
	2	1.33 ± 0.02	2.15 ± 0.03
	3	0.43 ± 0.02	0.86 ± 0.02
Vela Luka	1	0.51 ± 0.02	0.43 ± 0.01
	2	1.21 ± 0.02	0.89 ± 0.02
	3	0.43 ± 0.01	1.29 ± 0.02
Control-Chloramphenicol		5.15 ± 0.02	11.37 ± 1.50

Results are expressed as mean value ± SD; 1 = early flowering stage, 2 = early fruiting stage, 3 = late fruiting stage.

The results of statistical analysis of the effects of EOs obtained from different locations and PSs showed that the greatest inhibitory effect on both tested microorganisms was achieved with EO from mastic tree leaves harvested on Hvar during the second phenological phase (Table 7). These results are consistent with previous studies [50–53], which showed that the antimicrobial effect of EOs is due to their composition, specifically the proportion of components from chemical groups such as monoterpenes and sesquiterpenes (myrcene, α -pinene, limonene, and germacrene D), which possess cytotoxic properties against various microorganisms. The highest proportions of these components were determined in PS2 samples from the Hvar location (Tables 3 and 4). Similar findings were reported by Knobloch et al., who demonstrated that monoterpenes (α - and β -pinene) exhibit a moderate to strong inhibitory effect on microorganisms, as confirmed by other studies [40,54].

Table 7. The differences in antimicrobial activity of mastic tree leaf essential oils against Gram-negative bacteria *E. coli* strain AB1157 and *E. amylovora* strain EaED determined by disk diffusion method due to location and PS.

Source of Variation	<i>E. coli</i> Strain AB1157 Inhibition Zone (mm)	<i>E. amylovora</i> Strain EaED Inhibition Zone (mm)
Location	$p = 0.48$	$p = 0.01^*$
Barbariga	0.58 ± 0.08 ^a	0.86 ± 0.13 ^{a,b}
Pag	0.57 ± 0.10 ^a	0.87 ± 0.13 ^{a,b}
Hvar	0.78 ± 0.14 ^a	1.43 ± 0.19 ^{a,b}
Vela Luka	0.72 ± 0.12 ^a	0.87 ± 0.12 ^{a,b}
Phenological stage	$p < 0.01^*$	$p = 0.22$
1	0.51 ± 0.02 ^{a,b}	0.86 ± 0.13 ^a
2	0.98 ± 0.10 ^b	1.19 ± 0.17 ^a
3	0.50 ± 0.07 ^{a,b}	0.97 ± 0.11 ^a
Grand mean	0.66	1.01

Results are expressed as mean ± SE. * Statistically significant variable at $p \leq 0.05$. Values with different letters within column are statistically different at $p \leq 0.05$.

The antimicrobial effect of the EOs on the selected microorganisms was demonstrated by clear halos extending beyond the disk edge, with measured halo widths ranging from 0.24 mm to 1.33 mm against *E. coli* and from 0.21 mm to 2.15 mm against *E. amylovora*. In testing the antimicrobial activity of mastic tree EO on *E. coli* using the disk diffusion method, Gkogka et al. [55] measured an inhibition zone of 10 mm. According to Mharti et al. [56], the EO of the mastic tree leaves showed strong antimicrobial activity against the Gram-negative bacterium *Klebsiella pneumoniae*, with a measured inhibition zone of 16 mm and stronger activity than the antibiotics levofloxacin and ampicillin, but no activity against *Pseudomonas aeruginosa*.

To examine the possible grouping of *P. lentiscus* leaf essential oils based on chemical composition and antimicrobial activity with respect to location and PS, PCA was performed, and the results are shown in Figure 2.

