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Research Article

ENHANCED ANTIBACTERIAL EFFECTIVENESS THROUGH THE SYNERGISTIC ACTION OF THYMOL AND MELALEUCA ESSENTIAL OILS ABUNDANT IN 1,8-CINEOLE

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ABSTRACT

This work is a significant contribution to antibacterial research, specifically investigating the synergistic performance of thymol-combined Melaleuca essential oil with high 1,8−cineole content against multi-drug-resistant bacteria. The two Melaleuca species used in this study, Melaleuca sp2-Myrtaceae (MM) and Melaleuca cf. Quinquenervia (MQ), were chosen for their potential antibacterial properties. Simultaneously, the four bacteria, including Bacillus cereus, Staphylococcus aureus, Escherichia coli, and Salmonella Typhimurium, were selected for the antibacterial experiment series. The results, which demonstrate the highest antibacterial efficiency at the combination of the MM essential oil with 9.5 wt.% of thymol, are not only significant but also offer hope for the future. The minimal inhibitory concentration (MIC) and the antibacterial inhibition zone data further validate the synergistic performance. These findings are promising for the future of pharmaceutical synthesis, suggesting a potential avenue for developing effective treatments against multi-drug-resistant bacteria, a significant and growing health concern.

Keywords: 1,8-cineole; *Melaleuca* essential oils; synergistic antibacterial activity; Thymol

1. Introduction

Antimicrobial resistance, a significant global health crisis (Tappero et al., 2017), has been reported with high resistance in bacteria such as *Klebsiella pneumoniae, Staphylococcus aureus*, Nontyphoidal *Salmonella, Escherichia coli*, and others, which are the leading causes of common infections in various places or transmitted through the food chain (Chong & Lee, 2000; Zhang et al., 2022). The health and economic impacts of this crisis are severe. The use of natural essential oils (EOs) or binary combinations that blend two essential oils or an essential oil with a synthetic chemical is a novel and promising approach to reducing antimicrobial resistance (Ambrosio et al., 2017; Solórzano-Santos &

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Miranda-Novales, 2012; Souza, 2016). Studies have shown synergistic, additive, and no antagonistic effects of binary combinations (Ayari et al., 2020; Chraibi et al., 2021; Soulaimani et al., 2021). The authors, based on related literature, have combined *Thymus pallidus* with ρ-cymene, γ-terpinene, and thymol (Mseddi et al., 2020); *Lavandula maroccana* (Murb.) with carvacrol (Soulaimani et al., 2019); *Rosmarinus officinalis* L. with 1,8-cineole, camphor and α-pinene (Wang et al., 2008) to achieve synergistic efficiency, resulting in an effectively decrease in the minimal inhibitory concentration (MIC) values. These findings are particularly significant because the concentrations of binary combinations are typically lower than those of purified antibacterial components, suggesting the potential for more effective and economical treatments. *Melaleuca* essential oil (MEO), or the incorporation of MEO with other active ingredients, has been studied and identified to be potential in pharmaceutical synthesis against bacteria (Carson et al., 2006; Corona-Gómez et al., 2022; Jafri & Ahmad, 2020; Rapper et al., 2023). The combination of thymol with MEOs that have a significant amount of 1,8−cineole, obtained from commonly cultivated *Melaleuca* sp2-Myrtaceae (MM) and *Melaleuca* cf. *Quinquenervia* (MQ) in Vietnam, has not been documented in previous research. Hence, this study contributes to the scientific data on binary combinations by investigating the synergistic antibacterial activity of the binary combination of MM or MQ EOs with thymol. This research is significant as it contributes to the global effort to combat antimicrobial resistance, providing a potential solution to this pressing public health challenge.

2. Materials and methods

2.1. Materials

2.1.1. Chemicals and bacteria

Thymol (Ghtech, China), anhydrous sodium sulfate, ampicillin, and chloramphenicol (Merk, Germany) are commercially available chemicals.

