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Antimicrobial Activity of Selected Essential Oils against *Staphylococcus aureus* from Bovine Mastitis

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Abstract: *Staphylococcus aureus* is a major cause of subclinical mastitis in dairy cows, and its development of antibiotic resistance has limited treatment efficacy. Essential oils (EOs) are natural products with a wide range of antimicrobial properties that could be used to treat bovine mastitis. This study aims to investigate the antimicrobial activity of EOs against *S. aureus* isolated from subclinical bovine mastitis cases in the State of São Paulo—Brazil. A total of 14 *S. aureus* isolates were selected, based on the presence of biofilm-forming genes (*ica*A, *ica*D, and *bap*), and were cultured to a final concentration of 10^3 CFU.mL^{-1} for the minimum inhibitory concentration (MIC) and minimum bactericidal concentration (MBC) analysis of five EOs (*Citrus aurantium bergamia*—bergamot, *Copaifera reticulata*—copaiba, *Foeniculum vulgare*—fennel, *Zingiber officinale*—ginger, and *Ocimum basilicum*—basil). The chemical compositions of the EOs were characterized using gas chromatography coupled with a mass-selective detector (GC/MSD). Basil and bergamot EOs exhibited the highest antimicrobial activity against *S. aureus* strains, with mean MIC/MBC values of 1.561 ± 0.223/2.806 ± 0.255 mg.mL^{-1} and 2.782 ± 0.228/4.396 ± 0.198 mg.mL^{-1}, respectively. The primary compounds in basil EO were methyl-chavicol, linalool, and α-humulene, while bergamot EO predominantly contained linalyl acetate, limonene, and linalool. This research highlights the potential of basil and bergamot EOs as natural antimicrobial agents for treating bovine mastitis caused by *S. aureus*, offering a potential alternative to traditional antibiotics and contributing to animal welfare and public health. In addition, it emphasizes the need for further studies to validate the long-term effects, optimal dosages, and application methods.

Keywords: herbal medicine; antimicrobial effect; dairy cows; MIC; MBC; bovine mastitis; antibiotic resistance

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1. Introduction

Bovine mastitis, one the most prevalent and costly diseases of dairy cows, is primarily caused by *Staphylococcus aureus* [1]. This pathogen is a Gram-positive coccus, characterized by its catalase-positive and facultative anaerobic nature, and can grow in a wide range of temperatures as well as pH and water activity levels [2]. It is notably prevalent in Brazil, where it is a leading cause of foodborne outbreaks [3]. It possesses a range of virulence factors, including enzymes, toxins, and survival strategies that facilitate the evasion of host defenses, such as the ability to adhere to surfaces and form biofilms [2,4,5].

Mastitis can be classified into two forms: clinical mastitis, which shows evident signs, such as udder inflammation, redness, warmth, and swelling, representing the cardinal signs of inflammation. Additionally, it leads to visible changes in milk, such as clots and discoloration [6]. The second form is subclinical mastitis (SM), which lacks noticeable signs; however, due to the silent development of the disease, SM has a tendency to progress into chronic infections [7,8]. The effect of mastitis on dairy farms is significant, leading to reduced milk production, increased culling rates, and decreased milk quality [6,9]. Conventional treatments for mastitis primarily depend on antibiotic usage [10]. Unfortunately, the excessive and inappropriate use of antibiotics has contributed to antibiotic resistance in *S. aureus*, leading to significant hindrances in mastitis treatment. As a result, the efficacy of conventional treatment protocols has been compromised [6,11]. Consequently, there is a growing need to find effective alternatives for mastitis treatments and to reduce the antibiotic resistance problem [8,12].

Plant extracts have emerged as significant therapeutic alternatives, particularly due to the increasing antimicrobial resistance to conventional antibiotics [13]. Throughout the years, common knowledge has proven indispensable for gaining insight into the healing effects of essential oils (EOs). EOs are composed of a complex mixture of volatile molecules, which are specific to each plant, including their range of bioactivities; these molecules include alkaloids, monoterpenes, carotenoids, flavonoids, isoflavones, phenolic acids, oxygen-containing and non-oxygenated terpene hydrocarbons, and aldehydes [13,14]. The mechanisms of antimicrobial activity of EOs include the degradation of the cell wall and cytoplasmic membrane, cytoplasm coagulation, the inhibition of toxic bacterial metabolites, and the inhibition of the bacterial efflux system. However, the efficacy of antimicrobial activity can vary depending on the pathogen and the composition of the EO [15–17]. For example, these compounds have a hydrophobic characteristic, which confers greater effectiveness against Gram-positive bacteria. On the other hand, Gram-negative bacteria present lipopolysaccharides that cause rejection of EOs, hindering their absorption and antimicrobial activity [13,16].

