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Original Article

Formulation and Compound Identification of *Citrus hystrix* DC Essential Oil by GC-MS for Mosquito Repellent Activity Against *Aedes aegypti*

Formulasi dan Identifikasi Senyawa Minyak Atsiri Citrus hystrix DC. dengan GC-MS untuk Aktivitas Repelan terhadap Nyamuk Aedes aegypti

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Abstract

Plant-based mosquito control offers a promising alternative to reduce the health and environmental risks associated with chemical insecticides. *Citrus hystrix* DC. (kaffir lime), a member of the Citrus genus, produces essential oils rich in bioactive compounds such as limonene, citronellal, and terpinolene, which are known for antimicrobial and insecticidal properties. This study focused on extracting essential oil from kaffir lime fruit, identifying its chemical constituents using gas chromatography–mass spectrometry (GC-MS), and evaluating the repellent efficacy of its spray formulation against *Aedes aegypti*. Essential oil was obtained through steam distillation and analyzed by GC-MS to determine its chemical profile. Repellent activity was assessed experimentally using 100 adult mosquitoes divided into five treatment groups, with spray formulations prepared at concentrations of 5%, 10%, and 15%. Data was analyzed using one-way ANOVA followed by post-hoc tests. GC-MS analysis revealed 90 compounds, with five major constituents: D-limonene (14.57%), limonene (13.92%), citronellal (6.66%), terpinolene (6.47%), and α-terpineol (5.74%). Repellent testing demonstrated that the 15% concentration provided the highest protection against mosquito landings. These findings confirm the potential of kaffir lime essential oil as an effective natural mosquito repellent and provide a scientific basis for developing eco-friendly, plant-derived vector control products. This research contributes to reducing dependence on synthetic insecticides and supports sustainable strategies for vector management that prioritize human health and environmental safety.

Abstrak

Pengendalian nyamuk berbasis tanaman merupakan alternatif yang menjanjikan untuk mengurangi risiko toksisitas insektisida kimia. *Citrus hystrix* DC. (jeruk purut) adalah anggota genus Citrus yang menghasilkan minyak atsiri dengan potensi bioaktivitas, termasuk limonena, sitronelal, dan terpinolena yang diketahui memiliki sifat antimikroba dan insektisida. Penelitian ini bertujuan untuk mengekstraksi minyak atsiri dari buah jeruk purut, mengidentifikasi komponen kimia menggunakan gas chromatography–mass spectrometry (GC-MS), serta mengevaluasi efektivitas sediaan *spray* minyak atsiri sebagai repelan terhadap *Aedes aegypti*. Minyak atsiri diekstraksi melalui metode destilasi uap-air, kemudian dianalisis dengan GC-MS untuk menentukan profil senyawa. Uji repelan dilakukan secara eksperimental menggunakan 100 ekor nyamuk *Aedes aegypti* yang dibagi ke dalam lima kelompok perlakuan, dengan tiga variasi konsentrasi minyak atsiri (5%, 10%, dan 15%). Analisis data dilakukan menggunakan uji ANOVA satu arah dan uji lanjut post-hoc. Hasil GC-MS menunjukkan 90 senyawa teridentifikasi, dengan lima komponen dominan yaitu D-limonene (14,57%), limonene (13,92%), sitronelal (6,66%), terpinolena (6,47%), dan α-terpineol (5,74%). Uji efektivitas menunjukkan bahwa konsentrasi 15% memberikan daya proteksi tertinggi terhadap pendaratan nyamuk. Temuan ini menegaskan potensi minyak atsiri jeruk purut sebagai repelan alami yang efektif dan memberikan dasar ilmiah untuk pengembangan produk pengendalian nyamuk yang ramah lingkungan dan berkelanjutan. Hasil penelitian ini berkontribusi pada upaya pengurangan ketergantungan terhadap insektisida sintetis dan mendukung strategi pengendalian vektor yang lebih aman bagi kesehatan dan lingkungan.

