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Orthogonal Stimulus Response Method Optimization of Essential Oil Combinations from *Magnolia alba*, *Cryptocarya massoia*, and *Melaleuca alternifolia* for Antioxidant and Anti-*Cutibacterium acnes* Activities

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Abstract: This study aimed to optimize a combination of white champaca oil (*Magnolia alba*), massoia bark oil (*Cryptocarya massoia*), and tea tree oil (*Melaleuca alternifolia*) as antibacterial and antioxidant agents using the Orthogonal Stimulus Response Method (OSRM) with a Taguchi L4 (2^3) design. Antibacterial activity was evaluated using the well diffusion method against C. acnes, while antioxidant activity was assessed using the DPPH radical scavenging assay. The results demonstrated that all variations of the CMT oil combinations exhibited moderate antibacterial activity and strong antioxidant activity, with IC_{50} values ranging from 50 to 100 µg/mL. Based on the Taguchi analysis, the optimal combination for antibacterial activity was obtained at concentrations of 6% champaca oil, 0.4% massoia oil, and 5% tea tree oil. Meanwhile, the most optimal and stable antioxidant activity was achieved with the combination at concentrations of 75:100:100 ppm. In both assays, champaca oil was identified as the most influential factor affecting the observed biological responses. These findings indicate that the CMT oil combination has promising potential as a multifunctional therapeutic candidate for cosmetic and pharmaceutical applications, particularly as a topical nanoemulsion face mist for the management of acne while simultaneously preventing skin damage caused by free radicals.
Keywords: Essential oils synergy; Taguchi design; antibacterial activity; antioxidant activity

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INTRODUCTION

Premature aging and skin problems such as acne have become increasingly prevalent health concerns in modern society. Modern lifestyle changes—including increased workloads, technological demands, and unbalanced daily routines—contribute to chronic stress, which disrupts the body's homeostasis and promotes oxidative stress. Oxidative stress, defined as an imbalance between the production of free radicals and the body's antioxidant defense capacity, is further exacerbated by environmental exposures such as ultraviolet radiation, pollution, and cigarette smoke, which increase the generation of reactive oxygen species that damage skin cells and tissues (Baldin & Tan, 2021; Chen et al., 2021). As a consequence, this cascade of events accelerates premature skin aging, characterized by the appearance of fine wrinkles, skin laxity, and hyperpigmentation (Prakoeswa et al., 2023), but also

exacerbate skin conditions such as acne through inflammatory processes and tissue damage.

Acne is one of the most common dermatological disorders affecting the general population, particularly adolescents. This condition generally occurs due to increased sebum production during puberty, which leads to the obstruction of skin pores. The accumulation of sebum, dead skin cells, and impurities may promote the growth of bacteria such as *Staphylococcus aureus*, *Staphylococcus epidermidis*, and *Cutibacterium acnes*, which subsequently trigger inflammatory responses in the skin (Wardani, 2020; Tirani et al., 2024). Globally, the prevalence of acne is estimated to reach approximately 9.4% and has continued to increase from 1990 to 2021, with the highest incidence reported among individuals aged 15–19 years (Vasam et al., 2023; Zhu et al., 2025). In Indonesia, acne represents one of the most frequently encountered dermatological conditions in clinical practice, with a prevalence of approximately 83–85% in adolescent females and 95–100% in adolescent males (Amania, 2023).

Various strategies have been developed to address premature aging and acne, including the use of antioxidants and antibacterial therapies. Antioxidants play an important role in neutralizing free radicals, thereby reducing cellular damage and slowing the skin aging process (Neves et al., 2022). However, the use of synthetic antioxidants such as butylated hydroxyanisole (BHA), butylated hydroxytoluene (BHT), and tert-butylhydroquinone (TBHQ) has gradually declined due to reports of potential adverse effects, including allergic reactions, digestive disorders, DNA damage, increased cancer risk, and hepatotoxicity (Nusaibah et al., 2019; Ren et al., 2025). Furthermore, long-term acne treatment using antibiotics may also lead to side effects such as skin irritation, dryness, and the development of bacterial resistance due to irrational or prolonged use (Munira et al., 2021). These concerns highlight the need for safer and more effective alternatives derived from natural sources.

