A NATURAL MOSQUITO REPELLENT FORMULATION AGAINST *AEDES* SPECIES FROM HERBAL ESSENTIAL OILS

Nguyen Thi Minh Xuan*, Nguyen Thi Minh Nguyet, Le Thi Kieu My
The University of Science and Technology - The University of Danang

ARTICLE INFO

Received: 21/4/2025
Revised: 28/5/2025
Published: 28/5/2025

KEYWORDS

Mosquito repellent essential oil
Piper betel essential oil
Java citronella essential oil
Aedes aegypti
Aedes albopictus

ABSTRACT

Essential oil-based repellents provide a sustainable alternative to synthetic mosquito control methods and help combat diseases like dengue and Zika. This study developed a natural repellent using essential oils from betel leaf, Java citronella, and cinnamon to effectively target *Aedes aegypti* and *Aedes albopictus*. The formulation included 0.8% poly (ethylene glycol) as a fixative and was evaluated using a volunteer-free cage test model with an artificial skin attractant, ensuring ethical compliance. Gas chromatography-mass spectrometry (GC-MS) identified key repellent compounds, including chavibetol, citronellal, and cinnamaldehyde. The repellent achieved complete protection times of 3.93 ± 0.21 hours against *A. aegypti* and 5.10 ± 0.78 hours against *A. albopictus*, significantly longer than the control group. These results highlight the formulation's potential for effective mosquito control, with poly(ethylene glycol) enhancing protection duration. Future field studies are recommended to confirm its real-world efficacy.

NGHIÊN CỨU CHẾ PHẨM ĐUỔI MUỖI *AEDES* TỪ TINH DẦU TỰ NHIÊN

Nguyễn Thị Minh Xuân*, Nguyễn Thị Minh Nguyệt, Lê Thị Kiều My
Trường Đại học Bách khoa - Đại học Đà Nẵng

THÔNG TIN BÀI BÁO

Ngày nhận bài: 21/4/2025
Ngày hoàn thiện: 28/5/2025
Ngày đăng: 28/5/2025

TỪ KHÓA

Tinh dầu đuổi muỗi
Tinh dầu lá trầu
Tinh dầu sả Java
Aedes aegypti
Aedes albopictus

TÓM TẮT

Sản phẩm đuổi muỗi từ tinh dầu cung cấp một giải pháp thay thế bền vững cho các phương pháp kiểm soát muỗi từ hoá chất tổng hợp và giúp chống lại các bệnh như sốt xuất huyết và Zika. Nghiên cứu này đã phát triển một sản phẩm đuổi muỗi tự nhiên sử dụng tinh dầu từ lá trầu không, sả Java và quế để nhắm mục tiêu vào muỗi *Aedes aegypti* và *Aedes albopictus*. Công thức này có chứa 0,8% poly(ethylene glycol) làm chất cố định và được đánh giá bằng mô hình thử nghiệm lồng không có tình nguyện viên với chất dẫn dụ da nhân tạo, đảm bảo tuân thủ nguyên tắc về y đức. Sắc ký khí khối phổ (GC-MS) đã xác định các hợp chất chính trong sản phẩm, bao gồm chavibetol, citronellal và cinnamaldehyde. Sản phẩm đạt được thời gian bảo vệ hoàn toàn là 3,93 ± 0,21 giờ đối với *A. aegypti* và 5,10 ± 0,78 giờ đối với *A. albopictus*, dài hơn đáng kể so với nhóm đối chứng. Những kết quả này làm nổi bật tiềm năng kiểm soát muỗi hiệu quả của công thức này, với poly(ethylene glycol) giúp tăng cường thời gian bảo vệ. Mặc dù vậy, các nghiên cứu thực địa trong tương lai cần được thực hiện để xác nhận hiệu quả thực tế của nó.

DOI: https://doi.org/10.34238/tnu-jst.12648

* Corresponding author. *Email: ntmxuan@dut.udn.vn*

1. Introduction

Mosquitoes are significant carriers of diseases such as dengue fever, malaria, and the Zika virus, presenting substantial public health challenges, especially in tropical and subtropical regions. The World Health Organization estimates that mosquito-borne diseases affect over 700 million people each year, with dengue alone causing about 390 million infections annually [1]. Although conventional control strategies like DEET (N,N-diethyl-meta-toluamide) based repellents and insecticide-treated nets remain effective, they are challenged by resistance, environmental toxicity, and health concerns [2]. Consequently, there is increasing interest in safer, eco-friendly alternatives that align with global trends toward sustainability [3].

