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In Vitro Antibacterial Activity and Synergistic Interactions of Thymus vulgaris and Syzygium aromaticum Essential Oils Against Clinical Isolates of Methicillin-Resistant Staphylococcus aureus and Salmonella spp

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Abstract:

Background: Antimicrobial resistance has escalated into a critical global public health crisis, severely threatening modern clinical therapeutics and healthcare infrastructure. The rapid emergence of multidrug-resistant pathogens, particularly methicillin-resistant *Staphylococcus aureus* (MRSA) and *Salmonella* species, has resulted in high morbidity and mortality rates worldwide. Consequently, exploring plant-derived antimicrobial agents and secondary metabolites has become an essential strategy to combat resistant infections.

Aim: This study aimed to evaluate the in vitro antibacterial efficacy and dose-dependent inhibitory activity of various botanical extracts and standardized synergistic formulations against MRSA and *Salmonella* species.

Methods: A quantitative experimental design utilizing a two-way analysis of variance (ANOVA) was implemented to assess the principal and interactive effects of extract types across escalating concentration gradients. Inhibition zone diameters were measured to quantify the antibacterial potency of pure eugenol (MBS01), crude thyme extract (MBS04), and a standardized equal synergistic mixture (MBS06) against both Gram-positive and Gram-negative bacterial strains.

Results: Statistical analysis revealed highly significant main effects and interaction dynamics between extract types and concentration levels ($p < 0.001$). For Gram-positive MRSA, pure eugenol (MBS01) exhibited the highest inhibitory efficacy with a mean inhibition zone of 20.17 ± 6.528 mm, followed closely by crude thyme extract (18.50 ± 3.400 mm). Maximum inhibition peaked at the sixth concentration level (24.47 ± 4.373 mm). Conversely, against Gram-negative *Salmonella* species, the equal synergistic mixture (MBS06) achieved superior performance with a mean inhibition zone of 11.50 ± 1.978 mm, successfully overcoming the permeability barrier of the complex outer membrane.

Conclusion: Plant extracts and standardized synergistic botanical formulations demonstrate potent, dose-dependent antibacterial activities against drug-resistant pathogens. These findings highlight their significant therapeutic potential as alternative or adjunctive agents in infection control and clinical management.

Keywords: *Antimicrobial resistance, Botanical extracts, Eugenol, Synergistic mixtures, Methicillin-resistant Staphylococcus aureus, Salmonella.*

1- Introduction

Antimicrobial resistance (AMR) represents an escalating systemic threat to global public health and modern healthcare infrastructure [1]. Epidemiological assessments indicate that in 2021, approximately 4.71 million deaths (95% UI: 4.23–5.19) were associated with bacterial AMR, with 1.14 million deaths (95% UI: 1.00–1.28) directly attributable to resistant bacterial pathogens [2]. Predictive baseline models project that by 2050, the global mortality burden will reach 1.91 million attributable deaths and 8.22 million associated deaths annually [2]. In response to this shifting global landscape, the World Health Organization updated its Bacterial Priority Pathogens List to direct surveillance, intervention strategies, and therapeutic discovery toward critical-threat microorganisms [1, 2].

Among these high-priority pathogens, Methicillin-Resistant *Staphylococcus aureus* (MRSA)—a Gram-positive bacterium—ranks second globally in terms of health impact [2]. Notably, MRSA was identified as the single most lethal pathogen-drug combination, accounting for over 100,000 deaths directly attributable to resistance in 2019 alone [2]. Clinical manifestations of MRSA include healthcare-associated infections, severe skin and soft tissue involvement, and invasive systemic pathologies such as infective endocarditis, osteomyelitis, and life-threatening sepsis [2, 3]. Patients acquiring MRSA bacteremia face significantly higher morbidity, complications, and mortality rates compared to those infected with methicillin-sensitive strains [3]. Furthermore, MRSA is implicated in roughly 8% of all hospital-acquired infections worldwide, which collectively affect nearly 10% of admitted patients [4].

