

## Article

# Sustainable Antioxidant Production for Hygienic Disinfection Using Bioextractants from Lavender and Oregano Distillation Process

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**Abstract:** In the current study, the production of novel antioxidants for hygienic disinfection against common pathogenic bacteria, based on the incorporation of bioextractant oils/waters from either lavender or oregano distillates is proposed in the framework of circular economy. For the first time, the main compounds found in distillation products (oils/waters), specifically of lavender *Lavandula angustifolia* (linalyl acetate and linalool) and of oregano *Oreganum vulgare* (carvacrol, thymol, and p-cymene) are presented. The analyses of both the lavender and oregano essential oils/waters indicate excellent physicochemical properties and microbial absence. Moreover, the antioxidant activity of all distillates as DPPH radical scavengers is assessed. The results confirm that the essential oils of both oregano and lavender possess superior antioxidant activity to their corresponding waters, while the oregano oil exhibited far better antioxidant activity than the lavender oil, as 1 mL of oregano oil was able to consume 45 μmoles of DPPH radicals. Overall, our research findings suggest that the particular lavender and oregano bioextractants produced possess important potential to address the resistance of bacteria from the perspective of their wider exploitation in therapeutic or preventive medicine, thus contributing to enhancing public health.

**Keywords:** sustainable; antioxidants; bioextractants; *Lavandula angustifolia*; *Oreganum vulgare*; distillation process; hygienic disinfection; circular economy



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## 1. Introduction

Lavender is one of the most famous among the medicinal and aromatic plants [1]. It belongs to the *Lamiaceae* family; the most well-known species are *Lavandula angustifolia*, *Lavandula latifolia*, *Lavandula intermedia*, *Lavandula dentata*, and *Lavandula stoechas*, but only the first three of them are commonly used in distillation for essential oil production. The most valuable essential oil comes from *L. angustifolia* (LA) Miller, which is also known as true lavender or English lavender. The name “lavender” comes from the Latin word “lavare”, meaning “to wash”, since in ancient Arabia, Greece, and Rome, it was mainly used in washing [2,3].

Lavender *Lavandula angustifolia* Miller (*L. angustifolia*) is indigenous to the Mediterranean area and has been cultivated for thousands of years in Greece, Bulgaria, and France, with the latter two countries being the biggest European lavender cultivators [4,5]. The

cultivation of lavender and the extraction of its essential oil is a growing enterprise in Mediterranean countries [2]. The Mediterranean area offers a suitable phytosociological environment for lavender to grow and obtain its characteristics and increased concentration of secondary metabolites, due to which, the medicinal properties and the multiple benefits of lavender to human health have been recognized even from the ancient times [6]. It is regarded that the medicinal attributes of lavender and its products to human health originated from its physicochemical composition, particularly from the large amount of volatile aromatic compounds and non-volatile phenolic compounds [4]. Lavender oil can be used topically or orally, or it can be inhaled to cure or mitigate edema or pain caused by a variety of medical conditions because of its apparent disinfecting, carminative, cicatrizing, calming, and sedative effects [3]. In addition, lavender also contains high quantities of phenolic acids and flavonoids, which are known for their anti-inflammatory, antiproliferative, and antimicrobial properties [7,8], as well as their sedative and anxiolytic activities known to treat stress and depression [9]. Nowadays, lavender oil is often used by different industries in scenting cosmetic products, in perfume production, and in food flavoring [10].

Several studies have also reported on the effects of lavender oil as a means to mitigate oxidative stress in humans. Free radicals that are considered to be the main responsible oxidative stress factors in humans can be combated by various antioxidants [11]. Practically, the role of antioxidants is the capacity to prevent or delay cellular damage caused by the effect of free radicals [12]. They can act either by interfering with the oxidative process by reacting themselves with free radicals, or by acting as oxygen scavengers [13].

Indeed, the strong free radical scavenging ability of lavender oil was previously reported by Bouyahya et al. [14], while other studies have reported on its antioxidant properties, as lavender oil showed protective properties against mutagen-induced DNA damage by increasing the levels of enzymatic and non-enzymatic antioxidants in the human hepatoma cell line HepG2 in vitro and in rat hepatocytes ex vivo [9]. Moreover, certain compounds, such as linalool, linalyl acetate, eucalyptol, and camphor, all found in lavender essential oil, are reported to show anticancer and anti-mutagenic properties [15], through bacterial reverse mutation assay [16], or via cytotoxic actions [10,17,18].

Two *Oreganum vulgare* subspecies are especially valued from an economic viewpoint, *Origanum vulgare* L. subsp. *vulgare* and *Origanum vulgare* ssp. *hirtum*. The first one (common oregano) occurs in the Northern and Central Europe area, while the second one (*Origanum vulgare* ssp. *hirtum*), native to Greece, is known as Greek oregano worldwide, and is especially important due to the presence of pure carvacrol (up to 80% of the essential oil is carvacrol) [19]. Oregano may adapt and develop in various soils and climates, in rich and poor soils, from coastal to mountainous areas, from islands to mainland Greece. Oregano can be grown in arid areas and withstand drought. However, in prolonged drought, especially during spring, it should be watered once or twice in order to enhance its efficiency along with its quality. The dry form of oregano (leaves, flowers) is mainly used in cooking, and in small quantities in the production of essential oil and water. Moreover the essential oil is used as a natural antibiotic in feed for pigs, poultry, and lamb. Furthermore, it possesses strong antioxidant, antibacterial, and antiseptic properties but also diuretic, expectorant, stimulative, carminative, antispasmodic, and anticancer activities [20]. The antioxidant and antimicrobial activity of oregano essential oil is known to be related to the high quantities of carvacrol and/or thymol, followed by rosmarinic acid and its derivatives within the non-volatile fraction [21,22]. The chemical composition of the genus *Origanum* (Lamiaceae) is dominated by phenolic compounds in both the volatile and non-volatile fractions [23].

