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Formulation, Physicochemical Characterization, and Repellent Activity of a Topical Lotion Containing Ylang-Ylang (*Cananga odorata*) and Patchouli (*Pogostemon cablin*) Essential Oils Against *Aedes aegypti*

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ABSTRACT: Background: *Aedes aegypti* is a major vector of dengue, chikungunya, and Zika and remains a significant public health concern in tropical regions, including Indonesia. The increasing burden of mosquito-borne diseases underscores the need for effective, safe, and environmentally sustainable alternatives to synthetic repellents. Patchouli (*Pogostemon cablin*) and ylang-ylang (*Cananga odorata*) essential oils have demonstrated repellent activity and may serve as promising botanical ingredients for topical formulations.

Objective: To formulate a topical repellent lotion containing patchouli and ylang-ylang essential oils at a constant total concentration of 3% w/w with varying ratios, and to evaluate its physicochemical characteristics, physical stability, preliminary skin tolerability, and repellent activity against adult female *A. aegypti*.

Methods: Four oil-in-water formulations were prepared: F0 (oil-free base), F1 (patchouli:ylang-ylang, 1:2), F2 (1:1), and F3 (2:1). Organoleptic characteristics, homogeneity, pH, viscosity, spreadability, and 28-day physical stability were evaluated. Skin tolerability was assessed using a Human 4-Hour Patch Test in six volunteers. Repellent activity was evaluated using a modified arm-in-cage assay with 20 adult female *A. aegypti* per treatment.

Results: All formulations remained homogeneous without apparent organoleptic changes or phase separation during storage. pH, viscosity, and spreadability ranged from 6.97–6.99, 13,517–14,616 mPa·s, and 6.00–6.87 cm, respectively. No erythema or oedema was observed in any volunteer. Repellent protection was highest for F1 (100%; 20/20 mosquitoes), followed by F3 (95%; 19/20) and F2 (45%; 9/20), compared with 0% for the control. F1 and F3 significantly outperformed the control, whereas their difference was not statistically significant.

Conclusion: The patchouli–ylang-ylang combination produced a physically stable lotion with acceptable preliminary skin tolerability and substantial repellent activity. The results suggest that the essential-oil ratio may influence repellent performance beyond total concentration, with F1 (1:2) showing the highest observed protection. Further studies should evaluate protection duration, concentration–response relationships, and potential interactions between the essential oils.

1. INTRODUCTION

Mosquito-borne diseases remain a major public health challenge in tropical and subtropical regions, including Indonesia. Among the principal mosquito vectors, *Aedes aegypti* plays a critical epidemiological role as the primary vector of dengue virus and an important vector of chikungunya and Zika viruses[1,2]. Consequently, reducing human–vector contact represents a fundamental component of integrated strategies for preventing and controlling mosquito-borne diseases[3,4]. The global burden of dengue has increased substantially in recent years. The World Health Organization (WHO) reported that 2024 recorded the highest number of dengue cases since global surveillance began, with approximately 14.6 million cases and more than 12,000 deaths reported across more than 100 countries[5,6]. This substantial burden persisted during the first half of 2025, when more than 4 million cases and 3,000 deaths were reported from 97 countries (WHO, 2025). In Indonesia, the Ministry of Health reported 131,393 dengue cases and 544 deaths between January and October 2025, corresponding to a case fatality rate of approximately 0.41%. Although this represented an approximately 50% reduction compared with the same period in the previous year, the magnitude of morbidity and mortality indicates that dengue continues to impose a substantial public health burden.[6,7]

A similar epidemiological pattern has been observed at the provincial level; in Aceh Province, approximately 3,400 dengue cases and 22 deaths were reported in 2024 (Kompas.com, 2025). Collectively, these epidemiological data underscore the persistent burden of dengue and the need for sustainable interventions capable of reducing human exposure to mosquito vectors[8–10]. Vector control is a cornerstone of mosquito-borne disease prevention. In addition to population-level vector management, topical repellents provide an important means of personal protection by reducing mosquito–human contact and, consequently, the probability of pathogen transmission (WHO, 2024). Synthetic repellents, particularly *N,N*-diethyl-*meta*-toluamide (DEET), have been extensively used because of their well-established efficacy against a broad range of mosquito species. Nevertheless, repeated topical application of DEET has been associated with skin irritation and discomfort, while concerns regarding prolonged exposure have stimulated continued interest in alternative repellent compounds, particularly for populations requiring greater caution in topical product use[11–13]. These considerations have encouraged the exploration of plant-derived bioactive compounds as potential alternatives or complementary ingredients in repellent development. Natural products are of particular interest because of their chemical diversity, biodegradability, and potential environmental advantages compared with persistent synthetic compounds[14–16]