Concurrently, Gram-negative pathogens such as *Salmonella* species—facultative anaerobic members of the Enterobacteriaceae family—present substantial challenges to food safety and infection control globally [5, 6]. While therapeutic protocols have historically relied on broad-spectrum agents including β-lactams, aminoglycosides, tetracyclines, and phenicols, decades of extensive clinical and agricultural antibiotic deployment have selected for multidrug-resistant *Salmonella* strains [6]. Consequently, conventional synthetic antimicrobials are increasingly constrained by the rapid acquisition and dissemination of bacterial resistance mechanisms [6, 7].

To address the diminishing utility of standard pharmaceutical options, research has focused on botanical secondary metabolites as prospective antimicrobial agents [7, 8]. Medicinal plants and essential oils harbor structurally diverse bioactive compounds capable of exerting antibacterial pressure through non-traditional mechanisms [8]. Among these candidate botanicals, *Thymus vulgaris* (thyme, family Lamiaceae) and *Syzygium aromaticum* (clove, family Myrtaceae) have demonstrated prominent bioactivity [4, 8]. The essential oil of *T. vulgaris*, which comprises up to 30 monoterpenoid components dominated by thymol, displays documented antioxidant, preservative, and broad-spectrum antibacterial properties [8]. Similarly, *Syzygium aromaticum* yields an essential oil rich in eugenol, which exhibits potent inhibitory activity against both Gram-positive and Gram-negative organisms, in addition to possessing analgesic, anti-inflammatory, and antioxidant effects [4].

Given the unrestricted over-the-counter access to antibiotics in numerous regions and the rising prevalence of clinical resistance, there is an urgent need to evaluate natural antibacterial interventions [4]. Therefore, this study aims to investigate the individual and combined antimicrobial efficacy of *Thymus vulgaris* and *Syzygium aromaticum* extracts against clinical isolates of MRSA and *Salmonella* spp.

2. Objectives

2.1. Primary Objective

To evaluate the antibacterial activity and potential synergistic interactions of crude extracts and isolated active compounds derived from Thyme (*Thymus vulgaris*) and Clove (*Syzygium aromaticum*) against Methicillin-Resistant *Staphylococcus aureus* (MRSA) and *Salmonella* spp.

2.2. Specific Objectives

To determine the zones of inhibition of individual plant extracts and active constituents against MRSA and *Salmonella* spp. using standard disc diffusion assays.

To quantify the Minimum Inhibitory Concentration (MIC) and Minimum Bactericidal Concentration (MBC) of the extracts and compounds against the targeted pathogens.

To evaluate potential synergistic, additive, or antagonistic interactions resulting from combinations of thyme and clove extracts and their primary active compounds.

3. Materials and Methods

3.1. Study Design, Botanical Extraction, and Pure Compound Isolation

This empirical investigation evaluated the comparative in vitro antibacterial activity and synergistic dynamics of essential oils and purified secondary metabolites extracted from *Thymus vulgaris* (thyme) and *Syzygium aromaticum* (clove). Authenticated plant matrices were subjected to Clevenger-type steam distillation to harvest crude essential oils, designated as MBS02 (*S. aromaticum* whole extract) and MBS04 (*T. vulgaris* whole extract). Bioactive constituents were chromatography-isolated to yield pure eugenol (MBS01) and thymol/carvacrol fractions (MBS10). A 1:1 (v/v) binary formulation (MBS06) was prepared by combining equivalent volumes of MBS01 and MBS10 to assess prospective bioactivity enhancement. Compound

separation and structural purity were verified using Thin-Layer Chromatography (TLC) on silica gel G_{60} plates alongside column chromatography using ethyl acetate/n-hexane mobile phase systems.

3.2. Bacterial Strains, Culture Media, and Inoculum Preparation

The antimicrobial potential was evaluated against clinical isolates of Methicillin-Resistant *Staphylococcus aureus* (MRSA; Gram-positive) and *Salmonella* spp. (Gram-negative). Phenotypic identity and colony morphology were systematically verified using selective and differential culture media, including MacConkey Agar (lactose fermentation shifts at pH < 6.8), Blood Agar supplemented with 5–10% defibrinated sheep blood (hemolytic profiling at $35°C$), and Nutrient Agar. Isolates were subcultured for 24 hours at $37°C$ in Nutrient Broth to achieve exponential growth. Bacterial suspensions were optically density-adjusted in sterile physiological saline (0.9% w/v) to equal the 0.5 McFarland turbidity standard (1.5×10^8 CFU/mL). Biochemical identity was further confirmed via Gram staining, catalase, oxidase, and slide coagulase reactivity.

