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Variation in the Antimicrobial Activity of Essential Oils from Cultivars of *Lavandula angustifolia* and *L.* × *intermedia*

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Abstract: The antimicrobial properties of essential oil from *Lavandula* sp. raise hopes related to its use in phytotherapy. This study aimed to evaluate the antimicrobial activity of essential oils from cultivars of *L. angustifolia* ('Hidcote Blue Strain', 'Hidcote Blue') and *L.* × *intermedia* ('Phenomenal', 'Grosso') grown in central-eastern Poland, that is, at the border of the northern lavender cultivation range. The chemical composition of the essential oils was determined by GC/MS. Essential oil concentrations (20, 10, 5, 2.5, 1.25, 0.6, 0.3, 0.16, 0.08, and 0.04 mg/mL) were tested to determine the minimum inhibitory concentration (MIC), minimum bactericidal concentration (MBC) or minimum fungicidal concentration (MFC) towards ten strains of Gram-positive bacteria, five Gram-negative bacteria, and eight yeasts in vitro culture. Essential oils from the *Lavendula* cultivars showed antimicrobial activity against all microorganisms analysed. The yeasts were characterised by higher sensitivity to lavender oil compared to bacteria, while Gram-positive bacteria were more sensitive than Gram-negative bacteria. The lowest MIC values for bacteria and fungi were obtained for 'Grosso'. Furthermore, the 'Grosso' oil showed the highest fungicidal activity, while the highest bactericidal activity was found in 'Hidcote Blue' and 'Grosso'. Using *Staphylococcus aureus* as an example, it was shown that different bacterial strains of the same species show varying sensitivity to the essential oil. A higher oil content was noted for the cultivars *L.* × *intermedia*, especially for the 'Phenomenal'. Linalyl acetate and linalool were the main components of the essential oil in all cultivars. However, in the 'Grosso' oil, a high content of terpinen-4-ol (18.08%) was also recorded. An analysis of the relationships between the content of the main components in the analysed essential oils and the antimicrobial activity of essential oils suggested that linalool and terpinen-4-ol were compounds potentially responsible for antimicrobial activity. The obtained results allow us to conclude that essential oil with significant antimicrobial activity can be obtained from *Lavandula* sp. plants harvested in the northern part of the cultivation range.

Keywords: gram-positive bacteria; gram-negative bacteria; yeasts; lavender; lavandula; linalool; terpinen-4-ol

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1. Introduction

Lavender is a plant belonging to the lavender family found on the Mediterranean coast and is valued mainly for its pleasant aroma [1]. Lavender (genus *Lavandula* L.) comprises 41 species of flowering plants in the family Lamiaceae [2], has a long history of its use in medicine, and has been used for this purpose as far back as Ancient Greece and Rome [3,4]. The therapeutic effect of lavender 'aromas' is also mentioned by Hildegard of Bingen [5], and the antimicrobial properties of essential oil were used to treat wounds during the First

World War, for example [6,7]. Lavender essential oil also exhibits antioxidant, sedative, carminative, antiplatelet, antithrombotic, antidepressive, and anti-inflammatory properties and positive effects on the digestive and nervous systems [7–10]. The antibacterial and antifungal properties of the essential oil of the *Lavandula* sp. raise hopes related to its use in phytotherapy [11,12] and its supportive role in antibacterial combination treatment [8]. However, studies conducted to date regarding its antimicrobial activity have not yielded conclusive results. Some authors report that lavender essential oils do not show much antimicrobial activity [13], while others show bactericidal activity even against some antibiotic-resistant microorganisms [8,14]. These discrepant results may be due to the variability in essential oil composition [12,15–18]. Potent antimicrobial and antifungal effects have been attributed to linalool and linalyl acetate [19]. However, other studies have not found a correlation between the contribution of linalool and linalyl acetate and the bioactivity of lavender oil [14,15]. According to Jianu et al. [19], the antibacterial and antifungal effects of *Lavandula* sp. oils are due to the properties of their many components. Therefore, the varying results of the antibacterial and antifungal activity of the essential oil depend on many factors that affect its composition, e.g., the technology of growing, harvesting, and drying, and even the method of oil distillation [1,11–13,16,17,20–24]. However, the factors that most influence oil composition and antimicrobial activities are genetic variation and growing conditions [8,11,16,20,25,26]. Therefore, it was decided to evaluate the antibacterial and antifungal effects of essential oils from *L. angustifolia* and *L.* × *intermedia* plants cultivated at the border of their northern growing range on a group of 23 microorganisms (15 bacteria and eight yeasts) that live on and within human beings and play an important role in human health and disease. It was assumed that the variation in the composition of the essential oils of the studied lavender species and cultivars would affect their bactericidal and fungicidal activity and that the use of multiple strains of Gram-positive and Gram-negative bacteria and fungi in the study would allow the use of these oils in phytotherapy to be assessed. Furthermore, it was hypothesised that the variation within plants and the genotypic variation of five strains of one microorganism (*Staphylococcus aureus,* including methicillin-sensitive and methicillin-resistant strains) might influence the effects of essential oil application.

2. Materials and Methods

2.1. Description of the Station's Location

Plant material was obtained from the 'Lavendel Natur Haus' plantation ('Hidcote Blue', 'Phenomenal', 'Grosso') located in Rejowiec, Lublin region (51°13′72″ N, 23°29′74″ E) and from the Agricultural Experimental Farm Felin ('Hidcote Blue Strain') of the University of Life Sciences in Lublin (51°13′37″ N 22°37′58″ E). The experimental fields were located in central-eastern Poland, a region at the border of the northern range of *Lavendula* sp. cultivation.

2.2. Details of Field Experiment

The experimental material consisted of lavender (*Lavandula angustifolia* Mill.) 'Hidcote Blue Strain' and 'Hidcote Blue', and lavandin (*Lavandula* × *intermedia*) 'Phenomenal' and 'Grosso'. The plants were grown in rows every 80–100 cm, with a plant spacing of 120 cm, on agrotextiles; weeding was carried out manually without chemical pesticides. The raw material was harvested in July 2018 during sunny weather. The inflorescence shoots of the 'Hidcote Blue', 'Phenomenal', and 'Grosso' varieties were obtained from three-year-old plants, dried naturally in bunches in a shady, airy place. On the other hand, the inflorescences of the 'Hidcote Blue Strain' (also from three-year-old plants) were dried in a drying room at 35 °C. After being dried, the flowers were separated from the inflorescence stems by rubbing them on sieves.

2.3. Essential Oil Isolation and Analyses

The essential oils were obtained by hydrodistillation performed in the Clevenger apparatus for 3 h. To obtain oil from the flowers of individual lavender cultivars, samples weighing 20 g of each combination were prepared in triplicate.