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INTRODUCTION

Aedes aegypti is the primary vector of dengue virus, which causes Dengue Hemorrhagic Fever (DHF), a major public health concern in tropical countries including Indonesia.[1,2] Indonesia's tropical climate supports high mosquito density, contributing to recurrent dengue epidemics with significant morbidity and mortality.[3,4] Over the past five decades, the incidence of dengue hemorrhagic fever (DHF) in Indonesia surged from 0.05 cases per 100,000 person-years in 1968 to 77.96 cases in 2016, following a cyclical pattern with peaks every 6–8 years. Recently, Bali recorded the highest incidence with a case fatality rate below 1%, whereas non-endemic regions such as Papua experienced outbreaks marked by elevated fatality rates.[5] Mosquito control is considered the most reliable strategy to reduce human exposure to vectors.[6] Chemical repellents containing N,N-diethyl-meta-toluamide (DEET) are widely used but associated with skin irritation, hypersensitivity, and environmental concerns, while prolonged insecticide use can lead to resistance and ecological impact.[7,8] These limitations underscore the need for plant-based repellents that are safer and biodegradable.[9,10]

Indonesia is a major producer of essential oils, which are volatile compounds widely applied in perfumery, cosmetics, food, and pharmaceuticals.[11] Essential oils, also known as ethereal or volatile oils, are characterized by their distinctive aroma, volatility at room temperature, and solubility in organic solvents.[12] Among essential oil sources, *Citrus hystrix* DC. (kaffir lime) is notable for its high oil content and traditional culinary and medicinal uses. Its essential oil contains monoterpenes and sesquiterpenes such as limonene, citronellal, linalool, α-pinene, and terpinolene, which exhibit antimicrobial, antioxidant, and insecticidal properties.[13,14] Previous studies have identified limonene and citronellal as major constituents, but composition varies with plant part and extraction method.[15,16]

Steam distillation is a conventional and efficient technique for extracting essential oils from citrus matrices.[17,18] For chemical profiling, gas chromatography-mass spectrometry (GC-MS) is considered the gold standard, enabling separation and identification of complex mixtures with high accuracy, including minor components that influence aroma and bioactivity.[19,20] Standardized chemical characterization is critical to link composition with biological efficacy and ensure reproducible formulations.

Spray formulations offer practical advantages for repellent delivery, including ease of application, reduced microbial contamination, and prolonged contact time compared with other dosage forms.[21,22] Although kaffir lime oil has been explored for insecticidal and aromatherapy applications,[23,24] data on its effectiveness as a standardized spray repellent against *A. aegypti* remain limited. Furthermore, few studies integrate GC-MS-based profiling with repellent efficacy testing across multiple concentrations.

This study addresses these gaps by extracting essential oil from *C. hystrix* fruit via steam distillation, characterizing its chemical constituents using GC-MS, and evaluating the repellent activity of spray formulations at 5%, 10%, and 15% concentrations against *A. aegypti* under controlled laboratory conditions. The novelty lies in combining comprehensive chemical profiling with formulation and efficacy assessment, providing a scientific basis for developing eco-friendly, plant-derived mosquito repellents.

RESEARCH METHOD

Tools and Materials.

The equipment used included a steam distillation apparatus, analytical balance (Ohaus Pa 214), digital scale (ACIS BC-500), homogenizer (IKA T25 Digital Ultra Turrax®, Germany), Ostwald viscometer (Silberbrand, Germany), and universal pH indicator (Macherey-Nagel, Germany). Fresh *Citrus hystrix* (kaffir lime) fruits were sourced from Battal, Panji District, Situbondo, East Java (Identification: No. ID ELSA 60450). Additional materials included propylene glycol (PT. Karunia Sejahtera Abadi SABA KIMIA, Indonesia), 96% ethanol (PT. Brataco, Indonesia), and spray containers, obtained from the Faculty of Pharmacy, Universitas Mahasaraswati Denpasar.

Study Design

This study employed a mixed-method approach combining descriptive and experimental designs. GC-MS analysis was conducted to characterize the chemical composition of kaffir lime essential oil.[25,26] Subsequently, an experimental evaluation assessed the physical properties and repellent efficacy of spray formulations against *Aedes aegypti* mosquitoes.[22,27]

Plant Determination

Botanical identification of kaffir lime (*Citrus hystrix* DC.) was performed at the Characterization Laboratory of the Eka Karya Bali Botanical Garden (BRIN). Morphological characteristics were compared with descriptions in standard botanical references to confirm species identity.