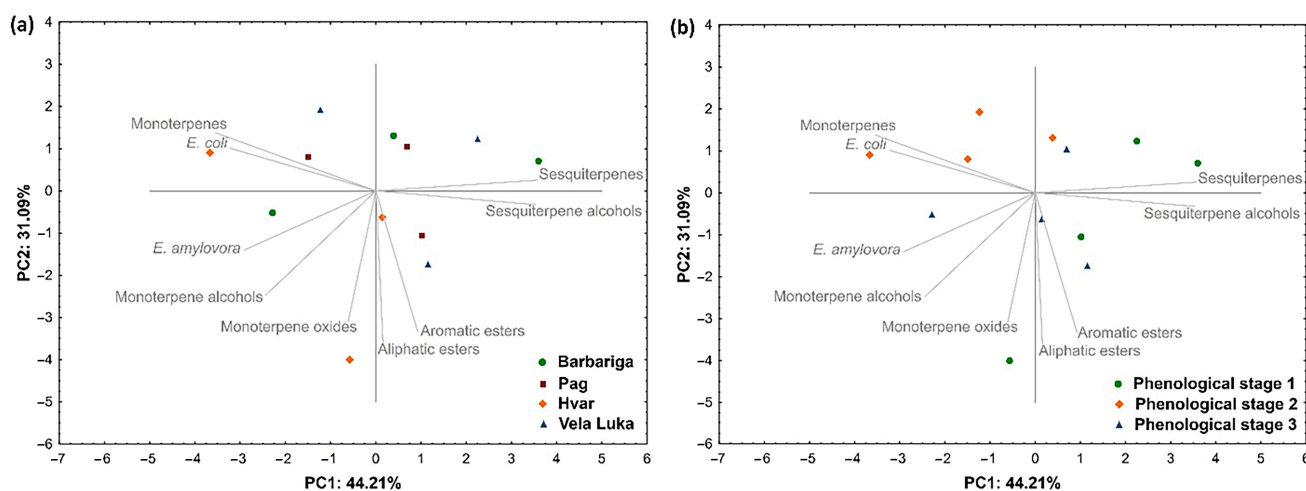


Figure 2. Distribution of essential oils from mastic tree leaves in a two-dimensional coordinate system defined by the first two principal components (PC1 and PC2) in relation to the (a) location and (b) phenological stage.

The communality values for monoterpenes, monoterpene alcohols, monoterpene oxides, sesquiterpenes, sesquiterpene alcohols, aliphatic esters, aromatic esters, and antimicrobial activity against *E. coli* and *E. amylovora* exceeded 0.5 and were therefore included in the analysis. The distribution of the samples was examined in the space defined by the first two principal components (PC1 and PC2), both of which had eigenvalues greater than 1. Together, PC1 and PC2 explained 75.30% of the total variance in the data. A very strong correlation was observed between PC1 and monoterpenes ($r = -0.88$), sesquiterpenes ($r = 0.89$), sesquiterpene alcohols ($r = 0.88$), and antimicrobial activity against *E. coli* ($r = -0.80$). There was also a strong correlation between PC1 and monoterpene alcohols ($r = -0.61$) and antimicrobial activity against *E. amylovora* ($r = -0.73$). PC2 was very strongly correlated with aliphatic esters ($r = -0.90$) and aromatic esters ($r = -0.83$), while a strong correlation was present between PC2 and monoterpene alcohols ($r = -0.62$) and monoterpene oxides ($r = -0.77$). As shown, there was no particular grouping of the samples with respect to location (Figure 2a). However, the samples showed some separation according to PS (Figure 2b). Most samples obtained in PS1 were located at positive PC1 values and were characterized by high contents of sesquiterpenes, sesquiterpene alcohols, and aliphatic and aromatic esters. Almost all samples from PS2 were distributed at negative PC1 values, showing high amounts of monoterpenes and antimicrobial activity against *E. coli*. Most samples from PS3 were found at negative PC2 values, with high levels of monoterpene alcohols and aliphatic and aromatic esters.

The calculated Pearson's correlation coefficient (Table 8) shows a possible connection in inhibitory activity between monoterpenes and *E. coli*, as well as a possible inhibitory effect of monoterpene alcohols against *E. amylovora*. In contrast, sesquiterpenes and sesquiterpene alcohols showed a moderate negative correlation with both tested microorganisms.

