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RESEARCH ARTICLE

Enhanced Antibacterial Activity of Blended Essential Oils against Staphylococcus Aureus and Methicillin-Resistant Staphylococcus Aureus

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Abstract: We aimed to evaluate the antibacterial effects of four blended and four single essential oils on the gram-positive bacterium *Staphylococcus aureus* (*S. aureus*) and methicillin-resistant *S. aureus* (MRSA). Minimum inhibitory concentration (MIC) testing was performed using disk diffusion and broth microdilution methods according to the Clinical and Laboratory Standards Institute criteria. The blended essential oils exhibited more powerful antibacterial activity than the single oils, and Relaxation Aroma Blending Oil exhibited a stronger inhibitory effect against *S. aureus* and MRSA than the Bergamot single oil. The average inhibition zones of Relaxation Aroma Blending Oil against *S. aureus* and MRSA were 19.6 ± 0.2 mm and 18.4 ± 0.2 mm, respectively, indicating that Relaxation Aroma Blending Oil produced inhibition zones that were 30% and 58% larger than those formed by Bergamot single oil against *S. aureus* and MRSA, respectively. These findings highlight blended essential oils as promising natural antibacterial agents.

Keywords: Antibacterial agents; Antibacterial activity; Essential oils; Methicillin-resistant *Staphylococcus aureus* (MRSA)

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Introduction

Essential oils are natural compounds extracted from various plants and have long been used as antimicrobial agents in traditional medicine. The widespread use of antimicrobial agents for therapeutic and preventive purposes in various fields, including medicine and pharmacy, has led to the emergence of antibiotic resistance, which is currently considered to be one of the biggest health problems worldwide [1]. The spread of antibiotic resistance emphasizes the need for new treatment strategies and interest in natural antimicrobials is rapidly increasing [2]. Essential oils are attracting attention as a solution for antibiotic resistance and have the potential to be effective against antibiotic-resistant strains, unlike existing antibiotics. Furthermore, essential oils exhibit antimicrobial activity against resistant strains such as methicillin-resistant Staphylococcus

aureus (S. aureus) (MRSA), with the mechanism of action suggested to be increased cell membrane permeability and inhibition of protein synthesis [3]. One of the major mechanisms of MRSA resistance is its ability to form biofilms. Biofilms limit drug penetration, promote horizontal gene transfer, and alter metabolic activity, thereby increasing antibiotic resistance and evasion of host immune responses. This makes MRSA-related infections particularly persistent and difficult to eradicate. Recent studies have shown that certain plant-derived essential oils possess antibiofilm properties by either preventing biofilm formation or disrupting pre-formed biofilms [4].

S. aureus is an important and well-known pathogen due to its combination of invasiveness, antibiotic resistance, and toxin-mediated virulence [5]. It is a common cause of food poisoning due to the *S. aureus* enterotoxin (SE) and is a serious opportunistic pathogen present in one-third of the healthy human population. Additionally, *S. aureus* is the primary cause of bacterial infections in developed countries, causing a variety of diseases, ranging from benign skin infections to fatal infective endocarditis and necrotizing pneumonia [5]. MRSA is a multidrug-resistant bacterium causing serious hospital-acquired infections resistant to conventional treatment [6]. This contributes to high mortality rates and increased treatment costs, making MRSA a significant global health concern [7].

The treatment of bacterial, fungi, viruses, and parasitic infections present a major challenge in the medical field. To overcome these challenges, nanoencapsulation of essential oils and synergy between the oils, their components, and antibiotics has been recommended [8]. A previous study found that combining two antimicrobial agents resulted in partial synergistic or additive effects against S. aureus and Escherichia coli, as evaluated using the disk diffusion and minimum inhibitory concentration (MIC) methods. Furthermore, the antioxidant and antimicrobial activities of essential oils and their binary combinations and synthetic compounds were enhanced [9].

