horticulturae

MDPI

Article

Shade-Induced Effects on Essential Oil Yield, Chemical Profiling, and Biological Activity in Some *Lamiaceae* Plants Cultivated in Serbia

Dragana Lalević [1], Zoran S. Ilić [1,*], Ljiljana Stanojević [2], Lidija Milenković [1], Ljubomir Šunić [1], Renata Kovač [3], Dragan Kovačević [4], Bojana Danilović [2], Aleksandra Milenković [2], Jelena Stanojević [2] and Dragan Cvetković [2]

[1] Faculty of Agriculture, University of Priština in Kosovska Mitrovica, 38219 Lešak, Serbia
[2] Faculty of Technology, University of Niš, 16000 Leskovac, Serbia
[3] Institute of Food Technology, University of Novi Sad, 21000 Novi Sad, Serbia
[4] Faculty of Agriculture, University of Novi Sad, 21000 Novi Sad, Serbia
* Correspondence: zoran.ilic63@gmail.com; Tel.: +381-63-8014966

Abstract: Thyme, mint, and lemon balm were used to determine whether shading conditions could improve the yield, composition, antioxidant, and antimicrobial activity in plant essential oils (EOs) in comparison with non-shaded plants from an open field. The yield of the EOs of non-shaded thyme, mint, and lemon balm, was 3.44, 3.96, and 0.21 mL/100 g, respectively. Plants covered by nets produced different levels of EOs (3.46, 2.20, and 0.45 mL/100 g) after 120 min of hydrodistillation. The main components of the thyme essential oil are thymol (44.2–43.9%), γ-terpinene (18.3–16.8%), and p-cymene (16.5–17.4%). The predominant components of mint essential oil are piperitenone oxide (52.6–64.8%) and 1,8 cineole (25.9–16.3%), while lemon balm essential oil consists of the following main components: geranial (34.0–32.8%); neral (21.3–24.9%); and piperitenone oxide (17.2–16.7%). The EOs from non-shaded thyme and mint plants have the highest antioxidant activity (EC_{50} value 0.54 mg/mL and 3.03 mg/mL). However, shaded lemon balm showed a stronger antioxidant activity (EC_{50} 3.43 mg/mL) than non-shaded plants (12.85 mg/mL) after 60 min of incubation. The EOs from all plants showed significant effects against *Escherichia coli*, *Proteus vulgaris*, *Bacillus subtilis*, *Staphylococcus aureus*, and *Candida albicans*. The most active EOs against most of the isolates originated from *Thymus vulgaris* L., plants. Adequate cultivation techniques, such as shading for Lamiaceae plants, has positive effects, especially in *Melissa officinalis* L. Shading can achieve a higher content and components in terms of the specific biological activity (antioxidant and microbial) of EOs.

Keywords: *Thymus vulgaris* L.; *Mentha piperita* L.; *Melissa officinalis* L.; essential oil; composition; antioxidant; microbial activity

Citation: Lalević, D.; Ilić, Z.S.; Stanojević, L.; Milenković, L.; Šunić, L.; Kovač, R.; Kovačević, D.; Danilović, B.; Milenković, A.; Stanojević, J.; et al. Shade-Induced Effects on Essential Oil Yield, Chemical Profiling, and Biological Activity in Some *Lamiaceae* Plants Cultivated in Serbia. *Horticulturae* **2023**, *9*, 84. https://doi.org/10.3390/horticulturae9010084

Academic Editor: Alessandra Durazzo

Received: 5 December 2022
Revised: 3 January 2023
Accepted: 3 January 2023
Published: 9 January 2023

1. Introduction

Plants from the Lamiaceae family such as thyme (*Thymus vulgaris* L.), mint (*Mentha piperita* L.), and lemon balm (*Melissa officinalis* L.) are used in all Balkan countries and are represented in folk medicine and food preparation, but also as herbal tea or beverages [1]. The herbal parts (leaves and flowers) have been used in traditional medicine for the treatment of a variety of diseases including gastroenteric and bronchopulmonary disorders, anthelmintic, carminative, sedative, diaphoretic, wounds, cough, skin, and gastrointestinal problems [2].

These plants are rich sources of minerals (especially K, Ca, and Mg), proteins, and other bioactive compounds. In addition, these plants could contribute to the production of EOs and other aromatic extracts as a source of natural antioxidant compounds [3]. As the main compounds from thyme essential oils (TEOs), thymol and carvacrol inhibit lipid peroxidation and manifest a strong antimicrobial activity against different kinds of microorganisms. The polyphenolic phytochemicals in thyme act as powerful antioxidants,

which have aroused an increasing interest in their application in functional food. There is a possibility of the future use of TEOs in extending the shelf life of bakery products and it could also find an application in the storage of root vegetables. Based on the antimicrobial activity of the vapor phase, it has been found that the essential oil has the potential to be used to protect packaged foods [4].

Mint essential oils (MEOs) are rich in monoterpenes and sesquiterpenes, having as major constituents menthone and menthol, which are potently exerted to treat muscle pain and neuralgic pain, as well as pains correlated with gastrointestinal disorders (the relief of abdominal pain, flatulence, repletion, obstipation, and diarrhea). Their use via inhalation is effective for relieving symptoms such as nausea, vomiting, and anorexia from patients receiving chemotherapy. The other biological effects of MEOs are their insecticidal, antibacterial, antiviral, antiallergenic, antioxidant, and cytotoxic activities [5].

According to various biological studies, *Melissa officinalis* L possesses a high amount of antioxidant activity through its chemical compounds, including flavonoids, rosmarinic acid, gallic acid, and their phenolic contents. Geranial, neral, citronellal, and geraniol are the main components of lemon balm essential oils (LEOs). The pharmacological effects of the extracts are mainly assigned to the presence of large amounts of polyphenolic compounds, such as antioxidant, antimicrobial, antiproliferative, and cytotoxic effects, and so on [6].

The importance of these plants and their medicinal value is reflected in the chemical composition and content of their essential oils (EOs) [7]. The EO content largely depends on the different geographical origins, chemo-types, environment, production method, plant parts from which the EO was obtained, the time of harvest, technique of isolation, storage method, etc. [8,9].

The biosynthesis of the secondary metabolites is mainly conditioned by the internal factors (the genetic basis, development stage, and plant tissue), but at the same time is affected by external, ago-ecological parameters such as the environmental conditions, including light [10], temperature, humidity, and techniques of production such as irrigation, soil type, and nutrition [8,9]. The intensity and quality of light can cause the accumulation and distribution of EOs in plants to differ. Shading plants with changes in the spectral composition of light can increase the content and modify the Eos' profile in medicinal plants [11,12].

The chemical composition of plant EOs differ among species; this is directly correlated to differences in the biological activities. Mostly two or three main components make up over 70% of EOs, while a large number of other components are present in the traces. The essential oil of dried herb of *Melissa officinalis* L. grown in Cuba was consisted mainly of neral (29.9%) and geranial (41.0%) [13]. The main constituents of the thyme and oregano EOs are thymol and carvacrol, with a strong antioxidant activity [14]. These components determine the biological properties of EOs and are conditioned by these constituents belonging to different groups due to a different biosynthetic origin. The essential oils from Lamiaceae plants have a natural antimicrobial activity.

Compared to most plants from this Family, thyme (*T. vulgaris*) EOs exhibit the strongest antimicrobial and antifungal activities. The thyme and mint EOs showed a strong activity against *C. albicans* [15]. The EOs from mint species are also used as an oral liquid in dental hygiene against pathogenic bacteria [16]. EOs are used in the food industry against food-borne pathogens [17], but also in pharmaceutical and cosmetic products. Eugenol, which is the most dominant EO component in basil grown under blue shade nets, exhibits a good activity in suppressing *Staphylococcus aureus, Escherichia coli*, and *Proteus vulgaris* [18]. Plant species from the Lamiaceae family have shown a significantly stronger antifungal activity than the standard antifungal molecules in our earlier research [19]. Due to insufficient experience and poor data from the existing literature on the influence of shading and plant density on the biological and chemical activity of cultivated medicinal plants, various experiments were carried out. The aim of this study was to investigate the influence of shading on the EO content and composition of thyme, mint, and lemon balm and their antioxidant and antimicrobial activities.

2. Material and Methods

2.1. Plant Material and Growing Conditions

The experiment was conducted throughout 2020–2021 in an experimental garden in the village of Moravac in South Serbia (21°42′ E, 43°30′ N, altitude 159 m). *T. vulgaris* (thyme), *M. piperita* (mint), and *Mofficinalis* (lemon balm) were used to determine whether shading conditions (plants covered by color nets) could improve the essential oils and antioxidant activity in plants.

The soil is weakly carbonated ($CaCP_3$—2.36%), and the neutral pH value in KCl is 6.94. It belongs to humus soils (3.69%). The content of the total nitrogen (N—0.18%) is within the limits of a medium security; it is phosphorus as well (P_2O_5—16.8 mg/100 g). It is well supplied with potassium (K_2O—24.0 mg/100 g, Table 1). The land is suitable for growing vegetables, medicinal plants, and herbs.

Table 1. Basic agrochemical properties of the soil.

$CaCO_3$ (%)	pH in KCl	Humus (%)	N—Total %	P_2O_5 mg/100 g	K_2O mg/100 g
2.36	6.94	3.69	0.18	16.8	30.8

The seeds were sown in the field with the task of achieving an optimal plant density of 50 plants/m2. Treatment combinations were replicated three times with one shading treatment (pearl nets with a shade index of 40%) and a non-shaded control treatment in a split-plot design. In the second year, after establishing the plant's production, the medicinal plants were harvested for the extraction of their essential oils (the main harvest took place in the middle of August).

Uniform shoots with leaves without any injuries or defects were selected and dried without the presence of light and ventilation at room temperature (about 25–30 °C) as air-dried herbs for the analysis.

2.2. Clevenger-Hydrodistillation

The growing of the medical plants, under shading and non-shading condition as well as the process of the production of EOs by hydrodistillation, was performed as described by Ilić et al. [9] and Milenković et al. [10]. The content of essential oil is displayed in% (*v*/*m*), which conforms to mL/100 g of air-dried plant material.

2.3. Gas Chromatography/Mass Spectrometry (GC/MS) and Gas Chromatography/Flame Ionization Detection (GC/FID) Analysis

The use of gas chromatography-mass spectrometry (GC-MS) and GC-flame ionization detection (GC-FID) for the characterization of the medical plants' essential oils is described in our previous research [10] following the methods of Sparkman et al. [20].