The samples were stored in the dark and at less than 4 °C. The chemical composition of the essential oils was determined by GC/MS. In the research, we used a GC apparatus (Shimadzu GC-2010 Plus, Kyoto, Japan) with a Zebron™ ZB-5MS column (length: 30 m; inner diameter: 0.25 mm; film thickness: 0.25 mm; Phenomenex Inc., Torrance, CA, USA). We used a temperature gradient of 50 °C for 3 min and then an increase of 5 °C up to 250 °C. Helium with a flow rate of 0.5 mL min^{-1} was used as the carrier gas. Automatic dosing with a sample division (1 µL) with a division ratio of 1:100 was used in the analysis. Compounds were detected with a Vatran 4000 MS/MS detector. The mass spectrometer worked in the mass scanning range of 50–400 amu, while the scanning speed was 0.2 s $scan^{-1}$. The mass spectrometer was operated in electron ionisation (El) mode with an ionisation energy of 70 eV. The ion source was maintained at 220 °C, while the interface and dispenser were maintained at 250 °C.

Retention indices (Kovats) were calculated using a series of n-alkanes C6-C40. The qualitative analysis was made based on MS spectra, comparing them with the spectra of the NIST library (62,000 spectrums) and the LIBR terpene library (TR), provided by the Finnigan MAT company. Literature data confirmed the identification of the compounds. In addition, the identification was also carried out based on a comparison of the obtained mass spectra with the NIST 2011 spectra library (National Institute of Standards and Technology, Gaithersburg, MD, USA), with the MassFinder 2.1 computer spectra library (http://www.massfinder.com, accessed on 17 September 2022), and with mass spectra of reference compounds. Essential oil components are reported as a relative percentage of the total oil by peak area. Values ≥ 0.5 were considered significant.

2.4. The Antimicrobial Activity of Essential Oils

The antimicrobial activity of the lavender essential oils was evaluated using the microdilution broth method according to the European Committee on Antimicrobial Susceptibility Testing (EUCAST) guidelines [27], as previously described [28]. The activity of lavender essential oils was determined for reference strains belonging to the American Type Culture Collection (ATCC), including ten Gram-positive bacteria (*Staphylococcus aureus* ATCC 29213, *Staphylococcus aureus* ATCC 6538P, and *Staphylococcus aureus* ATCC 25923—methicillin-sensitive strains, *Staphylococcus aureus* ATCC 43300 and *Staphylococcus aureus* ATCC BAA1707—methicillin-resistant strains, *Staphylococcus epidermidis* ATCC 12228, *Enterococcus faecalis* ATCC 29212, *Micrococcus luteus* ATCC 10240, *Bacillus subtilis* ATCC 6633, and *Bacillus cereus* ATCC 10876), five Gram-negative bacteria (*Salmonella* Typhimurium ATCC 14028, *Proteus mirabilis* ATCC 12453, *Bordetella bronchiseptica* ATCC 4617, *Escherichia coli* ATCC 25922, and *Pseudomonas aeruginosa* ATCC 27853), and eight fungi called yeasts (*Candida albicans* ATCC 2091, *Candida albicans* ATCC 10231, *Candida auris* CDC B11903, *Candida glabrata* ATCC 90030, *Candida parapsilosis* ATCC 22019, *Candida krusei* ATCC 14243, *Candida lusitaniae* ATCC 34449, and *Candida tropicalis* ATCC 1369). All of the used microbial strains were first subcultured on Mueller–Hinton Agar for bacteria or RPMI Agar for yeasts and incubated at 35 °C for 24 h. Microbial colonies were collected and suspended in sterile physiological saline to obtain inoculum of 0.5 McFarland standard, corresponding to 1.5×10^8 CFUs (colony forming units) mg/mL for bacteria and 5×10^6 CFUs mg/mL for fungi. The two-fold dilutions of the essential oils in Mueller–Hinton Broth for bacteria or by RPMI Broth for fungi were prepared in 96-well polystyrene plates. The essential oils were dissolved in DMSO to obtain a final concentration of 100 mg/mL. The final concentrations tested were 20, 10, 5, 2.5, 1.25, 0.6, 0.3, 0.16, 0.08, and 0.04 mg/mL. Subsequently, 2 µL of each bacterial or fungal inoculum was added to each well containing 200 µL of the serial dilution of the essential oils in the appropriate culture medium. This method allows for determining the minimum inhibitory concentration (MIC), minimum bactericidal

concentration (MBC) or minimum fungicidal concentration (MFC) of each essential oil towards 23 microorganisms in in vitro culture. Appropriate DMSO, growth, and sterile controls were carried out. The experiments were performed in triplicate. The standard antimicrobial agents, fluconazole (0.06–16 µg/mL), ciprofloxacin (0.015–16 µg/mL), and vancomycin (0.06–16 µg/mL), were used. MIC values were: 1 µg/mL of fluconazole for *Candida albicans* ATCC 10231, 1 µg/mL of vancomycin for *Staphylococcus aureus* ATCC 29213, and 0.015 µg/mL of ciprofloxacin for *Escherichia coli* ATCC 25922.

2.5. Data Analysis and Visualisations

Data analysis and visualisations were performed using the R 4.2.0 programming environment (https://www.r-project.org/, accessed on 20 October 2022) and appropriate packages. A radar plot presenting the percentage content of the main essential oil components was made using the fmsb 0.7.3 package (https://cran.r-project.org/web/packages/fmsb/index.html, accessed on 20 October 2022). A heatmap illustrating the antimicrobial activity of the analysed essential oils against tested microorganisms was made using packages pheatmap 1.0.12 (https://cran.r-project.org/web/packages/pheatmap/index.html, accessed on 20 October 2022) and ggplot2 3.3.6 (https://cran.r-project.org/web/packages/ggplot2/index.html, accessed on 20 October 2022). On the heatmap, data were arranged using hierarchical clustering of Canberra distances and the complete method applied to clustering. Correlation analysis was performed using the Pearson algorithm implemented in the cor function from the core stats package in R. A correlation plot was drawn using the corrplot 0.92 package (https://cran.r-project.org/web/packages/corrplot/index.html, accessed on 20 October 2022). Plots illustrating relationships between the percentage content of essential oil components and the antimicrobial activity of essential oils were generated using the ggplot2 3.3.6 package.

3. Results

3.1. Composition of Essential Oils

The flowers of the tested *Lavendula* sp. cultivars differed in their essential oil content, with *L.* × *intermedia* containing more essential oil than *L. angustifolia*. The highest essential oil content was found in *L.* × *intermedia* 'Phenomenal', which was as high as 8.1%, while the second cultivar, *L.* × *intermedia* 'Grosso', had much less essential oil (4.4%). The differences in essential oil content in the *L. angustifolia* flowers were smaller ('Hidcote Blue' 3.1%, 'Hidcote Blue Strain' 3.6%), although the raw material came from plants grown in different locations (Table 1).

Table 1. The oil content of the dried plant material (% *v*/*w*).