The chemical compositions of oregano essential oils may vary among several populations [24,25] and may be affected by various factors, such as the geographical environment and harvest period, thus affecting their antibacterial properties [26,27]. In the meantime, it has been proposed that the medicinal properties and the quality of lavender oil vary among the different species and areas of cultivation and are likely to depend on their chemical compositions [28]. The prefecture of Western Macedonia is one of the main areas

for lavender and oregano cultivation in Greece, but there are no studies available reporting on the quality characteristics of their essential oils in Greece.

Thus, in this research, we provide for the first time the characteristics and the chemical composition of the lavender *L. angustifolia* and the oregano *Origanum vulgare* ssp. *hirtum*, which are widely cultivated in the prefecture of Western Macedonia in Greece. A progression of information is introduced, including the specific kinds of development, the decision for developing boundaries (for example, a relative land examination of the area proposed for the specific harvest), as well as the refining system of lavender/oregano oil and lavender/oregano water. Regarding the essential oils, an examination of their synthetic structure and aromatic substances is introduced, while for the lavender and oregano water, an investigation of the normal compounds and microbial attributes is performed. Moreover, considering that a key mechanism through which antioxidants neutralize radicals is hydrogen atom transfer (HAT), where a proton ( $H^+$ ) and an electron ( $e^-$ ) are simultaneously transferred as a pair ( $H^+/e^-$ ), herein, we apply the DPPH method, which is reliable in both natural antioxidant and hybrid antioxidant materials [29], to assess the HAT antioxidant activity of the lavender and oregano extractants in oil and water. In addition, to explore the applicability and potential commercial use of lavender and/or oregano oil and water, we evaluate and present, for the first time in the literature to the best of our best knowledge, the effectiveness both of lavender oil- and oregano oil-based antiseptics against common bacteria that can present illnesses to humans. The resistance of bacteria against antiseptics has been observed by the scientific community in human health facilities, in animal production facilities and in food production plants. In accordance with the increased resistance to antibiotics, the resistance to antiseptics is recognized as a severe threat to public health and to food production [30]. Therefore, there is constant demand for the sustainable production of substances that could be used as antiseptics, and oregano and lavender distillation bioextractants could be useful from that perspective, for ensuring public health. Since the literature is scarce with references to lavender oil and oregano oil in hand sanitizer applications, the present study aims to investigate the potential of the particular lavender and oregano bioextractants in hand sanitizers to address their resistance to bacteria and potential as alternatives to commonly used alcohol-based hand sanitizers.

## 2. Materials and Methods

### 2.1. Distillation Process for the Production of Lavender and Oregano Bioextractant Oils and Chemical Analysis

A large cauldron was filled with 1000 kg of fresh lavenders and oregano, and water was then added to the top (Figure 1).



**Figure 1.** Presentation of the distillation process of lavender and/or oregano for the production of lavender and/or oregano oil, respectively.

The mixture was brought to a boil for around sixty minutes. After the boiling process was complete, the steam was moved from the cauldron to the refrigerator, where it was liquefied using a special conduit. Consequently, floral water, the distillation product, was obtained. This distillation mixture was transferred to a large container from the first Floridian container, where the oil and flower water were separated. The process of a second boiling step started in order to finish the distillation process once the large container was filled (this occurred after the aforementioned process occurred twice). At this point, the distillation traveled in a circle, going from the large container to an alternator and then back to the cauldron to boil it once more. After being moved to a second refrigerator, the steam was liquefied. It was separated from the remaining oil there, after passing through a second Floridian container. When the entire distillate from the large container was used up—roughly 45 min—the distillation process was declared finished. The lavender oil or oregano oil was extracted from the two Floridian containers once the distillation process was complete. After going through a sanitized filter to prevent any microbiological contamination, it was transferred into glass vials that were kept in a cold, shaded environment. Isolated from the aforementioned procedure were 12–22 kg of essential oil. The essential oil had a pale golden hue, was immaculately clean, and had a perfectly lovely, lingering scent. A sample of around 10 mL was diluted in 10% dichloromethane *w/v* and examined in a gas chromatograph–mass spectrograph (GC-MS) to determine its chemical composition and the presence of aromatic compounds [31], with the use of the Agilent 7890A Gas Chromatograph/5975C Mass Selective Detector System (Agilent 210GC-MS) (carrier gas: He, flow 0.8 mL/min, mode: split, split ratio: 100:1), a heater (240 °C, column: HP-5MS 5% phenyl methyl siloxane, max temperature: 325 °C, 30 m × 250 μm × 0.25 μm). The oven program was as follows: 40 °C for 20 min, then 4 °C/min to 240 °C for 20 min, followed by a Thermal Aux 2{MSD Transfer Line} at 280 °C.

### 2.2. Distillation for the Production of Lavender and Oregano Waters and Chemical Analysis

For the refining for the lavender water, 100 kg of lavender or oregano were inserted in the cauldron, and the required amount of water to fill the cauldron was added. The interaction was equivalent to the essential oil refining, with the distinction that the flower water was delivered following 4 h, and an amount of around 100 lt of lavender/oregano water was accumulated. An investigation to determine the compound attributes and microbial charge was performed by utilizing 300 mL of the last lavender/oregano water. The initial 100 mL of distillate was dissected for the presence of its microbial substance. A second 100 mL test was carried out to determine its physicochemical qualities. Finally, an examination of the lavender/oregano water was performed to identify the liquefying point and any remaining parts of the distillate.

### 2.3. Evaluation of the Antioxidant Radical Scavenging Capacity (RSC)

The antioxidant capacity of the oregano and lavender distillation products was determined using the DPPH method [32–34]. The UV-Vis spectra were recorded on a UV-Vis Hitachi U-2900 spectrophotometer equipped with a Unisoku cryostat. The cryostat was inserted inside the UV-Vis spectrophotometer beam chamber and used to keep the temperature constant at  $25 \pm 0.1$  °C. The chemicals used were methanol (purity > 99.8%) from Merck (Darmstadt, Germany), and 2,2-Diphenyl-1-picrylhydrazyl (DPPH) obtained from Sigma-Aldrich.