The utilization of natural products represents a promising approach due to their abundant availability and relatively favorable safety profiles. Several plant-derived materials have been reported to exhibit both antioxidant and antibacterial activities, including white champaca (*Magnolia alba*), massoia bark (*Cryptocarya massoia*), and tea tree oil (*Melaleuca alternifolia*). Champaca oil contains compounds such as linalool and caryophyllene, which have been reported to possess antioxidant and antibacterial activities (Batchu et al., 2017; Nasution et al., 2019). Massoia oil contains massoilactone, a compound known for its antioxidant and antimicrobial properties (Hertiani et al., 2018; Yuan et al., 2022). Meanwhile, tea tree oil contains terpinen-4-ol, which is widely recognized for its strong antibacterial activity against acne-causing bacteria (Nascimento et al., 2023; Iskandar et al., 2025). Although individual studies on each of these oils have been conducted, research on their combined formulation and systematic optimization remains limited. In particular, no study has yet applied a multi-response optimization approach to simultaneously evaluate their antioxidant and antibacterial potential as a combined formulation. Therefore, this study uniquely contributes by optimizing the combination of champaca, massoia, and tea tree oils (CMT) using the Orthogonal Stimulus Response Method (OSRM) with a Taguchi design to identify formulations with optimal antioxidant and antibacterial activities as a potential natural alternative for acne management.

METHOD

This laboratory-based experimental investigation was conducted from June to October 2025 at the Botany Laboratory of UIN Maulana Malik Ibrahim Malang and the Central Research and Animal Diagnostic Laboratory (Satwa Sehat), Batu City. The

selection of champaca, massoia, and tea tree essential oils was driven by their specific bioactive profiles, including linalool, massoilactone, and terpinen-4-ol, respectively, which offer established mechanisms for disrupting bacterial cell membranes and neutralizing oxidative stress. Consequently, this study aimed to determine the optimal composition of these three essential oils to achieve synergistic antioxidant and antibacterial activities against *Cutibacterium acnes*. The experimental design employed the Orthogonal Stimulus Response Method (OSRM) based on the Taguchi design with the assistance of Minitab 22 software. The OSRM–Taguchi approach was selected because it enables efficient multi-response optimization with a reduced number of experimental runs compared with conventional full-factorial designs, thereby minimizing resource expenditure while maintaining statistical rigor (). Within this framework, the concentrations of champaca, massoia, and tea tree essential oils served as independent variables, while antioxidant and antibacterial activities were defined as the dependent variables.

Different concentration scales were employed for the two assays due to their distinct methodological requirements and biological sensitivities. The DPPH antioxidant assay requires micro-concentrations (ppm) to accurately measure radical scavenging activity, whereas the antibacterial well diffusion method necessitates higher concentrations (%) to overcome agar diffusion barriers and ensure effective bacterial inhibition. Consequently, the Taguchi optimization for these dua responses was conducted separately. The specific concentration levels were selected based on their relevance to effective biological ranges established in previous studies, such as 60 and 75 ppm for champaca, 60 and 100 ppm for massoia, and 75 and 100 ppm for tea tree oil in the antioxidant assay (Batchu et al., 2017; Hertiani et al., 2018). For the antibacterial assay, levels were set at 6% and 12% for champaca, 0.2% and 0.4% for massoia, and 2.5% and 5% for tea tree oil to guarantee observable inhibition zones (Nasution et al., 2019; Iskandar et al., 2025). In the antioxidant activity assay, the samples were compared with the negative control group (DMSO) only on a qualitative basis. However, in the antibacterial activity assay, the sample groups were compared with the negative control group statistically using the Mann-Whitney test. To ensure the reliability of the data and minimize experimental bias, each experimental combination in the Taguchi array was performed in triplicate. Furthermore, the sequence of the experimental runs was fully randomized, and all procedures included appropriate positive and negative controls to maintain the integrity and stability of the results.