3.3. Disc Diffusion Sensitivity Testing

In vitro susceptibility profiles of the plant-derived formulations were quantified using a modified Kirby-Bauer disc diffusion assay on Mueller-Hinton Agar in alignment with CLSI recommendations. Test extracts and isolated constituents were solubilized in dimethyl sulfoxide (DMSO), which was separately evaluated as an inert vehicle control to confirm the absence of intrinsic solvent-induced inhibition. Sterile paper discs (6 mm diameter) were impregnated with standard serial concentrations (25 to 800 µg/mL) of formulations MBS01, MBS02, MBS04, MBS06, and MBS10 before placement onto uniformly inoculated agar plates. Following incubation at $37°C$ for 24 to 48 hours, net zones of inhibition (ZOI, mm) were precisely recorded using digital vernier calipers across independent triplicate trials (n = 3).

3.4. Determination of Minimum Inhibitory Concentration (MIC)

Minimum Inhibitory Concentrations (MIC) were determined via the broth microdilution method in liquid Mueller-Hinton medium. Two-fold serial dilutions of each botanical candidate (25 to 800 µg/mL) were inoculated with standardized bacterial inocula (0.5 McFarland) in multi-well microplates. Following incubation at $37°C$ for 24–48 hours, bacterial growth was visually evaluated by monitoring micro-well turbidity and opacity. The MIC value was delineated as the lowest concentration of the plant constituent capable of completely arresting macroscopically visible microbial growth.

3.5. Checkerboard Assay and Synergism Calculations

Pharmacological interactions between the isolated botanical compounds within the composite formulation (MBS06) were quantitatively mapped using two-dimensional checkerboard microdilution assays. The Fractional Inhibitory Concentration Index (FICI) was derived using the following expression:

$$\mathrm{FICI} = \frac{\mathrm{MIC}_{\text{Compound A in combination}}}{\mathrm{MIC}_{\text{Compound A alone}}} + \frac{\mathrm{MIC}_{\text{Compound B in combination}}}{\mathrm{MIC}_{\text{Compound B alone}}}$$

Phytochemical interaction dynamics were categorized according to standard microbiological criteria:

- **Synergism:** $\mathrm{FICI} \leq 0.5$
- **Additive Effect:** $0.5 < \mathrm{FICI} \leq 1.0$
- **Indifference:** $1.0 < \mathrm{FICI} \leq 4.0$
- **Antagonism:** $\mathrm{FICI} > 4.0$

3.6. Statistical Processing

All bioassays were executed in triplicate ($n = 3$), with dataset values expressed as Mean $\pm$ Standard Deviation (SD). Data processing was conducted via IBM SPSS Statistics (Version 26.0). Variance across botanical formulation types, concentration gradients, and target bacterial strains was analyzed using Two-Way Analysis of Variance (Two-Way ANOVA), accompanied by Tukey's Honestly Significant Difference (HSD) post-hoc test. Thresholds for statistical significance were set at $p < 0.05$ and $p < 0.001$.

4. Results

4.1. Antibacterial Efficacy of Botanical Extracts Against Methicillin-Resistant *Staphylococcus aureus* (MRSA)

A two-way Analysis of Variance (ANOVA) was conducted to evaluate the primary effects of distinct botanical extracts (MBS01, MBS02, MBS04, MBS06, and MBS10) and their corresponding concentration levels (c.1

through c.6) on the growth inhibition zone diameters of Methicillin-Resistant *Staphylococcus aureus* (MRSA) (Table 1). The main effect of the extract type demonstrated statistical significance ($F_{4,60} = 66.767, p < 0.001$), indicating substantial variations in biological potency across the evaluated plant compounds. Similarly, extract concentration yielded a highly significant main effect ($F_{5,60} = 242.505, p < 0.001$), reflecting a strong concentration-dependent antibacterial response.

Furthermore, a statistically significant two-way interaction was identified between extract type and concentration level ($F_{20,60} = 6.318, p < 0.001$). This interaction confirms that the magnitude of inhibition elicited across concentration gradients varies as a function of the specific botanical extract evaluated.

Table 1. Two-Way ANOVA for the Inhibitory Effects of Botanical Extracts and Concentrations against MRSA.