Table 8. Pearson's correlations between analyzed compounds and antimicrobial activity.

Group of Compounds	<i>E. coli</i> Strain AB1157	<i>E. amylovora</i> Strain EaED
Monoterpenes	0.76 *	0.45
Monoterpene alcohols	0.19	0.65 *
Monoterpene esters	0.06	−0.17
Sesquiterpenes	−0.55 *	−0.57 *
Sesquiterpenes alcohols	−0.68 *	−0.46
Aliphatic esters	−0.26	0.25
Aromatic esters	−0.31	0.18
Other	−0.64 *	−0.36

* $p \leq 0.05$.

This is consistent with the findings of Bakkali et al. [57] and Savoia [58], who demonstrated that oxygen-containing terpene derivatives have greater biological activity than hydrocarbon compounds.

4. Conclusions

The results of the study showed that pressure, PS, and location have a significant impact on the yield of mastic tree leaf essential oil obtained by steam distillation. The highest yield was achieved at a pressure of 1 bar, confirming a positive correlation between increased pressure and the efficiency of volatile compound isolation. The first phenological stage (early flowering stage) proved to be the most favorable period for obtaining higher essential oil yields, while differences between locations were associated with variations in climatic and microenvironmental conditions, especially precipitation and insolation. Response surface analysis confirmed that the combination of optimal pressure, appropriate phenological phase, and location conditions plays a key role in optimizing the isolation process. GC–MS analysis identified and quantified 56 components and determined a typical monoterpene chemotype for mastic leaf essential oil, with α -pinene, limonene, myrcene and β -pinene as the dominant components. Significant variability in individual components was observed, especially myrcene and sabinene, confirming the strong influence of environmental factors and phenological stage on the biosynthesis of secondary metabolites. Preliminary testing of antimicrobial activity by the disk diffusion method revealed a low inhibitory effect of the essential oils on the two tested Gram-negative bacterial strains, *E. coli* AB1157 and *E. amylovora* EaED. The observed variation in inhibitory activity among samples appears to be associated with differences in chemical composition; however, to establish and confirm causal relationship future work should include more specific and precise microbiological analyses. Overall, the research confirms that optimizing technological distillation parameters, together with appropriate selection of phenological stage and sampling location, is an important approach for increasing yield and standardizing the quality of mastic tree leaf essential oil. This provides a basis for its potential application not only in the pharmaceutical, food, and cosmetic industries, but also for the preparation of environmentally acceptable herbal preparations with biopesticide properties.

Author Contributions: Conceptualization, Z.Z. and S.D.; methodology, S.D., M.R., A.N.G., and Z.Z.; software, M.R., S.D., and Z.Z.; validation, S.D. and Z.Z.; formal analysis, S.D. and Z.Z.; investigation, S.D., M.M., B.M., and Z.Z.; resources, S.D. and B.M.; data curation, S.D. and M.M.; writing—original

draft preparation, S.D., M.R., A.N.G., and Z.Z.; writing—review and editing, S.D., M.R., A.N.G., M.M., and Z.Z.; visualization, M.R. and Z.Z.; supervision, all authors. All authors have read and agreed to the published version of the manuscript.

Funding: This work has been supported by the project “Natural agents for plant protection—an alternative to chemistry” (IP-UNIZD 2025-28090), funded by the European Union—NextGenerationEU. The views and opinions expressed are those of the authors only and do not necessarily reflect those of the European Union.

Institutional Review Board Statement: Not applicable.

Informed Consent Statement: Not applicable.

Data Availability Statement: The original contributions presented in this study are included in the article. Further inquiries can be directed to the corresponding authors.

