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Commercial Mentha Teas as Sources of Bioactive Volatile Compounds: Chemical Composition, Chemotypes, and Antimicrobial Activity

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Abstract

Mint (*Mentha* spp.) teas are widely consumed as functional herbal beverages and represent important dietary sources of bioactive volatile compounds. The present study comparatively evaluated the chemical composition and antimicrobial activity of essential oils (EOs) obtained from commercially available *Mentha* tea products purchased in Estonia, Lithuania, and Norway. In 10 EO samples studied, 85 compounds were identified by GC–MS. Carvone was the dominant constituent in the carvone chemotype (up to 58.1%), whereas menthol reached 30.7% in the menthol chemotype. Hierarchical clustering of CLR-transformed GC–MS data revealed three well-defined chemotypes among the analyzed *Mentha* EOs: carvone chemotype (three samples), menthol chemotype (five samples) and mixed, carvone-menthol chemotype (two samples). The antimicrobial activity of EOs was evaluated against 17 clinical antibiotic-sensitive and antibiotic-resistant microorganisms by the agar well diffusion method. The strongest antimicrobial activity was observed against resistant clinical *Staphylococcus aureus* strains, with MIC values as low as 0.10 mg/mL. EOs with a high carvone content consistently showed higher activity, especially against *S. aureus*, including resistant phenotypes. In contrast, menthol-chemotype EOs were associated with reduced antibacterial effectiveness. The obtained results demonstrate that the investigated commercial mint teas differ considerably in their phytochemical composition and biological properties, which may contribute to differences in their potential nutraceutical relevance as functional plant-derived beverages.

Keywords: *Mentha* spp.; mint tea; essential oils; GC–MS; chemotypes; antimicrobial activity

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1. Introduction

The genus Mint (*Mentha* L.) of the family *Lamiaceae* L. includes 25 species [1]. In medicine, the leaves of Peppermint (*Mentha* × *piperita*), a hybrid of spearmint (*M. spicata*) and water mint (*M. aquatica* L.), are used [2]. Considering the wide use of peppermint leaves in the food and perfumery-cosmetics industries and medicine, it is one of the most widely cultivated plants. The global mint oil market size was valued at USD 527.5 million in 2025 [3]. Not only is peppermint used to obtain essential oils (EOs), but also other species that are successfully cultivated, such as primarily spearmint (*M. spicata* L.), Japanese mint (*M. spicata* varietes 'Abura'), and bergamot mint (*M. citrata / M. aquatica* L. var. *citrata*).

M. piperita constitutes one of the most widely consumed single-ingredient herbal teas. For the production of mint tea, also wild mint (*M. arvensis* L.), apple mint, or pineapple mint (*M. suaveolens* Ehrh.) are cultivated.

According to the European Scientific Cooperative on Phytotherapy, whole-dried leaves of *M. piperita* contain not less than 12 mL/kg of essential oil. The cut dried leaves contain not less than 9 mL/kg of essential oil [4]. The content of EO in the leaves of peppermint grown in different regions of the planet varies significantly [5–10].

EOs of *Mentha* species are characterized by considerable chemical variability. In peppermint (*M. piperita*), the dominant constituents include menthol, menthone, carvone, and limonene [11–15]. *M. arvensis* is typically rich in menthol and menthone [16–20], whereas *M. spicata* is commonly characterized by carvone-rich chemotypes, although cineole- and piperitenone oxide-rich profiles have also been reported [12,20–24]. In contrast, *M. suaveolens* usually contains piperitenone, piperitenone oxide, pulegone, and related monoterpenoids rather than menthol or carvone [20,23,25–27]. Such compositional differences are largely determined by chemotype and may substantially influence the biological activity of mint essential oils.

The diverse chemical profiles of peppermint and other mint species, especially the different ratios of dominant EO components across various mint chemotypes, determine a wide range of biological activities, including antimicrobial, antioxidant, anti-inflammatory, and antispasmodic effects [28–30].

M. x piperita exhibited strong activities against *Escherichia coli, Bacillus subtilis, Candida albicans* [31], *Staphylococcus aureus*, *Staphylococcus epidermidis*, *Enterococcus faecalis*, *Listeria monocytogenes*, *Bacillus cereus*, and *Proteus mirabilis* [25]. *M. spicata* significantly inhibited *Salmonella typhi* and *B. subtilis* [31]. *M. suaveolens* inhibited *Klebsiella pneumoniae*, *C. albicans*, and *S. typhi* [31].

The EOs of *M. arvensis* show phytotoxic, genotoxic, cytotoxic and antimicrobial effects against eight medically important bacteria, including multidrug-resistant strains, such as *B. subtilis*, *E. faecalis*, *S. aureus*, methicillin-resistant *S. aureus*, *E. coli*, extended-spectrum β-lactamase-producing *E. coli*, *Pseudomonas aeruginosa* and *Salmonella enterica* subsp. *Enterica serovar Enteritidis* [17].

A high antibacterial effect was given by a carvacrol-thymol chemotype spearmint population. The least effective oil against Gram-negative bacteria was bergamot mint oil. In contrast, in the case of Gram-positive bacteria, oils containing dihydrocarvone as the main compound exhibited the weakest antibacterial effect [23].

According to Kapp et al. [32], *Mentha* spp. EOs showed antibacterial activity against *E. coli*, *S. aureus*, or both. In another study, the same authors [33] showed that *M. piperita* could serve as a potential source of health-promoting agents against *Chlamydia pneumoniae*. Even *M. piperita* candy flavor distillates showed antimicrobial activity against *Yersinia ruckeri*, *Bacillus cereus*, *Bacillus subtilis*, *Bacillus pumilus*, and *Micrococcus luteus*. The strongest bacteriostatic and bactericidal effects were observed on *B. cereus* and *B. subtilis* [34].

Peppermint and other *Mentha* species are widely consumed worldwide as herbal teas and are increasingly recognized as functional beverages with potential health-promoting properties. Beyond their traditional use for relieving gastrointestinal discomfort and symptoms of respiratory tract infections [4], mint teas are also valued as a dietary source of biologically active volatile compounds, particularly EOs rich in monoterpenes [11]. Regular consumption of *Mentha* infusions may modulate the oral and gastrointestinal microbiota and provide diet-derived antimicrobial support [32,33]. Such effects are especially relevant in the context of increasing antimicrobial resistance and growing consumer interest in plant-based preventive health approaches.

Although the chemical composition and antimicrobial activity of individual *Mentha* species have been extensively investigated, comparatively little attention has been paid to whether commercially available mint tea products differ systematically in their chemotypic composition and whether such differences are reflected in antimicrobial activity. Since commercial mint teas represent the form in which *Mentha* products are most commonly consumed, addressing this question is relevant from both phytochemical and nutraceutical perspectives. Thus, the novelty of the present study lies in the combined assessment of chemical composition, chemotype classification, and antimicrobial activity of commercially available mint tea products.

The aim of the present study was to comparatively characterize the chemical composition of EOs obtained from commercially available mint (*Mentha* spp.) teas and to evaluate their antimicrobial activity in relation to the dominant constituents of the oils, with particular emphasis on the potential nutraceutical relevance of commercially consumed mint teas as sources of diet-derived antimicrobial compounds.

2. Materials and Methods

2.1. Plant Materials

The samples were selected to represent commercially available mint tea products sold in supermarkets in Estonia, Lithuania, and Norway, including peppermint (*Mentha* × *piperita*), related *Mentha* species, and mixed mint formulations commonly available to consumers. Botanical authentication was not performed independently, and species identification was based on the information provided by the manufacturers on the product labels. However, the characteristic organoleptic properties, particularly the typical mint aroma, were consistent with the assignment of the products to the genus *Mentha*. Nevertheless, such observations cannot provide unequivocal species-level identification. Abbreviations of samples are shown in Table 1.

Table 1. Samples of Mint Teas Used for the Study.

No.	Product Name	Producer	Mass, Packaging, and Type of Drug	Country of Manufacture	Country Purchased From
1	*M. piperita* (M21)	'Kloster'	60 g pab, ch	Norway	Norway
2	*M. piperita* (M19)	'Natēja'	50 g cb, plb, ch	Lithuania	Lithuania
3	*M. piperita* (M25)	'Tamme Aiandustalu'	20 g pab, leaves	Estonia	Estonia
4	*M. piperita* (M13)	'Apotheka'	20 g cb, plb, ch	Estonia	Estonia
5	*M. piperita* (M49)	'Ööbiku talu'	10 g pab, leaves	Estonia	Estonia
6	*M. piperita* (M50)	'Energia talu'	20 g pab, ch	Estonia	Estonia

Table 1. *Cont.*

No.	Product Name	Producer	Mass, Packaging, and Type of Drug	Country of Manufacture	Country Purchased From
7	*M. suaveolens* (M24)	'Põhjala teetalu'	20 × 1.2 g cb, plb, tb, ch	Estonia	Estonia
8	*M. spicata* (M26)	'Tamme Aiandustalu'	20 g pab, leaves	Estonia	Estonia
9	*M. spicata* + *M. piperita* + *M. arvensis* (M10)	'Pukka'	20 × 1.6 g cb, tipa, ch	UK	Norway
10	*M. spicata* + *M. suaveolens* + *M. piperita* (M47)	'Tamme Aiandustalu'	20 g plb, ch	Estonia	Estonia

Packaging types: cb—cardboard box, tb—teabags, tipa—teabags in paper bags, pab—paper bag, plb—plastic bag. Herb types: ch—chopped herb, leaves.

2.2. Hydrodistillation of EOs

The EOs were hydrodistilled from the mint samples using the modified method described in the European Pharmacopoeia monograph "Peppermint leaf/*Menthae piperita folium*" [35]. The plant materials (20 g of crushed herbal drug) with 200 mL of purified water were hydrodistilled in a 1000 mL round-bottom flask, and hexane (0.5 mL) was added to a graduated tube to remove the distilled EO. The yields of EOs were found by the method described earlier [36].

2.3. Gas Chromatography-Mass Spectrometry

The EO samples were analyzed on an Agilent 6890/5973 GC–MS system, using MSD Chemstation. One microliter of the sample was introduced into the Agilent HP-5MSUI column (30 m length, 0.25 mm inner diameter, 0.25 µm film thickness) using split mode (50–100:1). The injector temperature was 280 °C, and the carrier gas (He) flow was kept constant at 1 mL/min throughout the whole analysis. The oven was held at 50 °C for 2 min, followed by a ramp of 4 °C/min to a final temperature of 280 °C, which was maintained for 5 min.