The use of EOs as an alternative to treat *S. aureus* bovine mastitis has not been extensively studied. There is limited research on the topic [17], and further studies are needed to confirm the safety and efficacy of EOs for use in dairy cows. In this context, studies regarding the antimicrobial activity of new compounds, including EOs, especially to develop new treatments against mastitis, are increasingly important [18].

Based on the abovementioned reasons, this study aims to evaluate the antimicrobial activity of five EOs that could be considered potential antimicrobial agents for treating subclinical mastitis caused by *S. aureus*. It seeks to identify natural compounds relevant to the dairy industry by offering effective and sustainable mastitis treatments.

2. Materials and Methods

2.1. Preparation of Bacterial Strains

Before initiating this study, ethical approval was secured from the Ethical Committee on Animal Use at the School of Veterinary Medicine and Animal Science, University of São Paulo, Brazil, with protocol number 3020/2013. A total of 14 *S. aureus* strains were initially sourced from raw milk. Samples were collected from cows with SM from commercial dairy herds in the Midwest of São Paulo State, Brazil. The selection criteria for cows were specifically designed to focus on those exhibiting signs of chronic SM, whilst excluding cows with a history of clinical mastitis. Identification of SM was based on consecutive biweekly somatic cell count (SCC) tests and culture-positive results. Specifically, cows were considered to have SM if they had at least two out of three SCC tests with counts exceeding 200,000 cells/mL. These *S. aureus* strains were sourced from a prior companion study conducted at the Milk Quality Research Laboratory of the University of São Paulo. A comprehensive description is available in previous studies [7,9]. Therefore, the strains were selected based on the presence of biofilm-forming genes (*ica*A, *ica*D, and *bap*). The cultures were stored on nutrient agar slopes at 4 °C until microbiological analysis. Then, bacterial

suspensions were prepared by subculturing 100 µL of each strain in 9 mL of brain–heart infusion broth (BHI, Kasvi, Brazil) and incubated at 35 ± 1 °C for 24 h. After this period, inoculum was standardized in sterile 0.85% saline solution to a turbidity of 0.5 on the McFarland scale (equivalent to 1.5×10^8 CFU/mL). For the purpose of this study, bacterial suspensions were adjusted to contain 10^3 colony-forming units (CFU)/mL.

2.2. Essential Oils

A total of five commercial EOs were purchased from BioEssência® (São Paulo, Brazil). Table 1 describes the characteristics of the EOs evaluated in our study.

Table 1. Botanical and geographical characteristics of EOs.

Botanical Origins	Plant Family	Common Names of EOs	Geographical Origin
Citrus aurantium bergamia	Rutaceae	Bergamot	Brazil
Copaifera reticulata	Fabaceae	Copaiba	Brazil
Foeniculum vulgare	Apiaceae	Fennel	Brazil
Zingiber officinale	Zingiberaceae	Ginger	Brazil
Ocimum basilicum	Lamiaceae	Basil	Brazil

2.3. GC/MSD Analysis of EOs

Evaluation of the chemical composition of EOs was performed at the Multidisciplinary Center of Chemical, Biological and Agricultural Research (CPQBA) of UNICAMP using an HP-6890 gas chromatograph coupled with an HP-5975 mass-selective detector (GC/MSD). The analyses were carried out using the following analytical conditions: HP-5MS capillary column (30 m × 0.25 mm × 0.25 µm); injector temperature of 220 °C; column temperatures of 60 °C, 3 °C/min, and 240 °C; and detector temperature of 250 °C. The injected volume was 1 µL, and the carrier gas was helium at a 1 mL/min^{-1} flow rate and a 40:1 split ratio. Identification of the analytes was performed by comparing their retention indices (IR) using coinjection of a mixture of hydrocarbon standards (C-8 to C-24) with the electronic equipment's database from the National Institute of Standards and Technology (NIST-11) [19] and available literature data [20].