2.4. DPPH Assay

The plants' essential oils were diluted with ethanol, and a series dilution was performed (0.002–0.2 mg/mL). The procedure was done in two probes. The ability of the essential oils to scavenge free DPPH radicals was determined using the DPPH assay.

The absorption was measured at 517 nm immediately after adding the DPPH radical and after 20 min of incubation with the radical for thyme and mint and after 60 min for lemon balm at room temperature in the dark (*As*—absorbance of the sample). All of the other relevant details of the assay used are provided by Stanojević et al. [21,22].

2.5. Antimicrobial Activity

Microorganisms. Eight microorganisms were selected to determine the antimicrobial activity of the analyzed EOs: (seven bacterial strains) *Escherichia coli* (ATCC 25922), *Pseudomonas aeruginosa* (ATCC 27853), *Proteus vulgaris* (ATCC 8427), *Bacillus subtilis* (ATCC

6633), *Staphylococcus aureus* (ATCC 25923), *Klebsiella pneumoniae* (ATCC 700603), *Listeria monocytogenes* (ATCC 15313), and (fungal strain) *Candida albicans* (ATCC 2091). The microorganisms are from the collection of the Laboratory for Microbiology, the Faculty of Technology, Leskovac.

Disc-diffusion method. The agar disc-diffusion method was used for testing the antimicrobial activity of the obtained EOs [23]. All details about the sterilization, prepared initial sus- 111 pensions, inoculation, and incubation are described in our previous publications [9].

All experiments were carried out in three replicates and the results are expressed as the mean value ± standard deviation.

2.6. Statistical Methods

In case of differences between shaded and non-shaded conditions, Student's t-test was used, while for the effect of the essential oils on the antimicrobial activity, an ANOVA was used to analyze the significance with Duncan's multiple range tests (with a level of 0.05). For the explanatory data analysis, principal component analysis was used. All of the statistical calculations were performed with STATISTICA software (TIBCO software Inc. Palo Alto, CA, USA. 2020, version 14.0.015.)

3. Results and Discussion

3.1. Climatic Condition

The production method as well as the environmental conditions (the light, temperature, pre-capitation, and air humidity) significantly affected the production, yield, and quality of the medicinal plants.

Based on the data from Table 2, it can be seen that, especially in 2021, there is an increase in the temperatures and solar radiation during the summer months compared to the 30-year average, which is also a confirmation of global warming in the world; because of this, the protection of plants by shading becomes more important and their application becomes necessary.

Table 2. Temperature (°C), solar radiation (h), and precipitation (mm) during the growing season.

Month	TS 2020. 2021.	TOD 2020. 2021.	TX 2020. 2021.	TM 2020. 2021.	MSR 2020. 2021.	MSRA	RR 2020. 2021.	RO
May	16.4 17.2	−0.2 0.6	22.9 24.5	11.0 11.0	173.2 226.8	219.0	67.0 29.4	66.7
June	19.8 21.5	0.3 2.0	25.7 28.9	14.9 14.6	183.7 245.7	237.2	186.7 30.3	69.7
July	22.3 25.5	1.0 4.2	29.1 32.8	15.9 18.6	290.9 293.2	289.0	78.6 39.8	43.6
August	22.6 23.5	1.5 2.4	30.0 31.8	16.5 16.1	265.5 302.1	276.0	78.6 39.6	43.3
September	20.3 18.0	3.1 0.8	28.0 25.8	13.6 14.1	224.8 200.9	210.0	14.9 21.0	43.6

TS—mean monthly air temperature (°C); TOD—temperature deviation for 30-year average (°C); TX—mean daily temperature maximum for month (°C); TM—mean daily temperature minimum for month (°C); MSR—monthly solar radiation (h). MSRA—monthly solar radiation (h) for 30-year average; RR—total monthly precipitation (mm); RO—monthly precipitation (mm) for 30-year average.

Certain plant species have specific requirements regarding light intensity. In Table 3, it can be noted that in the hottest part of the year in July, solar radiation in the midday hours can reach a value of 864.9 W m^{-2}. At the same time, the value of these parameters under the shading nets is significantly reduced (463.2 V m^{-2}).

Table 3. Influence of shading on growing environment (average day in July) 2021.

Time (h)	PAR * (µmol m^{-2} s^{-1})		Solar Radiation (W m^{-2})		Temperature °C		Relative Humidity%	
	Non-Shading	Shading Reduction%	Non-Shading	Shading	Non-Shading	Shading Reduction °C	Non-Shading%	Shading %
6:00	172.4	39.2	167.2	48.4	17.0	0.0	69.0	70.1
9:00	1349.1	48,8	520.1	276.3	27.2	0.4	41.2	41.6
12:00	2085.7	45.7	864.9	463.2	34.9	2.0	36.3	36.6
15:00	1747.2	48.8	770.5	343.7	37.1	2.5	19.8	20.6
18:00	513	50.3	354.3	92.1	34.2	0.9	26.2	26.6

* PAR.—photosynthetically active radiation.

Some of them tolerate shading, while others require a lot of light during growth. Light also affects the content of the bioactive components in plants. In our previous work, we found that medical plants species responded positively to cover by shade nets and synthesized more EOs compared to plants without shading [18]. Some of the medical plants in full sunlight showed the lowest levels of EOs [7]. The EOs from shaded oregano showed a higher antioxidant activity than the non-shaded control of plants [6]. Modified light with the application of shading nets affects the content of the antioxidant capacity more significantly than the plant species [10].

Shading nets can be used as cultivation methods during the production of medicinal plants, which results in a higher content of essential oils and a higher antioxidant and antimicrobial potency.

3.2. Yield of Essential Oils

3.2.1. Thyme Essential Oil (TEO) Yield

Thyme is a significant aromatic plant widely used for medicinal purposes as well as in culinary dishes, with therapeutic properties due to their essential oils. Its properties are due to its main component, thymol. No significant differences in the production of EOs were observed between the shaded and non-shaded thyme plants. The EO content in thyme (TEOs) was 3.44 mL/100 g of p.m. from non-shaded open field and 3.46 mL/100 g of plant material (p.m) from the shaded condition (Table 4).

Table 4. Yield of essential oil from shaded and non-shaded plants (*T. vulgaris* L., *M. piperita* L., and *M. officinalis* L.).

Sample	Essential Oil Yield, mL/100 g p.m. *
Thyme—non-shaded	3.44 ^{a} ± 0.01
Thyme—shaded	3.46 ^{a} ± 0.03
Mint—non-shaded	3.96 ^{a} ± 0.08
Mint—shaded	2.20 ^{b} ± 0.02
Lemon balm—non-shaded	0.21 ^{c} ± 0.01
Lemon balm—shaded	0.45 ^{c} ± 0.02

* p.m.—plant material (dry). Values followed by different letters are significantly different at $p < 0.05$.

Moreover, these results are in agreement with Milenković and colleagues [10] who showed the slightly lower production of TEOs from non-shaded compared to shaded plant. Depending on the environment, growth region, and cultivation practices, the EO content of the thyme plant also differs. A higher oil content was obtained in indigenous plants from Jordan, (3.7–5.6% of dried material) than in cultivated plants (1.1–2.0%). The plants grown at the highest altitude from Jordan produced the highest oil content (~5.4%), [24]. Choi et al. [25] reported that the content of the TEO was 2.62%, and this level of oil content is similar compared to the essential oils of other herbal plants.

The content of thyme essential oil differed by the region of origin. The main component of the TEO determined specifics thyme type. The TEOs from Casola and Modena, Italy,

were characterized by thymol [26], but thyme from Palmaria Island contained carvacrol as the main constituent [27].

The content of TEOs depends on the chemotype, origin, and part of the plant. *T. vulgaris* 'thymol' chemotype obtained EO yields which ranged from 0.47% to 1.2%, while the 'linalool' chemotype had between 1.20% and 2.8% of EOs [28]. As reported by Andolfi et al., *T. vulgaris* flowers are characterized by a higher EO content, followed by the leaves, while in the stems, EOs are present only in traces [29].

The TEOs' content ranged from 0.3% [30] to 4.0% [31]. The TEOs' content from the *T. vulgaris* cultivated in Romania was 1.25% [32]. A significantly different content of TEOs between shaded and non-shaded plants was recognized in our previous studies [8].

3.2.2. Mint Essential Oil (MEO) Yield

Domestic Mentha (*M. piperita*) is the most important type of mint as it is a naturally cultured hybrid of *M. aquatic* and *M. spicata* [33]. The content of menthe essential oil (MEO) obtained by steam distillation from mint grown in an open field was 3.96 mL/100 g of plant material. Mint grown in shading conditions has a significantly lower content of MEO (2.20 mL/100 g of plant material) than non-shaded control plants (Table 4).

Based on the literature data, it is known that mint and thyme are plant species from the Lamiaceae family that are characterized by a higher EO content than other plants, for example lemon balm, which contains an EO content which is up to several times [7]. The mint species grew better under long-day conditions. The mint plant species did not tolerate shading well, and the nets provide the lower presence of secretory cells of the glandular trichomes and the biosynthesis of essential oil. This work confirms the significant differences in the oil production of mint under a full sun and shading condition.

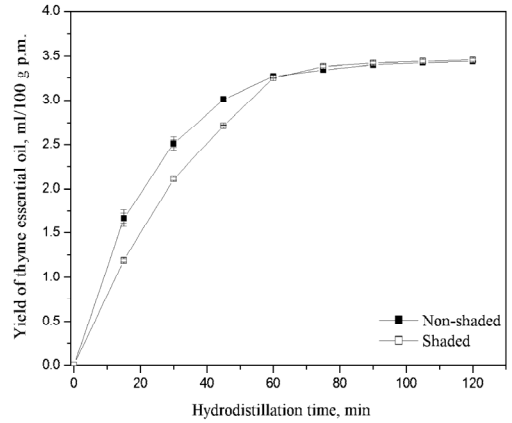
3.2.3. Lemon Balm Essential Oil (LEO) Yield

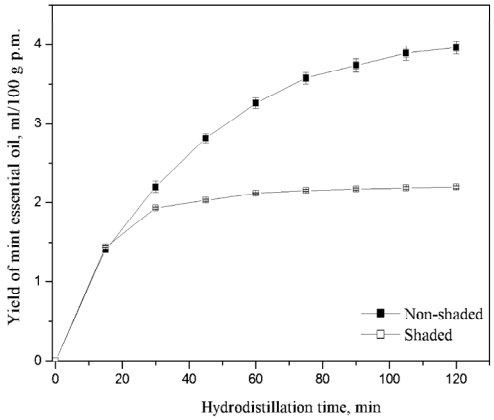
The contents of the EOs from the lemon balm (LEOs) grown in an open field was 0.21 mL/100 g of p.m. A significantly higher LEOs content (0.45 mL/100 g) was recorded in the plants covered by nets than from non-shaded plants (Table 4).