Conflicts of Interest: Author Sanja Dragović was employed by the company Genera Research Ltd. The remaining authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

References

1. Kozhoridze, G.; Orlovsky, N.; Orlovsky, L.; Blumberg, D.G.; Golan-Goldhirsh, A. Geographic Distribution and Migration Pathways of *Pistacia*—Present, Past and Future. *Ecography* **2015**, *38*, 1141–1154. [\[CrossRef\]](#)
2. Beghlal, D.; El Bairi, K.; Marmouzi, I.; Haddar, L.; Mohamed, B. Phytochemical, Organoleptic and Ferric Reducing Properties of Essential Oil and Ethanolic Extract from *Pistacia lentiscus* (L.). *Asian Pac. J. Trop. Dis.* **2016**, *6*, 305–310. [\[CrossRef\]](#)
3. Bougherra, H.H.; Bedini, S.; Flamini, G.; Cosci, F.; Belhamel, K.; Conti, B. *Pistacia lentiscus* Essential Oil Has Repellent Effect against Three Major Insect Pests of Pasta. *Ind. Crops Prod.* **2015**, *63*, 249–255. [\[CrossRef\]](#)
4. Bouchfara, A.; Zerrad, H.; Ez-zari, A.; Laglaoui, A.; Nechar, M.; Souhail, B. Antibacterial and Antioxidant Activities of *Pistacia lentiscus* Essential Oils: Impact of Total Phenolic Content on Antioxidant Efficacy. *Biocatal. Agric. Biotechnol.* **2025**, *64*, 103532. [\[CrossRef\]](#)
5. Silva, J.; Abebe, W.; Sousa, S.M.; Duarte, V.G.; Machado, M.I.L.; Matos, F.J.A. Analgesic and Anti-Inflammatory Effects of Essential Oils of Eucalyptus. *J. Ethnopharmacol.* **2003**, *89*, 277–283. [\[CrossRef\]](#) [\[PubMed\]](#)
6. Magiatis, P.; Melliou, E.; Skaltsounis, A.-L.; Chinou, I.; Mitaku, S. Chemical Composition and Antimicrobial Activity of the Essential Oils of *Pistacia lentiscus* Var. *Chia*. *Planta Med.* **1999**, *65*, 749–752. [\[CrossRef\]](#) [\[PubMed\]](#)
7. Vidrich, V.; Fusi, P.; Graziano, A.; Silvestrini, E.; Michelozzi, M.; Marco, F. Chemical Composition of the Essential Oil of *Pistacia lentiscus* L. *J. Essent. Oil Res.* **2004**, *16*, 223–226. [\[CrossRef\]](#)
8. Beraich, A.; El Farissi, H.; Belbachir, Y.; Cacciola, F.; Yahyaoui, M.I.; Choukoud, A.; Talhaoui, A. Traditional and Modern Extraction Methods for *Pistacia lentiscus* Essential Oil. *Sustain. Chem. Pharm.* **2024**, *40*, 101638. [\[CrossRef\]](#)