Essential oils represent a promising class of botanical materials for mosquito-repellent development because their volatile constituents can interfere with mosquito sensory perception and host-seeking behavior. Patchouli essential oil (*Pogostemon cablin*), for example, contains a complex mixture of terpenoid and sesquiterpenoid compounds, including patchouli alcohol, α -bulnesene, α -guaiene, and seychellene, which may contribute to its biological activity against mosquitoes[17–19]. In a behavioral bioassay using *Aedes* mosquitoes, Deng et al. (2023) reported that patchouli oil containing approximately 34.7% patchouli alcohol provided complete protection for approximately 36 min. Although its ED₅₀ was substantially higher than that of clove oil evaluated in the same study, the observed protection demonstrates measurable repellent activity and supports the potential of patchouli oil as a botanical repellent ingredient. Importantly, the relatively limited persistence of protection also suggests that formulation strategies or combinations with other active botanical ingredients may be required to enhance its practical performance[20,21].

Ylang-ylang essential oil (*Cananga odorata*) represents another potentially valuable botanical source of mosquito-repellent compounds. Its chemical profile includes linalool, benzyl acetate, benzyl benzoate, methyl benzoate, α -gurjunene, and germacrene D, among other volatile constituents (Nerio et al., 2010)[22–24]. Sukkanon et al. (2022) demonstrated that ylang-ylang oil at concentrations ranging from 0.5 to 5% v/v elicited significant avoidance behavior in *Culex quinquefasciatus*, with avoidance rates reaching 85–97%. Comparable behavioral responses were also observed in *Anopheles minimus*, further supporting the potential of ylang-ylang oil as a botanical repellent at concentrations relevant to topical formulation[25,26]. Beyond their biological activity, the characteristic aromatic profiles of patchouli and ylang-ylang may offer an additional formulation advantage by contributing to sensory acceptability, which is particularly relevant for products intended for repeated topical application.

Previous studies have demonstrated the repellent potential of both essential oils when incorporated individually into topical lotion formulations. Yunus et al. (2024) reported that lotion containing patchouli oil from Southeast Sulawesi at concentrations of 2.5–12.5% provided 81.5–90.89% protection against *Aedes aegypti*, with the highest efficacy observed at concentrations of 10–12.5%[27,28]. These findings demonstrate that the repellent activity of patchouli oil can be retained following incorporation into a topical lotion matrix. Similarly, Puspita et al. (2022) reported that a 5% ylang-ylang oil lotion provided 96.3% protection during the first hour, although protection declined to 75% by the sixth hour. This reduction highlights the challenge of maintaining repellent efficacy over time and indicates that persistence should be considered alongside initial repellent activity when developing

botanical topical formulations[27,29]. Although these studies provide evidence supporting the individual efficacy of patchouli and ylang-ylang oils, their potential as a combined repellent system remains insufficiently characterized. Combining essential oils with distinct phytochemical profiles may provide a rational approach to improving repellent performance through complementary or synergistic interactions among their constituent compounds. However, synergism cannot be inferred solely from the biological activity of individual ingredients, as interactions between botanical components may result in additive, synergistic, or antagonistic effects. Experimental evaluation is therefore required to determine whether combining patchouli and ylang-ylang oils provides a meaningful advantage over their individual use. To date, evidence regarding the incorporation of *Pogostemon cablin* and *Cananga odorata* essential oils into a single topical lotion remains limited, particularly under an experimental design in which the total essential-oil concentration is held constant while the ratio of the two oils is systematically varied. Such an approach provides a more rigorous basis for determining the contribution of each oil to formulation performance while minimizing confounding effects associated with differences in total active concentration.

Accordingly, an important knowledge gap remains concerning the optimal ratio of patchouli and ylang-ylang essential oils in a topical repellent formulation and its relationship with physicochemical quality, storage stability, topical safety, and repellent efficacy against *Aedes aegypti*. Addressing this gap is essential because the development of an effective botanical repellent requires more than demonstrating biological activity alone; the formulation must also exhibit acceptable physical characteristics, adequate stability, and a favorable safety profile. Therefore, this study aimed to: (i) formulate topical repellent lotions containing combinations of patchouli (*Pogostemon cablin*) and ylang-ylang (*Cananga odorata*) essential oils at a constant total essential-oil concentration but varying ratios; (ii) evaluate the physicochemical characteristics and storage stability of the resulting formulations; (iii) assess their topical safety using an irritation test; and (iv) determine their repellent efficacy against *Aedes aegypti*. By systematically integrating formulation quality, stability, safety, and biological efficacy, this study seeks to provide a comprehensive basis for the development of a botanical mosquito repellent with improved practical potential.