Essential oil-based repellents have emerged as effective alternatives due to their biodegradability, low toxicity, and compatibility with green living trends. Essential oils derived from plants such as betel leaf (*Piper betel*), Java citronella (*Cymbopogon winterianus*), and cinnamon (*Cinnamomum verum*) contain bioactive compounds like chavibetol, citronellal, and cinnamaldehyde, respectively. These compounds have demonstrated repellent activity against mosquito species, including *Aedes aegypti* and *Aedes albopictus* [4], [5]. However, a significant limitation of essential oils is their high volatility, which leads to a brief duration of protection—often less than two hours under standard conditions [6]. To mitigate this issue, fixatives such as poly(ethylene glycol) (PEG) have been investigated to decrease evaporation rates. Research has shown that certain formulations can extend the efficacy of essential oil repellents by up to 50% when fixatives are included, compared to those without fixatives [7], [8].

Evaluating the effectiveness of essential oil-based repellents usually involves several testing methods, including field tests, arm-in-cage tests, and laboratory assays like cage tests. Field tests assess the repellents' performance in real-world conditions but can be influenced by environmental factors such as humidity and temperature. Meanwhile, arm-in-cage tests measure complete protection time (CPT) by exposing volunteers' arms to mosquitoes in a controlled environment [9]. However, these methods, especially those involving human volunteers, often face significant ethical challenges due to medical ethics regulations in scientific research. For example, arm-in-cage tests raise concerns about informed consent, the possibility of allergic reactions, and the risk of disease transmission, particularly in areas where mosquito-borne pathogens are common [10]. These ethical issues can limit the feasibility of conducting tests with human volunteers, highlighting the need for alternative evaluation methods that adhere to ethical standards [11]. To address this, laboratory models such as standardized cage tests using artificial attractants have been developed. These models simulate mosquito behavior without human participation, although they may not completely replicate conditions found in the field [12].

This study aimed to develop and evaluate a mosquito repellent formulation that combines betel leaf, Java citronella, and cinnamon essential oils, using PEG-600 as a fixative. The goal was to achieve broad-spectrum efficacy against *Aedes aegypti* and *Aedes albopictus*. A primary objective was to standardize a volunteer-free cage test model utilizing an artificial skin attractant, ensuring compliance with medical ethics while maintaining scientific rigor. The standardized model was validated by demonstrating significant mosquito attraction to artificial skin treated with the attractant (e.g., a 77.7% mean landing proportion for latex + mini warmer + attractant), providing a reliable baseline for repellent testing without human involvement. Using this model, we assessed the formulation's efficacy, finding that PEG-600 significantly extended the complete protection time (CPT). Formulation 1 offered an average of 3.93 ± 0.21 hours of protection against *Aedes aegypti* and 5.10 ± 0.78 hours against *Aedes albopictus*, in contrast to less than 1.5 hours for the control (Formulation 2). Additionally, gas chromatography-mass spectrometry (GC-MS) analysis confirmed the presence of key volatile compounds. These findings contribute to the development of effective, natural mosquito repellents with an enhanced duration of action, addressing the need for sustainable mosquito control while adhering to ethical research practices.

2. Materials and methods

2.1. Materials

Essential oils from betel leaf (*Piper betle*), Java citronella (*Cymbopogon winterianus*), and cinnamon (*Cinnamomum burmannii*) were procured from a certified supplier (Hoa Thom Co La Co., Vietnam) who provided detailed GC-MS analyses confirming the composition and purity of each oil [13] – [15]. Poly(ethylene glycol) (PEG-600, molecular weight ~600 Da) was obtained from Sigma-Aldrich (USA). Coconut oil, used as a carrier, was sourced from a local supplier (Vietcoco Co., Vietnam). *Aedes aegypti* and *A. albopictus* mosquitoes (laboratory-reared, 7-10 days old, non-blood-fed females) were provided by the Center for Science and Technology Services**,** Ho Chi Minh city, Vietnam. The artificial skin attractant (a mixture of lactic acid, $CaCO_3$, distill water 1:1:1 w/w) was prepared in-house following standard protocols. All other reagents and materials were of analytical grade unless specified otherwise.

2.2. Preparation of repellent formulations

Two mosquito-repellent formulations were prepared using betel leaf (*Piper betle*), Java citronella (*Cymbopogon winterianus*), and cinnamon (*Cinnamomum zeylanicum*) essential oils, with coconut oil as the carrier. Betel leaf and Java citronella oils were each used at 10% concentration, based on their proven repellent efficacy at these levels [16], [17]. Cinnamon oil was included at 5% to minimize skin irritation while supporting repellent activity. Polyethylene glycol (PEG) 600 was added at 0.8% (w/v) to slow the evaporation of essential oils and enhance CO_2 absorption (~7.7 mg/g [18]), complementing the oils' action on mosquito CO_2 receptors. The combination of oils with varying molecular weights (cinnamaldehyde: 132.16 g/mol; citronellal/citronellol/citral: ~152–156 g/mol; chavibetol/chavibetol acetate: ~164.2 g/mol) ensures sustained release of active compounds.