The MSD was operated in EI mode at 70 eV, scanning across the mass range of 29–400 m/z with a delay time of 4 min and a scan speed of 3.8 scans per second. The data were analyzed using the Agilent MassHunter Software (B.07.04) package, applying a deconvolution algorithm at different window size factors. The resulting compounds were identified by using the NIST23 library with Match Factor $\geq$ 85 and by retention indexes (relative to n-alkanes C8–C30) or obtained by the analysis of the reference compounds. The area percentages of each peak were calculated from the total areas in the chromatograms without using correction factors. The same GC–MS method has previously been successfully used in the analysis of EOs [36,37].

2.4. Bacterial Strains and Media

The antimicrobial activity of EOs was evaluated against clinical antibiotic-sensitive and antibiotic-resistant microorganisms, including Gram-positive bacteria (*Staphylococcus aureus*, *Streptococcus sanguinis* (α-hemolytic), *Streptococcus pyogenes* (β-hemolytic, Group A), *Streptococcus agalactiae* (β-hemolytic, Group B), *Enterococcus faecalis*), Gram-negative bacteria (*Escherichia coli*, *Escherichia fergusonii*, *Klebsiella pneumoniae*, *Klebsiella ozaenae*, *Pseudomonas aeruginosa*, *Acinetobacter baumannii*), and yeast strains (*Candida albicans*, *Candida tropicalis*). Reference strains obtained from the American Type Culture Collection (ATCC) were also included: *Staphylococcus aureus* subsp. *aureus* (ATCC® 29213™), *Escherichia coli* (ATCC® 25922™), and *Klebsiella pneumoniae* (ATCC® 1705™).

Clinical bacterial isolates were identified based on morphological, culture, and biochemical characteristics in accordance with Bergey's Manual of Systematics of Archaea and Bacteria [38], using URiSelect medium, MacConkey agar, and blood agar, as well as STAPHYtest 16, STREPTOtest 16, ENTEROtest 24, and NEFERMENTtest 24 biochemical test systems (Erba Lachema, Brno, Czech Republic), in the Laboratory of Microbiological Research, Ivano-Frankivsk National Medical University. Clinical *E. coli* isolates were further identified by MALDI-TOF mass spectrometry at the bacteriological laboratory of St George's University of London. Fungal isolates were identified using the VITEK 2 system with the VITEK 2 YST ID card (bioMérieux, Lyon, France).

Bacterial strains were cultivated and maintained on Mueller–Hinton agar (MHA) and stored at −20 °C in accordance with EUCAST recommendations. Sabouraud agar was used for *Candida* spp., and blood agar for streptococci. Mueller–Hinton broth (MHB) and Yeast Extract Peptone Dextrose (YPD) broth (HiMedia Laboratories Pvt. Ltd., Mumbai, India) were used for antimicrobial susceptibility testing.

Antimicrobial susceptibility testing was performed by broth microdilution according to EUCAST guidelines. Results were interpreted according to EUCAST clinical breakpoints: version 15.0 (2025) for bacteria and version 11.0 (2024) for yeasts.

2.5. Antimicrobial Activity Testing

The agar well diffusion method was employed for the preliminary evaluation of the antimicrobial activity of 10 EO samples against medically relevant Gram-positive (G+) and Gram-negative (G−) bacteria, as well as yeast strains, in order to select the most active samples for subsequent analysis. Petri dishes were filled with 30 mL of microorganism specific agar medium, and after solidification, wells with a diameter of 4.0 mm were aseptically punched. The agar surface was inoculated as follows: 100 µL of a freshly prepared microbial suspension adjusted to 1×10^7 CFU/mL was applied onto the surface of the agar and evenly spread using a sterile cotton swab to obtain a confluent lawn of growth. After inoculation, the plates were allowed to dry at room temperature for 10–15 min. Subsequently, 20 µL of each EO sample (previously diluted 1:10 in 90% ethanol) was introduced into the wells, while 20 µL of the solvent (90% ethanol) served as the negative control. Inhibition zone diameters (IZDs) were measured after 24 h of incubation for bacterial cultures. The antifungal assay on solid medium was performed using the same agar well diffusion method as applied for bacterial strains. The procedure, including inoculation, well preparation, and sample application, was identical. The only difference was the use of Sabouraud agar as the growth medium for yeast strains. Fungistatic activity was recorded after 48 h, and fungicidal activity after 96 h of incubation [39]. Digital images of the plates were captured and analyzed using UTHSCSA ImageTool version 2.0 software.

Minimum inhibitory concentrations (MICs) and minimum bactericidal/fungicidal concentrations (MBCs/MFCs) of selected EO samples (those producing IZD > 10 mm in the agar well diffusion assay) were determined by a two-fold serial microdilution method in accordance with EUCAST guidelines. The agar diffusion assay was used as a preliminary screening method to identify EO samples exhibiting antimicrobial activity, whereas MIC/MBC/MFC determinations were subsequently performed to obtain quantitative measures of antimicrobial efficacy. In the first well of a sterile 96–well microplate, 190 µL of broth previously inoculated with a microbial suspension (1.0×10^6 CFU/mL) at a ratio of 1:10 (resulting in a final inoculum of approximately 1.0×10^5 CFU/mL) and 10 µL of EO sample (diluted 1:10 in 90% ethanol) were added. The remaining wells were filled with 100 µL of inoculated broth, followed by serial two-fold dilutions. Wells containing broth with microbial inoculum and solvent (90% ethanol) served as growth controls. Microbial growth was assessed by monitoring changes in optical density at 495 nm (OD_{495}) using a

Synergy™ HTX S1LFTA multimode microplate reader (BioTek Instruments, Inc., Winooski, VT, USA) at 2 h intervals. Growth curves were constructed to determine MIC values. The MIC was defined as the lowest EO concentration that inhibited growth by 100% relative to the control. MBCs/MFCs were determined by subculturing 100 µL from wells with concentrations above the MIC onto agar plates, incubating for 24 h at 37 °C, and defining the lowest concentration that completely inhibited microbial growth.

2.6. Statistical Analyses

Statistical analyses were conducted to explore differences in EO composition among samples and species. Relative abundances of identified compounds (Table S1) were grouped into classification groups (monoterpene hydrocarbons, oxygenated monoterpenes, sesquiterpene hydrocarbons, oxygenated sesquiterpenes, and others). Multivariate patterns in chemical composition were evaluated using principal component analysis (PCA) based on centered log-ratio (CLR)–transformed GC–MS data. PCA was applied as an exploratory tool to visualize similarities and differences among EO samples and to assess species-related clustering of EO profiles.

All microbiological experiments were performed in triplicate. Inhibition zone diameters are presented as mean ± standard deviation. Statistical significance relative to the solvent control was assessed using the non-parametric Kruskal–Wallis test, followed by Dunn's post hoc test with Holm correction. A p-value < 0.05 was considered statistically significant. Microbial growth curves were analyzed using BioTek Gen5 software, version 2.0 (BioTek Instruments, Inc., Winooski, VT, USA).

Correlation analyses were performed to identify associations between the relative abundance of EO constituents and antimicrobial activity parameters (IZD, MIC, and MBC/MFC). Quantitative GC–MS profiles were analyzed as raw relative percentages (raw%) and as centered log-ratios (CLR) to reduce compositional effects. For each microbial culture, Spearman's rank correlation (ρ) was used to assess univariate relationships between individual compounds (or compound groups) and antimicrobial activity, with two-sided p-values. Multiple testing was controlled using the Benjamini–Hochberg false discovery rate (BH-FDR) procedure, applied both per culture and globally across all tests, with statistical significance interpreted at $\alpha = 0.05$ (q-values).

For multivariate integration, PLS2 regression was performed with the CLR-scaled compound matrix as predictors (X) and the panel of microbial cultures, excluding missing values, as responses (Y). Variable importance was evaluated using VIP scores, with VIP ≥ 1 considered relevant. For interpretability, scatter plots were generated for selected compound–culture associations, including linear trend lines, 95% confidence intervals, annotated ρ and p-values.

Hierarchical clustering was performed on the same CLR-transformed data using Ward's linkage and Euclidean distance. All statistical analyses were conducted in the Python 3.10 environment using *pandas*, *SciPy*, *scikit-learn*, and *seaborn/matplotlib*. All scripts, processed datasets, and graphical outputs were archived to ensure reproducibility.

3. Results

3.1. Chemical Composition of Essential Oils

In 10 studied EOs, 85 compounds were identified (Tables 2 and S1). The GC–MS chromatograms of the EO of *M. piperita* (sample M19), *M suaveolens* (sample 24), and the mixture of *M. spicata* + *M. piperita* + *M. arvensis* (sample M10) are presented in Figures S1–S3, respectively.

Table 2. The average, minimum and maximum content of identified compounds in commercial mint teas.

Compound	RI Exp	RI Lib	Content, %		
			Average	Minimum	Maximum
(*E*)-2-Hexenal	849	854	0.06	0.03	0.15
α-Thujene	926	929	0.04	0.00	0.13
α-Pinene	932	932	1.11	0.12	1.76
α-Sabinene	972	974	0.81	0.05	1.30
β-Pinene	975	978	1.54	0.17	2.51
1-Octen-3-ol	978	980	0.11	0.00	0.24
β-Myrcene	990	991	0.63	0.05	1.80
3-Octanol	995	993	0.69	0.16	1.83
α-Terpinene	1016	1017	0.20	0.00	0.40
p-Cymene	1024	1025	0.19	0.02	0.46
Limonene	1029	1031	2.02	0.15	6.38
Eucalyptol	1030	1032	2.58	0.55	4.29
(*Z*)-β-Ocimene	1037	1038	0.32	0.00	0.99
Benzeneacetaldehyde	1043	1045	0.31	0.05	0.60
(*E*)-β-Ocimene	1048	1049	0.03	0.00	0.18
γ-Terpinene	1058	1060	0.40	0.00	0.81
(*E*)-Sabinene hydrate	1066	1070	1.11	0.03	2.57
Isoterpinolene	1088	1086	0.03	0.00	0.14
Terpinolene	1088	1088	0.05	0.00	0.13
Linalool	1100	1099	0.25	0.04	0.48
2-Methylbutyl isovalerate	1108	1107	0.11	0.01	0.25
3-Octanol acetate	1125	1123	0.24	0.02	0.81
Menthone	1154	1154	6.67	0.41	14.11
Isomenthone	1158	1157	12.44	0.00	33.85
Menthofuran	1164	1164	2.19	0.03	7.04
δ-Terpineol	1168	1166	0.33	0.05	0.45
Menthol	1173	1170	12.49	0.24	30.73
Isomenthol	1177	1179	5.31	0.00	13.64
Isoneomenthol	1186	1188	0.61	0.00	5.40
α-Terpineol	1191	1189	0.33	0.00	0.67
Dihydrocarveol I	1195	1192	0.36	0.00	1.51
Dihydrocarveol II	1195	1196	0.52	0.00	2.28
Myrtenal	1197	1203	0.34	0.00	1.63
Estragole	1199	1196	0.12	0.00	1.06
trans-Dihydrocarvone	1198	1201	1.93	0.00	8.38
trans-Carveol	1220	1217	0.03	0.00	0.32
cis-Carveol	1225	1229	0.16	0.00	0.61
Citronellol	1228	1228	0.04	0.00	0.12
(*Z*)-3-Hexenyl valerate	1238	1239	0.16	0.00	1.26
Pulegone	1240	1237	1.79	0.01	6.06
Carvone	1248	1245	20.23	0.00	58.14
Piperitone	1255	1253	5.22	0.00	17.71
Neomenthyl acetate	1277	1277	0.05	0.00	1.32
Carvone oxide	1278	1279	0.22	0.00	0.65
Anethole	1286	1287	0.14	0.00	1.01
Thymol	1292	1291	0.48	0.00	1.28
Dihydroedulan	1295	1293	0.36	0.18	0.71
Menthyl acetate	1295	1295	1.90	0.00	9.65
Dihydrocarvyl acetate	1309	1305	0.02	0.00	0.17
Pulespenone	1329	1330	0.18	0.00	0.77
Piperitenone	1342	1340	0.08	0.00	0.38
Eugenol	1358	1357	0.12	0.04	0.51