One year before these researches in the same location, the content of LEOs from plants grown in an open field was 0.18 mL/100 g, which is significantly less than the content of LEOs obtained from shaded plants (0.22 mL/100 g) [7].

The maximum content of EOs in the budding phase (Budapest) or during flowering (Poznań) from lemon balm was (0.08–0.46 mL/100 g dry weight) [34]. Lemon balm grown in Poland contains an LEO content which ranges from 0.08 to 0.25 mL/100 g. The LEO content is higher in plants from the experimental field than in commercial production. The LEO content is slightly higher in fresh leaves than in dried ones [35]. The dependence of the yield of thyme, mint and lemon balm essential oil from the hydrodistillation time was presented in Figure 1.

The use of colored shade nets during the growth of different medicinal plants provides spectral changes, resulting in a higher content of EOs in sweet basil [10,18], mint, oregano, marjoram, thyme [7,10], lemon balm [7,36], and sage [37]. The EO composition of mint is affected in a differential way by different wavelengths.





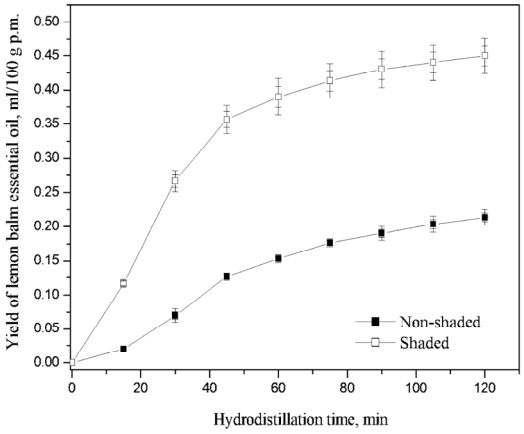


Figure 1. The dependence of the yield of thyme, mint, and lemon balm essential oil.

3.3. Essential Oils Composition

3.3.1. Thyme Essential Oil (TEO) Composition

The analysis of thyme essential oils (TEOs) ranging from 29 to 31 compounds which represent 99.8 to 100% of the essential oils (Table 5).

The main component in TEOs is thymol ranging from 43.9% in shaded plants to 44.2% in non-shaded plants. The second main TEO constituent is γ-terpinene ranging from16.8% in shaded plants to 18.3% in non-shaded plants Other significant constituents in TEOs included p-cymene (16.5–17.4%), myrcene (2.4–2.6%), α-terpinene (2.3–2.4%), and linalool (2.2–2.3%). Other components are present at a level below 1%. Some components, such as octanol acetate and isobornyl acetate, are present only in the TEO isolated from non-shaded plants. (E)-β-ocimene is present only in plants covered by shade nets, evidenced in Table 5.

The compounds below belong to different groups, namely aromatic compounds (66.3–66.8%), hydrocarbon monoterpenes (25.2–26.1%), oxygen-containing monoterpenes (5.1–5.5%), etc. Structures of the most common components of *Th. vulgaris* essential oils are presented in Figure 2.

Table 5. Thyme essential oil (TEO) isolated from non-shaded and shaded plants.

N^0	$t_{ret.}$, min	Compound	RI^{exp}	RI^{lit}	Method of Identification	c%	
						Non-Shaded	Shaded
1.	6.70	α-Thujene	923	924	RI, MS	1.3 ± 0.010	1.4 ± 0.010
2.	6.92	α-Pinene	931	932	RI, MS	0.8 ± 0.003	0.9 ± 0.002
3.	7.40	Camphene	947	946	RI, MS	0.4 ± 0.002	0.4 ± 0.002
4.	8.15	Sabinene	972	969	RI, MS	tr	Tr
5.	8.28	β-Pinene	977	974	RI, MS, Co-I	0.3 ± 0.001	0.3 ± 0.001
6.	8.61	1-Octen-3-ol	977	974	RI, MS	0.8 ± 0.003	1.1 ± 0.003 **
7.	8.70	Myrcene	991	988	RI, MS	2.4 ± 0.008	2.6 ± 0.007 *
8.	9.12	3-Octanol	994	988	RI, MS	tr	Tr
9.	9.25	α-Phellandrene	1007	1002	RI, MS	0.2 ± 0.001	0.2 ± 0.001
10.	9.40	δ-3-Carene	1011	1008	RI, MS	tr	0.1 ± 0.000
11.	9.67	α-Terpinene	1018	1014	RI, MS	2.3 ± 0.009	2.4 ± 0.009
12.	10.08	p-Cymene *	1021	1020	RI, MS	16.5 ± 0.05	17.4 ± 0.06
13.	10.18	1,8-Cineole *	1023	1026	RI, MS, Co-I	tr	Tr
14.	10.85	(E)-β-Ocimene	1041	1044	RI, MS	tr	Tr
15.	11.33	γ-Terpinene	1054	1054	RI, MS	18.3 ± 0.06	16.8 ± 0.05
16.	11.90	*cis*-Sabinene hydrate	1069	1065	RI, MS	1.0 ± 0.008	0.6 ± 0.004 **
17.	12.43	Terpinolene	1083	1086	RI, MS	0.1 ± 0.000	0.1 ± 0.000
18.	13.22	Linalool	1103	1095	RI, MS, Co-I	2.3 ± 0.008	2.2 ± 0.008
19.	14.85	Camphor	1142	1141	RI, MS, Co-I	0.1 ± 0.000	Tr
20.	16.15	Borneol	1173	1165	RI, MS, Co-I	0.8 ± 0.003	0.9 ± 0.004
21.	16.49	Terpinen-4-ol	1182	1174	RI, MS	0.8 ± 0.003	1.0 ± 0.004 *
22.	17.28	α-Terpineol	1200	1196	RI, MS	0.3 ± 0.002	0.3 ± 0.002
23.	17.56	Octanol acetate	1207	1211	RI, MS	0.2 ± 0.002	-
24.	18.74	Thymol, methyl ether	1235	1232	RI, MS	0.4 ± 0.003	0.7 ± 0.006 **
25.	19.13	Carvacrol, methyl ether	1244	1241	RI, MS	0.5 ± 0.003	0.4 ± 0.003
26.	20.75	Isobornyl acetate	1283	1283	RI, MS	tr	-
27.	22.59	Thymol	1299	1289	RI, MS, Co-I	44.2 ± 0.120	43.9 ± 0.110
28.	22.96	Carvacrol	1307	1298	RI, MS	4.7 ± 0.015	4.4 ± 0.015
29.	26.49	(E)-Caryophyllene	1421	1417	RI, MS	0.9 ± 0.004	1.4 ± 0.008 **
30.	28.59	Geranyl propanoate	1474	1476	RI, MS	0.2 ± 0.002	0.1 ± 0.001 *
31.	33.13	Caryophyllene oxide	1592	1582	RI, MS	0.2 ± 0.002	0.2 ± 0.002
					Total identified	100.0 ± 0.322	99.8 ± 0.300
		Grouped components (%)					
		Monoterpene hydrocarbons (1–5, 7, 9–11, 14, 15, 17)				26.1	25.2
		Oxygen-containing monoterpenes (13, 16, 18–22, 26, 30)				5.5	5.1
		Sesquiterpene hydrocarbons (29)				0.9	1.4
		Oxygenated sesquiterpenes (31)				0.2	0.2
		Aromatic compounds (12, 24, 25, 27, 28) *				66.3 *	66.8 *
		Phenolics (24, 25, 27, 28)				49.8	(49.4)
		Others (6, 8, 23)				1.0	1.1

Non-shaded plants—octanol acetate; isobornyl acetate. Shaded plants—(E)-β-ocimene. Difference in compound percentage is marked * for $p < 0.05$ or with ** for $p < 0.01$.

Our works similarly shows that thymol and γ-terpinene are the main components in the oil isolated from thyme, followed by p-cymene and caryophyllene oxide [10].

The European Pharmacopoeia proposes that only plants belonging to the thymol chemotype should be used for the production of TEOs. Thymol, carvacrol, and p-cymene are the main constituents in the TEOs, followed by γ-terpinene, linalool, β-myrcene, and terpinen-4-ol [38].

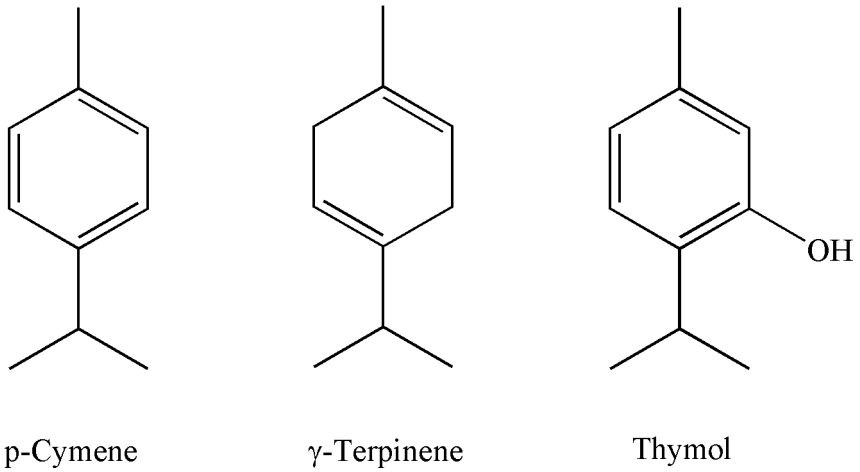


Figure 2. Structures of the most abundant components identified in TEOs.

3.3.2. Mint Essential Oils (MEOs) Composition

The analysis of mint essential oils (MEOs), which range from 31 to 34 compounds, representing 99.5 to 100% of the total EOs (Table 6). These compounds belong to different groups, namely hydrocarbon monoterpenes (10.8–13.9%), oxygenated monoterpenes (81.0–84.9%), hydrocarbon sesquiterpenes (2.2–3.3%), etc.

Piperitenone oxide is the main component of MEOs ranging between 52.6% in non-shaded and 64.8% in shaded plants. The second main MEO constituent is 1,8-cineole, ranging from 16.3% in shaded plants to 25.9% in non-shaded plants. Other significant constituents in MEOs included myrcene (6.2–7.1%), β-pinene (1.9–2.8%), sabinene (0.9–1.4%), and α-pinene (0.8–1.4%). The remaining components are present at below the 1% level. Some components such as α-thujene and camphene are present only in non-shaded mint plants. Dihydroedulan I, piperitenone, and (Z)-jasmone are detected only in plants cover by shade nets (Table 6). Structures of the most common components of *M. piperita* essential oils are presented in Figure 3.