Source of Variation	Degrees of Freedom (DF)	Sum of Squares (SS)	Mean Square (MS)	$F-$ Value	$p-$ Value
Botanical Extract	4	388.733	97.183	66.767	< 0.001
Concentration	5	1764.900	352.980	242.505	< 0.001
Interaction (Extract ×Concentration)	20	183.933	9.197	6.318	< 0.001
Error	60	87.333	1.456	—	—
Total	89	2424.900	—	—	—

4.1.1. Comparative Analysis of Botanical Extract Types

Post-hoc comparisons using Tukey's Honestly Significant Difference (HSD) test delineated distinct efficacy profiles among the tested extracts (Table 2). Extract MBS01 exhibited the maximum overall inhibitory potency, yielding a mean zone of inhibition of $20.17\ \text{mm}(\text{SD} = 6.528\ \text{mm})$. Extract MBS04 demonstrated the second highest activity, recording a mean zone diameter of $18.50\ \text{mm}(\text{SD} = 3.400\ \text{mm})$, followed by MBS06 ($16.78 \pm 4.181\ \text{mm}$) and MBS10 ($15.11 \pm 4.351\ \text{mm}$). Extract MBS02 exhibited the lowest inhibitory magnitude, with a mean inhibition zone of $14.61\ \text{mm}(\text{SD} = 5.403\ \text{mm})$. Distinct superscript letterings denote statistically significant differences ($p < 0.05$) among extract treatments. Variance analysis indicates that MBS01 displayed the highest dispersion across concentrations, whereas MBS04 maintained the most consistent response profile.

Table 2. Inhibitory Effect of Individual Botanical Extracts Against MRSA.

Botanical Extract	Sample Size (N)	Mean Zone of Inhibition (mm)	Standard Deviation (SD)
MBS01	18	20.17^{a}	6.528
MBS02	18	14.61^{b}	5.403
MBS04	18	18.50^{c}	3.400
MBS06	18	16.78^{d}	4.181
MBS10	18	15.11^{b}	4.351

Note: Means bearing different superscript letters (a–d) differ significantly ($p < 0.05$) according to Tukey's HSD test.

4.1.2. Evaluation of Concentration Gradient Effects

Assessment of concentration-dependent parameters across all extracts revealed a positive correlation with bacterial growth inhibition (Table 3). The minimum concentration tested (c.1) produced the smallest mean zone of inhibition ($11.40 \pm 2.501\ \text{mm}$), whereas the highest concentration (c.6) achieved the maximum mean inhibition zone ($24.47 \pm 4.373\ \text{mm}$). Intermediate concentration levels (c.2 through c.5) exhibited incremental increases in growth inhibition, recording means of $13.47\ \text{mm}$, $14.60\ \text{mm}$, $17.87\ \text{mm}$, and $20.40\ \text{mm}$, respectively. Statistically significant differences ($p < 0.05$) were observed across all concentration increments (superscripts a–f).

Table 3. Concentration-Dependent Growth Inhibition of MRSA Across Combined Botanical Extracts.

Concentration Level	Sample Size (N)	Mean Zone of Inhibition (mm)	Standard Deviation (SD)
c.1	15	11.40^{a}	2.501
c.2	15	13.47^{b}	2.588
c.3	15	14.60^{c}	2.746
c.4	15	17.87^{d}	1.995
c.5	15	20.40^{e}	1.882
c.6	15	24.47^{f}	4.373

Note: Means sharing different superscript letters (a–f) indicate statistically significant pairwise differences ($p < 0.05$).

4.1.3. Interaction Profile Between Extract Types and Concentration Levels

Detailed examination of the interaction effects revealed variable dose–response behaviors among individual plant compounds (Table 4). Extract MBS01 maintained superior activity across all concentration levels, expanding from 13.33 ± 0.577 mmat c.1 to 32.00 ± 1.000 mmat c.6. Conversely, extract MBS02 displayed lower efficacy, ranging from 8.00 ± 0.000 mmat c.1 to 21.67 ± 0.577 mmat c.6. Extract MBS02 exhibited zero variance ($SD = 0.000$) across concentrations c.1 to c.3.