Distillation of Kaffir Lime Essential Oil

Kaffir lime (*Citrus hystrix* DC.) essential oil was obtained using the steam–water distillation method, a commonly applied and efficient technique for isolating volatile compounds from plant materials.[28] A total of 24 kg of fresh kaffir limes were sorted and washed to remove impurities, then cut into small pieces and reweighed. The prepared plant material was placed in the distillation chamber, while the lower compartment of the apparatus was filled with water and boiling chips prior to heating. Steam–water distillation was carried out for approximately 6–7 hours, and the extraction was terminated once no additional oil droplets were observed. The collected distillate was treated with 20 g of anhydrous sodium sulfate (Na_2SO_4) to remove residual moisture, and the resulting essential oil was stored in airtight, light-protected containers to prevent oxidative and photolytic degradation.[12,29] The chemical composition of the essential oil was subsequently analyzed using GC–MS to obtain an accurate profile of its volatile constituents.[30]

GC–MS Analysis of Essential Oil

Chemical profiling of the essential oil was performed using Gas Chromatography–Mass Spectrometry (GC–MS) in accordance with established analytical procedures for Citrus essential oils. The analysis was conducted with helium as the carrier gas at a flow rate of 1 mL/min and an injection temperature of 250 °C. The oven temperature program was set as follows: an initial temperature of 50 °C with a 2-minute hold, followed by a ramp of 2 °C/min to 80 °C, 5 °C/min to 150 °C, 10 °C/min to 200 °C, and 20 °C/min to 300 °C, with a final hold of 5 minutes. The MS interface was operated at a source temperature of 230 °C and a quadrupole temperature of 150 °C. For sample preparation, 25 µL of the essential oil was diluted in 1 mL of n-hexane, filtered through a 0.22 µm syringe filter, and transferred into GC vials, from which a 1 µL aliquot was injected for analysis. Compound identification was achieved by comparing retention times and mass spectra with reference spectra from the instrument's library database, using similarity indices, molecular weights, and peak intensities as confirmation criteria.[16,31] GC–MS is widely recognized as a robust and validated technique for the characterization and chemometric evaluation of Citrus essential oils.

Spray Formulation and Preparation

Four formulations with varying essential oil concentrations (including 0%, 5%, 10%, and 15%) were prepared (**Table 1**). Ethanol (96%) was used as a volatile solvent and vehicle in the spray formulation to facilitate solubilization of essential oil components and rapid evaporation upon skin application. Ethanol concentrations of 60–80% v/v are commonly employed in topical antiseptic and repellent sprays and are considered safe for use on intact skin. Previous studies have shown that ethanol at these concentrations is well tolerated, causing at most mild and reversible dryness without significant disruption of the skin barrier.[32–34] The inclusion of a humectant (propylene glycol) in the formulation helps maintain skin hydration and enhances user comfort.[35]

Table 1 Spray repellent Formula

Material	Quantity (ml)				Function
	F1	F2	F3	F4	
Kaffir lime fruit essential oil	-	1	2	3	Active Ingredients
Propylene Glycol	4	4	4	4	Co-solvent/ humectant
Ethanol 96%	ad 20	ad 20	ad 20	ad 20	Solvent

Remarks: Formulation codes represent increasing concentrations of kaffir lime fruit essential oil: F1 (0%), F2 (5%), F3 (10%), and F4 (15%).

The essential oil was first transferred into a beaker, followed by the addition of propylene glycol as a solvent. The mixture was homogenized until a uniform solution was obtained. Ethanol was then added gradually while maintaining mixing with the homogenizer. The resulting spray solution was transferred into a calibrated container and adjusted to a final volume of 20 mL using 70% ethanol.[22,36]

Physical Evaluation of Spray

Organoleptic evaluation was performed by observing the color, odor, and texture of the preparation. Homogeneity was assessed using glass slides to ensure uniform distribution of the active ingredients and solvents; a homogeneous preparation is indicated by the absence of precipitation and visible contamination.[37] The pH of the formulation was measured using universal pH paper to ensure its suitability for topical application, as the ideal skin pH ranges from 4.5 to 6.[38] Viscosity was determined using an Ostwald viscometer to characterize the flow properties of the preparation, as viscosity is closely related to its resistance to deformation and flow.[39]

Anti-Mosquito Repellency Assay

The anti-mosquito spray assay was conducted to evaluate the repellent activity of Citrus hystrix DC. essential-oil spray against Aedes aegypti. All procedures involving animals were carried out in accordance with the guidelines of the Universitas Udayana Animal Ethics Committee and were approved under ethical clearance No: B/280/UN14.2.9/PT.01.04/2022.

