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Comparative In Vitro Evaluation of Selected Essential Oils and Commercial Blends Against Skin-Associated Pathogens

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Abstract

Essential oils are widely used in cosmetic products and are valued for their antimicrobial properties. In this study, the in vitro antimicrobial activity of five pure essential oils (EOs) and five commercially available EO blends was comparatively evaluated against six skin-associated pathogens: *Staphylococcus aureus*, *Streptococcus pyogenes*, *Escherichia coli*, *Pseudomonas aeruginosa*, *Candida lusitaniae* and *Candida guilliermondii*. Chemical profiling of volatile constituents was performed using gas chromatography–mass spectrometry; antimicrobial activity was assessed by broth microdilution to determine minimum inhibitory concentration (MIC), as well as spot-inoculation to determine minimum bactericidal concentration (MBC). Results revealed microorganism-dependent inhibitory and bactericidal activity of the tested essential oils. *Candida* spp. and *E. coli* were the most susceptible microorganisms, whereas *P. aeruginosa* exhibited the lowest susceptibility to essential oils. *Pinus sylvestris* showed comparatively lower MIC and MBC values across most tested microorganisms. Commercial EO blends showed comparable antimicrobial activity to individual essential oils. Overall, this study provides a comparative in vitro screening of selected essential oils and commercial blends relevant to cosmetic applications, indicating that EO blends do not exhibit superior antimicrobial activity over pure oils under the tested conditions.

Keywords: essential oils; skin-associated pathogens; *Staphylococcus aureus*; *Pseudomonas aeruginosa*; antimicrobial; essential oil blends

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1. Introduction

Skin and soft tissue infections are common worldwide and represent a clinical and public health challenge. The human skin functions as a complex physical, chemical and immunological barrier, which limits colonization and invasion by a wide range of microorganisms. Disruption of this barrier, alterations in skin microbiota, or immune system dysregulation can facilitate infection by opportunistic and pathogenic bacteria and fungi, including *Staphylococcus aureus*, *Streptococcus pyogenes*, *Pseudomonas aeruginosa* and *Candida* species [1,2]. These microorganisms are frequently associated with superficial infections,

chronic wounds and nosocomial infections, which remain challenging due to their different susceptibility to antimicrobial agents [2,3].

Essential oils are complex mixes of volatile aromatic compounds such as terpenes, terpenoids and other constituents, which exert antimicrobial activity through multiple mechanisms [4]. Extracts from medicinal and aromatic plants have long been used in traditional practices and are widely incorporated into cosmetic and topical products [5,6]. In recent decades, essential oils have gained scientific interest due to their antimicrobial and antioxidant properties. The antimicrobial activity of EOs could be attributed to disruption of the cell membrane or alteration of its permeability, interference with metabolism, inhibition of *quorum sensing* and biofilm formation [7]. Numerous in vitro studies have demonstrated inhibitory activity of individual essential oils against common skin pathogens. Oils derived from *Melaleuca alternifolia* (tea tree) [8–10], *Lavandula angustifolia* (lavender) [11–13], *Thymus vulgaris* (thyme) [14,15], *Eucalyptus globulus* [16,17], *Cinnamomum* sp. [18,19] and other plants have shown antimicrobial activity against Gram-positive and Gram-negative bacteria and yeasts, including drug-resistant strains [19,20]. However, the efficacy of EOs varies greatly depending on biological source, extraction method and type of microorganisms they were tested on, highlighting the importance of standardized evaluation [5].

Blending essential oils are widely marketed and used in cosmetic and topical applications. However, systematic comparative data evaluations of blends and pure essential oils remain limited. The topical application of essential oils is relevant in dermatological and cosmetic contexts. Due to their lipophilic nature, essential oils can penetrate the *stratum corneum*, potentially inhibiting microorganisms at the site of infection [21]. Moreover, essential oils exhibit anti-inflammatory, antioxidant and wound-healing properties, which may provide additional therapeutic benefits [6,9]. Nevertheless, skin sensitization, allergic reactions and variability in oil composition demand careful evaluation to ensure both efficacy and safety.