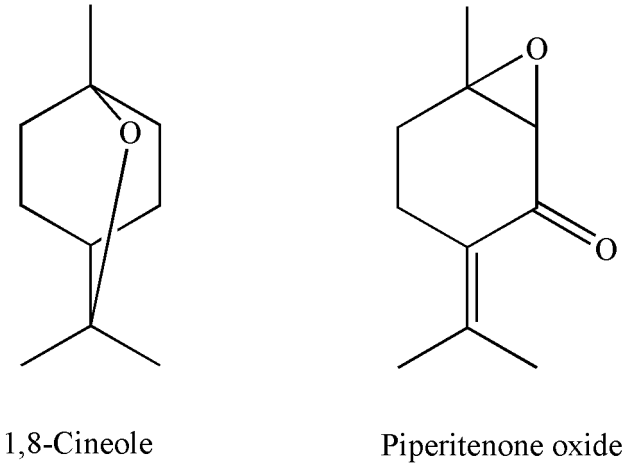


Figure 3. Structures of the most abundant components identified in MEO.

Similar results regarding the composition of MEOs have been previously reported [39–41]. The major constituents are oxygenated monoterpenes, followed by the oxygenated sesquiterpenes. The main constituents of the MEOs from China (menthol 30.69%, menthone 14.51%, and methyl acetate 12.86%) are different from the compositions of mint plant from other regions of the world [42]. Yadegarinia et al. [43] reported that the major component of the MEO from Iran was α-terpene (19.7%) and piperitenone oxide (19.7%), followed by isomenthone

(10.3%). The main compound in MEO isolated by hydro-distillation from the aerial mint parts from India was limonene (18.4%), α-pinene (17.3%), and β-pinene (13.9%) [44]. This variation in the chemical composition may be attributed to various climate conditions, the geographic location, and cultivated variety.

The results from other studies are characterized by the presence of a considerable level of carvone (62.7 to 75.5%) [45,46]. Some studies have reported limonene as the main component from Moroccan MEO (10.5–17.9%) [45,46].

Table 6. Mint essential oil (MEO) composition isolated from non-shaded and shaded plants.

N^0	$t_{ret.}$, min	Compound	RI^{exp}	RI^{lit}	Method of Identification	c%	
						Non-Shaded	Shaded
1.	6.70	α-Thujene	924	924	RI, MS	tr	-
2.	6.92	α-Pinene	932	932	RI, MS	1.4 ± 0.009	0.8 ± 0.006 **
3.	7.40	Camphene	947	946	RI, MS	tr	-
4.	8.15	Sabinene	972	969	RI, MS	1.4 ± 0.008	0.9 ± 0.006 **
5.	8.28	β-Pinene	976	974	RI, MS, Co-I	2.8 ± 0.008	1.9 ± 0.009 **
6.	8.61	1-Octen-3-ol	977	974	RI, MS	tr	Tr
7.	8.70	Myrcene	980	988	RI, MS	7.1 ± 0.021	6.2 ± 0.018 *
8.	9.12	3-Octanol	994	988	RI, MS	0.4 ± 0.003	0.3 ± 0.003
9.	9.67	α-Terpinene	1010	1014	RI, MS	tr	Tr
10.	10.08	p-Cymene	1021	1020	RI, MS	tr	Tr
11.	10.18	Limonene	1023	1024	RI, MS, Co-I	tr	Tr
12.	10.23	1,8-Cineole	1025	1026	RI, MS, Co-I	25.9 ± 0.080	16.3 ± 0.051 **
13.	10.44	(Z)-β-Ocimene	1030	1032	RI, MS	0.8 ± 0.010	0.7 ± 0.010
14.	10.85	(E)-β-Ocimene	1041	1044	RI, MS	tr	Tr
15.	11.28	γ-Terpinene	1054	1054	RI, MS	0.2 ± 0.002	0.2 ± 0.002
16.	11.90	*cis*-Sabinene hydrate	1069	1065	RI, MS	tr	Tr
17.	12.43	Terpinolene	1083	1086	RI, MS	0.2 ± 0.002	0.1 ± 0.001
18.	13.12	Isopentyl 2-methyl butanoate	1101	1100	RI, MS	tr	Tr
19.	13.22	Linalool	1103	1095	RI, MS, Co-I	tr	0.3 ± 0.002
20.	13.75	3-Octanol acetate	1116	1120	RI, MS	tr	tr
21.	16.22	δ-Terpineol	1172	1162	RI, MS	0.7 ± 0.003	0.6 ± 0.003
22.	16.49	Terpinen-4-ol	1182	1174	RI, MS	0.2 ± 0.001	0.2 ± 0.001
23.	17.04	Myrtenal	1195	1195	RI, MS	0.2 ± 0.001	0.2 ± 0.001
24.	17.28	α-Terpineol	1200	1196	RI, MS	0.6 ± 0.005	0.6 ± 0.005
25.	18.58	(3Z)-Hexenyl 3-methyl butanoate	1232	1232	RI, MS	tr	tr
26.	20.14	(4E)-Decen-1-ol	1268	1259	RI, MS	0.6 ± 0.005	0.7 ± 0.006
27.	20.48	(+)-Isopiperitenone	1276	-	MS	0.3 ± 0.002	0.3 ± 0.002
28.	22.57	Thymol	1299	1289	RI, MS, Co-I	1.4 ± 0.005	0.3 ± 0.002 **
29.	24.74	Piperitenone oxide	1376	1366	RI, MS	52.6 ± 0.120	64.8 ± 0.139 *
30.	25.94	Nepetalactone	1403	1393	MS	0.5 ± 0.003	0.9 ± 0.006 *
31.	26.49	(E)-Caryophyllene	1421	1417	RI, MS	1.1 ± 0.008	1.4 ± 0.009 *
32.	27.92	α-Humulene	1457	1452	RI, MS	tr	0.2 ± 0.001
33.	29.04	Germacrene D	1485	1484	RI, MS	1.1 ± 0.007	1.5 ± 0.008 *
34.	29.62	Bicyclogermacrene	1500	1500	RI, MS	tr	tr
					Total identified	99.5 ± 0.296	100.0 ± 0.841
		Grouped components (%)					
		Monoterpene hydrocarbons (1–5, 7, 9, 11, 13–15, 17)				13.9	10.8
		Oxygen-containing monoterpenes (12, 16, 19, 21–24, 27, 29, 30)				81.0	84.9
		Sesquiterpene hydrocarbons (31–34)				2.2	3.3
		Aromatic compounds (10, 28)				1.4	tr
		* Phenolics (28)				* 1.4	
		Others (6, 8, 18, 20, 25, 26)				1.0	1.0

Present in non-shaded plants—α-thujene; camphene. Present only in shaded plants—dihydroedulan I; piperitenone; (Z)-jasmone. Difference in compound percentage is marked * for $p < 0.05$ or with ** for $p < 0.01$.

3.3.3. Lemon Balm Essential Oil (LEO) Composition

Seventy-eight constituents (100% of the total oil composition) were identified in the LEO. The majority of LEO compounds from non-shaded and shaded plants were oxygen-containing monoterpenes (88.1%), sesquiterpene hydrocarbons (4.8%), and oxygen-containing sesquiterpenes (3.6%). No significant differences in the composition were observed between the non-shaded and shaded plants. Only the monoterpene hydrocarbons varied between shaded (3.6%) and non-shaded (0.5%) plants.

The components obtained by hydrodistillation from lemon balm were geranial (32.8–34.0%); neral (21.3–23.9%); piperitenone oxide (16.7–17.2%); and caryophyllene oxide (2.6–3.6%). Neral is more highly present in shaded plants compared to the plants from the open field. The components that have been only registered in non-shaded plants were also observed in the non-shaded plants in our study, 1-terpineol; isobornyl formate; 2,3-epoxygeranial; and undecanal. Similarly, α-thujene; rose furan; and borneol were detected only in shaded plants. The components present below the 1% level were defined as non-identified substances (Table 7).

Structures of the most common components of *M. officinalis* essential oils are presented in Figure 4.

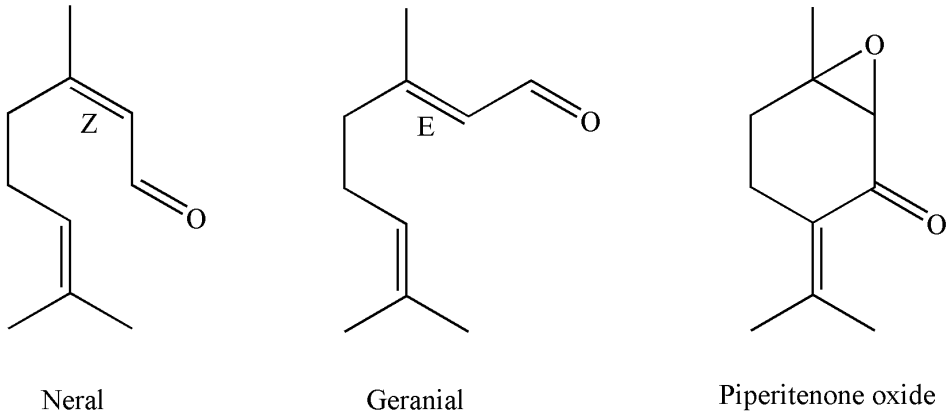


Figure 4. Structures of the most abundant components identified in LEO.

According to the literature, the major components of the LEOs are mono-, sesquiterpenes, and aliphatic aldehydes, alcohols such as geranial, neral, and citronellal, geranyl acetate, (E)-caryophyllene, caryophyllene oxide, geraniol, pinene, sabinene, thymol, carvacrol and muurolene, decadienal, and trans-carveol [47,48].

In our previous research [7,15] the main components of the lemon balm EOs were geranial, neral, piperitenone oxide, and caryophyllene oxide. Geranial and neral are more represented in shaded than in non-shaded plants. Based on the major LEO components from thirty *M. officinalis* samples from different origins [49], the components have been divided into four different classes: (I) geranial/neral; (II) geraniol/caryophyllene oxide; (III) citronellal; and (IV) α-pinene/caryophyllene oxide chemotypes.

The content of LEOs (0.01 to 0.3%) is lowest compared to the EO content from other plants of the Lamiaceae family because of the limited number of peltate trichomes in the leaf. This is the mean reason behind these EOs being very expensive [50]. The major constituents of the LEOs produced in northern India were geranial (42.3–44.9%), neral (30.7–32.6%), (*E*)-caryophyllene (2.8–3.5%), geranyl acetate (0.7–3.3%), geraniol (0.9–2.6%), piperitone (0.6–2.5%), nerol (0.8–2.4%), caryophyllene oxide (0.8–2.3%), (*E*)-isocitral (0.5–2.1%), and citronellal (0.4–2.1%). Citral (73.0–77.5%) was the main components of the LEOs from India [51].