Bacillus cereus (B. cereus), Staphylococcus aureus (S. aureus), Escherichia coli (E. coli), and Salmonella Typhimurium (*Salmonella* T.*)* were used in this study. They were taken from the microbe culture collection of the Faculty of Biology and Biotechnology, University of Science, Ho Chi Minh City, Vietnam.

2.1.2. Plant material and EO extraction

MM and MQ leaves were collected in Vinh Phuc province, 280000, Vietnam. The oils from MM and MQ leaves were extracted by steam distillation and dried over anhydrous sodium sulfate and, after filtration, stored at 4 °C until further analysis.

2.2. Chemical analysis

2.2.1. Gas chromatography analysis

Gas chromatography (GC) was conducted using the Hewlett 5890 Packard series II plus gas chromatograph apparatus, which was equipped with a Flame Ionization Detector (FID) and a capillary column Econo-Cap EC^{-5} (30 m x 0.25 mm i.d., film thickness 0.25

μm). The carrier gas, Nitrogen, was precisely controlled at a flow rate of 1 $mL \cdot min^{-1}$, split ratio 1:1. The oven temperature was programmed with an initial temperature of 50 °C (isothermal for two minutes), then rising at 2 $°C \cdot min^{-1}$ to 80 °C, then rising at 5 $°C \cdot min^{-1}$ to 150 °C, then rising at 10 $°C \cdot min^{-1}$ to 300 °C (isothermal for five minutes). The injected volume was a 25 μL sample of essential oil in purified hexane. The relative concentration was calculated using the software HP Chemstation, which allows the assimilation of the percentages of the peak areas to the percentages of the various constituents.

2.2.2. Gas chromatography-mass-spectrometry analysis

All the essential oils were analyzed on the Shimadzu GC/MS-QP2020 NX gas chromatograph equipped with a capillary column (30.0 m x 0.25 mm i.d., film thickness 0.25 μm). Helium was used as a carrier gas. The mass spectrometer operating conditions were ionization voltage, 0.88 kV, and an ion source of 260 °C. The GC/MS parameters were identical to those for the GC analysis. The retention indices were obtained by running a series of aliphatic hydrocarbons (C8-C20), increasing the number order of carbon atoms on the capillary column (30.0 m x 0.25 mm i.d., film thickness 0.25 μm).

2.2.3. Compound identification

The compounds were identified by comparing retention indices (relative to aliphatic hydrocarbons C8-C20) with authentic references, ensuring the highest level of accuracy. This was done using NIST 14 and WILEY7 Mass Spectral Library and by comparing their mass spectra with data already available in the literature in our databases by Adam (2007).

2.3. Antimicrobial bioassays

2.3.1. Determination of antibacterial activity

The antibacterial inhibition zones of all tested samples were evaluated using the disk diffusion method and the agar well diffusion method, while MIC values were determined by the broth microdilution method in accordance with EUCAST, Clinical and Laboratory Standards Institute guidelines (2017), and ISO 20776-1 (Arendrup et al., 2014; Clinical Laboratory Standards Institute, 2017; International Standard, 2019). Muller-Hinton broth (Merck, Germany) was prepared following the manufacturer‘s instructions.

Bacterial suspensions were prepared from a 24-hour broth culture for aerobes and a 48-hour broth culture for anaerobes, then diluted to match the 0.5 standard on the McFarland scale (10^8 $CFU \cdot ml^{-1}$). This inoculum was used as the bacterial suspension, which was further diluted to achieve a final concentration of 10^6 $CFU \cdot ml^{-1}$.

The sterile 90 mm diameter petri dish was used for the disk diffusion method and the agar well diffusion method. The samples with two-fold serial dilutions were made with a range from 100% to 0.35%. An inoculum matching the 0.5 standard of the MacFarland scale (10^8 $CFU \cdot ml^{-1}$) was swabbed (sterile cotton swabs) in susceptible media. For the disk diffusion method, the sterile 6 mm diameter disks impregnated with 10 μL of each sample were placed on the inoculated agar. For the agar well diffusion method, agar wells (8-mm diameter) were made in each of these plates using a sterile cork borer, and then 0.1 ml of serial dilutions of the sample were added. After incubation for 24 hours at 37 °C, the inhibition zone was measured in mm. Ampicillin (100 $\mu g \cdot ml^{-1}$) and Chloramphenicol (10 $\mu g \cdot ml^{-1}$) were used as positive controls, and DMSO (Dimethyl sulfoxide) 10 % was used as a negative control. All assays were performed in triplicate.