Two formulations were prepared to evaluate the efficacy of the essential oil blend with and without PEG-600. Formulation 1 consisted of 10% betel leaf essential oil, 10% Java citronella essential oil, 5% cinnamon essential oil, 0.8% PEG-600, and 74.2% coconut oil (w/w). Formulation 2 (control) contained the same essential oil blend (10% betel leaf, 10% Java citronella, 5% cinnamon) and 75% coconut oil, without PEG-600. The essential oils were mixed with coconut oil at 40°C using a magnetic stirrer (IKA C-MAG HS 7, Germany) at 300 rpm for 30 minutes to ensure homogeneity. For Formulation 1, PEG-600 was added and stirred for an additional 15 minutes at the same conditions. The formulations were stored in airtight amber glass containers at 25 °C until use.

2.3. Mosquito culture

For all experiments conducted in this study, female *Aedes aegypti* and *A. albopictus* were used. Batches of approximately 500 eggs were incubated in 33 × 51 × 5 cm plastic trays containing three liters of dechlorinated water and covered with gauze to facilitate hatching into larvae. Cat food pellets were fed to the larvae. Pupae were sorted into 200 mL plastic cups and transferred to an insect cage (30 × 30 × 30 cm). A 100 mL Erlenmeyer flask containing 20% sucrose solution with a cotton wick on top was placed in the cage and changed weekly. The cage was kept in an insect room maintained at 27 °C and 80% humidity with a 14/10 light/dark cycle [16]. Ten-day-old adult mosquitoes were used for subsequent experiments.

2.4. Standardized cage test model for repellent efficacy

The study was conducted using a standardized cage test model to evaluate mosquito attraction under controlled conditions [10]. Adult female mosquitoes were reared from three distinct larval batches and starved for 12–16 hours prior to testing to enhance responsiveness. Each experimental trial involved 10 or 15 mosquitoes placed in a 30 × 30 × 30 cm cage. To minimize stress-induced effects, a rotational testing protocol was employed using three separate cages, with a minimum interval of 3–5 days between experiments for each mosquito cohort.

Six experimental conditions were tested: (1) latex alone (< 0.15 mm), (2) latex with a mini warmer (maintained at 37 °C), (3) latex with attractant solution, (4) latex with both mini warmer and attractant solution, (5) latex with mini warmer and Coca cola (6) silicone (1 mm thickness) with both mini warmer and attractant solution. The attractant solution was prepared by mixing 1 g of calcium carbonate ($CaCO_3$), 1 mL of distilled water, and 1 mL of lactic acid, producing CO_2 through a chemical reaction. The solution was applied to the latex or silicone surface, and mosquito landings were recorded over a 10-minute period, starting from the initiation of the CO_2-generating reaction.

Three independent trials were conducted for each condition, and the number of mosquitoes landing on the treated surface was recorded. The total number of mosquitoes per trial (10 or 15) was used to calculate the proportion of mosquitoes landing (landings/total). The cage test model was illustrated in Figure 1.

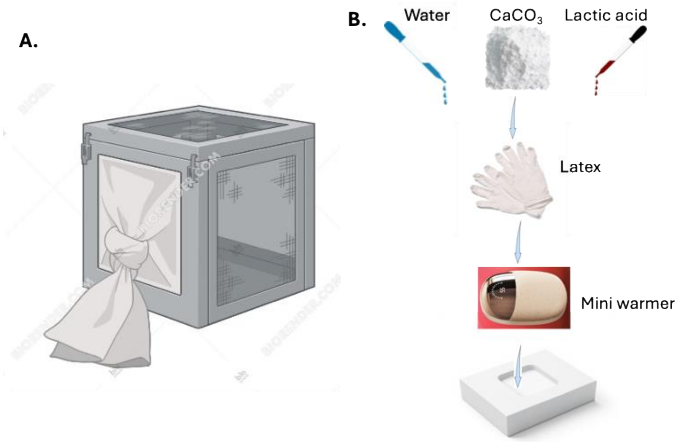

Figure 1. *Model for evaluating the odor effectiveness of essential oils* [19]

For repellent testing, the latex with a mini warmer and attractant was placed in the cage with 10-15 female starved mosquitoes, if there were mosquitoes landing on the latex within 1 minutes, the essential oil product was applied on the latex membrane. 100 μL of each formulation was applied evenly to the treated latex sheet around atractant and placed again in the cage. The time was recorded, and the new attractant was added every 30 minutes until the first landing occurred, defining the complete protection time (CPT). Tests were conducted at 27 ± 1 °C and 70 ± 5% relative humidity, with three replicates per formulation and mosquito species.