Variety	Oil Content
L. angustifolia 'Hidcote Blue Strain'	3.6
L. angustifolia 'Hidcote Blue'	3.1
L. × *intermedia* 'Phenomenal'	8.1
L. × *intermedia* 'Grosso'	4.4

Linalool and linalyl acetate were the predominant essential oil constituents in all cultivars tested; terpinen-4-ol, lavandulyl acetate, α-terpineol, 1,8-cineole, camphor, and caryophyllene oxide also had a large share (mean content above 2.5%) (Figure 1). The main components mentioned above had the lowest share in the essential oil of 'Hidcote Blue Strain' (56.07%); in the other cultivars, their share exceeded 70% ('Hidcote Blue' 71.14%, 'Phenomenal' 76.70%, 'Grosso' 77.06%) (Figure 1). The *L.* × *intermedia* cultivars' oil contained more linalool and terpinen-4-ol, and less caryophyllene oxide. The highest content of terpinen-4-ol (18.08%) was found in 'Grosso' and caryophyllene oxide in 'Hidcote Blue Strain' (6.42%). The highest linalyl acetate content was recorded for *L. angustifolia* 'Hidcote Blue' (34.22%), while the 'Hidcote Blue Strain' essential oil contained only 16.87% of this component. Lavender 'Hidcote Blue Strain', of all the essential oils tested, showed

the highest content of lavandulyl acetate (8.26%), and 'Phenomenal' the highest content of camphor (9.57%); the camphor content in the other cultivars' oils was in the range of 0.14–0.56%), and the content of 1.8-cineole was 9.1% (Table 2).

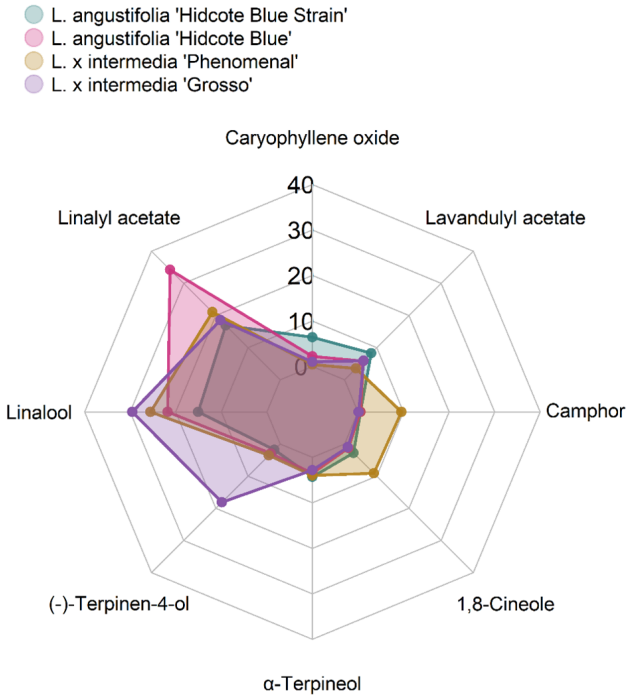

Figure 1. Radar plot presenting the percentage content of the most abundant constituents of the analysed essential oils. The plot was generated using the fmsb 0.7.3 package in R version 4.2.0.

Table 2. Identification and content (%) of compounds present in the essential oil obtained from lavender flowers by GC/MS.

	Name	Retention Time	Retention Index	*L. angustifolia* 'Hidcote Blue Strain'	*L. angustifolia* 'Hidcote Blue'	*L.* × *intermedia* 'Phenomenal'	*L.* × *intermedia* 'Grosso'
1.	β-Pinene	8.103	941	0.51	-	0.54	-
2.	1-Octen-3-ol	8.170	944	0.06	-	0.32	0.57
3.	3-Oktanone	8.243	946	0.56	1.04	-	0.83
4.	β-Myrcene	8.337	949	0.84	0.52	0.61	0.51
5.	n-Hexyl acetate	8.827	965	0.90	0.15	0.29	0.33
6.	o-Cymene	9.143	971	0.17	-	-	0.68
7.	p-Cymene	9.153	975	0.57	0.33	0.25	-
8.	Limonene	9.253	978	0.64	0.53	0.80	0.32
9.	1.8-Cineole	9.320	981	2.77	1.19	9.10	0.98
10.	(E)-β-Ocimene	9.363	982	0.48	0.84	-	0.53
11.	*trans*-Linalool oxide	10.147	1007	4.19	0.77	0.62	1.00
12.	6-Methyl-2-(2-oxiranyl)-5-hepten-2-ol	10.477	1018	3.22	0.51	0.24	0.32
13.	Linalool	10.757	1027	15.10	21.77	25.53	29.56
14.	1-Octene-3-ol acetate	10.833	1029	1.99	1.51	0.97	2.22
15.	Camphor	11.757	1058	0.56	0.55	9.57	0.14
16.	Cyclohexanone	11.770	1059	1.25	-	-	-
17.	Lavandulol	12.033	1067	1.08	1.19	0.52	2.46

Table 2. *Cont.*

	Name	Retention Time	Retention Index	*L. angustifolia* 'Hidcote Blue Strain'	*L. angustifolia* 'Hidcote Blue'	*L.* × *intermedia* 'Phenomenal'	*L.* × *intermedia* 'Grosso'
18.	Borneol	12.290	1075	2.45	1.92	4.58	0.71
19.	Terpinen-4-ol	12.423	1079	1.79	2.91	3.52	18.08
20.	n- Hexyl butyrate	12.503	1058	-	0.71	0.32	0.53
21.	Cryptone	12.530	1083	2.78	1.01	0.26	-
22.	α-Terpineol	12.713	1088	4.30	3.50	3.97	2.82
23.	2-Pinene-4-on	12.920	1095	-	0.07	-	0.60
24.	Cis-Geraniol	13.213	1104	0.76	0.46	0.46	0.45
25.	Linalyl acetate	13.607	1117	16.87	34.22	21.05	18.56
26.	Geraniol	13.670	1119	1.71	1.47	1.36	1.24
27.	Lavandulyl acetate	14.203	1137	8.26	5.80	3.52	5.90
28.	3-Nonanol	15.290	1173	0.70	0.38	0.10	-
29.	Neryl acetate	15.317	1180	1.07	1.14	0.79	0.73
30.	Geranyl acetate	15.850	1191	2.17	1.90	1.51	1.36
31.	Santalene	16.637	1218	0.24	0.87	0.16	0.24
32.	Caryopyllene	16.713	1221	0.46	1.67	0.74	1.47
33.	Cis- β-Farnesene	17.107	1234	0.09	0.63	0.43	1.46
34.	γ-Cadinene	18.813	1294	0.53	0.18	-	-
35.	Caryophyllene Oxide	19.300	1311	6.42	2.20	0.44	1.02
36.	γ-Muurolene	20.153	1343	2.10	0.65	0.98	-
37.	Androstan-17-on	20.598	1359	0.85	-	-	-
38.	Bisabolol	20.727	1364	-	-	1.87	-
Sum				88.44	91.55	95.42	95.62

3.2. Antimicrobial Activity

The results of an antimicrobial analysis performed using four essential oils obtained from different species and varieties of lavender are presented in Table 3 and are visualised on a heatmap with hierarchical grouping (Figure 2). It can be seen that all essential oils showed differential activity against the tested reference bacteria (MIC = 2.5–10 mg/mL) and yeasts (MIC = 0.3–1.25 mg/mL). The essential oils were characterised by the somewhat higher activity against Gram-positive bacteria compared to Gram-negative species.