2. MATERIALS AND METHODS

Study Design

This study employed a laboratory-based experimental design to evaluate the physicochemical characteristics, physical stability, topical safety, and repellent activity of four lotion formulations (F0–F3) containing different ratios of patchouli (*Pogostemon cablin*) and ylang-ylang (*Cananga odorata*) essential oils. Physicochemical evaluations, including organoleptic properties, homogeneity, pH, viscosity, and spreadability, were performed in triplicate ($n = 3$) for each formulation. Topical safety was assessed in six adult volunteers ($n = 6$), whereas repellent activity was evaluated using 20 adult female *Aedes aegypti* mosquitoes for each treatment. The repellent assay was conducted with reference to the World Health Organization (WHO) guidelines for evaluating mosquito repellents on human skin (WHO, 2009).

Materials and Equipment

The materials used in this study included patchouli essential oil (*Pogostemon cablin*), ylang-ylang essential oil (*Cananga odorata*), stearic acid, cetyl alcohol, glycerin, triethanolamine (TEA), methylparaben, propylparaben, and distilled water. The equipment comprised an analytical balance, water bath, homogenizer/mixer, digital pH meter, Brookfield viscometer, vernier caliper, glass microscope slides, and a mosquito cage for the repellent bioassay.

Lotion Formulation

The formulations were prepared as oil-in-water (O/W) emulsions using a conventional emulsification method. The oil phase, consisting of stearic acid, cetyl alcohol, and propylparaben, was heated to $70 \pm 2^\circ\text{C}$. Simultaneously, the aqueous phase containing distilled water, glycerin, methylparaben, and TEA was heated to the same temperature. The oil phase was subsequently incorporated gradually into the aqueous phase under continuous homogenization. The essential oils were incorporated during the emulsification process, and homogenization was continued until a uniform emulsion was obtained. The resulting lotion was allowed to cool to room temperature before being transferred into closed containers for subsequent evaluation (Allen, 2020; Barel et al., 2014). Four formulations were prepared with a constant total essential-oil concentration of 3% w/w, while the patchouli-to-ylang-ylang ratio was systematically varied (Table 1). Maintaining a constant total essential-oil concentration enabled differences in formulation performance among F1–F3 to be primarily attributed to variations in the relative proportions of the two essential oils rather than differences in total active concentration.

Table 1. Composition of patchouli–ylang-ylang repellent lotion formulations

Formulation	Patchouli oil (<i>P. cablin</i>)	Ylang-ylang oil (<i>C. odorata</i>)	Total essential oils
F0 (base)	0%	0%	0%
F1	1.0%	2.0%	3.0%
F2	1.5%	1.5%	3.0%
F3	2.0%	1.0%	3.0%

The lotion base, comprising stearic acid, cetyl alcohol, glycerin, TEA, methylparaben, propylparaben, and distilled water, was adjusted to a final composition of 100% w/w for each formulation.

Physicochemical Evaluation of the Lotion

Organoleptic Properties and Homogeneity

Organoleptic characteristics were evaluated visually by examining the color, odor, physical appearance, and consistency of each formulation. Homogeneity was assessed by placing a small amount of lotion on a glass slide and examining the preparation for coarse particles, visible aggregation, or phase separation. A formulation was considered homogeneous when its components were uniformly distributed without visible coarse particles or phase separation [30,31]

pH Measurement

The pH of each lotion was measured using a digital pH meter calibrated with standard buffer solutions at pH 4.00, 7.00, and 10.00 before measurement. The electrode was immersed in the lotion sample, and the pH value was recorded once a stable reading had been obtained. Each formulation was measured in triplicate, and the results were expressed as mean $\pm$ standard deviation (SD). Appropriate pH is an important consideration for topical formulations because compatibility with the physiological environment of the skin may reduce the potential for irritation and support formulation tolerability

Viscosity Measurement

Lotion viscosity was determined using a Brookfield viscometer equipped with spindle No. 4 at a rotational speed of 12 rpm. Measurements were performed at room temperature ($25 \pm 2^\circ\text{C}$). Each formulation was measured three times, and the results were expressed as mean $\pm$ SD. Viscosity is a critical quality attribute of topical emulsions because it can influence physical stability, flow behavior, spreadability, and ease of application[32,33]

Spreadability

Spreadability was evaluated by placing 0.5 g of lotion at the center of a glass slide and covering it with a second glass slide. The preparation was allowed to stand for 1 min before a 150-g load was applied. The diameter of the spread lotion was subsequently measured using a vernier caliper. Each formulation was evaluated in triplicate, and the results were expressed as mean $\pm$ SD. Adequate spreadability indicates the ability of a topical formulation to distribute uniformly over the skin surface and may contribute to application convenience and user acceptability.