2.5. Chemical Composition Analysis

The volatile components of the formulations were analyzed using gas chromatography-mass spectrometry (GC-MS) [20] on a Thermo Trace GC Ultra system equipped with a TG-5QC column (30 m × 0.25 mm, 0.25 μm film thickness). Headspace sampling was employed by equilibrating 1 g of each formulation in a 20 mL headspace vial at 80 °C for 15 minutes. A 0.25 mL aliquot of the headspace vapor was injected in split mode (split ratio 10:1). Helium was used as the carrier gas at a flow rate of 0.74 mL/min, with a column pressure of 43.3 kPa. The column temperature was programmed from 60°C (held for 2 minutes) to 280 °C at a rate of 10 °C/min. The injector, interface, and ion source temperatures were set at 250 °C, 260°C, and 250 °C, respectively. Mass spectra were acquired in electron ionization (EI) mode with a scan range of m/z 50–500. Compounds were identified by comparing their mass spectra and retention indices with the NIST 17 library and published data. Relative abundances of compounds were calculated based on peak areas, with a threshold of > 2% for major components.

2.6. Statistical analysis

The proportion of mosquitoes landing was analyzed using a binomial generalized linear model (GLM) to assess the effects of experimental condition and trial number. The model was fitted with the glm() function, using a binomial distribution, where the response variable was the number of landings out of the total mosquitoes per trial (cbind(landings, total - landings)). The model included experimental condition and trial number as predictors, with the formula glm(cbind(landings, total - landings) ~ condition + trial, family = binomial). Pairwise comparisons between conditions were conducted using Fisher's exact test to determine significant differences in mosquito attraction, with p-values adjusted for multiple comparisons using the Bonferroni correction.

CPT data were analyzed using one-way analysis of variance (ANOVA) followed by Tukey's post-hoc test to compare the efficacy of Formulations 1 and 2 against each mosquito species. Results were expressed as mean ± standard deviation (SD). Statistical significance was set at $p < 0.05$.

All Statistical analyses were conducted in R (version 4.4.1)

3. Results and Discussion

3.1. Validation of a standardized cage test model

The cage test mitigates ethical concerns associated with direct testing on human volunteers. However, a key challenge of the cage test is replicating the attractant conditions (prior to demonstrating repellent efficacy) that mimic those of a human host. Previous studies have identified temperature and carbon dioxide (CO_2) emissions from animal skin as critical cues for female mosquitoes to locate a host [21]. In this study, we standardized a cage test model (illustrated in Figure 1) by incorporating these attractant factors. The results of the mosquito attraction efficacy of the standardized model are presented in Table 1, based on three cohorts of mosquitoes reared from distinct larval batches.

Table 1. *Validation of a standardized cage test model*

Experiments	**Number of mosquitoes landing/ total number of mosquitoes**		
	Trial 1	**Trial 2**	**Trial 3**
Latex	0	0	
Latex + mini warmer	0	0	
Latex + mosquito-attractant **	4/10	5/10	
Latex + mini warmer + mosquito-attractant ***	7–8/10	9/10	11/15
Latex + mini warmer + Coca Cola	0	0	
Silicone (1mm of thickness) membrane + mini warmer + mosquito-attractant solution***		3/10	6/15

*** $p < 0.005$, *** $p < 0.001$ vs. Latex (Fisher's exact test).*

Missing trials (e.g., Trial 3 for Latex, Latex + mini warmer, Trial 1 for Silicone + warmer + attractant) were excluded from analysis

The standardized cage test model was evaluated for its ability to attract *Aedes aegypti* mosquitoes under various conditions across three trials (Table 1). Mosquitoes began landing on the treated surface within 1 minute after the initiation of the CO_2-generating reaction, with attraction sustained for 8–9 minutes before mosquitoes dispersed (9–10 minutes post-reaction). Latex alone or with a mini warmer (37 °C) attracted no mosquitoes (0/20 across all trials). The addition of the attractant solution to latex significantly increased attraction, with 4/10 and 5/10 mosquitoes landing in Trials 1, 2, respectively (mean proportion = 0.45, range = 0.4 – 0.5). When combined with a mini warmer, the latex + attractant condition attracted 7/10, 9/10, and 11/15 mosquitoes (mean proportion = 0.777, range = 0.7 – 0.9). For silicone (1 mm) with a mini warmer and attractant solution, attraction was observed lower nubmer at 3/10, and 6/15 mosquitoes (mean proportion =

0.35, range = 0.3–0.4), probably ralated to the thickness of the silicone membrane (Table 1). Interestingly, we replaced the mosquito attractant with Coca Cola, a liquid containing CO_2 bubbles and sugar, but no mosquitoes flew to it during the 10-minute test. This may mean that the smell produced by lactic acid is also an attractive factor for mosquitoes.