Repellency testing was performed in a 30 × 30 cm laboratory mosquito cage following standard entomological bioassay protocols.[40,41] Five rabbits (2.5–3.0 kg; 4–5 months old) obtained from Dusun Dukuh Pulu Tengah, Mambang Village, East Salamadeg, were used due to their sufficiently large dorsal surface area, which enables consistent and standardized exposure during repellency evaluation. All animals underwent a one-week acclimatization period under controlled conditions with ad libitum access to food and water.

On the day of testing, the dorsal hair of each rabbit was shaved evenly to ensure a uniform exposure area. The shaved skin was then cleansed using sterile gauze moistened with physiological saline. The mosquito spray formulation was applied evenly to the exposed dorsal area, after which each rabbit was placed into an individual mosquito cage containing adult Aedes aegypti. A control group received no treatment or a formulation containing 0% essential oil.

Observations were performed hourly from 0 to 6 hours following formulation application. During each interval, mosquito landing attempts, biting behavior, and avoidance responses were recorded. These parameters were used to quantify the repellency rate and overall protection efficacy of each test formulation. The study included five experimental groups: Group I served as the negative control (untreated animals), while Groups II, III, IV, and V received spray formulations containing 0%, 5%, 10%, and 15% essential oil, respectively. Repellent efficacy was assessed by comparing the proportion of mosquitoes repelled from animals treated with the test formulations to that of the control groups, which either received no treatment or a formulation lacking the active essential oil.[42,43] Mosquito repellency was calculated using the following formula:[44]

$$\%\text{Protection} = \frac{(C-T)}{C} X\ 100 \quad (1)$$

where C represents the number of mosquito landings or bites on the control area and T denotes the number of landings or bites on the treated skin area.

Statistical Analysis

Data was analyzed using SPSS version 21. Normality was assessed using the Shapiro–Wilk test, with $p > 0.05$ indicating a normal distribution. Differences among groups were evaluated using one-way ANOVA followed by appropriate post-hoc tests.[45] GC–MS results were presented descriptively based on chromatogram peak areas.

RESULTS AND DISCUSSION

This study aimed to characterize the chemical composition of kaffir lime (*Citrus hystrix* DC) essential oil using gas chromatography-mass spectrometry (GC-MS). Fruits were collected from Situbondo, East Java, and identified at the Characterization Laboratory of the Eka Karya Bali Botanical Garden (BRIN). Essential oil was extracted by steam distillation and subsequently analyzed using GC–MS under predetermined operational parameters. The steam-distilled essential oil yield obtained in this study was 0.363%. This value is lower than many published yields for Citrus hystrix reported in the literature, where yields typically range from approximately 0.9% to 1.8% depending on plant part and extraction conditions. For example, hydrodistillation of kaffir lime leaves has been reported to produce yields of ~0.98–1.38% (fresh vs. dried material), while steam-distillation of fruit peel/leaf samples in other studies reached yields up to ~1.8%.[15,29] Several factors likely account for this discrepancy, including the plant part used (fruit flesh vs. peel or leaves), pre-treatment (fresh vs. dried), harvest location and plant maturity, steam-distillation parameters (sample mass, distillation time, condenser efficiency), and possible post-harvest losses during storage. Meta-analyses and comparative studies highlight that peel and leaf material generally yield higher essential-oil percentages than whole fruit pulp, and optimized pre-treatments (e.g., drying, fermentation, steam-explosion) can substantially increase recoverable oil. Thus, the lower yield observed here does not negate the sample's chemical relevance but indicates differences in raw material and/or processing that should be noted when comparing across studies.[24,46]