Antioxidant Activity Assay

Antioxidant activity was evaluated using the 2,2-diphenyl-1-picrylhydrazyl (DPPH) method. A 0.1 mM DPPH solution was prepared by dissolving 4 mg of DPPH in 100 mL of ethanol and allowing it to stand for 30 minutes in the dark. The CMT oil combination samples were prepared at several concentrations, while ascorbic acid was used as the positive control. A total of 50 µL of the sample solution was mixed with 50 µL of DPPH solution in a microplate and incubated for 30 minutes at room temperature in the dark. Absorbance was measured at a wavelength of 517 nm using an ELISA reader. The percentage of inhibition was calculated from the difference in absorbance between the control and the sample, and the IC_{50} value was determined through linear regression analysis using the equation y = a + bx. The response data were analyzed using the Taguchi method with the signal-to-noise ratio (SNR) approach of the smaller-is-better type to determine the formulation combination with the highest antioxidant activity.

Antibacterial Activity Assay

The antibacterial activity assay was conducted against *Cutibacterium acnes* using the well diffusion method on Mueller–Hinton Agar (MHA) medium. The bacterial suspension was prepared in 0.9% NaCl solution until its turbidity reached the 0.5 McFarland standard, and then evenly inoculated onto the surface of the MHA medium. Wells were created using a sterile puncher and filled with 30 µL of the test solution, a positive control (clindamycin), and a negative control (4% DMSO). The plates were then incubated at 37 °C for 18–24 hours. Antibacterial activity was evaluated based on the diameter of the inhibition zone formed around the wells and categorized as weak (<5 mm), moderate (5–10 mm), strong (10–20 mm), and very strong (>20 mm). The inhibition zone data were analyzed using the Taguchi method with the larger-is-better signal-to-noise ratio (SNR) approach. Statistical analysis was performed using IBM SPSS version 20 to validate the experimental findings and complement the Taguchi optimization results. Linear regression analysis was utilized to determine IC_{50} values for the antioxidant activity. To evaluate the significance of differences between the CMT oil combinations, the positive control, and the negative control, a non-parametric approach using the Kruskal-Wallis test followed by the Post Hoc Mann-Whitney test was employed. A significance level of $p < 0.05$ was applied to all tests.

RESULTS AND DISCUSSION

Antioxidant Activity Assay

The results of the optimization of CMT oil concentrations based on OSRM optimization are presented in the following table.

Table 1. Sample concentrations based on OSRM optimization

Group	Champaca Oil (ppm)	Massoia Oil (ppm)	Tea Tree Oil (ppm)
A	60	100	75
B	60	60	100
C	75	100	100
D	75	60	75

Each sample group was evaluated for antioxidant activity using the DPPH method and compared with a positive control (ascorbic acid) and a negative control (4% DMSO). The absorbance values and percentage of inhibition (% inhibition) were obtained for each sample group. Subsequently, the IC_{50} values were calculated from the % inhibition data. The IC_{50} values for the sample groups and control solutions are presented in Table 2.

Table 2. IC_{50} values of sample groups and control solutions

Group	CMT Oil Sample (ppm)	IC_{50} (µg/mL)	Category	R^2 Value
A	Combination 60:100:75	76.74	Strong	0.9962
B	Combination 60:60:100	74.39	Strong	0.9955
C	Combination 75:100:100	54.06	Strong	0.9413
D	Combination 75:60:75	60.52	Strong	0.9905
K+	Ascorbic Acid	49.64	Very Strong	0.9163
K-	4% DMSO	638.90	Weak	0.7279

Based on Table 2, all samples and the positive control exhibited R^2 values ≥ 0.9, indicating a good fit of the linear regression model used to calculate the IC_{50} values. It should be noted that R^2 reflects the goodness of fit of the regression equation and does

not directly indicate antioxidant strength; antioxidant activity is properly determined by the IC_{50} values, where lower values correspond to stronger activity. This suggests that the CMT oil samples possess good antioxidant activity, as demonstrated by the positive correlation between increasing concentration and antioxidant activity (Nurnawati et al., 2025). In contrast, the negative control (4% DMSO) showed a relatively low R^2 value because it did not exhibit antioxidant activity.