An important biological property of essential oils is their antimicrobial function, which is often due to the presence of active monoterpene components [10]. Essential oils such as oregano and cinnamon demonstrate inhibitory effects on bacteria, fungi, and viruses, such as clinical isolates of P. aeruginosa, using the disk diffusion method [11]. The mechanism of action is related to the hydrophobic nature of essential oils, which have been proposed to interact with cell membranes and to have a destructive effect on the outer structures of cells [13]. Various essential oils, such as tea tree and oregano oils, have potent antimicrobial effects against various pathogenic microorganisms, including E. coli, P. aeruginosa, and S. aureus [13]. Moreover, blended essential oils have the potential to produce synergistic effects that are stronger than those of the individual oils; blending can amplify the interaction between each component, further enhancing the antibacterial effect against resistant strains [14]. However, research comparing the effectiveness of mixed essential oils against multidrug-resistant strains, such as MRSA, remains insufficient. The chemical compositions of various essential oils differ, and there is a lack of standards for evaluating antibacterial activity, making it difficult to compare previously reported results; thus, further research is required [15, 16].

In this study, the antimicrobial efficacy of four single essential oils and their blends against Staphylococcus aureus (S. aureus) and methicillin-resistant Staphylococcus aureus (MRSA) was evaluated according to CLSI criteria to investigate the potential of mixed essential oils as alternative treatments for antibiotic-resistant strains.

To address the selection rationale and blending process, four essential oils were selected based on their previously reported antimicrobial properties and unique phytochemical profiles supported by existing literature. Each oil contains constituents known to exert antimicrobial activity through different mechanisms.

The blending process was strategically conducted to explore potential synergies between chemically diverse constituents. We demonstrated that mixed essential oils may exhibit enhanced antimicrobial properties compared to individual oils, suggesting their potential for the development of natural antimicrobials and may provide an alternative approach to the problem of antimicrobial resistance.

Materials and Methods

Essential Oils

We included four single essential oils (S1–S4) and their blends (B1–B4), as shown in Table 1. Each oil was purchased from a commercial supplier (Herb Island Agricultural Cooperative Association, Pocheon-si, Republic of Korea) and stored in sealed vials at 4°C in the dark until use.

Bergamot fruit oil, the primary component of blend B1, was designated as S1. We then designated peppermint leaf oil (highest content of B2), rosemary leaf oil (highest content of B3), and orange peel oil (highest content of B4) as S2 to S4, respectively. Oils with a concentration of <10% were marked as other oils.

Table 1. Names and contents of the components of oils B1–B4

Oil	Ingredients	ICID Name	Content (%)
B1	1	Bergamot fruit oil	45
	2	Lavender flower oil	35
	3	Scented geranium oil	10
	4	Other oils	10
B2	1	Peppermint leaf oil	67
	2	Lavender flower oil	10
	3	Pine needle oil	10
	4	Other oils	13
B3	1	Rosemary leaf oil	26
	2	Scented geranium flower oil	20
	3	Fennel oil	15
	4	Pine berry oil	15
	5	Bergamot fruit oil	11
	6	Other oils	13
B4	1	Orange peel oil	48
	2	Bergamot fruit oil	20
	3	Scented geranium oil	16
	4	Lavender flower oil	14
	5	Other oils	2

Target Strains and Medium

To evaluate the antibacterial activity, S. aureus (ATCC 25923) and MRSA (ATCC 33591) strains were used in the disk diffusion method. To determine the MIC, S. aureus (ATCC 29213) and MRSA (ATCC 33591) strains were used.

According to the CLSI guidelines M100 ED34: 2024, Mueller–Hinton agar (MHA) was used as a medium suitable for the disk diffusion method, and MHB was used for the MIC. Sterilization was performed before use.

Disk Diffusion

S. aureus and MRSA were cultured on MHA medium at 37°C for 24 h before the experiment. The colonies of the cultured strains were measured using a Densicheck turbidimeter (bioMerieux, Marcy L'Etoile, France) in 0.5 McFarland nephelometer tubes (bacterial count approximately 10^8 CFU/mL) to adjust the bacterial density. The adjusted bacterial suspension was evenly spread on the surface of the MHA medium using a sterile cotton swab and left at 22°C until the medium surface was completely dry. A sterilized 6-mm paper disk was placed in a sterilized Petri dish, and 20 µL of each essential oil (blended and single essential oil) was evenly dispensed, allowed to absorb for 15 min, dried at room temperature for 1 h, and then lightly placed on MHA with sterilized tweezers, gently pressed, and cultured in an oxygenated incubator at 35°C for 18–24 h with the medium lid down. After culturing, the diameter of the inhibition zone formed around each disk was measured (mm) using a digital caliper. Disks soaked in sterile saline were used as negative controls, and erythromycin (15 µg), oxacillin (1 µg), vancomycin (30 µg), and clindamycin (2 µg) disks were used as positive controls. *S. aureus* quality control was performed by confirming that the Tier 1 and 2 antibiotics were within the disk diffusion quality control range (erythromycin 22–30 mm, oxacillin 18–24 mm, vancomycin 17–21 mm, and clindamycin 24–30 mm) specified in the CLSI M100 ED34: 2024 guidelines. MRSA was confirmed to be resistant to erythromycin, oxacillin, and clindamycin, and showed a 21 mm inhibition zone for vancomycin, confirming the quality control of the test. All measurements were repeated three times at two-day intervals to derive average values. These procedures were performed in strict accordance with the CLSI M100 ED34: 2024 guidelines.