Increasing interest in plant-derived antimicrobials led to this study, which investigates the antimicrobial effects of selected pure EOs and commercially available blends against several skin-associated pathogens. Although the antimicrobial activity of individual essential oils has been extensively studied, direct and standardized comparisons remain limited. In this study, a side-by-side comparison of pure essential oils and commercially available blends was performed under identical experimental conditions against the same panel of skin-associated microorganisms, which allows an objective evaluation of measurable antimicrobial advantages between pure essential oils and EO blends. The findings may contribute to the development of essential oil formulations and their application in dermatological and clinical practice.

2. Materials and Methods

2.1. Essential Oil Composition

A total of 10 essential oils were used for this study, including five pure EOs and five commercial blends (Table 1) provided by JSC "Kvapų namai" (Vilnius, Lithuania) as 100% stock oils. Gas chromatography–mass spectrometry (GC/MS) was applied to determine the chemical composition of pure essential oils and their mixtures. An amount of 10 µL of each sample was dissolved in 1 mL of pentane and diethyl ether (1:1) mixture. An amount of 1 µL of the prepared solution was injected into the GC/MS system. Analyses were performed using Shimadzu GC/MS-Q2010 PLUS (Shimadzu Corporation, Kyoto, Japan) interfaced to a Shimadzu GC-MS-QP2010 ULTRA mass spectrometer (Shimadzu Corporation, Kyoto, Japan) and fitted with a non-polar Rxi-5MS (30 m × 0.25 mm × 0.25 µm) capillary column (Restek, Bellefonte, PA, USA). Mass spectra in electron impact mode were generated at 70 eV, 0.97 scans per second, at a mass range of 33–400 m/z. The oven

temperature of the gas chromatograph was set at 50 °C (for 1 min) and then increased by 5 °C per minute to reach 160 °C, then held for 2 min and programmed to reach 250 °C at the increased rate of 10 °C/min and held at the final temperature for 4 min. A flow rate of 1.0 mL/min was used for the carrier gas. Detector and injector temperatures were 250 °C and the ions' source temperature was 220 °C.

Table 1. List of essential oil blends and pure essential oils included in the study, with corresponding plant sources.

Code	Source
M1	*Picea mariana*, *Citrus limonum*, *Thymus zygis*, *Eucalyptus globulus*, *Melaleuca alternifolia*
M2	*Melaleuca alternifolia*, *Pinus sylvestris*, *Cimbopogon martini* var. *motia*, *Eucalyptus globulus*, *Thymus hyemalis*
M3	*Melaleuca alternifolia*, *Pinus sylvestris*, *Boswellia carterii*, *Ravensara aromatica*, *Rosmarinus officinalis*, *Cimbopogon citratus*
M4	*Eucalyptus globulus*, *Abies balsamea*, *Eucalyptus radiata*, *Cinnamonum camphora linaloolifera*, *Backhousia citriodora*, *Eucalyptus staigerania*
M5	*Cinnamonum camphora linaloolifera*, *Rosmarinus officinalis*, *Citrus limonum*, *Eucalyptus globulus*, *Eugenia caryophylus*, *Cinnamonum cassia*
P1	*Melaleuca alternifolia*
P2	*Eucalyptus globulus*
P3	*Pinus sylvestris*
P4	*Citrus limonum*
P5	*Thymus hyemalis*

The qualitative analysis and identification of compounds were based on the comparison of retention times and indices of the column, with corresponding data in the literature [22], as well as computer libraries of mass spectra (Wiley and NIST) using "GC/MS solution" v. 2.71 software (Shimadzu Corporation, Kyoto, Japan). The identification of the compound was approved if mass spectra library data matched the computer data with a probability equal to 90% or above. The retention indices were determined regarding retention times of a series of n-alkanes (C7–C30) with linear interpolation. The relative percentage of essential oil constituents was computed from the chromatogram peak areas with none of the correction factors.

2.2. Preparation of Essential Oil Working Solutions

A water and essential oil emulsion was obtained as previously described by Man et al. (2021) with some modifications [23]. An amount of 500 µL of essential oil stocks (100%) was subsequently diluted with distilled water at a 1:1 (*v*/*v*) ratio and 1% (*v*/*v*) of dimethyl sulfoxide (DMSO, Sigma-Aldrich, Hamburg, Germany) was added to facilitate dispersion [24]. The mixtures were subjected to sonication at 40 kHz for 20 min at room temperature using a VibraCell™ (Sonics & Materials, Inc., Newtown, CT, USA) ultrasonic processor until uniform emulsions were formed. These suspensions were considered as stock working solutions of the respective essential oils and were freshly prepared prior to each experiment.