2.3.2. Determination of minimal inhibitory concentration (MIC)

The samples were dissolved in 10 % DMSO and considered as the stock solution (100%). In a series of microtubes, 100 µl of a bacterial suspension at 10^6 $CFU \cdot ml^{-1}$ was added to 100 µl of each stock solution, and then two-fold serial dilutions were made with a range from 100 % to 0.0026 %. A microtube was used as a positive control, and antibiotics were added. A microtube negative control contains 100 µl of a broth dilution of DMSO 10 %. The microplates were incubated for 24 hours at 37 °C. The absorbance was measured by a BMG spectrophotometer at a wavelength of 600 nm. MIC was defined as the lowest concentration of essential oils for preventing visible bacterial growth. All experiments were done in triplicate.

3. Results and discussion

3.1. Chemical components and antibacterial activity of MEOs

Chromatographic analysis, specifically Gas Chromatography-Mass Spectrometry (GC-MS) and Gas Chromatography-Flame Ionization Detection (GC-FID), of MM and MQ essential oils (EOs) identified 51 components representing 97.13% and 98.38%, respectively (Appendix 1). These techniques are widely used in the analysis of EOs due to their high sensitivity and specificity. Fourteen significant compounds were detected in the samples (Table 1). The MQ EO, with its 1,8-cineole content of 69.7 %, is classified as a Grade 1 oil, a significant distinction. In contrast, the MM EO, with a 1,8-cineole content of 58.8%, is classified as a Grade 2 oil (Brophy & Doran, 1996). The main components of these tested MEOs were minor indifference in terms of the antibacterial constituents proportion, compared to seven *Melaleuca* species grown in Brazil including α-pinene (2.8-4.7%), β-pinene (0.9-1.6%), 1,8-cineole (7.2-80.2%), γ-terpinene (0.3-18.9%), terpinene-4-ol (1.0-53.7%), α-Terpineol (2.2-22.6%), viridiflorol (0.2-71%) (Barbosa et al., 2013). The proportion differences were influenced by environmental conditions, geographic variations, social conditions, and the type of EOs cultivated (Figueiredo et al., 2008).

Table 1. *Chemical compositions of Melaleuca EOs by GC/MS analysis*

No.	**Compounds**	**MM EO (%)**	**MQ EO (%)**
1	α-pinene	1.51	1.9
2	β-pinene	1.53	1.75
3	**1,8-cineole**	**58.77**	**69.66**
4	γ-terpinene	**-**	0.33

5	terpinen-4-ol	2.67	0.72
6	α-terpineol	8.45	4.73
7	Terpinolene	1.35	-
8	Linalool	0.32	0.02
9	α-terpinyl acetate	5.18	-
10	caryophyllene oxide	0.11	0.31
11	Thymol	-	-
12	Viridiflorol	0.28	0.09
13	Eugenol	0.04	-
14	*trans*-caryophyllene	2.00	-
Total		**82.21**	**79.51**

Table 1 shows that the main chemical component in MEOs is 1,8-cineole, an antibacterial compound reported in previous literature (Simsek & Duman, 2017). Therefore, both MM and MQ are potential antibacterial essential oils.

Table 2 details the antibacterial inhibition zones of MEOs, compared with 1,8-cineole, against four specific bacterial strains: *B. cereus*, *S. aureus*, *E. coli*, and *Salmonella* T. Based on the width of the inhibition zone diameter expressed in millimeters, the ranking system provides a clear and comprehensive understanding of the results. It categorizes the sensitivity of the bacterial strains as follows: not sensitive (-) for zone diameters equal to 8 mm or below, sensitive (+) for zone diameters between 8 and 14 mm, and susceptible (++) for zone diameters between 14 and 20 mm and extremely sensitive (+++) for zone diameters equal or larger than 20 mm (Banerjee et al., 2022).