Table 3. MIC [mg/mL], MBC [mg/mL], and MFC [mg/mL] values of *L. angustifolia* 'Hidcote Blue Strain', 'Hidcote Blue', *L.* × *intermedia* 'Grosso' and 'Phenomenal' essential oils against reference strains of microorganisms.

Microorganisms	*L. angustifolia* 'Hidcote Blue Strain'		*L. angustifolia* 'Hidcote Blue'		*L.* × *intermedia* 'Phenomenal'		*L.* × *intermedia* 'Grosso'	
Gram-positive bacteria	**MIC**	**MBC**	**MIC**	**MBC**	**MIC**	**MBC**	**MIC**	**MBC**
Staphylococcus aureus ATCC 29213	10	20	5	10	5	10	5	20
Staphylococcus aureus ATCC 6538P	2.5	10	2.5	5	2.5	5	2.5	5
Staphylococcus aureus ATCC 25923	5	20	2.5	5	5	10	2.5	5
Staphylococcus aureus ATCC 43300	5	10	5	5	5	10	5	10
Staphylococcus aureus ATCC BAA-1707	5	10	5	5	5	10	5	10
Staphylococcus epidermidis ATCC 12228	10	20	2.5	20	5	20	2.5	20
Enterococcus faecalis ATCC 29212	10	20	10	20	10	20	5	20
Micrococcus luteus ATCC 10240	2.5	20	2.5	20	2.5	20	2.5	10
Bacillus subtilis ATCC 6633	5	20	5	5	5	5	2.5	2.5
Bacillus cereus ATCC 10876	5	10	5	5	2.5	5	2.5	2.5

Table 3. *Cont.*

Microorganisms	*L. angustifolia* 'Hidcote Blue Strain'		*L. angustifolia* 'Hidcote Blue'		*L.* × *intermedia* 'Phenomenal'		*L.* × *intermedia* 'Grosso'	
Gram-negative bacteria	**MIC**	**MBC**	**MIC**	**MBC**	**MIC**	**MBC**	**MIC**	**MBC**
Salmonella Typhimurium ATCC 14028	10	10	10	10	5	10	5	5
Proteus mirabilis ATCC 12453	10	10	10	10	5	10	5	5
Bordetella bronchiseptica ATCC 4617	2.5	5	5	5	2.5	5	2.5	5
Escherichia coli ATCC 25922	10	10	10	10	5	5	5	5
Pseudomonas aeruginosa ATCC 27853	10	20	10	10	10	10	10	10
Yeasts	**MIC**	**MFC**	**MIC**	**MFC**	**MIC**	**MFC**	**MIC**	**MFC**
Candida albicans ATCC 2091	0.3	1.25	1.25	2.5	0.6	1.25	0.3	0.6
Candida albicans ATCC 10231	0.6	1.25	1.25	2.5	1.25	2.5	0.6	1.25
Candida auris CDC B11903	1.25	1.25	1.25	2.5	1.25	2.5	0.6	1.25
Candida glabrata ATCC 90030	1.25	2.5	1.25	2.5	1.25	2.5	0.6	1.25
Candida parapsilosis ATCC 2019	0.16	2.5	0.16	5	0.3	2.5	0.3	2.5
Candida krusei ATCC 14243	1.25	2.5	0.6	1.25	0.6	2.5	0.3	1.25
Candida lusitaniae ATCC 3449	1.25	2.5	1.25	2.5	1.25	2.5	0.6	1.25
Candida tropicalis ATCC 1369	0.6	2.5	1.25	2.5	1.25	2.5	0.3	1.25

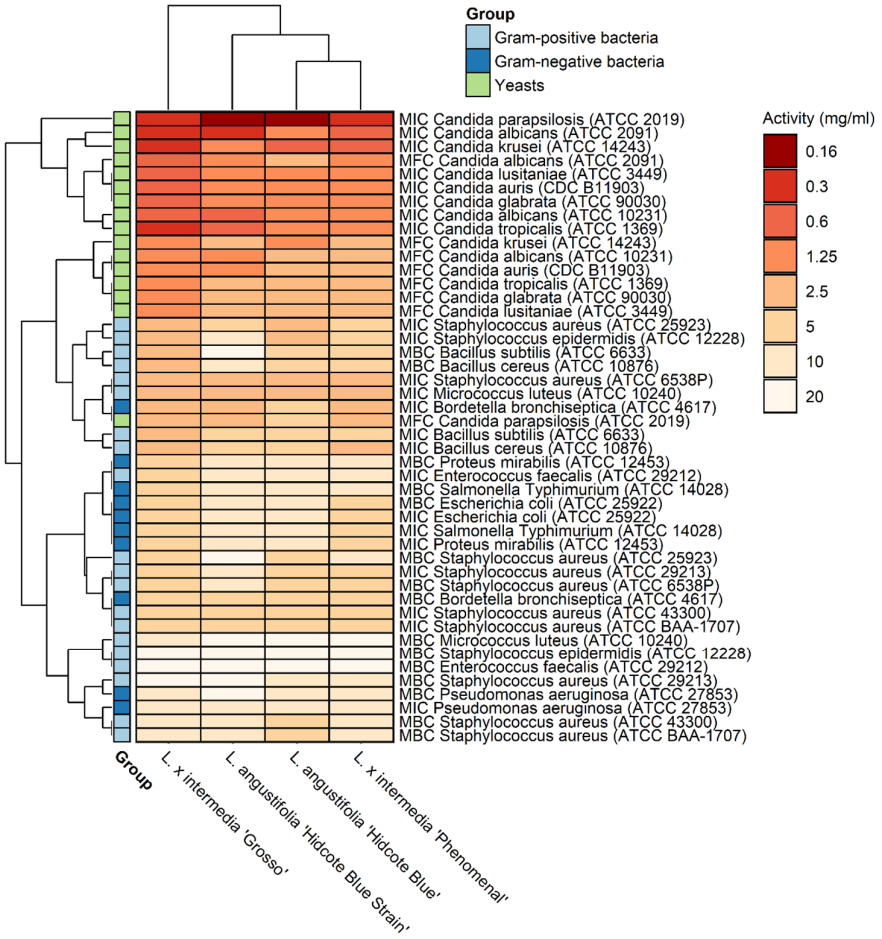

Figure 2. Heatmap illustrating antimicrobial activity (MIC, MBC, and MFC doses) of analysed essential oils against tested microorganisms. Hierarchical clustering was performed using the complete method applied to Canberra distances. Heatmap was made using pheatmap 1.0.12 and ggplot2 3.3.6 packages in R version 4.2.0.