The growing method (open field or shading) and harvest time have been show to influence the EO content composition in lemon balm produced from Cuba. The plants cultivated in an open field and collected in April contained more EOs [52].

The major constituents of LEOs were β-caryophyllene (23.06%), *E*-citral (17.61%), *Z*-citral (13.64%), and caryophyllene oxide (10.83%). The antioxidant activity of the essential

oil was moderate (EC_{50} = 749.60 µg/g), but was lower compared to butylated hydroxytoluene (BHT) [53]. The major constituents of the LEOs from the wild plants from Greece were β-pinene, sabinene, (E)-caryophyllene, and caryophyllene oxide without citral and citronellal [54].

Table 7. Lemon balm essential oil (LEO) composition from non-shaded and shaded plants.

N⁰	$t_{ret.}$, min	Compound	RI^{exp}	RI^{lit}	Method of Identification	c%	
						Non-Shaded	Shade
1.	8.17	Sabinene	962	969	RI, MS	tr	0.4 ± 0.003
2.	8.28	β-Pinene	967	974	RI, MS, Co-I	tr	0.5 ± 0.003
3.	8.61	6-Methyl-5-hepten-2-one	977	981	RI, MS	1.5 ± 0.009	1.3 ± 0.008
4.	8.70	Myrcene	980	988	RI, MS	0.5 ± 0.003	2.0 ± 0.008 **
5.	10.18	1,8-Cineole	1023	1026	RI, MS, Co-I	1.1 ± 0.007	4.4 ± 0.018 **
6.	10.43	(Z)-β-Ocimene	1030	1032	RI, MS	tr	0.3 ± 0.002
7.	10.85	(E)-β-Ocimene	1041	1044	RI, MS	tr	0.2 ± 0.002
8.	10.97	Benzene acetaldehyde	1044	1036	RI, MS	0.3 ± 0.002	Tr
9.	13.22	Linalool	1103	1095	RI, MS, Co-I	0.7 ± 0.006	0.6 ± 0.006
10.	14.62	1-Terpineol	1130	1137	RI, MS	0.2 ± 0.001	-
11.	15.17	Citronellal	1150	1148	RI, MS	4.1 ± 0.016	2.4 ± 0.009 **
12.	15.69	(Z)-Isocitral	1162	1160	RI, MS	0.3 ± 0.002	0.9 ± 0.004 **
13.	16.43	(E)-Isocitral	1180	1177	RI, MS	0.4 ± 0.003	1.6 ± 0.009 **
14.	17.26	α-Terpineol	1186	1196	RI, MS	0.4 ± 0.003	0.3 ± 0.002
15.	18.28	Isobornyl formate	1225	1235	RI, MS	0.3 ± 0.002	-
16.	18.71	2,3-Epoxygeranial	1235	-	MS	1.1 ± 0.007	-
17.	19.11	Neral	1244	1235	RI, MS, Co-I	21.3 ± 0.080	24.9 ± 0.085
18.	19.72	*cis*-Piperitone epoxide	1258	1250	RI, MS	3.5 ± 0.014	0.1 ± 0.000 **
19.	20.44	Geranial	1274	1264	RI, MS, Co-I	34.0 ± 0.070	32.8 ± 0.060
20.	21.32	Undecanal	1296	1305	RI, MS	1.1 ± 0.007	-
21.	22.48	Methyl geranate	1324	1322	RI, MS	0.7 ± 0.006	0.5 ± 0.003 *
22.	24.56	Piperitenone oxide	1374	1366	RI, MS	17.2 ± 0.100	16.7 ± 0.008
23.	24.91	Geranyl acetate	1383	1379	RI, MS	2.3 ± 0.008	1.2 ± 0.008 **
24.	25.96	Nepetalactone	1403	1393	RI, MS	0.5 ± 0.003	0.4 ± 0.003
25.	26.49	(E)-Caryophyllene	1421	1417	RI, MS	4.0 ± 0.017	4.1 ± 0.016
26.	27.92	α-Humulene	1457	1452	RI, MS	0.4 ± 0.003	0.3 ± 0.002
27.	29.03	Germacrene D	1485	1484	RI, MS	0.4 ± 0.003	0.7 ± 0.006 **
28.	33.13	Caryophyllene oxide	1592	1582	RI, MS	3.6 ± 0.015	2.6 ± 0.010 *
					Total identified	100.0 ± 0.387	100.0 ± 0.271
Grouped components (%)							
		Monoterpene hydrocarbons (1, 2, 4, 6, 7)				0.5	3.6
		Oxygen-containing monoterpenes (5, 9–19, 21–24)				88.1	87.2
		Sesquiterpene hydrocarbons (25–27)				4.8	5.1
		Oxygenated sesquiterpenes (28)				3.6	2.6
		Aromatic compounds (8)				0.3	tr
		Others (3, 20)				2.6	1.7

Present only in non-shaded plants: 1-terpineol; isobornyl formate; 2,3-epoxygeranial; and undecanal. Present only in shaded plants: α-thujene; rose furan; and borneol. Difference in compound percentage is marked * for $p < 0.05$ or with ** for $p < 0.01$.

3.4. Antioxidant Activity

Thyme and mint plants covered by shade nets showed a lower antioxidant activity compared to the non-shaded, control plants. Based on the results given in Table 8, the highest antioxidant activity was observed in the thyme EOs from non-shaded plants.

The EC_{50} values (efficient concentration of the oil) increased in the following order (the smaller the EC_{50} value, the better the antioxidant activity): non-shaded thyme (0.54) > shaded thyme (0.92) > non-shaded mint (3.03) shaded lemon balm (3.43) > shaded mint (5.43) > non-shaded lemon balm (12.85). The incubation time was different depending

on the plant species. The incubation time in these explorations for thyme and mint was 20 min and for lemon balm was 60 min.

Table 8. EC_{50} values of essential oil from the different parts of aromatic plants.

Species/Production Methods	EC_{50}, mg/mL Incubation Time			
	Without Incubation	20 min	40 min	60 min
Thyme—non-shaded	/	0.54 ^{c} ± 0.003		
Thyme—shaded	/	0.92 ^{c} ± 0.014		
Mint—non-shaded	/	3.03 ^{b} ± 0.027		
Mint—shaded	/	5.43 ^{b} ± 0.237		
Lemon balm—non-shaded	/			12.85 ^{a} ± 0.199
Lemon balm—shaded	/			3.43 ^{b} ± 0.010

Values followed by different letters are significantly different at $p < 0.05$.

In our previous experiments, all the medicinal plants covered by shade nets showed a higher antioxidant activity compared to the non-shaded, control plants [15].

Figures 5 and 6 shows the percentage of DPPH radical neutralization with increasing extract concentration with incubation (20 min) of essential oil from non-shaded and shaded thyme and mint plants.

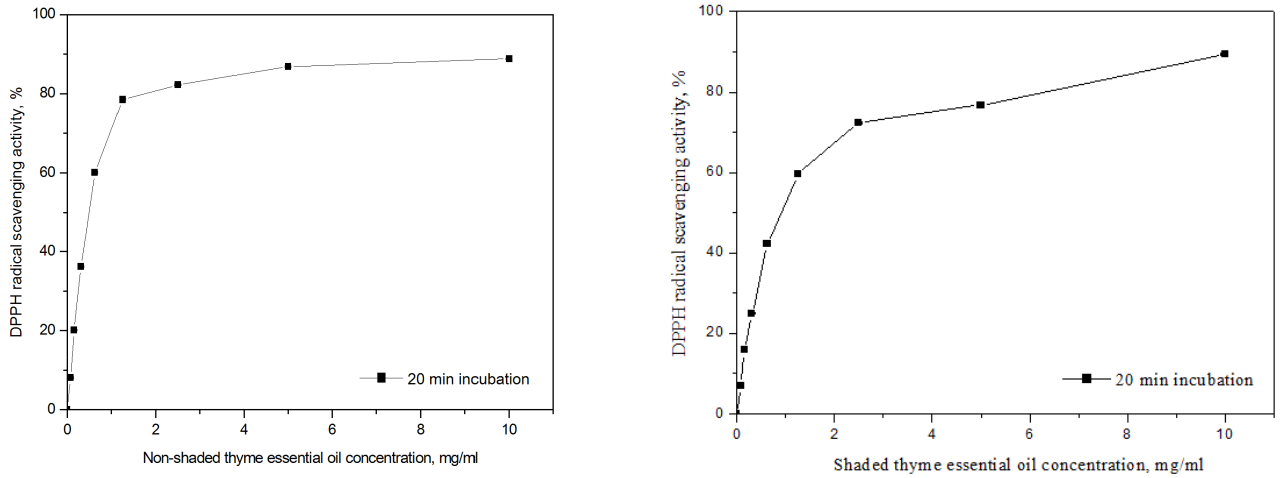


Figure 5. Antioxidant activity of non-shaded and shaded thyme essential oil.

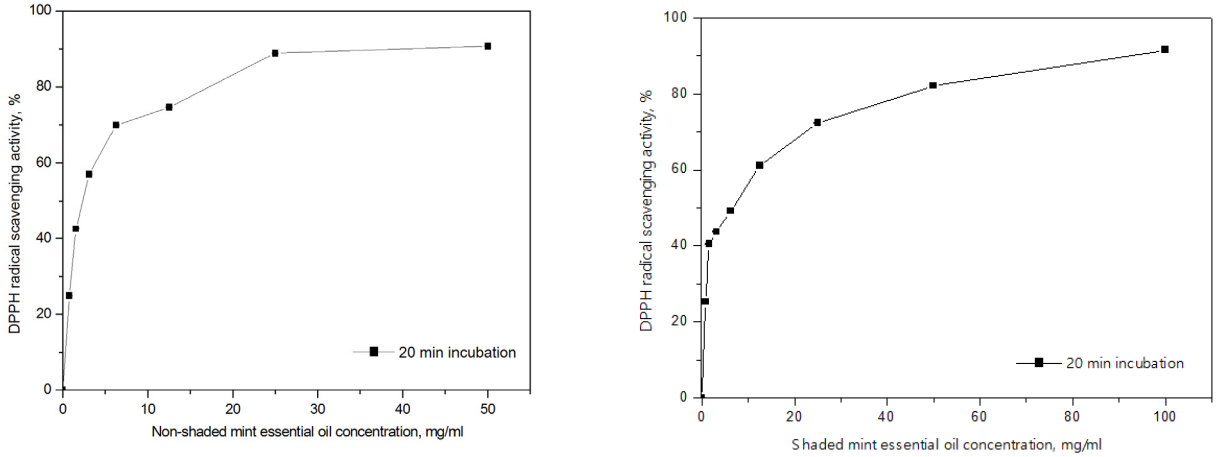


Figure 6. Antioxidant activity of non-shaded and shaded mint essential oil.