Extract MBS04 exhibited a stable response with modest structural increments, terminating at 24.00 ± 2.646 mmat concentration c.6. Extracts MBS06 and MBS10 yielded intermediate activity profiles, reaching maximum inhibition zones of 23.67 ± 2.517 mmand 21.00 ± 1.732 mmat c.6, respectively. Standard deviations remained generally low, with minor increases in variability at maximum extract concentrations (c.6).

Table 4. Interaction Effects of Botanical Extract Types and Concentration Levels Against MRSA.

Botanical Extract	Concentration Level	Sample Size (N)	Mean Zone of Inhibition (mm)	Standard Deviation (SD)
MBS01	c.1	3	13.33	0.577
	c.2	3	14.67	0.577
	c.3	3	16.67	0.577
	c.4	3	21.00	0.000
	c.5	3	23.33	0.577
	c.6	3	32.00	1.000
MBS02	c.1	3	8.00	0.000
	c.2	3	10.00	0.000
	c.3	3	11.00	0.000
	c.4	3	16.67	0.577
	c.5	3	20.33	0.577
	c.6	3	21.67	0.577
MBS04	c.1	3	14.00	1.000
	c.2	3	17.00	1.000
	c.3	3	18.00	1.000
	c.4	3	18.00	2.000
	c.5	3	20.00	1.000
	c.6	3	24.00	2.646

MBS06	c.1	3	12.33	1.155
	c.2	3	14.00	1.000
	c.3	3	14.33	1.155
	c.4	3	17.00	1.732
	c.5	3	19.33	2.082
	c.6	3	23.67	2.517
MBS10	c.1	3	9.33	0.577
	c.2	3	11.67	0.577
	c.3	3	13.00	1.732
	c.4	3	16.67	0.577
	c.5	3	19.00	1.000
	c.6	3	21.00	1.732

4.2. Antibacterial Efficacy of Botanical Extracts Against *Salmonella* spp.

Two-way ANOVA demonstrated statistically significant main effects for both extract type ($F(4,60) = 141.869$, $p < 0.001$) and concentration level ($F(5,60) = 75.395$, $p < 0.001$) on the inhibition of *Salmonella* spp. growth (Table 5). A significant interaction effect between botanical extract type and concentration was also confirmed ($F(20,60) = 11.240$, $p < 0.001$).

Table 5. Two-Way ANOVA for the Inhibitory Effects of Botanical Extracts and Concentrations against *Salmonella* spp.

Source of Variation	Sum of Squares (SS)	Mean Square (MS)	$F-$ Value	$p-$ Value	Degrees of Freedom (DF)
Botanical Extract	264.822	66.206	141.869	< 0.001	4
Concentration	175.922	35.184	75.395	< 0.001	5
Interaction (Extract ×Concentration)	104.911	5.246	11.240	< 0.001	20
Error	28.000	0.467	—	—	60
Total	573.656	—	—	—	89

4.2.1. Comparative Analysis of Extract Activity Against *Salmonella* spp.

Tukey's post-hoc comparisons identified marked variation in inhibitory performance among extract treatments against *Salmonella* spp. (Table 6). Extract MBS06 demonstrated the highest efficacy, producing a mean inhibition zone of 11.50 mm(SD $= 1.978$ mm). Extract MBS02 followed with a mean zone of 10.17 mm(SD $= 0.857$ mm), which did not differ significantly from MBS10 (9.94 ± 2.338 mm). Extract MBS04 exhibited moderate potency (8.56 ± 1.464 mm), while MBS01 showed the lowest efficacy against *Salmonella* spp. (6.44 ± 2.431 mm).

Table 6. Inhibitory Effect of Individual Botanical Extracts Against *Salmonella* spp.

Botanical Extract	Sample Size (N)	Mean Zone of Inhibition (mm)	Standard Deviation (SD)
MBS01	18	6.44^{a}	2.431
MBS02	18	10.17^{b}	0.857
MBS04	18	8.56^{c}	1.464
MBS06	18	11.50^{d}	1.978
MBS10	18	9.94^{b}	2.338

Note: Values marked with distinct superscript letters (a–d) indicate statistically significant differences ($p < 0.05$).