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MIC

Wells 1–9 were serially diluted two-fold, starting from 100% essential oil concentration. For the bacterial suspension, the turbidity of the bacteria was adjusted to a 0.5 tube (bacterial count approximately 108 CFU/mL) of a McFarland nephelometer with MHB, and then diluted 1:20 using saline solution to obtain a final inoculum count of 5×106 CFU/mL. Next, 180 µL of the bacterial suspension was inoculated into each well from wells 1–9 of the 96-well plate; 200 µL of the bacterial suspension was used for well 10 as a positive growth control, and 200 µL of MHB was used for well 11 as a negative growth control. The inoculated 96-well plate was cultured in an oxygenated incubator at 35 °C for 18–24 h, and the minimum concentration that inhibited growth was defined as the MIC. The MIC was defined as the minimum concentration that resulted in visually observable minimal turbidity or growth inhibition with the naked eye.

The absorbance of the culture suspension was measured at 600 nm using a spectrophotometer (SpectraMax M2 / M2e Microplate Readers, e-Innotech, Republic of Korea), and the results were interpreted (Fig. 1). For antimicrobial agents, 128 µg/mL was diluted two-fold using a sterilized pipette, starting from wells 1–9 of the 96-well plate, with 20 µL. According to the CLSI guideline M100 ED34:2024, appropriate reference strains were tested simultaneously according to the test antimicrobial agent or bacteria, using solvents and dilutions to confirm whether the results were within the acceptable range. Quality control was performed by confirming that each antimicrobial agent specified in CLSI guidelines M100 ED34: 2024 was within the MIC quality control range.