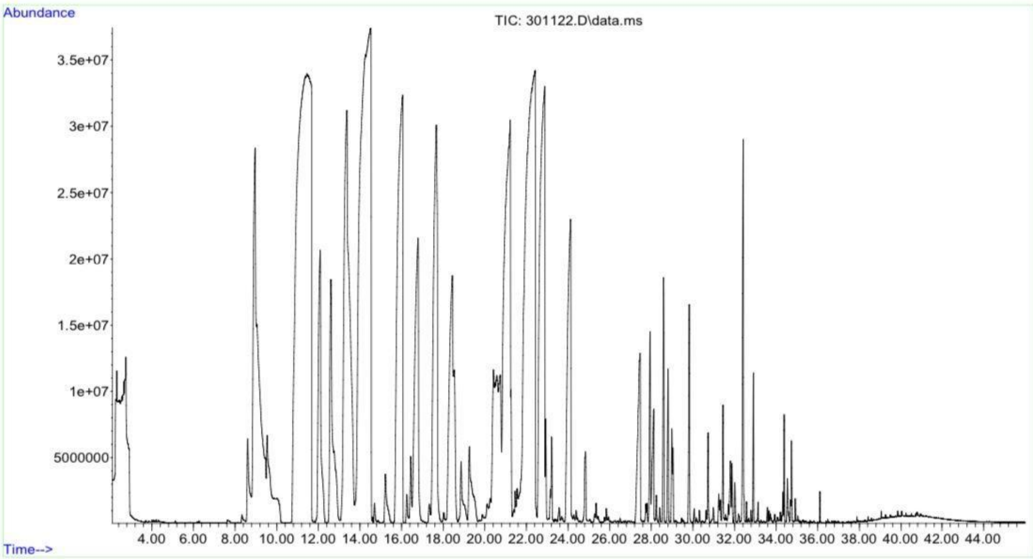

Figure 1. GC-MS chromatogram of kaffir lime (*Citrus hystrix* DC) essential oil showing the distribution of identified volatile constituents.

The resulting chromatogram (**Figure 1**) revealed 90 distinct peaks corresponding to 90 identified compounds, all exhibiting quality values above 80.[47,48] A summary of the 20 most abundant compounds among the 90 identified constituents of kaffir lime essential oil is presented in **Table 2**. Relative abundances were determined based on peak area percentages. The five most abundant constituents were D-limonene (14.57%, retention time 14.268 min), limonene (13.92%, retention time 11.376 min), citronellal (6.66%, retention time 21.222 min), terpinolene (6.47%, retention time 13.372 min), and α-terpineol (5.74%, retention time 22.876 min). Compared with previous studies analyzing peel or twig-derived oils, this investigation identified a greater number of compounds, while key constituents such as limonene, sabinene, and citronellal remained consistent with earlier findings. Notably, the essential oil also contained linalool (46%), a compound recognized for its mosquito-repellent properties and its role as a contact toxin in insects. Overall, the GC–MS chromatogram (**Figure 1**) provides a comprehensive chemical profile of kaffir lime fruit essential oil, underscoring its potential as a natural and effective mosquito repellent.

The compound profiling results obtained in this study show notable differences from previous reports analyzing kaffir lime peel essential oil. The number of detected compounds was substantially higher than that reported by Iryani and Deka,[49] who identified only 27 constituents, although several major compounds—such as limonene, sabinene, and citronellal—were consistently observed in both studies. Simanjuntak et al.,[50] who examined essential oil derived from kaffir lime twigs, reported 12 compounds with citronellal as the major component. It is well known that essential-oil composition may vary according to plant part, cultivation location, and distillation conditions.[51,52] In this study, the kaffir lime fruits were sourced from Situbondo, a different geographical region from those investigated in previous studies, which may account for the identification of 90 distinct compounds. Furthermore, temperature control during the steam distillation process can influence the characteristics of the essential oil. Excessive temperature may cause oil droplets from the fruit to rise, resulting in a slightly yellowish coloration. This represents one of the limitations of the present study. Nevertheless, the number of identified secondary metabolites remained greater than that reported in several earlier investigations.

Table 2. Top 20 Chemical Constituents Identified by GC–MS from a Total of 90 Compounds in Kaffir Lime (*Citrus hystrix* DC) Fruit Essential Oil.