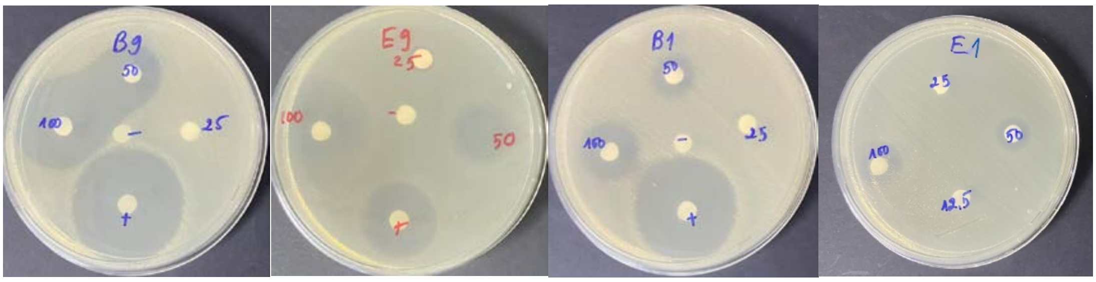
Although MQ EO contains a higher content of 1,8-cineole, its antibacterial activity is low. Considering the chemical compositions of MEOs (Table 1), it can be seen that the proportion of alcohols (terpinene-4-ol, α-terpineol) and ester in the MM EO is higher than those in the MQ EO, totaling about 10%, which aids in the dispersion of 1,8-cineole in the bacterial culture. In addition, observing the samples tested by the disk diffusion, we noticed the appearance of insoluble oil scum on the surface when using MQ EO. As a result, the dispersion level of the MQ EO into the agar medium significantly impacts antibacterial effectiveness. Table 2 shows that the results are consistent with Figure 1, showing a clear difference in the inhibition diameters when using MQ EO and 1,8-cineole. For both *B. cereus* and *E. coli* bacteria, the inhibition diameters corresponding to MQ EO concentrations of 100% and 50% were minimal, significantly lower than the inhibition diameters of the positive control and 1,8-cineole.

Table 2. *The antibacterial inhibition zones (mm) of 1,8-cineole and EO applied alone against B. cereus (1), E. coli (2), Salmonella T. (3), and S. aureus (4)*

Concentration %	**MM EO**				**MQ EO**				**1,8-cineole**			
(v/v)	***1***	***2***	***3***	***4***	***1***	***2***	***3***	***4***	***1***	***2***	***3***	***4***
100%	GI	GI	GI	GI	19	12	-	-	GI	GI	GI	GI
50%	GI	GI	24	GI	12	11	-	-	GI	GI	GI	GI
25%	17	24	16	18	-	-	-	-	18	GI	24	GI
12.5%	13	18	10	16	-	-	-	-	10	24	14	24
6.25%	13	9	8	14	-	-	-	-	9	21	10	24
3.1%	7	8	7	11	-	-	-	-		8	8	17
1.5%	-	-	-	-	-	-	-	-	-	7		
0.75%	-	-	-	-	-	-	-	-	-	-	-	-
0.4%	-	-	-	-	-	-	-	-	-	-	-	-
Positive control (mm)	29	27	29	35	24	22	21	25	28	27	22	30

Oil volume/medium volume: v/v

Growth inhibition: GI



(a) (b) (c) (d)

Figure 1. *The antibacterial inhibition zones (mm) of 1,8-cineole against B. cereus (a) and E. coli (b), MQ EO against B. cereus (c) and E. coli (d) at the concentrations of 25, 50, and 100 %*