The IC_{50} calculation revealed that all CMT oil samples fell within the strong antioxidant category (50–100 µg/mL), with the best activity observed in group C (75:100:100 ppm), which showed the lowest IC_{50} value of 54.06 µg/mL. Meanwhile, the negative control produced a very high IC_{50} value (638.90 µg/mL), confirming that 4% DMSO does not possess free radical scavenging activity. These results indicate that the antioxidant activity observed in the CMT oil samples originates from the active compounds present in the essential oils rather than from the solvent.

The IC_{50} values obtained were then used as response variables in the Taguchi analysis to determine the optimal combination of oil concentrations. The results of the optimal combination based on the Taguchi analysis are presented in Table 3.

Table 3. Taguchi response analysis results

Level	Response Based on Mean			Response Based on S/N Ratio		
	Champaca Oil (ppm)	Massoia Oil (ppm)	Tea Tree Oil (ppm)	Champaca Oil (ppm)	Massoia Oil (ppm)	Tea Tree Oil (ppm)
1	74.37	67.45	67.44	-37.43	-36.53	-36.53
2	57.29	64.20	64.22	-35.15	-36.04	-36.04
Delta	17.08	3.25	3.21	2.28	0.49	0.49
Rank	1	2	3	1	2	3

As shown in Table 3, the mean and S/N ratio analyses using the “smaller-is-better” approach indicate that all factors achieved optimal performance at level 2. The lowest mean value, representing the smallest IC_{50} and thus the strongest antioxidant activity, was observed for champaca oil, massoia oil, and tea tree oil at level 2. Therefore, the optimal combination based on the mean response is 75:100:100 ppm. Similarly, the highest S/N ratio values for all three factors were also observed at level 2, indicating that this combination is not only the most effective but also the most stable, with the lowest response variability.

The delta analysis further confirmed that champaca oil had the greatest contribution to the response variation, both in the mean and S/N ratio analyses. This finding suggests that variations in the concentration of champaca oil have the most significant impact on antioxidant activity compared with massoia oil and tea tree oil. The higher sensitivity of the response to changes in champaca oil concentration indicates that its phytochemical components play a more dominant role in influencing free radical scavenging activity.

Overall, the evaluations based on mean values, S/N ratio, and delta analysis consistently indicate that the combination of 75:100:100 ppm represents the most optimal, effective, and stable formulation for producing antioxidant activity in the CMT oil samples.

Antibacterial Activity Assay

Table 4. Diameter of inhibition zones

Experimental Group	Factors (%)			Inhibition Zone Diameter (mm)			Mean ± SD	Category
	C	M	T	R1	R2	R3		
A	6	0.2	2.5	6.32	6.24	5.95	6.17 ± 0.19	Moderate
B	6	0.4	5	7.98	8.47	8.19	8.21 ± 0.25	Moderate
C	12	0.2	5	5.70	6.16	5.92	5.93 ± 0.23	Moderate
D	12	0.4	2.5	6.10	5.67	5.87	5.88 ± 0.22	Moderate
K+ (Clindamycin)				11.28	11.13	10.90	11.10 ± 0.19	Strong
K− (4% DMSO)				0	0	0	0.00 ± 0.00	-

Based on Table 4, the antibacterial assay results indicate that all CMT oil combinations were able to inhibit the growth of *Cutibacterium acnes*, producing inhibition zones ≥5 mm, which fall into the moderate activity category, while the negative control showed no antibacterial activity with an inhibition zone of 0 mm (Ernawati & Jannah, 2021). Treatment B produced the largest inhibition zone with an average diameter of 8.21 mm, whereas treatment D showed the smallest inhibition zone (5.88 mm). The positive control, clindamycin, produced an inhibition zone of 11.10 mm, which falls into the strong category (Ernawati & Jannah, 2021).