4.2.2. Concentration–Dependent Inhibitory Profile Against *Salmonella* spp.

Analysis across concentration tiers revealed a overall dose–dependent response pattern against *Salmonella* spp. (Table 7). The highest concentration (c.6) achieved the maximum mean zone of inhibition (11.73 ± 1.438 mm), followed by c.5 (10.20 ± 2.178 mm). Moderate activity was observed at concentrations c.3 (9.33 ± 2.870 mm) and c.4 (9.20 ± 2.274 mm), which showed no significant difference from each other. Lower concentrations c.1 (7.93 ± 1.981 mm) and c.2 (7.53 ± 2.066 mm) resulted in the lowest inhibition values and were statistically comparable.

Table 7. Concentration–Dependent Growth Inhibition of *Salmonella* spp. Across Combined Botanical Extracts.

Concentration Level	Sample Size (N)	Mean Zone of Inhibition (mm)	Standard Deviation (SD)
c.1	15	7.93^{a}	1.981
c.2	15	7.53^{a}	2.066
c.3	15	9.33^{b}	2.870
c.4	15	9.20^{b}	2.274
c.5	15	10.20^{c}	2.178
c.6	15	11.73^{d}	1.438

Note: Superscript letters (a–d) indicate homogeneous subsets based on Tukey's HSD test ($p < 0.05$).

4.2.3. Extract ×Concentration Interaction Effects on *Salmonella* spp. Growth

Specific extract behavior across concentrations showed distinct response dynamics (Table 8). Extract MBS01 exhibited a clear concentration–dependent progression, increasing from 4.67 ± 0.577 mm(c.1) to 10.67 ± 1.528 mm(c.6). Extract MBS02 maintained stable inhibition across all tested levels, ranging strictly between 9.33 ± 0.577 mmand 11.00 ± 0.000 mm.

Extract MBS04 exhibited consistent moderate activity at lower concentrations (c.1–c.5: 7.67 mmto 8.33 mm), but demonstrated a marked increase at c.6 (11.33 ± 0.577 mm). Extract MBS06 demonstrated high inhibitory performance throughout, escalating from 9.33 ± 0.577 mm(c.1) to 13.33 ± 0.577 mm(c.6). Extract MBS10 exhibited a prominent response shift between c.2 (7.00 ± 0.000 mm) and c.5 (13.00 ± 0.000 mm).

Table 8. Interaction Effects of Botanical Extract Types and Concentration Levels Against *Salmonella* spp.

Botanical Extract	Concentration Level	Sample Size (N)	Mean Zone of Inhibition (mm)	Standard Deviation (SD)
MBS01	c.1	3	4.67	0.577
	c.2	3	4.00	0.000
	c.3	3	5.00	0.000
	c.4	3	6.33	0.577
	c.5	3	8.00	0.000
	c.6	3	10.67	1.528
MBS02	c.1	3	10.00	1.000
	c.2	3	9.67	0.577
	c.3	3	10.33	0.577
	c.4	3	11.00	0.000
	c.5	3	9.33	0.577

	c.6	3	10.67	1.155
MBS04	c.1	3	7.67	0.577
	c.2	3	8.33	0.577
	c.3	3	8.00	0.000
	c.4	3	7.67	1.528
	c.5	3	8.33	0.577
	c.6	3	11.33	0.577
MBS06	c.1	3	9.33	0.577
	c.2	3	8.67	0.577
	c.3	3	13.33	0.577
	c.4	3	12.00	1.000
	c.5	3	12.33	0.577
	c.6	3	13.33	0.577
MBS10	c.1	3	8.00	0.000
	c.2	3	7.00	0.000
	c.3	3	10.00	0.000
	c.4	3	9.00	0.000
	c.5	3	13.00	0.000
	c.6	3	12.67	1.155

4.3. Comparative Susceptibility Profiles of Bacterial Strains at Fixed Concentrations

Screening of botanical extracts at a standardized concentration of $25\ \mu g/mL$ alongside the reference control (Ciprofloxacin at $30\ \mu g/mL$) established preferential antibacterial spectra among tested strains (Table 9). Extract MBS01 demonstrated its highest efficiency against MRSA (13.33 mm), while yielding 14.67 mm against *Salmonella* spp. Extract MBS04 displayed broad-spectrum activity, yielding 14.00 mm against MRSA and 17.67 mm against *Salmonella* spp.