2.4. Determination of Minimum Inhibitory Concentrations (MIC)

The determination of broth microdilution was performed using the microdilution technique. Initially, an evaluation of EO density was performed in triplicate to determine the volume of EO stock solution needed to achieve the desired concentration based on the relationship between mass and volume. For the MIC procedures, 360 µL of TSB broth (Tryptone Soy Broth) was dispensed into the first column of a 96-well acrylic plate, along with 0.5% Tween 80 and 40 µL of essential oil, reaching a final concentration of 10% of essential oil in the first well. Then, 200 µL of TSB broth with 0.5% Tween 80 was distributed into the wells of the following columns. After homogenization of the contents in the wells of the first column, two-fold serial dilution was performed. An aliquot of 200 µL of the mixture from the first well of the row was transferred into the second well, and so on, consecutively. Subsequently, the strains were standardized to the McFarland scale and adjusted with sterile saline to a concentration of 10^5 CFU/mL, and 2 µL of the microbial suspension obtained was inoculated, reaching a final concentration of 10^3 CFU/mL per well. The microplates were incubated at 37 °C for 24 h. Afterward, growth confirmation was carried out in the wells by applying 50 µL of resazurin solution with a concentration of 0.01%, and then, waiting ~5 to 10 min to perform a visual reading of the occurrence of a colorimetric reaction, and an evaluation of the growth inhibition of microorganisms [21,22]. All tests were performed in triplicate. The MIC was calculated according to the well with the lowest concentration of essential oil that presented a blue color, which indicates the absence of microbial growth.

2.5. Determination of the Minimum Bactericidal Concentration (MBC)

To determine the minimum bactericidal concentration, 10 µL was removed from the MIC and the three previous wells and subsequently deposited on nutrient agar plates using the micro-drop technique. Later, the plates were incubated at 37 °C for 24 h. At the end of incubation, a reading was performed, and the following criteria were considered: sections with microbial growth indicated bacteriostatic activity of the essential oil in question at the analyzed concentration, and sections with no microbial growth showed bactericidal activity. The well with the lowest concentration that exhibited a bactericidal effect was recorded as having MBC [23].

2.6. Statistical Analysis

Data are reported as the mean ± standard deviation (SD) of three measurements. Statistical analysis to evaluate the difference between groups was performed using the analysis of variance (ANOVA), followed by Tukey's range test to analyze data, with the significance level set at $p < 0.05$. All statistical analyses were performed using SPSS Statistics 21 software (IBM Corp., Armonk, NY, USA) [24].

3. Results

3.1. Chemical Composition of the Essential Oils

The compositions of the EOs obtained through GC/MSD analysis are presented in Table 2, and their chromatograms are shown in Figure 1. Among the several chemical components found in bergamot EO, linalyl acetate, limonene, and linalool were the most abundant compounds (accounting for 39.18%, 29.88%, and 16.91%, respectively). Sesquiterpenes were found to be the major compound of ginger EO, containing mainly alpha-zingiberene (32.76%), ar-curcumene (14.03%), and β-sesquiphellandrene (12.95%). Basil EO presented phenylpropanoids, monoterpenes, and sesquiterpenes, especially methyl-chavicol (72.86%), linalool (18.76%), and α-humulene (1.55%), as its major compounds. Copaiba oil was rich in sesquiterpenes, containing mainly trans-caryophyllene (47.43%), α-humulene (7.95%), α-trans-bergamotene (7.59%), and α-copaene (6.81%). Fennel EO contained mainly a combination of monoterpenes and phenylpropanoids, with trans-anethole (81.36%), followed by fenchone (5.84%) and α-pinene (4.56%), being the primary compounds.

Table 2. Chemical composition of EOs identified by GC/MSD.