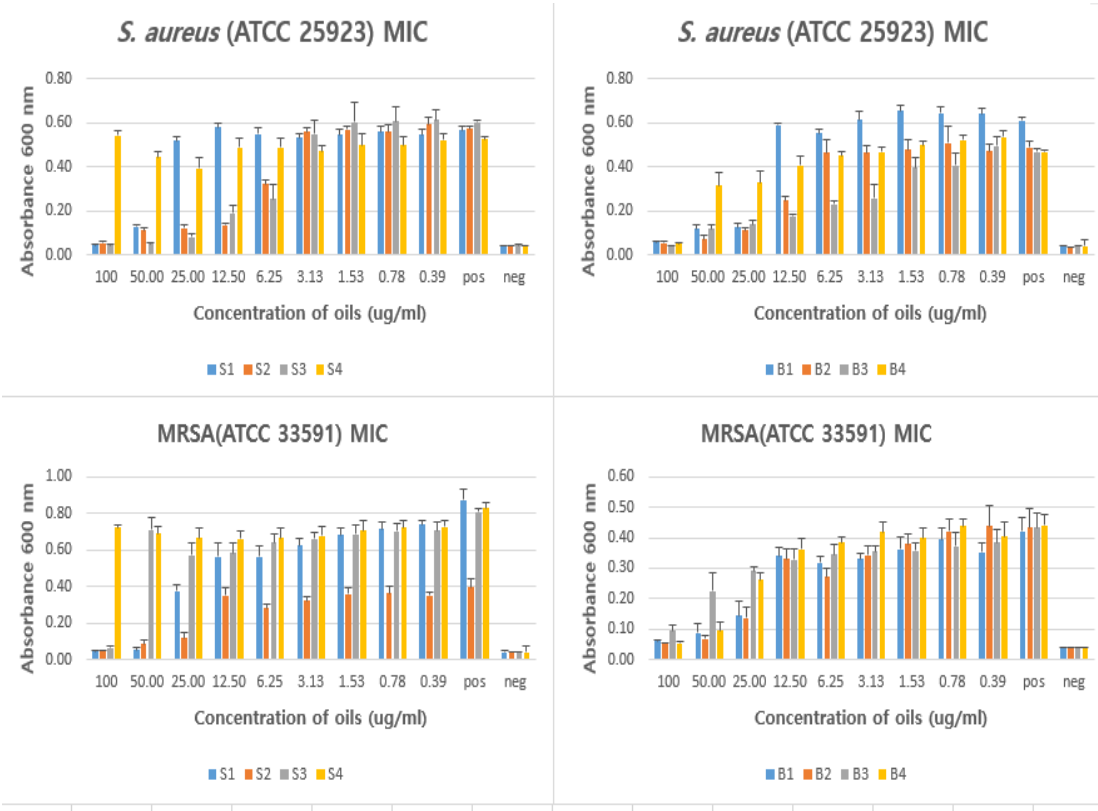

Fig. 1: MIC of S1, S2, S3, S4, B1, B2, B3, and B4 against Staphylococcus aureus (ATCC 25923) and methicillin-resistant S. aureus (MRSA; ATCC 33591). Abbreviations: S1, Bergamot single oil 1; S2, Peppermint single oil 2; S3, Rosemary single oil 3; S4, Orange single oil 4; MIC, minimum inhibitory concentration; Pos, positive control; Neg, negative control

Results

Antibacterial Effect

The antibacterial effect of each blended essential oil and its main components (each essential oil) against S. aureus was evaluated using the disk diffusion method. The results showed that the blended essential oils formed a wider inhibition zone

than the individual essential oils (Table 2). Among them, S2, B1, B2, and B4 showed superior antibacterial effects than the other essential oils.

Table 2: Antimicrobial activity of 20 µL of essential oils determined by disk diffusion method against Staphylococcus aureus (ATCC 25923) and methicillin-resistant S. aureus (MRSA; ATCC 33591)

Bacteria	Inhibition zone (mm)							
	S1	S2	S3	S4	B1	B2	B3	B4
S. aureus	11.2 ± 0.2	11.2 ± 0.2	13.2 ± 0.1	ND	19.6 ± 0.2	20.2 ± 0.2	12 ± 0.1	17.2 ± 0.1
MRSA	11.2 ± 0.1	14.8 ± 0.1	7 ± 0.1	ND	18.4 ± 0.2	16.0 ± 0.1	13.1 ± 0.1	16.6 ± 0.2

Abbreviations: ND, not detected; S1, Bergamot single oil 1; S2, Peppermint single oil 2; S3, Rosemary single oil 3; S4, Orange single oil 4

The antibacterial activity results of each essential oil by the disk diffusion method against S. aureus (ATCC 25923) for S1, S2, and S3 were 11.2 ± 0.2 mm, 15.2 ± 0.1 mm, and 13.2 ± 0.1 mm, respectively. S4 did not show any antibacterial activity. B1 was 19.6 ± 0.2 mm, forming an inhibition zone 30% larger than that of S1. B2 was 20.2 ± 0.2 mm, forming an inhibition zone 11% larger than that of S2. B3 was 12 ± 0.1 mm, showing an antibacterial effect similar to that of S3. B4 was 17.2 ± 0.1 mm, showing an antibacterial effect that was absent in S4.

The antibacterial effect against MRSA was evaluated in the same manner as for the existing *S. aureus* strain. For MRSA (ATCC 33591), the average inhibition zones for S1, S2, and S3 were 11.2 ± 0.1 mm, 14.8 ± 0.1 mm, and 7.0 ± 0.1 mm, respectively; S4 showed no antibacterial activity. B1 was 18.4 ± 0.2 mm, forming an inhibition zone 58% larger than that of S1. B2 was 16.0 ± 0.1 mm, forming an inhibition zone that was larger than that of S2. B3 was 13.1 ± 0.1 mm, forming an inhibition zone 48% larger than S3. B4 was 16.6 ± 0.2 mm, showing an antibacterial effect that was absent in S4 (Fig. 2), visually confirming this contrast.