Table 3 shows the MICs of the MEOs against the bacteria, including *B. cereus*, *E. coli*, *Salmonella* T., and *S. aureus*. The MIC is the lowest concentration of an antimicrobial compound that inhibits the visible growth of a microorganism after a specific incubation period. For the MM EO, MIC values are low, varying from 0.170-0.350%, confirming the potential antibacterial activity of the essential oil. On the other hand, MIC values when using MQ EO are low for two bacteria, *B. cereus* and *E. coli*, consistent with the higher inhibition zone data shown in Table 2. It is important to note that the antimicrobial properties of EOs are not arbitrary but strictly associated with the chemical structure of their antibacterial constituents, such as hydrogen bonding and molecular size, a system of delocalized electrons, and the presence of an oxygen function in the framework (Ultee et al., 2002). This understanding of the role of chemical structure in antimicrobial properties is crucial for further research and development in this field.

Table 3. *Minimal inhibitory concentration (MIC) of EO applied alone against B. cereus (1), E. coli (2), Salmonella* T. *(3), and S. aureus (4)*

Concentration % (v/v)	**MM EO**				**MQ EO**			
	1	***2***	***3***	***4***	***1***	***2***	***3***	***4***
50%	0.008	0.793	-0.115	-0.061	0.078	0.09	0.077	0.081
25%	-0.043	0.076	0.009	0.014	0.057	0.085	0.057	0.065
12.5%	0.002	0.084	0.001	0.003	0.061	0.077	0.089	0.246
6.25%	-0.083	0.049	-0.026	0.006	0.073	0.076	0.087	0.290
3.1%	-0.008	0.079	0	0.001	0.071	0.073	0.092	0.334
1.5%	-0.008	0.057	0.019	0.006	0.068	0.065	0.063	0.369
0.75%	0.005	0.047	0.001	0.003	0.060	0.059	0.134	0.495
0.4%	-0.005	0.05	0.09	0.066	0.079	0.056	0.170	0.639
0.2%	0.002	0.408	0.171	0.496	0.072	0.05	0.192	0.830
0.1%	0.231	0.514	0.259	0.639	0.053	0.059	0.201	0.942
0.05%	0.710	0.712	0.411	0.966	0.048	0.084	0.432	0.922
0.025%	1.463	0.704	1.14	0.957	0.439	0.372	0.792	0.934
MIC%	**0.170**	**0.350**	**0.350**	**0.350**	**0.043**	**0.043**	**1.5**	**25**

3.2. Chemical components and synergistic antibacterial impact of the binary combinations of thymol and MEOs

To determine the appropriate amount of thymol to add to MEOs, the antibacterial activity of purified thymol against the four bacteria was evaluated through the antibacterial inhibition zones (mm) and the minimum inhibitory concentration (%) methods (Table 4). The results show that at a thymol concentration of 12.5% (v/v), the antibacterial activity of thymol was higher than the positive control. However, at a concentration of 6.25% (v/v), the antibacterial activity of thymol decreased significantly. Therefore, the amount of thymol added to the two EOs, MM and MQ, was calculated from the average values of 12.5% and 6.25%. Adding thymol changes the relative proportions of the substances present in the original EO samples, so to maintain the original proportion of 1,8-cineole (Table 1), a calculated amount of 1,8-cineole was added simultaneously with thymol. The results of the chemical composition analysis of EOs after adding thymol and 1,8-cineole are presented in Table 5 (Appendix 2). Compared to the original composition of the two MM and MQ essential oils, the mass ratios of other compounds remain largely unchanged, aside from the presence of thymol.

Table 4. *The antibacterial inhibition zones (mm) and MIC (%) of thymol against B. cereus (1), E. coli (2), Salmonella* T. *(3), and S. aureus (4)*