Essential Oil	RI	RI (Lit.)	Compound	% Rel.
Bergamot	932	932	α-pinene	0.56
	971	969	sabinene	0.59
	975	974	β-pinene	3.49
	989	988	β-myrcene	0.77
	1023	1020	p-cymene	0.69
	1029	1029	Limonene	29.88
	1057	1054	γ-terpinene	3.83
	1102	1095	Linalool	16.91
	1227	1227	nerol (cis-geraniol)	1.06
	1239	1238	neral (cis-citral)	0.69
	1258	1257	Linalyl acetate	39.18
	1269	1267	geranial (trans-citral)	0.86
	1372	1423	linalyl butyrate	1.48
Copaiba	1335	1335	δ-elemen	0.67
	1347	1495	α-cubebene	1.36
	1374	1374	α-copaene	6.81
	1388	1387	β-cubebene	0.44
	1390	1389	β-elemene	1.75
	1396	1398	cyperene	0.54

Table 2. *Cont.*

Essential Oil	RI	RI (Lit.)	Compound	% Rel.
Copaiba	1421	1419	trans-caryophyllene	47.43
	1432	1434	γ-elemene	2.04
	1435	1432	α-trans-bergamotene	7.59
	1452	1452	α-humulene	7.95
	1456	1454	trans-β-farnesene	0.36
	1458	1458	allo-aromadendrene	0.43
	1475	1478	γ-muurolene	2.43
	1479	1484	germacrene D	5.69
	1483	-	M = 204	1.59
	1492	1493	epi-cubebol	1.04
	1493	1500	bicyclogermacrene	0.75
	1497	1500	α-muurolene	0.66
	1507	1505	β-bisabolene	4.11
	1511	1513	γ-cadinene	0.6
	1521	1522	δ-cadinene	4.11
	1541	-	M = 204	0.59
	1579	1582	caryophyllene oxide	0.59
	1614	1618	junenol	0.46
Fennel	932	932	α-pinene	4.56
	975	974	β-pinene	0.55
	989	988	β-myrcene	0.59
	1004	1002	α-phellandrene	3.55
	1023	1020	p-cymene	0.34
	1027	1029	limonene	1.2
	1056	1054	γ-terpinene	0.21
	1088	1086	fenchone	5.84
	1197	1195	p-allylanisole (estragole)	0.97
	1251	1249	cis-anethole	0.63
	1291	1282	trans-anethole	81.36
	1478	1484	germacrene D	0.21
Ginger	800	801	hexanal	0.28
	932	932	α-pinene	1.66
	947	946	camphene	5.01
	984	981	6-methyl-5-hepten-2-one	0.31
	989	988	β-myrcene	0.57
	1028	1029	limonene	4.88
	1030	1026	1,8-cineole (eucalyptol)	2.27
	1164	1165	endo-borneol	1.13
	1174	-	M = 166	0.24
	1189	1186	α-terpineol	0.54
	1373	1374	α-copaene	0.74
	1382	1379	geranyl-acetate	0.42
	1390	1389	β-elemene	1.17
	1404	1405	sesquitujene	0.37
	1431	1434	γ-elemen	0.45
	1455	1454	trans-β-farnesene	0.42
	1478	1478	γ-muurolene	1.75
	1483	1479	ar-curcumene	14.03
	1490	1496	valenceno	1.58
	1498	1493	α-zingiberene	32.76
	1509	1505	β-bisabolene	11.72
	1516	1520	7-epi-α-selenene	0.62
	1525	1521	β-sesquiphellandrene	12.95

Table 2. *Cont.*

Essential Oil	RI	RI (Lit.)	Compound	% Rel.
Ginger	1531	1529	trans-γ-bisabolene	0.39
	1547	1548	elemol	0.51
	1562	1561	trans-nerolidol	0.56
	1587	-	M = 222	0.34
	1611	-	M = 222	0.77
	1628	-	M = 222	0.53
	1685	-	M = 222	0.56
	1692	-	M = 220	0.48
Basil	971	969	sabinene	0.57
	1015	1014	α-terpinene	0.32
	1023	1020	p-cymene	0.71
	1056	1054	γ-terpinene	0.69
	1087	-	n.i.	0.37
	1101	1095	linalool	18.76
	1171	1167	menthol	0.61
	1176	1174	terpin-4-ol	0.82
	1202	1195	p-allylanisole (estragole)	72.86
	1269	1264	trans-citral (geranial)	0.64
	1433	1432	α-trans-bergamotene	0.7
	1541	1452	α-humulene	1.55
	1563	1562	trans-methoxycinnamaldehyde	0.76
	1565	-	M = 164	0.64

RI: retention index. RI (Lit.): retention index from literature data [20].