Of all the bacteria tested, the most sensitive were *S. aureus* ATCC 6538P and *M. luteus* ATCC 10240, with MIC values of 2.5 mg/mL, belonging to Gram-positives bacteria, and *B. bronchiseptica* ATCC 4617, with MIC values in the range of 2.5–5 mg/mL, belonging to Gram-negative bacteria. At the same time, *P. aeruginosa* ATCC 27853 was the most insensitive strain to all lavender essential oils.

The highest activity of all the essential oils was shown against yeasts, with MIC values ranging from 0.16 to 1.25 mg/mL. *C. parapsilosis* ATCC 2019 was the most sensitive to all lavender essential oils (MIC = 0.16–0.3 mg/mL). The sensitivity of *C. albicans* ATCC 2091, *C. krusei* ATCC 14234, and *C. tropicalis* ATCC 1369 was dependent on the essential oils used (MIC = 0.3–0.6 mg/mL). It is interesting to notice that essential oil from *L.* × *intermedia* 'Grasso' and *L. angustifolia* 'Hidcote Blue Strain' showed the strongest activity against yeasts and Gram-positive bacteria.

Antimicrobial substances are usually regarded as bactericidal or fungicidal if the MBC/MIC or MFC/MIC ratio is $\leq$4. If the MBC/MIC or MFC/MIC ratio is >4, antimicrobial substances are typically regarded as bacteriostatic or fungistatic [27]. Based on the presented results, it can be concluded that the lavender essential oils showed bactericidal (MBC/MIC = 1–4) and fungicidal effects (MFC/MIC = 1–4) for most tested microorganisms. The bacteriostatic effect was noted against *S. epidermidis* ATCC 12228, *M. luteus* ATCC 10240 (MBC/MIC = 8), and *C. parapsilosis* ATCC 2019 (MFC/MIC = 16–32). However, it is worth noting that the MBC values in Gram-negative bacteria were, in most cases, equal to the MIC values (except for *B. bronchiseptica* ATCC 4617), while in Gram-positive bacteria, the differences between the MIC and MBC values were large. Only for *B. subtilis* ATCC 6633 (for three oils) and *B. cereus* ATCC 10876 (for two oils) and for 'Hidcote Blue' oil used on *S. aureus* ATCC 43300 and *S. aureus* ATCC BAA-1707, were these values equal (Table 3).

In a further step of the study, an attempt was made to determine which of the dominant components of the essential oils could be responsible for the observed antimicrobiological effects. A correlation analysis was performed to obtain an initial picture of the relationship between the percentage content of the dominant components and the values of MIC, MBC, and MFC (Figure 3). However, it should be clearly emphasised that, due to the small number of samples (four oils), the correlation coefficients only allow a preliminary assessment of the directions of correlation (positive, negative) and the strength of the potential antimicrobial effect. In the analysis, particular attention was paid to negative correlations, i.e., when a higher percentage of the active ingredient corresponds to a stronger antimicrobial effect (lower MIC, MBC, and MFC values). The analysis shows that it is likely that linalool and terpinen-4-ol show the most potent biological activity, as the most negative correlations were obtained for them. Interestingly, in the case of 1,8-cineol and camphor, a high similarity in their biological action was observed (Figure 3), which may be related to the structural similarity of the two compounds, because both compounds belong to the cyclic monoterpenes.

To gain further insight into the correlations between the percentage of dominant components and the MIC, MBC, and MFC values, scatter plots were made for individual correlations and visually assessed, and then those representing the most likely correlations were selected (Figure S1). The selected correlations include those between the percentage of linalool and terpinen-4-ol and the MBC values for *B. cereus* (ATCC 10876), *B. subtilis* (ATCC 6633) and the MIC values for *C. krusei* (ATCC 14243). The relationships between the contents of these flocculants in oils and the biological activity values of the oils against these strains are shown in Figure 4.

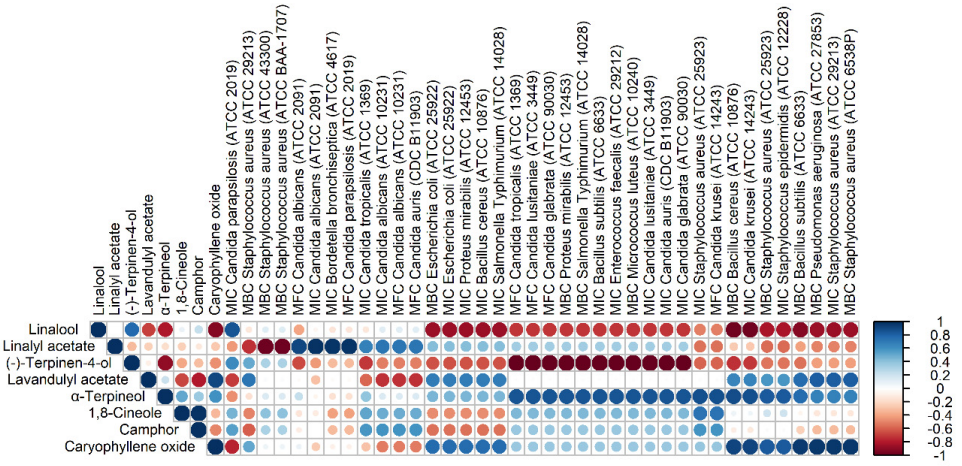

Figure 3. Corrplot presenting Pearson correlation coefficients for percentage content of eight primary constituents of studied essential oils and MIC/MBC/MFC doses determined for tested microorganisms.

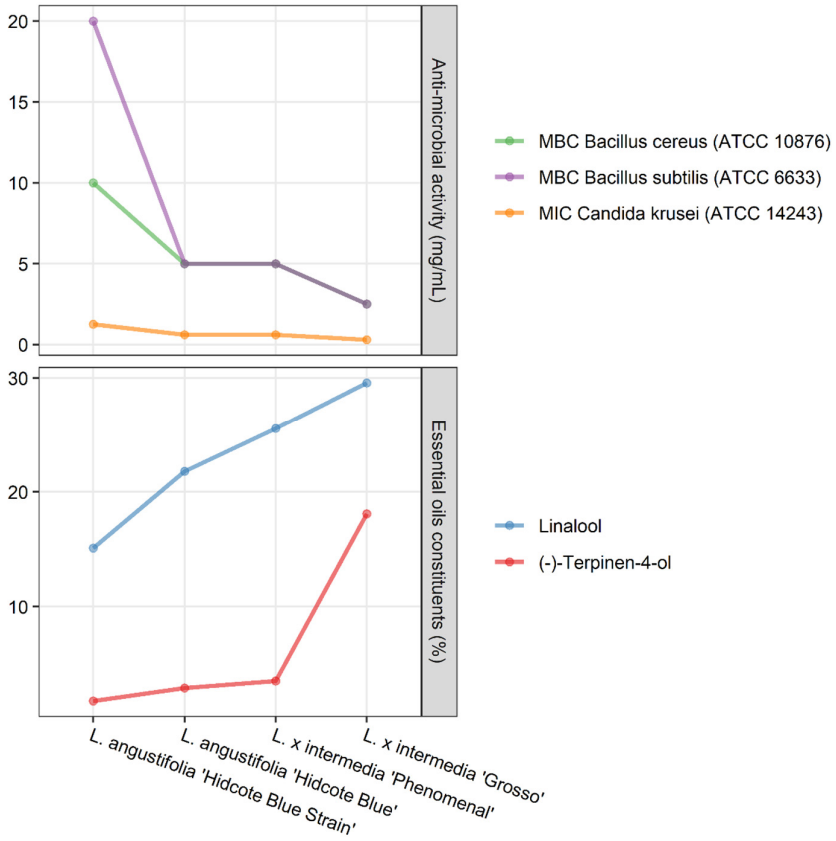

Figure 4. The most probable relationships between the percentage content of essential oil constituents and the biological activity of studied essential oils. The higher content of linalool and terpinen-4-ol is potentially related to lower doses of MBC for *B. cereus* (ATCC 10876), MBC for *B. subtilis* (ATCC 6633), and MIC for *C. krusei* (ATCC 14243).