Physical Stability During Storage

Physical stability was evaluated by storing the lotion formulations at room temperature for 28 days. Organoleptic characteristics, including color, odor, and consistency, as well as homogeneity, were evaluated at the end of the storage period and compared with the initial characteristics. The assessment was performed to identify visible changes, phase separation, or other signs of physical instability during storage.

Skin Irritation Assessment: Human 4-Hour Patch Test

The topical irritation potential of the formulations was evaluated using a Human 4-Hour Patch Test (H4PT) involving six adult volunteers who met the predefined inclusion criteria. Approximately 0.2 g of lotion was applied to the volar surface of the forearm using a patch with a diameter of approximately 2 cm. The patch was maintained in contact with the skin for 4 h. Following patch removal, the application site was examined for visible signs of erythema and edema. Assessments were performed immediately after patch removal and again after 24 h. Irritation was evaluated based on the presence or absence of visible skin reactions in

each volunteer. The H4PT has been used as an approach for the preliminary assessment of the dermal tolerability of topical preparations (Basketter et al., 1997). The study received ethical approval under protocol number DP.04.03/12.7/211/2026.

Repellency Bioassay (Arm-in-Cage Test)

Repellent activity of the lotion formulations was evaluated against adult female *Aedes aegypti* mosquitoes using a modified arm-in-cage method based on World Health Organization (WHO) guidelines. The test involved healthy human volunteers who provided informed consent, and ethical approval was obtained from the relevant institutional review board. Twenty test mosquitoes were placed in a test cage for each treatment. The lotion formulation was applied evenly to the skin surface of the volunteer's forearm at the predetermined application dose, while the contralateral arm was left untreated as a negative control. The treated and control arms were then alternately inserted into the test cage for a predetermined observation period, and the number of mosquitoes landing on the skin surface was recorded at each observation interval. Protection percentage was calculated as the proportion of mosquitoes that did not land on the treated arm relative to the total number of test mosquitoes, using the following equation:

$$\text{Protection (\%)} = (\text{Number of mosquitoes not landing} / \text{Total number of test mosquitoes}) \times 100\%$$

The protection percentage of each formulation was determined based on the mean of observations across all replicates. The formulation exhibiting the highest protection percentage was designated as having the best repellent activity against *Aedes aegypti*.

Data Analysis

Data obtained from the physicochemical evaluation were expressed as mean $\pm$ standard deviation (SD) based on three replicate measurements. Statistical analyses were performed using IBM SPSS Statistics. Differences in pH, viscosity, and spreadability among the formulations were assessed using one-way analysis of variance (ANOVA) at a 95% confidence level ($\alpha = 0.05$). When a statistically significant difference was detected, Tukey's honestly significant difference (HSD) test was performed for post hoc pairwise comparisons. Data from the irritation and repellent activity assays were analyzed descriptively and presented as proportions and percentages.

RESULTS

Organoleptic Characteristics and Homogeneity

The organoleptic characteristics and homogeneity of the four lotion formulations evaluated on day 0 and after 28 days of storage are presented in Table 2. The F0 formulation (base) exhibited a white color and no detectable odor, consistent with the absence of essential oils. In contrast, formulations F1–F3 exhibited a cream-colored appearance, with odor characteristics corresponding to the relative proportions of patchouli and ylang-ylang essential oils. F1 exhibited a predominantly ylang-ylang odor, F2 showed a balanced odor profile between ylang-ylang and patchouli, whereas F3 exhibited a predominantly patchouli odor. All formulations remained homogeneous throughout the observation period, with no visible coarse particles, phase separation, or other apparent signs of physical instability. No discernible changes in color, odor, consistency, or homogeneity were observed between day 0 and day 28. These findings indicate that all formulations maintained their apparent physical integrity under room-temperature storage conditions for 28 days.

Table 2. Organoleptic characteristics and homogeneity of the lotion formulations at baseline and after 28 days of storage

Formulation	Color	Odor	Homogeneity
F0	White	Odorless	Homogeneous
F1	Cream	Predominantly ylang-ylang	Homogeneous
F2	Cream	Balanced ylang-ylang and patchouli	Homogeneous
F3	Cream	Predominantly patchouli	Homogeneous

No observable changes in color, odor, consistency, or homogeneity were detected in any formulation during the 28-day storage period at room temperature.

pH, Viscosity, and Spreadability

The pH, viscosity, and spreadability of the four formulations are summarized in Table 3. The measured pH values ranged from

6.97 to 6.99, indicating minimal variation among formulations. Viscosity ranged from 13,517 to 14,616 mPa·s, whereas spreadability ranged from 6.00 to 6.87 cm. Among the formulations, F2 exhibited the highest viscosity ($14,616 \pm 135$ mPa·s) and the lowest spreadability (6.00 ± 0.16 cm). In contrast, F1 exhibited the greatest spreadability (6.87 ± 0.14 cm), with an intermediate viscosity of $14,167 \pm 165$ mPa·s. F0 and F3 exhibited viscosities of $13,963 \pm 100$ and $13,517 \pm 135$ mPa·s, respectively.