Concentration % (v/v)	**The antibacterial inhibition zones (mm)**				**Minimal inhibitory concentration (%)**			
	1	***2***	***3***	***4***	***1***	***2***	***3***	***4***
100%	GI	GI	GI	GI	-	-	-	-
50%	GI	GI	GI	GI	0.092	0.044	0.048	0.067
25%	37	GI	31	GI	0.044	0.007	0.012	0.076
12.5%	33	GI	30	GI	0.076	0.086	0.084	0.051
6.25%	24	27	23	18	0.062	0.075	0.056	0.023
3.1%	21	26	14	16	0.072	0.026	0.045	0.069
1.5%	15	15	12	14	0.079	0.025	0.080	0.096
0.75%	13	14	11	13	0.094	0.046	0.121	0.094
0.4%	-	-	-	-	0.051	0.063	0.283	0.062
0.2%	-	-	-	-	0.087	0.064	0.112	0.095
0.1%	-	-	-	-	0.136	0.094	0.110	0.123
0.05%	-	-	-	-	0.174	0.187	0.121	0.389
0.025%	-	-	-	-	0.345	0.028	0.105	0.670
Positive control	33	35	29	43	0.046	0.056	0.059	0.049
MIC (%)	-	-	-	-	0.17	0.09	0.70	0.17

Table 5. *Chemical compositions of the binary combinations by GC/MS analysis*

No.	**Compounds**	**Thymol and MM EO**	**Thymol and MQ EO**
1	α-thujene	0.25	0.06
2	α-pinene	1.0	1.03
3	β-pinene	0.92	0.97
4	1,8-cineole	57.64	71.22
5	γ-terpinene	0.52	-
6	Terpinen-4-ol	0.94	0.33
7	α-Terpineol	2.42	2.44
8	Geraniol	-	-
9	Linalool	0.03	0.14
10	Caryophyllene oxide	0.17	0.16
11	Thymol	9.51	2.61
12	Viridiflorol	0.33	0.06
Total		**73.73**	**79.02**

The synergistic impacts of thymol and MM EO are effective on all the bacteria tested (Table 6), particularly superior to the positive control. When combined with thymol, the MIC values of MM EO and MQ EO were 2 to 141-fold lower than the MICs of pure EOs. As mentioned, there is limited diffusion of MQ EO into the agar medium, affecting the antibacterial performance of the EO. However, when combined with thymol, the antibacterial activity of the binary combinations was significantly improved, including gram-negative and gram-positive bacteria among the four bacteria. The diameter of the antibacterial ring against the gram-negative bacteria is lower than that of the gram-positive bacteria. This can be explained based on the difference in cell wall structure between these two types of bacteria. Gram-positive bacterial cell walls contain approximately 90-95% peptidoglycan, allowing hydrophobic molecules to quickly move across the cell wall. Meanwhile, the cell wall of gram-negative bacteria is more complex, with an outer membrane covering the peptidoglycan layer in a thin size of 2-3 nm. The outer membrane is composed of a bilayer of phospholipids firmly bound to peptidoglycan by lipopolysaccharides, which makes gram-negative bacteria more resistant to essential oils than gram-positive bacteria (Nazzaro et al., 2013).

The combination of thymol at the appropriate concentration of 9.51% with MM EO resulted in an antibacterial cycle that completely inhibited the growth of the three bacterial strains investigated, except *Salmonella* T., which was 24 mm lower than the result of thymol at 12.5% for this bacteria it is 30 mm. Hence, there is a synergistic effect between the antibacterial ingredients in the MM EO and thymol combinations. In addition, the antibacterial efficacy depends on the bacterial strain. As reported, the mode of antibacterial action of essential oils and thymol on microbial cells is a complex process. It involves antimicrobial constituents causing changes in the internal pH of the cellular membrane, leakage of cytoplasmic contents, and tolerance of NaCl through a damaged cytoplasmic membrane. Then, it inhibits microbial oxygen uptake, leading to morphological changes and reduced viability of bacteria (Carson et al., 2002; Cox et al., 1998).