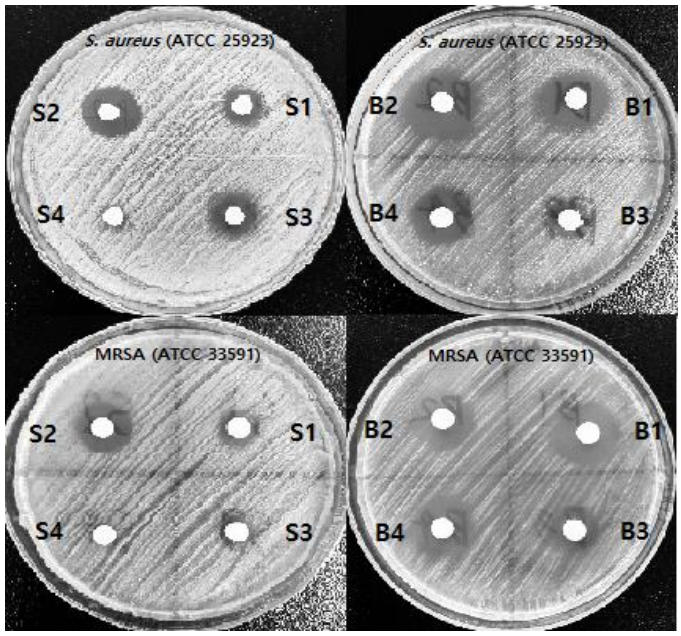

Fig. 2: Antibacterial inhibition zones of S1, S2, S3, S4, B1, B2, B3, and B4 oils against Staphylococcus aureus and methicillin-resistant S. aureus (MRSA) by disk diffusion method. Abbreviations: S1, Bergamot single oil 1; S2, Peppermint single oil 2; S3, Rosemary single oil 3; S4, Orange single oil 4

According to CLSI guideline M100-S22, the zones of inhibition for 15 µg erythromycin, 1 µg oxacillin, 30 µg vancomycin, and 2 µg clindamycin disks corresponding to Tier 1 and Tier 2 against S. aureus (ATCC 25923) and MRSA (ATCC 33591) were used as positive controls. The gram-positive strain S. aureus (ATCC 25923) results were 27, 22, 18, and 27 mm, respectively, which is within the CLSI guideline range for erythromycin (22–30 mm), oxacillin (18–24 mm), vancomycin (17–21 mm), and clindamycin (24–30 mm), confirming the quality control of the test. The zone of inhibition for MRSA (ATCC

33591) was also measured. Resistance to erythromycin, oxacillin, and clindamycin was confirmed, and a 21-mm inhibition zone was observed for vancomycin, confirming the quality control of the test. Compared to antibacterial agents, the inhibition zones of mixed oils showed similar antibacterial effects, suggesting that mixed essential oils may possess antibacterial activities similar to those of some antibiotics.

MIC Evaluation

The MIC was calculated by serially diluting the oils two-fold from a concentration of 100 to determine the MIC of oils S1, S2, S3, S4, B1, B2, B3, and B4 against S. aureus (ATCC 29213) and MRSA (ATCC 33591). For the positive control, a solution adjusted to McFarland 0.5 (approximately 1.5×108 CFU/mL) in Mueller–Hinton broth (MHB) was used. MHB was used as the negative control. As a control, gram-positive bacteria were treated with oxacillin. Table 3 shows the MIC and turbidity of essential oils at various concentrations after 24 h of inoculation with S. aureus and MRSA in 96-well plates. According to the CLSI guidelines M100-S22, MRSA was confirmed to be resistant to oxacillin, and the MIC of oxacillin used for gram-positive cocci (0.5 µg/mL) was used for S. aureus (ATCC 25923), which is within the range of 0.12–0.50 µg/mL suggested in the CLSI guidelines M100 ED34: 2024, confirming the quality control of the test.

Table 3: Minimum inhibitory concentration and turbidity for various concentrations of essential oils after 24 h

Strain	Oils	Concentration of oils (µg/mL)								
		100	50	25.00	12.50	6.25	3.13	1.53	0.78	0.39
S. aureus	S1	-	-	+	+	+	+	+	+	+
	S2	-	-	-	+	+	+	+	+	+
	S3	-	-	-	+	+	+	+	+	+
	S4	+	+	+	+	+	+	+	+	+
	B1	-	-	-	+	+	+	+	+	+
	B2	-	-	-	+	+	+	+	+	+
	B3	-	-	-	-	+	+	+	+	+
	B4	-	+	+	+	+	+	+	+	+
MRSA	S1	-	-	+	+	+	+	+	+	+
	S2	-	-	-	+	+	+	+	+	+
	S3	-	+	+	+	+	+	+	+	+
	S4	+	+	+	+	+	+	+	+	+
	B1	-	-	+	+	+	+	+	+	+
	B2	-	-	+	+	+	+	+	+	+
	B3	-	+	+	+	+	+	+	+	+
	B4	-	-	+	+	+	+	+	+	+