Table 2. *Cont.*

Compound	RI Exp	RI Lib	Content, %		
			Average	Minimum	Maximum
cis-Carvyl acetate	1364	1362	0.06	0.00	0.16
Piperitenone oxide	1368	1367	0.04	0.00	0.22
Copaene	1377	1376	0.04	0.00	0.17
β-Bourbonene	1387	1384	1.49	0.32	3.70
β-Elemene	1394	1391	0.17	0.06	0.37
(*Z*)-Jasmone	1399	1394	0.21	0.00	0.72
α-Gurjunene	1412	1409	0.03	0.00	0.17
Caryophyllene	1422	1419	1.34	0.53	4.13
cis-β-Copaene	1431	1432	0.23	0.07	0.58
Isogermacrene D	1447	1448	0.19	0.00	1.41
trans-Muurola-3,5-diene	1448	1451	0.44	0.00	2.97
Humulene	1456	1454	0.31	0.06	0.72
(*E*)-β-Farnesene	1458	1457	0.21	0.06	0.69
cis-Muurola-4(15),5-diene	1466	1463	0.94	0.00	5.38
Germacrene D	1484	1481	2.35	0.00	6.27
epi-Bicyclosesquiphellandrene	1486	1482	0.92	0.00	9.23
Bicyclogermacrene	1499	1496	0.36	0.06	0.80
γ-Cadinene	1516	1513	0.10	0.00	0.47
trans-Calamenene	1525	1529	0.47	0.00	1.40
cis-Calamenene	1525	1530	0.42	0.00	3.32
δ-Cadinene	1526	1524	0.14	0.08	0.38
α-Cadinene	1540	1538	0.10	0.00	0.48
Spatulenol	1580	1576	0.17	0.04	0.60
Caryophyllene oxide	1586	1581	0.22	0.02	0.82
Viridiflorol	1594	1591	0.36	0.04	0.72
diepi-Cubenol	1618	1614	0.22	0.00	0.80
α-Cadinol	1657	1653	0.19	0.07	0.47
Aromadendrene oxide	1676	1678	0.03	0.00	0.15
ent-Germacra-4(15),5,10(14)-trien-1β-ol	1690	1690	0.08	0.01	0.29
Shyobunol	1701	1699	0.02	0.00	0.13
α-Mintsulfide	1741	1742	0.05	0.01	0.38
Farnesyl acetone	1919	1918	0.03	0.01	0.11
Phytol	2108	2114	0.04	0.00	0.20

The highest EO content was found in tea samples of *M. piperita* 'Apotheka' (22.40 mL/kg), *M. piperita* 'Ööbiku' (19.83 mL/kg), and *M. piperita* 'Tamme' (15.58 mL/kg). The lowest EO content was observed in the mixture of *M. spicata* + *M. piperita* + *M. arvensis* (1.99 mL/kg) (Table 3).

In studying the relationships between the dominant components of EOs, it was established that menthol and eucalyptol have a correlation coefficient of 0.88; menthol and isomenthone—0.83; carvone and piperitone—0.83; and eucalyptol and isomenthone—0.67.

A negative correlation was found between the pairs menthol and carvone——0.79; eucalyptol and pulegone——0.72; eucalyptol and menthone——0.67; eucalyptol and carvone——0.65; and menthol and menthone——0.63.

The main groups of compounds that make up peppermint EOs are monoterpenes, monoterpenoids, sesquiterpenic acids, sesquiterpenoids, phenylpropanoids, diterpenoids, apocarotinoids, phenols, aliphatic alcohols and esters. The dominant group of compounds in all studied sample EOs is monoterpenoids (Figure 1).

Table 3. EO yield and content of dominant compounds in EOs of commercial mint teas.

Sample	No.	Yield of EOs, ml/kg	Content, %											
			Limonene	Eucalyptol	Menthone	Menthol	Isomenthone	Isomenthol	Mentho-furan	Menthyl acetate	Pulegone	Carvone	Piperitone	*trans*-Dihydrocarvone
M. piperita 'Kloster'	M21	13.75	0.30	2.49	14.11	15.51	15.20	13.51	4.54	2.07	3.93	0.95	4.16	0
M. piperita 'Nateja'	M19	8.86	0.44	1.55	7.47	8.38	19.90	8.83	7.04	9.65	6.06	13.97	1.53	0
M. piperita 'Tamme Aiandustalu '	M25	15.58	0.67	2.50	11.66	8.61	0.01	0.01	0.34	0.26	0.09	25.88	15.4	4.44
M. piperita 'Apotheka'	M13	22.40	1.35	4.29	4.72	22.19	30.16	8.25	4.47	0.76	0.76	0.01	1.41	0
M. piperita 'Ööbiku talu'	M49	19.83	1.44	3.99	4.28	20.92	25.25	5.77	2.50	3.21	1.98	0.29	1.41	0
M. piperita 'Energia talu'	M50	9.53	1.90	4.12	4.42	30.73	33.85	0.01	2.42	0.92	0.28	0.01	1.43	0
M. suaveolens	M24	7.85	6.38	2.72	0.41	0.24	0.01	0.01	0.04	0	0.01	24.57	17.71	0.93
M. spicata	M26	12.13	4.57	1.72	0.58	0.42	0.01	0.01	0.03	0.05	0.13	58.14	0	8.38
M. spicata + *M. piperita* + *M. arvensis*	M10	1.99	0.15	0.55	13.17	14.03	0.01	13.64	0.33	1.92	3.72	37.12	0	0.36
M. spicata + *M. piperita* + *M. suaveolens*	M47	11.65	2.97	1.88	5.86	3.87	0.01	3.11	0.18	0.14	0.93	41.41	9.19	5.15

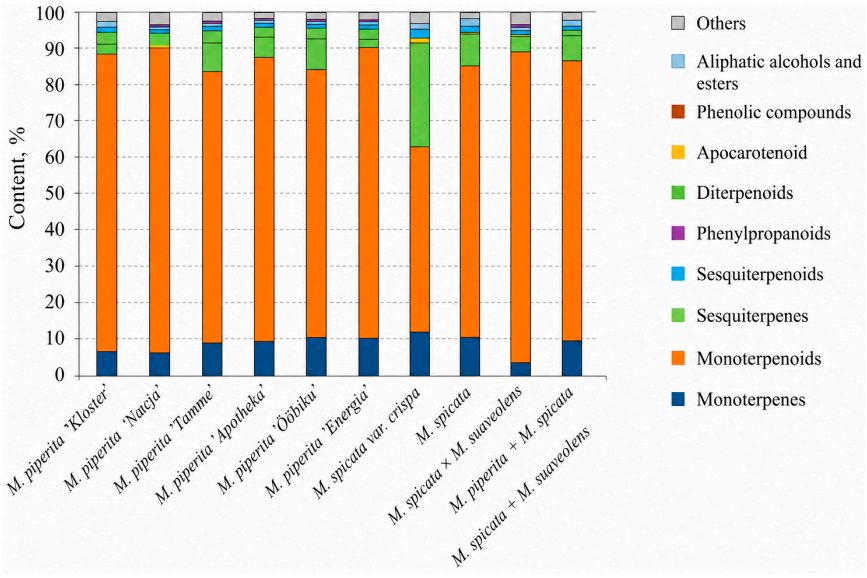


Figure 1. Distribution of essential oil constituents in commercial *Mentha* spp. teas according to classification groups.

3.2. Antimicrobial Activity

The antimicrobial activity of the EO samples was evaluated against clinical bacterial isolates with different resistance phenotypes and ATCC reference strains. Preliminary screening using the agar well diffusion assay was performed to distinguish EO samples with measurable antimicrobial activity (inhibition zone diameter, IZD > 10 mm) from inactive ones. The antimicrobial effects of the tested EO samples against bacterial and fungal strains are summarized in Table 4.

Table 4. Antimicrobial activity of commercial *Mentha* spp. EOs, 1:10 dilution (IZD, mm).