2.3. Bacterial Strains

A total of six human pathogens were used in this study in order to evaluate their susceptibility to the essential oils, and their blends were kindly provided by Prof. Dr. Eglė Lastauskienė and Dr. Kotryna Čekuolytė from the Department of Microbiology and Biotechnology, Institute of Biosciences, Life Sciences Centre, Vilnius University, Lithuania. The following microorganisms were used in this study, two Gram-positive

bacteria strains, *Staphylococcus aureus* ATCC29213 and *Streptococcus pyogenes* ATCC12384; two Gram-negative bacteria, *Escherichia coli* and *Pseudomonas aeruginosa* ATCC27853; and two yeast strains, *Candida lusitaniae* (accession No. KJ396345) and *C. guilliermondii* (accession No. KJ396344), previously isolated from the skin of patients with atopic dermatitis and identified by 26S rDNA sequence analysis [25]. No specific antimicrobial resistance profiles were determined prior to the determination of MIC or MBC.

2.4. Determination of the Minimum Inhibitory Concentrations

For each microbial strain, two sterile 96-well microplates were prepared. In the first well of each row, 200 µL of the working solution was added, while 100 µL of double-concentrated Mueller–Hinton II broth (Biolab Zrt., Budapest, Hungary) (for bacteria) or Yeast Peptone Dextrose (YPD, Biolab Zrt., Budapest, Hungary) medium (for yeasts) was distributed into wells 2–12. Two-fold serial dilutions were performed by transferring 100 µL sequentially between wells using a multichannel pipette, discarding 100 µL from the final well.

Bacterial strains were subcultured on tryptic soy agar (TSA) (Biolab Zrt., Budapest, Hungary) and yeasts on Sabouraud agar (Biolab Zrt., Budapest, Hungary) 48 h prior to testing. A standardized inoculum of 0.5 McFarland was prepared for each strain. An amount of 10 µL of inoculum was added to 9.9 mL of double-concentrated media (Mueller–Hinton II broth for bacteria and YPD for yeasts). An amount of 100 µL of the prepared inoculum was added to each well in order to achieve approximately 2×10^5 CFU per well in a final volume of 200 µL. The working solutions contained 500 µL of essential oil in total and 1 mL volume of emulsion. Therefore, after mixing 100 µL of the emulsion with 100 µL of inoculated medium, the highest final tested EO concentration corresponded to 250 µL/mL, followed by two-fold serial dilutions: 125 µL/mL, 62.5 µL/mL, 31.25 µL/mL, 15.63 µL/mL, 7.8 µL/mL, 3.9 µL/mL, 1.95 µL/mL, 1 µL/mL, 0.5 µL/mL, 0.25 µL/mL and 0.125 µL/mL (alternatively, concentrations could be expressed as 25%, 12.5%, 6.25%, 3.125%, 1.56%, 0.78%, 0.39%, 0.2%, 0.1%, 0.05%, 0.025% and 0.012% of essential oil in the final suspension). Negative controls of each microorganism inoculum in corresponding media containing 1% (*v*/*v*) DMSO, as well as controls without DMSO, were included. Plates were incubated at 35 °C for 18 h under aerobic conditions. Growth controls containing 1% (*v*/*v*) DMSO showed no antimicrobial effect, indicating that DMSO did not influence the results. The MIC was defined as the lowest essential oil concentration with no visible microbial growth.

2.5. Determination of the Minimum Bactericidal Concentrations

Minimum bactericidal concentrations (MBCs) were determined by subculturing aliquots from MIC assay wells that showed no visible microbial growth. Briefly, 5 µL from the last five growth-negative wells of each microplate row were transferred on Mueller–Hinton II agar plates, labelled according to the corresponding 96-well plate coordinates, and spot-inoculated. The plates were then incubated at 35 °C overnight under aerobic conditions. Following incubation, colony formation was assessed visually. The MBC was defined as the lowest essential oil concentration that resulted in no bacterial colony growth on the agar surface [23].

Standard reference antimicrobial agents were not included as positive controls because the objective of this study was to compare the relative antimicrobial performance of pure essential oils and commercial blends rather than to benchmark their activity against conventional therapeutics.