This graph clearly shows that, the higher the content of linalool and terpinen-4-ol in the oils tested, the lower the MBC doses obtained for the *B. cereus* (ATCC 10876) and *B. subtilis* (ATCC 6633) strains and the lower the MIC for *C. krusei* (ATCC 14243). The results obtained are relatively consistent, as the essential oil obtained from the flowers of *L.* × *intermedia* 'Grosso', which contained the highest amount of linalool and terpinen-4-ol (Figure 1), probably with the most potent antimicrobial activity (Figure 3), also showed

the highest overall biological activity (Figure 2). However, the conclusion that linalool and terpinen-4-ol are mainly responsible for the antimicrobial activity of the oils, with particular emphasis on the activity against *B. cereus* (ATCC 10876), *B. subtilis* (ATCC 6633), and *C. krusei* (ATCC 14243), is a hypothesis and needs to be confirmed under experimental conditions.

4. Discussion

Lavender is an ornamental plant that provides a valuable essential oil with applications in cosmetics and medicine [11,12,24]. According to data collected by Aprotosoaie et al. [12], the oil content in dried flowers or flowering aerial parts of *L. angustifolia* ranges from 0.21 to 8.85%, and in *L.* × *intermedia*, 0.2–9.9%. Despite similar values, *L.* × *intermedia* is considered more productive in terms of essential oil content [16,21,24,29,30]. In the present study, the *L.* × *intermedia* cultivars had a higher oil content than *L. angustifolia* cultivars. Studies conducted by other authors also indicate the influence of genotype variability on the content of essential oils [8,16,20,25].

The main compounds in the essential oil obtained from the dry flowers of *L. angustifolia* and *L.* × *intermedia* are linalool, linalyl acetate, 1,8-cineole, camphor, borneol, lavandulyl acetate, terpinen-4-ol, and α-terpineol [12]. In this study, similar results were obtained; the main components of *L. angustifolia* essential oils were linalyl acetate, linalool, and lavandulyl acetate, and in *L.* × *intermedia*, they were linalyl acetate, linalool, terpinene-4-ol, and lavandulyl acetate. Many studies have shown a high variability in the essential oil composition of *L. angustifolia* and *L.* × *intermedia* depending on the cultivars [8,12,16,17,20,25]. According to some researchers, the most important constituents of *Lavandula* sp. essential oil are linalyl acetate, linalool, and camphor [10,12,24]. In the present experiment, only 'Phenomenal' had a high content of camphor in the essential oil. In the case of *L.* × *intermedia* 'Grosso', the content of camphor in the essential oil was lower than in *L. angustifolia* 'Hidcote Blue'. The essential oil from *L.* × *intermedia* was characterised by a higher linalool content, a lower linalyl acetate content compared to 'Hidcote Blue' grown at the same site, and slightly higher linalyl acetate content than 'Hidcote Blue Strain' from a farm located in Felin.

The antibacterial and antifungal effects of lavender and lavandin essential oils are the result of the antimicrobial properties of their major and minor components. Potent antimicrobial and antifungal effects are attributed to linalool and linalyl acetate [19]. The statistical analysis of the presented experiment suggests the intense antimicrobial activity of linalool. This analysis also indicates that terpinen-4-ol may be a potent antimicrobial compound.

In the study conducted, the different strains of *S. aureus* showed varying sensitivity to lavender oil; *S. aureus* ATCC 6538P was the most sensitive, while relatively high MIC and MBC doses were required for *S. aureus* ATCC 29213. It may explain the varying results regarding the bactericidal effect of the oil on the different microorganisms when their different strains were used for the study [17,18,31,32]. Adaszynska et al. [25] also found differential antimicrobial activity of the oils of the tested strains against *S. aureus*, but the effects on the two tested strains were minor. The different sensitivity of individual strains of *S. aureus* may be due to their genetic diversity, confirmed by different sensitivities to agents with antibacterial activity [33].

The antimicrobial activity of essential oils from different *Lavandula* sp. cultivars varies [8,25]. Furthermore, in the present study, variability was found in the antimicrobial activity of *Lavandula* sp. oils from different cultivars of *L. angustifolia* and *L.* × *intermedia*. The essential oil extracted from *L.* × *intermedia* 'Grosso' showed the highest MIC activity in all strains tested. However, the oils of all cultivars showed bactericidal activity. *E. faecalis* (ATCC 29212) and *P. aeruginosa* (ATCC 27853) were the most resistant to the analysed essential oils. In a study by Danh et al. [17], lavender essential oil did not limit the growth of *P. aeruginosa* (ATCC 27853), but in the case of *E. faecalis* (ATCC 19433), the MIC (μg/μL) was ≤2.5. Similarly, in a study by Adaszyńska et al. [25], only the essential

oil of two of the five varieties of *L. angustifolia* limited the growth of *P. aeruginosa*. The antimicrobial activity of plant essential oils depends not only on the content of active substances, but also on the type of bacteria. Gram-negative bacteria are more resistant because their membrane contains hydrophilic lipopolysaccharides (LPS) that form a barrier to macromolecules and hydrophobic compounds [34–36]. The same activity against Gram-positive and Gram-negative bacteria was previously reported for essential oils obtained from *L. angustifolia* [37,38]. In addition, in the present study, Gram-positive bacteria were more sensitive than Gram-negative bacteria. However, lower doses of essential oil caused the inhibition of their growth in Gram-positive bacteria, but bactericidal activity was found at higher oil concentrations than in Gram-negative bacteria. When tested, essential oils obtained from different species and varieties of lavender showed more vigorous antifungal activity than antimicrobial activity. Data from the literature also indicate that lavender oil shows antifungal activity, especially against *Candida* strains [17,31]. The variation in sensitivity between bacteria and yeasts results from the different mechanisms of action of essential oils on these microorganisms [39]. The study indicates that the essential oil obtained from *L.* × *intermedia* 'Grosso' has the highest antimicrobial activity, probably due to its high linalool and terpinen-4-ol content. The literature data indicate the validity of this statement because, in a study by Fisher and Phillips [40], linalool showed the most effective antimicrobial activity, also against *B. cereus*.