Micro-Organisms	Source	Resistance Phenotype	*M. piperita* 'Kloster' M21	*M. piperita* 'Natēja' M19	*M. piperita* 'Tamme Aiandustalu' M25	*M. piperita* 'Apotheka' M13	*M. piperita* 'Ööbiku talu' M49	*M. piperita* 'Energia talu' M50	*M. suaveolens* M24	*M. spicata* M26	*M. spicata* + *M. piperita* + *M. arvensis* M10	*M. spicata* + *M. suaveolens* + *M. piperita* M47
S. aureus	ATCC 29213	Penicil-linase +	11.85 ± 1.68 *	13.55 ± 1.31 *	11.45 ± 0.76 *	0	9.90 ± 0.74 *	8.30 ± 0.91 *	12.54 ± 0.44 *	12.18 ± 1.21 *	13.68 ± 2.53 *	9.75 ± 0.45 *
S. aureus	Wound pus	MLS, ind-	22.87 ± 0.65 *	19.61 ± 0.76 *	24.87 ± 0.63 *	16.78 ± 0.98	22.98 ± 0.87 *	20.87 ± 0.72 *	13.78 ± 0.60 *	26.67 ± 0.75 *	21.67 ± 0.40 *	23.01 ± 1.05 *
S. aureus	Wound	MRSA	20.86 ± 2.45 *	18.78 ± 1.25 *	24.41 ± 2.07 *	16.52 ± 0.85	24.75 ± 0.86 *	19.73 ± 0.82 *	19.83 ± 1.78 *	26.23 ± 0.65 *	22.56 ± 0.92 *	22.67 ± 0.97 *
α-hemolytic *St. sanguinis*	Oral cavity	S	10.96 ± 0.45 *	10.76 ± 0.75 *	0	0	0	0	0	0	0	0
β-hemolytic Group A *St. pyogenes*	Throat	S	0	0	0	0	0	0	0	0	0	0
β-hemolytic Group B *St. agalacticae*	Throat	S	0	0	0	0	0	0	0	0	0	0
E. faecalis	Urethra	MDR	0	9.04 ± 1.31 *	8.55 ± 0.87 *	0	0	0	8.36 ± 0.94 *	8.40 ± 0.63 *	0	8.94 ± 0.77 *
E. coli	ATCC 25922	S	8.79 ± 0.76 *	8.63 ± 0.78 *	10.07 ± 0.51 *	8.13 ± 1.33 *	0	9.13 ± 0.75 *	9.00 ± 0.63 *	11.42 ± 0.86 *	8.91 ± 0.80 *	11.32 ± 0.86 *
E. coli	Urine	MDR	8.39 ± 0.75 *	9.39 ± 0.79 *	10.10 ± 0.54 *	10.27 ± 1.07 *	8.63 ± 0.76 *	9.07 ± 1.78 *	0	10.08 ± 1.08 *	0	0
E. fergusonii	Stool	MDR	0	9.25 ± 0.26 *	0	0	0	0	0	0	0	8.72 ± 1.01 *
K. pneumonie	ATCC 1705	KPC	0	0	10.63 ± 0.58 *	0	0	8.64 ± 0.54 *	0	0	8.07 ± 1.09 *	8.67 ± 1.11 *
K. ozaneae	Nasal swab	TEM	0	0	8.47 ± 0.56 *	0	0	0	0	0	0	0
P. aeruginosa	Wound pus	ESβL	0	0	0	0	0	0	0	0	0	0
A.baumannii	Sputum	ESβL	0	8.27 ± 0.39 *	8.05 ± 0.79 *	7.38 ± 0.80 *	0	0	7.99 ± 0.90 *	0	8.00 ± 0.41 *	0
C. albicans	Oral cavity	FCZ-S	8.67 ± 0.43 [11.54 ± 0.39]	9.67 ± 0.75 [12.84 ± 0.90]	9.45 ± 0.75 [11.6 ± 1.19]	7.22 ± 0.98 [9.77 ± 2.02]	8.98 ± 0.43 [10.51 ± 0.15]	9.78 ± 0.12 [12.23 ± 1.48]	0 [8.82 ± 0.72]	0	7.56 ± 0.76 [8.63 ± 0.62]	9.88 ± 0.32 [12.23 ± 0.84] *
C. albicans	Oral cavity	FCZ-R	9.98 ± 0.76 * [14.15 ± 0.96] *	9.56 ± 0.78 * [17.28 ± 0.97] *	8.67 ± 0.76 * [11.09 ± 0.96] *	0 [10.25 ± 0.90] *	8.72 ± 0.32 * [17.38 ± 1.92] *	8.89 ± 0.78 * [11.91 ± 0.69] *	0 [8.5 ± 0.93] *	11.34 ± 0.98 * [22.44 ± 0.97] *	0 [9.39 ± 0.26] *	11.89 ± 0.54 * [15.54 ± 1.82] *
C. tropicalis	Sputum	FCZ-R	0 [8.65 ± 0.89]	8.23 ± 0.72 [10.69 ± 1.23] *	0 [14.04 ± 1.08] *	0 [11.87 ± 0.57] *	0 [8.39 ± 0.78]	0 [9.19 ± 0.94]	0 [11.68 ± 0.44] *	0 [11.38 ± 0.37]	0 [9.63 ± 1.68]	0

Note: 1. S—antibiotic-susceptible strains; MDR—multidrug-resistant strains; MRSA—methicillin-resistant *S. aureus*; MLS resistance (macrolides, lincosamides, streptogramin B); ind—-absence of induced lincosamide resistance; ESβL—extended spectrum β-lactamases; TEM-β-lactamase—resistance to penicillins and early-generation cephalosporins; KPC—carbapenemase; FCZ-R—fluconazole resistant; FCZ-S—fluconazole susceptible.; Penicillinase +—β-lactamase resistance to penicillins. 2. Square brackets [] indicate zones of partial growth inhibition (bacteriostatic/fungistatic effect). 3. * $p < 0.01$ compared to the control (90% ethanol) in Kruskal–Wallis test with Dunn's post hoc analysis (Holm correction).

The antifungal-susceptible *C. albicans* strain and *C. tropicalis* exhibited limited sensitivity to the solvent control (90% ethanol), with IZDs of 10.12 ± 0.11 mm and 8.15 ± 0.42 mm, respectively. No inhibitory effect of ethanol was observed against the remaining tested microorganisms.

The most pronounced antimicrobial activity of the EO samples was observed against clinical *S. aureus* strains (Figure 2). Strong growth inhibition was detected particularly for MRSA and MLS-resistant phenotypes, with maximum inhibition zones reaching 26.67 ± 0.75 mm, exceeding those observed for the reference strain *S. aureus* ATCC 29213 (β-lactamase producer). In contrast, all tested EO samples exhibited weak or no activity against other Gram-positive bacteria, including *Streptococcus* spp. and multidrug-resistant *E. faecalis*.

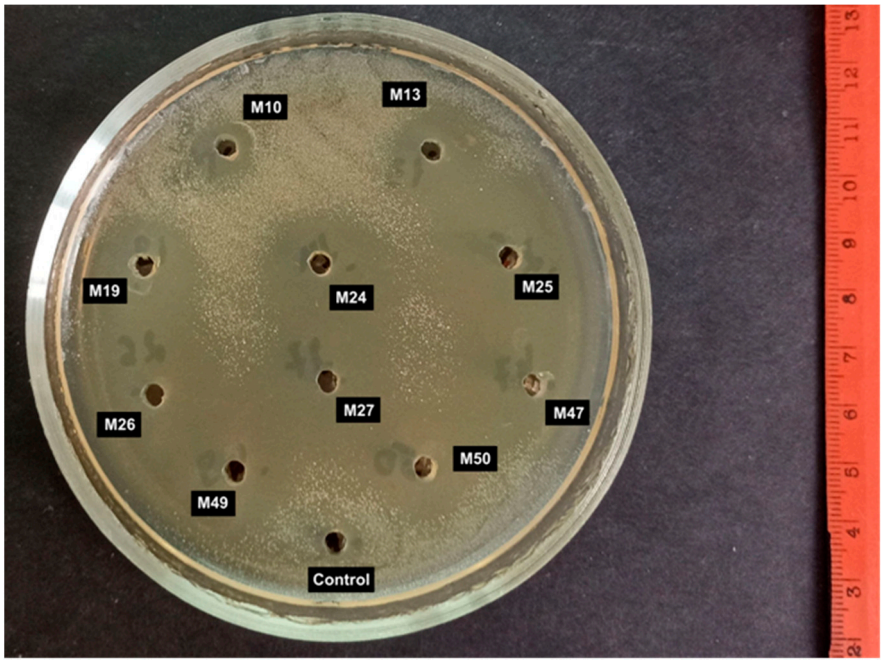


Figure 2. Representative agar well diffusion assay showing growth inhibition zones of *Staphylococcus aureus* (clinical MRSA strain) produced by essential oils from commercial *Mentha* teas. EO samples are indicated by their corresponding codes (see Table 1). The solvent (90% ethanol) was used as the negative control.

Among Gram-negative bacteria, clinical *Escherichia* spp. isolates, the reference strains *E. coli* ATCC 25922, and *K. pneumoniae* were moderately inhibited by selected EO samples. In particular, *M. piperita* 'Tamme Aiandustalu' (M25), *M. spicata* (M26), and the EO mixture of *M. spicata* + *M. suaveolens* + *M. piperita* (M47) exhibited inhibitory activity against *E. coli* ATCC 25922, with IZDs reaching 11.32 ± 0.86 mm.

Almost all EO samples and their mixtures displayed a fungistatic effect against both fluconazole-susceptible and fluconazole-resistant *C. albicans* strains. Notably, selected samples (*M. spicata* (M26) and the EO mixture *M. spicata* + *M. suaveolens* + *M. piperita* (M47)) also exerted a fungicidal effect. In contrast, fluconazole-resistant *C. tropicalis* showed lower sensitivity to the investigated EO samples.

The antimicrobial concentrations of selected EO samples were determined using the two-fold serial dilution method. Mean values of MICs and MBCs/MFCs expressed in mg/mL are presented in Table 5.

Table 5. MIC and MBC values (mg/mL) and corresponding dilution factors of commercial *Mentha* spp. EOs.