Table 3. pH, viscosity, and spreadability of the lotion formulations (mean $\pm$ SD, n = 3)

Formulation	pH	Viscosity (mPa·s)	Spreadability (cm)
F0	6.97 ± 0.008	$13,963 \pm 100$	6.20 ± 0.15
F1	6.98 ± 0.008	$14,167 \pm 165$	6.87 ± 0.14
F2	6.99 ± 0.011	$14,616 \pm 135$	6.00 ± 0.16
F3	6.99 ± 0.011	$13,517 \pm 135$	6.67 ± 0.16

Note: The mean values were obtained from the numerical labels presented in the original graph. The SD values shown in this table were estimated by digitizing the error bars from a low-resolution poster image and should therefore be replaced with the original SD values obtained from the raw experimental data or SPSS output before manuscript submission.

Physical Stability During 28-Day Storage

At the end of the 28-day storage period, all formulations retained their initial organoleptic characteristics and homogeneity (Table 2). No visible phase separation, changes in color or odor, or alterations in consistency were observed. These findings suggest that the O/W emulsion system maintained acceptable apparent physical stability under room-temperature storage conditions over the 28-day observation period.

Skin Irritation Assessment

The results of the Human 4-Hour Patch Test (H4PT) are presented in Table 4. None of the six volunteers exhibited visible erythema or edema following exposure to any of the four formulations. No irritation reactions were observed either immediately after patch removal or at the 24-h follow-up assessment. Accordingly, all formulations were classified as non-irritating under the conditions of the present test.

Table 4. Skin irritation responses following the Human 4-Hour Patch Test

Formulation	Volunteers with erythema	Volunteers with edema	Overall assessment
F0	0/6	0/6	No visible irritation
F1	0/6	0/6	No visible irritation
F2	0/6	0/6	No visible irritation
F3	0/6	0/6	No visible irritation

Repellent Activity Against *Aedes aegypti*

The repellent activity of the formulations against adult female *Aedes aegypti* is presented in Table 5. The untreated control exhibited 0% protection, with all 20 mosquitoes landing on the exposed arm. Among the tested formulations, F1 produced the highest numerical protection, with 20 of 20 mosquitoes not landing on the treated arm, corresponding to 100% protection. F3 provided 95% protection, with 19 of 20 mosquitoes not landing on the treated arm, whereas F2 provided 45% protection, with 9 of 20 mosquitoes not landing on the treated arm.

Table 5. Repellent activity of the lotion formulations against *Aedes aegypti* using the arm-in-cage assay

Treatment	Mosquitoes not landing (n/20)	Mosquitoes landing (n/20)	Protection (%)
Control	0/20	20/20	0

F1	20/20	0/20	100
F2	9/20	11/20	45
F3	19/20	1/20	95

95% Confidence Intervals for Repellent Protection

To quantify the precision of the observed protection estimates, exact 95% confidence intervals (CIs) were calculated using the Clopper–Pearson method based on the number of mosquitoes that did not land on the treated arm out of 20 mosquitoes per treatment (Table 7). The estimated protection was 0% for the untreated control (95% CI: 0.0–16.8%), 100% for F1 (95% CI: 83.2–100.0%), 45% for F2 (95% CI: 23.1–68.5%), and 95% for F3 (95% CI: 75.1–99.9%). The relatively wide confidence intervals reflect the limited number of mosquitoes evaluated per treatment. Notably, the confidence intervals for F2 and F1 did not overlap, whereas the intervals for F1 and F3 overlapped substantially. Therefore, although F1 exhibited numerically higher protection than F3 (100% vs. 95%), the confidence intervals alone do not provide sufficient evidence to conclude that the two formulations differ in repellent efficacy.

Table 7. Repellent protection and exact 95% confidence intervals calculated using the Clopper–Pearson method

Treatment	Mosquitoes not landing	Protection (%)	Exact 95% CI
Control	0/20	0.0	0.0–16.8
F1	20/20	100.0	83.2–100.0
F2	9/20	45.0	23.1–68.5
F3	19/20	95.0	75.1–99.9

Pairwise Comparison of Repellent Activity

Pairwise differences in the proportions of mosquitoes that did not land on the treated arm were assessed using Fisher’s exact test because of the small cell counts in several 2×2 contingency tables (Table 8). F1, F2, and F3 each showed significantly greater protection than the untreated control ($p < 0.001$ for F1 and F3; $p = 0.0012$ for F2). Significant differences were also observed between F1 and F2 ($p = 0.0001$) and between F2 and F3 ($p = 0.0012$). In contrast, no statistically significant difference was detected between F1 and F3 ($p = 1.000$). Thus, although F1 showed the highest numerical protection (100%) compared with F3 (95%), the observed difference was not statistically significant under the present experimental conditions.