Abbreviations: S1, Bergamot single oil 1; S2, Peppermint single oil 2; S3, Rosemary single oil 3; S4, Orange single oil 4; *S. aureus*, *Staphylococcus aureus*; MRSA, methicillin-resistant *Staphylococcus aureus*. Positive (+), Turbidity indicating growth; Negative (-), Turbidity that does not indicate growth

Discussion

We evaluated the antibacterial effects of four blended and single essential oils on S. aureus and MRSA. The blended essential oils showed greater antibacterial activity than the single essential oils.

The superior antimicrobial efficacy of the blended essential oils observed in this study suggests that the synergistic effect of the combination of individual components may be more potent than that of the individual constituents when used alone. This has been confirmed in previous studies and indicates that the interaction between various essential oils can enhance the antibacterial effect through mechanisms such as the destruction of bacterial cell membranes, inhibition of protein synthesis, or inhibition of intracellular metabolic processes [14]. When multiple individual antibacterial components are present

in an essential oil, a synergistic effect is more likely to occur, and the overall antibacterial activity of the essential oil is amplified [14, 17]. The blended essential oils used in this study showed significant inhibitory effects against both S. aureus and MRSA, suggesting that the individual oil components act complementarily to inhibit bacterial survival. This synergistic effect can be achieved by components that complement the relatively weak antibacterial activity during the blending process, or by simultaneously attacking different targets [18].

The antibacterial effect was evaluated using the disk diffusion and liquid microdilution methods. The disk diffusion test is one of the oldest susceptibilities testing methods and is widely used in many clinical laboratories, especially in resource-limited environments [19]. This method is inexpensive, reproducible, and easy to interpret [20-22]. It uses commercially manufactured paper disks containing fixed concentrations of antimicrobial agents. The clearing diameter, or inhibition zone, created around the disk is related to the rate of drug diffusion through the agar medium and the susceptibility of the isolate to the drug. If the zone diameter is correlated with the MIC for a given bacterial–antimicrobial combination, it can be converted into an interpretation category [23].

The MIC is internationally recognized as the lowest concentration (expressed in μg/mL or mg/L) that causes visual growth inhibition in comparison to a control group not exposed to the antimicrobial agent under identical test conditions for all bacterial species. MIC measurements are used to assess the distribution of clinical isolates according to their antimicrobial activity, and this distribution is necessary to characterize the epidemiological cutoff value [24].

MRSA is a multidrug-resistant bacterium that is resistant to methicillin and is a major pathogen that is difficult to treat using existing antibiotics. Our results showed that the blended essential oils exhibited strong antibacterial activity against MRSA, suggesting that they can complement the effects of existing antibiotics or serve as an effective alternative against antibiotic-resistant bacteria [25]. The effectiveness of essential oils against MRSA has been demonstrated; they act by binding to the bacterial cell membrane to increase permeability, or by inhibiting nucleic acid and protein synthesis within the cell [26]. In addition, tea tree and oregano oils show strong antibacterial activity against MRSA, which supports our findings [27].

Multidrug resistance has become a major challenge for the medical community worldwide and remains one of the leading causes of human death in recent years [3]. The use of synthetic chemicals to control microorganisms remains limited due to their carcinogenic effects, acute toxicity, and environmental hazards [28]. Antibiotic resistance is a serious public health problem worldwide; therefore, there is an urgent need to develop new antibiotics to address multidrug resistance [29]. However, the development of new synthetic antibiotics is expensive and time-consuming, whereas natural substances such as essential oils have the advantage of being relatively inexpensive and can be developed quickly [30]. The antimicrobial effect of blended essential oils on multidrug-resistant bacteria such as MRSA suggests a new therapeutic alternative for the rapidly increasing resistance to existing antibiotics. This provides an important foundation for the study of antibiotic resistance and the development of natural product-based antimicrobial agents and shows that essential oils can effectively suppress resistant bacteria through multi-target mechanisms [13]. This is an important factor in enhancing their effect against antibiotic-resistant bacteria.