No.	Sample	EO Concentration Before Serial Dilution, mg/mL	Antimicrobial Concentrations	
			MIC, mg/mL	MBC, mg/mL
1	2	3	4	5
		S. aureus ATCC 29213		
1.	*M. piperita* 'Kloster'	1080	2.70	2.70
2.	*M. piperita* 'Nateja'	1070	2.68	2.68
3.	*M. piperita* 'Tamme Aiandustalu'	1090	2.73	2.73
4.	*M. piperita* 'Apotheka'	1190	>5.95	>5.95
5.	*M. piperita* 'Ööbiku talu'	1050	>5.25	>5.25
6.	*M. piperita* 'Energia talu'	1290	>6.45	>6.45
7.	*M. suaveolens*	1040	2.60	2.60
8.	*M. spicata*	1000	2.50	2.50
9.	*M. spicata* + *M. piperita* + *M. arvensis*	410	0.21	0.21
10.	*M. spicata* + *M. suaveolens* + *M. piperita*	1030	>5.15	>5.15
		S. aureus MLS ind^{-}		
1.	*M. piperita* 'Kloster'	1080	2.70	2.70
2.	*M. piperita* 'Nateja'	1070	1.34	5.35
3.	*M. piperita* 'Tamme Aiandustalu'	1090	2.28	2.28
4.	*M. piperita* 'Apotheka'	1190	>5.95	>5.95
5.	*M. piperita* 'Ööbiku talu'	1050	2.63	2.63
6.	*M. piperita* 'Energia talu'	1290	3.23	3.23
7.	*M. suaveolens*	1040	2.60	5.20
8.	*M. spicata*	1000	1.25	5
9.	*M. spicata* + *M. piperita* + *M. arvensis*	410	0.21	0.21
10.	*M. spicata* + *M. suaveolens* + *M. piperita*	1030	2.58	2.58
		S. aureus MRSA		
1.	*M. piperita* 'Kloster'	1080	2.70	5.40
2.	*M. piperita* 'Nateja'	1070	2.68	2.68
3.	*M. piperita* 'Tamme Aiandustalu'	1090	5.45	>5.45
4.	*M. piperita* 'Apotheka'	1190	3.00	5.95
5.	*M. piperita* 'Ööbiku talu'	1050	1.32	5.25
6.	*M. piperita* 'Energia talu'	1290	3.23	6.45
7.	*M. suaveolens*	1040	2.60	2.60
8.	*M. spicata*	1000	1.25	1.25
9.	*M. spicata* + *M. piperita* + *M. arvensis*	410	0.10	0.10
10.	*M. spicata* + *M. suaveolens* + *M. piperita*	1030	2.58	5.15
		α-hemolytic *S. sanguinis* S		
1.	*M. piperita* 'Kloster'	1080	<1.35	<1.35
2.	*M. piperita* 'Nateja'	1070	>5.35	>5.35
3.	*M. piperita* 'Tamme Aiandustalu'	1090	>5.45	>5.45
4.	*M. piperita* 'Apotheka'	1190	>5.95	>5.95
5.	*M. piperita* 'Ööbiku talu'	1050	>5.25	>5.25
6.	*M. piperita* 'Energia talu'	1290	>6.45	>6.45
7.	*M. suaveolens*	1040	>5.20	>5.20
8.	*M. spicata*	1000	>5.00	>5.00
9.	*M. spicata* + *M. piperita* + *M. arvensis*	410	0.21	0.21
10.	*M. spicata* + *M. suaveolens* + *M. piperita*	1030	>5.15	>5.15
		E. coli ATCC 25922		
1.	*M. piperita* 'Kloster'	1080	5.40	>5.40
2.	*M. piperita* 'Nateja'	1070	5.35	>5.35
3.	*M. piperita* 'Tamme Aiandustalu'	1090	5.45	5.45
4.	*M. piperita* 'Apotheka'	1190	5.95	5.95
5.	*M. piperita* 'Ööbiku talu'	1050	5.25	>5.25

Table 5. *Cont.*

No.	Sample	EO Concentration Before Serial Dilution, mg/mL	Antimicrobial Concentrations	
			MIC, mg/mL	MBC, mg/mL
6.	*M. piperita* 'Energia talu'	1290	6.45	6.45
7.	*M. suaveolens*	1040	5.20	5.20
8.	*M. spicata*	1000	2.50	2.50
9.	*M. spicata* + *M. piperita* + *M. arvensis*	410	>0.21	>0.21
10.	*M. spicata* + *M. suaveolens* + *M. piperita*	1030	2.58	>5.15
	K. pneumonie ATCC 1705 KPC β-lactamase			
1.	*M. piperita* 'Kloster'	1080	>5.40	>5.40
2.	*M. piperita* 'Nateja'	1070	>5.35	>5.35
3.	*M. piperita* 'Tamme Aiandustalu'	1090	>5.45	>5.45
4.	*M. piperita* 'Apotheka'	1190	>5.95	>5.95
5.	*M. piperita* 'Ööbiku talu'	1050	>5.25	>5.25
6.	*M. piperita* 'Energia talu'	1290	6.45	6.45
7.	*M. suaveolens*	1040	>5.20	>5.20
8.	*M. spicata*	1000	>5.00	>5.00
9.	*M. spicata* + *M. piperita* + *M. arvensis*	410	>0.205	>0.205
10.	*M. spicata* + *M. suaveolens* + *M. piperita*	1030	>5.15	>5.15
	C. albicans ATCC 885-65 FCZ-S			
1.	*M. piperita* 'Kloster'	1080	>5.40	>5.40
2.	*M. piperita* 'Nateja'	1070	5.35	>5.35
3.	*M. piperita* 'Tamme Aiandustalu'	1090	>5.45	>5.45
4.	*M. piperita* 'Apotheka'	1190	>5.95	>5.95
5.	*M. piperita* 'Ööbiku talu'	1050	5.25	5.25
6.	*M. piperita* 'Energia talu'	1290	6.45	6.45
7.	*M. suaveolens*	1040	>5.20	>5.20
8.	*M. spicata*	1000	>5.00	>5.00
9.	*M. spicata* + *M. piperita* + *M. arvensis*	410	0.21	0.21
10.	*M. spicata* + *M. suaveolens* + *M. piperita*	1030	>5.15	>5.15
	C. tropicalis FCZ-R			
1.	*M. piperita* 'Kloster'	1080	5.40	5.40
2.	*M. piperita* 'Nateja'	1070	>5.35	>5.35
3.	*M. piperita* 'Tamme Aiandustalu'	1090	>5.45	>5.45
4.	*M. piperita* 'Apotheka'	1190	1.48	>5.95
5.	*M. piperita* 'Ööbiku talu'	1050	>5.25	>5.25
6.	*M. piperita* 'Energia talu'	1290	>6.45	>6.45
7.	*M. suaveolens*	1040	>5.20	>5.20
8.	*M. spicata*	1000	>5.00	>5.00
9.	*M. spicata* + *M. piperita* + *M. arvensis*	410	0.05	0.21
10.	*M. spicata* + *M. suaveolens* + *M. piperita*	1030	5.15	5.15

Note: Initial concentration refers to the concentration of the essential oil before the serial two-fold dilution.

Overall, the most pronounced antimicrobial activity of the EO samples was observed against all tested *S. aureus* strains. The EO mixture composed of *M. spicata* + *M. piperita* + *M. arvensis* (M10) completely inhibited the growth of the antibiotic-susceptible reference strain *S. aureus* ATCC 25923 and the clinical MLS-resistant isolate at a dilution of 1:2000 (0.205 mg/mL), while inhibition of MRSA was achieved at a dilution of 1:4000 (0.1025 mg/mL).

EO samples of *M. piperita* 'Nateja' (19), *M. piperita* 'Kloster' (M21), *M. piperita* 'Tamme Aiandustalu' (M25), *M suaveolens* (M24), and *M. spicata* (M26) exhibited inhibitory activity against antibiotic-susceptible *S. aureus* at dilutions of 1:200, corresponding to concentrations of 2.5–2.7 mg/mL. Against antibiotic-resistant clinical staphylococcal isolates, inhibitory effects were observed at dilutions ranging from 1:400 to 1:800 (concentrations 1.36–5.35 mg/mL). In contrast, EO samples of *M. piperita* ('Ööbiku talu' (M49), 'Energia

talu' (M50), and the mixture of *M. spicata* + *M. suaveolens* + *M. piperita* (M47) demonstrated activity against antibiotic-resistant staphylococci within the tested concentration range but did not inhibit the growth of the reference strain *S. aureus* ATCC 25923 (Table 5).

The antimicrobial activity of EO samples against other Gram-positive bacteria was generally limited, showing weak effects against α-hemolytic streptococci and enterococci and no detectable activity against β-hemolytic streptococci.

Several EO samples derived from *M. piperita* ('Apotheka' (M13), 'Natēja' (M19), ''Kloster' (M21), 'Ööbiku talu' (M29) 'Energia talu' (M50), and *M. suaveolens* (M24) displayed weak antimicrobial activity against Gram-negative bacteria, particularly antibiotic-susceptible and antibiotic-resistant *E. coli* strains. In addition, EO samples of *M. piperita* ('Energia talu' (M50), 'Tamme Aiandustalu' (M25) and both tested EO mixtures (M10 and M47) showed weak inhibitory activity against KPC-producing *K. pneumoniae* ATCC 1705, while EO samples of *M. piperita* ('Apotheka' (M13), 'Natēja' (M19), 'Tamme Aiandustalu' (M25), *M. suaveolens* (M24), and the mixture *M. spicata* + *M. piperita* + *M. arvensis* (M10) exhibited weak activity against ESβL-producing *A. baumannii*.

No consistent differences were detected between fluconazole-susceptible and fluconazole-resistant *Candida* isolates. Likewise, no systematic differences in susceptibility were detected between antibiotic-susceptible and multidrug-resistant *E. coli* strains.

4. Discussion

A comparative analysis of the composition of EOs (Table S1) revealed pronounced differences between the *M. piperita* samples and other mint species. The observed differentiation was mainly associated with differences in the relative amounts of the dominant compounds. Thus, menthol, menthone, isomenthone, and isomenthol predominated in the EO composition of *M. piperita* 'Kloster', 'Apotheka', 'Ööbiku', and 'Energia'. However, the samples of *M. piperita* 'Nateja' and 'Tamme' differ significantly in their carvon content. Carvone-containing profiles were characteristic of *M. spicata*, *M. suaveolens*, and mixed samples (Tables 3 and S1). Previously, chemotypes of *M. spicata* rich in carvone (62.1–67.4%) were found [40].

The present results demonstrate considerable variability in EO composition and antimicrobial activity among the investigated commercial mint teas, including products marketed under the same species designation. Such variation may contribute to substantial differences that influence the biological properties and potential nutraceutical value of mint teas consumed on a daily basis, highlighting the importance of phytochemical characterization and quality control of commercial herbal products.

The antimicrobial activity observed in the present study should also be considered in the broader context of other widely consumed herbal teas. Numerous medicinal plant infusions have been reported to exhibit antibacterial and antifungal properties, although their efficacy varies substantially depending on phytochemical composition and extraction conditions. Among herbal teas, thyme (*Thymus vulgaris*) and oregano (*Origanum vulgare*) are generally regarded as possessing particularly strong antimicrobial activity due to their high contents of thymol and carvacrol, whereas sage (*Salvia officinalis*), lemon balm (*Melissa officinalis*), chamomile (*Matricaria chamomilla*), and mint (*Mentha* spp.) are typically characterized by moderate but well-documented antimicrobial effects [41]. In a comparative study evaluating 31 commonly consumed herbal teas, peppermint tea was among the preparations exhibiting antimicrobial activity against clinically relevant microorganisms, including *Staphylococcus aureus*, *Escherichia coli*, and *Candida albicans*, although thyme-based preparations generally showed broader and stronger inhibitory effects [42]. Similarly, comparative analyses of selected herbal teas demonstrated measurable antibacterial activity in infusions of *Mentha piperita*, *Melissa officinalis*, and *Salvia officinalis*, with antimicrobial efficacy closely

associated with the content of phenolic and terpenoid constituents [43]. Collectively, these findings indicate that the antimicrobial potential of herbal teas is strongly determined by their dominant bioactive compounds and their relative abundance. In contrast to many studies that compare different medicinal plant species, the present work demonstrates that substantial differences in antimicrobial activity may also occur among commercial tea products belonging to the same botanical genus, depending on their EO chemotype.