A binomial generalized linear model (GLM) revealed significant effects of condition on mosquito attraction ($\chi^2 = 62.403$, df = 5, $p < 0.001$), but no significant effect of trial ($\chi^2 = 0.456$, df = 2, $p = 0.796$). Pairwise comparisons showed that latex with attractant solution attracted significantly more mosquitoes than latex alone (Fisher's exact test, $p = 0.0012$). The addition of heat to the latex + attractant condition significantly enhanced attraction compared to latex + attractant only ($p = 0.021$). Similarly, silicone with a mini warmer and attractant solution attracted significantly more mosquitoes than latex alone ($p < 0.001$), but its performance was lower comparable to the latex + warmer + attractant condition ($p = 0.00286$).

The model's ability to reliably attract mosquitoes within a 1–10-minute window validated its utility for repellent testing.

3.2. Development and evaluation of an essential oil-based mosquito repellent formulation

3.2.1. Development and evaluation of repellent efficacy

Essential oils from betel leaf (*Piper betle*), Java citronella (*Cymbopogon winterianus*), and cinnamon (*Cinnamomum verum*) were combined to formulate a mosquito repellent effective against *Aedes aegypti* and *Aedes albopictus*. Previous studies have shown that betel leaf essential oil, containing chavibetol and chavibetol acetate, repels *A. aegypti* effectively [13], but is less potent against *A. albopictus*. Java citronella essential oil exhibits stronger efficacy against *A. albopictus*, while cinnamon essential oil was included for its warming properties and ability to soothe mosquito bite irritation. To reduce the volatility of essential oils, poly(ethylene glycol) (PEG)-600 was incorporated at 0.8% (w/w) in Formulation 1, with Formulation 2 (lacking PEG-600) serving as a control.

Repellent efficacy was assessed using a standardized cage test model previously validated for mosquito attraction (Figure 1, Table 1). In this model, an artificial skin treated with an attractant solution was exposed in a mosquito rearing chamber, and complete protection time (CPT) was defined as the duration during which no mosquitoes landed on the surface after repellent application. The attraction model established baseline landing rates (e.g., 77.7% mean landing proportion for latex + warmer + attractant; Table 1), providing a robust framework for evaluating repellent performance.

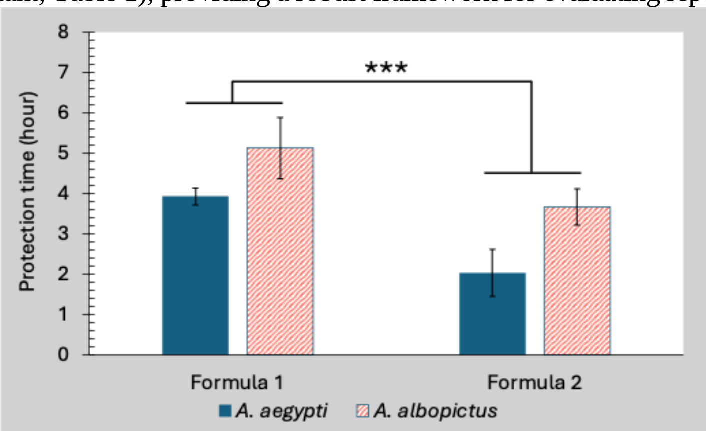

Figure 2. *Complete protection time (CPT) of repellent formulations against Aedes aegypti and Aedes albopictus. Bars represent mean ± SD. ANOVA test indicated significant main effects of formulation (*** p < 0.001)*

A two-way analysis of variance (ANOVA) was conducted to assess the effects of formulation (Formulation 1 vs. Formulation 2) and mosquito species (*A. aegypti* vs. *A. albopictus*) on CPT.