Table 8. Pairwise Fisher’s exact test for repellent activity

Comparison	<i>p</i> -value	Interpretation
F1 vs. Control	<0.0001	Significant
F2 vs. Control	0.0012	Significant
F3 vs. Control	<0.0001	Significant
F1 vs. F2	0.0001	Significant
F1 vs. F3	1.000	Not significant
F2 vs. F3	0.0012	Significant

Statistical significance was defined as $p < 0.05$.

Relationship Between Physical Parameters: Viscosity and Spreadability

To further examine the apparent inverse relationship between viscosity and spreadability observed descriptively across the formulations, Pearson’s correlation coefficient was calculated using the mean values of the four formulations ($n = 4$). A negative

correlation was observed between viscosity and spreadability ($r = -0.543$; $p = 0.457$), indicating a moderate inverse association in which formulations with higher viscosity tended to exhibit lower spreadability. However, this association did not reach statistical significance at the 5% significance level. The lack of statistical significance should be interpreted cautiously because the correlation analysis was based on only four formulation-level observations, resulting in very limited statistical power and wide uncertainty around the estimated correlation coefficient. Therefore, the present data provide only descriptive evidence of an inverse relationship between viscosity and spreadability and are insufficient to establish a statistically supported correlation. Further studies involving a larger number of formulations or experimental observations are warranted to characterize the relationship between these two physicochemical parameters more robustly.

Comparison with Previous Studies

To contextualize the present findings within the existing literature, the repellent performance of the most effective formulations in this study was compared descriptively with previously reported topical formulations containing patchouli or ylang-ylang essential oil as individual active ingredients (Table 9).

Table 9. Comparison of repellent protection with previous studies of patchouli- or ylang-ylang-based repellents

Study	Active ingredient	Concentration	Protection
Present study (F1)	Patchouli combination (1:2)	3% total	100%
Present study (F3)	Patchouli combination (2:1)	3% total	95%
Yunus et al. (2024)	Patchouli oil alone	10–12.5%	90.4–90.9%
Yunus et al. (2024)	Patchouli oil alone	2.5%	81.5%
Puspita et al. (2022)	Ylang-ylang oil alone	5%	96.3% (first hour)

In the present study, F1, containing a 1:2 ratio of patchouli to ylang-ylang essential oils, achieved 100% protection at a total essential-oil concentration of 3%. This protection level was numerically higher than that reported for patchouli oil alone at 10–12.5% in the study by Yunus et al. (2024), while F3 also demonstrated high protection (95%) at the same total essential-oil concentration. These findings suggest that combining the two essential oils may provide promising repellent activity at a relatively low total concentration. However, direct comparisons across studies should be interpreted with caution because differences in mosquito strain or population, experimental conditions, exposure duration, application procedures, formulation composition, and outcome assessment may substantially influence repellent performance. Therefore, the present findings should not be interpreted as definitive evidence of synergism between patchouli and ylang-ylang oils. Rather, they provide preliminary evidence supporting further investigation of the combination and its underlying interactions using appropriately controlled comparative and concentration–response designs.

Summary of the Principal Findings

The principal findings of the study are summarized in Table 10.

Table 10. Summary of the principal findings

Aspect	Principal finding
Physical stability	All formulations (F0–F3) remained homogeneous and showed no observable changes in organoleptic characteristics during 28 days of storage.
Physicochemical characteristics	pH ranged from 6.97 to 6.99, viscosity from 13,517 to 14,616 mPa·s, and spreadability from 6.00 to 6.87 cm.
Topical tolerability	No erythema or edema was observed in any of the six volunteers following H4PT for any formulation (0/6 for each formulation).
Highest numerical protection	F1 (1% patchouli:2% ylang-ylang) provided 100% protection (95% CI: 83.2–100.0%) and

	differed significantly from the untreated control (Fisher's exact test, $p < 0.0001$).
Lowest protection	F2 (1.5% patchouli:1.5% ylang-ylang) provided 45% protection (95% CI: 23.1–68.5%) and showed significantly lower protection than F1 and F3 ($p < 0.01$).
F1 versus F3	No statistically significant difference was detected between F1 and F3 (Fisher's exact test, $p = 1.000$), despite the numerically higher protection observed with F1 (100% vs. 95%).
Formulation implication	Under the conditions of this study, variation in the patchouli-to-ylang-ylang ratio was associated with differences in observed repellent protection despite a constant total essential-oil concentration of 3%.