Variations in inhibition zone size may be influenced by the limited diffusion capacity of essential oils, which are hydrophobic in nature, as well as by the thickness of the agar medium that may slow the diffusion of active compounds (Puxeddu et al., 2025). Nevertheless, antibacterial activity was still observed due to the presence of active compounds such as linalool and caryophyllene in white champaca oil, massoilactone in massoia oil, and terpinen-4-ol in tea tree oil, which act by disrupting bacterial cell membranes (Hamzah et al., 2022).

Table 5. Response table for means and Signal-to-Noise (S/N) ratio

Level	Response Based on Mean			Response Based on S/N Ratio		
	Champaca Oil (ppm)	Massoia Oil (ppm)	Tea Tree Oil (ppm)	Champaca Oil (ppm)	Massoia Oil (ppm)	Tea Tree Oil (ppm)
1	7.192	6.048	6.025	17.04	15.62	15.59
2	5.903	7.047	7.070	15.41	16.83	16.86
Delta	1.288	0.998	1.045	1.63	1.21	1.28
Rank	1	3	2	1	3	2

Based on the results presented in Table 5, the analysis of the mean and S/N ratio values indicates that champaca oil was the most influential factor in inhibiting the growth of *C. acnes*. This is demonstrated by the highest delta value observed both in the mean response analysis (Δ = 1.288) and the S/N ratio analysis (Δ = 1.63), followed by tea tree oil and massoia oil (Rashid, 2023).

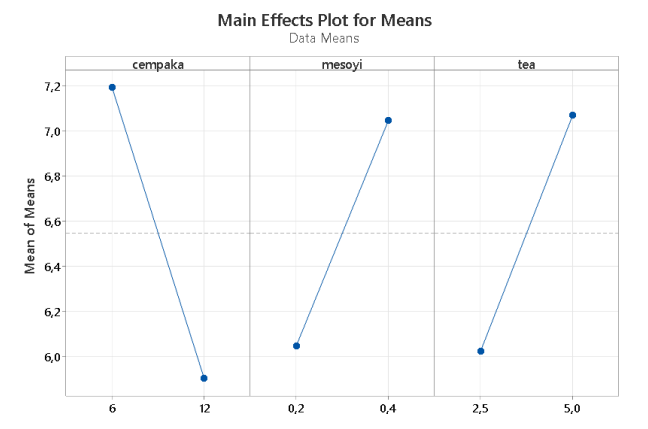

Figure 1. Taguchi orthogonal array response graph based on mean values

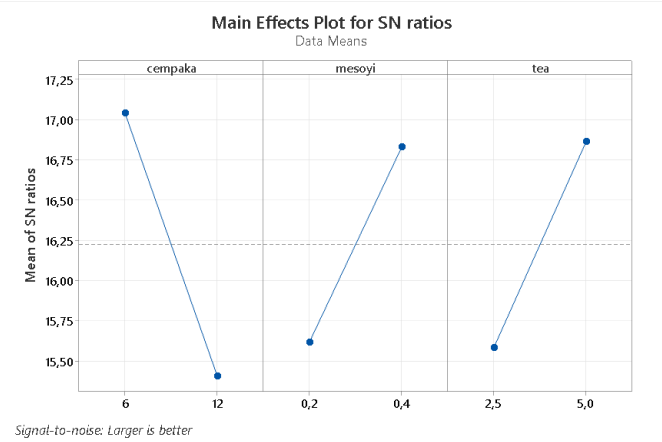

Figure 2. Taguchi orthogonal array response graph based on Signal-to-Noise (S/N) ratio values

Figures 1 and 2 illustrate the Taguchi orthogonal array response analysis based on the mean values and S/N ratio. Both graphical analyses showed a similar pattern. Using the “larger-is-better” criterion, the optimal combination was observed at champaca oil level 1, massoia oil level 2, and tea tree oil level 2. This combination produced the highest S/N ratio values (17.04; 16.83; 16.86) as well as the largest mean values (7.192; 7.047; 7.070), indicating the most effective antibacterial inhibition response with minimal variability. Therefore, the optimal ratio was obtained in combination B, consisting of 6% champaca oil, 0.4% massoia oil, and 5% tea tree oil, which was identified as the most optimal formulation according to the Taguchi method (Rashid, 2023).