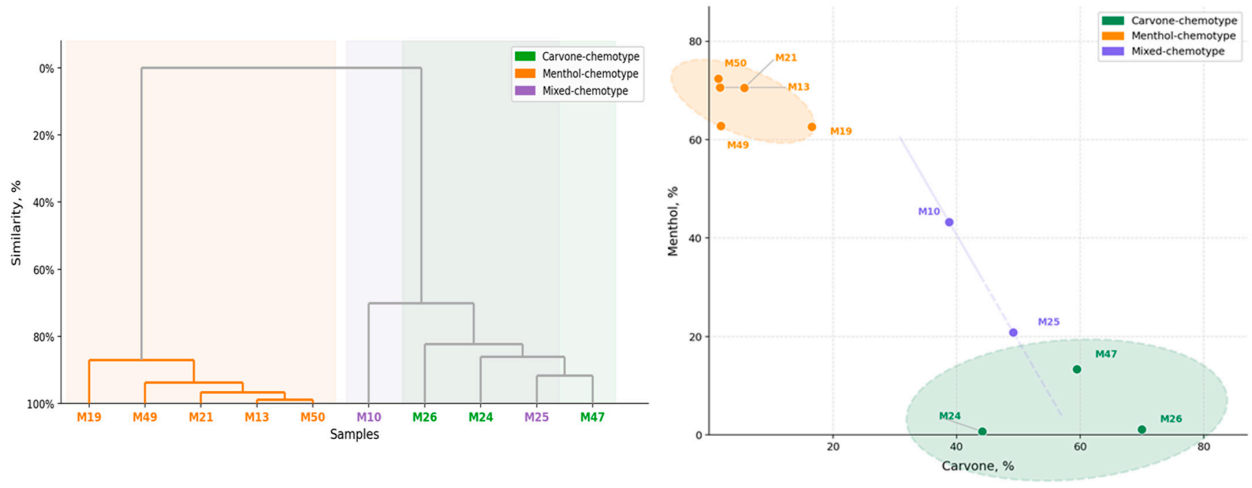
Significant levels of toxic pulegone were identified in the EO samples of *M. piperita* 'Nateja' (6.06%), *M. piperita* 'Kloster' (3.93%), and the mixture of *M. spicata* + *M. piperita* + *M. arvensis* (3.72%), which partially correlates with data from previous studies [44]. Pulegone can be metabolized into menthofuran, which is toxic to the liver and other organs [45,46]. Maximum limits on the use of pulegone and menthofuran in foodstuffs are regulated by the European Union (EU) [47]. However, various studies have shown that pulegone is a monoterpene with strong insecticidal and antibacterial activities [48].

Trans-carveol was detected only in the mixture of *M. spicata* + *M. piperita* + *M. arvensis*, which gives grounds for assuming its presence in the composition of *M. arvensis* EO. *Epi*-bicyclosesquiphellandrene was found, even in significant amounts (9.23%), only in the EO of *M. suaveolens*, and may be a marker compound of this species. The EO of *M. suaveolens* differs significantly from the other understudied samples in having the highest content of sesquiterpenes (33.88%), monoterpenes (11.07%), noticeable content of sesquiterpenoids (2.66%), and the lowest content of monoterpenoids (49.39%). Attention is also drawn to the highest content of piperitone in the EO composition of *M. suaveolens* (17.71%) compared to other studied samples and its significant content (9.19%) in the EO composition of the mixture *M. spicata* + *M. piperita* + *M. suaveolens*. Piperitone and its derivatives have demonstrated a range of biological activities, including antimicrobial, insecticidal, anti-inflammatory, and antioxidant effects [49].

Thus, chemotype-based clustering derived from chemical composition analysis was directly mirrored by biologically relevant differences in antimicrobial activity, providing experimental validation of the proposed chemotype–activity map. Integration of hierarchical chemotype clustering with MIC distribution analysis indicates that antimicrobial efficacy in EO samples is governed neither by individual compounds nor by total terpene abundance per se, but rather by chemotype-level compositional architecture. In this framework, the carvon chemotype, dominated by carvone- and piperitone-related profiles, was associated with enhanced antimicrobial activity in the investigated samples, whereas the menthol chemotype was consistently associated with attenuated antimicrobial potency. Because EOs are complex multicomponent mixtures, these associations should not be interpreted as evidence that carvone or any other individual constituent alone is responsible for the observed antimicrobial effects. Synergistic and antagonistic interactions among constituents may also contribute to the observed activity patterns.

Hierarchical clustering of CLR-transformed GC–MS data revealed three well-defined chemotypes among the analyzed *Mentha* EOs (Figure 3). In carvone-chemotype EOs (spicy-balsamic aroma), the sum of carvone, piperitone, piperitenone, piperitenone oxide, carvone oxide, dihydrocarvyl acetate, *cis*-carvyl acetate, dihydrocarveol I, dihydrocarveol II, *trans*-dihydrocarvone, *trans*-carveol, and *cis*-carveol ranges from 44 to 70%: *M. suaveolens* (M24)—44.15%, *M. spicata* (M26)—70.07%, mixture *M. spicata* + *M. piperita* + *M. suaveolens* (M47)—59.42%.

In menthol-chemotype EOs (menthol scent), the sum of menthone, isomenthone, menthol, isomenthol, isoneomenthol, menthofuran, Menthyl acetate, and neomenthyl acetate ranges from 62 to 73%: *M. piperita* (M13)—70.60%, *M. piperita* (M19)—62.59%, *M. piperita* (M49)—62.80%, *M. piperita* (M50)—72.41%, *M. piperita* (M21)—70.51%.



(**a**) Hierarchical clustering dendrogram (**b**) PCA score plot

Figure 3. Integrated chemotype map of *Mentha* spp. EOs: (**a**) Hierarchical clustering dendrogram of EO samples constructed from GC–MS compositional profiles (same input data as for PCA); (**b**) principal component analysis (PCA) score plot based on CLR-transformed GC–MS compositional data.

In carvone-menthol mixed-chemotype EOs (herbaceous-mint scent), the sum of compounds of the carvone group and the sum of compounds of the menthol group were as follows: mixture *M. spicata* + *M. piperita* + *M. arvensis* (M10) 38.80–43.25%, respectively, and *M. piperita* (M25) 49.15–20.87%, respectively.

Principal component analysis (PCA) of CLR-normalized GC–MS data resulted in a clear separation of EO samples in the score space, corresponding closely to the three chemotypes identified by hierarchical clustering (Figure 3a). The dendrogram reveals a clear chemotype-level organization, separating carvon-chemotype samples from menthol-chemotype samples and the carvone-menthol mixed chemotype, thereby confirming the stability of the chemotypic classification suggested by multivariate ordination. Ellipses (Figure 3b) indicate the 95% confidence regions for each chemotype, illustrating unsupervised separation in chemical space along the first two principal components (PC1 and PC2).

Mapping MIC values onto the chemotype framework (Figure 4) demonstrated that EOs of the carvone chemotype were associated with a marked shift toward lower MIC/MBC values and larger inhibition zones across the tested microorganisms. In contrast, the menthol chemotype exhibited predominantly higher MIC values and reduced inhibition zones, indicating comparatively lower antimicrobial potency.

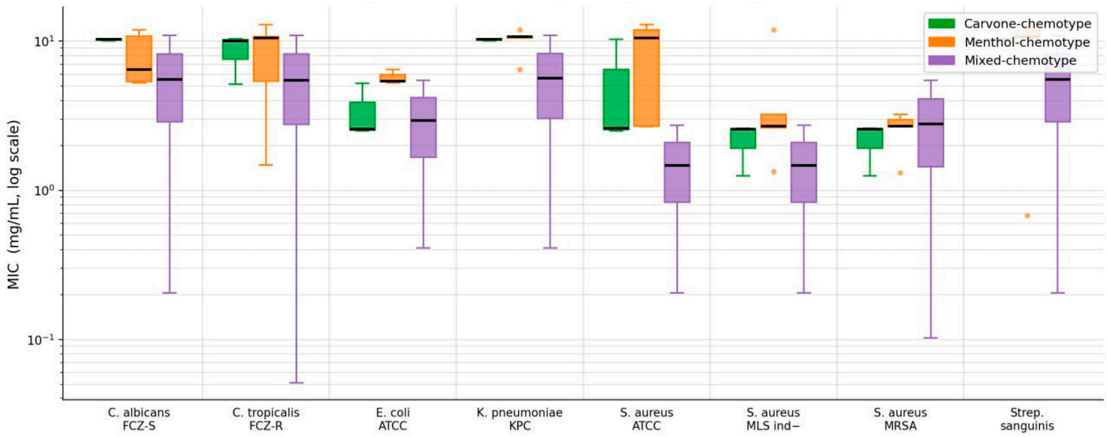
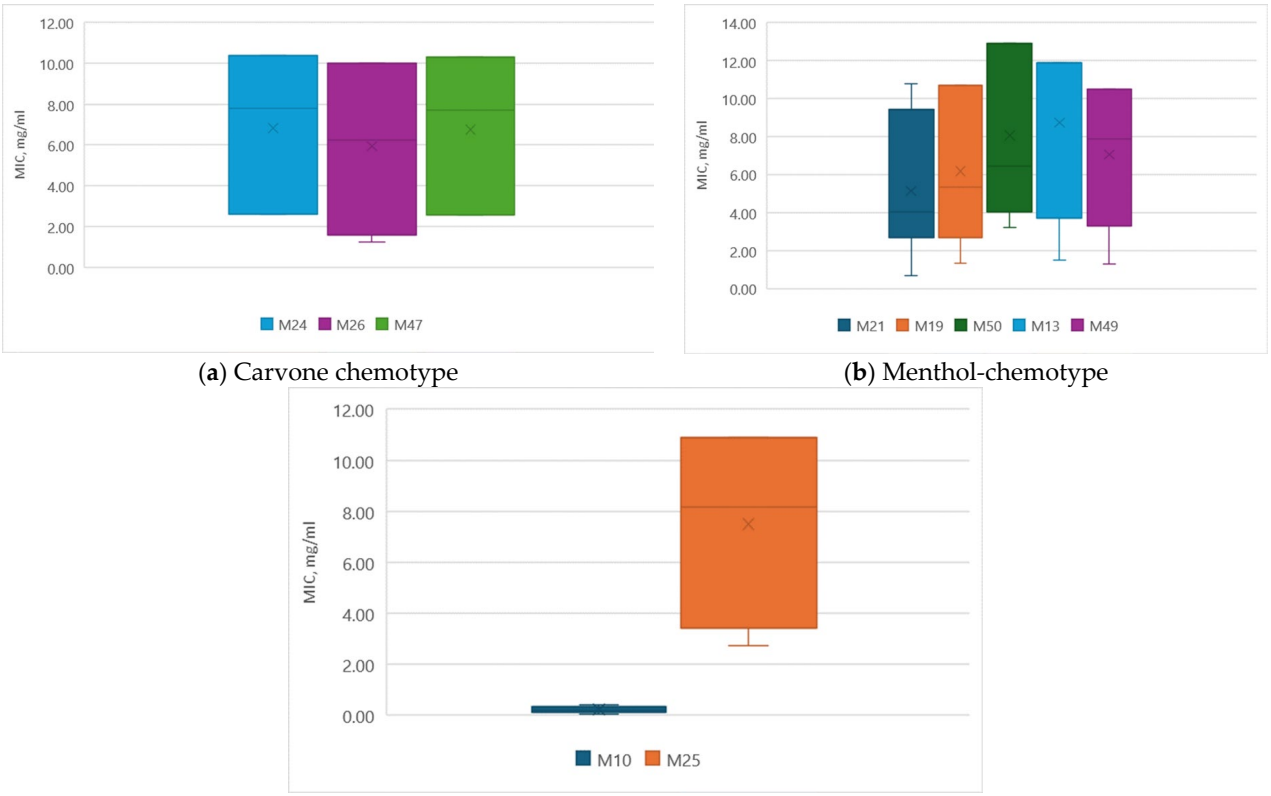