Assumptions of normality (Shapiro-Wilk test, $p > 0.05$) and homogeneity of variances (Levene's test, $p > 0.05$) were satisfied. The ANOVA revealed significant main effects of formulation (F = 29.2, df = 1, $p < 0.001$) and species (F = 20.7, df = 1, $p = 0.0018$), with no significant interaction (F = 0.484, df = 1, $p > 0.05$). Formulation 1 yielded mean CPTs of 3.93 ± 0.21 hours against *A. aegypti* and 5.10 ± 0.78 hours against *A. albopictus*, whereas Formulation 2 provided CPTs of less than 1.5 hours for both species (Figure 2). Additionally, CPTs for *A. albopictus* were 1.3 to 1.8 times longer than those for *A. aegypti* across both formulations (Figure 2).

These findings demonstrate that PEG-600 significantly enhances the duration of repellent efficacy and highlight species-specific responses to the essential oil blend. The standardized cage test model effectively validated the formulations, supporting their potential for mosquito control applications.

3.2.2. Chemical composition of the repellent product

The mosquito repellent product, formulated from a blend of betel leaf (*Piper betle*), Java citronella (*Cymbopogon winterianus*), and cinnamon (*Cinnamomum verum*) essential oils, exhibited a clear, pale yellow appearance with a characteristic aroma dominated by betel leaf, moderated by the milder notes of Java citronella and cinnamon. The chemical composition of the product was analyzed using gas chromatography-mass spectrometry (GC-MS), with results presented in Figure 3 and Table 2.

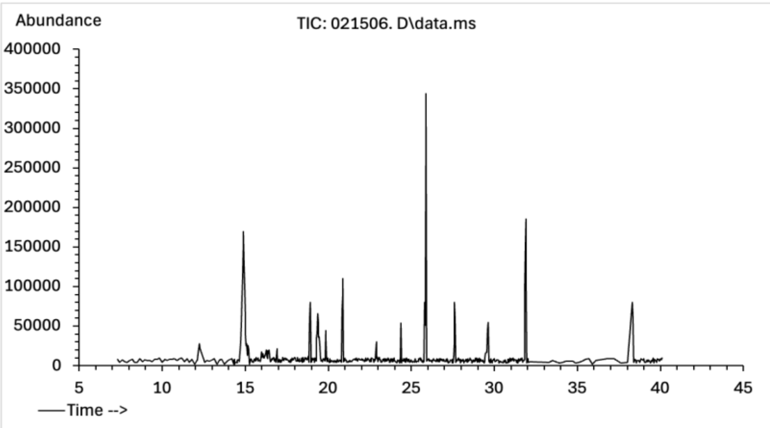

Figure 3. *Gas chromatography-mass spectrometry (GC-MS) chromatogram of the mosquito repellent product, showing 15 major volatile compounds (relative abundance >2%)*

Table 2. *Major chemical compounds in the mosquito repellent product (GC-MS Analysis)*

Peak No.	Retention Time (min)	Compound Name	Relavtive Abundance (%)
1	12.23	Linalool	2.00
2	14.97	Citronellal	13.80
3	18.89	Citronellol	5.97
4	19.37	Geranial	5.31
5	19.84	Neral	3.20
6	20.93	Geraniol	8.97
7	22.96	Chavicol	2.12
8	24.37	Safrole	4.22
9	25.86	Cinnamaldehyde	27.43
10	27.60	Chavibetol	6.90
11	29.61	Chaviryl acetate	4.63
12	31.84	Eugenol	15.88
13	38.26	Lauric acid	6.61
14	40.86	Myristic acid	6.94
15	42.85	Palmitic acid	5.71

GC-MS analysis identified 15 major volatile compounds (relative abundance > 2%), including three fatty acids derived from coconut oil used as a carrier. The number of detected compounds was substantially lower than that reported for individual essential oils in previous studies (references cited). This reduction can be attributed to the diluted concentrations of essential oils in the formulation—10% betel leaf, 10% Java citronella, and 5% cinnamon—compared to the concentrated single-essential-oil compositions analyzed in prior research. Nevertheless, the product retained the characteristic chemical profiles of the constituent essential oils, confirming the presence of their key bioactive components. Key compounds included chavibetol (from betel leaf), and citronellal (from Java citronella) consistent with their reported repellent properties.