2.6. Statistical Analysis

Principal component analysis (PCA) was employed in XLSTAT software (version 2020.1, Addinsoft, New York, NY, USA). PCA was applied as a descriptive tool to visualize differences in volatile composition among samples and was not used to predict or quantitatively correlate chemical profiles with antimicrobial activity.

All data were expressed as mean values (±standard error) of at least three independent replicates. MIC and MBC values were plotted on a $\log_2$-scaled y-axis due to a wide concentration range. The normality of data distribution was assessed using the Shapiro–Wilk test. Because the data did not follow a normal distribution, non-parametric tests were applied. Differences among microbial species and essential oil formulations were analyzed using the Kruskal–Wallis test, followed by Dunn's post hoc test with Bonferroni adjustment for multiple comparisons. Statistical significance was set at $p < 0.05$. Descriptive statistics, boxplots and rank comparisons were generated to visualize MIC and MBC distributions. All statistical analyses were performed using Microsoft Excel (version 1808, Microsoft®, Redmond, WA, USA) and IBM SPSS Statistics for Windows (version 30.0.0.0. (172), IBM Corp., Armonk, NY, USA).

3. Results

3.1. Chemical Composition of Essential Oils and Principal Component Analysis

The detailed chemical composition of all the essential oils and their blends used in this study was determined by gas chromatography–mass spectrometry. Only those compounds with a content exceeding 1% in at least one of the samples are listed in the Supplementary Material (Table S1). Compounds with less than this amount or with only traces detected are listed below the table in the order of their exit from the column.

The richest by volatile constituents was sample M5, in which 131 compounds were identified. Half as many compounds (62) were identified in samples P1 and P4. 1,8-cineole was identified as the first dominant compound in the three samples (M4, M5 and P2) used in this study. Three more samples (M1, M3 and P1) were characterized as containing the highest amount of terpinen-4-ol. *σ*-3-Carene, *para*-cymene, limonene and geraniol were the major contributors in one sample each (P3, P5, P4 and M2, respectively). Of the 42 compounds listed in Table S1, as many as fourteen were detected at greater than 1% in only one of the eleven samples. In the other samples, the levels of these compounds ranged from not detected at all to almost 1%. *d*-3-carene (37.56% in P3), limonene (66.27% in P4), 1,8-cineole (42.97% and 47.97% in M4 and P2) and terpinen-4-ol (44.08% in P1) were the predominant compounds with more than a third of the samples. It should be pointed out that α-pinene was the only compound present at levels greater than 1% in all samples used in the study.

Principal component analysis (PCA) was applied to visualize volatile compound profiles. The first two principal components explained 48.5% of the total variance, with PC1 contributing 27.83% and PC2 contributing 20.67% (Figure 1). Separation along PC1 was primarily associated with differences in monoterpene hydrocarbons and oxygenated monoterpenes, including terpinen-4-ol, γ-terpinene, p-cymene, α-terpinene, α-pinene, β-pinene, and δ-3-carene. PC2 was influenced mainly by compounds such as linalool, eucalyptol, bornyl acetate, and geraniol. The PCA score plot showed distinct clustering of the samples. P1 showed separation along the positive PC1 axis, while P3 and M4 clustered on the negative side, indicating differences in volatile composition. M1 exhibited a pronounced positive score along PC2, while most other samples clustered near the origin, reflecting more similar chemical profiles. Overall, PCA revealed compositional variability among the samples, with specific volatile compounds playing a crucial role in distinguishing the sample groups.