Although bacterial resistance mechanisms are generally focused on defending against a single target, the complex components of essential oils can simultaneously attack various physiological pathways [11]. This could also be a potent strategy for providing effective antimicrobial agents against drug-resistant bacteria [13]. The discovery of blended essential oils that can effectively suppress multidrug-resistant bacteria such as MRSA could lead to the development of new disinfectants or treatments for infection control and prevention in hospitals and public places. This may be effective in improving healthcare and reducing medical costs [2].

Our findings suggest that blended essential oils have high practical application potential as antibacterial agents. These oils can be used in various industrial fields, such as food preservatives, cosmetics, pharmaceuticals, and medical device disinfectants. The effectiveness of essential oils against multidrug-resistant bacteria such as MRSA makes them an important tool for infection control and prevention in hospital settings [31]. In addition, the antibacterial effect of blended essential oils can be continuously exerted under various environmental conditions, which is an important advantage for practical applications. For example, the antibacterial activity of essential oils does not change significantly under different temperatures, humidity, and pH, which enables their use in real environments outside the laboratory [32].

This study demonstrated the antibacterial effects of blended essential oils against S. aureus and MRSA. However, some limitations should be acknowledged. The chemical composition of the blended essential oils was not analyzed, which limits our ability to identify the specific phytochemicals responsible for the observed antibacterial activity. Essential oils are complex

mixtures of various compounds, and the ratios and interactions among these constituents can significantly influence their biological effects. Without component-level profiling using techniques such as GC-MS, it is difficult to determine which constituents contribute most to the efficacy. Future research will include detailed chemical analyses to explore structure–activity relationships.

No quantitative synergy analysis was performed. Although the blended oils showed greater antibacterial activity than individual oils, the study did not include methods such as the fractional inhibitory concentration index (FICI) or checkerboard assay to evaluate synergistic effects quantitatively. Thus, only qualitative inferences could be made. Future studies will address this by incorporating standardized synergy testing.

The antibacterial activity was assessed at a single concentration of essential oils, without systematic evaluation across a concentration range. Since concentration plays a key role in determining both efficacy and potential toxicity, the lack of optimal dosing data is a limitation. Additionally, volatility and stability of the oils can vary with concentration, and such factors were not considered in this study.

The study focused exclusively on two representative gram-positive bacterial strains (S. aureus and MRSA), which limits the generalizability of the findings to other pathogens. While these strains are clinically significant and serve as important models for antibacterial evaluation, the results cannot be extended to gram-negative bacteria, fungi, or viruses without further testing. Future studies should explore the broader antimicrobial spectrum of blended essential oils.

The study evaluated only the phenotypic antibacterial effects of the oil blends and did not investigate the underlying mechanisms by which resistance in MRSA may be overcome. Previous studies suggest that synergistic effects may arise from mechanisms such as membrane disruption, efflux pump inhibition, or interference with multiple metabolic pathways. Follow-up research at the molecular level is needed to clarify these mechanisms.

Conclusion

We evaluated the antibacterial effects of essential oils on S. aureus and MRSA according to the CLSI criteria. Our results show that blended essential oils exhibited strong antibacterial effects against S. aureus and MRSA, suggesting the potential of using blended essential oils as an alternative approach to address the problem of antibiotic resistance and a new direction for the development of natural antibacterial agents. Therefore, antibacterial activity can be maximized through the combination of natural compounds, providing an important alternative to antibiotic resistance. In addition, our data confirmed the potential of essential oils as natural antibacterial agents and revealed the possibility of their application in various industrial fields. In the modern industrial environment, where the demand for natural product-based products is increasing, our results may provide practical and economic value and contribute to the development of environmentally sustainable products preferred by consumers.

Authors' Contributions

Min-Gi Kwon: Writing - original draft.
Ji-Hyuk Kang: Formal analysis.
Jae Kyung Kim: Writing - review & editing.

Ethics

This study was approved by the Dankook University Institutional Review Board (IRB file No. DKU NON2024-004). There was no personal patient information. Therefore, the Dankook University Institutional Review Board waived the requirement for informed consent.

Data Availability Statement

The datasets used and analyzed during the current study are available from the corresponding author upon reasonable request.

Disclosure Statement

The authors report there are no competing interests to declare.

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