To carry out the kinetics experiments, a 30 μM DPPH solution was put in a quartz cell, 1 × 1 × 3 cm, (Hellma Suprasil quartz glass, 100-OS), under stirring. To enable the quantification of the concentration of DPPH radicals, the intensity of the peak at 515 nm was measured [32]. The absorption of 30 μM of DPPH solution in MeOH is  $I_{515} = 0.32$  [29]. Three (3) repetitions were conducted for the DPPH evaluation.

To study the antioxidant activity of the four aromatic liquids, different amounts of them were added into the cuvette, which contained a 30 μM solution of DPPH in MeOH; in any case, the final volume in cuvette was 3 mL. For the study of the oregano oil, 100 μL of

the pristine oil was dissolved in 100 mL of methanol. From this solution, 3 different amounts (250  $\mu$ L, 500  $\mu$ L, and 1000  $\mu$ L) were evaluated. Regarding the study of the antioxidant effect of oregano water, 3 different amounts (250  $\mu$ L, 500  $\mu$ L, and 1000  $\mu$ L) were directly studied. Accordingly, for the lavender essential oil, 300  $\mu$ L was dissolved in 15 mL of methanol. From this solution, the amounts of 250  $\mu$ L, 500  $\mu$ L, and 1000  $\mu$ L were studied. Finally, 2.5 mL of the lavender water was dissolved in 2.5 mL of methanol; from this solution, the amounts of 250  $\mu$ L, 500  $\mu$ L, and 1000  $\mu$ L were evaluated.

#### 2.4. Production of Disinfectants

To investigate the potential use of essential oils for disinfectant production, lavender oil-based and three oregano oil-based disinfectants have been produced for the purposes of the current study, each of which was formulated with three different concentrations of ethanol as per 80% *v/v*, 75% *v/v*, and 70% *v/v*. Disinfectants were prepared according to ISO 22716:2007 (LGC easi-tab™ Reference Materials, Beijing, China, 2022) Guidelines on Good Manufacturing Practices (GMPs) and were formulated with the inclusion of 0.5% *v/v* of either lavender oil or oregano oil, respectively, in addition to glycerol (3.5% *v/v*) and H<sub>2</sub>O<sub>2</sub> (3.0% *v/v*). Lavender water or oregano water was also included, as per QSD. The efficacy of all disinfectants was evaluated against *Pseudomonas aeruginosa* and *Staphylococcus aureus* based on a quantitative suspension test according to EU standards EN 1040 for disinfectant evaluation [35]. Moreover, both essential oils (lavender and oregano) were tested separately against *Pseudomonas aeruginosa* and *Staphylococcus aureus*.

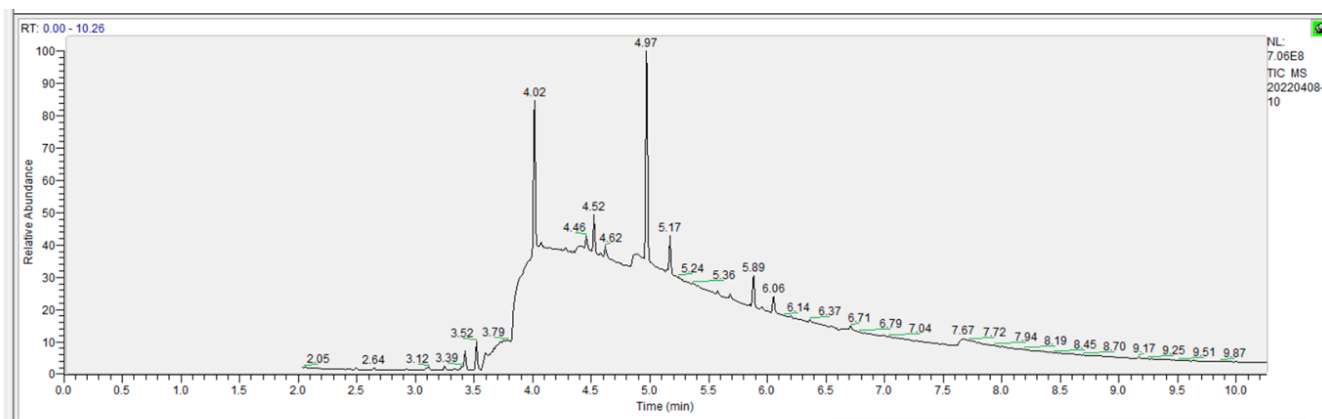
### 3. Results

#### 3.1. Lavender Oil Analysis

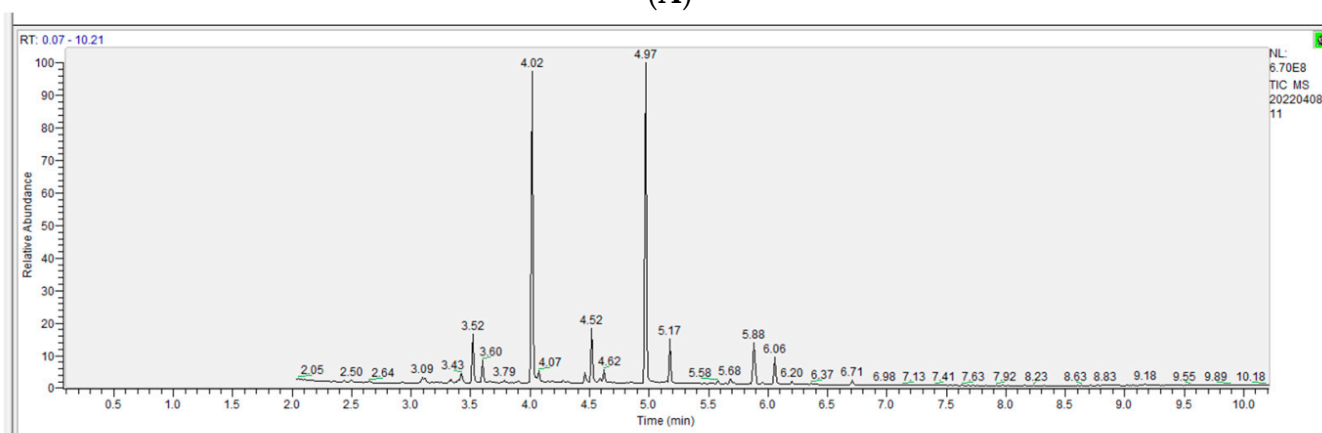
The results of the lavender oil composition analysis are presented in Table 1 and include the spectrums of all detectable compounds obtained by the GC-MS analysis (Figure 2). The analysis of the two samples showed that the lavender essential oil consisted of 14 different compounds, with linalyl acetate as the main compound, with concentrations of 26.98 and 33.61%, followed by linalool (21.73 and 26.57%). The other compounds detected were lavandulyl acetate (4.04 and 5.46%), caryophyllene (3.79 and 5.24%), terpinen-4-ol (5.12 and 5.33%), cis and trans-ocimene (3.85 and 3.99%, and 1.80 and 2.53%, respectively), and 1. cineole (eucalyptol) (0.97 and 2.61%). Trace amounts of other chemical compounds were also identified (not presented).