Figure 4. Integrated chemotype activity map of *Mentha* spp. EOs.

The complex antimicrobial activity of mint chemotaxones depends on their chemotypes (Figure 5). Consequently, the highest total antibacterial activity in relation to the studied strains was shown by M10 (Figure 5c) (mixed type—carvone group 80%, menthol group 43.25%), the average value was shown by carvone chemotypes M26, M47, and M24 (Figure 5a), as well as M25 (mixed type—carvone group 49.15%, menthol group 20.87%). The menthol chemotype shows decreasing antibacterial activity in the following order: M21, M19, M49, M13, and M50 (Figure 5b).



(**a**) Carvone chemotype (**b**) Menthol-chemotype

(**c**) Carvone-menthol mixed chemotype

Figure 5. Complex antimicrobial activity of mint chemotaxones by carvone chemotype, menthol chemotype and mixed carvone-menthol chemotype.

Comprehensive correlation analysis between EO chemical composition (CLR-transformed relative abundances) and antibacterial activity against *S. aureus* revealed clear and reproducible patterns across the three tested strains. The strongest associations were predominantly linked to mono- and sesquiterpene constituents.

For the susceptible strain *S. aureus* ATCC 29213, the most pronounced negative correlations were observed between MIC values and the abundance of carvone, α-cadinene, and diepi-cubenol ($\rho = -0.879$; $p = 0.000814$), indicating enhanced antibacterial efficacy with increasing concentrations of these compounds. In the structure of the compounds carvone, α-cadinene, and diepi-cubenol, a common feature is the presence of a double bond in the ortho-position with a methyl radical, which may serve as a basis for explaining the pronounced antimicrobial activity against the strain *S. aureus* ATCC 29213 (Figure 6). Carvone, caryophyllene oxide, α-cadinene, and ent-germacra-4(15),5,10(14)-trien-1β-ol have unsaturated double bonds in their structure (caryophyllene oxide—one exocyclic, cadinene—two endocyclic, ent-germacra-4(15),5,10(14)-trien-1β-ol—two exocyclic and one endocyclic, carvone—one exocyclic, one endocyclic), which can determine pronounced antibacterial activity against methicillin-resistant *S. aureus*—MRSA; moreover, these compounds induce apoptosis in cancer cells and exhibit cytotoxicity [50–53].

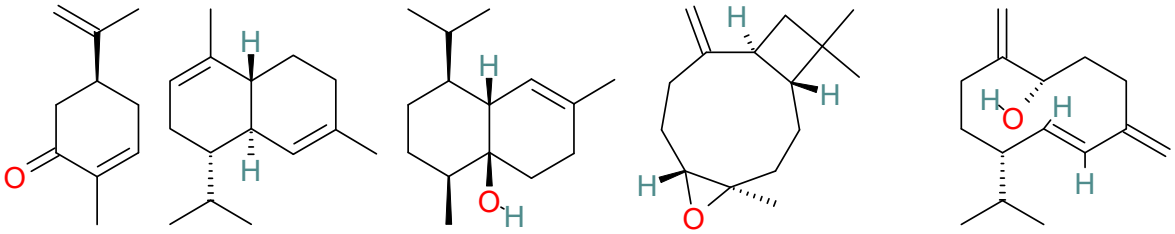


Figure 6. Structural formulas of individual compounds.

In contrast, *cis*-sabinene hydrate, (Z)-β-ocimene, linalool, and α-terpinene showed positive correlations with MIC, suggesting reduced antibacterial activity at higher abundances.

The MLS-ind phenotype exhibited similar trends. A marked reduction in MIC was associated with elevated carvone levels ($\rho = -0.830$; $p = 0.00294$), whereas increased levels of 2-methylbutyl isovalerate, α-sabinene, piperitone, and several other monoterpenes correlated with higher MIC values. This pattern indicates a largely preserved chemosensitivity profile in the MLS-ind^{-} strain relative to ATCC 29213, particularly toward structurally related monoterpenes.

In contrast, the clinically challenging MRSA strain displayed a distinct correlation pattern. For MIC, piperitone exhibited a significant positive correlation ($\rho = +0.661$; $p = 0.0376$), indicating reduced antibacterial potency, whereas carvone ($\rho = -0.648$), together with caryophyllene oxide, α-cadinene, and ent-germacra-4(15),5,10(14)-trien-1β-ol, showed negative associations linked to enhanced antibacterial activity. Analysis of MBC values in MRSA further highlighted carvone as a key contributor ($\rho = -0.818$; $p = 0.00381$), accompanied by α-cadinene, cubenol, and caryophyllene oxide, all associated with lower bactericidal concentrations.

Overall, aggregated analysis across *S. aureus* strains indicated that carvone, α-cadinene, caryophyllene oxide, and related sesquiterpenoids consistently correlated with reduced MIC and MBC values. In contrast, piperitone, 2-methylbutyl isovalerate, α-terpinene, and several monoterpene hydrocarbons were more frequently associated with diminished antibacterial performance. This biphasic behavior between terpene classes is consistent with known mechanistic differences, whereby oxygenated mono- and sesquiterpenes typically exert stronger membranotropic and/or enzyme-inhibitory effects than hydrocarbon monoterpenes.

In *C. albicans*, the FCZ-R phenotype showed predominantly negative correlations with oxygenated monoterpenes (menthofuran, δ-terpineol, and isomenthone), whereas in FCZ-S isolates, several monoterpene hydrocarbons exhibited moderate positive associations. In *E. coli* ATCC 25922, (E)-2-hexenal and carvyl/carvone-related structures emerged as positive predictors of activity, whereas oxygenated monoterpenes were more often negatively associated.

Multivariate PLS2/VIP analysis based on CLR-transformed data supported these findings. Compounds with the strongest influence (VIP $\gtrsim 1$) included δ-terpineol, *cis*-β-copaene, α-cadinol, *trans*-dihydrocarvone, δ-cadinene, β-bourbonene, menthofuran, isomenthone, caryophyllene, and bicyclogermacrene. These constituents predominantly acted as negative modulators of microbial growth, whereas carvone-type compounds and selected sesquiterpene hydrocarbons served as positive drivers of antimicrobial activity.

As the majority of GC–MS-detected compounds represented minor EO constituents, subsequent correlation analyses focused on the top 10 quantitatively dominant compounds to reduce noise and improve interpretability.

Several compound–organism-specific associations were identified. For MIC, the strongest and most consistent positive correlations—corresponding to reduced antimi-

crobial potency—were observed for eucalyptol, isomenthone, menthol, and δ-terpineol, particularly in *S. aureus* strains and *E. coli*. These associations remained significant after BH-FDR correction ($q < 0.10$). A similar pattern was evident for MBC/MFC, where eucalyptol, isomenthone, and menthol again showed the most consistent positive correlations, most prominently in *S. aureus* ATCC 29213. In contrast, carvone exhibited negative correlations with both MIC and MBC/MFC across several organisms, most notably *S. aureus*, *E. coli*, and FCZ-R *C. tropicalis*, indicating a potential contribution to lower (more favorable) inhibitory and bactericidal/fungicidal concentrations. Taken together, these results suggest that highly abundant oxygenated monoterpenes (e.g., eucalyptol, isomenthone, menthol) are generally associated with reduced antimicrobial potency, whereas carvone consistently contributes positively to antimicrobial activity across both MIC and MBC/MFC endpoints.

Compositional GC–MS data, aggregated into 10 chemical classes, were further analyzed following centered log-ratio (CLR) transformation; additional representations included aggregated sums and differentiated classification groups. Within this expanded classification scheme, the apocarotenoid fraction showed the most consistent associations. For MIC, several robust negative correlations were detected across bacterial models (multiple *S. aureus* strains and *E. coli*), and these associations remained significant after BH-FDR correction.

For other broad chemical classes (monoterpenes/monoterpenoids, sesquiterpenes/sesquiterpenoids, phenolics, phenylpropanoids, and diterpenoids), associations with antimicrobial activity were organism-specific and predominantly trend-level, with most not retained after BH-FDR adjustment. This pattern supports the notion that biologically relevant signals are driven by narrower chemical subgroups, whereas grouping compounds too broadly dilutes signals.

Following further subdivision of the oxygenated monoterpene fraction into alcohols, ketones, esters, and oxides, and subsequent multivariate modeling (PLS2; seven-group scheme), the largest integrated contributions to antimicrobial variability (MIC/MBC) were observed for oxides (VIP $\approx$ 1.53) and monoterpene hydrocarbons (VIP $\approx$ 1.51), with a moderate contribution from sesquiterpene hydrocarbons (VIP $\approx$ 1.29). Alcohols and esters were the least informative. Pairwise Spearman correlations (BH-FDR adjusted) were concordant with the multivariate model: the relative abundance of oxides correlated positively with MIC/MBC (i.e., reduced activity) in staphylococci, α-hemolytic *S. sanguinis*, and *E. coli* ATCC 25922 (Table 6). In contrast, ketones predominantly showed negative correlations, consistent with improved antimicrobial activity. Collectively, these findings suggest that enhancing antibacterial efficacy may be achieved by increasing the fraction of ketones (e.g., menthone, camphor, carvone), while limiting oxides, particularly 1,8-cineole (eucalyptol), which are associated with elevated MIC/MBC values across several bacterial models.