9. Ait Said, S.; Torre, F.; Derridj, A.; Gauquelin, T.; Mevy, J.P. Gender, Mediterranean Drought, and Seasonality: Photosystem II Photochemistry in *Pistacia lentiscus* L. *Photosynthetica* **2013**, *51*, 552–564. [\[CrossRef\]](#)
10. Carvalho, S.; Macel, M.; Mulder, P.P.J.; Skidmore, A.; van der Putten, W.H. Chemical Variation in *Jacobaea vulgaris* Is Influenced by the Interaction of Season and Vegetation Successional Stage. *Phytochemistry* **2014**, *99*, 86–94. [\[CrossRef\]](#)
11. Boelens, M.H.; Jimenez, R. Chemical Composition of the Essential Oils from the Gum and from Various Parts of *Pistacia lentiscus* L. (Mastic Gum Tree). *Flavour Fragr. J.* **1991**, *6*, 271–275. [\[CrossRef\]](#)
12. Buil, P.; Garnero, J.; Guichard, G. Contribution à La Connaissance Chimique de l'essence de Lentisque de Provence. *Riv. Ital. Essenze Profum. Piante Off. Aromi Sapon. Cosmet. Aerosol.* **1974**, *56*, 245–252.
13. Djenane, D.; Yangüela, J.; Montañés, L.; Djerbal, M.; Roncalés, P. Antimicrobial Activity of *Pistacia lentiscus* and *Satureja montana* Essential Oils against *Listeria monocytogenes* CECT 935 Using Laboratory Media: Efficacy and Synergistic Potential in Minced Beef. *Food Control* **2011**, *22*, 1046–1053. [\[CrossRef\]](#)
14. Fernández, A.; Camacho, A.; Fernández, C.; Altarejos, J.; Pérez, P. Composition of the Essential Oils from Galls and Aerial Parts of *Pistacia lentiscus* L. *J. Essent. Oil Res.* **2000**, *12*, 19–23. [\[CrossRef\]](#)
15. Picci, V.; Scotti, A.; Mariani, M.; Colombo, E. Composition of the Volatile Oil of *Pistacia lentiscus* L. of Sardinian Origin. In *Flavour Science and Technology*; Martens, M., Dalen, G.A., Russwurm, H., Eds.; John Wiley & Sons: Chichester, UK, 1987; pp. 75–78.
16. Zrira, S.; Elamrani, A.; Benjilali, B. Chemical Composition of the Essential Oil of *Pistacia lentiscus* L. from Morocco—A Seasonal Variation. *Flavour Fragr. J.* **2003**, *18*, 475–480. [\[CrossRef\]](#)
17. De Pooter, H.; Schamp, N.; Aboutabl, E.; El Tohamy, S.; Doss, S. Essential Oils from the Leaves of Three *Pistacia* Species Grown in Egypt. *Flavour Fragr. J.* **1991**, *6*, 229–232. [\[CrossRef\]](#)