**Table 1.** The major 14 compounds of lavender oil as analyzed with gas chromatograph–5975C Mass Selective Detector.

A/A	Parameter	Unit	Result Sample 1 (Retention Time: 0.00–10.26)	Result Sample 2 (Retention Time: 0.07–10.21)
1	$\alpha$ -Terpineol	% <i>w/w</i>	1.43	1.27
2	$\beta$ -Farnesene	% <i>w/w</i>	3.19	2.74
3	Borneol	% <i>w/w</i>	1.80	1.58
4	Camphor	% <i>w/w</i>	0.73	0.53
5	Caryophyllene	% <i>w/w</i>	5.24	3.79
6	Limonene	% <i>w/w</i>	0.43	0.22
7	1,8 Cineole (Eucalyptol)	% <i>w/w</i>	2.61	0.97
8	Octanone-3	% <i>w/w</i>	0.94	0.61
9	Cis- $\beta$ -Ocimene	% <i>w/w</i>	3.85	3.99
10	Trans- $\beta$ -Ocimene	% <i>w/w</i>	2.53	1.80
11	Linalool	% <i>w/w</i>	21.73	26.57
12	Terpinen-4-ol	% <i>w/w</i>	5.12	5.33
13	Linalyl acetate	% <i>w/w</i>	33.61	26.98
14	Lavandulyl acetate	% <i>w/w</i>	5.46	4.04



(A)



(B)

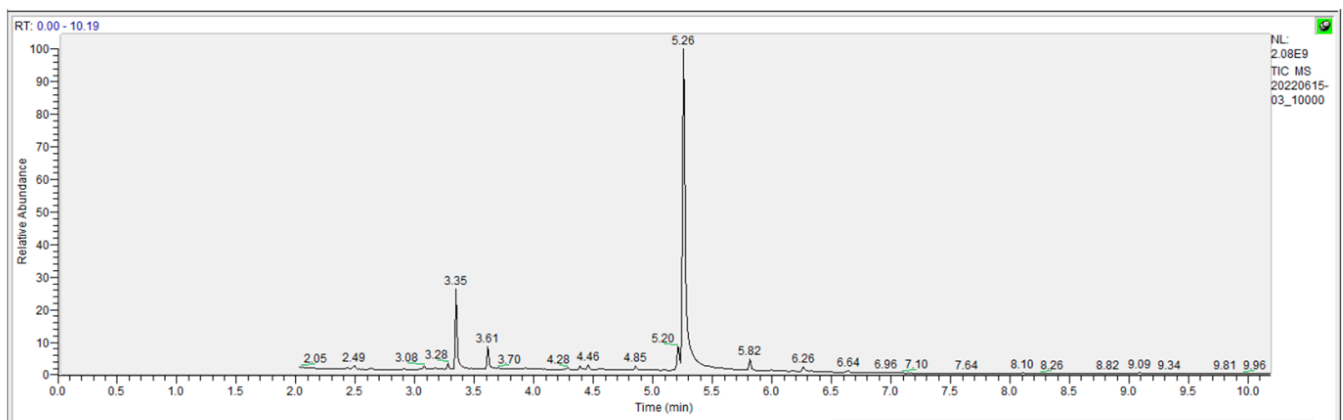
**Figure 2.** Agilent 7890A Gas Chromatograph/5975C Mass Selective Detector System analysis of two samples of lavender oil (products of lavender distillation of plant *Lavandula angustifolia*). (A) First sample. (B) Second sample. Each peak corresponds to an organic compound that was found in the sample and determines the constitution of the sample as an essence oil. It is assumed that the contributions of organic compounds in the final sample were different.

### 3.2. Oregano Oil Analysis

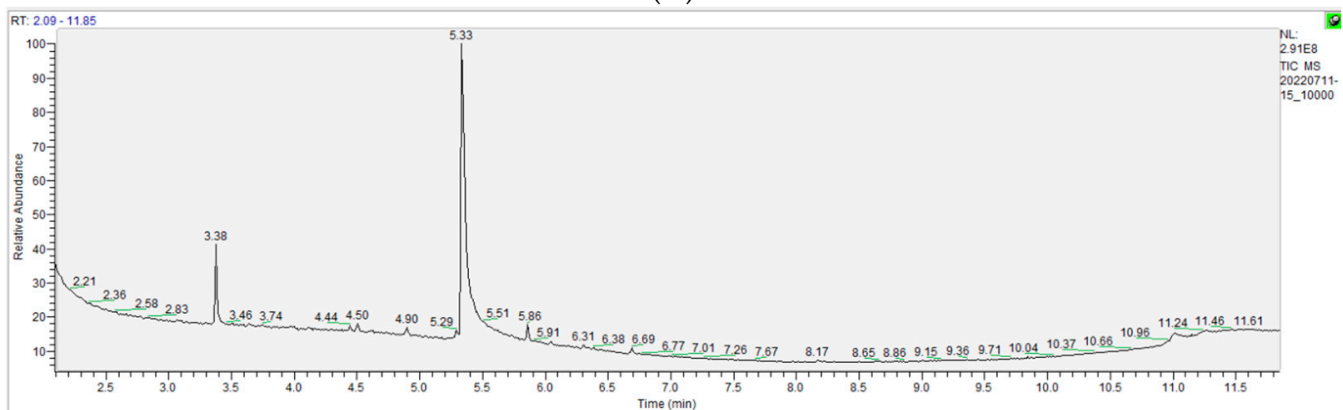
The results of the oregano oil composition analysis are presented in Table 2 and include the spectrums of all detectable compounds obtained by the GC-MS analysis (Figure 3). The analysis of the two samples showed that the oregano essential oil consisted of 11 different compounds, with carvacrol as the main compound, with concentrations of 65.97 and 78.56%, followed by p-cymene (9.6 and 11.29%), and thymol (3.36 and 1.24%). Other compounds detected were caryophyllene (1.33 and 1.69%), and  $\gamma$ -terpinene (2.9 and 0.87%). Trace amounts of other chemical compounds were also identified (not presented). Carvacrol and thymol are aromatic oxygenated monoterpenes, with a phenyl group, which is capable of donating hydrogen atoms or electrons and protons, and may be connected to the antioxidant activity of the essential oils of aromatic plants [36].