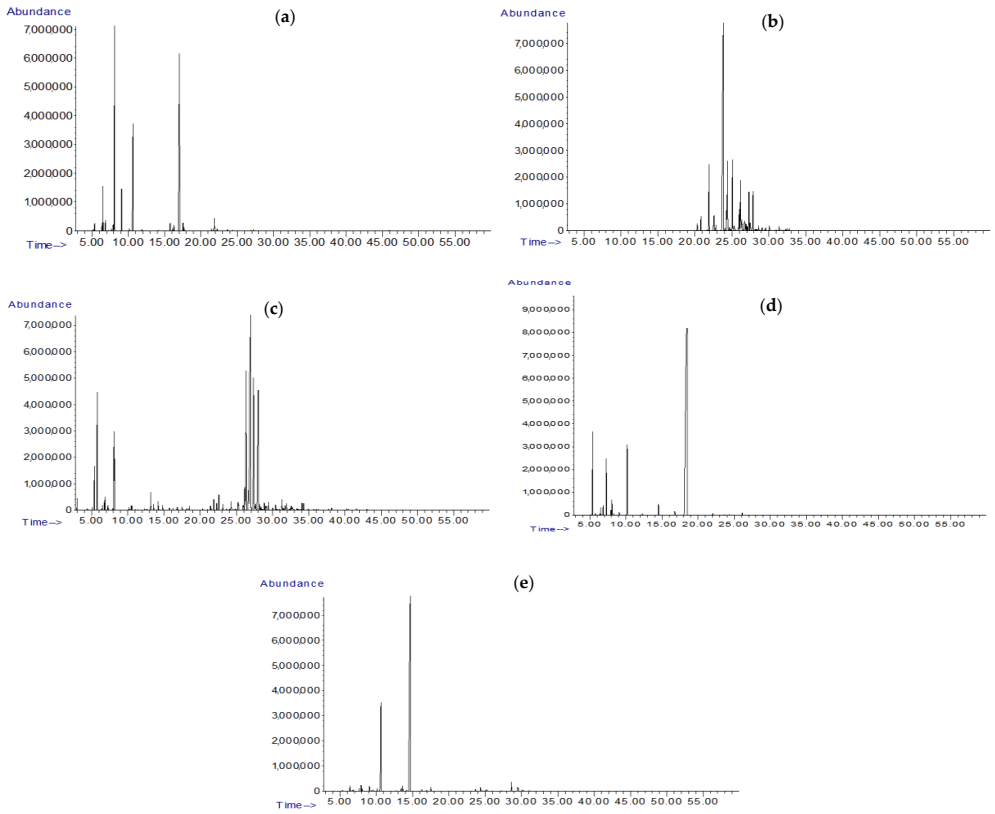

Figure 1. Chromatographic profiles of EOs tested via GC/MSD. The area represented by the peaks corresponds to each component's proportions in the mixture. (**a**) Bergamot EO; (**b**) copaiba EO; (**c**) ginger EO; (**d**) fennel EO; (**e**) basil EO.

In order to provide a comprehensive overview of the chemical compositions of the essential oils examined in this study, we grouped the identified compounds into their respective chemical classes based on the information delineated in Table 2. For Bergamot EO, monoterpenes emerged as the most abundant class, making up roughly 56.72% of the total composition. This was followed by esters, which contributed around 40.66%. Alcohols and aldehydes were present but less abundant, constituting approximately 1.06% and 1.55%, respectively. Copaiba EO was characterized by sesquiterpenes, accounting for 86.36% of the overall composition. Oxygenated sesquiterpenes made up a smaller fraction, approximately 0.59%. Compounds designated as 'M = 204' comprised about 2.18% of the EO composition. In the case of Fennel EO, phenylpropanoids were overwhelmingly dominant, contributing to about 82.62% of the total composition. Monoterpenes followed, accounting for roughly 11.00%, while sesquiterpenes were minimally present, constituting about 0.21%. For Ginger EO, sesquiterpenes were the major class, making up approximately 73.09% of the total oil composition. Monoterpenes were also significant, contributing about 13.89%. Oxygenated monoterpenes and aldehydes were found in smaller amounts, representing 2.27% and 0.28%, respectively. Lastly, basil EO was primarily composed of phenylpropanoids, which accounted for 73.62% of the total composition. Monoterpenes comprised the second largest category, contributing about 21.57%, while sesquiterpenes were found in smaller proportions, around 2.25% of the total composition.