18. Fleisher, Z.; Fleisher, A. Volatiles of the Mastic Tree—*Pistacia lentiscus* L. *J. Essent. Oil Res.* **1992**, *4*, 663–665. [\[CrossRef\]](#)
19. Burt, S. Essential Oils: Their Antibacterial Properties and Potential Applications in Foods—A Review. *Int. J. Food Microbiol.* **2004**, *94*, 223–253. [\[CrossRef\]](#) [\[PubMed\]](#)
20. Carson, C.F.; Mee, B.J.; Riley, T.V. Mechanism of Action of *Melaleuca alternifolia* (Tea Tree) Oil on *Staphylococcus aureus* Determined by Time-Kill, Lysis, Leakage, and Salt Tolerance Assays and Electron Microscopy. *Antimicrob. Agents Chemother.* **2002**, *46*, 1914–1920. [\[CrossRef\]](#)
21. Aouinti, F.; Imelouane, B.; Tahri, M.; Wathelet, J.P.; Amhamdi, H.; Elbachiri, A. New Study of the Essential Oil, Mineral Composition and Antibacterial Activity of *Pistacia lentiscus* L. from Eastern Morocco. *Res. Chem. Intermed.* **2014**, *40*, 2873–2886. [\[CrossRef\]](#)
22. Dragović, S.; Dragović-Uzelac, V.; Pedisić, S.; Čošić, Z.; Friščić, M.; Elez Garofulić, I.; Zorić, Z. The Mastic Tree (*Pistacia lentiscus* L.) Leaves as Source of BACs: Effect of Growing Location, Phenological Stage and Extraction Solvent on Phenolic Content. *Food Technol. Biotechnol.* **2020**, *58*, 303–313. [\[CrossRef\]](#)
23. Adams, R.P. *Identification of Essential Oil Components by Gas Chromatography/Mass Spectrometry*; Allured Business Media: Carol Stream, IL, USA, 2017; ISBN 978-1-93263321-4.
24. Sparkman, O.D. Identification of Essential Oil Components by Gas Chromatography/Quadrupole Mass Spectroscopy Robert P. Adams. *J. Am. Soc. Mass Spectrom.* **2005**, *16*, 1902–1903. [\[CrossRef\]](#)
25. Bauer, A.W.; Kirby, W.M.M.; Sherris, J.C.; Turck, M. Antibiotic Susceptibility Testing by a Standardized Single Disk Method. *Am. J. Clin. Pathol.* **1966**, *45*, 493–496. [\[CrossRef\]](#) [\[PubMed\]](#)
26. Koutsoudaki, C.; Krsek, M.; Rodger, A. Chemical Composition and Antibacterial Activity of the Essential Oil and the Gum of *Pistacia lentiscus* Var. *Chia*. *J. Agric. Food Chem.* **2005**, *53*, 7681–7685. [\[CrossRef\]](#) [\[PubMed\]](#)
27. Bouakline, H.; Brahmi, M.; Ziani, I.; Rhizlan, A.; Yahyaoui, M.I.; Angioni, A.; Talhaoui, A.; Bnouham, M.; Abdeslam, A.; Tahani, A.; et al. Influence of Air-Drying Temperature on Yield, Volatilome Content, Antioxidant, Antidiabetic and Antimicrobial Activities of *Pistacia lentiscus* Leaf Oil: Experimental and Modeling Aspects. *Food Biosci.* **2025**, *63*, 105773. [\[CrossRef\]](#)
28. Babu, K.G.D.; Singh, B.; Joshi, V.P.; Singh, V. Essential Oil Composition of Damask Rose (*Rosa Damascena* Mill.) Distilled under Different Pressures and Temperatures. *Flavour Fragr. J.* **2002**, *17*, 136–140. [\[CrossRef\]](#)
29. Maizi, Y.; Meddah, B.; Tir Touil Meddah, A.; Gabaldon Hernandez, J.A. Seasonal Variation in Essential Oil Content, Chemical Composition and Antioxidant Activity of *Teucrium polium* L. Growing in Mascara (North West of Algeria). *J. Appl. Biotechnol. Rep.* **2019**, *6*, 151–157. [\[CrossRef\]](#)