Figure 7 shows the percentage of DPPH radical neutralization with increasing extract concentration with incubation (60 min) of essential oil from non-shaded and shaded lemon balm plants.

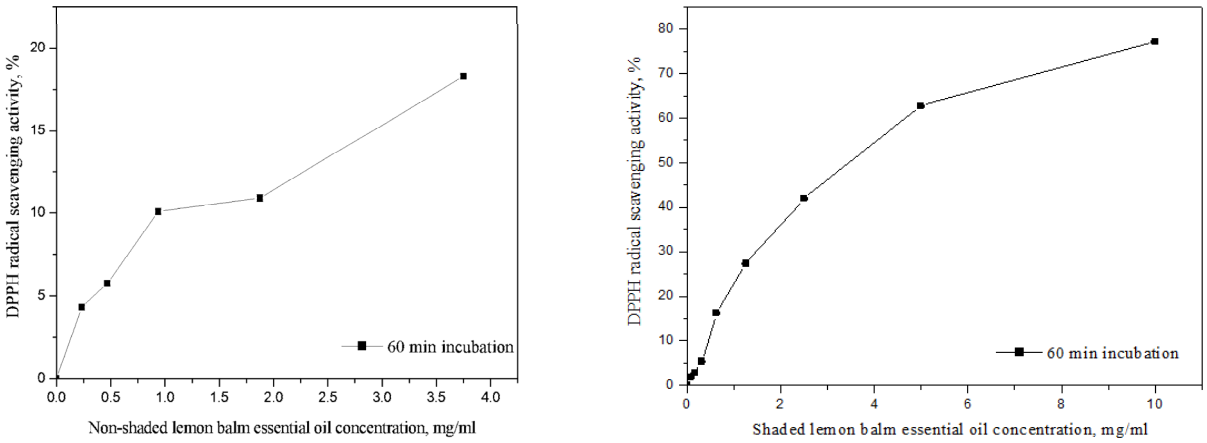


Figure 7. Antioxidant activity of non-shaded and shade lemon balm essential oil.

3.5. Antimicrobial Activity

The EOs of the medicinal plants from the Lamiaceae family in our exploration exhibited an efficacy against the analyzed pathogenic microorganisms. The EOs from *T. vulgaris* L. proved to be most active against all isolates with a strong inhibitory effect. The EOs from *T. vulgaris*, *M. piperita*, and *M. officinalis* showed significant anti-candida activity (from 42 mm with lemon balm EOs to 55 mm with mint EOs) (Table 9).

Table 9. Antimicrobial activity (inhibition zone, mm) of essential oils from shaded and non-shaded medicinal plants.

Species/Production Methods	*Escherichia coli*	*Pseudomonas aeruginosa*	*Proteus vulgaris*	*Staphylococcus aureus*	*Bacillus cereus*	*Bacillus subtilis*	*Klebsiella pneumoniae*	*Listeria monocytogenes*	*Candida albicans*
Thyme—non-shaded	44.3 ^{a}	0.0	26.0 ^{a}	38.6 ^{a}	0.0	62.0 ^{a}	0.0	0.0	51.6 ^{a}
Thyme—shaded	41.3 ^{b}	0.0	23.3 ^{a}	33.3 ^{b}	0.0	64.0 ^{a}	0.0	0.0	51.3 ^{a}
Mint—non-shaded	19.3 ^{c}	0.0	0.0 ^{e}	23.3 ^{c}	0.0	30.3 ^{b}	0.0	0.0	55.0 ^{a}
Mint—shaded	20.6 ^{c}	0.0	15.7 ^{c}	17.0 ^{d}	0.0	32.3 ^{b}	0.0	0.0	42.3 ^{b}
Lemon balm—shaded	14.6 ^{d}	0.0	12.3 ^{d}	0.0 ^{e}	0.0	20.6 ^{c}	0.0	0.0	42.0 ^{b}

Values followed by different letters are significantly different at $p < 0.05$.

TEO exhibits the most expressed inhibition in *B. subtilis* (62–64 mm). TEO, unlike the other two plant essential oils, manifest a significant effect against *S. aureus*, *E. coli*, and a somewhat weaker effect on the growth of *P. vulgaris*. MEO exhibits slightly stronger antibacterial effects compared to thyme, but it is still significantly successful against *B. subtilis*, *S. aureus*, and *E. coli* (Table 9).

The EOs extracted from all three plant species has no effect on *P. aeruginosa*, *B. cereus*, and *L. monocytogenes*. The plants' species has a greater influence on the zone of inhibition, while shading has a weaker effect.

Different essential oils inhibited the growth of *E. coli*, *P. vulgaris*, *S. aureus*, *B. subtilis*, and *C. albicans* (Table 9). Shading made a difference in the essential oil activity in the case of *S. aureus*, where the essential oils from shaded plants reduced the zone of inhibition. Mint seems to be more sensitive to shading since the essential oils from shaded mint plants had a significantly different reaction in the case of *P. vulgaris*. To better explain the effect of essential oils on microorganisms, a principal component analysis (PCA) was performed (Figure 8).

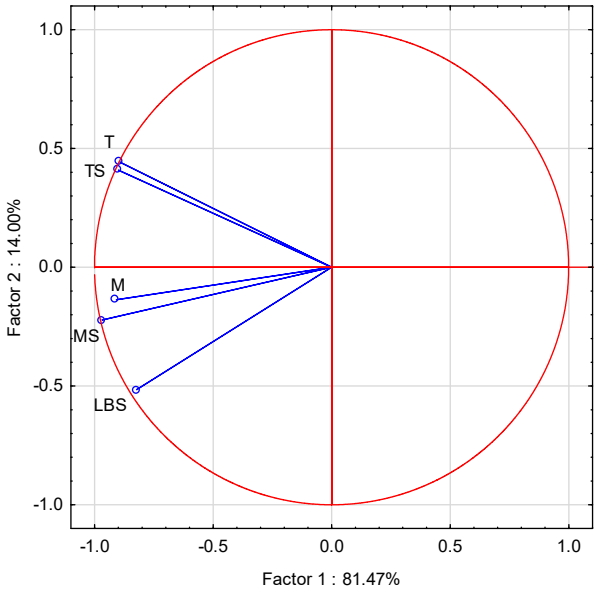


Figure 8. Bi-plot of principal component analysis of antimicrobial potential of essential oils from selected medicinal plants: thyme—shaded—TS, thyme—non-shaded—T; mint—non-shaded—M, mint—shaded—MS; lemon balm—shaded—LBS.

In Figure 8, We observe that essential oils are not separated by major factor one, which shows that the essential oils have a similar reducing effect on microorganisms. However, the separation of essential oils by factor two shows similar results as the ANOVA, by clearly separating the essential oils by their plant species, and to a lesser extent by shading.

On the second factor, the effect of the essential oils on microorganisms is presented in Figure 9. A separation by major factor one (81.47%) is seen between *B. subtilis* and *C. albicans* and on the right side of *S. aureus*, *E. coli*, and *P. vulgaris.* The separation of the microorganisms on factor one is probably due to shading while a minor separation was observed, on factor two, based on a separation by the origin of the essential oils, i.e., in the case of a *C. albicans* and *P. vulgaris* overlap between mint and lemon balm.

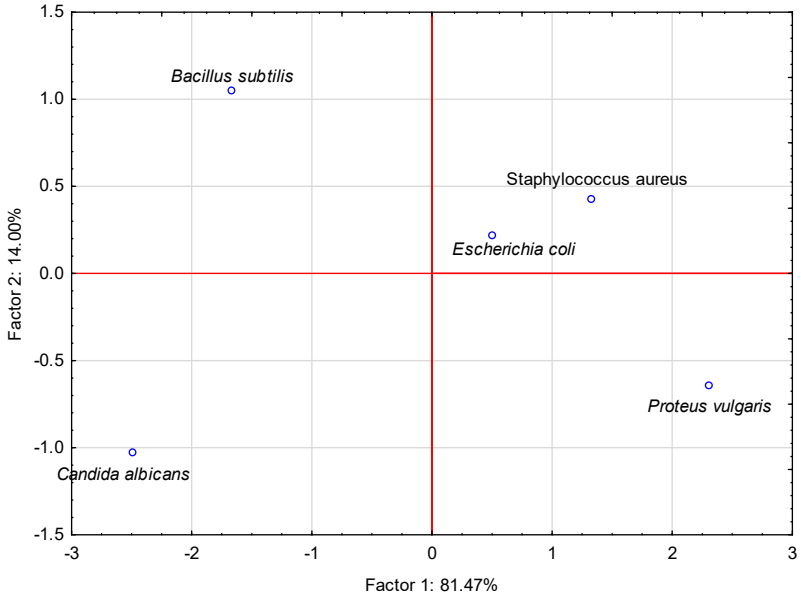


Figure 9. Bi-plot of principal component analysis of antimicrobial potential of essential oils *E coli*; *P vulgaris*; *B. subtilis*; *S. aureus*; and *C. albicans*.

A very effective replacement for the nitrates in meat products can be TEOs with very strong antilisterial effects [55]. TEO has been used to treat wound infections as an antibacterial agent in oral hygiene [56]. Due to the presence of menthol and menthone in MEO, it manifests a significant antimicrobial activity against *S. aureus*, *M. flavus*, *B. subtilis, S. enteritidis*, and *S epidermidis* [57]. MEO possesses a strong activity against *C. albicans* with inhibition zones ranging from 38.0 to 45.0 mm and, together with TEO, showed much larger inhibition zones (16.0–30.0 mm) in comparison to other oils and streptomycin (0–20.0 mm) [58].

Due to their antimicrobial properties against numerous food pathogens such as *S. typhimurium*, *C. perfringens*, *L. monocytogenes, P. putida*, and *S. aureus*, EOs are increasingly present in the food industry [59], as well as in meat products [60]. In order to promote the healthy growth of animals, the use of EOs in animal feed as a substitute for antibiotics is increasing [61].