5. Conclusions

Lavender is a valuable aromatic plant with beautiful flowers from which a pleasant-smelling oil is obtained. Of the two lavender species tested, the cultivars of *L.* × *intermedia* were more productive in terms of essential oil content, but differences in essential oil content between the *L.* × *intermedia* cultivars were greater. However, in *L.* × *intermedia* essential oil, camphor and 1,8-cineole had a high share in the 'Phenomenal', and terpinen-4-ol in the 'Grosso'.

Lavandula sp. essential oil was characterised by antimicrobial activity against all the microorganisms analysed. The composition of the oil influenced its antimicrobial activity, and linalool and terpinen-4-ol were considered the main components influencing such activity. The lowest MIC values, for bacteria and fungi, were obtained for *L.* × *intermedia* 'Grosso'. Furthermore, the oil obtained from this cultivar showed the highest fungicidal activity. The essential oil obtained from flowers of the 'Hidcote Blue' and 'Grosso' had the highest bactericidal activity, but the susceptibility of the microorganisms analysed to the oil obtained from the tested cultivars varied.

Individual bacterial strains of *S. aureus* show varying sensitivity to lavender oil. *S. aureus* ATCC 6538P was the most sensitive, while relatively high doses of oil were required by *S. aureus* ATCC 0 to achieve MIC and MBC effects.

Growth inhibition in Gram-positive bacteria was observed at lower concentrations than in Gram-negative bacteria, whereas the bactericidal effect required the use of higher concentrations of essential oil in Gram-positive bacteria.

Yeast-like fungi showed significantly greater sensitivity to *Lavandula* sp. essential oil than Gram-positive and Gram-negative bacteria.

Studies indicate that it is possible to cultivate different varieties of *L. angustifolia* and *L.* × *intermedia* in central-eastern Poland, i.e., at the border of the northern range of lavender cultivation. Furthermore, the essential oils obtained from *Lavandula* sp. show a composition and antimicrobial activity similar to those obtained from the traditional growing areas of this plant. The results of antimicrobial activity also confirm that the areas of central and eastern Poland allow the field cultivation of various species and cultivars of *Lavandula* sp., not only with good ornamental qualities, but also with significant antimicrobial activity.

Supplementary Materials: The following supporting information can be downloaded at: https://www.mdpi.com/article/10.3390/agronomy12122955/s1, Figure S1: Plots illustrating selected correlations. Blue lines indicate the trend line (fitting line of the simple linear regression model).

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References

1. Erbaş, S.; Baydar, H. Effects of harvest time and drying temperature on essential oil content and composition in lavandin (*Lavandula* × *intermedia* Emerice × Loisel.). *Turk. J. Field Crops.* **2008**, *13*, 24–31.
2. Royal Botanic Gardens, Kew. Plants of the World Online. Available online: https://powo.science.kew.org/taxon/urn:lsid:ipni.org:names:20960-1 (accessed on 13 November 2022).
3. Cavanagh, H.M.A.; Wilkinson, J.M. Biological activities of lavender essential oil. *Phytother. Res.* **2002**, *16*, 301–308. [CrossRef] [PubMed]
4. Dioscorides, P. *De Materia Medica—Being an Herbal with Many Other Medicinal Materials, Written in Greek in the First Century of the Common Era: A New Indexed Version in Modern English*; Osbaldeston, T.A., Wood, R.P.A., Eds.; Ibidis Press: Johannesburg, South Africa, 2000.
5. Bingen, H. *Physica–Uzdrawiające Dzieło Stworzenia. Naturalna Siła Oddziaływania Rzeczy*; Polskie Centrum Świętej Hildegardy: Legnica, Poland, 2014.
6. Gattefosse, R.-M. *Gattefosse's Aromatherapy*; Random House: New York, NY, USA, 2012.
7. Cavanagh, H.M.; Wilkinson, J.M. Lavender essential oil: A review. *Aust. Infect. Control* **2005**, *10*, 35–37. [CrossRef]
8. Adaszyńska, M.; Swarcewicz, M.; Dzięcioł, M.; Dobrowolska, A. Comparison of chemical composition and antibacterial activity of lavender varieties from Poland. *Nat. Prod. Res.* **2013**, *27*, 1497–1501. [CrossRef]
9. Prusinowska, R.; Smigielski, K.B. Composition, biological properties and therapeutic effects of lavender (*Lavandula angustifolia* L.). A review. *Herba Pol.* **2014**, *60*, 56–66. [CrossRef]
10. Barreca, S.; La Bella, S.; Maggio, A.; Licata, M.; Buscemi, S.; Leto, C.; Pace, A.; Tuttolomondo, T. Flavouring extra-virgin olive oil with aromatic and medicinal plants essential oils stabilizes oleic acid composition during photo-oxidative stress. *Agriculture* **2021**, *11*, 266. [CrossRef]
11. Herraiz-Peñalver, D.; Cases, M.Á.; Varela, F.; Navarrete, P.; Sánchez-Vioque, R.; Usano-Alemany, J. Chemical characterization of *Lavandula latifolia* Medik. essential oil from Spanish wild populations. *Biochem. Syst. Ecol.* **2013**, *46*, 59–68. [CrossRef]
12. Aprotosoaie, A.C.; Gille, E.; Trifan, A.; Luca, V.S.; Miron, A. Essential oils of *Lavandula* genus: A systematic review of their chemistry. *Phytochem. Rev.* **2017**, *16*, 761–799. [CrossRef]
13. Zheljazkov, V.D.; Astatkie, T.; Hristov, A.N. Lavender and hyssop productivity, oil content, and bioactivity as a function of harvest time and drying. *Ind. Crops Prod.* **2012**, *36*, 222–228. [CrossRef]
14. Sienkiewicz, M.; Głowacka, A.; Kowalczyk, E.; Wiktorowska-Owczarek, A.; Jóźwiak-Bębenista, M.; Łysakowska, M. The biological activities of cinnamon, geranium and lavender essential oils. *Molecules* **2014**, *19*, 20929–20940. [CrossRef]
15. Lis-Balchin, M.; Deans, S.G.; Eaglesham, E. Relationship between the bioactivity and chemical composition of commercial plant essential oils. *Flavour Fragr. J.* **1998**, *13*, 98–104. [CrossRef]
16. Lis-Balchin, M. (Ed.) *Lavender: The Genus Lavandula*; CRC press: Boca Raton, FL, USA, 2002; Available online: www.books.google.pl (accessed on 20 October 2022).
17. Danh, L.T.; Han, L.N.; Triet, N.D.A.; Zhao, J.; Mammucari, R.; Foster, N. Comparison of chemical composition, antioxidant and antimicrobial activity of lavender (*Lavandula angustifolia* L.) essential oils extracted by supercritical CO_2, hexane and hydrodistillation. *Food Bioprocess Tech.* **2013**, *6*, 3481–3489. [CrossRef]
18. Andrys, D.; Kulpa, D.; Grzeszczuk, M.; Bihun, M.; Dobrowolska, A. Antioxidant and antimicrobial activities of *Lavandula angustifolia* Mill. field-grown and propagated in vitro. *Folia Hortic.* **2017**, *29*, 161–180. [CrossRef]
19. Jianu, C.; Pop, G.; Gruia, A.T.; Horhat, F.G. Chemical composition and antimicrobial activity of essential oils of lavender (*Lavandula angustifolia*) and lavandin (*Lavandula* × *intermedia*) grown in Western Romania. *Int. J. Agric. Biol.* **2013**, *15*, 772–776.
20. Lammerink, J.; Wallace, A.R.; Porter, N.G. Effects of harvest time and postharvest drying on oil from lavandin (*Lavandula* × *intermedia*). *N. Z. J. Crop Hortic. Sci.* **1989**, *17*, 315–326. [CrossRef]