3.2. Antimicrobial Activity of EOs against S. aureus

The antimicrobial activity of the EOs was evaluated using MIC and MBC tests. The results of the mean values are shown in Figure 2.

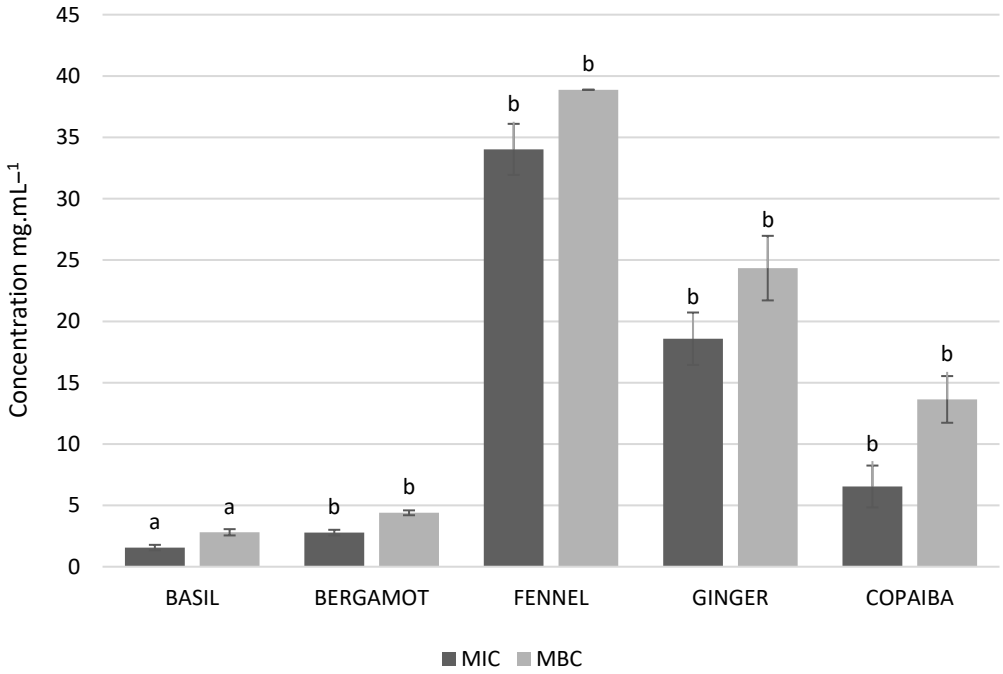

Figure 2. Minimum inhibitory concentration and minimum bactericidal concentration results of EOs tested against *S. aureus* strains. Bars represent mean ± SD. Means with different letters are significantly different ($p < 0.05$).

Based on the antimicrobial activity results, the EOs displayed varying levels of effectiveness against *S. aureus* strains, with a clear decreasing order of activity: basil EO > bergamot EO > copaiba EO > ginger EO > fennel EO.

The two most effective EOs were basil and bergamot, with mean MIC/MBC values of 1.561 ± 0.223/2.806 ± 0.255 and 2.782 ± 0.228/4.396 ± 0.198 mg.mL^{-1}, respectively. These EOs exhibited higher antimicrobial activity against *S. aureus* isolates than copaiba

and ginger EOs, which had mean MIC/MBC values of 6.541 ± 1.705/13.637 ± 1.903 and 18.58 ± 2.138/24.341 ± 2.631 mg.mL^{-1}, respectively. Fennel EO had the highest MIC values, with a mean of 34.02 ± 2.083 mg.mL^{-1}, and did not exhibit a bactericidal effect, even at the highest concentration applied.

Concerning the results of the MIC/MBC tests for each *S. aureus* strain, they are displayed in Supplementary Figures S1 and S2, respectively. The MIC/MBC values of basil and bergamot EOs ranged between 0.149–2.378/0.298–4.755 and 0.277–4.435/1.109–8.87 mg.mL^{-1}, respectively. Copaiba and ginger EOs had MIC/MBC values ranging between 1.11–17.76/4.44–35.52 and 4.605–36.84/9.21–36.84 mg.mL^{-1}, respectively. The MIC values of fennel EO ranged from 19.44 to 38.88 mg/mL^{-1}.