30. Souto-Bachiller, F.A.; De Jesus-Echevarría, M.; Cárdenas-González, O.E.; Acuña-Rodríguez, M.F.; Meléndez, P.A.; Romero-Ramsey, L. Terpenoid Composition of *Lippia Dulcis*. *Phytochemistry* **1997**, *44*, 1077–1086. [\[CrossRef\]](#)
31. Palá-Paúl, J.; Pérez-Alonso, M.J.; Velasco-Negueruela, A.; Palá-Paúl, R.; Sanz, J.; Conejero, F. Seasonal Variation in Chemical Constituents of *Santolina rosmarinifolia* L. ssp. *Rosmarinifolia*. *Biochem. Syst. Ecol.* **2001**, *29*, 663–672. [\[CrossRef\]](#) [\[PubMed\]](#)
32. Naghdi Badi, H.; Yazdani, D.; Ali, S.M.; Nazari, F. Effects of Spacing and Harvesting Time on Herbage Yield and Quality/Quantity of Oil in Thyme, *Thymus vulgaris* L. *Ind. Crops Prod.* **2004**, *19*, 231–236. [\[CrossRef\]](#)
33. Ben Farhat, M.; Jordán, M.J.; Chaouch-Hamada, R.; Landoulsi, A.; Sotomayor, J.A. Phenophase Effects on Sage (*Salvia officinalis* L.) Yield and Composition of Essential Oil. *J. Appl. Res. Med. Aromat. Plants* **2016**, *3*, 87–93. [\[CrossRef\]](#)
34. Oliveira, M.J.; Campos, I.F.P.; Oliveira, C.B.A.; Santos, M.R.; Souza, P.S.; Santos, S.C.; Seraphin, J.C.; Ferri, P.H. Influence of Growth Phase on the Essential Oil Composition of *Hyptis suaveolens*. *Biochem. Syst. Ecol.* **2005**, *33*, 275–285. [\[CrossRef\]](#)
35. Sefidkon, F.; Abbasi, K.; Jamzad, Z.; Ahmadi, S. The Effect of Distillation Methods and Stage of Plant Growth on the Essential Oil Content and Composition of *Satureja Rechingeri* Jamzad. *Food Chem.* **2007**, *100*, 1054–1058. [\[CrossRef\]](#)
36. Sellami, I.H.; Maamouri, E.; Chahed, T.; Wannes, W.A.; Kchouk, M.E.; Marzouk, B. Effect of Growth Stage on the Content and Composition of the Essential Oil and Phenolic Fraction of Sweet Marjoram (*Origanum majorana* L.). *Ind. Crops Prod.* **2009**, *30*, 395–402. [\[CrossRef\]](#)
37. Castelo, A.V.M.; Del Menezzi, C.H.S.; Resck, I.S. Seasonal Variation in the Yield and the Chemical Composition of Essential Oils from Two Brazilian Native Arbustive Species. *J. Appl. Sci.* **2012**, *12*, 753–760. [\[CrossRef\]](#)
38. Meier, C.; Mediavilla, V. Factors Influencing the Yield and the Quality of Hemp (*Cannabis sativa* L.) Essential Oi. *J. Int. Hemp Assoc.* **1998**, *5*, 16–20.
39. Gardeli, C.; Vassiliki, P.; Athanasios, M.; Kibouris, T.; Komaitis, M. Essential Oil Composition of *Pistacia lentiscus* L. and *Myrtus communis* L.: Evaluation of Antioxidant Capacity of Methanolic Extracts. *Food Chem.* **2008**, *107*, 1120–1130. [\[CrossRef\]](#)
40. Derwich, E.; Manar, A.; Benziane, Z.; Boukir, A. GC/MS Analysis and In Vitro Antibacterial Activity of the Essential Oil Isolated from Leaf of *Pistacia lentiscus* Growing in Morocco. *World Appl. Sci. J.* **2010**, *8*, 1267–1276.
41. Feijó, E.V.R.d.S.; de Oliveira, R.A.; Costa, L.C.d.B. Light Affects *Varronia Curassavica* Essential Oil Yield by Increasing Trichomes Frequency. *Rev. Bras. De Farmacogn.* **2014**, *24*, 516–523. [\[CrossRef\]](#)