Generally, EOs are more efficient against Gram-positive bacteria than Gram-negative bacteria, whose membranes contain protective lipopolysaccharides successfully in prohibit ing the diffusion of the lipophilic compounds [62]. The EOs from all medicinal plants in our studies expressed an antibacterial activity against *B. subtilis*, *L. monocytogenes*, *S. aureus*, *E. coli*, and *P. aeruginosa* and strong antifungal effects against *C. albicans*.

4. Conclusions

The effect of using shading nets in achieving optimal conditions for the production of more essential oils with a better antioxidant activity is necessary in growing seasons with higher temperatures and intense radiation. Among these three plant species, thyme is known as the species with the strongest antioxidant activity. Physiologically, the shading of plants can improve the antioxidant properties of *M. officinalis.* It is evident that the modification of the light intensity can act as a physiological tool via shade nets to improve the yield of essential oils and their constituents, phytochemical quality, and antioxidant activity. These plant species tolerate shading well, so it is recommended to grow them under shading nets. The EOs have an antimicrobial activity against a wide range of microorganisms. The TEOs are the most active in terms of their antimicrobial and antifungal activity. The use of EOs is very important because as they are natural substances and therefore easily biodegradable, they could be a promising alternative to replace synthetic materials to prevent microbial spoilage and a wide range of pathogenic microorganisms in food, pharmaceutical, and cosmetic industries. The practical applications of EOs are numerous, such as an antimicrobial agent in the food, the processing industry, as anti-sprouting agents in the storage of fresh products such as root vegetables and potato, but also as insecticide during plant growth.

Author Contributions: Conceptualization, D.C. and D.L.; methodology, L.Š. and R.K.; investigation, L.M., D.K. and A.M.; data curation, J.S. and A.M.; writing—original draft preparation.; writing—review and editing, Z.S.I. and L.S.; visualization, J.S. and B.D.; supervision, Z.S.I. All authors have read and agreed to the published version of the manuscript.

Funding: This research received external funding from a program for financing scientific research work, with grant numbers 451-03-68/2022-14/200133 and 451-03-68/2022-14/200189 was financially supported by the Ministry of Education Science and Technological Development of the Republic of Serbia.

Institutional Review Board Statement: Not applicable.

Informed Consent Statement: Not applicable.

Conflicts of Interest: The authors declare no conflict of interest.

References

1. Tmušić, N.; Ilić, Z.S.; Milenković, L.; Šunić, L.; Lalević, D.; Kevrešan, Ž.; Mastilović, J.; Stanojević, L.; Cvetković, D. Shading of medical plants affects the phytochemical quality of herbal extracts. *Horticulturae* **2021**, *7*, 437. [CrossRef]
2. Stojanović, N.M.; Samardžić, L.; Ranđelović, P.J.; Radulović, N.S. Prevalence of self-medication practice with herbal products among nonpsychiatric patients from southearstern Serbia: A cross-secional study. *Saudi Pharm. J.* **2017**, *25*, 884–890. [CrossRef] [PubMed]

3. Ait Bouzid, A.; Oubannin, S.; Ibourki, M.; Bijla, L.; Hamdouch, A.; Sakar, E.H.; Harhar, H.; Majourhat, K.; Koubachi, J.; Gharby, S. Comparative evaluation of chemical composition, antioxidant capacity, and some contaminants in six Moroccan medicinal and aromatic plants. *Biocatal. Agric. Biotechnol.* **2023**, *47*, 102569. [CrossRef]
4. Galovičová, L.; Borotová, P.; Valková, V.; Vukovic, N.L.; Vukic, M.; Štefániková, J.; Ďúranová, H.; Kowalczewski, P.Ł.; Čmiková, N.; Kačániová, M. *Thymus vulgaris* essential oil and its biological activity. *Plants* **2021**, *19*, 1959. [CrossRef] [PubMed]
5. Efe Ertürk, N.; Ta¸scı, S. The Effects of peppermint oil on nausea, vomiting and retching in cancer patients undergoing chemotherapy: An open label quasi-randomized controlled pilot study. *Complement. Ther. Med.* **2021**, *56*, 102587. [CrossRef]
6. Petrisor, G.; Motelica, L.; Craciun, L.N.; Oprea, O.C.; Ficai, D.; Ficai, A. *Melissa officinalis*: Composition, pharmacological effects and derived release systems-A review. *Int. J. Mol. Sci.* **2022**, *23*, 3591. [CrossRef]
7. Ilić, S.Z.; Milenković, L.; Tmušić, N.; Stanojević, L.J.; Cvetković, D. Essential oils content, composition and antioxidant activity of lemon balm, mint and sweet basil from Serbia. *LWT Food Sci. Technol.* **2022**, *153*, 112210. [CrossRef]
8. Milenković, L.; Stanojević, J.; Cvetković, D.; Stanojević, L.; Lalević, D.; Šunić, L.; Fallik, E.; Ilić, S.Z. New technology in basil production with high essential oil yield and quality. *Ind. Crops Prod.* **2019**, *140*, 111718. [CrossRef]
9. Ilić, Z.; Stanojević, L.; Milenković, L.; Šunić, L.; Milenković, A.; Stanojević, J.; Cvetković, D. The yield, chemical composition, and antioxidant activities of essential oils from different plant parts of the wild and cultivated oregano (*Origanum vulgare* L.). *Horticulturae* **2022**, *8*, 1042. [CrossRef]
10. Milenković, L.; Ilić, S.Z.; Šunić, L.; Tmušić, N.; Stanojević, L.; Cvetković, D. Modification of light intensity influence essential oils content, composition and antioxidant activity of thyme, marjoram and oregano. *Saudi J. Biol. Sci.* **2021**, *28*, 6532–6543. [CrossRef]
11. Giannoulis, K.D.; Kamvoukou, C.A.; Gougoulias, N.; Wogiatzi, E. *Matricaria chamomilla* L. (*German chamomile*) flower yield and essential oil affected by irrigation and nitrogen fertilization. *Emir. J. Food Agric.* **2020**, *32*, 328–335. [CrossRef]
12. Ninou, E.; Cook, C.M.; Papathanasiou, F.; Aschonitis, V.; Avdikos, I.; Tsivelikas, A.L.; Stefanou, S.; Ralli, P.; Mylonas, I. Nitrogen effects on the essential oil and biomass production of field grown Greek oregano (*Origanum vulgare* subsp. *hirtum*) populations. *Agronomy* **2021**, *11*, 1722. [CrossRef]
13. Pino, J.A.; Rosado, A.; Fuentes, V. Composition of the Essential Oil of *Melissa officinalis* L. from Cuba. *J. Essen Oil Res.* **1999**, *11*, 363–364. [CrossRef]
14. Rodriguez-Garcia, I.; Silva-Espinoza, B.A.; Ortega-Ramirez, L.A.; Leyva, J.M.; Siddiqui, M.W.; Cruz-Valenzuela, M.R.; Gonzalez-454 Aguilar, G.A.; Ayala-Zavala, J.F. Oregano essential oil as an antimicrobial and antioxidant additive in food products. *Crit. Rev. Food Sci. Nutr.* **2016**, *56*, 1717–1727. [CrossRef] [PubMed]
15. Milenković, L.; Šunić, L.J.; Mastilović, J.; Kevrešan, Ž.; Kovač, R.; Cvetković, D.; Stanojević, L.; Danilović, B.; Stanojević, J.; Ilić, S.Z. Antimicrobial activity of essential oils from medical plants grown in light modified environment. In Proceedings of the Food, Nutrients and Nutrition of the Future: 31st Food Technology Days 2022 dedicated to Prof. F. Bitenc, Ljubljana, Slovenia, 15 June 2022; pp. 73–89.
16. Shanaida, M.; Hudz, N.; Białon, M.; Kryvtsowa, M.; Svydenko, L.; Filipska, A.; Wieczorek, P. Chromatographic profiles and antimicrobial activity of the essential oils obtained from some species and cultivars of the Mentheae tribe (Lamiaceae). *Saudi J. Biol. Sci.* **2021**, *28*, 6145–6152. [CrossRef]
17. Kosakowska, O.; Czupa, W. Morphological and chemical variability of common oregano (*Origanum vulgare* L. subsp. *vulgare*) occurring in eastern Poland. *Herba Polon.* **2013**, *64*, 11–21. [CrossRef]
18. Ilić, S.Z.; Milenković, L.; Šunić, L.; Tmušić, N.; Mastilović, J.; Kevrešan, Ž.; Stanojević, L.; Danilović, B.; Stanojević, J. Efficiency of basil essential oil antimicrobial agents under different shading treatments and harvest times. *Agronomy* **2021**, *11*, 1574. [CrossRef]
19. Helal, I.M.; El-Bessoumy, A.; Al-Bataineh, E.; Joseph, M.R.P.; Rajagopalan, P.; Chandramoorthy, H.C.; Ben Hadj Ahmed, S. Antimicrobial efficiency of essential oils from traditional medicinal plants of Asir Region, Saudi Arabia, over drug resistant isolates. *BioMed Res. Int.* **2019**, *2019*, 8928306. [CrossRef]
20. Sparkman, D.O.; Penton, Z.E.; Fulton, K.G. *Gas Chromatography and Mass Spectrometry: A Practical Guide*, 2nd ed.; Elsevier Inc.: Oxford, UK, 2011.
21. Stanojević, J.S.; Stanojević, L.P.; Cvetković, D.J.; Danilović, B.R. Chemical composition, antioxidant and antimicrobial activity of the turmeric essential oil (*Curcuma longa* L.). *Adv. Technol.* **2015**, *4*, 19–25. [CrossRef]
22. Stanojevic, L.J.; Marjanovic-Balaban, Z.; Kalaba, V.; Stanojevic, J.; Cvetkovic, D.; Cakic, M. Chemical composition, antioxidant and antimicrobial activity of basil (*Ocimum basilicum* L.) essential oil. *J. Essent. Oil Bear. Plants* **2017**, *20*, 1557–1569. [CrossRef]
23. Kiehlbauch, J.A.; Hannett, G.E.; Salfinger, M.; Archinal, W.; Monserrat, C.; Carlin, C. Use of the national committee for clinical laboratory standards guidelines for disk diffusion susceptibility testing in New York State Laboratories. *J. Clin. Microb.* **2000**, *38*, 3341–3348. [CrossRef] [PubMed]
24. Hudaib, M.; Aburjai, T. Volatile components of *Thymus vulgaris* L. from wild-growing and cultivated plants in Jordan. *Flavour Fragr. J.* **2007**, *22*, 322–327. [CrossRef]
25. Choi, I.Y.; Song, Y.J.; Choi, D.C.; Lee, W.H. A comparative study for obtaining maximum essential oil from six herbs on the basis of harvesting time, cultivation regions & type, and drying methods. *Korean J. Hortic. Sci. Technol.* **2010**, *28*, 492–496.
26. Zani, F.; Massimo, G.; Benvenuti, S.; Bianchi, A.; Albasini, A.; Melegari, M.; Vampa, G.; Bellotti, A.; Mazza, P. Studies on the genotoxic properties of essential oils with *Bacillus subtilis* recassay and salmonella/microsome reversion assay. *Planta Med.* **1991**, *57*, 237–241. [CrossRef] [PubMed]