Furthermore, when comparing the antimicrobial activity of the EOs, basil emerged as the most effective EO, demonstrating significantly lower MIC and MBC against *Staphylococcus aureus*. Bergamot, fennel, ginger, and copaiba showed no significant difference in their antimicrobial activities.

4. Discussion

Bovine mastitis, associated with *S. aureus* infection, requires preventive measures in dairy farming [6,9,10]. Managing bovine mastitis presents a formidable challenge, mainly due to the biofilm-forming capabilities of *S. aureus*, facilitated by genes such as *ica*A, *ica*D, and *bap*. These genes enable the bacteria to firmly attach to the bovine mammary epithelium and form biofilms, which can enhance bacterial resistance to antimicrobial agents, often leading to persistent and recurrent infections [25,26]. In light of this, our isolate selection was strategic, aiming to assess the antimicrobial potency of EOs as agents against *S. aureus*, even in the presence of genes capable of forming biofilms.

The overuse of antibiotics has led to resistance in pathogens [11,27], driving the search for alternative treatments without exacerbating antibiotic resistance [8,18]. Several studies have shown that the chemical constituents of EOs are abundant in aromatic compounds, which can be classified into two structural groups: terpenoids and phenylpropanoids [13,17]. These phenolic compounds of EOs can modify the permeability of the cell membrane by penetrating the phospholipids bilayer of the bacterial cell wall [28]. In our study, it was observed that among the tested EOs, basil EO exhibited the highest antimicrobial activity, with low MIC/MBC, which indicates their ability to inhibit and kill *S. aureus* at relatively low concentrations. The phenolic compounds and monoterpenes found as the major components of these EOs must be associated with their antimicrobial properties (72.86%). Supporting our findings, Falowo et al. [29] reported that basil EO contains bioactive chemicals such as estragole (41.40%), 1,6-octadien-3-ol, 3.7-dimethyl (29.49%), and bergamotene (5.32%). Based on these observations, we hypothesize that the presence of estragole and other bioactive compounds in basil EO plays a crucial role in its remarkable antimicrobial activity against *S. aureus* strains.

Terpenes and terpenoids represent the most active phytochemicals studied, with properties for treating or preventing diseases, followed by polyphenols (such as phenolic acids and flavonoids) [16,30]. Our findings support these claims, as these compounds, constituting up to 90% of most EO compositions, exhibit diverse chemical and biological properties [28]. Our study reported that bergamot EO was found to be the second most effective antimicrobial agent against *S. aureus* strains according to the MIC/MBC results. Among the several chemical components contained in this EO, monoterpenes such as linalyl acetate (39.18%), limonene (29.88%), and linalool (16.91%) were found to be the most abundant. A study performed to discover the biological activities of bergamot EO suggested good antimicrobial activity against *S. aureus*, and identified d-limonene (60.44%) and γ-terpinene (20.28%) as the major compounds contributing to this activity [24]. The significant presence of these active compounds, particularly the monoterpenes, in bergamot EO may account for its notable antimicrobial properties.

It is important to note that the antimicrobial activity of EOs can vary depending on the specific pathogen and the composition of the EO. For instance, EOs of basil and bergamot

were found to be active against Gram-positive bacteria (*S. aureus* and *B. subtilis*). On the other hand, perilla EO strongly inhibited yeast growth [31], adding complexity to the application of EOs as antimicrobial agents. While our results are promising, indicating that basil and bergamot EOs could be helpful in treating *S. aureus* infections in subclinical bovine mastitis, more research is needed. Their low MIC and MBC values indicate that they could help control the growth of *S. aureus* in milk, thereby reducing the risk of mastitis in dairy cows. However, further research must corroborate these findings, and the safety and efficacy of using EOs in dairy cows must be thoroughly evaluated. Future studies should focus on assessing the long-term effects of EO treatment, including appropriate dosage, application methods, and potential interactions with other medications or treatments. It is essential to ensure these compounds' safe and effective management in bovine mastitis, without negatively impacting milk quality or human health.