**Table 2.** The major 11 compounds of oregano oil as analyzed with Gas Chromatograph/5975C Mass Selective Detector.

A/A	Parameter	Unit	Result Sample 1 (Retention Time: 0.00–10.19)	Result Sample 2 (Retention Time: 2.09–11.85)
1	$\alpha$ -Thujene	% w/w	0.29	0.23
2	$\alpha$ - Pinene	% w/w	0.58	0.12
3	Myrcene	% w/w	0.63	0.88
4	$\alpha$ -Terpinene	% w/w	0.78	0.28
5	p-Cymene	% w/w	9.60	11.29
6	$\gamma$ -Terpinene	% w/w	2.90	0.87
7	Borneol	% w/w	0.47	0.39
8	Terpinen-4-ol	% w/w	0.49	0.80
9	Thymol	% w/w	3.36	1.24
10	Carvacrol	% w/w	65.97	78.56
11	Caryophyllene	% w/w	1.33	1.69



(A)



(B)

**Figure 3.** Agilent 7890A Gas Chromatograph/5975C Mass Selective Detector System analysis of two samples of oregano oil (products of oregano distillation of plant *Oreganum vulgare*). (A) First sample. (B) Second sample. Each peak corresponds to an organic compound that was found in the sample and determines the constitution of sample as essence oil. It is credited that the contribution of organic compounds in the final sample is different.

### 3.3. Lavender Water and Oregano Water Analysis

The results of the physicochemical attributes analyses of the two samples of 100 mL of lavender water are presented in Table 3, and those of the two samples of 100 mL of oregano water are shown in Table 4.

**Table 3.** Results of physicochemical attributes analysis of two samples of lavender water (products of lavender distillation of plant *Lavandula angustifolia*). (A) First sample. (B) Second sample.

A			
A/A	Parameter	Units	Result
1	Density at 20 °C	gr/mL	0.9971
2	PH at 20 °C	-	3.88
3	Conductivity (EC) at 20 °C	μS/cm	97.0
4	Refractive Index at 20 °C	-	1.3329
5	Flash Point (FP)	°C	>215.0
B			
A/A	Parameter	Units	Result
1	Density at 20 °C	gr/mL	0.9972
2	PH at 20 °C	-	3.85
3	Conductivity (EC) at 20 °C	μS/cm	88.2
4	Refractive Index at 20 °C	-	1.3327
5	Flash Point (FP)	°C	>215.0

**Table 4.** Results of physicochemical attributes of analysis of two samples of oregano water (products of oregano distillation of plant *Oreganum vulgare*). (A) First sample. (B) Second sample.

A			
A/A	Parameter	Units	Result
1	Density at 20 °C	gr/mL	0.9982
2	PH at 20 °C	-	3.5
3	Conductivity (EC) at 20 °C	μS/cm	214.1
4	Refractive Index at 20 °C	-	1.3329
5	Flash Point (FP)	°C	>215.0
B			
A/A	Parameter	Units	Result
1	Density at 20 °C	gr/mL	0.9981
2	PH at 20 °C	-	4.05
3	Conductivity (EC) at 20 °C	μS/cm	429.2
4	Refractive Index at 20 °C	-	1.3329
5	Flash Point (FP)	°C	>215.0

The results of the microbial content analysis for both lavender water samples are shown in Table 5, and those of both oregano water samples are shown in Table 6, indicating that there is no concern for these parameters. It should be stressed that the analyses were also repeated after a one-and-a-half-year period for the same sample, with precisely the same results.



**Table 5.** Results of lavender water microbial analysis.

A/A	Parameter	Units	Result	Analysis Method
1	<i>Escherichia coli</i>	cfu/gr	<10	[37]
2	Total Coliforms	cfu/gr	<10	[37]
3	<i>Pseudomonas aeruginosa</i>	cfu/gr	<10	[38]
4	<i>Staphylococcus aureus</i>	cfu/gr	<20	[39]
5	Total Mesophilic Flora	cfu/gr	<100	[40]
6	Yeasts	cfu/gr	<20	[41]
7	Fungi	cfu/gr	<20	[41]
8	<i>Candida albicans</i>	cfu/gr	Absence	[42]