A more detailed examination of Figure 1 (mean response) reveals that champaca oil exhibited the steepest slope among all three factors, declining from a mean inhibition zone of 7.192 mm at level 1 (6%) to 5.903 mm at level 2 (12%). This sharp decline indicates that a lower champaca oil concentration (6%) is more effective for antibacterial inhibition, likely because excessive concentrations of linalool may reduce diffusion efficiency through the agar medium. In contrast, massoia oil and tea tree oil showed upward trends from level 1 to level 2, meaning that higher concentrations of these oils (0.4% and 5%, respectively) contributed more favorably to antibacterial activity. This divergent behavior among the three factors highlights the complexity of formulation optimization and underscores the necessity of a systematic experimental design such as Taguchi to identify the true optimal combination.

Figure 2 (S/N ratio) confirms and reinforces this trend. Champaca oil at level 1 yielded the highest S/N ratio (17.04 dB), while massoia and tea tree oils both achieved their peak S/N ratios at level 2 (16.83 dB and 16.86 dB, respectively). The convergence of both mean and S/N ratio analyses toward the same optimal levels strengthens the robustness of this conclusion. Notably, the relatively flat slopes for massoia and tea tree oils in Figure 2, compared to the steeper slope for champaca oil, further support the delta analysis finding that champaca oil is the most sensitive factor with the greatest impact on response variability. These graphical trends collectively confirm that combination B (6% champaca, 0.4% massoia, 5% tea tree oil) represents the most effective and consistent formulation for antibacterial activity against *C. acnes*.

Table 6. Post Hoc Mann–Whitney test results

Groups	K+	K-	A	B	C	D
K+	-	0,046*	0,050*	0,050*	0,050*	0,050*
K-	0,046*	-	0,046*	0,046*	0,046*	0,046*
A	0,050*	0,046*	-	0,050*	0,127**	0,127**
B	0,050*	0,046*	0,050*	-	0,050*	0,050*
C	0,050*	0,046*	0,127**	0,050*	-	0,513**
D	0,050*	0,046*	0,127**	0,050*	0,513**	-

A non-parametric approach was selected because preliminary normality testing (Shapiro-Wilk) indicated that the inhibition zone data did not satisfy the normality assumptions required for parametric analyses such as one-way ANOVA. The Mann–Whitney test is therefore the appropriate statistical method for comparing independent groups with non-normally distributed data and small sample sizes (n = 3 replicates per group). The results of the Post Hoc Mann–Whitney test showed that the positive control differed significantly from the negative control ($p = 0.046$), confirming the validity of the method since clindamycin exhibited antibacterial activity while DMSO did not (Rizki et al., 2022; Chezar et al., 2025). The positive control also showed significant differences compared with all treatment groups ($p = 0.050$), indicating that the inhibition zones produced by the CMT oil combinations were smaller than those of clindamycin, although antibacterial activity was still observed. From a biological standpoint, the significantly smaller inhibition zones of the CMT oil combinations compared to clindamycin are expected, given that clindamycin is a clinically optimized antibiotic with a well-defined mechanism targeting bacterial ribosomal protein synthesis, whereas the essential oil combinations act through a broader, membrane-disruption-based mechanism that is inherently less concentrated when dissolved in agar. Nevertheless, the statistically significant inhibition observed across all CMT groups compared to the negative control ($p = 0.037$) confirms genuine biological activity, suggesting that these formulations warrant further development as complementary or alternative anti-acne agents.

All treatment groups differed significantly from the negative control ($p = 0.037$), demonstrating that the CMT oil combinations exerted a significant inhibitory effect on *C. acnes*. Comparisons among treatment groups revealed that groups A, C, and D did not differ significantly from each other, whereas group B showed significant differences compared with the other groups ($p = 0.050$). These findings are consistent with the OSRM analysis, in which group B produced the largest inhibition zone diameter among the experimental groups. Therefore, it can be concluded that formulation B represents the most optimal combination for inhibiting the growth of *C. acnes*.