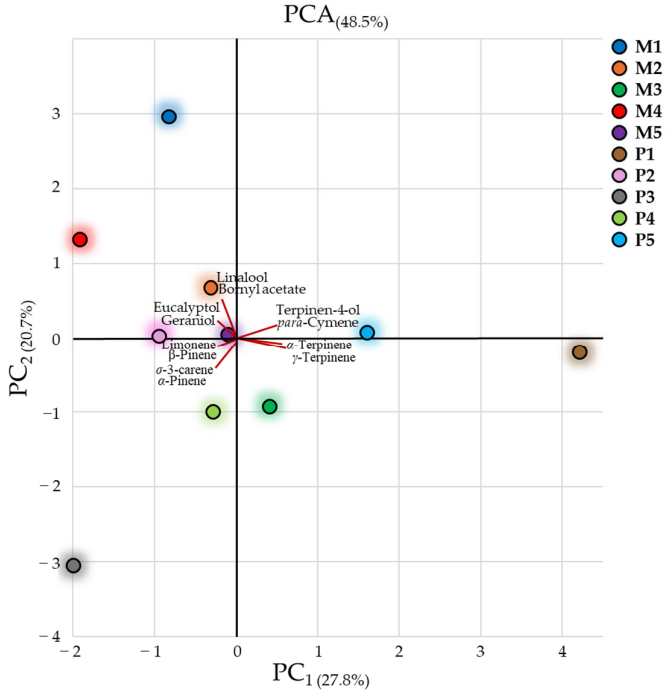

Figure 1. Principal component analysis of predominant compounds in tested essential oils and their blends.

3.2. Minimum Inhibitory Concentrations

In total five pure essential oils and five commercial EO blends were tested against six skin-associated pathogens: *S. aureus*, *S. pyogenes*, *E. coli*, *P. aeruginosa*, *C. lusitaniae* and *C. guilliermondii*. The lowest MIC values among the tested oils were shown by the pure EO of *Pinus sylvestris* (P3), followed by oil blend M2 containing *Melaleuca alternifolia* and *Pinus sylvestris* (Table 2).

Table 2. Mean MIC and MBC values of essential oil blends (M1–M5) and pure EOs (P1–P5) against tested microorganisms (µL/mL), from at least three independent replicates. Full table with standard errors provided in Supplementary Material Table S2.

Microorganism	Means of Essential Oil Blends					Means of Pure Essential Oils				
	M1	**M2**	**M3**	**M4**	**M5**	**P1**	**P2**	**P3**	**P4**	**P5**
					MIC					
S. aureus	3.43	1.75	3.43	1.75	3.43	1.75	1.00	0.63	4.88	2.95
S. pyogenes	6.83	1.75	2.95	1.50	2.48	2.48	1.50	0.88	3.27	4.88
P. aeruginosa	23.44	23.44	39.06	31.25	23.44	23.44	46.88	23.44	35.16	70.31
E. coli	0.80	0.45	0.45	0.45	0.65	0.65	1.00	0.80	0.70	0.40
C. lusitaniae	0.50	0.33	0.50	0.25	1.00	0.50	0.50	0.25	0.25	0.25
C. guilliermondii	0.50	0.25	0.50	0.50	0.42	0.50	0.50	0.25	0.25	0.25
Grand mean	5.91	4.66	7.81	5.95	5.23	4.89	8.56	4.37	7.42	13.17
					MBC					
S. aureus	54.69	46.88	62.50	31.25	23.44	31.25	62.50	5.38	19.53	11.71
S. pyogenes	54.69	39.06	39.06	19.53	23.44	27.34	46.88	2.48	8.78	10.41
P. aeruginosa	54.69	54.69	54.69	78.13	78.13	66.41	101.56	78.13	39.06	109.38
E. coli	1.30	0.70	0.70	0.75	0.80	1.10	1.20	1.10	0.70	0.55
C. lusitaniae	1.33	0.67	1.33	0.67	0.67	0.67	1.33	0.42	0.42	0.25
C. guilliermondii	0.83	0.42	0.67	0.50	0.42	0.67	0.50	0.25	0.42	0.25
Grand mean	27.92	23.73	26.49	21.80	21.15	21.24	35.66	14.62	11.48	22.09

Pure essential oils and commercial blends showed good antimicrobial activity against *E. coli* and *Candida* species. However, elevated essential oil concentrations were required to inhibit *Staphylococcus*, *Streptococcus* and especially *Pseudomonas aeruginosa*. Pure essential oils from *Eucalyptus globulus* (P2) and *Pinus sylvestris* (P3) showed similar activity and MIC values against *S. aureus* and *S. pyogenes*. The lowest MIC value against *P. aeruginosa* was achieved by the pure essential oil from *Pinus sylvestris*. Although the pure essential oil of *Thymus hyemalis* (P5) showed great activity against *E. coli* and *Candida* species, it required the highest concentration of 70 µL/mL against *P. aeruginosa* (Figure 2).