**Table 6.** Results of oregano water microbial analysis.

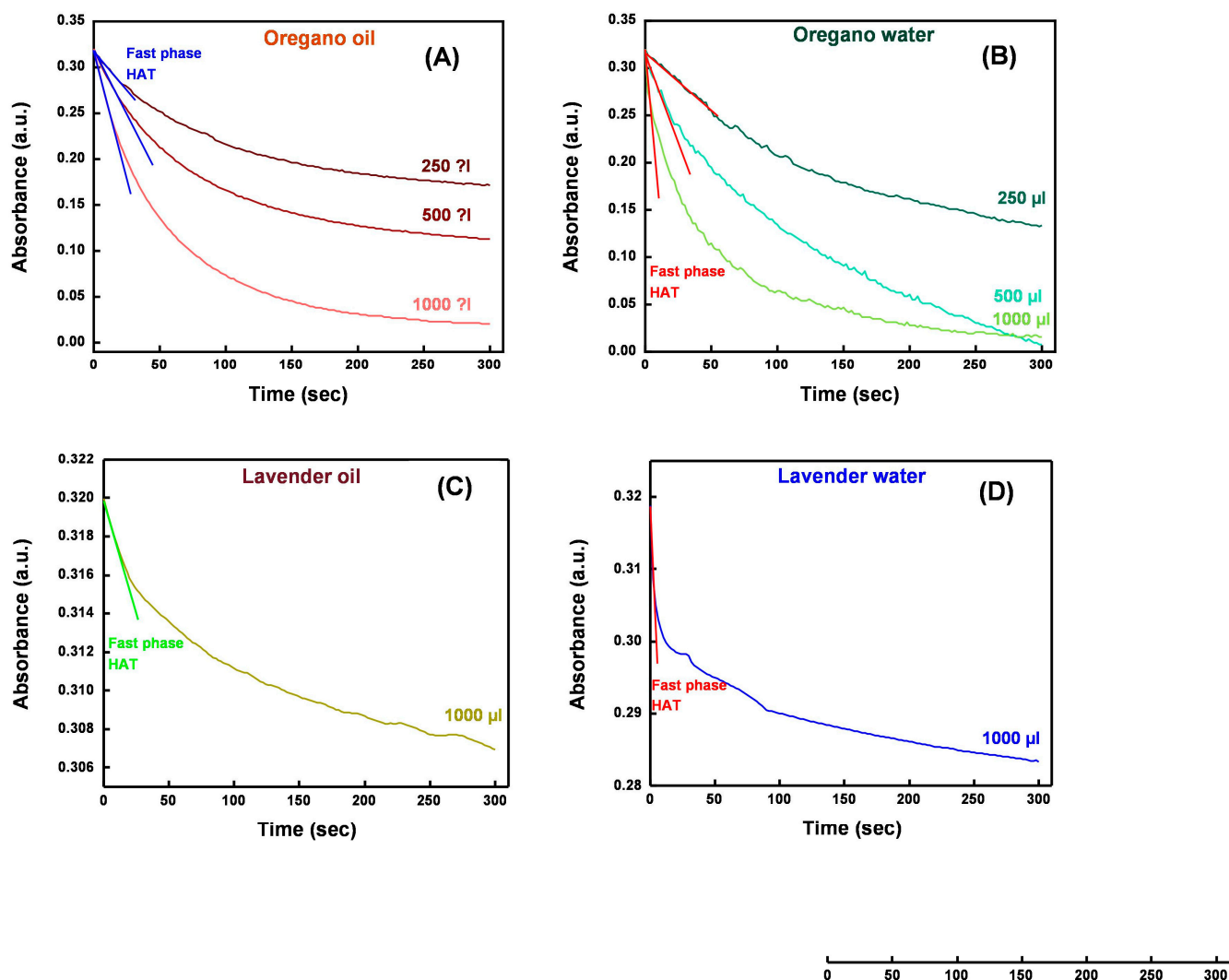
A/A	Parameter	Units	Result	Analysis Method
1	<i>Escherichia coli</i>	cfu/gr	<10	[37]
2	Total Coliforms	cfu/gr	<10	[37]
3	<i>Pseudomonas aeruginosa</i>	cfu/gr	<10	[38]
4	<i>Staphylococcus aureus</i>	cfu/gr	<20	[39]
5	Total Mesophilic Flora	cfu/gr	<100	[40]
6	Yeasts	cfu/gr	<20	[41]
7	Fungi	cfu/gr	<20	[41]
8	<i>Candida albicans</i>	cfu/gr	Absence	[42]

### 3.4. Evaluation of Antioxidant Activity

The DPPH in the MeOH solution had a characteristic purple color and a characteristic absorbance at 515 nm, which is equal to 0.32. With the addition of the antioxidant, the purple color of the solution began to fade and turn yellow, while the absorbance from 0.32 began to decrease. In the present work, the oregano and lavender extractants were evaluated as scavengers for the DPPH radical.

To carry out the kinetic study of the antioxidant activity of oregano oil, the original solution had to be diluted, and thus, 100  $\mu$ L of the pristine solution was taken and diluted in 100 mL of methanol. Figure 4A shows the antioxidant activity of three different volumes of the above-mentioned diluted oregano oil vs. the same amount of DPPH contained in 3 mL of a 30  $\mu$ M solution. As shown in Figure 4A, there was a clear antioxidant effect of the oregano oil, which is mass-dependent. Figure 4B shows the activity of the oregano water as an antioxidant against the DPPH radical by using a standard 30  $\mu$ M solution of DPPH. For this set of experiments, the oregano water was used as received since no further dilution was required. Also, a decay of the absorbance of DPPH was observed, indicating the antioxidant activity of the oregano water.

Consequently, Figure 4C,D shows the activity of the lavender oil and lavender water, respectively, as antioxidants. It is worth noting that from the pristine solution of lavender oil, 300  $\mu$ L was taken and diluted in 15 mL of methanol, while for the as-received lavender water, a 1:1 (*v/v*) solution with MeOH was prepared. Different amounts of the above-described diluted solutions were evaluated to check their antioxidant activity against a standard 30  $\mu$ M DPPH solution (the final volume was 3 mL). In Figure 4C,D, the antioxidant effects of 1000  $\mu$ L of the diluted lavender oil and lavender water distillates are shown, respectively.