21. Sadowska, U. The influence of the lavender and lavendine drying method on the plant material quality. *J. Res. Appl. Agric. Eng.* **2012**, *57*, 83–85.
22. Zheljazkov, V.D.; Cantrell, C.L.; Astatkie, T.; Jeliazkova, E. Distillation time effect on lavender essential oil yield and composition. *J. Oleo Sci.* **2013**, *62*, 195–199. [CrossRef] [PubMed]
23. Mirjalili, M.H.; Salehi, P.; Vala, M.M.; Ghorbanpour, M. The effect of drying methods on yield and chemical constituents of the essential oil in *Lavandula angustifolia* Mill. (*Lamiaceae*). *Plant Physiol. Rep.* **2019**, *24*, 96–103. [CrossRef]
24. Kuş, Ç.; Duru, M.E. Effects of post-harvest drying times of *Lavandula angustifolia* and *L. intermedia* species on chemical components of their essential oils. *Avrupa Bilim Teknol. Derg.* **2021**, *21*, 501–505. [CrossRef]
25. Adaszyńska, M.; Swarcewicz, M.; Markowska-Szczupak, A. Porównanie składu chemicznego i aktywności przeciwdrobnoustrojowej olejku eterycznego otrzymanego z różnych krajowych odmian lawendy wąskolistnej *(Lavandula angustifolia* L.). *Post Fitoter* **2013**, *2*, 90–96. Available online: http://polona.pl/item/46238610 (accessed on 20 October 2022).
26. Bombarda, I.; Dupuy, N.; Le Van Da, J.P.; Gaydou, E.M. Comparative chemometric analyses of geographic origins and compositions of lavandin var. Grosso essential oils by mid infrared spectroscopy and gas chromatography. *Anal. Chim. Acta* **2008**, *613*, 31–39. [CrossRef]
27. European Committee for Antimicrobial Susceptibility Testing (EUCAST) of the European Society of Clinical Microbiology and Infectious Diseases (ESCMID). Determination of minimum inhibitory concentrations (MICs) of antibacterial agents by broth dilution. *Clin. Microbiol. Infect. Dis.* **2003**, *9*, ix–xv. [CrossRef]
28. Malm, A.; Grzegorczyk, A.; Biernasiuk, A.; Baj, T.; Rój, E.; Tyśkiewicz, K.; Dębczak, A.; Stolarski, M.J.; Krzyżaniak, M.; Olba-Zięty, E. Could supercritical extracts from the aerial parts of *Helianthus salicifolius* A. Dietr. and *Helianthus tuberosus* L. be regarded as potential raw materials for biocidal purposes? *Agriculture* **2021**, *11*, 10. [CrossRef]
29. Wells, R.; Lis-Balchin, M. Perfumery uses of lavender and lavandin oils. In *Lavender: The Genus Lavandula*; Lis-Balchin, M., Ed.; CRC Press: Boca Raton, FL, USA, 2002; pp. 208–213.
30. Charles, D.J.; Renaud, E.N.; Simon, J.E. Comparative study of essential oil quantity and composition from ten cultivars of organically grown lavender and lavandin. In *Lavender: The Genus Lavandula*; Lis-Balchin, M., Ed.; CRC Press: Boca Raton, FL, USA, 2002; pp. 246–256.
31. Raut, J.S.; Karuppayil, S.M. A status review on the medicinal properties of essential oils. *Ind. Crops Prod.* **2014**, *62*, 250–264. [CrossRef]
32. Kokina, M.; Salević, A.; Kalušević, A.; Lević, S.; Pantić, M.; Pljevljakušić, D.; Nedović, V. Characterization, antioxidant and antibacterial activity of essential oils and their encapsulation into biodegradable material followed by freeze drying. *Food Sci. Biotechnol.* **2019**, *57*, 282. [CrossRef] [PubMed]
33. Lindsay, J.A.; Holden, M.T. *Staphylococcus aureus*: Superbug, super genome? *Trends Microbiol.* **2004**, *12*, 378–385. [CrossRef] [PubMed]
34. Burt, S. Essential oils: Their antimicrobial properties and potential application in foods—A review. *Int. J. Food Microbiol.* **2004**, *94*, 223–253. [CrossRef] [PubMed]
35. Hyldgaard, M.; Mygind, T.; Meyer, R.L. Essential oils in food preservation: Mode of action, synergies, and interactions with food matrix components. *Front. Microbiol.* **2012**, *3*, 12. [CrossRef] [PubMed]
36. Trombetta, D.; Castelli, F.; Sarpietro, M.G.; Venuti, V.; Cristani, M.; Daniele, C.; Saija, A.; Mazzanti, G.; Bisignano, G. Mechanisms of antibacterial action of three monoterpenes. *Antimicrob. Agents Chemother.* **2005**, *49*, 2474–2478. [CrossRef] [PubMed]
37. De Rapper, S.; Viljoen, A.; van Vuuren, S. The in vitro antimicrobial effects of *Lavandula angustifolia* essential oil in combination with conventional antimicrobial agents. *Evid.-Based Complement. Altern. Med.* **2016**, *2016*. [CrossRef] [PubMed]
38. Malm, A. Olejki eteryczne w profilaktyce i leczeniu chorób infekcyjnych (Essential oils in the prevention and treatment of infectious diseases). *PZWL Wydaw. Lek.* **2021**, *25–28*, 42–48.
39. Marín, I.; Sayas-Barberá, E.; Viuda-Martos, M.; Navarro, C.; Sendra, E. Chemical composition, antioxidant and antimicrobial activity of essential oils from organic fennel, parsley, and lavender from Spain. *Foods* **2016**, *5*, 18. [CrossRef] [PubMed]
40. Fisher, K.; Phillips, C.A. The effect of lemon, orange and bergamot essential oils and their components on the survival of *Campylobacter jejuni*, *Escherichia coli* O157, *Listeria monocytogenes*, *Bacillus cereus* and *Staphylococcus aureus* in vitro and in food systems. *J. Appl. Microbiol.* **2006**, *101*, 1232–1240. [CrossRef] [PubMed]