Table 6. *MIC (%) of the binary combinations against B. cereus (1), E. coli (2), Salmonella T. (3), and S. aureus (4)*

Concentration % (v/v)	**Thymol and MM EO**				**Thymol and MQ EO**			
	1	***2***	***3***	***4***	***1***	***2***	***3***	***4***
50%	-0.057	0.061	0.003	-0.022	0.075	0.047	0.094	0.067
25%	-0.050	0.053	-0.015	-0.067	0.08	0.057	0.070	0.081
12.5%	-0.007	0.050	-0.010	-0.041	0.064	0.061	0.060	0.150
6.3%	-0.005	0.068	-0.015	-0.012	0.077	0.062	0.071	0.263
3.1%	-0.007	0.044	-0.011	-0.003	0.086	0.079	0.062	0.313
1.5%	-0.006	0.044	-0.004	-0.002	0.065	0.071	0.057	0.582
0.8%	-0.014	0.040	-0.008	0.014	0.058	0.068	0.065	0.690
0.4%	-0.006	0.047	-0.004	0.002	0.049	0.065	0.075	0.820
0.2%	-0.004	0.047	-0.006	0.165	0.046	0.048	0.048	0.933
0.1%	-0.002	0.054	0.001	0.228	0.053	0.047	0.047	0.980
0.05%	0.253	0.045	1.024	0.763	0.043	0.044	0.048	0.913
0.025%	0.500	0.524	1.104	0.763	0.252	0.050	0.053	0.974
MIC%	**0.085**	**0.043**	**0.085**	**0.350**	**0.043**	**0.005**	**0.011**	**25**

4. Conclusions

This study has investigated the antibacterial activity of two essential oils from *Melaleuca* leaves in the binary combination of thymol. The synergistic effect of thymol and other antibacterial components in the EOs, especially 1,8-cineole, has been investigated via the antibacterial inhibition zone and MIC methods. Combining thymol with MM EO has antibacterial activity against all bacteria tested, including *B. cereus, E. coli, Salmonella* T., *and S. aureus*. Meanwhile, the synergistic activity of thymol with MQ EO was almost ineffective for *S. aureus* bacteria, with a very high MIC value (25%). The results served as a crucial preliminary step toward combining locally available essential oils with commercial antibacterial active ingredients to create binary combinations with enhanced antibacterial activity.

❖ ***Conflict of Interest:*** *Authors have no conflict of interest to declare.*

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HIỆU QUẢ KHÁNG KHUẨN NÂNG CAO NHỜ VÀO TÁC DỤNG HIỆP ĐỒNG CỦA THYMOL VÀ TINH DẦU TRÀM LOẠI GIÀU 1,8-CINEOLE

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TÓM TẮT

Nghiên cứu này đã chỉ ra hiệu quả hiệp đồng chống lại vi khuẩn kháng nhiều loại thuốc của thymol kết hợp tinh dầu tràm chứa hàm lượng 1,8−cineole. Hai loài Melaleuca được sử dụng trong nghiên cứu này, Melaleuca sp2-Myrtaceae (MM) và Melaleuca cf. Quinquenervia (MQ), đã được chọn vì đặc tính kháng khuẩn tiềm tàng của chúng. Đồng thời, bốn loại vi khuẩn, bao gồm Bacillus cereus, Staphylococcus aureus, Escherichia coli và Salmonella Typhimurium, đã được chọn cho loạt thí nghiệm kháng khuẩn. Kết quả chứng minh hiệu quả kháng khuẩn cao nhất khi kết hợp tinh dầu MM với 9,5 wt.% thymol không chỉ có ý nghĩa mà còn mang lại hi vọng cho tương lai về lĩnh vực này. Nồng độ ức chế tối thiểu (MIC) và dữ liệu vùng ức chế kháng khuẩn xác nhận thêm hiệu quả hiệp đồng. Những phát hiện này có ý nghĩa đầy hứa hẹn đối với tương lai của tổng hợp dược phẩm, gợi ý một hướng đi tiềm năng để phát triển các phương pháp điều trị hiệu quả chống lại vi khuẩn kháng nhiều loại thuốc, một mối quan tâm đáng kể và ngày càng tăng đối với sức khỏe. Tiềm năng ứng dụng thực tế này cho thấy nhiều tiến triển khả quan trong lĩnh vực nghiên cứu kháng khuẩn.

Từ khóa: 1,8-cineole; tinh dầu tràm *Melaleuca*; hoạt tính kháng khuẩn hiệp đồng; Thymol