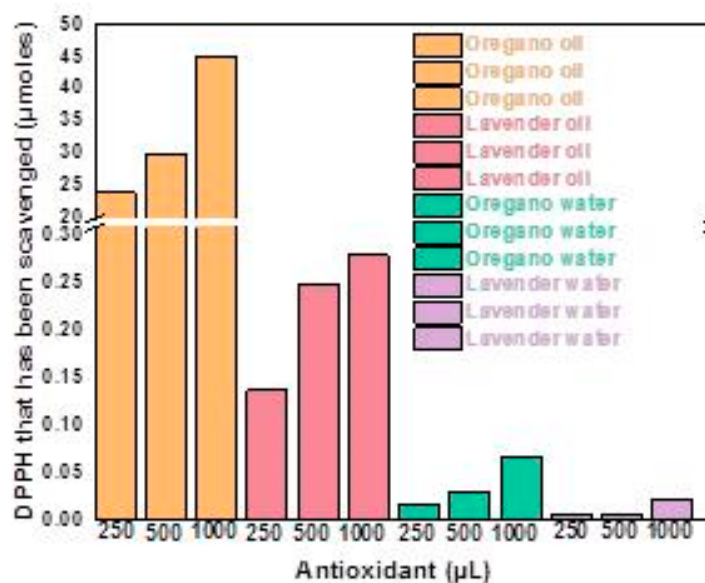


**Figure 4.** Representation of kinetics decay of the absorbance at 515 nm for DPPH radicals ( $[DPPH]_0 = 30.0 \pm 0.1 \mu M$ ) reacting with four different aromatic extractants: (A) oregano oil, (B) oregano water, (C) lavender oil, (D) lavender water.

The antioxidant activity of each aromatic distillation product against the DPPH radicals contained in 3 mL of a standard 30  $\mu M$  DPPH solution was calculated considering the achieved dilutions and expressed in  $\mu moles$  of DPPH scavenged by a given volume of each distillation product (see Figure 5).

### 3.5. Potential Use of Essential Oils for Disinfectant Production

The results of the analysis from the six different disinfectant formulas are depicted in Figures S1–S6 (Supplementary Materials). The evaluation of the essential oils (lavender and oregano) against *Pseudomonas aeruginosa* and *Staphylococcus aureus* are shown in Figures S7 and S8, respectively (Supplementary Materials).



**Figure 5.** Antioxidant activity of each aromatic extractant for 3 different volumes expressed in the  $\mu\text{moles}$  of DPPH consumed. (Orange color for oregano oil, red for lavender oil, green and purple for oregano and lavender water, respectively).

#### 4. Discussion

The results of the present work on lavender oil composition are in agreement with previous studies. Recently, Pokajewicz et al. [43] analyzed the Ukrainian *L. angustifolia*, and reported linalool and linalool acetate as the main components (ranging from 26.14 to 57.07% and from 9.08 to 24.45% respectively), followed by terpinen-4-ol (2.16–22.44%) and lavandulyl acetate (2.12–10.23%). However, Tarakemeh et al. [44] identified lower levels of linalool (6.8–19.2%) in Iranian *L. angustifolia* and reported 1,8-cineole as the major compound (29.0–38.0%) of the essential oil. In terms of linalool concentrations, similar results to our study have been obtained for another lavender species, i.e., *L. officinalis*, with linalool as the main component in Egyptian [15], Tunisian [45], Algerian [46], and Jordanian [47] essential lavender oil. Moreover, in Tunisian essential oil obtained from *L. latifolia* species, the linalool concentration was found to be 32.3% [48], while in Moroccan essential oil obtained from *L. dentate* species, linalool was detected in 45.06% [49].

According to Héral et al. [4], the previously mentioned volatile components that were isolated from plants during the distillation process in order to obtain the essential oils, are regarded as the main source of the pleasant aroma of lavender plants. The results of the present study are in compliance with previous studies on lavender essential oils, supporting the findings that show they contain mainly oxygenated monoterpenes, such as linalyl acetate and linalool, which constitute 4–57% and 1–54% of true lavender oil, respectively [50].

Indeed, our results show that linalyl acetate was determined at levels as high as (26.98 and 33.61%), followed by linalool (21.73 and 26.57%). According to Duskova et al. [51], the floral aromatic and pharmaceutical quality of the oils is mainly attributed to the high concentration of the oxygenated monoterpenes, and it is common that the concentration of these compounds does not fall below 20% of the total substances in the lavender essential oils. More oxygenated monoterpenes are usually detected, such as terpineols, including terpinen-4-ol (mainly in *L. angustifolia*) and  $\alpha$ -terpineol, with contents of 2–14% and 2–9%, respectively, and borneol. Eucalyptol (1,8-cineole) and camphor were also identified in the essential lavender oil in a study (0.97–2.61% and 0.53–0.73%), with the usual contents of 0.1–44% and traces of about 28%, respectively [51]. These high values of eucalyptol and camphor are usually found in essential oils from lavender leaves and stems or flowers from some unique special chemotypes [52], increasing antimicrobial action, while deteriorating

the oil quality in the perfumery industry [53,54]. Moreover, unique irregular oxygenated monoterpenes, such as lavandulyl acetate, provide a characteristic herbal rosy scent [36,51], a compound that was also found in the lavender oil in this study by GS-MS analysis. The other terpene groups found in our study, such as monoterpene hydrocarbons ( $\beta$ -ocimene) and sesquiterpene hydrocarbons ( $\beta$ -farnesene and caryophyllene), usually constitute a lesser fraction of lavender oils [55].

In accordance with our results on oregano oil, a previous study by Azizi et al. [56] showed that the predominant components of the essential oil from 42 oregano accessions were carvacrol (up to 66%), thymol (up to 66%),  $\gamma$ -terpinene (up to 11%), and p-cymene (up to 5.7%). D'Antuono et al. [57] analyzed native populations of *Origanum vulgare* L. from the Liguria and Emilia regions of northern Italy for their essential oil contents and composition, where they found sixty-four compounds. According to the essential oil composition, the samples were divided into three groups, where the first one had high contents of carvacrol and thymol, the second one had different sesquiterpenes and linalool, and the third one had abundant sesquiterpenes. Our results are in compliance with the first group, which included the seven Ligurian accessions from Finale, Savona, Recco, Sestri, Levanto, Monterosso, and Cerri, where these two monoterpene phenols, their main precursors  $\gamma$ -terpinene and p-cymene, and other related compounds represent the bulk of the essential oil, which has a low content of many of the sesquiterpenes [56]. An older report by Kokkini et al. [58] found thymol to be the main component in oils of *O. vulgare* ssp. *hirtum* plants from the Northern part of Greece, whereas carvacrol prevailed in oils from the Southern part of the country. Samples rich in carvacrol were also found in Bulgaria, with a maximum carvacrol content of 73.4% [59]. Kosakowska et al. [60] analyzed *Origanum vulgare* ssp. *hirtum* and found 22 compounds, including  $\gamma$ -terpinene (17.31%), p-cymene (11.13%), thymol (0.58%), and phenol monoterpenes (39.79%) with a clear domination of carvacrol (37.21%). Shafiee-Hajiabad et al. [61] analyzed Greek oregano oil and found that carvacrol had the highest content among all components, while Baranauskiene et al. [62] analyzed Lithuanian *Origanum vulgare* ssp. *hirtum* and found carvacrol as the dominant component (72–88%).