No	Compound name	Qual	%Area	Total
1	*D-limonene*	96	8.29%	14.57%
	D-limonene	96	6.28%	
2	*Limonene*	87	11.21%	
	Limonene	87	1.90%	13.92%
	Limonene	87	0.81%	
3	*Citronellal*	93	6.66%	6.66%
4	*Terpinolene*	98	6.47	6.47%
5	*Gamma terpinene*	97	6.16%	6.16%
6	*Alpha terpineol*	91	5.74%	
7	*Alpha pinene*	94	2.61%	5.16%
	Alpha pinene	96	2.55%	
8	*Isoterpinolene*	98	4.14%	4.14%
9	*Linalool*	96	2.51%	3.22%
	Linalool	97	0.69%	
10	*Ethyl propan carbonate*	94	2.85%	2.85%
11	*Citronellol*	97	2.66%	2.66%
12	*Alpha phallandrene*	90	1.55%	2.11%
	Alpha phallandrene	93	0.55%	
13	*Beta myrcene*	94	2.06%	2.06%
14	*Cyclofenchene*	87	2.00%	2.00%
15	*3-carane*	91	1.79%	1.79%
16	*Menthoglycol*	91	1.20%	1.20%
17	*Camphene*	97	0.87%	1.09%
	Camphene	97	0.87%	
18	*Neo-isopulegol*	99	1.01%	1.01%
19	*isoregol*	98	0.88%	0.88%
20	*Copaene*	99	0.62%	0.62%

Prior to assessing mosquito-repellent efficacy, the spray formulations underwent a four-week physical stability screening, conducted as a short-term preliminary evaluation to examine the integrity of the formulations during storage. The physical quality assessments included organoleptic evaluation, homogeneity testing, pH measurement, and viscosity determination. The organoleptic assessment aimed to characterize the formulations in terms of physical appearance, color, and odor, as well as to evaluate their sensory acceptability for topical use.[53] All formulations appeared as clear liquids with the characteristic aroma of kaffir lime, and no changes in appearance, color, or odor were observed throughout the four-week storage period. These findings indicate organoleptic stability under the short-term screening conditions.

The pH measurement was included because pH is a critical chemical parameter for topical preparations that affects both formulation stability and skin tolerability. Maintaining an appropriate pH is crucial to prevent skin irritation, with the recommended range for topical products being 4.5–6.0.[38] The measured pH of the mosquito-repellent spray was 6.0, which falls within the acceptable range and suggests that the manufacturing and storage conditions maintained formulation integrity at room temperature. Homogeneity testing assessed the presence of undispersed particles or agglomerates; a formulation is considered homogeneous if no solid particulates or clumps are detectable.[53] No particulate matter or agglomeration was observed in the spray formulations, indicating satisfactory homogeneity and uniform dispersion of ingredients. Viscosity testing was carried out to determine the formulation's rheological properties, which influence sprayability and spreadability. All formulations exhibited viscosities below 150 cP (**Table 3**), a level considered suitable for easy dispensing via pump or trigger spray bottles and for achieving good spray distribution.[54] Taken together, the physical quality evaluations demonstrate that the spray formulations exhibited satisfactory short-term physical stability and retained appropriate characteristics for topical administration during the four-week storage period.

Table 3. Viscosity Measurements of Kaffir Lime Essential Oil Spray Formulations

Formula	Replication	Viscosity (cPs) on Week-			
		1	2	3	4
F1	1	1.849	1.444	1.775	1.459
	2	1.565	1.595	1.793	1.644
	3	1.793	1.778	1.729	1.694
	mean ± SD	1.736±0.150	1.606±0.167	1.766±0.033	1.599±0.124
F2	1	1.698	1.581	1.606	1.548
	2	1.596	1.669	1.718	1.557
	3	1.609	1.487	1.686	1.672
	mean ± SD	1.634±0.056	1.579±0.091	1.670±0.058	1.592±0.069
F3	1	1.683	1.873	1.805	1.668
	2	1.795	1.669	1.610	1.664
	3	1.787	1.646	1.825	1.714
	mean ± SD	1.755±0.062	1.729±0.125	1.747±0.119	1.682±0.028
F4	1	1.548	1.624	1.629	1.6293
	2	1.737	1.649	1.718	1.6300
	3	1.693	1.625	1.693	1.6884
	mean ± SD	1.659±0.099	1.633±0.014	1.680±0.046	1.649±0.034

Remarks: Formulation codes represent increasing concentrations of kaffir lime fruit essential oil: F1 (0%), F2 (5%), F3 (10%), and F4 (15%).