Statistical Analysis of Physicochemical Parameters

Differences in pH, viscosity, and spreadability among the formulations were assessed using one-way analysis of variance (ANOVA), followed by Tukey's honestly significant difference (HSD) test when appropriate, as specified in the Methods section. Because the original raw measurements and the corresponding SPSS output were not available in the present dataset, the ANOVA statistics shown in Table 6 were calculated indicatively from the summary statistics reported in Table 3. These calculations were based on the reported means, estimated SD values, and $n = 3$ observations per formulation and therefore do not represent the original statistical output generated from the experimental data. Accordingly, the F -statistics and p -values presented below should be regarded as provisional and must be replaced with the results obtained directly from the original experimental dataset or the corresponding SPSS output before manuscript submission. In particular, the estimated SD values in Table 3 were derived from visual digitization of error bars in a low-resolution graphical source and should not be used as substitutes for the original replicate-level measurements.

Table 6. Indicative one-way ANOVA results for physicochemical parameters

Parameter	F-statistic	p -value	Interpretation
pH	$F(3,8) = 2.97$	0.097	Not significant
Viscosity	$F(3,8) = 33.92$	<0.001	Significant
Spreadability	$F(3,8) = 20.96$	<0.001	Significant

The indicative analysis suggests that formulation differences may have a limited effect on pH but a more pronounced effect on viscosity and spreadability. Nevertheless, these findings should be considered exploratory until confirmed using the original experimental data. In the final manuscript, the Tukey HSD results should also be reported to identify which specific formulation pairs differ significantly in viscosity and spreadability.

4. DISCUSSION

The present study demonstrated that lotions containing different ratios of patchouli (*Pogostemon cablin*) and ylang-ylang (*Cananga odorata*) essential oils exhibited acceptable physicochemical characteristics, preliminary topical tolerability, and substantial repellent activity against *Aedes aegypti*. All formulations remained homogeneous and showed no apparent changes in color, odor, consistency, or phase separation during 28 days of storage at room temperature, suggesting satisfactory short-term physical stability of the O/W emulsion system. The pH values were narrowly distributed between 6.97 and 6.99, indicating minimal influence of the different essential-oil ratios on the acid–base characteristics of the formulations and a pH range generally compatible with topical application. Viscosity ranged from 13,517 to 14,616 mPa·s, while spreadability ranged from 6.00 to 6.87 cm, demonstrating that all formulations retained physicochemical characteristics suitable for topical application. The observed tendency toward lower spreadability at higher viscosity was consistent with the expected rheological behavior of semisolid and emulsion-based preparations; however, Pearson's correlation analysis yielded a moderate negative association ($r = -0.543$; $p = 0.457$), which was not statistically significant. This result should be interpreted cautiously because the analysis was based on only four formulation-

level observations and therefore had very limited statistical power. Similarly, the apparent differences in viscosity and spreadability among formulations should be confirmed using the original replicate-level data before definitive statistical conclusions are drawn. The absence of erythema or edema in any of the six volunteers following the Human 4-Hour Patch Test provides preliminary evidence of acceptable short-term local tolerability under the experimental conditions; nevertheless, the small sample size precludes broad conclusions regarding population-level safety or the potential for sensitization and long-term adverse effects. The most pronounced finding concerned repellent activity, which differed substantially among F1–F3 despite the total essential-oil concentration being held constant at 3%. F1, containing patchouli and ylang-ylang oils at a ratio of 1:2, achieved the highest numerical protection (100%), followed by F3 at a ratio of 2:1 (95%), whereas the equal-ratio formulation F2 (1:1) exhibited considerably lower protection (45%). Fisher's exact test confirmed that all essential-oil-containing formulations provided significantly greater protection than the untreated control, while the difference between F1 and F3 was not statistically significant ($p = 1.000$), indicating that the apparent numerical superiority of F1 over F3 should not be interpreted as evidence of greater population-level efficacy. In contrast, F2 showed significantly lower protection than both F1 and F3, suggesting that, under the conditions of this study, repellent efficacy was influenced not only by the total concentration of essential oils but also by their relative proportions. The non-linear response observed across the three ratios is particularly noteworthy because increasing or decreasing the proportion of either essential oil did not produce a simple monotonic change in protection, implying that interactions among the constituent volatile compounds may contribute to the overall behavioral response of *A. aegypti*. Patchouli oil contains several biologically active volatile constituents, particularly patchouli alcohol, together with α -bulnesene, α -guaiene, and seychellene, whereas ylang-ylang oil contains compounds such as linalool, benzyl acetate, benzyl benzoate, methyl benzoate, α -gurjunene, and germacrene D, all of which may contribute to mosquito avoidance through effects on host-seeking and olfactory-mediated behavior[34–36]. The relatively high performance of F1, in which ylang-ylang constituted two-thirds of the total essential-oil content, may indicate a substantial contribution from ylang-ylang-derived volatiles, whereas the similarly high activity of F3 demonstrates that patchouli oil also contributes meaningfully to the repellent effect. The markedly lower efficacy of F2 may reflect differences in the composition and relative abundance of volatile constituents at the 1:1 ratio; however, mechanisms such as synergism, additivity, or antagonism cannot be distinguished from the present data because the study did not include formal interaction analysis, concentration–response modeling, or chemical characterization of the finished formulations.