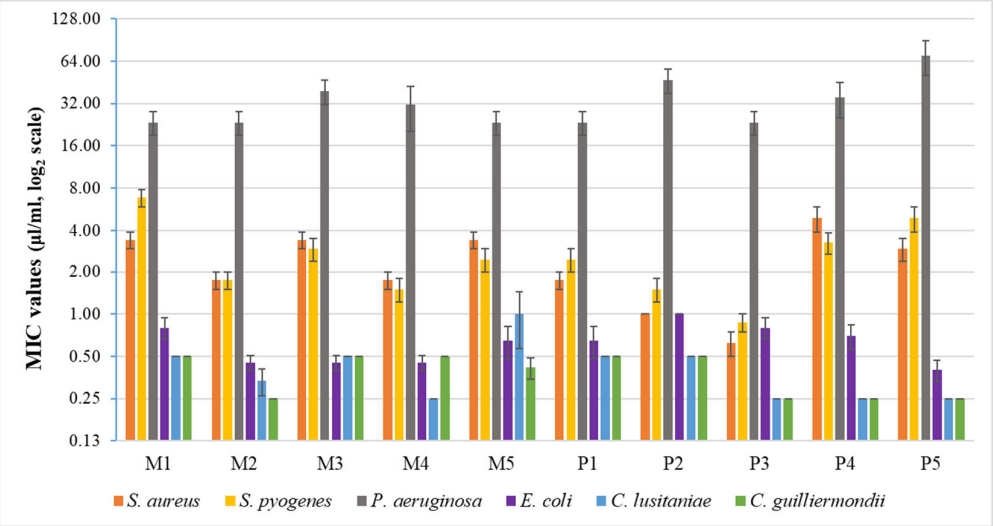

Figure 2. Minimum inhibitory concentrations of pure essential oils (P1–P5) and oil blends (M1–M5) against *Staphylococcus aureus*, *Streptococcus pyogenes*, *Pseudomonas aeruginosa*, *Escherichia coli*, *Candida lusitaniae* and *Candida guilliermondii*. Data presented as mean of MIC values ± standard error from at least three independent replicates on a $\log_2$-scaled y-axis.

Oil blend M2 containing *Melaleuca alternifolia* and *Pinus sylvestris* oils showed better antimicrobial activity than M3, suggesting that the formulation of oil blends may influence antimicrobial efficacy. When we compared essential oils M2 and M3 to the P1 pure *Melaleuca alternifolia* EO, the overall results were similar ($p > 0.05$). M4 blend containing *Eucalyptus globulus* performed slightly better than P2 pure oil against most pathogens, including *P. aeruginosa* and *S. pyogenes*; however, pure oil showed better MIC values against *S. aureus*. The *Citrus limonum* EO blend M1 showed slightly worse overall results than pure oil P4, except against *S. aureus*.

Commercial essential oil blends exhibited MIC ranges comparable to those of pure essential oils. Some blends showed slightly lower mean MIC values against selected microorganisms compared with individual oils. However, these differences were not statistically significant following correction for multiple comparisons. Overall, no consistent trend indicating superior inhibitory activity of blends over pure oils was observed.

3.3. Minimum Bactericidal Concentrations

Bactericidal activity of EOs was tested with spot-inoculation of the five last wells from 96-well plate where no visible growth was observed. The most effective of the tested oils was the pure EO of *Pinus sylvestris* (P3) (Figure 3), followed by the pure oil of *Citrus limonum* (P4) (Table 2). The P2 and P5 pure essential oils were the least effective against *Pseudomonas aeruginosa* of all the tested oils, with MBCs in the range of 100–110 µL/mL.