3.3. Discussion

The present study focused on developing and evaluating a mosquito repellent formulation that combines essential oils from betel leaf (*Piper betel*), Java citronella (*Cymbopogon winterianus*), and cinnamon (*Cinnamomum burmannii*), using PEG-600 as a fixative. The goal was to achieve broad-spectrum efficacy against *Aedes aegypti* and *Aedes albopictus*. The formulation demonstrated significant effectiveness, with Formulation 1 (containing 0.8% PEG-600) providing complete protection times (CPTs) of 3.93 ± 0.21 hours against *A. aegypti* and 5.10 ± 0.78 hours against *A. albopictus*. In contrast, Formulation 2, which did not include PEG-600, offered less than 1.5 hours of protection. These results are consistent with previous research showing that PEG-based fixatives can extend the duration of essential oil repellents by reducing their volatility [7], [8]. For example, Navayan *et al.* [7] reported a 50% increase in protection time with the incorporation of PEG, which aligns with our findings of a 1.4- to 1.93-fold improvement in CPT for Formulation 1 compared to the control. This enhancement is likely due to PEG-600's ability to create a matrix that slows the evaporation of volatile compounds such as chavibetol, citronellal, and cinnamaldehyde. These components were confirmed as key ingredients in our formulation through GC-MS analysis.

The differences in complete protection time (CPT) between species indicate that *Aedes albopictus* provides 1.3 to 1.8 times longer protection compared to *Aedes aegypti*, supporting previous studies on the effectiveness of essential oils. *A. albopictus* is generally more sensitive to plant-based repellents, which may be attributed to variations in olfactory receptor sensitivity between the two species [22], [23]. Our gas chromatography-mass spectrometry (GC-MS) analysis identified 15 major volatile compounds, each with greater than 2% abundance, including chavibetol, citronellal, and cinnamaldehyde, which are known to affect mosquito olfactory pathways [4], [6]. However, PEG-600 was not detected in the headspace GC-MS analysis, consistent with its low volatility and limited partitioning into the vapor phase at the equilibration temperature of 80°C. This finding aligns with previous studies on the analytical behavior of PEG in GC-MS, where its high molecular weight and polarity restrict detection under standard conditions [24]. Although PEG-600 did not contribute to the volatile profile, its role as a fixative was evident in the extended CPT, suggesting that its primary function is to stabilize the release of active compounds rather than to act as a repellent itself.

A key contribution of this study is the development and standardization of a volunteer-free cage test model that uses an artificial skin attractant to evaluate the efficacy of repellents. The model was validated by achieving a 77.7% mean landing proportion for the combination of latex, a mini warmer, and the attractant, demonstrating its reliability in simulating mosquito attraction without the involvement of human participants. This approach addresses ethical concerns related to human-based testing, such as arm-in-cage tests, which raise issues of informed consent and the risk of disease transmission [10], [11]. By eliminating the need for volunteers, our method complies with medical ethics regulations and provides a controlled, reproducible framework for repellent testing. Additionally, the use of artificial skin helps to reduce environmental variability present in field tests, ensuring that our results reflect the intrinsic efficacy of the formulation.

4. Conclusion

This study successfully developed a mosquito repellent formulation using betel leaf, Java citronella, and cinnamon essential oils, enhanced by PEG-600. The formulation achieved complete protection times (CPTs) of 3.93 ± 0.21 hours against *Aedes aegypti* and 5.10 ± 0.78 hours against *Aedes albopictus*, significantly outperforming the control formulation, which had a CPT of less than 1.5 hours. The standardized volunteer-free cage test model, validated with a 77.7% landing proportion, provided an ethical and reliable method for evaluating repellent efficacy, addressing the ethical challenges associated with human-based testing. Gas chromatography-mass spectrometry (GC-MS) analysis confirmed the presence of key repellent compounds. These findings underscore the potential of natural repellents, especially those that incorporate fixatives like PEG-600, to provide sustainable and effective mosquito control. Future research should focus on field validation, the temporal analysis of volatile release, and testing against a broader range of mosquito species to further optimize the formulation for practical applications.

Acknowledgement

This work was supported by The University of Danang – University of Science and Technology, code number of Project: T2023-02-08MSF.

REFERENCES

[1] World Health Organization, "Vector-borne diseases fact sheet," 2024. [Online]. Available: https://www.who.int/news-room/fact-sheets. [Accessed April 20, 2025].

[2] D. R. Swale *et al.*, "Neurotoxicity and Mode of Action of N, N-Diethyl-Meta-Toluamide (DEET)," *PLOS ONE*, vol. 9, no. 8, Aug. 2014, Art. no. e103713.

[3] K. Jetter and T. D. Paine, "Consumer preferences and willingness to pay for biological control in the urban landscape," *J. Biol. Con.*, vol. 30, no. 2, pp. 312-322, June 2004.

[4] T. M. X. Nguyen *et al.*, "Herbal essential oils as the alternative repellent against mosquitoes," *Univ. Danang - J. Sci. Technol.*, vol. 21, no. 8.2, pp. 24–30, Aug. 2023.