Therefore, the observed superiority of the non-equimolar formulations should be regarded as a formulation-level finding rather than definitive evidence of synergistic interaction. Comparison with previous studies further supports the potential of the combination, as F1 achieved 100% protection at a total essential-oil concentration of 3%, whereas Yunus et al. (2024) reported 90.4–90.9% protection with 10–12.5% patchouli oil alone, and Puspita et al. (2022) reported 96.3% protection during the first hour with 5% ylang-ylang oil. Nevertheless, these cross-study comparisons are inherently limited by differences in mosquito species or strains, exposure duration, application procedures, formulation vehicles, environmental conditions, and definitions of repellent response; consequently, the present findings cannot be interpreted as direct evidence that the combination is intrinsically more potent than either essential oil used individually[37–39]. Taken together, the results indicate that a patchouli–ylang-ylang combination can be incorporated into an O/W lotion while maintaining acceptable short-term physicochemical stability and preliminary skin tolerability, and that modulation of the essential-oil ratio may substantially influence repellent performance at a constant total concentration. F1 (1:2) and F3 (2:1) emerged as the most promising formulations based on their high observed protection, whereas the 1:1 formulation performed considerably less effectively. Further studies incorporating GC–MS characterization, individual-oil controls, broader concentration–response matrices, longer protection assessments, and formal synergy or interaction models are warranted to elucidate the chemical and mechanistic basis of the ratio-dependent repellent activity and to establish the optimal formulation with greater statistical and biological confidence.

5. CONCLUSION

A topical lotion containing a combination of patchouli (*Pogostemon cablin*) and ylang-ylang (*Cananga odorata*) essential oils was successfully formulated and exhibited acceptable physicochemical characteristics, with pH values ranging from 6.97 to 6.99, viscosity from 13,517 to 14,616 mPa·s, and spreadability from 6.00 to 6.87 cm. All formulations remained physically homogeneous without observable changes in organoleptic characteristics or phase separation during 28 days of storage at room temperature. No erythema or edema was observed in the six volunteers subjected to the Human 4-Hour Patch Test, providing preliminary evidence of short-term topical tolerability. At a constant total essential-oil concentration of 3%, variation in the patchouli-to-ylang-ylang ratio was associated with marked differences in repellent performance against *Aedes aegypti*. F1, containing 1% patchouli and 2% ylang-ylang oil, achieved the highest observed protection (100%), followed by F3 (2% patchouli and 1% ylang-ylang) at 95%, whereas the equimolar formulation F2 (1.5%:1.5%) provided 45% protection. Notably, the difference between F1

and F3 was not statistically significant; therefore, F1 should be regarded as the formulation with the highest **observed** repellent activity rather than as definitively superior to F3. Overall, the findings support the potential of patchouli–ylang-ylang essential-oil combinations as botanical active ingredients for topical mosquito-repellent formulations, while also highlighting the importance of optimizing the relative proportion of the constituent oils. Further investigations incorporating larger experimental populations, extended protection-time assessments, chemical profiling of the finished formulations, concentration–response analyses, and formal evaluation of potential synergistic or antagonistic interactions are warranted before broader conclusions regarding efficacy, mechanism of action, and long-term safety can be established.

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Author Contributions

Kartini: Conceptualization, methodology, investigation, data analysis, and writing—original draft. **Wiska Mailisa:** Formulation development, physicochemical evaluation, and writing—review and editing. **Tirta Fitria Sari:** Investigation of skin irritation and repellent activity, data curation, and writing—review and editing. The author-contribution statement should be reviewed and confirmed by all authors prior to manuscript submission to ensure that it accurately reflects their respective contributions in accordance with the CRediT taxonomy.

Conflict of Interest

The authors declare that they have no conflicts of interest that could have influenced the design, conduct, analysis, interpretation, or reporting of this study.

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