The mosquito-repellent activity of kaffir lime (*Citrus hystrix* DC) essential oil spray formulations was evaluated over a 6-hour observation period. The total number of mosquito landings and the calculated hourly protection percentages are presented in **Table 4** and **Table 5**, respectively. The base formulation (F1; 0% essential oil) demonstrated a low mean protection rate of 12.46%. Increasing concentrations of essential oil produced a clear concentration-dependent improvement in repellent efficacy: formulations containing 5% (F2), 10% (F3), and 15% (F4) essential oil achieved mean protection rates of 35.89%, 57.76%, and 93.76%, respectively. The F4 formulation provided the highest level of protection, maintaining 100% protection during the first three hours and 93.33% at hour four before a gradual decline occurred.

Table 4. Total Number of Mosquito Landings for Each Treatment Group During 6-Hour Observation

Treatment groups	Total Number of Mosquito Landings at Hour–					
	1st Hour	2nd Hour	3rd Hour	4th Hour	5th Hour	6th Hour
C	8	8	16	16	18	27
F1	7	6	14	15	16	25
F2	3	3	11	12	13	13
F3	2	2	5	8	10	10
F4	0	0	0	1	3	3

Remarks: F1–F4 correspond to spray formulations containing 0%, 5%, 10%, and 15% kaffir lime essential oil, respectively. C represents the negative control.

Statistical evaluation confirmed these observations. Shapiro–Wilk tests indicated normal distributions for all groups ($p > 0.05$), and Levene's test showed homogeneity of variance ($p = 0.150$). One-way ANOVA detected a significant effect of formulation on protection ($p < 0.001$). Post-hoc pairwise comparisons revealed significant differences between all treatment pairs ($p < 0.05$), indicating that each incremental increase in essential-oil concentration produced a statistically meaningful improvement in protection.

Table 5. Protection Percentage of Kaffir Lime Essential Oil Spray Formulations Over 6 Hours

Treatment	Protection percentage (%)						Average (%)
	1st Hour	2nd Hour	3rd Hour	4th Hour	5th Hour	6th Hour	
F1*	12.50	25.00	12.50	6.25	11.11	7.41	12.46
F2*	57.14	50.00	21.43	20.00	18.75	48.00	35.89
F3*	71.43	66.67	64.29	46.67	37.50	60.00	57.76
F4*	100.00	100.00	100.00	93.33	81.25	88.00	93.76

Remarks: F1–F4 correspond to spray formulations containing 0%, 5%, 10%, and 15% kaffir lime essential oil, respectively. The asterisks on the formulations indicate significant differences.

The observed concentration-dependent efficacy is consistent with reports for other botanical repellents (e.g., citronella), where higher active concentrations yield greater protection.[44,55] The activity of kaffir lime oil likely reflects the combined effects of major volatile constituents identified in this study—citronellal, limonene, linalool, and α-pinene—which are known to disrupt mosquito olfactory and sensory pathways. In particular, linalool has documented repellent and contact-toxic properties that can provoke avoidance or knockdown in insects.[56,57]

Female *Aedes aegypti* (2–5 days old) were used in accordance with standard testing guidelines to ensure biological relevance.[58,59] Testing conditions (controlled lighting, standardized spray volume) were applied to minimize procedural variability and support comparability across formulations.

Temporal decline in protection is attributable to volatilization of active components—a recognized limitation of essential-oil–based repellents. While regulatory guidance ideally targets ≥90% protection for six hours, many plant-derived repellents fail to sustain that duration because of rapid evaporation.[55,60] In this study, F4's maintenance of >90% protection for ≈4 hours nevertheless represents superior short-term performance relative to several reported botanical formulations. To advance product development, further work should focus on extending residual activity (e.g., microencapsulation, use of fixatives or extender matrices), testing across larger sample sizes and additional mosquito species, and conducting comprehensive stability and safety assessments (including skin-irritation and microbial tests) to support commercialization and regulatory claims.

CONCLUSION

Based on the findings of this study, the essential oil of kaffir lime was identified to contain 90 distinct compounds, with the five most abundant constituents being D-limonene (14.57%), limonene (13.92%), citronellal (6.66%), terpinolene (6.47%), and α-terpineol (5.74%). The formulated kaffir lime essential oil spray demonstrated effective mosquito repellent activity. Among all formulations, F4—containing 15% kaffir lime essential oil—exhibited the highest repellent efficacy, providing 93.76% protection. These results indicate that kaffir lime essential oil possesses significant potential as a natural, plant-based mosquito repellent and may serve as a promising alternative to conventional synthetic repellents.

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CONFLICT OF INTEREST

The authors declare that they have no conflicts of interest related to this publication.

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