[5] H. A. Luker *et al.*, "Repellent efficacy of 20 essential oils on *Aedes aegypti* mosquitoes and *Ixodes scapularis* ticks in contact-repellency assays," *Sci. Rep.*, vol. 13, no. 1, Jan. 2023, Art. no. 1705.

[6] Y. Trongtokit *et al.*, "Comparative repellency of 38 essential oils against mosquito bites," *Phytother Res.*, vol. 19, no. 3, pp. 303–309, Apr. 2005.

[7] A. Navayan *et al.*, "Evaluation of the Mosquito Repellent Activity of Nano-sized Microemulsion of *Eucalyptus globulus* Essential Oil Against *Culicinae*," *Jundishapur J. Nat. Pharm. Prod.*, vol. 12, no. 4, Nov. 2017, Art. no. e55626.

[8] S. Songkro *et al.* "Effects of Glucam P-20, Vanillin, and Fixolide on Mosquito Repellency of Citronella Oil Lotion," *J. Med. Entomol.*, vol. 49, no. 3, pp. 672-677, May 2012.

[9] H. A. Luker, "A critical review of current laboratory methods used to evaluate mosquito repellents," *Front. Insect Sci.*, vol. 4, Jan. 2024, Art. no. 1320138.

[10] M. Moreno-Gómez *et al.*, "Two New Alternatives to the Conventional Arm-in-Cage Test for Assessing Topical Repellents," *J. Med. Entomol.*, vol. 20, no. 10, pp. 1-13, Jan. 2021.

[11] N. L. Achee, "Considerations for the Use of Human Participants in Vector Biology Research: A Tool for Investigators and Regulators," *Vector Borne Zoonotic Dis.*, vol. 15, no. 2, pp. 89-102, Feb. 2015.

[12] Y. Morimoto *et al.*, "New mosquito repellency bioassay for evaluation of repellents and pyrethroids using an attractive blood-feeding device," *Parasites & Vectors*, vol. 14, March 2021, Art. no. 151.

[13] Hoa Thom Co La, "Certificate of Analysis - *Piper Betle essential oil – Physical and Chemical properties*," 2020. [Online]. Available: https://hoathomcola.vn [Accessed May 19, 2025].

[14] Hoa Thom Co La, "Certificate of Analysis - *Citronella essential oil – Physical and Chemical properties*," 2019. [Online]. Available: https://hoathomcola.vn [Accessed May 19, 2025].

[15] Hoa Thom Co La, "Certificate of Analysis - *Cinnamon essential oil – Physical and Chemical properties*," 2019. [Online]. Available: https://hoathomcola.vn [Accessed May 19, 2025].

[16] T. M. N Nguyen and T. M. X. Nguyen, "Prediction of chemical components that affect the mosquito-repellent ability of essential oils using multiple linear regression techniques," *TNU J. Sci. Tech.*, vol. 229, no. 14, pp. 271-279, Nov. 2024.

[17] F. Bencsits, "Composition containing citronella java oil and use thereof for repelling insects," U. S. Patent 7144591B2 , 05 Dec. 2006.
[18] O. Aschenbrennera and P. Styring, "Comparative study of solvent properties for carbon dioxide absorption," *Energy Environ. Sci.*, vol. 3, pp. 1106-1113, Jun. 2010.
[19] Biorender, "Insect rearing cage," 2025. [Online]. Available: https://www.biorender.com/ [Accessed May 26, 2025].
[20] R. P. Adams, "Identification of Essential Oil Components by Gas Chromatography/Mass Spectrometry," *J. Am. Soc. for Mass Spectrom.*, vol. 18, no. 5, pp. 803–806, May 2007.
[21] C. J. McMeniman *et al*., "Multimodal integration of carbon dioxide and other sensory cues drives mosquito attraction to humans," *Cell*, vol. 156, no. 5, pp. 1060-1071, Feb. 2014.
[22] A. A. Costa *et al.*, "Repellent and Larvicidal Activity of the Essential Oil From *Eucalyptus nitens* Against *Aedes aegypti* and *Aedes albopictus* (Diptera: Culicidae)," *J. Med. Entomol.*, vol. 54, no. 3, pp. 670-676, May 2017.
[23] M. Wheelwright, C. R. Whittle, and O. Riabinina., "Olfactory systems across mosquito species," *Cell Tissue Res.*, vol. 383, no. 1, pp. 75-90, Jan. 2021.
[24] Z. Lin *et al.*, "Comparative studies on low-temperature pyrolysis products of pure PEG and PEG/nano-Co_3O_4 by Py-GC/MS," *J. Anal. Appl. Pyrolysis*, vol. 81, no. 1, pp. 121–126, Jan. 2008.