The present study provides an integrated evaluation of the antimicrobial potential of *Mentha* spp. EOs by combining detailed chemical profiling with phenotypic susceptibility testing across a diverse panel of bacterial and fungal strains. By applying multivariate compositional analyses alongside conventional microbiological assays, we sought to move beyond single-compound interpretations and to conceptualize antimicrobial activity as an emergent property of complex EO systems. Such a systems-level perspective is increasingly recognized as essential for understanding EO bioactivity, given the multicomponent nature of plant volatiles and their nonlinear biological effects [54–57].

Table 6. Stable Spearman correlations (BH-FDR adjusted) between oxides, ketones and antimicrobial activity (MIC and MBC/MFC), calculated across 10 commercial *Mentha* spp. EO samples.

Strain	Oxides			Ketones		
	ρ	*p*	q	ρ	*p*	q
S. aureus ATCC 29213	+0.794	0.006	0.039	−0.758	0.011	0.039
S. aureus MLS ind$^-$	+0.879	0.001	0.006	−0.673	0.033	0.116
S. aureus MRSA	+0.745	0.013	0.088	−0.697	0.025	0.088
S. sanguinis	+0.758	0.011	0.078	−0.527	0.117	0.323
E. coli ATCC 25922	+0.782	0.008	0.053	−0.697	0.025	0.088
K. pneumoniae ATCC 1705 KPC β lactamase	+0.552	0.098	0.689	−0.333	0.347	0.853
C. albicans ATCC 88565 (FCZ S)	−0.564	0.090	0.344	−0.552	0.098	0.344
C. tropicalis (FCZ R)	+0.758	0.011	0.078	−0.564	0.090	0.314

Notes: 1. Negative correlation (ρ < 0): larger proportion corresponds to lower (better) MIC/MBC values. Positive correlation (ρ > 0): larger proportion corresponds to higher (worse) MIC/MBC values. 2. Multiple comparisons corrected using the Benjamini–Hochberg False Discovery Rate (BH-FDR).

Among the tested microorganisms, staphylococcal strains emerged as the most susceptible targets, highlighting *S. aureus* as a particularly informative model for delineating chemotype-dependent activity patterns within *Mentha* spp. EOs. The comparable susceptibility of antibiotic-resistant phenotypes, including MRSA and MLS-resistant isolates, to that of antibiotic-susceptible *S. aureus* suggests that EO-mediated effects are largely independent of classical resistance mechanisms. Similar observations have been reported for *M. piperita* and other monoterpene-rich EOs, whose antibacterial activity persists across resistant and susceptible strains, suggesting mechanisms distinct from conventional antibiotic targets [54,58,59].

In the fungal context, the predominantly fungistatic activity observed against *Candida* spp., regardless of fluconazole susceptibility, is consistent with previous reports indicating that *Mentha* spp. EOs typically interfere with fungal growth rather than inducing rapid fungicidal effects. Samber et al. showed that *M. piperita* EO and its major constituents perturb membrane integrity and ergosterol-associated processes, leading mainly to growth inhibition [60]. Similar fungistatic patterns have been described for other monoterpene-rich EOs [55,57].

The generally weak activity observed against Gram-negative bacteria and yeasts underscores the significance of cell envelope architecture as a major determinant of susceptibility to EOs. The outer membrane of Gram-negative bacteria and the multilayered fungal cell wall represent effective permeability barriers that restrict the intracellular accumulation of hydrophobic EO constituents [61].

From a practical perspective, this constraint suggests that while *Mentha* spp. EOs may have limited standalone efficacy against Gram-negative bacteria and yeasts, their activity profiles remain relevant in the context of combination strategies or chemotype-guided optimization, especially when contrasted with the markedly higher efficacy observed against *S. aureus*.

Several studies have shown that peppermint EO and its major constituents can modulate bacterial susceptibility to commonly used antibiotics, rather than acting solely as independent antimicrobial agents. In particular, *M. piperita* and *M. arvensis* EOs have been shown to enhance the activity of aminoglycosides and fluoroquinolones against *S. aureus* and *E. coli* [59,62,63]. Samber et al. further demonstrated that *M. piperita* EO and its major constituents, including carvone, menthol, and menthone, exhibited synergistic interactions with fluconazole against *C. albicans*, *C. tropicalis*, and *C. glabrata* [60]. Comparable synergistic and additive interactions with conventional antibiotics have been reported for piperitenone epoxide and related ketone-rich fractions against clinical *S. aureus* isolates,

including resistant strains [58]. Importantly, the lack of a clear antibiotic-class-specific effect pattern in these interactions suggests that the observed effects are unlikely to result from direct interference with antibiotic targets. Instead, existing evidence supports the hypothesis that monoterpene ketones and related constituents alter cytoplasmic membrane properties, including membrane fluidity and permeability, thereby facilitating antimicrobial uptake [60,64]. In this context, the demonstrated synergistic potential of *Mentha* spp. EOs indicates that their principal value may lie not in direct bactericidal activity, but in combination strategies aimed at enhancing the efficacy of conventional antimicrobials in infections involving multidrug-resistant pathogens [56,65].

At the compositional level, the consistently higher antimicrobial efficacy associated with carvone chemotypes, compared with menthol chemotypes, emphasizes the importance of qualitative compositional architecture over simple abundance-based metrics. Although oxygenated monoterpenes are often regarded as broadly bioactive, accumulating evidence indicates that high proportions of menthol and eucalyptol are frequently associated with attenuated antimicrobial potency [60,64]. In contrast, ketone-dominated profiles, particularly those enriched in carvone and piperitone, were associated with a more favorable antimicrobial profile in the investigated samples, reconciling discrepancies reported in the literature regarding *Mentha* EO efficacy [54,58].

Taken together, the concordant separation observed in chemical space (PCA), compositional topology (hierarchical clustering), and functional antimicrobial responses provide strong evidence for a robust chemotype–activity relationship. These findings support the view that the antimicrobial potency of *Mentha* spp. EOs emerges as a systems-level property of chemotypes, rather than as the additive effect of individual constituents. This framework has important implications for chemotype-guided selection, standardization, and rational formulation of plant-based antimicrobials, particularly in the context of infections involving multidrug-resistant pathogens.

Although the present study used isolated EOs, the results remain relevant because volatile compounds are partially transferred into mint tea preparations during infusion [36]. Therefore, chemotype-related differences may contribute to variation in the biological properties and potential nutraceutical value of commercially consumed mint teas.

The present study has several limitations. The antimicrobial activity was evaluated in vitro using isolated EOs, which may not fully reflect the biological effects of consumed aqueous infusions. In addition, the number of commercial products analyzed was limited and may not represent the entire variability of *Mentha* products available on the European market. Future studies should include infusion-based analyses, sensory characterization, and evaluation of microbiome-related effects under physiologically relevant conditions.

Overall, the present findings support the view that commercially available mint teas represent chemically heterogeneous plant-derived products with markedly different biological properties. Chemotype-dependent variation in antimicrobial activity suggests that the nutraceutical value of mint teas may be closely linked to their volatile composition and highlights the importance of phytochemical characterization for evidence-based use of herbal beverages.

Limitations of the study: The antimicrobial activity reported in the present study was determined using essential oils isolated from commercial mint teas and therefore reflects the intrinsic in vitro activity of the volatile fraction rather than the biological effects achieved after consumption of a tea infusion. During the preparation of herbal teas, only a portion of the volatile constituents is transferred into the aqueous extract, while additional losses may occur due to evaporation and subsequent metabolism after ingestion. Consequently, the MIC and MBC values reported here should not be interpreted as concentrations attainable in vivo through normal tea consumption. Nevertheless, these results provide useful

comparative information on the antimicrobial potential of different commercial mint tea products and their chemotypes, and may contribute to assessing their nutraceutical value.

A limitation of this study is the relatively small number of commercial mint tea products investigated. Therefore, the results should not be considered fully representative of the European market but rather of the analyzed sample set.

5. Conclusions

In conclusion, commercially available *Mentha* teas demonstrated substantial variability in EO composition and antimicrobial activity, reflecting pronounced chemotype-dependent differences among products. Carvone-dominated chemotypes were generally associated with stronger antimicrobial effects, particularly against *Staphylococcus aureus* strains, including resistant phenotypes, whereas menthol-rich chemotypes showed lower activity.

The observed relationship between the component composition of essential oils and antimicrobial efficacy suggests that the biological characteristics and potential nutraceutical value of peppermint teas may depend on the chemotypes of different species of mint.

These findings highlight the importance of phytochemical characterization and quality control of commercially distributed mint teas intended for regular consumption as functional plant-derived beverages. Among the investigated products, *M. spicata* tea (M26) and the mixture of *M. spicata*, *M. piperita*, and *M. arvensis* (M10) showed the most promising antimicrobial profiles against resistant *Staphylococcus aureus* strains and may therefore warrant further investigation from a nutraceutical perspective.

Supplementary Materials: The following supporting information can be downloaded at: https://www.mdpi.com/article/10.3390/nutraceuticals6030045/s1, Figure S1: GC–MS chromatogram of the essential oil of *Mentha piperita* (Sample M19); Figure S2: GC–MS chromatogram of the essential oil of *Mentha suaveolens* (Sample M24); Figure S3: GC–MS chromatogram of the essential oils from *Mentha spicata* + *M. piperita* + *M. arvensis* (M10); Table S1: Content of essential oils from commercial mint teas analyzed by GC–MS.

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Abbreviations

The following abbreviations are used in this manuscript:

M.	*Mentha*
EOs	Essential oils
GC–MS	Gas chromatography-mass spectrometry
MICs	Minimum inhibitory concentrations
MBCs	Minimum bactericidal concentrations

MFCs	Minimum fungicidal concentrations
IZD	Inhibition zone diameter
PCA	Principal component analysis
S	Antibiotic-susceptible strains
MDR	Multidrug-resistant strains
MRSA	Methicillin-resistant *S. aureus*
MLS	Resistance (macrolides, lincosamides, streptogramin B)
ESβL	Extended spectrum β-lactamases
TEM-β-lactamase	Resistance to penicillins and early-generation cephalosporins
KPC	Carbapenemase
FCZ-R	Fluconazole resistant
FCZ-S	Fluconazole susceptible

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