42. Serralutzu, F.; Stangoni, A.; Amadou, B.; Tijan, D.; Re, G.A.; Marceddu, S.; Dore, A.; Bullitta, S. Essential Oil Composition and Yield of a *Rosmarinus officinalis* L. Natural Population with an Extended Flowering Season in a Coastal Mediterranean Environment and Perspectives for Exploitations. *Genet. Resour. Crop Evol.* **2020**, *67*, 1777–1793. [\[CrossRef\]](#)
43. Taarit, M.B.; Msaada, K.; Hosni, K.; Marzouk, B. Changes in Fatty Acid and Essential Oil Composition of Sage (*Salvia officinalis* L.) Leaves under NaCl Stress. *Food Chem.* **2010**, *119*, 951–956. [\[CrossRef\]](#)
44. Sarmoum, R.; Haid, S.; Biche, M.; Djazouli, Z.; Zebib, B.; Merah, O. Effect of Salinity and Water Stress on the Essential Oil Components of Rosemary (*Rosmarinus officinalis* L.). *Agronomy* **2019**, *9*, 214. [\[CrossRef\]](#)
45. Barra, A.; Coroneo, V.; Dessi, S.; Cabras, P.; Angioni, A. Characterization of the Volatile Constituents in the Essential Oil of *Pistacia lentiscus* L. from Different Origins and Its Antifungal and Antioxidant Activity. *J. Agric. Food Chem.* **2007**, *55*, 7093–7098. [\[CrossRef\]](#)
46. Lakušić, B.; Ristić, M.; Slavkovska, V.; Stojanović, D.; Lakušić, D. Variations in Essential Oil Yields and Compositions of *Salvia officinalis* (Lamiaceae) at Different Developmental Stages. *Bot. Serb.* **2013**, *37*, 127–139.
47. Douissa, F.B.; Hayder, N.; Chekir-Ghedira, L.; Hammami, M.; Ghedira, K.; Mariotte, A.-M.; Dijoux-Franca, M.-G. New Study of the Essential Oil from Leaves of *Pistacia lentiscus* L. (Anacardiaceae) from Tunisia. *Flavour Fragr. J.* **2005**, *20*, 410–414. [\[CrossRef\]](#)
48. El-Sherei, M.; Khaleel, A.; Motaal, A.A.; Abd-Elbaki, P. Effect of Seasonal Variation on the Composition of the Essential Oil of *Solidago canadensis* Cultivated in Egypt. *J. Essent. Oil Bear. Plants* **2014**, *17*, 891–898. [\[CrossRef\]](#)
49. Boira, H.; Blanquer, A. Environmental Factors Affecting Chemical Variability of Essential Oils in *Thymus piperella* L. *Biochem. Syst. Ecol.* **1998**, *26*, 811–822. [\[CrossRef\]](#)
50. Cole, R.A.; Haber, W.A.; Setzer, W.N. Chemical Composition of Essential Oils of Seven Species of *Eugenia* from Monteverde, Costa Rica. *Biochem. Syst. Ecol.* **2007**, *35*, 877–886. [\[CrossRef\]](#)
51. Abdallah, H.M.; Ezzat, S.M. Effect of the Method of Preparation on the Composition and Cytotoxic Activity of the Essential Oil of *Pituranthos tortuosus*. *Z. Für Naturforschung C* **2011**, *66*, 143–148. [\[CrossRef\]](#) [\[PubMed\]](#)
52. Salvador, M.J.; de Carvalho, J.E.; Wisniewski, A., Jr.; Kassuya, C.A.L.; Santos, É.P.; Riva, D.; Stefanello, M.É.A. Chemical Composition and Cytotoxic Activity of the Essential Oil from the Leaves of *Casearia lasiophylla*. *Rev. Bras. De Farmacogn.* **2011**, *21*, 864–868. [\[CrossRef\]](#)
53. Satyal, P.; Woods, K.E.; Dosoky, N.S.; Neupane, S.; Setzer, W.N. Biological Activities and Volatile Constituents of *Aegle marmelos* (L.) Corrêa from Nepal. *J. Med. Act. Plants* **2012**, *1*, 114–122.
54. Hiji, F.; Tétouani, S.F.; Elaraki, A.T. Antimicrobial Activity of Twenty-One Eucalyptus Essential Oils. *Fitoterapia* **1993**, *64*, 71–77.
55. Gkogka, E.; Hazeleger, W.C.; Posthumus, M.A.; Beumer, R.R. The Antimicrobial Activity of the Essential Oil of *Pistacia lentiscus* Var. *Chia*. *J. Essent. Oil Bear. Plants* **2013**, *16*, 714–729. [\[CrossRef\]](#)
56. Mharti, F.; Lyoussi, B.; Abdellaoui, A. Antibacterial Activity of the Essential Oils of *Pistacia lentiscus* Used in Moroccan Folkloric Medicine. *Nat. Prod. Commun.* **2011**, *6*, 1505–1506. [\[CrossRef\]](#)
57. Bakkali, F.; Averbeck, S.; Averbeck, D.; Idaomar, M. Biological Effects of Essential Oils—A Review. *Food Chem. Toxicol.* **2008**, *46*, 446–475. [\[CrossRef\]](#) [\[PubMed\]](#)
58. Savoia, D. Plant-Derived Antimicrobial Compounds: Alternatives to Antibiotics. *Future Microbiol.* **2012**, *7*, 979–990. [\[CrossRef\]](#) [\[PubMed\]](#)

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