The chemical composition of the Greek oregano essential oil from Western Macedonia analyzed in the present investigation allowed us to classify the oil as a mixed carvacrol/p-cymene/thymol/ $\gamma$ -terpinene chemotype. Regarding industrial applications, essential oils with up to 80% of carvacrol are considered the most valuable due to the proven biological activity of this phenolic monoterpene [63]. In addition, according to European Pharmacopeia recommendations, the sum of thymol and carvacrol in Greek oregano essential oil should not be lower than 60% [60,64].

The results of the microbial content analysis suggest that the probability of growth of microbiological charge does not exist even during the storage of lavender/oregano oil in a special plastic small bottle of 100 mL.

The data shown in Figure 5 demonstrate the far superior antioxidant activity of oregano oil, 1 mL of which is able to consume 45  $\mu$ moles of DPPH radicals. In terms of activity, lavender oil follows, with 1 mL consuming 0.28  $\mu$ moles of DPPH radicals. Finally, 1 mL of oregano water and 1 mL of lavender water neutralize 0.067 and 0.022  $\mu$ moles DPPH, respectively. Overall, the oregano and lavender oils presented much better antioxidant effects compared to the oregano and lavender water. Between lavender and oregano oil, the best antioxidant activity by far is that of oregano oil, with the others in the following order: antioxidant activity of oregano oil >>> lavender oil >> oregano water > lavender water. Moreover, all the studied distillates showed antioxidant activity, which is mass-dependent, against the DPPH radical through the HAT mechanism, where one  $H^+$  and one  $e^-$  are simultaneously transferred as  $\{H^+/e^-\}$  from the OH group of the antioxidant to the DPPH radical, resulting in its inactivation.

Nowadays, there is an increased usage of antiseptics by consumers, which drives the industry towards the increased production of such products, but on the other hand, there are concerns about the deleterious effects of disinfectant and sanitizer use on humans,

animals, and the environment [65]. The resistance to disinfectants leads to an increase in the bacterial load and can undermine food safety in food production plants or biosecurity status in medical facilities. Bacteria can combat chemical stress either by intrinsic mechanisms or, usually, by the transfer of mobile genetic elements [66,67].

Thus, there is a need to fulfill consumers' needs but also to control the side effects of disinfectant use. Essential oil-based sanitizers for common everyday use could be an alternative, environmental, and human friendly approach in an attempt to mitigate the adverse effects that chemical-based disinfectants may cause.

Our results show that all the disinfectants tested possess excellent bactericidal activity under the conditions of the evaluation (EU protocol EN 1040), irrespective of the amount of ethanol used for the disinfectant preparation (i.e., 70% *v/v*, 75% *v/v*, and 80% *v/v*) and of the origin of the essential oil (lavender or oregano). It should be noted that it was beyond the scope of the current study to carry out sophisticated experimentation in order to further evaluate to which extent the antibacterial properties of these disinfectants are attributed to the inclusion of the essential oils. It has been shown earlier that ethanol, as a chemical substance, is capable of inactivating *Staphylococcus aureus* and *Pseudomonas aeruginosa* when used as such or as a substance in alcohol-based hand sanitizers [68,69].

Although the literature is scarce with references to lavender oil and oregano oil, some recent studies have demonstrated that alcohol-free hand sanitizers prepared either with 1.25% (*v/v*) clove oil [70] or 10% tea tree oil [71] are effective for hand disinfection and could serve as a potential alternative to commonly used alcohol-based hand sanitizers. In addition, the antimicrobial properties of essential oils also are known for their antioxidant attributes and have been extensively used for skincare formulations [72]. Nevertheless, the antibacterial and antioxidant dynamics of lavender oil and oregano oil as substances in hand sanitizers remain to be evaluated.

## 5. Conclusions

The qualitative and quantitative analyses of the particular lavender and oregano distillate oils produced revealed the presence of 14 and 11 principal compounds, respectively, which contribute to the aromatic characteristics of the essential oils. The essential oils of both oregano and lavender possessed superior antioxidant activity to their corresponding waters, while the oregano oil exhibited far better antioxidant activity than the lavender oil. Moreover, the compounds of the essential oils probably enhance the efficacy of the novel alcohol-based disinfectants prepared containing lavender or oregano oil against common pathogenic bacteria to contribute to ensuring public health. Our research findings are encouraging, suggesting that sustainable antioxidant production for hygienic disinfection using bioextractants from the lavender and oregano distillation process is feasible, as the distillate products prepared possess important dynamics for wider industrial and commercial use in therapeutic or preventive medicine hygienic applications.

**Supplementary Materials:** The following supporting information can be downloaded at: <https://www.mdpi.com/article/10.3390/en16227534/s1>, Figure S1. Bactericidal activity of Formula #1 Disinfectant 80% with Lavender Oil and Water. Figure S2. Bactericidal activity of Formula #1 Disinfectant 75% with Lavender Oil and Water. Figure S3. Bactericidal activity of Formula #1 Disinfectant 70% with Lavender Oil and Water. Figure S4. Bactericidal activity of Formula #1 Disinfectant 80% with Oregano Oil and Water. Figure S5. Bactericidal activity of Formula #1 Disinfectant 75% with Oregano Oil and Water. Figure S6. Bactericidal activity of Formula #1 Disinfectant 70% with Oregano Oil and Water. Figure S7. Bactericidal activity of Lavender Oil. Figure S8. Bactericidal activity of Oregano Oil.

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