27. Cioni, P.L.; Tomei, P.E.; Catalano, S.; Morelli, I. Study of variation in individual essential oils in a micropopulation of *Thymus vulgaris* L. plants. *Riv. Ital.* **1990**, *1*, 3–6.
28. Najar, B.; Pistelli, L.; Ferri, B.; Angelini, L.G.; Tavarini, S. Crop yield and essential oil composition of two *Thymus vulgaris* chemotypes along three years of organic cultivation in a hilly area of central Italy. *Molecules.* **2021**, *23*, 5109. [CrossRef]
29. Andolfi, L.; Flamini, G.; Ceccarini, L.; Cioni, P.L.; Macchia, M.; Morelli, I. Indagine agronomica su un ecotipo di *Thymus vulgaris* L. e composizione dell'olio essenziale. *Agric. Ric.* **2000**, *186*, 3–10.
30. Ozguven, M.; Tansi, S. Drug yield and essential oil of *Thymus vulgaris* L. as in influenced by ecological and onto genetical variation. *Turk. J. Agric. For.* **1998**, *22*, 537–542.
31. Carlen, C.; Schaller, M.; Carron, C.A.; Vouillamoz, J.F.; Baroffio, C.A. The new *Thymus vulgaris* L. hybrid cultivar (Varico 3) compared to five established cultivars from Germany, France and Switzerland. *Acta Hort.* **2018**, *60*, 161–166. [CrossRef]
32. Borugă, O.; Jianu, C.; Mişcă, C.; Goleţ, I.; Gruia, A.T.; Horhat, F.G. Thymus vulgaris essential oil: Chemical composition and antimicrobial activity. *J. Med. Life* **2014**, *7*, 56–60.
33. Iscan, G.; KIrimer, N.; Kürkcüoglu, M.; Baser, H.C.; Demirci, F. Antimicrobial screening of *Mentha piperita* essential oils. *J. Agric. Food Chem.* **2002**, *50*, 3943–3946. [CrossRef] [PubMed]
34. Németh-Zámboriné, É.; Seidler-Łożykowska, K.; Szabó, K. Effect of harvest date on yield and secondary compounds of lemon balm (*Melissa officinalis* L.). *J. Appl. Bot. Food Qual.* **2019**, *92*, 81–87.
35. Patora, J.; Majda, T.; Gora, J.; Klimek, B. Variability in the content and composition of essential oil from lemon balm (*Melissa officinalis* L.) cultivated in Poland. *J. Endocrinol. Invest.* **2003**, *26*, 950–955.
36. Oliveira, G.C.; Vieira, W.L.; Bertolli, S.C.; Pacheco, A.C. Photosynthetic behavior, growth and essential oil production of *Melissa officinalis* L. cultivated under colored shade nets. *Chil. J. Agric. Res.* **2016**, *76*, 123. [CrossRef]
37. Li, Y.; Craker, L.E.; Potter, T. Effect of light level on the essential oil production of sage (*Salvia officinalis*) and thyme (*Thymus vulgaris*). *Acta Hortic.* **1996**, *426*, 419–426. [CrossRef]
38. Kim, M.; Sowndhararajan, K.; Kim, S. The chemical composition and biological activities of essential oil from Korean native thyme Bak-Ri-Hyang (*Thymus quinquecostatus* Celak.). *Molecules* **2022**, *27*, 4251. [CrossRef]
39. Karousou, R.; Balta, M.; Hanlidou, E.; Kokkini, S. Mints, smells and traditional uses in Thessaloniki (Greece) and other Mediterranean countries. *J. Ethnopharmac.* **2007**, *109*, 248–257. [CrossRef]
40. Mounira, M.; Mohamed, B.; Najeh, B.F. Variability of volatiles in Tunisian *Mentha pulegium* L. (Lamiaceae). *J. Essent. Oil Res.* **2007**, *19*, 211–214.
41. Telci, I.; Demirtas, I.; Bayram, E.; Arabaci, O.; Kacar, O. Environmental variation on aroma components of pulegone/piperitone rich spearmint (*Mentha spicata* L.). *Ind. Crops Prod.* **2010**, *3*, 588–592. [CrossRef]
42. Lu, C.G.; Liang, C.Y.; Li, W.L. Analysis on chemical components of volatile oil from *Mentha piperita* L. *J. Anhui Agric. Sci.* **2008**, *36*, 400–425.
43. Yadegarinia, D.; Gachkar, L.; Rezaei, M.B.; Taghizadeh, M.; Astaneh, S.A.; Rasooli, I. Biochemical activities of Iranian *Mentha piperita* L. and *Myrtus communis* L. essential oils. *Phytochemistry* **2006**, *67*, 1249–1255. [CrossRef] [PubMed]
44. Tyagi, A.K.; Malik, A. Antimicrobial potential and chemical composition of *Mentha piperita* oil in liquid and vapour phase against food spoilage microorganisms. *Food Control* **2011**, *22*, 1707–1714. [CrossRef]
45. Eddaya, T. Gestion Intégrée des Ravageurs de la Menthe Verte (*Mentha spicata* L. ou Huds) au Centre-Sud du Maroc. Ph.D. Thesis, l'université de Moulay Ismail, Meknes, Morocco, 2015; 285p.
46. Soilhi, Z.; Rhimi, A.; Heuskin, S.; Fauconnier, M.L.; Mekki, M. Essential oil chemical diversity of Tunisian Mentha spp. collection. *Ind. Crops Prod.* **2019**, *131*, 330–340. [CrossRef]
47. Alizadeh Behbahani, B.; Shahidi, F. *Melissa officinalis* essential oil: Chemical compositions, antioxidant potential, total phenolic content and antimicrobial activity. *Nutr. Food Sci. Res.* **2019**, *6*, 17–25. [CrossRef]
48. Shakeri, A.; Sahebkar, A.; Javadi, B. *Melissa officinalis* L.—A review of its traditional uses, phytochemistry and pharmacology. *J. Ethnopharm.* **2016**, *188*, 204–228. [CrossRef]
49. Sharopov, F.S.; Wink, M.; Khalifaev, D.R.; Zhang, H.; Dosoky, N.S.; Setzer, W.N. Composition and bioactivity of the essential oil of *Melissa officinalis* L. growing wild in Tajikistan. *Int. J. Tradit. Nat. Med.* **2013**, *2*, 86–96.
50. Moradkhani, H.; Sargsyan, E.; Bibak, H.; Naseri, B.; Fayazi-Barjin, A.; Meftahizade, H. *Melissa officinalis* L., a valuable medicine plant: A review. *J. Med. Plants Res.* **2010**, *4*, 2753–2759.
51. Verma, R.S.; Padalia, R.C.; Chauhan, A. Evaluation of essential oil quality of lemon balm (*Melissa officinalis* L.) grown in two locations of northern India. *J. Essent. Oil Res.* **2015**, *27*, 412–416. [CrossRef]
52. León-Fernández, M.; Sánchez-Govín, E.; Quijano-Celis, C.E.; Pino Jorge, A. Effect of planting practice and harvest time in oil content and its composition in *Melissa officinalis* L. cultivated in Cuba. *J. Essent. Oil Bear. Plants* **2008**, *11*, 62–68. [CrossRef]
53. Mohamadpoor, H.; Pirbalouti, A.G.; Malekpoor, F.; Hamedi, B. Chemical composition and bioactivity of the essential oil of *Melissa officinalis* L., cultivated in southwestern Iran. *J. Herb. Drug.* **2018**, *8*, 213–218. [CrossRef]
54. Basta, A.; Tzakou, O.; Couladis, M. Composition of the leaves essential oil of *Melissa officinalis* L. from Greece. *Flavour Fragr. J.* **2005**, *20*, 642–644. [CrossRef]
55. Salvaneschi, S.; Iriti, M.; Vitalini, S.; Vallone, L. *Thymus vulgaris* L. as a possible effective substitute for nitrates in meat products. *Ital. J. Food Saf.* **2020**, *9*, 7739. [CrossRef] [PubMed]

56. Nikolić, M.; Jovanović, K.K.; Marković, T.; Marković, D.; Gligorijević, N.; Radulović, S.; Soković, M. Chemical composition, antimicrobial, and cytotoxic properties of five Lamiaceae essential oils. *Ind. Crops Prod.* **2014**, *61*, 225–232. [CrossRef]
57. Kot, B.; Wierzchowska, K.; Piechota, M.; Czerniewicz, P.; Chrzanowski, G. Antimicrobial activity of five essential oils from lamiaceae against multidrug-resistant *Staphylococcus aureus*. *Nat. Prod. Res.* **2019**, *33*, 3587–3591. [CrossRef] [PubMed]
58. Soković, M.D.; Vukojević, J.; Marin, P.D.; Brkić, D.D.; Vajs, V.; Van Griensven, L.J.L.D. Chemical composition of essential oils of Thymus and Mentha species and their antifungal activities. *Molecules* **2009**, *14*, 238–249. [CrossRef] [PubMed]
59. Burt, S. Essential oils: Their antibacterial properties and potential applications in foods—A review. *Int. J. Food Microbiol.* **2004**, *94*, 223–253. [CrossRef]
60. Oussalah, M.; Caillet, S.; Saucier, L.; Lacroix, M. Inhibitory effects of selected plant essential oils on the growth of four pathogenic bacteria: *E. coli* O157: H7, *Salmonella typhimurium*, *Staphylococcus aureus* and *Listeria monocytogenes*. *Food Control* **2007**, *18*, 414–420. [CrossRef]
61. Castanon, J.I. History of the use of antibiotic as growth promoters in European poultry feeds. *Poult Sci.* **2007**, *86*, 2466–2471. [CrossRef]
62. El Abed, N.; Kaabi, B.; Smaali, M.I.; Chabbouh, M.; Habibi, K.; Mejri, M.; Mazouki, M.N.; Ahmed, S.B.H. Chemical composition and atibacterial activities of *Thymus capitata* essential oil with its preservative effects against *Listeria monocytogenesis* inoculated in minced beef meat. *Evid.-Based Complement. Altern. Med.* **2014**, *2014*, 152487. [CrossRef]