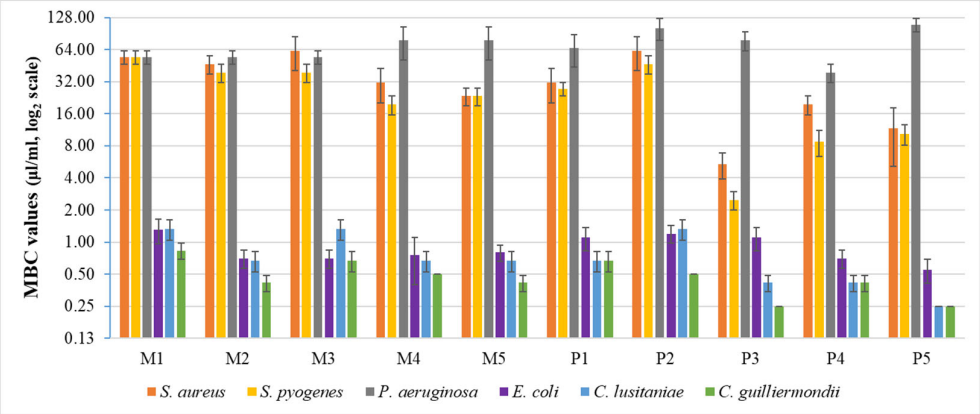

Figure 3. Minimum bactericidal concentrations of pure essential oils (P1–P5) and oil blends (M1–M5) against *Staphylococcus aureus*, *Streptococcus pyogenes*, *Pseudomonas aeruginosa*, *Escherichia coli*, *Candida lusitaniae* and *Candida guilliermondii.* Data presented as mean of MBC values ± standard error from at least three independent replicates on a $\log_2$-scaled y-axis.

Melaleuca alternifolia- and *Pinus sylvestris*-containing oil blend M2 overall had slightly better MBC values than M3. However, comparing M2 and M3 blends to pure P1 *Melaleuca alternifolia*, antimicrobial activity was similar, with M2 showing slightly lower MBC values against *E. coli* and *Pseudomonas* compared to pure oil. Essential oil blends, compared to the pure oil of *Pinus sylvestris*, were not as effective. The M4 mix containing *Eucalyptus globulus* showed lower MBC values compared to P2 pure oil against all pathogens except *C. lusitaniae*, where results were similar. The pure essential oil of *Thymus hyemalis* (P5) tested moderately in our experiment, showing the lowest MBC values against *E. coli*, *C. lusitaniae* and *C. guilliermondii*. Despite showing the highest MBC values against *P. aeruginosa*, P5 MBC values were one of the lowest against *Staphylococcus* and *Streptococcus* compared to other tested oils. *Eucalyptus globulus* (P2) showed good inhibitory activity against the tested *Staphylococcus* and *Streptococcus*; however, it was not as effective in bacteria eradication.

Similarly to MIC results, commercial blends showed bactericidal activity profiles comparable to those of pure essential oils. No statistically significant differences in MIC or MBC values were observed between essential oil blends and pure oils across the tested microorganisms (adj. $p > 0.05$). Based on the post hoc Dunn–Bonferroni test, significant differences in the antimicrobial activity of EOs M2, M4, P2, and P3 compared with other oils were observed only within the *S. aureus* and *S. pyogenes* groups (adj. $p < 0.05$).

Statistical analysis confirmed significant differences in MIC values among tested microorganisms ($p < 0.001$). *Candida guilliermondii*, *C. lusitaniae* and *E. coli* exhibited significantly lower MIC values compared with *Staphylococcus aureus*, *Streptococcus pyogenes* and *Pseudomonas aeruginosa*. Results were similar significantly within pairs of *S. aureus* and *S. pyogenes*, as well as *Candida* spp. and *E. coli* (adj. $p < 0.05$). Differences in the MIC values among the remaining microbial groups were statistically significant (adj. $p < 0.05$). Similarly, MBC values differed significantly among the tested microorganisms ($p < 0.001$), with similar values detected between *S. aureus*, *S. pyogenes* and *P. aeruginosa* (adj. $p > 0.05$). These bacteria were significantly less susceptible to the tested essential oils than *Candida* spp. and *E. coli* ($p < 0.001$).

4. Discussion

The present study provides a comparative in vitro evaluation of selected pure essential oils and commercially available essential oil blends against several skin-associated pathogens. PCA highlighted compositional differences among pure essential oils and blends and no quantitative correlation between PCA and antimicrobial activity was performed; therefore, any interpretations should be considered descriptive.

The results demonstrate clear species-dependent susceptibility patterns, reflecting known structural and biochemical differences among microorganisms [9]. Among the tested pathogens, *P. aeruginosa* showed the lowest susceptibility to all tested essential oils, consistent with previous reports describing its intrinsic resistance mechanisms, including low membrane permeability and active efflux pumps [7,15,19,26,27]. In contrast, *Candida* spp. and *E. coli* showed the highest susceptibility to the essential oils, consistent with earlier reports highlighting the strong antifungal and antibacterial activities of tea tree, thyme, pine and eucalyptus oils against yeasts and Gram-negative rods [9,28–30]. *S. aureus* and *S. pyogenes* required higher MIC and MBC values, in agreement with previous studies [4,9,12,16,28].