Overall, the results of this study indicate that the combination of champaca oil, massoia oil, and tea tree oil exhibits both antioxidant and antibacterial activities, although the optimal formulations differ between the two assays. In the antioxidant assay, the best combination was observed in group C (75:100:100 ppm) with an IC_{50} value of 54.06 µg/mL, which falls into the strong antioxidant category. Meanwhile, in the antibacterial assay against *Cutibacterium acnes*, the optimal combination was observed in group B, consisting of 6% champaca oil, 0.4% massoia oil, and 5% tea tree oil, which produced the largest inhibition zone diameter (8.21 ± 0.25 mm).

This difference in optimal formulations indicates that the concentration requirements of active compounds for antioxidant and antibacterial activities are not necessarily the same, as the underlying mechanisms differ. Antioxidant activity is related to the ability to scavenge free radicals, whereas antibacterial activity is also influenced by compound diffusion and interactions with bacterial cell membranes. Mechanistically, the superiority of the 75:100:100 ppm combination for antioxidant activity may be attributed to the elevated concentration of champaca oil (75 ppm), which provides a higher amount of linalool — a monoterpene alcohol with well-characterized electron-donating capacity that enhances free radical scavenging. The elevated massoilactone content from massoia oil at 100 ppm further amplifies this effect, as the lactone moiety in massoilactone can stabilize reactive oxygen species intermediates through electron donation (Hertiani et al., 2018; Yuan et al., 2022). For antibacterial activity, the optimal 6%:0.4%:5% combination prioritizes effective membrane-disrupting concentrations of terpinen-4-ol from tea tree oil and linalool from champaca oil, with massoilactone serving an auxiliary penetration-enhancing role rather than direct antimicrobial action at these concentrations (Hamzah et al., 2022). This mechanistic divergence explains why a single formulation cannot simultaneously maximize both biological responses and why separate optimization was necessary.

Based on the Taguchi analysis, champaca oil was identified as the most influential factor affecting both antioxidant and antibacterial activities, as indicated by the highest delta values in both the mean and signal-to-noise ratio analyses. This finding suggests that variations in the concentration of champaca oil contributed the most significant effect on the observed biological responses compared with massoia oil and tea tree oil. The presence of active compounds such as linalool and caryophyllene in champaca oil plays an important role as free radical scavengers and antimicrobial agents capable of disrupting bacterial cell membranes.

Therefore, champaca oil appears to be the dominant component in the CMT oil formulation, while massoia oil and tea tree oil function as supporting components that may enhance the potentially synergistic antioxidant and antibacterial effects of the formulation. However, as individual oil controls were not included in this study design, direct confirmation of synergism requires further investigation through established methods such as the checkerboard assay or fractional inhibitory concentration (FIC) index analysis.

CONCLUSION

Based on the Taguchi analysis, champaca oil was identified as the most influential factor affecting both antioxidant and antibacterial activities, as indicated by the highest delta values in the mean and signal-to-noise ratio analyses. Active compounds such as linalool and caryophyllene play important roles as free radical scavengers and also exhibit antimicrobial activity. The combination of champaca oil (*Magnolia alba*), massoia oil (*Cryptocarya massoia*), and tea tree oil (*Melaleuca alternifolia*) demonstrated strong antioxidant activity, with the optimal combination

identified as 75:100:100 ppm in the DPPH assay. Meanwhile, the best antibacterial activity was obtained at a composition of 6% champaca oil, 0.4% massoia oil, and 5% tea tree oil, which showed a moderate inhibition category against the tested bacteria. These findings indicate that champaca oil acts as the dominant component in the CMT formulation, while massoia oil and tea tree oil function as supporting components that enhance the combined effect of the formulation.

RECOMMENDATION

Further research is required to investigate the molecular mechanisms underlying the antibacterial and antioxidant activities of the CMT oil combination. Such studies may include the evaluation of bacterial cell membrane damage, alterations in membrane permeability, and the inhibition of biofilm formation by *Cutibacterium acnes*. In addition, the free radical scavenging capacity of the active compounds should be further assessed using complementary antioxidant assays, such as ABTS, FRAP, and ORAC.

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