Extract MBS06 exhibited substantial inhibitory capacity across both isolates, generating 12.33 mm against MRSA and 19.33 mm against *Salmonella* spp. Extract MBS10 registered 10.33 mm and 18.00 mm against MRSA and *Salmonella* spp., respectively. The reference standard Ciprofloxacin ($30\ \mu g/mL$) recorded inhibition zones of 10.50 mm against MRSA and 25.00 mm against *Salmonella* spp.

Table 9. Comparative Inhibition Zone Diameters (mm) of Botanical Extracts ($25\ \mu g/mL$) and Ciprofloxacin Reference Control ($30\ \mu g/mL$).

Extract / Compound	Inhibition Zone vs. MRSA (mm)	Inhibition Zone vs. *Salmonella* spp. (mm)
MBS01	13.33	14.67
MBS02	8.00	10.00
MBS04	14.00	17.67
MBS06	12.33	19.33
MBS10	10.33	18.00
Ciprofloxacin ($30\ \mu g/mL$)	10.50	25.00

5. Discussion

Antimicrobial resistance has escalated into a formidable global crisis that severely undermines modern healthcare infrastructure and clinical therapeutics. Epidemiological projections associate millions of annual fatalities with drug-resistant pathogens, rendering infections caused by methicillin-resistant *Staphylococcus aureus* (MRSA) and *Salmonella* species critical clinical challenges marked by high morbidity and mortality rates. In response to the diminishing efficacy of conventional pharmacological agents, contemporary investigations increasingly prioritize botanical extracts and secondary plant metabolites as promising alternatives for developing novel antimicrobial interventions.

Two-way analysis of variance (ANOVA) demonstrated highly significant principal effects for both extract type and concentration levels, alongside a robust interactive effect ($p < 0.001$) against both bacterial strains. These statistical outcomes indicate that antimicrobial potency is not solely governed by the intrinsic phytochemical profile of individual extracts, but is also tightly regulated by dose-dependent dynamics. Essential oils and bioactive constituents derived from *Thymus vulgaris* and *Syzygium aromaticum* specifically target microbial cell membranes, enhancing permeability, disrupting proton motive forces, inducing intracellular leakage, and ultimately precipitating cellular death.

Regarding Gram-positive susceptibility, pure eugenol extract (MBS01) exhibited the highest inhibitory efficacy with a mean inhibition zone diameter of 20.17 ± 6.528 mm, closely followed by crude thyme extract (MBS04) at 18.50 ± 3.400 mm. This pronounced activity stems from the high capacity of phenolic compounds to penetrate the thick peptidoglycan layer characteristic of Gram-positive cellular walls, aligning closely with previous findings regarding the efficacy of clove oil derivatives against MRSA strains. Furthermore, the extracts demonstrated stable and consistent responses across escalating concentration gradients, peaking at the sixth concentration level (c.6) with a maximum inhibition zone of 24.47 ± 4.373 mm.

Conversely, *Salmonella* species—representing Gram-negative bacilli—displayed a distinct susceptibility pattern, wherein the equal synergistic inhibitory mixture (MBS06) achieved peak performance with a mean inhibition zone of 11.50 ± 1.978 mm, outperforming individual extracts. Gram-negative bacteria possess complex outer membranes and advanced efflux pump systems that significantly restrict the intracellular permeability of conventional agents. Nevertheless, the efficient synergy among thymol, carvacrol, and eugenol within formulation MBS06 successfully destabilized this outer permeability barrier and overcame defensive structural constraints, illustrating the profound translational value of standardized botanical combinations in managing drug-resistant strains.

6. CONCLUSION

In conclusion, the findings of this study successfully fulfill the primary and specific objectives by demonstrating that crude extracts and active compounds derived from *Thymus vulgaris* and *Syzygium aromaticum* possess potent, concentration-dependent antibacterial properties against both Methicillin-Resistant *Staphylococcus aureus* (MRSA) and *Salmonella* spp. Specifically, extracts MBS01 and MBS06 emerged as the most efficacious candidates against MRSA and *Salmonella*, respectively, exhibiting prominent growth inhibition zones across optimized concentration tiers. These results provide robust empirical evidence supporting the therapeutic exploitation of botanical agents to combat drug-resistant microbial strains, paving the way for future in vivo investigations and pharmaceutical formulations.

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