Across the tested EOs, *Pinus sylvestris* (P3) showed the lowest MIC and MBC values. This corresponds with results found in the literature, demonstrating strong antimicrobial activity of the main constituents of pine oils [31,32]. *Thymus hyemalis* (P5) exhibited strong activity against *E. coli* and *Candida* spp. but was not as effective against *P. aeruginosa*, consistent with prior reports indicating variable effectiveness of thymol-rich oils [23,31,33]. *Eucalyptus globulus* oil showed moderate inhibitory effects against Gram-positive bacteria, while higher concentrations were required to achieve bactericidal activity, highlighting differences between growth inhibition and microbial eradication.

Comparison between pure essential oils and commercial blends revealed largely comparable antimicrobial activity profiles, with no statistically significant differences in MIC or MBC values across the tested microorganisms. Some blends containing *Pinus sylvestris*, *Melaleuca alternifolia* or *Eucalyptus* species showed slightly lower mean inhibitory concentrations compared with individual oils. The lack of superior antimicrobial activity of commercial blends compared with pure essential oils may be explained by several factors. Blending multiple oils can lead to the dilution of key bioactive constituents that are present at higher concentrations in single oils. In addition, interactions between individual components may result in additive, synergistic, or antagonistic effects. While synergism can enhance efficacy, antagonistic interactions may reduce overall antimicrobial activity, likely contributing to comparable results of the tested blends and pure oils [34,35].

It is often assumed that essential oils are not dangerous because they are obtained from plant raw materials. However, certain essential oils may cause dose-dependent skin reactions, irritation of the eyes and mucous membranes, other allergic reactions, photosensitization phenomena or even exhibit potentially carcinogenic effects [36–39]. The 1% concentration of the formulation (comparable to 10 µL/mL concentration used in this study), typically considered safe for dermal application, is based on the established guidelines from the International Fragrance Association (IFRA) on allergenic components and permissible concentrations to enhance product safety [37], as well as the guidelines addressed by the Council of Europe for cosmetic manufacturers [38]. The MIC and MBC values reported in this study provide in in vitro reference ranges for the relative antimicrobial potential of essential oils. However, translation to finished cosmetic products must consider dilution, formulation, volatility, skin tolerance, and regulatory constraints. Notably, bactericidal concentrations, particularly against *S. aureus*, *S. pyogenes* and *Pseudomonas aeruginosa*, exceed the 10 µL concentration which is considered safe [37,38]. Therefore, from a cosmetic formulation perspective, these findings suggest that essential oils and blends may be selected primarily for sensory characteristics and formulation stability as preservative agents, rather bactericidal, based on marketing considerations rather than enhanced antimicrobial efficacy [35,36,40,41].

Limitations. Several limitations of this study must be acknowledged. Commercially available blends were evaluated strictly as commercial end products rather than as defined experimental formulations, which limits reproducibility and precludes detailed mecha-

nistic interpretation of potential synergistic or antagonistic interactions among individual constituents. The present study did not assess synergistic, additive, or antagonistic interactions between individual components of EOs or blends. Therefore, potential interaction effects cannot be excluded and should be addressed in future studies using dedicated synergy assays. Standard reference antimicrobial agents were not included as positive controls because the objective of this study was to compare the relative antimicrobial performance of pure essential oils and commercial blends, not to compare their activity against conventional therapeutics.

Supplementary Materials: The following supporting information can be downloaded at: https://www.mdpi.com/article/10.3390/cosmetics13010039/s1, Table S1: Chemical composition of tested essential oils and their blends determined by gas chromatography–mass spectrometry; Table S2: Mean minimum inhibitory concentrations (MICs) and minimum bactericidal concentrations (MBCs) (± standard error) of essential oil blends (M1–M5) and pure essential oils (P1–P5) against the tested microorganisms (μL/mL), calculated from at least three independent replicates.

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Abbreviations

The following abbreviations are used in this manuscript:

AMR	Antimicrobial resistance
EO	Essential oil
DMSO	Dimethyl sulfoxide
MIC	Minimum inhibitory concentration
MBC	Minimum bactericidal concentration
MDR	Multidrug resistance
TSA	Tryptone soy agar
YPD	Yeast peptone dextrose

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