Sesquiterpenes are well known for their bioactive properties that display good inhibitory activity against *S. aureus* [32]. Copaiba EO varies in its phytochemical composition depending on its species. The primary components include sesquiterpenes, diterpenes, and β-caryophyllene [33]. In line with this, our results demonstrated a high concentration of sesquiterpenes in the tested copaiba EO, containing mainly trans-caryophyllene (47.43%), α-humulene (7.95%), α-trans-bergamotene (7.59%), and α-copaene (6.81%). These findings support the research conducted by de Faria et al. [34], who also identified sesquiterpenes as the major compound in Copaiba EO and showed its antimicrobial activity against coagulase-negative *Staphylococcus*. Overall, our results reinforce that sesquiterpenes are bioactive compounds responsible for the observed antimicrobial activity against the strains we tested.

Studies have shown that Ginger EO can help prevent the growth of various pathogens by targeting the bacterial cell membrane and genetic material [13,35]. Although Ginger EO exhibits some antimicrobial activity against *S. aureus* strains, its efficacy appears to be comparatively lower when compared to the other EOs tested in our study. Sesquiterpenes were the primary compound of this EO, mainly containing α-zingiberene, ar-curcumene, and β-sesquiphellandrene. In line with our findings, Dal Pozzo et al. [36] evaluated the activity of EOs from various plants, including ginger, basil, rosemary, and sage EO, along with the major compound cineole, and reported no antimicrobial activity against *S. aureus*. While ginger EO exhibits some antimicrobial effects, our study confirms that it has lower efficacy compared to the other tested EOs.

Based on the MIC results, fennel EO, predominantly composed of monoterpenes and phenylpropanoids, exhibited minimal activity and showed no activity in the MBC test against most of the tested *S. aureus* strains. Interestingly, our results concerning fennel EO diverge from some of the existing literature. In comparison, some studies have reported its antimicrobial potential [37,38]. Likewise, Kwiatkowski et al. [39] demonstrated that trans-anethole at a concentration of 4% displayed antistaphylococcal effects. However, our results are consistent with those of Rani et al. [40], who determined that fennel EO has an insignificant impact on *S. aureus.* Overall, these findings suggest that fennel EO may not be a potent solution for fighting against *S. aureus* that have biofilm-forming genes (e.g., *ica*A, *ica*D, and *bap*, such as in the current study).

Above all, and based on previous studies that report that EO compounds represent the main source of chemical diversity [13,16,32], we support the relevance of the current finding that includes natural compounds' biological properties. The discovery of these antimicrobial agents against *S. aureus*, which causes bovine mastitis, may have several benefits not only in the dairy industry but also for the pharmaceutical and medical industries. In addition, this study offers practical benefits for dairy breeders. Using EOs as an antibiotic alternative could align with sustainable farming practices. This is especially valuable in the current context of increased antibiotic resistance, and consumer preference for natural treatments. These insights contribute to improved herd health, milk quality, and overall farm sustainability. Given the rich source of chemical diversity in EOs, future studies should focus on key bioactive elements in EOs with proven efficacy against *S. aureus*. Additionally,

further studies are needed to confirm the safety and effectiveness of EOs for use in dairy cows with bovine mastitis, and evaluate appropriate dosages and application methods. In this context, ensuring that EOs do not negatively impact milk quality or human health becomes essential.

5. Conclusions

In conclusion, our study reported that basil and bergamot are the most efficient antimicrobial EOs among the five EOs tested against *S. aureus* causing bovine mastitis. The phenylpropanoid compounds and monoterpenes found to be the major components of these EOs must be associated with their antimicrobial properties. This study contributes to the development of potential alternatives to conventional antibiotics, as an approach to mitigate the challenges posed by antibiotic-resistant *S. aureus*.

Supplementary Materials: The following supporting information can be downloaded at: https://www.mdpi.com/article/10.3390/dairy5010005/s1, Figure S1: Minimum inhibitory concentration results of EOs tested against S. aureus strains, Figure S2: Minimum bactericidal concentration results of EOs tested against *S. aureus* strains.

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Abbreviations

The following abbreviations are used in this manuscript:

EO	Essential oil
MIC	Minimum inhibitory concentration
MBC	Minimum bactericidal concentration
GC/MSD	Gas chromatography coupled with a mass-selective detector
CFU/mL	Colony-forming units per milliliter
SM	Subclinical mastitis
SCC	Somatic cell count
BHI	Brain–heart infusion broth
SD	Standard deviation
ANOVA	Analysis of variance

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